# MyAssays Desktop User's Guide 

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## Introducing MyAssays Desktop

MyAssays Desktop is an easy-to-use data analysis tool for bioassays.
MyAssays Desktop is the only desktop tool with an integrated search-and-download feature providing direct access to the growing online database of protocols from myassays.com. The preconfigured data analysis protocols cover popular applications and support for commercial assay kits (backed by leading kit manufacturers); these are all ready to download and integrate into your lab's workflow.

Beyond this, if your application is not already supported, the software provides a powerful yet easy-to-use system to build and customize your own data analysis protocols.

MyAssays Desktop provides first-class tools for:

- Data Import
- Data Visualization
- Data Analysis (including advanced Curve Fitting)
- Reporting
- Export
- Workflow Integration


## How to Use This Guide

We have arranged this guide to help you get started quickly and to simplify how you can find the information and details that you need.

This Introduction contains a summary of the key features and editions of the software.
The Getting Started section covers installation and general orientation information. It also includes a Quick Start section to show you where the basics are and to walk you through your first data analysis. It also provides links to further information and online video guides.

The Working with MyAssays Desktop section dives into further detail, covering each element in more detail with the content arranged into logical sections. This includes:

- getting around the various applications
- protocols
- common tasks (such as importing, exporting, curve fitting, marking outliers to exclude, and much more)

The final Reference section provides a more complete description of each facet. Here the content is arranged to simplify quick retrieval.

## What Should I Read?

We encourage all new users to read through all of the Introduction and Getting Started sections.

You will find more information about the topics and get a better understanding from the Working with MyAssays Desktop section. Feel free to skip sections here that are not relevant to your applications. If you're working with particular protocols or need to perform specific tasks, be sure to follow the relevant sections under Protocols and Common Tasks.

We don't expect you to read through the Reference section in order; it is more useful to consider it a resource for providing further details as and when you need them.

## Features

## Supported Measurements

MyAssays Desktop is designed to process measurement data from any microplate or rackbased reader. In addition, it can import and analyze data from any scientific measurement device.

The software supports measurements from any type of detection technology (such as absorbance, luminescence, fluorescence, counts, counts-per-minute), with data collected from each sample as:

- Endpoint - A single measurement value
- Kinetic - Values measured over time (data can be processed with or without time stamps)
- Spectral - Data measured at various multiple wavelengths
- Multiplex - Multiple analyte measurements from each sample

Data can be collected and processed together in any combination. For example, dual endpoint measurements read at both 665 nm and 620 nm can be imported and analyzed (perhaps with a ratio calculation). Even more exotic measurement combinations are possible with features to process multiple endpoint, kinetic, and spectral data all together within the same analysis.

## Multiple Plate Measurements

Data can be captured and processed from measurements made from a single microplate or from across multiple plates. Data to process together can be arranged in a single file or in separate files that can be imported and merged together. Curve fitting can be performed with standards defined on some or all plates, with curves computed using averages of standard measurements across plates or from the measurements on the same plate.

## Data Import

For maximum flexibility, MyAssays Desktop provides a number of ways to get your data into the system. These are designed to help you get started quickly and to streamline your workflow:

- In the simplest case, copy and paste your data from any source for quick and convenient analysis.
- The included extensive Import Script library can automatically detect and import data from many proprietary instrument formats. Simply select the file to import; if it's supported, the data and any metadata (data accompanying the read such as measurement date/time, barcode, etc.) are imported automatically. (For the latest list of currently supported formats, see: https://www.myassays.com/supported-file-formats.html.)
- If your data is in an Excel document, the import wizard will guide you through the process of importing the relevant data and meta data from the cells in your workbook. Mark the cells to import the data (with these settings saved to your protocol).
- For other files, use the intuitive and familiar import wizard to specify how to process and import the data from your files.

If your data is spread across multiple files, the import wizard can import your data together for a combined analysis.

## Importing Other Types of Data

In addition to first-class support for handling your measurement data, MyAssays Desktop supports importing other types of data, including:

- Measurement metadata (data associated with your readings, such as date/time, plate ID, operator ID, etc.)
- Worklist (e.g., containing sample IDs, dilution factors, standard concentrations)
- Plate name/IDs, barcodes, analyte names, etc.

Beyond this, it is also possible to import any data analysis parameters (including layout, calculation settings, fit method, etc.) from data stored or produced by other systems. In this way, your data analysis process can be fully automated or controlled by other software.

## Data Visualization

MyAssays Desktop provides a rich modern user interface to clearly present data, configuration, and analysis results, including:

- 2D and 3D interactive views of your measurement data
- Heatmap
- Histogram
- Interactive charts
- Overlay charts (e.g., by fit, by sample, by replicate) in any combination for convenient visual comparison
- Zoom in/out
- Mark outliers (to exclude from analysis) directly from a chart or plate (including outliers in endpoint, spectral, kinetic, and multiplex data)
- Specify data analysis ranges interactively (e.g., mark subsets of data to process for kinetic or spectral analysis)
- Sample labels for data
- By name
- By colour
- By graphical value bars


## Data Analysis

Beyond the online database of ready-to-use preconfigured protocols, MyAssays Desktop provides a comprehensive data analysis engine supporting a wide range of applications.

The system provides a toolbox of preconfigured components (including over 30 transforms) ready to bolt together in any combination. Each transform performs a single mathematical task on measurement or calculated data. Transforms include endpoint, kinetic/spectral, and kinetic/spectral reduction.

Transforms include:

- Blank Correction - Subtract a specified background value (or values) from all samples
- \%CV - Calculate percentage coefficient of variation of replicates
- Standard Curve Fit - Calculate concentrations from a standard curve fit
- Maximum Slope - Find the maximum slope and report its values (such as slope/time to onset/response)

For a complete list of the 30+ available transforms, see Transforms.
Transforms are linked to your sample layout. If your sample layout changes between assay runs (or even to correct a preparation error), the analysis takes this into account. No reconfiguration of analysis is required to work with different layouts.

The transforms parameters are completely customizable. In addition, a rich expression-based language enables you to specify parameters and methods as mathematical operations using an expressive familiar (Excel-like) syntax.

## Results with Labels

Label your results using your own text and highlights. For example, perhaps samples with a response or calculated concentration greater than a specified amount should be clearly indicated as such; use labels to define your own rules accordingly.

## Validation

Add automatic checking of measurements and results. For example, check that your samples are within an expected range by defining your own validation rules. These are evaluated with each run of your assay, with the results clearly presented as pass or fail in the report.

## Curve Fitting

MyAssays Desktop provides the curve fitting tools you would expect, plus more, including:

- Quantitative tools - Weighted and unweighted 4-parameter logistic, 5-parameter logistic, and linear regression (plus legacy methods such as cubic spline, smoothed spline, cubic regression, point-to-point, and many more)
- Fit data to your own user-defined curve fitting equation
- Best Fit feature automatically compares multiple fit methods and applies the best (with customizable scoring methods)
- Goodness of fit measures reported - $\mathrm{R}^{2}$, $\mathrm{aR}^{2}$, SE, F-statistic, P-value, AIC, AICc, BIC and more (and available for further automatic scrutiny such as validation messages or best fit scoring)
- Automatic computation of LLOQ, ULOQ, and LOD based on customizable precision and accuracy thresholds
- View computed curve fit equations in mathematical symbolic notation


## Reporting

For some applications, the most convenient type of report to work with is sheet-based; for other scenarios, a page-based structure is more suitable.

- Sheet - A spreadsheet structure comprising of a workbook of worksheets of cells
- Page - Structured ties to a fixed sized printed format (e.g., A4)

For these reasons, MyAssays Desktop supports both. Easily switch between report views to best work with your content as you need to.

## Excel Objects

Exported Excel content features native Excel objects (e.g., chart content). This means that you can edit and extend the output using the full power of Excel.

MyAssays Desktop protocols reports can be configured to include power-features such as value bars for enhanced viewing and visualization of content.

## Report Templating with Word

For page-based reporting, use the full power of Word to define a reporting template to apply to your MyAssays Desktop output. This can include any content you can create in Word, such as headers, footers, custom images, fonts, macros, signature line, etc.

Analysis results are merged into your Word template for maximum usercustomizable reporting output.

## Export

MyAssays Desktop provides a wide variety of export options and settings:

- Export to XLS/XLSX/PDF/DOCX/TXT/CSV/HTML/XML.
- XSLT support is provided, enabling custom export definitions to be created for exacting output as required by your existing system.
- Specify the export destination folder.
- Specify the naming convention of the output files, including references to imported meta data (for example, name your output files to match a barcode imported along with your measurement data).
- Configure a protocol to automatically export with these settings so no user intervention is required.


## Workflow Integration

MyAssays Desktop has been designed with your workflow in mind and provides a number of features and tools to integrate with other systems and streamline your process.

For routine data analysis, your reports can be generated without any user interaction required at all. For example, MyAssays Desktop data analysis can be performed completely automatically, with data (including any combination of measurement data, sample IDs, parameters, etc.) being imported, analyzed, exported, or printed as soon as a new measurement file is detected from your reader software.

## Command Driven

MyAssays Desktop analysis provides a complete command-line interface. Launch the analysis directly from another application or process. Specify measurements files to process, worklists to use, output options. All data analysis parameters can be sent from the command line.

## Launcher

To facilitate working with the command line, the user-friendly Launcher utility simplifies building your command line with a point-and-click interface.

## Folder Poll

To integrate more effectively with other systems (such as instrument control software), use the integrated Folder Poll tool to automatically launch MyAssays Data analysis as soon as a new data file is detected.

## XSLT Export

For integration with other systems, such as LIMS which might have very specific data formatting requirements, utilize the XSLT feature to output data in the exact format required. Example XSLT files are installed to help you get started.

## Advanced XML Configuration and Tooling

Inside the system, a well-design XML structure is used to define the data analysis of every assay. Most users will not need know about its existence, but for those specialist scenarios, advanced users can configure data analysis using a powerful built-in XML editor complete with inline help and auto-complete features.

## Which Edition is Right for Me?

To satisfy different needs and budgets, MyAssays Desktop is available in both Basic and Pro editions. Also, optional Add-Ons are available to cater for working with specific applications and environments.

MyAssays Desktop Basic
The Basic version is suitable for working with the preconfigured assays that are installed as part of MyAssays Desktop or those available for download from the growing assay database at MyAssays.com. With the Basic edition, it is easy to:

- Find and use an assay protocol
- Provide measurement data (via copy-and-paste or import)
- Input sample IDs
- Tweak basic protocol parameters such as fit method, sample concentrations, layout, etc.
- Calculate results
- Mark outliers to exclude from analysis
- Generate reports
- Export to Word/Excel/PDF
- Save versioned results


## MyAssays Desktop Pro

The Pro edition includes all of the Basic features plus a wealth of tools and functions to simplify building and integrating powerful data analysis and reporting into the workflow of the most demanding laboratory.

A key benefit with the Pro edition is the capability to build data analysis protocols from scratch or by adapting existing protocols. This is achieved through the provision of a well-designed toolbox of data analysis components ready to bolt together to meet the needs of a wide range of data analysis applications. This toolbox includes:

- Transforms - Utilize a library of over 30 transforms.
- Matrices - Configure each layer of the analysis, including numerical formatting, sample labels, and value bars.
- Evaluation - Specify custom evaluations using powerful expression-based library.
- Validation - Define rules to verify each time analysis is performed (e.g., checking raw or computed control values are in range, checking the \%CV of samples). The results are clearly presented as pass or fail.
- Report Elements - Customize the report content by adding the report elements that you need, including histograms, multiple sample tables, matrices, text, macros, and many more.

Also, the Pro edition provides power-features designed for the busy lab professional to improve productivity, including:

- Best Fit - Perform multiple fit methods and weight models on a single assay and automatically pick the best (the definition of "what is the best" can be customized).
- Standard Curve Fit from Library - Utilize a standards curve measured in a previous assay run (to save costs of reagents).
- Folder Poll - Streamline your workflow so that data analysis (or other operations) are performed automatically as soon as new measurement files are detected using the integrated folder polling tool.
- Multiple Plate Support - Analyze measurement data that spans more than one plate.
- Custom Export - Configure the export for working with third-party systems such as LIMS.

Beyond that, Pro Add-Ons are available to target specific assay and instrument types, enhance data security, or streamline inter-assay quality control.

To view complete list of features available for each edition, see Feature Comparison Matrix.

## Licensing

Single, multiple seat, and site licenses are available for all editions and add-ons.

## Pro Add-Ons

MyAssays offers a range of optional tools that provide data analysis solutions tailored to your lab. Add-ons extend Pro functionality customizing it to fit your laboratory's exact needs.

## Enhanced Security

For those users working under GxP or 21CFR11, the Enhanced Security edition offers the features that are necessary to achieve compliance. The software provides protected and tracked access to system features inside a secure environment with:

- Access control - Authentication and authorization are used to permit or deny access to MyAssays Desktop Pro functions.
- Operating-system-level security-data storage protection - Only authorized users can make changes to MyAssays Desktop Pro data files and settings.
- Audit trail and login log - All activity is stored against the logged-in user with a date/time stamp.
- Data certification - Restrict the system to handle only certified data (eliminating the processing of tampered data).
- Password protected and locked outputs (e.g., locked Excel and Word outputs).


## Multiplex

Quickly and easily perform multiplex data analysis on your multiplex data:

- Calculate results from multiple analytes within a single analysis step.
- For quantitative analysis, apply the same curve-fitting method to each analyte or use the Best Fit option to automatically apply the most appropriate fit method for each analyte.
- Specify concentration values as a series (defining the starting concentration of each analyte and the series) or enter specific concentrations for each analyte.
- Specify units per analyte or common units.
- Paste in or import measurement data, analyte names, starting concentration values, or specific concentration values.
- Includes import methods to automatically extract multiplex measurement data and analyte names from Luminex ${ }^{\circledR}$ xPONENT and Meso Scale Discovery Workbench ${ }^{\circledR}$ CSV and TXT formats.


## QC

The QC option provides a range of inter-assay analysis features to support the continuousmonitoring automatic validation that your assay remains in control:

- Monitor, track, and chart observations across assay runs.
- Any numerical result can be tracked. For example, monitor raw or calculated control values, \%CVs, or curve $\mathrm{R}^{2}$ values.
- Include Levey-Jennings and CUSUM charts as part of each assay report to compare and visualize the latest results with historical data.
- Generate QC reports on-the-fly as and when needed.
- Automatic validation can ensure that the monitored observations are within defined acceptance parameters. Violations are clearly indicated in the report. For example, an acceptance rule can be defined to test whether a control result of a particular assay run is within a range of $+/-2 *$ SD of the historical observations.


## Rack Layouts

If your reader measures samples in tubes or vials (rather that microplates), then the Rack Add-On is for you:

- Rack Layout Editor - Define layouts in accordance with racks of tubes or vials.
- Dynamic sample layouts - Layouts are automatically adjusted based on the number of samples actually measured.
- Support for microplate layouts included (for the multifunctional lab).


## Feature Comparison Matrix

Please refer to the table below to learn which features are available with your edition.

| Feature | Basic | Pro |
| :---: | :---: | :---: |
| Downloadable Protocols (from myassays.com) | $\checkmark$ | $\checkmark$ |
| Installed Protocols: | $\checkmark$ | $\checkmark$ |
| - 4PL (Constrained by B0 and NSB) Quantification | $\checkmark$ | $\checkmark$ |
| - 4PL Quantification | $\checkmark$ | $\checkmark$ |
| - Alpha 384-well Screening | $\checkmark$ | $\checkmark$ |
| - ATP Lite | $\checkmark$ | $\checkmark$ |
| - Best-Fit Quantification | ص | $\checkmark$ |
| - Cell Confluency Assay Development | $\checkmark$ | $\checkmark$ |
| - DNA 260nm | $\checkmark$ | $\checkmark$ |
| - DNA Quantification | $\checkmark$ | $\checkmark$ |
| - Dual DPM | $\square$ | $\checkmark$ |
| - Dose-Response Curves for EC50 | ص | $\checkmark$ |
| - ELISA | $\checkmark$ | $\checkmark$ |
| - ELISA - Single Page Report | $\checkmark$ | $\checkmark$ |
| - LANCE cAMP | $\checkmark$ | $\checkmark$ |
| - Linearized (Logit-Log) Quantification | $\checkmark$ | $\checkmark$ |
| - Liquid Handling QC | $\checkmark$ | $\checkmark$ |
| - Multiplex | ص | $\checkmark$ |
| - OQ Absorbance | $\checkmark$ | $\checkmark$ |
| - Standards Curve with Titres and Parallelism Plots (Dual) | $\square$ | $\checkmark$ |
| - Z-Factor | $\checkmark$ | $\checkmark$ |
| Import Script Support | $\checkmark$ | $\checkmark$ |
| Excel Import | $\checkmark$ | $\checkmark$ |
| Worklist Import | $\checkmark$ | $\checkmark$ |
| Data Visualization (3D View/Heatmap/Kinetic Overlay/Spectral Plot) | $\checkmark$ | $\checkmark$ |
| Versioned Analysis | $\checkmark$ | $\checkmark$ |
| Export to XLSX/DOCX/PDF | $\checkmark$ | $\checkmark$ |
| Edit Protocols | Limited | $\checkmark$ |
| Report Customization | Limited | $\checkmark$ |
| Custom Export (e.g., for LIS/LIMS) | $\square$ | $\checkmark$ |
| Multiple Plate Support | $\square$ | $\checkmark$ |


| Feature | Basic | Pro |
| :---: | :---: | :---: |
| Create New Protocols (New Protocol Wizard) | $ص$ | $\checkmark$ |
| Matrix-Transform Data Analysis | ص | $\checkmark$ |
| Custom Validation Rules | ص | $\checkmark$ |
| Custom Evaluation Expressions | $\square$ | $\checkmark$ |
| Best Fit | $\square$ | $\checkmark$ |
| Fit to Library Curve | $\square$ | $\checkmark$ |
| Folder Poll | ص | $\checkmark$ |
| PLA | $\square$ | $\checkmark$ |

## Getting Started <br> Installation

## System Requirements

MyAssays Desktop runs successfully on the minimum system configuration listed below. If your computer does not meet the technical requirements, you may be not able to use MyAssays Desktop.

## CPU:

Memory:
Available disk space:
Operating systems:
Other software:
Display:

Intel/AMD 2 GHz or faster processor (32-
bit or 64-bit)
2GB RAM
300MB
Windows 10, Windows 8.x, Windows 7
Microsoft .NET 4.7.2 or later
$1024 \times 768$ or higher resolution

## Software Requirements

Microsoft .NET 4.7.2 or later must be installed (this is pre-installed on newer Windows 10 systems). For earlier versions of Windows, if it is not already installed on your system or you are not sure, please download and install from:
http://go.microsoft.com/fwlink/?Linkld=863262.
More information about the .NET 4.7.2 installation can be found at:
https://support.microsoft.com/en-gb/help/4054530/microsoft-net-framework-4-7-2-offline-installer-for-windows.

## Installation Process

The latest version of MyAssays Desktop can be downloaded here.

## Upon downloading the installation package:

1. Double-click the installation package file to launch MyAssays Desktop Setup Wizard and click Next to continue.
2. To proceed with installation on your computer, you must accept the terms of End-User License Agreement. Please read the license agreement carefully, then select I accept the terms of the license agreement and click Next.
If you do not agree with terms of the license agreement, click Cancel to exit the wizard.
3. If you are content with the default path, click Next. To pick another destination folder, click Change and set preferable root.
4. Select the choices you made and click Install. The process will take a few moments.
5. Click Finish to complete the installation. Select Launch MyAssays Explorer on exit to start working with the software immediately.
6. Activate MyAssays Desktop with your product serial code and activate your license in order to start working with the application.

MyAssays Desktop can be installed and run alongside the legacy software WorkOut.
Refer to Installing Alongside WorkOut for further details.

## Launching

After installation, you will see the MyAssays Desktop Explorer icon on your desktop. To launch the application, click it or:

## On Windows 10:

Select Start, then scroll down to and click the MyAssays Desktop Explorer icon.

## For earlier versions of Windows:

- Windows 8.1 or Windows 8: Swipe up or choose the arrow at the bottom of the Start screen to see a list of all your applications. You might need to scroll left or right to see the MyAssays Desktop Explorer.
- Window 7: Choose Start > All Programs to see a list of all your applications and click the MyAssays Desktop Explorer icon.

To access MyAssays Desktop Explorer more conveniently, you can pin it to your Start menu or taskbar. Right-click the desktop icon and click Pin to Start or Pin to Taskbar.

## Product Licensing

MyAssays Desktop requires a valid license to activate the application. You can obtain a trial, temporary, or permanent license from MyAssays Ltd.

A serial code represents your license or licenses (for multiple seat site licenses) to use the software. When you first launch the software, you must enter a valid serial code and activate your license.

A single license can be transferred between systems but can only be active on one system at a time.

## License Activation

On launching MyAssays Desktop, if there is no valid license already installed, then the Licensing Activation Wizard will appear.

An administrator account is required to activate (or deactivate) a license.
If you have a serial code, select I have a serial code to activate and click Next.

If you do not yet have a serial code, then you can obtain a trial serial code from
https://www.myassays.com/download-and-install-myassays-desktop.html by clicking the Claim Key option.

Paste in the serial code along with your email address into the Licensing Activation Wizard and click Next.

A working valid email address is required to administer your license. Access to this email account will be required to transfer your license to another system in the future.

## Online Activation

If your PC is connected to internet:

1. Select Use internet connection for activation and click Continue to proceed.
2. Fill in your personal details and click Activate.
3. When prompted to grant User Account Control permission to access the licensing settings, confirm by selecting Yes.
4. If the license activation is accessed, a confirmation window appears.
5. Click Close to exit the License Activation Wizard and start the application.

## Offline Activation

If your PC is not connected to internet, it is still possible to activate MyAssays Desktop using a separate internet-connected device. In this case:

1. Clear the Use internet connection for activation check box and click Continue to proceed.
2. A QR code will appear; if you have a network-connected device (such as a smartphone or tablet) capable of reading this, then scan the QR code and follow the instructions. Alternatively, copy and paste the provided link and open it on a system with internet connection (perhaps by transferring via USB memory device).
3. On successful activation of your serial code from your internet-connected device, a license key text string will be generated (with a copy sent to the provided email address).
4. Transfer this text string to your PC where MyAssays Desktop is installed and paste in the License Activation Wizard under the license key setting.
5. When prompted, grant User Account Control permission to access the licensing settings and confirm by selecting Yes.
6. If the license activation is accessed, a confirmation window appears.
7. Click Close to exit License Activation Wizard and start the application.

## Multiple Seat Licenses

A multiple seat license can be used to install and use the software on multiple PCs at the same time.

With a multiple seat license, the same serial code is used to activate MyAssays Desktop Pro on up to as many seats as you have purchased.

In this case, the email address used to activate the first seat will be treated as the primary email address. Supply this address when activating other seats with the same license. It's not possible to activate other seats with a different email address.

We recommend using a permanent email address of an administrator as the primary email address for activating each seat.

## Licensed Features

There are various editions of MyAssays Desktop as well as optional add-ons. The features available to you will depend on your license. To see which features are included in your license:

1. Launch MyAssays Desktop Explorer.
2. Click the File tab to open MyAssays Backstage view and click the Information tab.

The availability of licensed features is marked with the following icons:
for active features.
$\mathbb{X}$ for features not supported by your current license.
To learn more about additional features, which are not included in your existing MyAssays Desktop license, click the Upgrade License button.

## License Deactivation

If you need to transfer an activated license to another PC, it must first be deactivated from the PC where it is in use.

To deactivate MyAssays Desktop on your PC, launch the Deactivation Wizard as follows:

1. Launch MyAssays Desktop Explorer.
2. Click the File tab to open MyAssays Backstage view.
3. On the Information tab, click Deactivate License.

Follow the steps of the License Deactivation Wizard as described below to deactivate the license:

## Online Deactivation

If your PC is connected to internet:

1. Keep the Use internet connection for deactivation selected and click Continue to proceed with deactivation.
2. Confirm deactivation and click Deactivate.
3. When prompted to grant User Account Control permission to access the licensing settings, confirm by selecting Yes.
4. On successful deactivation, a copy of the license serial code will be sent to the email address it is associated with, ready for activation on another system.
5. Click Finish to close the License Deactivation Wizard.

## Offline Deactivation

If your PC is not connected to internet, it is still possible to deactivate MyAssays Desktop using a separate internet-connected device. In this case:

1. Clear the Use internet connection for deactivation check box and click Continue to proceed with deactivation.
2. Confirm deactivation and click Deactivate.
3. When prompted to grant User Account Control permission to access the licensing settings, confirm by selecting Yes.
4. A QR code will appear; if you have a network-connected device (such as a smartphone or tablet) capable of reading this, then scan the QR code and follow the instructions.

Alternatively, copy and paste the provided link and open it on a system with internet connection (perhaps by transferring via USB memory device).
5. On successful deactivation of your serial code, a confirmation message will appear in the browser and a copy of the license serial code will be sent to the email address it is associated with, ready for activation on another system.
6. Confirm that the email has been received in the License Deactivation Wizard and click Finish.

After successful deactivation, the application will be closed and will not be usable until it is reactivated. The deactivated license will be immediately available for activation on another system.

## Activate New License

When you obtain a new serial code (perhaps as the result of a purchased upgrade or support extension), it will be necessary to replace an existing license with a new one.

Follow these steps to activate your new license:

1. Launch MyAssays Desktop Explorer.
2. Click the File tab to open MyAssays Backstage view.
3. On the Information tab, click Activate New License.
4. Click Yes to confirm the removal of the existing license.
5. When prompted to grant User Account Control permission to access the licensing settings, confirm by selecting Yes.
6. Follow the steps of the License Activation Wizard as described in the License Activation section to activate new license.
7. On completion of the wizard, any new license updates will be applied to your installation and any new features or support extensions will be available.

## Update Existing License

If new features have been added to your existing license (perhaps as the result of a purchased upgrade or support extension), then it will be necessary to update your activated license with the latest information.

1. Launch MyAssays Desktop Explorer.
2. Click the File tab to open MyAssays Backstage view.
3. On the Information tab, click Update Existing License.
4. If your PC is connected to the internet, then select Update my license online in one click. Otherwise, select Get a unique link to my activation code and activate using a separate device that is online (as described in License Activation).
5. When prompted to grant User Account Control permission to access the licensing settings, confirm by selecting Yes.
6. On completion of the wizard, any new license updates will be applied to your installation and any new features or support extensions will be available.

## Quick Start

## MyAssays Desktop - The Basics

This section introduces the basics of the MyAssays Desktop system by providing a summary of the key features and functions and where to find them.

This section is intended to be short to help equip you with the basics so that you can start to be productive with the software quickly and feel comfortable to explore further functions yourself.
Firstly, MyAssays Desktop provides two key applications to help organise and work with your data:

- MyAssays Desktop Explorer (MADE) - For arranging, locating, discovering, and launching your assay data
- MyAssays Analysis (MAA) - For performing the assay data analysis

The following subsections provide orientation details for working with both of these applications.

- Introducing MyAssays Desktop Explorer
- Introducing MyAssays Analysis


## Introducing MyAssays Desktop Explorer

## Orientation

The Welcome screen is displayed on launching MADE. Its main area provides shortcut links to:

- Previously used protocol and results files
- Online resources including video tutorials
- The protocols and results folders
- The New Protocol wizard (Pro versions only)

Along the left side of the MADE window, you will see tabbed sections to conveniently access the folders that contain the various types of assay data.

Along the top of the MADE window, you will see the ribbon providing various commands and functions for navigating and managing your data.

Here you can find the Search MyAssays.com control, which provides a convenient function to search the online database of preconfigured protocols. Simply enter any appropriate search term such as assay or kit name, product or kit manufacturer name to search for available downloadable content.

## Assay Data

If you've just installed MyAssays Desktop, you will find some installed assay protocols and layouts under their respective tabs. The results and reports tabs will be empty until you've ran some analyses and created some reports.

As you run analyses and create various content, MyAssays Desktop will automatically store content in the associated folders.

## Where is My Data?

It is worth noting that all of your MyAssays data is stored under a single parent folder. This makes backing up your data a simple process of copying all of the data in this folder. The default location of MyAssays data is the subfolder located under your Windows Documents folder.

You may prefer to store your data in a location different to this default location. For example, you may want to:

- Use a common location shared with your colleagues
- Store your data in another location

To access this location setting, select File | Data, the Assay Data Folder Root section reports the current setting. Use the Select Folder Root option to change this as required. This setting is stored with your Windows login account (so will be remembered the next time you log in).

You can of course access all of your data outside of MADE and in Windows Explorer, simply navigate to assay data folder root directory.

## Introducing MyAssays Analysis

## Orientation

The MAA application is displayed when launching an assay protocol or opening an existing assay results file.

The Overview tab is typically selected when launching MAA. This tab displays name and any description of the launched assay. It might also include a link to online content (such as kit manufacturer's product insert).

Along the left side of the MAA window, you will see various tabbed sections for providing and reviewing the data and results for the analysis. All assays will contain the tabs:

## - Overview

- Measurements - From here you can provide and review your measurements (and any metadata). Various subtabs are provided for viewing your data in different formats.
- Results - After performing calculations, the results of the analysis will appear in this tab. This will include the report. If the report is arranged as multiple worksheets, you will see worksheet tabs at the bottom of this area for navigating between worksheets. Also, depending on the analysis performed, you might see various subtabs appearing to the right of a Report subtab; these provide interactive controls for working with your data in various ways (such as flagging outliers from a Standard Curve Fit).

In addition to the Overview, Measurements and Results tabs, your assay protocol might be configured to include further tabs such as:

- Microplate - For defining, reviewing, and managing the sample layout.
- Sample IDs - For providing ID values for each unknown sample.
- Dilution Factors - For providing dilution factors for each unknown sample. These will be applied to the concentration value computed from the curve.
- Standard Concentrations - For providing known concentration values for each standard (calibrator) sample.

There might also be further tabs displayed for providing data specific to the assay.

## Assay Protocol and Assay Results

When an assay protocol is launched, MAA operates in protocol mode. In protocol mode you can provide the data for your analyses and optionally save the settings to the protocol file.

Provide your analysis assay parameters along with your measurement data (or use the provided example data) and then click Calculate to create the first set of results.

For all assay protocols, typical example data is provided to help you familiarise yourself with the analysis when you don't have any data to hand.

## Clicking the Calculate button:

- Performs the calculations
- Generates the report
- Performs any configured export operations

For the first set of calculations, some additional steps are automatically performed as MAA transitions from protocol to results mode:

- The results appear in the Results tab.
- An assay results file is created. This contains a copy of the assay protocol that was launched and all of the data provided (measurements and analysis parameters).
- The assay results file is automatically named and saved on the first calculation.


## The file naming method can be changed under File | Save \| Results Naming.

## Results

The calculated results can be found under the Results tab. If the analysis generates charts, then these will also appear in subtabs under Results.

When there are multiple subtabs, the report can be found under the Report subtab. If the report is arranged into multiple worksheets (in Sheet View), then the sheet can be selected using the sheet names appearing at the bottom of the Report subtab.

## Page View or Sheet View

The report can be displayed as Sheet View or Page View; these settings are available through the Ribbon's Report tab.

When getting started, we suggest using the Sheet View so that your outputs are not confined by the limitations of a printed page. Use the Page View only if and when the layout of the printed page is important (For page-based reporting, you can define a reporting template to apply to your MyAssays Desktop output).

## Save the Changes to the Protocol?

Before the first calculation is performed (protocol mode) any interactions performed apply to the launched protocol. If you perform the Save operation at this point, then the changes will be saved, updating the original protocol file. Also, on the first set of calculations the settings are automatically saved, updating the protocol.
This workflow might be useful if you want your changes to apply to future runs of the protocol (so you don't need to repeat the configuration again next time). Alternatively, you might want the changes to only apply to this particular analysis.

For example, perhaps you are running a routine assay but have used a slightly different sample layout than you usually use. You might want to apply this change to the current run only, or you may decide to use this layout in future runs too.

To configure the behaviour that best suits you, select File \| Save and set the Save changes to the protocol option as you require. With this option set, any changes you make to the launched protocol are saved to the protocol. The changes are also save to the protocol on the first calculation (as the system transitions from protocol to results mode). Any further changes in results mode will then only apply to the results.

This Save changes to the protocol setting is stored across sessions so it will apply to any other protocols you launch in the future.

## Further Save Options

Under the File | Save area, you will see further self-explanatory save options:

- Save
- Save Results As
- Save Protocol As

Note that the Save Protocol As option can be used when viewing results to extract all of the current configuration settings to a new protocol file. This is useful to package up all of your recent changes made to a results file to a new protocol file.

## Results Naming

Before any calculations are performed (protocol mode), the Results Naming option is available to enable you to specify how the created assay results file is named and stored. For example, you could name the results file using metadata imported from the measurements data file and/or customize the subfolder where it is stored.

Save the changes to the protocol so any future runs of the protocol will follow the naming convention you have specified.

## Calculate

Click the Calculate button when you are ready to calculate the results. In case this is not obvious, if you make any changes to the data or parameters, you will be reminded to perform the calculations when you are ready.

MAA does not perform calculations automatically on every setting change because this could impact on your experience of working with the software. With larger data sets (such as multiplex, kinetic or multiple plate assays) it might take a few seconds to perform the analysis.

## Result Versions

Each time the Calculate button is clicked, a new version of results is created. You can find the version controls including the full version history of the results in the Home | Versions ribbon group.

To view a different version, use the Current Version selector. Changing a version will update the whole of the MAA application to reflect the newly selected version; all data views reflect the data and results for the selected version.

If you have made changes but not yet calculated the results of these changes, then the Current Version selector will display the new version number with the text (pending) following it. This is to indicate that the new results are pending calculation. Click Calculate to create the new results version.

If you change version when results are pending calculation, then those changes will be lost (a warning message will prompt for confirmation before this action is completed).


You cannot make changes to an earlier previously calculated version; any changes made will apply to a new result versions pending calculation.

In addition, you can remove unwanted versions using:

- Purge - Removes all versions except the one currently selected.
- Rollback - Removes all versions that follow the selected version.


## Video Tutorials

For the latest video content please visit https://www.myassays.com/myassays-desktop-training-course.html

Selected video links are provided here:

## Getting Started

- https://www.myassays.com/video/myassays-desktop-installation (3 min 23 sec )
- https://www.myassays.com/video/licensing-myassays-desktop-with-internet-connectivity (2 min 17 sec )
- https://www.myassays.com/video/licensing-myassays-desktop-without-internetconnectivity ( 3 min 18 sec )
- https://www.myassays.com/video/get-started-with-myassays-desktop-(concepts-andprocesses) ( 9 min 53 sec )
- https://www.myassays.com/video/get-started-with-myassays-desktop-(using-thesoftware) ( 5 min 24 sec )
- https://www.myassays.com/video/myassays-desktop-explorer (4 min 30 sec)
- https://www.myassays.com/video/download-protocols-for-myassays-desktop (3 min 47 sec)
- https://www.myassays.com/video/create-a-protocol-with-myassays-desktop (11 min 33 $\mathrm{sec})$
- https://www.myassays.com/video/introduction-to-assay-validation-(elisa) (12 min 13 sec )
- https://www.myassays.com/video/introduction-to-assay-validation-(ria) (6 min 58 sec )
- https://www.myassays.com/video/whats-new-in-myassays-desktop-r4 (8 min 7 sec )
- https://www.myassays.com/video/whats-new-in-myassays-desktop-r5 (6 min 20 sec )
- https://www.myassays.com/video/whats-new-in-myassays-desktop-r6 (8 min 12 sec )
- https://www.myassays.com/video/whats-new-in-myassays-desktop-r7 (7 min 8 sec )


## Essentials

- https://www.myassays.com/video/get-started-with-myassays-desktop-(concepts-andprocesses) ( 9 min 53 sec )
- https://www.myassays.com/video/get-started-with-myassays-desktop-(using-thesoftware) ( 5 min 24 sec )
- https://www.myassays.com/video/myassays-desktop-plate-layout-editor (4 min 57 sec )
- https://www.myassays.com/video/myassays-desktop-rack-layout-editor ( 5 min 46 sec )
- https://www.myassays.com/video/import-data-with-myassays-desktop (9 min 33 sec )
- https://www.myassays.com/video/create-a-protocol-with-myassays-desktop (11 min 33 sec)
- https://www.myassays.com/video/myassays-desktop-standard-curve-fit-transform (8 min 45 sec )
- https://www.myassays.com/video/myassays-desktop-fit-methods (9 min 16 sec )
- https://www.myassays.com/video/myassays-desktop-transforms (7 min 2 sec )
- https://www.myassays.com/video/best-fit-with-myassays-desktop (4 min 29 sec )
- https://www.myassays.com/video/reporting-with-myassays-desktop-(an-introduction) (2 $\min 20 \mathrm{sec})$
- https://www.myassays.com/video/data-export-with-myassays-desktop (5 min 14 sec )
- https://www.myassays.com/video/elisa-analysis-with-myassays-desktop (11 min 45 sec )
- https://www.myassays.com/video/ria-data-analysis-with-myassays-desktop (6 min 15 sec)
- https://www.myassays.com/video/qualitative-assays-and-data-qualification-with-myassays-desktop ( 3 min 33 sec )
- https://www.myassays.com/video/myassays-desktop-sample-labels (5 min 15 sec )
- https://www.myassays.com/video/myassays-desktop-expressions (7 min 29 sec )
- https://www.myassays.com/video/kinetic-and-spectral-data-analysis-with-myassaysdesktop (4 min 52 sec )
- https://www.myassays.com/video/myassays-desktop-with-folder-poll (4 min 33 sec )
- https://www.myassays.com/video/migrating-from-a-legacy-system-to-myassays-desktop ( 12 min 25 sec )


## Basics

- https://www.myassays.com/video/download-protocols-for-myassays-desktop (3 min 47 sec)
- https://www.myassays.com/video/import-data-with-myassays-desktop (9 min 33 sec )
- https://www.myassays.com/video/create-a-protocol-with-myassays-desktop (11 min 33
sec)
- https://www.myassays.com/video/myassays-desktop-standard-curve-fit-transform (8 min 45 sec )
- https://www.myassays.com/video/myassays-desktop-fit-methods (9 min 16 sec )
- https://www.myassays.com/video/myassays-desktop-transforms (7 min 2 sec )
- https://www.myassays.com/video/myassays-desktop-expressions (7 min 29 sec )
- https://www.myassays.com/video/myassays-desktop-sample-labels (5 min 15 sec )
- https://www.myassays.com/video/reporting-with-myassays-desktop-(an-introduction) (2 $\min 20 \mathrm{sec})$
- https://www.myassays.com/video/data-export-with-myassays-desktop (5 min 14 sec )
- https://www.myassays.com/video/quality-control-with-myassays-desktop (8 min 22 sec )
- https://www.myassays.com/video/myassays-desktop-with-folder-poll (4 min 33 sec )
- https://www.myassays.com/video/qualitative-assays-and-data-qualification-with-myassays-desktop ( 3 min 33 sec )
- https://www.myassays.com/video/myassays-desktop-dynamic-worklist-import (6 min 34 $\mathrm{sec})$
- https://www.myassays.com/video/introduction-to-assay-validation-(elisa) (12 min 13 sec )
- https://www.myassays.com/video/introduction-to-assay-validation-(ria) (6 min 58 sec )
- https://www.myassays.com/video/edit-overview-and-resource-references-in-myassaysdesktop ( 1 min 58 sec )


## Working with Microplate Assays

- https://www.myassays.com/video/myassays-desktop-plate-layout-editor (4 min 57 sec )
- https://www.myassays.com/video/myassays-desktop-plate-layout-editor-(for-multiple-plate-analysis) ( 7 min 54 sec )
- https://www.myassays.com/video/elisa-analysis-with-myassays-desktop (11 min 45 sec )
- https://www.myassays.com/video/multiple-plate-analysis-with-myassays-desktop (6 min 25 sec )
- https://www.myassays.com/video/multiple-plate-analysis-with-myassays-desktop-(different-standard-concentrations-per-plate) ( 3 min 16 sec )
- https://www.myassays.com/video/myassays-desktop-fit-methods (9 min 16 sec)
- https://www.myassays.com/video/troubleshooting-multiple-samples-outside-curve-limits-with-myassays-desktop ( 5 min 10 sec )


## Working with Multiplex Assays

- https://www.myassays.com/video/multiplex-data-analysis-with-myassays-desktop (9 min $25 \mathrm{sec})$
- https://www.myassays.com/video/luminex-xponent-multiplex-data-analysis-with-myassays-desktop ( 10 min 12 sec )


## Working with RIA

- https://www.myassays.com/video/migrating-from-a-legacy-system-to-myassays-desktop ( 12 min 25 sec )
- https://www.myassays.com/video/ria-data-analysis-with-myassays-desktop (6 min 15 sec)
- https://www.myassays.com/video/myassays-desktop-rack-layout-editor (5 min 46 sec )
- https://www.myassays.com/video/myassays-desktop-fit-methods (9 min 16 sec )
- https://www.myassays.com/video/wizard2-data-import-with-myassays-desktop (7 min 41 $\mathrm{sec})$
- https://www.myassays.com/video/introduction-to-assay-validation-(ria) (6 min 58 sec )
- https://www.myassays.com/video/quality-control-with-myassays-desktop (8 min 22 sec )
- https://www.myassays.com/video/wizard2-integration-with-myassays-desktop-(1-of-3) (2 $\min 48 \mathrm{sec}$ )
- https://www.myassays.com/video/wizard2-integration-with-myassays-desktop-(2-of-3) (7 $\min 9 \mathrm{sec})$
- https://www.myassays.com/video/wizard2-integration-with-myassays-desktop-(3-of-3) (7 $\min 28 \mathrm{sec})$


## Kinetic/Spectral Analysis

- https://www.myassays.com/video/kinetic-and-spectral-data-analysis-with-myassaysdesktop ( $4 \min 52 \mathrm{sec}$ )
- https://www.myassays.com/video/myassays-desktop-xy-data-analysis-range (3 min 36 sec)
- https://www.myassays.com/video/curve-fitting-to-xy-data-with-myassays-desktop (4 min

13 sec )

- https://www.myassays.com/video/myassays-desktop-xy-reduction-peak-transform (10 min 19 sec )
- https://www.myassays.com/video/enzyme-kinetics-with-myassays-desktop (11 min 55 sec)
- https://www.myassays.com/video/myassays-desktop-xy-reduction-transforms (17 min 16 $\mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-xy-plot-report-element (3 min 35 $\mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-xy-replicates-function-transform (2 $\min 16 \mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-xy-reduction-function-transform (3 $\min 23 \mathrm{sec})$


## Scan Well Analysis

- https://www.myassays.com/video/well-scan-data-analysis-with-myassays-desktop (7 min $23 \mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-scan-blank-correction-transform (2 min 33 sec )


## Configuring Data Calculations

- https://www.myassays.com/video/myassays-desktop-transforms (7 min 2 sec )
- https://www.myassays.com/video/myassays-desktop-matrices (5 min 35 sec )
- https://www.myassays.com/video/myassays-desktop-sample-labels (5 min 15 sec )
- https://www.myassays.com/video/myassays-desktop-evaluations (10 min 43 sec )
- https://www.myassays.com/video/validation-with-myassays-desktop (6 min 40 sec )
- https://www.myassays.com/video/myassays-desktop-expressions (7 min 29 sec)
- https://www.myassays.com/video/myassays-desktop-expression-transforms (9 min 29 $\mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-sample-label-expressions (6 min 48 $\mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-validation-expressions (7 min 11

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\text { User's Guide }
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sec)

- https://www.myassays.com/video/myassays-desktop-evaluation-expressions (7 min 51 sec)
- https://www.myassays.com/video/myassays-desktop-reference-curve-overlay (5 min 11 sec)
- https://www.myassays.com/video/myassays-desktop-custom-fit (2 min 55 sec )
- https://www.myassays.com/video/troubleshooting-multiple-samples-outside-curve-limits-with-myassays-desktop (5 min 10 sec)
- https://www.myassays.com/video/quality-control-with-myassays-desktop (8 min 22 sec)
- https://www.myassays.com/video/myassays-desktop-scatter-plot-xy-report-element (5 min 53 sec$)$
- https://www.myassays.com/video/myassays-desktop-multiple-standard-curve-fits-on-a-single-plate (14 min 37 sec )
- https://www.myassays.com/video/adding-conditional-transforms-with-myassays-desktop (3 min 47 sec )
- https://www.myassays.com/video/adding-ec-and-ic-values-with-myassays-desktop (4 min 57 sec$)$
- https://www.myassays.com/video/adding-ec-and-ic-values-with-myassays-desktop-r7 (4 min 37 sec )
- https://www.myassays.com/video/add-evaluations-to-dose-response-curves-in-myassays-desktop (4 min 40 sec)
- https://www.myassays.com/video/reference-limits-of-quantification-in-myassays-desktop ( 5 min 12 sec )
- https://www.myassays.com/video/myassays-desktop-posvaluebyindex-function (3 min 57 sec)

Reporting

- https://www.myassays.com/video/reporting-with-myassays-desktop-(an-introduction) (2 min 20 sec$)$
- https://www.myassays.com/video/myassays-desktop-report-elements (4 min 55 sec)
- https://www.myassays.com/video/sheet-reports-with-myassays-desktop (6 min 38 sec)
- https://www.myassays.com/video/page-reports-with-myassays-desktop (7 min 24 sec)
- https://www.myassays.com/video/sample-table-ordering-with-myassays-desktop (4 min $42 \mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-sample-labels (5 min 15 sec)
- https://www.myassays.com/video/myassays-desktop-matrix-references-in-sample-labels (4 min 28 sec )
- https://www.myassays.com/video/sample-labels-column-with-myassays-desktop (5 min $2 \mathrm{sec})$
- https://www.myassays.com/video/ordering-field-data-with-myassays-desktop (4 min 12 $\mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-reference-curve-overlay (5 min 11 $\mathrm{sec})$
- https://www.myassays.com/video/chart-appearance-editor-in-myassays-desktop (3 min $59 \mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-results-naming-with-meta-data (5 $\min 11 \mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-scatter-plot-xy-report-element (5 $\min 53 \mathrm{sec}$ )
- https://www.myassays.com/video/myassays-desktop-curve-overlay (6 min 27 sec)
- https://www.myassays.com/video/myassays-desktop-xy-plot-report-element (3 min 35 sec)
- https://www.myassays.com/video/editing-multiple-matrices-and-report-elements-with-myassays-desktop (3 min 21 sec )
- https://www.myassays.com/video/myassays-desktop-report-scaling-and-sheet-ordering ( 3 min 37 sec )


## Transforms

- https://www.myassays.com/video/myassays-desktop-standard-curve-fit-transform (8 min 45 sec )
- https://www.myassays.com/video/myassays-desktop-general-curve-fit-transform (7 min $2 \mathrm{sec})$
- https://www.myassays.com/video/dose-response-curve-analysis-with-myassays-desktop ( 6 min 3 sec )
- https://www.myassays.com/video/dose-response-curve-parameters-with-myassays-
desktop (2 min 19 sec )
- https://www.myassays.com/video/updates-to-dose-response-curves-in-myassaysdesktop ( 6 min 25 sec )
- https://www.myassays.com/video/curve-fitting-to-xy-data-with-myassays-desktop (4 min 13 sec )
- https://www.myassays.com/video/myassays-desktop-xy-reduction-peak-transform (10 $\min 19 \mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-expression-transforms (9 min 29 $\mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-xy-reduction-transforms (17 min 16 $\mathrm{sec})$
- https://www.myassays.com/video/adding-conditional-transforms-with-myassays-desktop ( 3 min 47 sec )
- https://www.myassays.com/video/myassays-desktop-dose-by-position (3 min 14 sec )
- https://www.myassays.com/video/myassays-desktop-import-to-expression-by-samplegroup ( 5 min 57 sec )
- https://www.myassays.com/video/add-evaluations-to-dose-response-curves-in-myassays-desktop (4 min 40 sec )
- https://www.myassays.com/video/myassays-desktop-xy-reduction-function-transform (3 $\min 23 \mathrm{sec})$
- https://www.myassays.com/video/well-scan-data-analysis-with-myassays-desktop (7 min $23 \mathrm{sec})$


## Automating Data Analysis

- https://www.myassays.com/video/myassays-desktop-with-folder-poll (4 min 33 sec )
- https://www.myassays.com/video/myassays-desktop-analysis-launcher (5 min 12 sec )
- https://www.myassays.com/video/myassays-desktop-analysis-launcher-with-tweak-files ( 3 min 11 sec )
- https://www.myassays.com/video/kaleido-3-0-data-analysis-automation-with-myassaysdesktop (4 min 43 sec )


## Protocols

- https://www.myassays.com/video/download-protocols-for-myassays-desktop (3 min 47 $\mathrm{sec})$
- https://www.myassays.com/video/create-a-protocol-with-myassays-desktop (11 min 33 sec)
- https://www.myassays.com/video/dose-response-curve-analysis-with-myassays-desktop ( 6 min 3 sec )
- https://www.myassays.com/video/dose-response-curve-parameters-with-myassaysdesktop (2 min 19 sec )
- https://www.myassays.com/video/liquid-handling-quality-control-with-myassays-desktop (1 min 52 sec )
- https://www.myassays.com/video/introduction-to-parallel-line-analysis-with-myassaysdesktop (4 min 33 sec )


## Parallel Line Analysis

- https://www.myassays.com/video/introduction-to-parallel-line-analysis-with-myassaysdesktop (4 min 33 sec )
- https://www.myassays.com/video/create-a-parallel-line-analysis-protocol-with-myassaysdesktop ( $9 \min 42 \mathrm{sec}$ )
- https://www.myassays.com/video/multiple-plate-parallel-line-analysis-with-myassaysdesktop ( 7 min 36 sec )
- https://www.myassays.com/video/myassays-desktop-parallel-line-analysis-acceptancecriteria ( 14 min 11 sec )


## Data

- https://www.myassays.com/video/import-data-with-myassays-desktop (9 min 33 sec )
- https://www.myassays.com/video/myassays-desktop-transforms (7 min 2 sec )
- https://www.myassays.com/video/myassays-desktop-matrices (5 min 35 sec )
- https://www.myassays.com/video/myassays-desktop-meta-data (5 min 46 sec )
- https://www.myassays.com/video/myassays-desktop-field-data (6 min 21 sec )
- https://www.myassays.com/video/data-flagging-with-myassays-desktop (7 min 50 sec )
- https://www.myassays.com/video/myassays-desktop-sample-labels (5 min 15 sec )
- https://www.myassays.com/video/sample-table-ordering-with-myassays-desktop (4 min
$42 \mathrm{sec})$
- https://www.myassays.com/video/custom-flag-labels-in-myassays-desktop (4 min 10 sec )
- https://www.myassays.com/video/myassays-desktop-matrix-references-in-sample-labels ( 4 min 28 sec )
- https://www.myassays.com/video/ordering-field-data-with-myassays-desktop (4 min 12 $\mathrm{sec})$
- https://www.myassays.com/video/multiplex-data-analysis-with-myassays-desktop (9 min $25 \mathrm{sec})$
- https://www.myassays.com/video/data-export-with-myassays-desktop (5 min 14 sec )
- https://www.myassays.com/video/myassays-desktop-results-naming-with-meta-data (5 $\min 11 \mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-numerical-field-data (8 min 46 sec )
- https://www.myassays.com/video/myassays-desktop-advice-feature (8 min 29 sec )
- https://www.myassays.com/video/constraining-curve-fits-with-myassays-desktop (6 min 53 sec )
- https://www.myassays.com/video/myassays-desktop-qc-observation-references-in-advice-conditions (2 2 min 20 sec )
- https://www.myassays.com/video/import-multiple-columns-from-a-csv-file-in-myassaysdesktop (4 min 32 sec )
- https://www.myassays.com/video/myassays-desktop-scripting-feature (3 min 49 sec )
- https://www.myassays.com/video/myassays-desktop-import-to-expression-by-samplegroup ( 5 min 57 sec )
- https://www.myassays.com/video/myassays-desktop-expression-by-sample-replicates (1 $\min 43 \mathrm{sec})$
- https://www.myassays.com/video/well-scan-data-analysis-with-myassays-desktop (7 min 23 sec )
- https://www.myassays.com/video/myassays-desktop-scan-blank-correction-transform (2 $\min 33 \mathrm{sec})$
- https://www.myassays.com/video/statistical-functions-in-myassays-desktop\%3A-confidence-intervals ( 3 min 33 sec )
- https://www.myassays.com/video/myassays-desktop-certificate-validator-tool (3 min 41 $\mathrm{sec})$


## Enhanced Security

- https://www.myassays.com/video/myassays-desktop-enhanced-security-introduction (8 $\min 45 \mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-enhanced-security-myassays-loginauthentication ( 6 min 57 sec )
- https://www.myassays.com/video/myassays-desktop-enhanced-security-configuration (7 $\min 50 \mathrm{sec}$ )
- https://www.myassays.com/video/securing-data-import-with-import-requires-nomodification (4 min 1 sec )
- https://www.myassays.com/video/securing-data-import-with-an-approved-data-folder (3 min 51 sec$)$
- https://www.myassays.com/video/myassays-desktop-enhanced-security-audit-trail (4 min $21 \mathrm{sec})$
- https://www.myassays.com/video/securing-data-import-with-data-certification (4 min 34 sec)
- https://www.myassays.com/video/myassays-desktop-enhanced-security-configuration-for-wizard2-pc-(1-of-4) (11 min 15 sec )
- https://www.myassays.com/video/myassays-desktop-enhanced-security-configuration-for-wizard2-pc-(2-of-4) (13 min 23 sec )
- https://www.myassays.com/video/myassays-desktop-enhanced-security-configuration-for-wizard2-pc-(3-of-4) ( 9 min 53 sec )
- https://www.myassays.com/video/myassays-desktop-enhanced-security-configuration-for-wizard2-pc-(4-of-4) (5 min 58 sec )
- https://www.myassays.com/video/myassays-desktop-pro-enhanced-security-withenvision ( 7 min 57 sec )
- https://www.myassays.com/video/introduction-to-adding-data-certification-to-enspire (3 $\min 40 \mathrm{sec})$
- https://www.myassays.com/video/configuring-and-using-enspire-with-myassays-desktop-pro-enhanced-security ( 16 min 8 sec )
- https://www.myassays.com/video/myassays-desktop-certificate-validator-tool (3 min 41 sec)

Working with EnSight/Kaleido 3.0

- https://www.myassays.com/video/introduction-to-kaleido-3-0-and-myassays-desktop (1 $\min 48 \mathrm{sec}$ )
- https://www.myassays.com/video/kaleido-3-0-data-export-and-import-into-myassaysdesktop ( 3 min 37 sec )
- https://www.myassays.com/video/create-a-new-protocol-with-kaleido-3-0-xml-data-in-myassays-desktop ( 6 min 12 sec )
- https://www.myassays.com/video/kaleido-3-0-data-analysis-automation-with-myassaysdesktop (4 min 43 sec )


## Working with WIZARD²

- https://www.myassays.com/video/wizard2-integration-with-myassays-desktop-(1-of-3) (2 $\min 48 \mathrm{sec}$ )
- https://www.myassays.com/video/wizard2-integration-with-myassays-desktop-(2-of-3) (7 $\min 9 \mathrm{sec}$ )
- https://www.myassays.com/video/wizard2-integration-with-myassays-desktop-(3-of-3) (7 $\min 28 \mathrm{sec}$ )
- https://www.myassays.com/video/wizard2-data-import-with-myassays-desktop (7 min 41 $\mathrm{sec})$
- https://www.myassays.com/video/ria-data-analysis-with-myassays-desktop (6 min 15 sec)
- https://www.myassays.com/video/myassays-desktop-enhanced-security-configuration-for-wizard2-pc-(1-of-4) ( 11 min 15 sec )
- https://www.myassays.com/video/myassays-desktop-enhanced-security-configuration-for-wizard2-pc-(2-of-4) (13 min 23 sec )
- https://www.myassays.com/video/myassays-desktop-enhanced-security-configuration-for-wizard2-pc-(3-of-4) (9 min 53 sec$)$
- https://www.myassays.com/video/myassays-desktop-enhanced-security-configuration-for-wizard2-pc-(4-of-4) (5 min 58 sec )


## IQ/OQ with MyAssays Desktop QT

- https://www.myassays.com/video/myassays-desktop-qualification-testing-tool (2 min 36 $\mathrm{sec})$
- https://www.myassays.com/video/myassays-desktop-qualification-testing-tool-setup-and-use ( 8 min 5 sec )

Integrating with LIMS

- https://www.myassays.com/video/import-sample-ids-from-lims-in-myassays-desktop (2 $\min 57 \mathrm{sec}$ )
- https://www.myassays.com/video/export-results-to-lims-with-myassays-desktop (5 min 0 sec)


## Working with MyAssays Desktop Key Concepts

Throughout MyAssays Desktop, various concepts and terms are used to communicate and reference the different types of data related to assay data analysis. This section introduces these fundamental concepts.

## Measurements

The measurements are the data points acquired from your scientific instrumentation (e.g., microplate reader).

The software supports measurements from any type of detection technology (such as absorbance, luminescence, fluorescence, counts, counts-per-minute), with data collected from each sample as:

- Endpoint - A single measurement value
- Kinetic - Values measured over time (data can be processed with or without time stamps)
- Spectral - Data measured at various multiple wavelengths
- Multiplex - Multiple analyte measurements from each sample

Measurement data is typically accompanied with other types of data; examples include date/time of measurements, plate ID, and user ID. We refer to this data as meta data. The software can import and report this alongside your measurement data.

## Import

The import process gets your measurement and any meta data into the software. The software supports a variety of methods including:

- Copy and paste
- Excel selection import
- Automated import for supported third-party proprietary formats


## Protocol

An assay protocol defines all of the parameters necessary for performing the data analysis required for any assay. This includes:

- Expected measurement data (e.g., endpoint, kinetic, spectral, multiplex, etc.)
- Layout - How samples are arranged
- Calculations
- Report content and configurations

Also, the protocol can define:

- Import settings (exactly how and what data to import)
- Export settings (how to export the data, e.g., to Excel/PDF or a custom file such as CSV/TXT)
- Quality control settings (intra and inter-assay calculations and settings)

With MyAssays Desktop, the settings for a particular assay protocol are stored in an assay protocol file. These files use the .assay-protocol file extension.

An assay protocol file is a self-contained file with no dependencies on any database, making it easy to transfer protocols between systems and to share protocols with colleagues.

## Results

Each time an assay protocol is launched and results are calculated, an assay results file is automatically created. The assay results file contains the results of the analysis including the measurements and meta data and any data specific to the run. This could include:

- Sample IDs
- User-entered notes
- Calculation log
- Any changes to the configuration such as:
- New sample layout
- Calculation settings
- Any flagged data (i.e., marked as outliers)

Assay result files use the .assay-results file extension.

An assay results file also contains its own copy of the assay protocol. This is important so that any changes to the original assay protocol file will not impact the results already calculated. This structure also simplifies sharing data and transferring

In addition, an assay results file can contain the whole version history of changes made to the analyses. Each time results are recalculated, a new set of results is created and stored within the same results file. The full-version history is available so you can go back to earlier versions of the results.

## Reports

The report is the primary output of interest of the data analysis. A report is generated each time the assay results are calculated.

Typically, the report will contain a table listing the measurement data and results for each sample. The report might also contain charts and other content. MyAssays Desktop Pro provides a wealth of features and functions for tailoring the report content to your exact needs.

## Report Views

Depending on your data or preferences there are two types of report, each with their own benefits:

- Sheet view
- Page view


## Exports

MyAssays Desktop can export the report content to a variety of formats, including Excel/Word/PDF. In addition, content can be easily output to popular text-based formats such as CSV using preconfigured export methods. Beyond this, XSLT support is provided to enable you to export your content in any format to exactly match you output requirements.

## Sample Layout

The sample layout (or simply layout) defines how the samples are arranged on the container or containers from where they were or will be measured.

Here we use the general term "container" because the samples could be measured in a microplate or a rack of vials; in fact, any vessel for your samples is supported.

## Getting Around

MyAssays Desktop provides two key applications to help organize and work with your data:

- MyAssays Desktop Explorer - For arranging, locating, discovering, and launching your assay data
- MyAssays Analysis - For performing the assay data analysis


## MyAssays Desktop Explorer (MADE)

MADE is the application launched when you click the MyAssays Desktop Explorer icon installed on your desktop. This application is the starting point for all MyAssays Desktop functionality. From here you can:

- Search and download preconfigured assays from MyAssays.com
- Access useful videos and training guides
- Access technical support
- Manage your product license
- Access your assay data
- Create new assay protocols (requires Pro version)


## MyAssays Desktop Analysis (MAA)

If you launch a protocol or open a results file, MAA opens. MAA provides the user interface for working with your assay data analysis. From here you can access all of the various options, including:

- Import/export data
- Provide assay parameters
- Enter sample IDs
- Generate report
- Calculate new results


## MyAssays Desktop Explorer

MyAssays Desktop Explorer is the first application of MyAssays Desktop you interact with.
With MADE, you can:

- Find, download, and launch assay protocols from MyAssays.com database
- View existing assay layouts, results, and reports
- Run assay protocols and open results
- Create or request an assay protocol

Assay protocols and results can also be launched directly from Windows Explorer

## MADE Ribbon

Ribbon extends across the top of the main area of the MADE.

## Ribbon Tabs:

## Home

This tab consists of several groups for operating with data files inside MADE directly.

- Navigation includes three main options for moving around tabs, folders, and subfolders inside the application.
As you continue working on your data analysis, you may prefer to divide and arrange your data in folders by its measurements or features. Therefore, the number of folders and subfolders in tabs will grow. You may use the Back, Forward, and Up navigation commands on Ribbon or Quick Access Toolbar to change your current location quickly.
- Clipboard provides better management of your stored data. You may prefer to rearrange the way your files or folders are stored by using Paste, Cut, and Copy commands. This way you can simply move around data files between various storage folders.

Clipboard data is automatically deleted when the computer shuts down or restarts, so remember to save important information.

- File consists of two commands: New, which launches New Protocol Wizard, and Open, which launches data from MADE's tabs.
- Search MyAssays.com can be of the maximum use for finding and downloading specific preconfigured assays from the MyAssays online database. This tab consists of Search text box and Search button.


## Tools

This tab embodies Utilities you may find useful.

- Analysis launcher is a tool for advanced launching of the MAA.
- Folder Poll Manager launches MyAssays Folder Poll Manager.


## Support

This tab provides connection between MyAssays and the end users.
Assistance group has a Request Protocol command, which you may find useful if the assay template you are looking for is not yet in the MyAssays database.

It also features a backstage view for additional use of application scenarios and configuration.

## Backstage Area:

## File Tab

File Tab gives you access to MADE's important tasks.

- Information contains details about your MyAssays Desktop personal use. This includes product version, current license details, and features.

You may want to change some features of your MyAssays Desktop (i.e., activate, deactivate, or update license). The Product Licensing describes further details for these options.

- View defines whether the Measurements Tab is displayed on the Folder Side Bar and the Launch Assay Protocol with Options setting is displayed on preview pane in the Protocols tab.
- Data displays the storage path for persisting your assay data files and Welcome Screen's Recent feature options.
- Drivers
- Exit closes the application.


## Quick Access Toolbar

Quick Access Toolbar contains shortcuts to commands and functions to make your work on MyAssays Desktop Explorer easier and personalized.

You can Customize the Work Area to fully adjust it to your needs.

## Application Button

Click the logo in the upper left corner of the MADE to:

- Restore - Return window size to the previous one
- Move - Change windows on-screen' locations, if they are not full screen
- Size - Widen or narrow the window
- Minimize - Put the window in taskbar
- Maximize - Make the window full screen
- Exit - Close the program


## Navigation Shortcuts

The other default commands of Quick Access Toolbar are the ones from Navigation group, namely Back, Forward, and Up, which are useful for switching between the tabs you currently work with quickly.

## Advanced Options

You can set the Quick Access Toolbar position in this drop-down menu. Click Show Quick
Access Toolbar Below/Above the Ribbon (depending on its current location), Minimize the Ribbon, and change the Quick Access Toolbar view by adding or excluding some options in More Commands menu.

## Folders Tab

MADE provides a hierarchical view of the organized data and presents its preview if it is available. MyAssays Desktop stores different types of data in separate subfolders in the MyAssays Data Folder Root. These tabs point to actual Windows folders.

In MADE, you can access information from every subdirectory easily in the appropriate left side bar tab.

MyAssays Desktop arranges your data automatically in correspondent folders so that you can easily manage, share, and back up your assets. You may prefer to customize how results files are named and where your data is stored.

## Folders Tab Content

| Tab | Contains Files |
| :--- | :--- |
| Search Results | Assay protocol titles that match search query |
| Protocols | Assay protocol files with .assay-protocol extension |
| Measurements | Measurement data files in supported file formats (*.TXT, *.CSV, <br> *.XML, *.DAT, *.XLS, *.XLSX, and others) |
| Results | Assay results files with .assay-results extension |
| $\underline{\text { Layouts }}$ | Layout files with .assay-samplelayout extension |
| $\underline{\text { Reports }}$ | Report files with supported report extension (*.DOC, *PPT, *.XLS, <br> *DOCX, *XLSX, *PPTX, *.MHT, *.HTM, *.PDF) |

Selecting a tab displays its content in the main window.

## File and Preview Panes

The File and Preview panes are populated with the content of the selected tab.

## File Pane

A list of available data files displays on the left side of the active tab. It is a visualization of the folder that persists data, that was used to launch, or is a result of performing a particular assay analysis.

In the File Pane, files can be selected using normal Windows procedures. Right-click to display the default Windows Explorer context-sensitive pop-up menu. Relevant MADE Ribbon options are available when a single file is selected.

## Sort and View

The default view of content is Details. To change it, right-click active tab's empty space > View > select preferable view from the drop-down list.

Files are sorted by name by default. To change the file sorting order:

- Right-click the empty space of the active tab > Arrange Icons By > choose preferable option form the drop-down list.


## OR

- If File List is displayed in default view, click the column headings Name, Size, and Date Modified.


## Subfolders

Tabs may contain subfolders (subdirectories). Creating subfolders in tabs comes in handy when working with big data sets or with multiple versions of analyses. All subfolders will be of the same type as their primary parent. To create a new subfolder, right-click anywhere on the File Pane and select New > Folder.

The File Pane may be navigated using the relevant buttons on the MADE Ribbon.

## Preview Pane

Select a single file from the list to see its preview and available actions on the right side of the active window.

The contents of the preview pane depend on the type of file selected:

| Folder Tab | Preview Pane Contents |
| :--- | :--- |
| Search Results | A summary of assay protocols, including the name, the instrument <br> settings, the position used and layout, and any configured transformations |
| Protocols | Measurements |
| Resecanned and custom raw data files |  |
|  | Two panes: <br> - Overview with the assay protocol summary <br> - Latest Report with the latest report of the results |
| Layouts | A microplate control showing each plate layout in the layout |
| $\underline{\text { Reports }}$ | The preview of the PDF reports |

## Welcome Screen

This is the screen that displays as you launch MADE. It contains some useful options for a proficient start. Get acquainted with MyAssays Desktop Explorer's basic functions and begin your work at full speed.

The left pane of Welcome screen consists of:

- Start - Shortcuts to basic data analysis options, particularly New Protocol, Launch Protocol, and Open Results
- Recent - A specified number of assay protocols and results you have worked with last
(the particular title will be launched in MAA upon selection)

You can customize a number of entries in the Recent list or clear the file history in Data options.

The right pane comprises of two subpanes:

- Upper subpane Discover MyAssays Desktop displays links to a detailed review of MyAssays tools and the desktop application specifically. Visit MyAssays Home Page or What's New in MyAssays Desktop? and get to know latest information about desktop version.
- Lower subpane contains:
- Product Videos - Links to video materials that will help you familiarize yourself with MyAssays Desktop and its components
- Support - A link to the MyAssays Support contact form and the Request an Assay Protocol dialog box


## Recent Used Files Feature

The number of files displayed under the Recent subpane of the Welcome Screen may vary from 0 to 20.

To change the number of Recent files, type it in the corresponding box or use the arrows to adjust it.

Press the Clear Recently Used File list button if you want to erase the history.

## Search Results Tab

This tab displays on the Folder Tab after you submit a Search MyAssays.Com. It displays a File List and Preview of the assay protocols that match search query.
When this tab is active, multiple search results options are available.

## Search and Download Preconfigured Protocols from MyAssays.com

Using MyAssays Desktop, you can access the constantly evolving database of preconfigured assay protocol templates from MyAssays.com.

Use the Search tool and explore search results options.

## About Preconfigured Assays

MyAssays.com allows users to quantify their data with growing base of online assay tools. Using MyAssays Desktop, you can search the protocol database and download ready-to-use data analysis protocols on your PC. The preconfigured assays include:

- Commercially available kits
- Common analysis methods
- Tailor-made protocols per request

The database supports wide range of microplate and rack-based instrumentation and contains high-quality templates that simplify assay data evaluation.

## Finding Assay by Search Term

You can search the database by any keyword or term such as kit manufacturer name, study type (in vivo, in vitro, qualitative, quantitative, etc.), assay type (ELISA, HTRF, etc.), or fit method (4PL, 5PL, etc.).

Find an Assay Protocol from MyAssays.com

1. Launch MADE.
2. On the Home ribbon tab, in Search MyAssays.com group, in the Enter Assay Name field, enter or paste the search term.

You can also use a CTRL + E keyboard shortcut.
3. Click Search or press Enter on keyboard.

The list of preconfigured assay protocols that match your query will appear in the Search Results tab.

## How the Search Results Are Displayed

Upon submitting the search, you will see the list of assay protocols that match the entered keyword. To help you choose a protocol that will suit your needs, select an item from the list to view a description of the analysis performed in the right-hand Preview pane.

Some assays also contain the Kit Booklet with the instructions and notes provided by the manufacturer. To launch the booklet with your default browser, click View Assay PDF link under the assay summary.

## Download and Launch Options

When you select an assay protocol, the following options are available:

- Download and Launch - To save a single assay protocol to your Protocols tab and automatically launch it in MAA.You can also double-click the file from the list to download and launch it in MAA automatically.
- Download - To save one or more assay protocols to your Protocols tab.

To select multiple protocols, use the (Ctrl+Shift+Arrow Keys) keyboard shortcut or Ctrl+A to select all search results.

When selecting multiple protocols from the list, only this option is available.

- Launch Assay Protocol - Appears if you have downloaded a particular assay protocol previously. The above actions are then not displayed. To enable redownloading, click the Click here hyperlink in the message below Visit on MyAssays.Com.
- Visit on MyAssays.Com - Directs you to the online MyAssays.Com webpage of the selected assay protocol.


## If No Assays Match Your Search

If the assay you are looking for is not yet in the MyAssays database, our experts can provide data analysis solutions specific to your needs. To learn more, see Request an Assay Protocol.

## Protocols Tab

The Protocols Tab displays a File List and Preview of the assay protocols that are persisted to your PC.

- 

Only data files with an .assay-protocol or .apr extension are visible through this folder.

Upon selecting a particular assay title from the list, you will see its summary on the Preview pane and, if it is available, a detailed Booklet Kit from the kit manufacturer.

In the Protocols Tab, you can:
MyAssays Desktop Pro edition allows creating new protocols for your data with the New Protocol Wizard.
To start the wizard, launch MADE and do one of the following:

- On the Welcome screen, click New Protocol.
- On the Protocols tab, in File group of the ribbon, click New.

You also can launch the wizard from command line. For further details, see Command Line Arguments.

How to: Launch Assay Protocol File
Select the Protocols tab, then select a single assay result file to open and do one of the following:

- Double-click its title.
- Right-click the title and click Run with MyAssays Desktop Analysis.
- Click Launch Assay Protocol in the lower right corner of the Preview pane.

By doing this, you will launch the file in MAA.

You can also launch an assay protocol directly from Search Results.

## Launch Assay Protocol with Options

This feature will open the Analysis Launcher tool with the selected protocol.
Click the Launch Assay Protocol with Options button to launch an assay protocol with command-line options for more control over the analysis.

How to: Launch Assay Protocol with Command Line Options

1. Launch MADE.
2. Click the File tab to open MyAssays Backstage view and click the View tab.
3. Select Show Launch Assay Protocol with Options.

(1)
The Show Launch Assay Protocol with Options state persists between sessions.

If it is selected, a corresponding option appears on the Preview pane of the Protocols tab upon protocol file selection.

How to Request Assay Protocol
Launch MADE and do one of the following:

- In the Welcome tab, on Support subpane, click Request an Assay protocol.
- On the ribbon, in the Support tab, click Request an Assay protocol.

When this option is selected, a Request Protocol dialog box will appear. Fill in the fields to specify what kind of an assay you want to be included in the MyAssays knowledge base.

Please mention any information that can be of use in Other Significant field.
You can and are welcome to attach any applicable files, such as Example Raw Data, Kit Insert PDF, and Other files (in zipped files).

After you have provided details of an assay, click the Submit Request button to send your requirements to MyAssays Support.

## Measurements Tab

## How to: View the Measurements Tab

1. Launch MADE.
2. Select the File tab of the MADE Ribbon.
3. Select the View screen.
4. Select the View Measurements check box.
(1) The View Measurements state persists between sessions.

If it is selected, an additional tab is displayed on the Folder Side Bar.
The measurements tab contains a default Examples folder with a $12 x 8$ Endpoint.txt file. For each assay, a text file containing your measurement settings data can be uploaded and processed for analysis. To upload a file, paste it in the Measurements folder in your MyAssays Folder Root.
The New protocol wizard can automatically identify and import the relevant readings from a file saved in many supported file formats, including TXT, CSV, or other proprietary formats.

Visit MyAssays Supported File Formats (http://www.myassays.com/supported-fileformats.html) to see list of supported measurements data formats.

## Results Tab

The Results Tab displays a File List and Preview of the results of previously run data analyses. Select a single Assay Results file from the File List to see the Preview of the report. Results files generate when you have launched a particular assay protocol and performed a data analysis. It is empty on your first time launching MADE and will fill as you work with the application. By default, these files are named after a protocol, but you can change the name (i.e., after incoming measurements, current date, etc.).
> (1)

> Only assay results data files with an .assay-results or .ars extension are visible through this folder.

When you select a title, two information insets appear on the Preview pane. The Overview shows an assay summary and may contain a link to the Booklet Kit from manufacturer. The Latest Report contains the report of the selected Assay Results file.

To enlarge the Preview view, hold down Ctrl button and push the mouse scroll wheel upward.

In the Results Tab, you can Open Results in MAA.

## Open Existing Results

## How to: Launch Assay Results File

On the Results tab, select a single assay result file and do one of the following:

- Double-click its title
- On the Home ribbon tab, in the File group, click Open
- On the Preview pane, in Latest Report inset, click Open

By doing this you will launch the file in MAA.

## Layouts Tab

The Layouts tab displays a File List and Preview of the assay layouts persisted to your PC. Vice versa, only files persisted to this tab may be used in running assays by MyAssays Desktop Analysis. Select a single Assay Layout file to view it on the Preview pane.

Only data files with an .assay-samplelayout or .mlo extension are visible through this folder.

Assays in MyAssays Desktop Analysis are initially set up to use a default layout for your samples. You will most likely prefer to use your own sample layouts in your data analyses. Use the Plate Layout Editor to edit existing or create new layouts.

## Reports Tab

All assay result data exported into a report file is stored to this folder. Double-click the title to open it in a relevant application.

## Customize the Work Area

You can configure Work Area to fully match your preferences and enhance your experience. Learning about its potential may be useful for taking full advantage of the features, tools, and options of the application.

## Customizing the Quick Access Toolbar

The Quick Access Toolbar displays tools that you have bookmarked. For making use of its full potential, you may prefer to rearrange and manage these tools.

## Location

You may prefer the Quick Access Toolbar to be displayed above or below the Ribbon. Rightclick any spot of the Ribbon and hit on the Show Quick Access Toolbar Below the Ribbon command. If the toolbar is already below, you can configure it to be vice versa.

## Adding or Excluding Tools

To add a command to the Quick Access Toolbar, choose the one you want from the Home,
Tools, or Support tab and right-click on it. In the drop-down menu that will appear, click Add to Quick Access Toolbar. Upon doing that, the command will be displayed in the Quick Access Toolbar.

To exclude a command from the Quick Access Toolbar, right-click on it and click Remove from Quick Access Toolbar.

## Advanced Customizing

You may consider customizing the Quick Access Toolbar to fully match your preferences. To call up a Customize Quick Access Toolbar options menu, do one of the following:

- Right-click anywhere on the Ribbon and click Customize Quick Access Toolbar on the context menu.
- Click Advanced on the Quick Access Toolbar and click the More Commands command. Upon doing this, a dialog box will appear. Select tools you prefer and add or remove them from the list.

You can add or remove commands from a list or from a specific tab. You can also determine their on-screen order by pressing the arrows on the right side of the Quick Access Toolbar list.

When you have finished customizing toolbar, click OK to save changes or Cancel to undo them.

To return the toolbar to its default configuration, click Reset in the Customize Quick
Access Toolbar dialog box.

## Customizing the Ribbon

The Ribbon is designed to make finding and working with commands convenient and easy. You may consider the capability to personalize and customize the Ribbon in the way you want useful. With this feature, you can change the default Ribbon and create custom tabs and groups with your preferred commands.

To customize the Ribbon, right-click anywhere on the Ribbon and then choose the
Customize Quick Access Toolbar context menu to bring out the dialog box. Select the Customize Ribbon option and personalize your Ribbon view for your convenience.

## Minimize Ribbon

This option may be useful to expand your on-screen view of the application. If the Ribbon is minimized, tabs appear on click and hide when you click on any other spot of the screen. To do this, right-click anywhere on the Ribbon and select Minimize Ribbon in the drop-down list. To undo minimizing, repeat these actions and clear the command.

## Layout Rules

Layout rules are requirements that can be defined as part of the layout. An assay of a particular type may require certain specifications to be complied. For example, a quantitative assay that uses a curve fit may require a certain number of standard samples to be measured (at least 2 would be required for linear regression, more for other fit methods). Similarly, an assay protocol developed by a kit manufacturer may insist on using a specific number of replicates.

When creating or editing a layout according to layout rules, the system will check that the new layout satisfies these rules on save. If these rules are not satisfied, then the new layout cannot be saved or used with the associated protocol.

## Layout Filtering

The layout rules are also used to filter out irrelevant layouts for selection; this helps to find only useful layouts that apply to the assay you are working with. For example, if there are many layouts defined on your system, when working with a specific protocol the system will automatically exclude any layout that does not satisfy these rules.

## Layout References in Expressions

Data analysis is linked to and relies on the sample layout to perform required calculations. The layout itself and its components can be referred to in expressions for more accurate and flexible coordination of the assay analysis.

## Sample Group References

In all cases (except Standard Curve Fit using the Curve option Average with multiple plates), a reference to a specific group implies to the replicates of that group on the container (analyte/plate) it is associated with. In configuration, the group numbering is always localbased.

For example, during configuration of microplate assays, Blank1 refers to the first blank group on the layout. It does not consider replicates on other plates/analytes. The Standard Curve Fit using the Curve option Average with multiple plates is a special case that enables the standard curve to be created using samples across plates. Thus, Blank1 will only refer to the replicates in the first group of the current container.

Note the distinction between group numbers that are reported (i.e., in the report and group numbers during configuration). They are different for multiple plate.

More details on referencing sample groups is provided in the Operands subsection.

## References of the Positions and Layout in Expressions

Over and above sample references, the layout itself and even single positions can be useful when configuring data analysis. For example, the layout function is used for By-Row Blank Correction Example.

Further information on this topic is provided in Layout Analysis Functions.

## Plate Layout Editor

Most assays allow you to specify your own layout for processing and distinguishing various types of measurement data. The editor is used to create new layouts or modify existing layouts.

You can freely position your samples and change the number of sample groups providing the requirements of the assay are met. Certain assays allow you to provide measurement data ran across multiple plates.

In addition to being used to define a layout before data is collected, the editor can be used after data collection to correct for preparation errors.

If your samples are measured in tubes or vials, then use the Rack Layout Editor to define the sample arrangement.

## Using the Plate Layout Editor

The Plate Layout Editor is displayed when you click Create to create a new layout or Edit to edit an existing layout on the Layout Selection pane of MAA Microplate tab. You can also create a new layout as a step of the New Protocol Wizard.

## Editor Flexibility

The actual flexibility available in your custom layout depends on an assay's requirements. Most assays require a certain number of positions of a particular type to be defined (e.g.,
Blank or Standard groups). In most cases, however, you may set up the layout as you prefer providing your layout meets an assay's requirements.

Once your new layout is created, you can select it to be applied to future calculations of an assay. By making calculations or saving settings, your new layout is saved under MyAssays/Layouts in your MyAssays folder root.

## Creating Multiple Plate Layouts

A layout can be defined for each real-world physical container. The Plate Layout Editor can be used to define layouts across multiple containers. The group numbering of samples is contiguous and sequential for each type.

There are a number of different ways that standards and samples of an assay might be arranged across multiple plates. Plate Layout Editor allows you to specify for each plate:

1. How samples are arranged
2. Which standards measurements should be used to construct a standard curve
3. Which curve to use to calculate concentrations from

## Plate Layout Editor Settings

With the following options, you can specify how samples are arranged on a plate.
There are two modes for specifying an assay layout:

- Erase - For removing samples that you are not measuring
- Fill - For defining the layout by selecting areas to fill



## Erase Edit Mode

Erase mode allows you to remove samples that you are not measuring (i.e., mark them Unused).

To remove a position:

1. Select the Erase icon on the toolbar.
2. Move the mouse pointer over a defined position.
3. Left-click to remove all positions of this sample group.

If the sample group contains more positions than you want to remove, select
Unused type on the type and group selector and fill the position(s) with it.
Sample numbering will be adjusted to ensure that the group numbering remains contiguous.

## Fill Edit Mode

Fill Edit mode allows greater flexibility in layout arrangement. To enter Fill Edit mode, click Fill. The Fill Settings button will appear, enabling you to specify how the positions you select are filled.

Also, the type and group selector can be used to select the next group to fill with your chosen settings.

To change the sample at a position, simply left-click your mouse pointer on the position. To fill an area, left-click at the first position and hold and drag to the last position. The Fill
Settings dialog window appears, allowing you to change how this fill operates. You can specify the fill direction and number of positions for your selected group. The following fill options are available:

## - By Row

- By Column
- Rectangle Mode

If Rectangle Mode is selected, you can choose whether the replicates fill By Row or By Column. You can specify the number of positions per group using the entry box.

Group numbering must start at 1 and be contiguous for each sample type.

## Undo/Redo

Use these buttons to cancel the last change or re-apply it.

## Save + Close

When you are satisfied with your changes, click Save + Close. This will save the new layout to your Layouts folder and make it available to relevant assay analyses.

## Multiple Plate Options

If you have selected the Multiple Plate option or are editing a multiple plate layout, the Plate Layout Editor's functionality expands with options applicable only to multiple plate layouts. Also, you can switch between plates and choose curve-fit generation properties.

## Options

The toolbar for multiple plate expands with following options:

- Add Plate - Adds an empty plate
- Remove Plate - Removes a selected plate
- Duplicate plate - Copies a selected plate with all defined changes

Curve Selection
Curve selection describes how the multiple layout is arranged and behaves (i.e., defines if and how a curve can be selected).

For multiplex assays, only single layouts can be applied.

## Multiple Plate Group Numbering

When defining the layout (in the Plate Layout Editor), the numbering for each sample group on every plate should always start at 1 .
This might seem counter-intuitive, however, this is intentional to make things easier when working with multiple plates. With this approach, if you decide to change the number of samples on the first plate you do not need to update the numbering on all plates that follow. For example, being able to refer to, for example, Control1 to reference all Control1 replicates is much simpler than having to remember or decode the measurement data.
To handle multiple plate assay operations efficiently, you can refer to sample group either on certain container(s) precisely or with regard to whole measured data. This is described further in the Sample References in Multiple Container Assays.
When the data analysis is performed, the software will apply the group numbers across plates contiguously. This assures the results (for non-calibrator groups types) are presented sequentially and each sample group has a unique number. For example, if the layouts for plate 1 and plate 2 are both defined with Unknown1-Unknown10, when the analysis is performed, plate 1 will include Unknowns1-10 and plate 2 would include Unknowns1120.

## Multiple Plate Standard/Calibrator

With Standard/Calibrator (Control) sample types, it does not make sense for numbering to be sequential across plates as this would imply that the data for a single curve is spanned across plates. Also, it is possible to configure standard replicates across plates (e.g., every plate has a Standard1, etc.). Therefore, for these sample types, numbering always starts at 1 for each plate.

(1)
The number of plates defined in the layout may or may not correspond to the actual number of plates measured. See Trimming and Padding subsection.

## Multiple Plate Rules

The number of plates defined in the layout may or may not be equivalent to the number of data containers measured. For example, the layout might only define one container whereas 50 containers' worth of data are provided. Equally, there may be less containers measured than there are defined in the layout. In these cases MAA will rely on the following rules:

## Trimming

## The layout contains more plates than data containers.

When there are more plates defined in the layout than containers measured, for each data container the layout with the same index will be applied. The extra layouts will be ignored.

Example: Let's assume that we have a layout made up of 3 plates and 2 containers of data. The analysis will be performed in the following way: Plate1 on Container1, Plate2 on Container2. The Plate3 will not be used for analysis.

## Padding

## The layout contains more plates than data containers.

When there are more containers measured than there are defined in the layout, the last defined layout plate will be reused. For each data container will be applied the layout with the same index. The excessive measurements data will be analyzed using the layout with the last index.

Example: Let's assume that we have layout made up of 3 plates and 5 containers of data. The analysis will be performed in the following way: Plate1 on Container1, Plate2 on Container2, Plate3 on Container3, Plate3 on Container4, and Plate3 on Container5.

(1)
The data analysis evaluation results are presented sequentially, with a unique number for each sample group.

## Multiple Plate Analysis

The multiple plate features greatly simplify processing measurement data when your samples span more than one plate.

For multiple plate layouts, the layout can contain one or more plates with different positioning (and curve-fitting options) of the samples on each. Each layout in a collection has the same dimensions (width and height). For example, all containers in a collection might be $12 \times 8$.

While different layouts may be applied on different containers, the same layout is applied on all matrices of a specific container unconditionally.

Plate Layout Editor allows you to freely define how your samples are arranged on each plate. For example, the following multiple plate layout uses a different sample arrangement on each plate:

For quantitative assays, your standards might be arranged on every plate in the batch or you may decide to measure standards only on some of the plates in the batch. You can specify how the standard curve is generated and which curve to use to calculate the concentrations for each plate.

## Plate 1



Plate 2


Plate 3


## Partial Standards

When running an assay across multiple plates, you might decide that it is not necessary to measure the full range of standards on every plate. Instead you could read the full range of standards on only one plate and measure a subset of the standards on other plates, then visually compare the curve fits to ensure they are usable. This allows you to:

- Save costs on standards
- Save preparation time
- Measure more samples per plate

MyAssays makes this process easy by allowing you to define layouts with partial standard groups. We use this term to define a layout that includes the full range of standards on one or
more plates of the layout with other plates featuring only a subset of the full range of standards.

## Multiple Plate Layout Curve Fit Settings

The Plate Layout Editor provides a method for selecting the curve to use for each plate. The layout configuration defines how the standard groups are arranged on each plate. This can be the curve generated from either:

- the average (of calibrator groups measured across separate plates)
- a specific plate


## Example

With a layout that comprises of 3 plates, the following options for creating a curve are available:

- Standard (Average Across Plates)
- Standard (Plate 1)
- Standard (Plate 2)
- Standard (Plate 3)

For more details about curve fitting on multiple plate assays, see Multiple Plate Standard Curve Fit Analysis.

## Rack Layout Editor

This section provides information about creating and editing custom rack layouts with the Rack Layout Editor.

The Rack Layout Editor is used to define the sequence of samples when assay data is measured from tubes or vials. The editor is used to create new layouts or modify existing layouts.

Racks are used to represent the sample data on-screen. The editor allows you to define and arrange the sequence of your samples.

In addition to being used to define a layout before data is collected, the editor can be used after data collection to correct for preparation errors.

If your samples are measured on a microplate, then use the Plate Layout Editor to define the sample arrangement.

## Rack Layout

With rack layouts, the number of samples that can be measured in each rack is fixed, this is the rack width and it is the same for all racks measured in an assay. Each rack is measured from left-to-right. For example, the following layout illustrates the racks used to measure 26 samples with each rack containing up to 10 samples.


With rack layouts the actual number of samples may vary at each run. To simplify the configuration of rack layouts, a sequence based editor is used to define the sequence of samples to measured. This is then converted to a layout to apply for samples that are actually read on each run of the assay.

## Using the Rack Layout Editor

The Rack Layout Editor is displayed when you click Create to create a new layout or Edit to edit an existing layout on the Layout Selection pane of MAA Layout (Racks) tab. You can also create a new layout as a step of the New Protocol Wizard.

## Rack Layout Editor Settings

The Rack Layout Editor is arranged into two panes:

- Definition - For defining the sequence of samples to measure and for specifying any dynamic section
- Preview - A visual representation of the rack layout using sample sequence with a specified number of samples to measure

Initially the Preview pane will not be displayed. It can be displayed by pressing the Preview button.

For example, here we can see the Rack Layout Editor showing a sequence definition of BO, Standards, Controls and Unknowns. The left-hand side shows the definition with the righthand side showing the preview for 48 samples:


## Sequence Definition

A rack assay might be fixed or dynamic:

- Fixed - The resulting sequence will contain only the samples specified in the definition pane
- Dynamic - The sample layout adjusts in accordance of number of positions measured

A fixed layout is only useful if you plan to run the exact same number of samples each time the assay is used.

A dynamic layout allows you to specify a common layout that can automatically be applied to different runs of the assay where a different number of samples is measured in each case. With a dynamic layout one or more adjacent sample groups are marked as dynamic; these appear highlighted in yellow in the definition pane. The resulting sequence will contain $n$ copies of the groups in the sample (where n depends on the total number of samples). More...

## Definition Pane

In this pane, you specify the order in which your samples will be measured.
Add Sample(s) settings
The Add Sample(s) controls are located on the bottom of the Sample Sequence Grid and are used to specify the type and number of samples to add. It comprises of:

- Type Selector - Select the sample type from a drop-down list
- Number of Groups - The number of sample groups to add
- Number of Replicates - The number of replicates in the sample group

After specifying the options, click Add Sample(s). The ordering in which samples are added reflects the order in which they will be added to the layout.

In case the grid already contains any samples specified, the Add Sample(s) expands with a drop-down menu of insertion settings. Select a sample on the grid and select Before
Selection or After Selection option to specify insertion of a new sample. To add a sample at the end of the list, select the Append option.

Group numbering must start at 1 and be contiguous for each sample type.

## Sample Sequence Grid

While the width of the racks is fixed (as defined by protocol settings), the actual number of samples may vary at each run. Thus, you can define the sequence in which the samples will be added by specifying state of sample sections:

- Fixed - The resulting sequence will contain these samples in the exact number as they are defined in the section
- Dynamic - Adjusts in accordance of number of positions measured. The resulting sequence will contain $n$ copies of the groups in the sample (where $n$ depends on the total number of samples). More...

The defined sequence of groups is listed in the grid, where each row corresponds to a sample. The attributes of each sample are editable.

## Set Dynamic

One or more adjacent rows can be set as dynamic. Select one or more adjacent rows in the table and click Set Dynamic. Rows in the dynamic selection are highlighted with a yellow background. To return the row to the fixed state, select it and click Remove Dynamic.

To select multiple rows to mark as dynamic, press and hold the Shift key when selecting rows with the mouse.

## Undo/Redo

Use these buttons to cancel the last change or reapply it.

## Clear

This button removes all Sample configurations (i.e. remove all Fixed and Dynamic sections)

## Delete

This button removes selected sample(s) from the table. Sample group numbering may be prompted to be adjusted to ensure that it remains contiguous.

## Save + Close

When you are satisfied with your changes, click Save + Close. This will save the new layout to your Layouts folder and make it available to relevant assay analyses.

## Preview Pane

The Preview Pane displays the layout arrangement sequenced using the specified number of samples. To display the preview settings, click Preview. The Number of Samples: edit-box will appear, enabling you to specify how many positions to display on the preview. To apply this setting click the $D$ icon to the right of the edit box.

## Racks

When working with assays that measure samples in tubes or vials (rather than microplates), MyAssays Desktop presents the measurement data on screen using "racks of samples".

Each rack is represented on screen with a fixed number of samples and are filled left-to-right. For example, the following graphic illustrates a rack-layout for 26 measured samples. These samples are represented across 3 racks.


The rack representation provides a convenient method for presenting data measured from multiple samples in a 2D view.

## Rack Width

Within an assay a fixed rack width is used. If your reader or workflow works with racks, then use a rack width that corresponds to the actual number of tubes or vials per rack.

The rack width is the number of samples measured in each rack.

## I'm Not Working With Racks

If your reader or workflow does not use racks but instead reads any number of tubes or vials, then adopt a rack width that is a practical size to present your data onscreen. A rack width of 10 is a good choice, but as you work with the software you may prefer a different width.

## Dynamic Layouts

When working with rack-based assays, the actual number of samples (tubes or vials) to measure may vary considerably between assay runs. Typically, there will be samples that are always read on each assay run (such as standards, controls, blanks) however there will also be sample types (such as unknowns) that will be measured in different volumes on each run of the assay.

For flexibility and ease-of-use, MyAssays Desktop supports the definition of "dynamic" layouts. The main benefit of working with a dynamic layout is that you do not need to redefine the sample layout each time the assay is ran. The sample layout is automatically inferred from the actual number of samples measured and the dynamic sample layout definition.

A dynamic layout defines one or more sample groups as dynamic. Each time the assay is ran, the number of samples allocated for the dynamic section on the layout will be automatically adjusted based on the total number of samples measured.

## Example 1:

In this example, the Unknown group (in duplicate) is marked as dynamic. This means that the number of Unknowns on the layout will depend on the number of samples measured. This particular layout will ensure that a Control group is read before and after the

## Unknowns.

| Type | Group | Replicates |
| :--- | :--- | :--- |
| B0 | 1 | 2 |
| Standard | 1 | 2 |
| Standard | 2 | 2 |
| Standard | 3 | 2 |
| Standard | 4 | 2 |
| Standard | 5 | 2 |
| Standard | 6 | 2 |
| Control | 1 | 2 |
| Unknown | 1 | 2 |
| Control | 2 | 2 |

For example, with 26 samples, the resulting layout comprises of 4 automatically assigned Unknown groups:


Using the same layout measured with 40 samples, 11 Unknown groups are automatically assigned.


Example 2:
In this example, the dynamic section defines a singlet Blank followed by an Unknown in duplicate.

| Type | Group | Replicates |
| :--- | :--- | :--- |
| B0 | 1 | 2 |
| Standard | 1 | 2 |
| Standard | 2 | 2 |
| Standard | 3 | 2 |
| Standard | 4 | 2 |
| Standard | 5 | 2 |
| Standard | 6 | 2 |
| Blank | 1 | 1 |
| Unknown | 1 | 2 |

To select multiple rows to mark as dynamic, press and hold the Shift key when selecting rows with the mouse.

For example, with 26 samples, the resulting layout comprises of 4 automatically assigned Unknown groups each with an associated blank.

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 3 | 3 | 4 |

Using the same layout measured with 38 samples, 8 Unknown groups each with an associated blank are automatically assigned.


## Analysis

## Versioned Analysis

## Overview

When you launch an assay protocol and click Calculate, an assay-results file is created with the first calculated version of results (Version 1). A result version contains all of the settings and results for a specific Calculation. You can select to view a different version of results at any point.

(1)
Each time the Calculate button is clicked, a new version of results is created and stored to the .assay-results.

## Changing Versions

When there is more than one version of results available, you can freely switch between them
How to: Switch Between Versions of the Results

1. Open a results file.
2. On the ribbon, in Home tab, hover to the Versions group.
3. From the Current version drop-down list, select the version you want to switch to.

Changing the version of results updates all settings and results views to display the data relevant for the selected results view. Any changes made to the currently selected version will be saved as a new version.

Clicking Calculate will create a new version (with version number $1+$ the current highest version number).

For example, if there are 3 versions of results and you go back to Version 1 and make changes, then Version 4 (pending) is created. If you then click Calculate, the Version 4 will be stored. This is the new set of results based on Version 1 with those changes made to Version 1 (as Version 4).

## Purge Versions and Rollback

## Purge Versions

The Purge operation will delete all version data except the current selected version. This setting is available if your assay contains more than one version.

## Rollback

The Rollback operation will delete all data that follows the selected version. It is active if the current version is not the newest version of the results.

(1)
The Purge Versions and Rollback operations cannot be undone; however, the changes are not committed to the file until the Save operation is performed.

## Uncalculated Version

When you make changes to a results file after the last set of calculations and before the Calculate button is clicked again, the Version $\{\mathbf{n}\}$ (pending) label is displayed in the Current Version selector.

## Unsaved Changes

If you made changes without calculating them (i.e., a pending version) and want to exit MAA, you will be advised to Save these settings on exit. These settings will be saved to the assay-results file as new uncalculated version and also to the assay protocol if that option is selected.

This also applies to assay protocol with no calculated changes.
When the assay-results file with uncalculated changes is opened in MAA, the first set of results will be calculated automatically.

## Save Options

You can save data you are working with as an:

- Assay protocol - To save only the settings representing the assay analysis to be performed
- Assay results - To save the settings representing the assay analysis and versioned results of its performance


## Save Changes To Protocol

This check box appears if you have launched MAA with an assay-protocol file. It indicates whether the changes (i.e., layout to use, measurements data, sample IDs) should be stored in the assay-protocol file, overwriting its default properties.

The Save Changes to Protocol check box state persists between sessions.

## Save Commands

## - Save

To simplify the different types of save options, there is one master Save command. The master Save command is accessible from:

- Quick Access Toolbar
- On the File MyAssays Backstage View, in the Save tab

This option saves results of the analysis to the directory it was launched from. It also optionally saves changes to a protocol, if the Save Changes to Protocol check box is selected.

## - Save Results As

With this command you can save the assay results with all changes to a new .assay-results file. When you click Save Results As, a dialog box appears. You can provide a new filename and a location for it to be stored in.

## - Save Protocol As

With this command you can save the modified assay protocol to a new .assay-protocol file. When you click Save Protocol As, a dialog box appears. You can provide a new filename and a location for it to be stored in.

## Naming Results

This setting is only available if MAA was launched with an assay-protocol file.
With this you can specify a custom target path to save a file to and how save filenames are automatically generated (e.g., to arrange all results into subfolders based on the protocol name).

When you click Results Naming, a dialog window with the following options appears:

1. Default (automatically named and stored in Results folder). This means a default file naming convention is used and applied by default; this default method is based on the protocol name of the related assay protocol. Example: For "ELISA.assay-protocol," the first time it is ran a results file "ELISA (1).assay-results" is created in the Results folder.

## 2. Custom

You can customize the naming method and target path manually, by using macros, or by combining these approaches.

## How to: Set Custom Results Naming

If you choose the Custom option in the Results Naming dialog window, the following text boxes will appear:

Folder. With this you can provide a custom path to store your files in. The options are:

- Provide a full path to the folder in the text box (manually or from clipboard).
- Click Browse to provide the full path to the folder in Browse for Folder tree view. Click OK to save the path or Cancel to close the dialog box without any updates.
- Click Manage to insert any number of supported macros from the list. Click Update to save changes or Discard to cancel.

Also, you can combine these options. Example: Provide path to a parent folder via the Browse option and use the <ProtocolName> <CurrentDateShort> macros from Manage to specify folder name.

Filename. This specifies the title of assay results files. The options are:

- Type a name in the textbox.
- Click Manage to insert any number of supported macros from the list. Click Update to save changes or Discard to cancel.

Also, you can combine these options. Example: type in "Experimental results for" and use <ProtocolName> <CurrentDateShort> macros from Manage to specify the analysis type and date it was performed.

(1)If the Test settings when OK is clicked check box is selected, MAA checks the accessibility of specified target path and verifies if the file can be created.

When these are provided, click OK to apply changes or Cancel to close the dialog box without any updates.

## New Protocol Wizard

How to: Launch New Protocol Wizard
Launch the MADE and do one of the following:

- On Welcome Screen, click New Protocol.
- On the Protocols tab, in the File ribbon group, click New.

The New Protocol Wizard provides a way to easily create a new assay protocol by entering only the minimum and most important information.

It guides you through the steps you can move between by clicking Next and Back to configure the following assay parameters:

- Measurement data specifics
- Layout and sample types settings
- Administrative properties (metadata)
- A name and (optionally) a description for the protocol

(1)
At any step, you can click the cross icon at the upper right of the New Protocol Wizard window to discard all changes and exit.

At the end of the wizard, the .assay-protocol file with defined configuration is added to your default Protocols folder.

## Analysis Launcher

The Analysis Launcher tool simplifies building up the command line arguments to launch a protocol with a specified set of options in MAA. It provides visual controls and validation to predetermine data analysis with no user intervention required. Also, it comes in handy when configuring multiple runs of a certain data analysis (via assay protocol) with various measurements data.

The Launcher includes:

- Controls for browsing and selecting an assay protocol, measurement data, Sample IDs, and protocol tweaks files
- Various user-friendly controls for selection of command line arguments
- A text box showing the full command line of the current selection
- The Copy to clipboard button, which copies a built command line with selected controls to the clipboard
- The Launch button, which launches the command line

The built command line is displayed as changes are made. When valid, the command line can be launched directly from Launcher.

How to: Open Analysis Launcher
You can open this tool in one of the following ways:

- On the Tools tab of MADE, in the Utilities group, click Analysis Launcher.
- In MADE, enable the Launch Assay Protocol with Options feature, and then click Launch


## Assay Protocol with Options.

- Launch the MyAssays.Desktop.Analysis.Launcher application under the MyAssays/MyAssays Desktop/ installation root folder.

If you run the application from the installation root, specify the protocol file path to see all applicable controls.

## Installed Protocols

The default MyAssays Desktop installation includes a number of preconfigured protocols; these protocols are stored in the Protocols folder.

## What About Commercial Kits?

If you are performing your assay using a commercially available kit, use Search to find a preconfigured assay protocol available at MyAssays.com. You can search by manufacturer or analyte, and if your protocol isn't currently available, you can request it using the Request
Protocol button.

## Tips for MyAssays Desktop Pro

If you are using Pro edition, you can review and edit the various settings of the installed protocols. This is a good way to see how the components of an assay protocol (including transforms, evaluations, validations, and report content) fit together to perform data analysis and reporting for real-world applications. To explore how an assay protocol is configured, follow these steps:

1. Launch an assay protocol.
(Optional) At this point, you might prefer to save a copy of the protocol to another location (or under a modified name), to keep both the original and modified versions available.
2. On the View group of the ribbon, click Properties.

This opens Assay Properties panel. If the tabs are not immediately visible, click << Show All Properties to expand.
3. Explore the assay configuration and then click Calculate to generate a report.
4. Review the report then make changes to the assay configuration. Click Calculate again to generate the report with your updates.

You also can create your own assay protocols with the New Protocol wizard.
MyAssays Desktop is available in multiple editions and configurations; certain
functions are only available if your current license supports this feature.

## Providing the Data

Each installed protocol contains Overview, Measurements and, after calculation, the
Results tab. Also, most protocols apply a layout definition when calculating results. On the Microplate tab (Rack (Layouts) tab for Dual DPM protocol), you can select or create a layout that represents your data's configuration.

Additional tabs may be available depending on the functionality of the assay protocol. Here the most general tabs—Standard Concentrations and Dilution Factors—are described. The tabs specific to a particular protocol are documented under their topics.

## Standard Concentrations

In the Standard Concentrations tab, you can provide concentration values for each Standard sample. The display and options depend on the way in which the standard concentration values are specified:

- A start concentration with a common dilution/concentration series
- Unique concentration values for each standard


## Concentration Modes

Click the Series button to switch the concentration modes: one designed for serially diluted standards, and one for standards with independent values. In the right Conc. (concentration) column of the Standard Concentrations grid, specify concentrations using:

- Series - With Series turned on, provide a value for the first calibrator group and a factor to apply to all groups. Use this if all calibrators share a common concentration or dilution series. For example, here the editor displays a start concentration and series to multiply by 2 for each Standard sample:

- Non-Serial - With Series turned off, you can specify concentration values for each calibrator separately. Use this if known concentrations do not share a common dilution series or if they are not in a simple mathematical series. For example, here the editor displays a non-serial concentration value for each Standard sample:

| Calibrator | Conc. | Series |
| :--- | ---: | ---: |
| Standard1 | 1 |  |
|  | Paste |  |
| Standard2 | 4 |  |
| Standard3 | 8 |  |
| Standard4 | 16 |  |
| Standard5 | 20 |  |
| Standard6 | 24 |  |

## Paste Option

You can copy and paste the clipboard contents to the Concentrations column. The content is pasted starting from the first row on a line by line basis. This is useful if you have your concentration values in another application (such as Excel or Notepad).

If you click Paste, the mode automatically switches to Non-Serial.

## Flashing Standard Samples

With Serial mode, all Standards on the layout flash on the Layout Preview pane. With Non-
Serial mode, only the selected group in the Standard Concentrations grid flashes.

## Dilution Factors

In the Dilution Factors tab, you can configure how the unknowns are diluted in your experiment. The dilution factors can be specified as:

- Serial dilution of all unknowns
- Serial dilutions repeated for subsets of any number of unknowns
- Unique dilution factors for any Unknown sample (for example, you can use this setting for samples that have been reassayed due to their original values falling outside the standard curve)

The two-column Dilutions table lists all unknowns as defined by the selected layout in the Sample column, and the applied dilution values in the Factor column. The available options will depend on the selected dilution mode.

## Dilution Modes

Click the Series button to switch the mode. In the Factor column, provide dilution factors as:

- Series - Use this mode if the dilution-fold is the same in each step, or if the dilutions follow a mathematical series.

With Series turned on, provide a factor for the Unknown1 and a common series to apply to all of the following unknowns. To define a series, select addition, subtraction, multiplication, or division icon operator along with a specified factor. With this, each dilution uses the previous dilution as the input for the next dilution step. For example, here the dilution factor for the Unknown1 is 1, all subsequent dilutions are multiplied by the factor of 2:


- Repeated Series - Use this mode if the same dilution steps are repeated for each subset of unknowns.

With Series turned on and its options defined (as described above), click Repeat and enter a number of contiguous Unknown samples each serial dilution will apply to. It will then be repeated from the beginning to the next subset of Unknowns. For example, if you set Repeat steps to 3, the specified Series will apply to Unknowns from 1 to 3, and then be repeated for Unknowns from 4 to 6, etc. and sequentially to the last subset:


- Non-Serial - Use this mode to dilute only specific unknowns or if your dilutions do not follow a simple mathematical series.

With Series turned off, you can assign different dilutions for any Unknown samples. For example, here the dilution is specified only to the Unknown4 and Unknown7 sample groups:

| Sample | Factor | (e.g. enter 2 for a dilution of 1:2) |
| :--- | ---: | :---: |
| Unknown1 | 1 | Series |
| Unknown2 | 1 | Paste |
| Unknown3 | 1 |  |
| Unknown4 | 2 |  |
| Unknown5 | 1 |  |
| Unknown6 | 1 |  |
| Unknown7 | 5 |  |
| Unknown8 | 1 |  |

## Paste Option

You can copy and paste the clipboard contents to the Factor column. The content is pasted starting from the first row on a line by line basis. This is useful if the dilution scheme for the assay is in another application (such as Excel or Notepad).

If you click Paste, the mode automatically switches to Non-Serial.

## Flashing Samples

With Serial mode, all Unknowns on the layout flash on the Layout Preview pane. With
Non-Serial mode, only the selected Unknown flashes.

## Results and Report

Most of the installed protocols provide quantitative analysis of samples, so they share a lot of typical report elements. These are described in the Quantitative Assay Report Contents topic. The report content specific for particular protocols is listed under their respective topics.

## Quantitative Assay Report Contents

This topic explains the contents of a typical report generated for a quantitative assay.
A quantitative assay uses a standard curve to calculate concentrations of samples. The report can contain a variety of report elements, including:

- Fit Chart - A graphic of the curve fit
- Sample Table - Showing the computed concentrations
- Calibrators Table - The points used in the fit
- Various Details about the Fit, including:
- Coefficients - The computed parameters of the fit equation
- Goodness Measures - Applicable measures to assess how good the fit models the data
- Fit Equations - Representations of the curve equations using mathematical notation
- Quantification Limits - Any computed limits of the quantification range

If you are working with MyAssays Desktop Pro edition, then you can configure how and whether to include the following report elements and how they will appear. For information about configuring your report, see Report Content. For specific report elements of endpoint curve-fitting transforms, see Curve Fit Transforms Report Contents.

## Fit Chart

The fit chart plots the standard data points as red dots with their average values shown as pink triangles. The chart displays the fit line and calibrators. The thick black line shows the curve calculated from these standards. It's also possible to display unknowns on the curve.


## Sample Table

The Sample Table lists the calculation results for each sample by row. Columns can include the sample ID, positions along with the measurement and computed values. The available columns depend on the configured transforms. The included columns can be specified using
the Matrices tab under the Properties control, and clicking the Include in Sample Tables check box.

Relevant column titles can also include any specified output units. The output units can be specified under Properties | Transforms.

Here is the snippet of results for Unknown1:

| Sample | Positions | Factor | Raw | Blank <br> Corrected | Concentration | Concentration (x <br> Dil. Factor) | \%CV |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| OUnknown1 | A3 | 1 | 0.347 | 0.3455 | 142.103 | 142.103 | 0.280766 |

The following columns are typically included:

- Sample - The sample ID, e.g., Unknown1. For unknown samples the default name will be replaced by any provided sample ID (provided in the Sample IDs tab).
- Positions - The positions on the layout designated as containing the sample (as selected in the Microplate or Layout (Racks) tab).
- Factor - The dilution factor applied to any unknowns (in the Dilution Factors tab). If the sample is not diluted or its type is not Unknown, then "1" will be displayed.
- Raw - The raw measurements data (as provided in the Measurements tab).
- Blank Corrected - The mean of each sample minus the average of included Blank group(s). If you select layout with no blanks, this column (and calculations) will be omitted.
- Concentration - The average concentrations for each sample (Raw or Blank Corrected) determined from the fit.
- Concentration (x Dil. Factor) - If unknowns are diluted, this lists concentration of each sample with any specified Factor applied.
- $\% \mathbf{C V}$ - If the samples are replicated (comprise more than 1 position), this column lists the percentage coefficient of variation between the replicates.

Samples outside the range of the standards (greater than the upper asymptote or below the lower asymptote) are highlighted in yellow. In this case, you might consider diluting these samples on the next assay run.

## Curve Data Table

The Calibrators Table lists the measurement and calculation details for each calibrator used in the fit. Here is the snippet of calibrators table for Standard1 and Standard2:

| Calibrator | Positions | Conc. <br> (Defined) | Blank <br> Corrected | Blank <br> Corrected <br> (Average) | Conc. <br> (Calc.) | \%CV <br> (Conc. <br> Calc.) | \% <br> Accuracy |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Standard1 | A1 | 400 | 0.191 | 0.1945 | (NA) <br> (NA) | (NA) | (NA) |
| A2 | 0.198 | (Nandard2 | B1 | 200 | 0.292 | 0.2955 | 186.523 <br> 178.336 |
| B2 | 3.17338 | 91.2147 |  |  |  |  |  |

Each row corresponds to the calibrator (typically a Standard) and typically includes the following columns:

- Calibrator - The sample IDs of the calibrator groups.
- Positions - The positions on the layout designated as containing standards.
- Conc. (Defined) - The known concentration value associated with the standard (as provided in the Standard Concentrations tab).
- Blank Corrected - The measurement values of each standard minus the average of included Blank group(s). If you select layout with no blanks, this column (and calculations) will be omitted.
- Blank Corrected (Average) - The average blank corrected concentration for each standard. If you select layout with no blanks, this column (and calculations) will be omitted.
- Conc. (Calc.) - The concentration values calculated from the curve for each standard.
- \%CV (Conc. Calc.) - The percentage coefficient of variation between the calculated concentrations.
- \% Accuracy - The ratio between the known concentration and the average of calculated concentration values.

Each row is highlighted based on whether it satisfies the precision and accuracy rules. The background colour is olive for rows that are within the defined thresholds and light red for those that are not. Calculated values that fall outside the range of the curve are reported with (NA) label.

## Coefficients Table

The three-column table with coefficients of the fit, their calculated value, and +/- error in correspondent columns.

## Goodness Measures Table

The two-column table with each calculated goodness measure followed by its value.

## Fit Equations

The formulae and calculated results for equation and inverse equation (if available) of the fit method.

## Quantification Limits

The two-column table that lists any additional values from the curve, such as lower and upper limits of quantification (LLOQ and ULOQ) or lower detection limit (LDL). It may also contain output units of the calculated values.

## 4PL (Constrained by B0 and NSB) Quantification

This protocol quantifies sample concentrations with constrained Four Parameter Logistic (4PL). With this analysis, the top and bottom asymptotes of the curve are constrained to nonspecific and maximal binding as determined by the measurements of BO and NSB samples.

## Assay Summary

This protocol is suitable for enzyme immunoassay (EIA) assays measured on 96 -well (12x8) microplates. It includes:

1. Background correction - If a Blank sample group is included on the selected layout, first it subtracts the mean of the blank replicates from the raw data measurements (then uses the corrected values in the fit).
2. $\mathbf{B} / \mathbf{B 0 \%}$ calculation - Calculates percentage value of each sample ranging between the measured $\mathbf{B 0}$ and $\mathbf{N S B}$ samples.
3. Curve generation - Fits Four Parameter Logistics (4PL) curve through the plotted standard data points (concentrations vs. $\mathrm{B} / \mathrm{BO} \%$ measurements) and constrains it so that upper and lower asymptotes equal B0 (100\% binding) and NSB (0\% binding). Then calculates concentrations of the samples from the curve.
4. Dilution factors application - Applies any specified dilution factors to Unknown samples.
5. \%CV calculation - If Unknowns on the selected layout are replicated, computes the percentage coefficient of variation for final concentration between the replicate values.

## Assay Protocol

To perform data analysis using this assay:

1. Launch 4PL Quantification assay protocol or results file.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Dilution Factors tab, optionally provide the dilution factors for your Unknowns.
5. Click Calculate to perform calculations and see the results.

## Results

The 4PL (Constrained by B0 and NSB) Quantification protocol reports results in a sample table and also provides additional fit results.

For a general description of report content for this assay, see Quantitative Assay Report Contents. However, the report for this assay does not include curve equations.

## 4PL Quantification

This protocol quantifies sample concentrations using a Four Parameter Logistic (4PL) curve fit.

## Assay Summary

This protocol is suitable for enzyme immunoassay (EIA) assays measured on 96 -well (12x8) microplates. It includes:

1. Background correction - If a Blank sample group is included on the selected layout, first it subtracts the mean of the blank replicates from the raw data measurements (then uses the corrected values in the fit).
2. $\mathbf{B} / \mathbf{B 0 \%}$ calculation - Calculates the percentage value of each sample ranging between the measured B0 (100\% binding) and NSB (0\% binding) or specified samples/values representative of $0 \%$ and $100 \%$ bound.
3. Curve generation - Fits Four Parameter Logistics (4PL) curve through the plotted standard data points (concentrations vs. $\mathrm{B} / \mathrm{BO} 0$ measurements). Then calculates concentrations of the samples from the curve.
4. Dilution factors application - Applies any specified dilution factors to Unknown samples.
5. \%CV calculation - If Unknowns on the selected layout are replicated, computes the percentage coefficient of variation for final concentration between the replicate values.

## Assay Protocol

To perform data analysis using this assay:

1. Launch 4PL Quantification assay protocol or results file.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Dilution Factors tab, optionally provide the dilution factors for your Unknowns.
5. Click Calculate to perform calculations and see the results.

## Results

The 4PL Quantification protocol reports results in a sample table and also provides additional fit results. The B/B0\% calculations are listed under the column in the same name in the sample table.

For a complete description of report content for this assay, see Quantitative Assay Report Contents.

## Alpha 384-well Screening

This protocol provides quantitative analysis of samples using a Four Parameter Logistic (4PL) curve fit with $1 / \mathrm{y}^{2}$ data weighting.

## Assay Summary

This protocol is provided for 384 -well $(24 \times 16)$ microplate layout. This assay:

1. Curve generation - Plots specified concentrations against the measurement data for each standard on log axes. Then fits Four Parameter Logistics (4PL) curve through the plotted standard data points using $1 / \mathrm{y}^{2}$ data weighting. After that calculates concentrations of the samples from the curve.
2. Dilution factors application - Applies any specified dilution factors to Unknown samples.
3. LLOQ and ULOQ determination - Computes upper and lower limits of quantification using precision and accuracy measures.
4. LDL calculation - Computes lower detection limit by interpolating the average background counts (blank wells without analyte) $+3 x$ standard deviation value on the standard curve.

## Assay Protocol

To perform data analysis using this assay:

1. Launch Alpha Screening assay protocol or results file.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Standard Concentrations tab, specify concentration values for Standards.
5. In the Dilution Factors tab, optionally provide the dilution factors for your Unknowns.
6. Click Calculate to perform calculations and see the results.

## Results

The Alpha 384-well Screening protocol reports results in a sample table and also provides additional fit results.

For a complete description of report content for this assay, see Quantitative Assays Report Contents.

## ATP Lite Protocol

This assay protocol provides quantitative analysis of samples using a standard curve fit to calculate ATP concentrations.

## Assay Summary

This protocol is suitable for ATP Lite 1-step luminescence assay system and is provided for 96-well (12x8) and 384-well (24x16) microplate layouts. It includes:

1. Curve generation - Fits the Linear Regression through the plotted standard data points (known concentrations vs. measurements). The concentrations of the samples are then calculated from the fit.
2. Dilution factors application - Applies any specified dilution factors to Unknown samples.
3. \%CV calculation - If Unknowns on the selected layout are replicated, computes the percentage coefficient of variation for final concentration between the replicate values.

## Assay Protocol

To perform data analysis using this assay:

1. Launch ATP Lite assay protocol or results file for your plate dimensions.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Standard Concentrations tab, specify concentration values for Standards.
5. In the Dilution Factors tab, optionally provide the dilution factors for your Unknowns.
6. Click Calculate to perform calculations and see the results.

## Results

The ATP Lite protocol reports results in a sample table and also provides additional fit results. For a general description of report content for this assay, see Quantitative Assays Report Contents.

## Best-Fit Quantification

This protocol performs multiple curve fits with different weighting models and automatically picks the Best Fit to quantify sample concentrations.

## Assay Summary

This protocol is suitable for enzyme immunoassays (EIA) measured on 96-well (12x8) microplates. It includes:

1. Background correction - If a Blank sample group is included on the selected layout, first it subtracts the mean of the blank replicates from the raw data measurements (then uses the corrected values in the fit).
2. B/B0\% calculation - Calculates the percentage value of each sample ranging betweenthe measured B0 (100\% binding) and NSB (0\% binding) or specified samples/values representative of $0 \%$ and $100 \%$ bound.
3. Curve generation - Determines the Best Fit curve and fits it through the plotted standard data points (concentrations vs. B/B0\% measurements). Then calculates concentrations of the samples from the curve.
4. Dilution factors application - Applies any specified dilution factors to Unknown samples.
5. \%CV calculation - If Unknowns on the selected layout are replicated, computes the percentage coefficient of variation for final concentration between the replicate values.

## Assay Protocol

To perform data analysis using this assay:

1. Launch 4PL Quantification assay protocol or results file.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Dilution Factors tab, optionally provide the dilution factors for your Unknowns.
5. Click Calculate to perform calculations and see the results.

## Results

The Best-Fit Quantification protocol reports results in a sample table and also provides additional fit results. It also contains best fit results table.

For a complete description of report content for this assay, see Quantitative Assays Report Contents.

## Cell Confluency Assay Development

Assay Development protocol preconfigured to import EnSight data:

- Confluency [\%]
- Foreground Roughness Mean
- Foreground Roughness SD
- Background Roughness Mean
- Background Roughness SD
- Total Roughness Mean
- Roughness Range Factor

This protocol is provided for 384 -well ( $24 \times 16$ ) microplate layout.
Assay Protocol
To perform data analysis using this assay:

1. Launch Cell Confluency Assay Development assay protocol or results file.
2. Import your measurement data.
3. Select or create a layout that matches your assay design.
4. Click Calculate to perform calculations and see the results.

## Results

This protocol imports data and automatically arranges it into 7 matrices. It does not perform any calculations on the data.

## DNA 260nm

This assay protocol provides quantitative analysis of samples using a standard curve fit to calculate DNA concentrations.

## Assay Summary

This protocol is suitable for assays measured on 96 -well (12x8) and 384 -well ( $24 \times 16$ ) microplates. It includes:

1. Background correction - If a Blank sample group is included on the selected layout, first it subtracts the mean of the blank replicates from the raw data measurements (then uses the corrected values in the fit).
2. Curve generation - Fits the Linear Regression through the plotted standard data points (known concentrations vs. measurements). Then calculates concentrations of the samples from the curve.
3. Dilution factors application - Applies any specified dilution factors to Unknown samples.
4. \%CV calculation - If Unknowns on the selected layout are replicated, computes the percentage coefficient of variation for final concentration between the replicate values.

## Assay Protocol

To perform data analysis using this assay:

1. Launch DNA 260 nm assay protocol or results file for your plate dimensions.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Standard Concentrations tab, specify concentration values for Standards.
5. In the Dilution Factors tab, optionally provide the dilution factors for your Unknowns.
6. Click Calculate to perform calculations and see the results.

## Results

The DNA 260 nm protocol reports results in a sample table and also provides additional fit results.

For a complete description of report content for this assay, see Quantitative Assays Report Contents.

## DNA Quantification

This assay protocol quantifies DNA in samples using a linear regression curve fit.

## Assay Summary

This protocol is suitable for assays measured on 96 -well (12x8) microplates. It includes:

1. Curve generation - Fits the Linear Regression model through the plotted standard data points (concentration vs. fluorescence). Then determines DNA concentrations of the samples from the linear model.
2. \%CV calculation - If Unknowns on the selected layout are replicated, computes the percentage coefficient of variation for final concentration between the replicate values.

## Assay Protocol

To perform data analysis using this assay:

1. Launch DNA Quantification assay protocol or results file for your plate dimensions.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Standard Concentrations tab, specify concentration values for Standards.
5. In the Dilution Factors tab, optionally provide the dilution factors for your Unknowns.
6. Click Calculate to perform calculations and see the results.

## Results

The DNA Quantification protocol reports results in a sample table and also provides additional fit results.

For a general description of report content for this assay, see Quantitative Assays Report Contents.

## Dose-Response Curves for EC50

This assay protocol computes dose-response curves for each sample group using the Four Parameter Logistics (4PL) regression. The fitted curves are plotted and overlaid on a single chart for visual comparison, and the fit results values are reported.

## Assay Summary

This protocol is provided for 96 -well ( $12 \times 8$ ) and 384 -well ( $24 \times 16$ ) microplate layouts. For each sample group, the measurement values are plotted against specified dose values. (This assumes that each sample group is dosed in the same way.)

Doses can be specified using:

- A start dose and a common dilution or concentration series
- Unique values for each dose


## Assay Protocol

To perform data analysis using this assay:

1. Launch Dose-Response Curves for EC50 assay protocol or results file.
2. Provide your required measurement data for the assay.
3. Select or create a layout that matches your assay design.

All sample groups are assumed to have the same number of positions defined on the layout (e.g., if Unknown1 has 10 replicates, then all Unknowns on the layout must also have 10 replicates).
4. In the Doses tab, specify dose values (as described below).
5. Click Calculate to perform calculations and see the results.

## Doses Configuration

The Doses editor specifies the doses ( $x$ values) to plot against the specified responses (measurements). The editor is displayed by clicking the Doses tab. Its display and options depend on the layout design and the mode in which the data has been specified.

## Dose Replicates

Select the number of adjacent positions each dose value applies to. Dose Replicates are determined automatically and include all possible divisors (i.e., all divisors excluding itself) of the number of positions in each sample.

For example, for a $12 \times 8$ layout with 8 Unknown sample groups, each group contains 12 positions. Thus, Dose Replicates options include 1, 2, 3, 4, and 6.

## Dose Concentrations

The left-hand Doses grid is used to select and indicate the dose value to edit. Select the dose to edit in a two-column grid.

The Dose Group column is numbered from 1 upwards, prefixed with "Dose". The number of rows is updated according to number of Dose Replicates selected.

In the Dose column, click the dose to edit its value. There are two modes for specifying dose values:

- Series - Provide a Dose value for the first dose group and common series to apply to all groups. Use this if all of the doses share a common dilution scheme or follow a simple mathematical series.

- Non-Serial - Provide a specific dose value for each dose group. Use this if your doses do not share a common concentration or dilution series or are not in a simple mathematical series.

| Dose Group |  | Dose | Series |  |
| :---: | :---: | :---: | :---: | :---: |
|  | Dose 1 | 1 | Paste |  |
|  | Dose 2 | 2 |  |  |
|  | Dose 3 | 3 | Dose Replicates: |  |
|  | Dose 4 | 4 | 2 | $\checkmark$ |
|  | Dose 5 | 6 |  |  |
|  | Dose 6 | 8 |  |  |
|  | Dose 7 | 12 |  |  |
|  | Dose 8 | 18 |  |  |

Click Series to switch the Series and Non-Serial options.

## Paste Option

You can copy and paste the clipboard contents to the Dose column. The content is pasted starting from the first row on a line by line basis. This is useful if you have your dose values in another application (such as Excel or notepad).

When you click Paste, the mode automatically switches to Non-Serial.

## Flashing Replicates

When a row is selected, the positions associated with the selection flash on the Layout Preview pane. With this, you can review to which replicates in each group a provided dose value is applied. For example, with the below layout:

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| B | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| C | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| D | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| E | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| F | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| G | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| H | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |

Unknown
If Dose Group 1 is selected and Dose Replicates is 1 , then all positions on row A flash. If Dose Group 1 is selected and Dose Replicates is 2 , then all samples on rows $A$ and $B$ flash.

## Results

This assay protocol fits a dose-response curve to each sample group of the Unknown type. It reports an overlaid chart, curve table that lists and a table with fit results values for each sample group.

## Dose-Response Curve Chart

The chart contains a curve using 4PL curve fitting plotted for each sample group, allowing you to review each fitting. For example, see the chart for example data plotted using the default layout:


## Dose-Response Fit Table

Here is a snippet that shows the Dose-Response Fit Table results for Unknown1 (using the
Unknown1 data from the chart above):

| Sample | Bottom | Top | Span | Hill Slope | EC50 | Log(EC50) | $\mathbf{R}^{\mathbf{2}}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| OUnknown1 | 350370 | 199779 | 150591 | 1.98321 | 9.05295 | 0.95679 | 0.999978 |

## Dose-Response Curves Data Tables

For each fitting, these tables list the measurement and calculation details for the calibration doses used in the fit. Here is a snippet of the Dose-Response Curves Data Table for Dose 1 and Dose 2 of Unknown3 (using the data from the chart above):

| Calibrator | Positions | Dose (Defined) | Raw | Dose (Calc.) | \%CV (Dose <br> Calc.) | \% Accuracy |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| OUnknown3 (Dose <br> 1) | A5 | 1 | 499806 | (NA) | (NA) | (NA) |
| OUnknown3 (Dose <br> $2)$ | B5 | 499550 | 0.852352 |  |  |  |

Each row corresponds to the dose and includes the following columns:

- Calibrator - The sample ID followed by the dose group of the calibrator.
- Positions - The positions on the layout designated as containing this dose.
- Dose (Defined) - The specified dose ( $x$ value) associated with the calibrator (as provided in the Doses tab).
- Raw - The measurement data (response) associated with positions of the calibrator.
- Dose (Calc.) - The dose values (y values) calculated from the curve for each specified dose.
- \%CV (Conc. Calc.) - The percentage coefficient of variation between the calculated dose values.
- \% Accuracy - The ratio between the specified dose and the average of calculated dose values.

Each row is highlighted based on whether it satisfies the precision and accuracy rules. The background colour is olive for rows that are within the defined thresholds and light red for those that are not. Calculated values that fall outside the range of the curve are reported with (NA) label.

## Dual DPM (10-vial rack)

This assay protocol computes disintegrations per minute from dual CPM measurements using the specified efficiency factor.

## Assay Summary

This protocol is suitable for rack-based and RIA assays measured on racks with 10 -vial width. It accepts dual spectrum data with common spectrum channel counts. For each data set, this protocol computes disintegrations per minute (DPM), counts, average counts per minute (CPM), and percentage error of CPM.

## Assay Protocol

To perform data analysis using this assay:

1. Launch Dual DPM assay protocol or results file.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Efficiency tab, specify the efficiency factor.
5. Click Calculate to perform calculations and see the results.

## Efficiency

In the Efficiency tab, provide the counting efficiency factor for use in calculating DPM.

## Results

The Dual DPM (10-vial rack) protocol reports results in a sample table and includes the defined efficiency factor.

Here is a snippet showing the Sample Table results for Unknown1 using the example data included in the protocol:

| Sample | Number | Spectrum | I-129 <br> Counts | I-129 <br> CPM | I-129 <br> Error <br> CPM \% | I-125 <br> Counts | I-125 <br> CPM | I-125 <br> Error <br> CPM \% | I-129 <br> DPM <br> $\mathbf{( 0 . 6 5 )}$ | I-125 <br> DPM <br> $\mathbf{( 0 . 6 5 )}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| CPknown1 | 1 |  | 17795 | 44551 | 0.47 | 19993 | 50058 | 0.45 | 28958.2 | 32537.7 |

## ELISA (96 well) - Single Page Report

This assay protocol provides quantitative analysis of samples using a Four Parameter Logistics (4PL) curve fit to calculate concentrations. The page-based report is designed for printing on a single page.

Assay Summary
This protocol is suitable for enzyme-linked immunosorbent assays measured on 96-well (12x8) microplates where samples are measured in replicate groups. It includes:

1. Background correction - If a Blank sample group is included on the selected layout, first it subtracts the mean of the blank replicates from the raw data measurements (then uses the corrected values in the fit).
2. Curve generation - Fits Four Parameter Logistics (4PL) curve through the plotted standard data points (known concentrations vs. measurements). Then calculates concentrations of the samples from the curve.
3. Dilution factors application - Applies any specified dilution factors to Unknown samples.
4. \%CV calculation - If Unknowns on the selected layout are replicated, computes the percentage coefficient of variation for final concentration between the replicate values.

## Assay Protocol

To perform data analysis using this assay:

1. Launch ELISA - Single Page Report assay protocol or results file.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Standard Concentrations tab, specify concentration values for Standards.
5. In the Dilution Factors tab, optionally provide the dilution factors for your Unknowns.
6. Click Calculate to perform calculations and see the results.

## Results

The report lists the computed concentrations and \%CV values for unknown and control sample groups. Calculated data points for Unknowns are plotted on the curve. Validation tests check for a good $R^{2}$ value (greater than 0.9 ) and that all samples meet the acceptable precision threshold (\%CV is less than 15).

Page-based report is designed to be printed on a single page.

## ELISA

This assay protocol provides quantitative analysis of samples using a Four Parameter Logistics (4PL) curve fit to calculate concentrations.

## Assay Summary

This protocol is suitable for enzyme-linked immunosorbent assays and is provided for 96well ( $12 \times 8$ ) and 384 -well ( $24 \times 16$ ) microplate layouts. It includes:

1. Background correction - If a Blank sample group is included on the selected layout, first it subtracts the mean of the blank replicates from the raw data measurements (then uses the corrected values in the fit).
2. Curve generation - Fits Four Parameter Logistics (4PL) curve through the plotted standard data points (known concentrations vs. measurements). Then calculates concentrations of the samples from the curve.
3. Dilution factors application - Applies any specified dilution factors to Unknown samples.
4. \%CV calculation - If Unknowns on the selected layout are replicated, computes the percentage coefficient of variation for final concentration between the replicate values.

## Assay Protocol

To perform data analysis using this assay:

1. Launch ELISA assay protocol or results file for your plate dimensions.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Standard Concentrations tab, specify concentration values for Standards.
5. In the Dilution Factors tab, optionally provide the dilution factors for your Unknowns.
6. Click Calculate to perform calculations and see the results.

## Results

The ELISA protocol reports results in a sample table and also provides additional fit results. For a complete description of report content for this assay, see Quantitative Assay Report Contents.

## LANCE cAMP

This assay protocol provides quantitative analysis of samples using a standard curve fit to calculate concentrations for a LANCE assay.

## Assay Summary

This protocol is suitable for LANCE assays and is provided for 96-well (12x8) and 384-well (24x16) microplate layouts. It includes:

1. Ratio calculation - Calculates the ratios of the 615 nm and 665 nm channels for each sample.
2. Curve generation - Fits the selected curve (Four Parameter Logistics (4PL) by default) through the plotted standard data points (concentration vs. ratio). Then calculates concentrations of the samples from the curve.
3. Dilution factors application - Applies any specified dilution factors to Unknown samples.
4. \%CV calculation - If Unknowns on the selected layout are replicated, computes the percentage coefficient of variation for final concentration between the replicate values.

## Assay Protocol

To perform data analysis using this assay:

1. Launch LANCE cAMP assay protocol or results file for your plate dimensions.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Standard Concentrations tab, specify concentration values for Standards.
5. In the Dilution Factors tab, optionally provide the dilution factors for your Unknowns.
6. Click Calculate to perform calculations and see the results.

## Results

The LANCE cAMP protocol reports results in a sample table and also provides additional fit results.

For a general description of report content for this assay, see Quantitative Assay Report Contents.

## Linearized (Logit-Log) Quantification

This assay protocol quantifies sample concentrations with the linearized (Logit-Log) method that plots logit $B / B 0$ versus log concentration using a linear fit.

This protocol is provided to support legacy requirements and methods. In general, the 4PL or 5PL methods are better suited for dose-response curves. The main weakness of $\operatorname{Logit}(\mathrm{B} / \mathrm{BO})$ is that the accuracy reduces towards the upper and lower limits.

## Assay Summary

This protocol is suitable for enzyme immunoassays (EIA) and is provided for 96 -well ( $12 \times 8$ ) microplate layouts. It includes:

1. Background correction - If a Blank sample group is included on the selected layout, first it subtracts the mean of the blank replicates from the raw data measurements (then uses the corrected values in the fit).
2. B/BO calculation - Calculates the value of each sample with respect to the measured B0 (100\% binding) and NSB (0\% binding) or specified samples/values representative of $0 \%$ and $100 \%$ bound; then multiplies the calculated values by 100 .
3. Curve generation - Fits the Linear Regression through the plotted standard data points (concentration vs. Logit $\mathrm{B} / \mathrm{BO}$ with log transform). Then calculates the concentrations of the samples from the fit.
4. Dilution factors application - Applies any specified dilution factors to Unknown samples.
5. \%CV calculation - If Unknowns on the selected layout are replicated, computes the percentage coefficient of variation for final concentration between the replicate values.

## Assay Protocol

To perform data analysis using this assay:

1. Launch Linearized (Logit-Log) Quantification assay protocol or results file.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Standard Concentrations tab, specify concentration values for Standards.
5. In the Dilution Factors tab, optionally provide the dilution factors for your Unknowns.
6. Click Calculate to perform calculations and see the results.

Results
The Linearized (Logit-Log) Quantification protocol reports results in a sample table and also provides additional fit results.
For a complete description of report content for this assay, see Quantitative Assay Report Contents.

## Liquid Handling QC

This protocol provides quantitative precision results (\%CV), and qualitative analysis of the results to determine if the measurements are within specified precision limits.

## Assay Summary

This protocol is suitable for assays measured on 96 -well (12x8) microplates. It includes:

1. Calculation of the mean and \%CV - Calculates the average and percentage coefficient of variation for all measurements by-plate, by-row, and by-column.
2. Comparison against limits - Compares the \%CV of the plate, each row, and each column against the \%CV limit. The \% difference of each position's measurements and the plate mean is compared against the specified $\pm \%$ limit.

## Assay Protocol

To perform data analysis using this assay:

1. Launch Liquid Handling QC assay protocol or results file.
2. Supply your measurement data.
3. In the Threshold tab, provide Plate/Row/Column \%CV and Each measurement's difference with plate mean ( $\mathbf{\pm}$ ) values as described below.
4. Click Calculate to perform calculations and see the results.

## Threshold

In the Threshold tab, provide acceptable limits for:

- Plate/Row/Column \%CV - The imprecision between positions in each row, column, and across the entire plate.
- Each measurement's difference with plate mean ( $\pm \%$ ) - The percentage difference between value of each position related to average value for the plate.


## Results

This report shows the quality control analysis of measurements from a 96-well microplate. The results are arranged in tables:

- Thresholds - The limits provided in the Threshold tab.

In each of the following tables, the results are reported with pass $(\mathbb{N})$ or fail $(\mathbb{N})$ icons in the Success column to indicate if they are within the acceptable limits.

- Plate Analysis - The calculated mean and \%CV of all measurements on the plate.
- Position Analysis - The position ID, value and \% difference between each position's measurement and the plate mean.
- Row Analysis - The mean and \%CV of all measurements in each row.
- Column Analysis - The mean and \%CV of all measurements in each column.

Also, the report contains the heatmap-based graphical representation of the measurements. For more information about heatmap view, see Heatmap.

For further details about this report element, see Matrix View.

## Multiplex

This assay protocol fits a standard curve and calculates concentrations, lower and upper limits of quantification (LLOQ and ULOQ), and limit of detection (LOD) for each analyte. It also applies specified dilutions and calculates \%CV between replicates.

## Assay Summary

This protocol is suitable for multiplex analysis on any number of analytes and is provided for 96 -well (12x8) microplate layouts. For each analyte:

1. Curve generation - Fits the Four Parameter Logistics (4PL) curve through the plotted standard data points. Calculates concentrations of the samples from the standard curve and computes with LLOQ, ULOQ and LOD.
2. Plate dilution factor application - Applies specified Plate Dilution Factor to the calculated concentrations and limits.
3. Sample dilution factors application - Applies any specified dilution factors to Unknown samples.
4. \%CV calculation - If Unknowns on the selected layout are replicated, computes the percentage coefficient of variation for final concentration between the replicate values.

## Assay Protocol

To perform data analysis using this assay:

1. Launch Multiplex assay protocol or results file.
2. Supply your measurement data.
3. Select or create a layout that matches your assay design.
4. In the Plate Dilution Factor tab, specify dilution to apply to calculated concentrations and limits.
5. In the Sample Dilution Factors tab, provide the dilution factors for your Unknowns.
6. Click Calculate to perform calculations and see the results.

## Plate Dilution Factor

In the Plate Dilution Factor tab, enter or paste a factor to multiply all samples by.

## Sample Dilution Factors

In the Sample Dilution Factors tab, specify dilution factors for your Unknown samples.

See Dilution Factors for a detailed description.
Results
The Multiplex protocol reports results per analyte in a sample table and also provides additional fit results. For a description of report content for each analyte, see Quantitative Assay Report Contents. Additionally, Quantification Limits table features calculated LOD, and all three values are multiplied by the specified Plate Dilution Factor (PDF).

## OQ Absorbance

This assay protocol provides operational qualification (OQ) analysis of an absorbance test.

## Assay Summary

This protocol operates on XY data and is suitable for absorbance assays measured on 96-well $(12 \times 8)$ microplates. In this case, a special test plate with two calibrated filters is used. The nominal Optical Density (OD) of these filters is different for each plate. It includes:

- Data reduction - Calculates the average, maximum, and minimum absorbance values for each position.
- OQ calculation - Calculates precision and accuracy for each sample.


## Assay Protocol

To perform data analysis using this assay:

1. Launch OQ Absorbance assay protocol or results file.
2. Supply your measurement data for $\mathbf{B 2}$ (negative control) and $\mathbf{G} \mathbf{2}$ (positive control) positions.
3. In the Parameters tab, provide High and Low values.
4. Click Calculate to perform calculations and see the results.

## Parameters

In the Parameters tab, specify the nominal optical density values for your plate:

- Low - This is low absorbance value.
- High - This is low absorbance value.


## Results

This assay protocol plots average and peak (maximum and minimum) XY analysis charts and reports results for each sample in a sample table. Here is the snippet of sample table for Control1 (B2 position on the layout):

The following details are reported in the columns from left to right:

- Sample - The sample ID, e.g., Unknown1. For unknown samples, the default name will be replaced by any provided sample ID (provided in the Sample IDs tab).
- Positions - The positions on the layout designated as containing the sample (as selected in the Microplate or Layout (Racks) tab).
- Absorbance - The XY plot for each position (in plate scale).
- Average Abs. - The average absorbance value for each position.
- Max Abs. - The minimum absorbance value for each position.
- Min Abs. - The maximum absorbance value for each position.
- Precision \% - The precision of the absorbance test in this position.
- Accuracy \% - The accuracy of the absorbance test in this position.


## Standards Curve with Titres and Parallelism Plots (Dual)

This protocol calculates difference between measurement and reference data, quantifies sample concentrations using a Four Parameter Logistic (4PL) curve fit, and then plots the fitted curves on an overlaid chart for line analysis.

## Assay Summary

This protocol is provided for dual endpoint data measured on 96 -well (12x8) microplates. It includes:

1. Dual matrix background correction - Corrects measurement data (Matrix 1 ) by subtracting reference data (Matrix 2) point-by-point.
2. Curve generation - Fits Four Parameter Logistics (4PL) standards curve (specified concentrations vs. corrected measurements). The concentrations of the samples are then calculated from the curve.
3. Mean, SD and \%CV calculation - Computes average, standard deviation and percentage coefficient of variation for all standards.
4. Titres calculation - Calculates titre values for each sample (calculated concentration $x$ specified dilution).
5. Curves comparison - Plots overlaid 4 PL curves of each sample group for parallel line analysis (specified dilution vs. measurement).

## Assay Protocol

To perform data analysis using this assay:

1. Launch Standards Curve with Titres and Parallelism Plots (Dual) assay protocol or results file.
2. Supply your measurements in the Matrix 1 and reference data in Matrix 2.
3. Select or create a layout that matches your assay design.

The layout must contain at least one Blank and two Standard sample groups.
4. In the Standard Concentrations tab, specify concentration values for Standards.
5. In the Sample Dilutions tab, provide the dilution factors for your Unknowns, Standards, and Controls.
6. Click Calculate to perform calculations and see the results.

## Sample Dilutions

In the Sample Dilutions tab, you can provide different dilutions for each replicate of Standard, Control, and Unknown samples. This tab displays grid-based view of the dilutions for the input data. The configuration of the grid depends on the selected layout, thus each position's background is coloured to match its sample type.

Within Sample Dilutions grid, you can select and specify dilution for:

- A single position
- Multiple positions to set to a same dilution

Multiple positions can be selected by:

- Dragging the mouse (for rectangular selection)
- Using Ctrl to select random positions
- Using Shift to select the end position (rectangular selection)


## (1) Copy and Paste keyboard commands are supported.

## Results

The Standards Curve with Titres and Parallelism Plots (Dual) protocol results in:

- Sample results for Standards, Controls and each Unknown sample separately. These are presented as tables that list all calculation results for each sample.
- Standards Chart and Fit results for Standards that include goodness of fit measures and $\pm$ errors.
- Parallelism curve chart that contains a curve using 4 PL curve fitting plotted for

Standards, Controls, and each Unknown sample group, allowing you to review each fitting. Under the chart, the goodness measures are included in the table for comparison.

## Z-Factor

Calculates Z-Factor in accordance with Zhang JH, Chung TD, Oldenburg KR, A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. J Biomol Screen. 1999;4(2):67-73.

## Assay Protocol

To perform data analysis using this assay:

1. Launch Z-Factor assay protocol or results file.
2. Provide your required measurement data for the assay.
3. Select or create a layout that matches your assay design.

## This assays requires a layout with at least one Pos Control and Neg

## Control groups.

4. Click Calculate to perform calculations and see the results.

## Results

This protocol calculates the Z-Factor for the given measurements. It also reports:

## Control Results table

The two-column table that lists evaluation results for positive and negative controls. For each control, the average (AVG), standard deviation (SD), and percentage coefficient of variation (\%CV) are reported.

## Heatmapped Raw Data

The heatmap-based graphical representation of the raw measurements data.
For more information about heatmap view, see Heatmap.
For further details about this report element, see Matrix View.

## Metadata

The MyAssays Desktop version, file names of the protocol and results files, current user and date/time stamp are also included in the report.

## Common Tasks

## Data Storage

## Storing Data

The default path for saving your data files lies in your Documents folder, under the MyAssays subfolder. You can open it in MyAssays Desktop Explorer, Windows Explorer, or any other content-managing application.

You may want to change the way your data files are stored. For example, you may prefer your data to be stored in a network location or in a shared folder that is shared with other users of the system.

## How to Set an Assay Data Folder Root

1. Click the File tab to open MyAssays Backstage view and then click Data.
2. Click Select Folder Root and choose the preferable folder.

Uninstalling MyAssays Desktop will not remove your data files.

## Backing Up Data

Periodical backing up of your assets is beneficial so you will always have a copy of all important data, and is a part of responsible PC administration. To facilitate your backup process, all data files are stored under a single parent data folder that should be backed up.

To determine which folder to backup, launch MyAssays Desktop Explorer and select File |
Data to obtain the current parent directory. All relevant data is stored in the MyAssays subdirectory of this folder.

## Adding Field Data Alongside Measurements

Using the Field Data feature, you can include additional details for each sample position in your assays alongside numerical measurements and calculated results. Field data can be imported from the same file as your measurement data or from a data file separate to the file containing measurement data.

## What Is the Field Data?

Field data is any textual or numerical data associated with a particular sample position on a field matrix. Field data matrices can be either:

- String (text)
- or Numerical

Textual field data matrices are not included in data analysis; they are imported into the protocol and reported in Sample Tables and Matrix Tables alongside measurements and calculation results. Typically, textual field data can include information such as label name, measurement time, detection parameters, and other background measurement data.

Numerical field data values are available for further data analysis, they can be used as transform inputs and can be referred to from expressions (such as in selected transform parameters, evaluations and validations).

Field data might be automatically imported alongside your measurement data for your instrumentation (e.g. WIZARD ${ }^{2}$ counters with the All data import option), or you can add field data matrices to your assay protocol configuration as needed.

## How to Include Field Data in Your Assay?

To include field data in your assay, first create and configure an assay protocol that includes the number of field data matrices you need.

1. Launch MADE.
2. In the upper-left corner of Welcome screen, click New Protocol to launch the New Protocol Wizard.
3. Supply your measurements data and:

- For RIA assays with measurements imported from WIZARD ${ }^{2}$ instrument readers, select My measurement data is in a file or spread across multiple files option. Click Next and select the Automatically import measurement data check box on the next step.
- When importing measurements from other instrumentation sources, select I will enter/paste in my data or I don't have any data yet option.

1. When selecting type of your measurements data, select the Other/Combination option and click Next.
2. On the next step, under the Data pane, click Add Data, select Field Data and select the Field Type (either String or Numeric). For Numeric field data also specify numerical formatting and precision.

To rename the matrix, select it and edit its Name on the Configuration pane.

Add at least one measurement data matrix to proceed.
4. Click Next to continue.

When creating an assay protocol with more than 5 field data matrices, for conciseness, these matrices will be excluded from the report by default. To learn how to include field data in the report, see Handling a Large Number of Raw and Field Matrices.

## Field Data with Multiple Plate Assays

With multiple plate assays, each plate will contain the configured number of field data matrices. In this case, the Field Data sub tab features the Plate drop-down selector, where you can choose the plate to view.

Field Data with Multiplex Assays
With multiplex data, the field data is common for all analytes.

## How to View and Edit Field Data?

You can view the field data on the Field Data sub-tab of the Measurements tab. It presents all field data matrices in a table structure, where each column corresponds to a matrix.

- For microplate assays, each row is preceded with a position ID (e.g., A1, B6, etc.) coloured according to its sample type.
- For rack assays, each row is preceded with the 1-based position number for each sample and coloured according to its sample type.

You have several options when adding, editing, or removing data. You can paste data into a field data matrix, enter it into a single cell, or into several cells.

## Paste Data in a Matrix

1. Right-click the field data matrix header.
2. Click Paste.

To remove values from all cells in the matrix, click Clear.

## Enter or Paste Data in a Cell

- To enter data manually, click a cell.

Type the text or numbers and then press Enter.

- To paste data from clipboard, right-click a cell and click Paste.


## Paste the Same Data into Multiple Cells

1. Select the cells to paste data into. These cells do not have to be adjacent.

To select adjacent cells, select the first cell, press Shift and select the last cell in the range. Or click the first cell, then hold and drag the pointer to the last cell in the range. To select cells that are not adjacent, press and hold Ctrl and select the cells to paste into.
2. Right-click an active cell, and then on the drop-down menu click Paste.

## What Can I Do with Field Data?

Apart from viewing and editing field data in your assay, you can:

- Configure how Field matrices are reported
- Label the Field data that matches specified word, phrase, or number

With Numerical field data matrices, the field data values can be used in the analysis. Numerical field data matrices are available as inputs to transforms (in the same way as endpoint measurement matrices)

## Referencing Numerical Field Data Values in Expressions

Numerical Field Data values can be referenced in expressions by specifying the field data matrix letter as part of the reference. The field data matrix lettering starts from A. View the Matrices tab to see the lettering of any field data matrices.

For example, the expression Unknown1.A refers to the value of the Unknown1 sample group for field data matrix with letter A (the first field data matrix). Similarly, the expression A1.B refers to the numerical field data value at position A1 of the second field data matrix (lettered B).

For more information see numerical field data operands.

## Visualizing Data

## Heatmap Plate View

The Heatmap View displays a plate or rack layout coloured in accordance with the measurement data numerical values. For example, data with smaller numerical values is depicted in dark green and data with larger numerical values is shown in red.

How to: Access the Heatmap View

1. In MAA, select the Measurements or Microplate (Layout (Racks) for rack assays) tab.
2. Check the Heatmap View option in the lower left corner of the Layout Preview pane.

Heatmap view is relevant for endpoint data only.

## Structure

Heatmap View comprises:

- Plate preview with the colour of each position based on its numerical value converted to a colour within a given scale

Data for Unused positions is not displayed; the wells appear white and empty.

- A heatmap scale (located under plate preview) with clickable minimum and maximum controls
- A scale function menu that determines the heatmap scale type (linear or Log10)
- The auto scale option
- 2D/3D view buttons



## Heatmap View Settings

## Minimum and Maximum Controls

These settings allow you to set the colour scheme range displayed on the heatmap plate preview. Each position on the layout is coloured according to its numerical value and the colour scale (using the colour range $\mathrm{min} / \mathrm{max}$ values). Values outside the range are rounded to the nearest min or max colour.

## How to: Change the Minimum Value

1. Hover to and click the minimum value on the left side of the colour bar to edit the heatmap minimum value.
2. Type in a minimum value for the heatmap (e.g., 0.05).
3. Click the check icon to save your changes or the cross icon to cancel them.

If you type in a minimum value that is greater than the maximum value, an error message will be displayed.

How to: Change the Maximum Value

1. Hover to and click the maximum value (right side of the colour bar) to edit the maximum value.
2. Type in a maximum value for the heatmap (e.g., 2.00).
3. Click the check icon to save your changes or the cross icon to cancel them.

(1)
For multiple plate multiplex assays, consider all used positions before you type in minimum and maximum values for the heatmap.

## Scale Function

With the Heatmap View, you can use one of the following scale settings for heatmap generation:

- Log 10 scale - Minimum and maximum control values change based on a logarithmic function
- Linear scale - Minimum and maximum control values change with a constant speed


## How to: Switch Between the Log10 and the Linear Scales

1. With the Heatmap View turned on, click on the Scale function button (from the
righthand side of the heatmap maximum control) to display the scale settings.
2. Select the scale you want the Heatmap View to display with:

- Log10 scale - Click the Log10 scale button (right one). The heatmap is refreshed to display a Log10 colour scale.
- Linear scale - Click the Linear scale button (left one). The heatmap is refreshed to display a linear colour scale.


## Auto Scale

The Auto Scale setting allows you to automatically scale the plate preview if the minimum and maximum values are changed for a set of measurement data. For example, if you type a custom maximum value in the heatmap maximum control and then change the measurement data, the Heatmap View will be updated with the original scale.

If you select the Auto Scale and there is a change in the measurement data, the Heatmap View is updated using the new scale. In this case, if the edited measurement data has a new $\mathrm{min} / \mathrm{max}$, then the heatmap scale is updated accordingly.

## How to: Access the Auto Scale

1. With the Heatmap View turned on, click the Auto Scale button (on the right side of the heatmap maximum control).

You can switch the Auto Scale setting on and off by clicking the Auto Scale button.

## 2D/3D View

Similar to the Layout View, you can view the heatmap in a 2D or a 3D format.
How to: View the Heatmap in a 3D Format

1. In MAA, select the Measurementsor Microplate tab.
2. Select the Heatmap View option in the lower left corner of right pane.
3. Click the 3D button.

You can switch between the 2D and 3D view using the corresponding buttons.

## 3D View

The 3D view projects single endpoint measurement data layout in three dimensions for a convenient visual comparison. The 3D view can use Layout or Heatmap colours.

How to: View Endpoint Measurement Data in 3D

1. Launch an assay protocol or open a results file.
2. Select the Measurements, Microplate (Layout (Racks) for rack assays), or Sample IDs tab.
3. For assays that contain multiple matrices and/or containers, select the matrix you want to view from the drop-down list.
4. In the lower left corner of the Layout Preview pane, click 3D.

Switch between 2D and 3D view using the corresponding buttons.
The 3D view is based on the default 2D view comprising of rows and columns corresponding to the $x$-axis and $y$-axis of the 3D model; the $z$-axis represents the measurement at each position.

All other actions applicable to 2D layout are also applicable to the 3D view.

## Change 3D View

To manipulate the 3D view:

- Rotate: Hold the mouse button down and drag in the required direction.
- Zoom In/Out: Use the mouse scroll wheel or hold down left and right mouse button and move up and down.
- Reset: Press the $\mathbf{r}$ key.


## Move

You can move the layout model by pressing the wheel mouse button and moving the mouse.

## Enable or Disable 3D View

The 3D view is available for layouts up to 384 positions. This setting can be modified under the 3D View subsection of View tab in the MAA's File backstage area.

## Fit Chart

When using assays with endpoint measurement data, the Fit Chart allows you to inspect your sample data alongside the fitted curve. Also, you can flag standard points directly from the chart; this is useful for excluding outliers from the curve fit.

The Fit Chart is included in the report as one of Transform Content report elements.


Displaying Samples
Click the sample name legend above the chart to show or hide the sample groups. For example, here the Unknown legend has been clicked to show where the Unknowns fall on the curve.

To plot non-calibrator sample types on the fit line in the report, you can use Points on Curve setting.


## Flag Outliers

Outlier standard points can be removed by simply clicking on the point. After marking the point or points to remove, click Calculate to generate a new report with the new data set. The flagged points are excluded from the calculations (i.e., not used in the curve fit).


When viewing a Fit Chart for Standard Curve Fit (From Library) or Dose-Response Curves transform, flagging Library Standards and Library Average points in not available.

## Chart Zoom

For a closer look at your data points, you can zoom in on the chart by clicking and holding the mouse whilst dragging an area to zoom. Release the mouse button to zoom the chart area.
Click the Reset Zoom button or double-click the chart to return to the full view.
Point Details
Hover the mouse over a point to view further information about that point. In the image below, the mouse has been positioned over the point of the third standard to display the point details.


## Curve Overlay Chart

When working with multiple fitted curves, it can be useful to overlay each curve on to a single chart for visual inspection and curve comparison. The Curve Overlay feature allows curves from different sources to be overlaid to a single chart. Also, points of interest can be interpolated and plotted on the curves. This topic explains how to work with a curve overlay chart.

To learn how to configure curve overlay charts in your assay protocol, see Curve Overlay report element topic.

The Curve Overlay chart shows the fitted curves together in a single chart. For example,


Displaying Curves
Click the curve name legend above the chart to show/hide the curves. For example, here the Curve 1 legend has been clicked to hide this curve.


## Chart Zoom

For a closer look at a specific area of the chart, zoom in by clicking and holding the mouse whilst dragging an area to zoom. Release the mouse button to zoom the chart area. Click the Reset Zoom button or double-click the chart to return to the full view.

## Curve Overlay Chart Evaluations

A curve overlay chart might include evaluations to indicate where certain values fall on each of the chart. See Curve Overlay Evaluations for the configuration steps.

Curve 1 ED20 ED50 ED80 Curve 2 ED20 ED50 ED80


## Kinetic/Spectral Overlay

When working with kinetic or spectral data, the Kinetic/Spectral Overlay Chart gives you a clear view of measurements at the position(s) or group(s). The $y$-axis of the chart corresponds to measurement values; the x-axis represents (depending on the assay) cycle, time, or wavelength values.


## View Selection Modes

Depending on the view selection, you can explore data ranging from a plot that belongs to a single position to the overall plate/analyte values.

How to: View XY Overlay Chart

1. In MAA, select the Measurements tab.
2. Select the Chart subtab.
3. Use the Select Group/Select Position controls to select the view mode.

There are two modes for Kinetic/Spectral Overlay:

- Position
- Group

For kinetic or spectral data, any specific position can be selected and viewed on a Kinetic/Spectral Overlay chart.


Moreover, any number of positions in any combination can be selected from the right-most View Selection pane. Select the check boxes corresponding to positions you want to view.

Alternatively, any sample group can be also viewed on the Kinetic/Spectral Overlay chart with the same actions as described above.


Additionally, you can click Show All or Hide All to display plots for all plate/analyte positions or for none, respectively.

## Zoom In/Out

Position the mouse pointer on the area you want to enlarge, press and hold Ctrl button, and scroll the middle mouse button up or down to zoom in or out. Alternatively, hold down left mouse button and drag a rectangular area to zoom in.

Double-click or click Reset zoom on the upper left of the enlarged area to reset the zoom.

## Flagging Outliers

Any data point can be flagged and unflagged interactively on this view. The chart will update accordingly.


Multiple Plate View
If your measurement data is spread across multiple containers, you can choose which plate/analyte to be displayed. Select the required container from the drop-down list in the left upper corner of the Kinetic/Spectral Overlay Chart.

## Kinetic/Spectral Analysis Chart

For kinetic/spectral assays, you can inspect the data analysis results on a Kinetic/Spectral Analysis Chart with respect to the configured XY data settings. Moreover, you can flag points and define subset of data points to consider directly on the chart.


The $y$-axis of the chart corresponds to the measurement values; the $x$-axis represents (depending on the assay) cycle, time, or wavelength values.

## Displaying Data Plots

In most cases, the results are presented in terms of each position and contain input data plot in red colour and calculated result represented in blue.For XY Replicates Average and XY Replicates Function, the results are presented for each sample group.

For XY Blank Correction transform results, the chart is not produced.

## Flagging Outliers

To flag an outlier data point, click it. After marking the point or points to remove, click
Calculate to generate a new report with the new data set. The flagged points are excluded from the calculations.


## Specifying XY Data Subset

For any position, you can define a subset of kinetic/spectral data points to include in calculations via the positioning of vertical dashed range selectors. The points outside the selected range are displayed as greyed out with no lines between them and are exlcluded from further calculations.

Recalculate to generate a new report with the new data set.


## Analysis Description

 the description of analysis performed and any non-matrix data for each plot is displayed immediately below the chart.


## Zoom Options

Position the mouse pointer on the area you want to enlarge, press and hold Ctrl button, and scroll the wheel mouse button up or down to zoom in or out. Alternatively, hold down left mouse button and drag a rectangular area to zoom in.

Double-click or click Reset zoom on the upper left of the enlarged area to reset the zoom.
Multiple Plate View
If your measurement data is spread across multiple containers, each plate/analyte is displayed in a separate Kinetic/Spectral Analysis Chart tab.

## Flagging/Outlier Exclusion

## Overview

Flagging is used to mark and indicate data to exclude from further analysis. This can be used to identify outliers to ignore issues such as poor measurements or data for incorrectly prepared or contaminated samples.

There are two types of data that can be flagged:

- Position
- Point

For all measurements types, any position can be marked as flagged (e.g., position A1). When the data at a position is flagged, any reference to the sample contained at that position will not include the flagged position's data.

For example, if Standard $\mathbf{1}$ comprises of 3 replicates measured at position $\mathbf{A 1}, \mathbf{A 2}$, and $\mathbf{A 3}$, if position $\mathbf{A} \mathbf{1}$ is flagged, Standard 1 would refer to only the non-flagged values (i.e., the average of the data at positions $\mathbf{A} 2$ and $\mathbf{A 3}$ ).

For kinetic or spectral data, any specific data point can be marked as flagged too (e.g., the data point for cycle 1 of position A1). Any flagged data points are not included in any further analysis. If all points of a position are flagged, then the position is considered to be flagged.

For example, if the kinetic measurement data at position $\mathbf{A 1}$ comprises of 10 cycles, if point 1 is flagged, then a kinetic reduction operation is performed (such as computing the average of kinetic cycles in XY Reduction Average) and the calculations would ignore the data of the flagged point.

Data can be flagged interactively or by calculations. Any flagged data will be indicated in the report and export accordingly. It is also possible to customize the labels for flagged data in the report.

## Interactive Flagging

Position or point data can be flagged (and "unflagged") interactively by left-clicking the mouse on the position or data point to flag. This is possible in:

- Measurements View (in the Measurements tab)
- Standards Chart (in the Results tab when working with Standard Curve Fit transform)
- Kinetic/Spectral Measurements Chart (in the Measurements tab|Charts subtab when working with kinetic/spectral data)
- Kinetic/Spectral Analysis Chart (in the Results tab when working with Kinetic/Spectral transforms)


## How to: Flag Data Position with Measurements View

1. In MAA, select the Measurements, Microplate, or Sample IDs tab.
2. Hover to the Plate Preview pane on the right of the selected tab.
3. Position the mouse pointer over the point to flag and left-click.
(1.) A flagged position appears on the Measurements View with a cross through it.


How to: Flag Data Position with Standards Chart

1. Add the Standard Curve Fit Transform:
2. Launch an assay protocol or open a results file.
3. If you cannot see the Assay Properties panel, click the Properties button to open it.
4. Click the Add button.
5. Select the Standard Curve Fit transform.
6. Click the Create button.
7. Review or edit the transform's input matrix and output matrix name as required.
8. Provide your required settings for the transform (see the Properties section above).
9. Click Calculate.
10. Select the Standard Curve Fit tab of the Results tab. If your assay comprises multiple plates, select the one that contains the analysis view for your data.
11. Select the sample type on the legend to view how the points are spread on the curve.
12. Position the mouse pointer over the point to flag and left-click.
13. Click Calculate to perform the calculations with the new settings and to see the results.
(1) Any flagged item can be "unflagged" by left-clicking on the flagged item.


## How to: Flag Kinetic/Spectral Data Point with Kinetic/Spectral

## Measurements Chart

1. In MAA, select the Measurements tab.
2. Select the Chart tab.
3. Use the Select Group/Select Position controls to select the required data.
4. Position the mouse pointer over the point to flag and left-click.


How to: Flag Kinetic/Spectral Data Point with Kinetic/Spectral Analysis Chart

If your data analysis includes an Analysis View (used to display further information about the performed data analysis), then you can flag XY points directly from this view.

1. In MAA, select the Results tab.
2. Select the tab that contains the analysis view for your data.
3. Position the mouse pointer over the point to flag and left-click.

Any flagged item can be "unflagged" by left-clicking on the flagged item.

XY Maximum Slope


## Flags View

If there is any flagged data, then the Flags option will appear in the View group of the
Home ribbon tab. The Flags view control will display a list of the currently flagged items (including both positions and point data if relevant).

| Flags |  |  |  |  | $\times$ |  |
| :---: | :--- | :--- | :--- | :--- | :--- | :--- |
| Status | Type | Position | Point | Plate | Matrix |  |
| $\square$ | Point | A5 | 7 | Plate 1 | Raw |  |
| $\square$ | Point | A5 | 6 | Plate 1 | Raw |  |
| $\square$ | Point | A5 | 5 | Plate 1 | Raw |  |
| $\square$ | Position | A1 | - | Plate 1 | Raw |  |
| $\square$ | Position | A2 | - | Plate 1 | Raw |  |
| $\square$ | Position | A3 | - | Plate 1 | Raw |  |

Select or clear the status of an entry to change its flagged status.

## Calculated Flagging

In addition to manually selecting which data to flag, calculations may result in flagged results.

For example, the Replicate Outlier Removal transform can be used to automatically flag sample replicates that are outside a definable expected range. For more flexibility, the Auto Flag transform enables you to define conditions to flag endpoint values.

When working with kinetic or spectral data, the XY Outlier Removal transform can be used to automatically detect specific data points that deviate from an expected pattern beyond an expected threshold.

Also, for certain calculations there might not be a valid result for the calculation. In those cases the result will be marked as flagged (as no further calculation is possible).

## Advanced

All transforms include a setting under the Advanced properties group for selecting which sample types the transform should calculate results for. For those samples that are not to be calculated, the results can be set to be flagged as either:

## - Flagged with Value

## - Flagged no Value

## Flagged Data in the Report

The report will indicate flagged data as strikethrough text (such as $\mathbf{4 . 2 3}$ or Flagged). Where data has been flagged and there is a value associated with it, the original value appears in strikethrough.

Where there is no value associated, the text Flagged appears (also in strikethrough).
For example,

| Sample | Positions | Raw | Concentration |
| :--- | :--- | :--- | :--- |
| Unknown1 | A3 | 1.599 | 1.53791 |
|  | A4 | 1.665 |  |

Here, position A3 has been flagged. In this case the calculation of the concentration will use only the value from position A4.

Here the Raw values in positions A3 and $\mathbf{A} 4$ have been flagged. This means that there is no result for the Concentration.

| Sample | Positions | Raw | Concentration |
| :--- | :--- | :--- | :--- |
| Unknown1 | A3 | 1.599 | Flagged |
|  | A4 | 1.665 |  |

## Flagged Data in Text-Based Exports

For text-based export (such as CSV, TXT) where strikethrough is not available, flagged data is identified by asterisk symbols surrounding each flagged data item, for example:
*1.23*
*Flagged*
For example, the following export text shows three data values flagged:
Sample, Positions, Raw, Concentration
Unknown1,A3 A4,*1.599* *1.665*,*Flagged*

## Custom Flag Label

If the default strikethrough text "Flagged" is not suitable, you can specify your own text to use instead. For example, here the text No result is used instead of Flagged.

| Sample | Positions | Raw | Concentration |
| :--- | :--- | :--- | :--- |
| Unknown1 | A3 | 1.599 | No result |
|  | A4 | 1.665 |  |

This is an advanced setting that can only be configured through the XML editor. For more information please refer to Custom Flag Label configuration.

## Custom Flag Label Configuration

The Custom Flag Label feature enables specific labels to be defined and used to indicate flagged results instead of the default strikethrough flagged text (such as $\mathbf{4 . 2 3}$ or Flagged).

The settings are available at different levels of the Matrix-Transform Analysis configuration; this provides a fine degree of control over how flagged results are reported. For example, you may prefer to label flagged raw data differently than flagged concentration results.

Additionally, the Standard Curve Fit transform supports conditional flagging and applying labels. This enables the definition of rules to apply that define how to flag and label results in accordance with calculations from the curve. For example, you may want to flag or use a custom label for samples that fall outside a defined concentration range. This is described further in Standard Curve Fit Custom Flag Labels.

About label sample results that satisfy specified conditions without flagging them, see Applying Labels to Endpoint Data.

## Custom Flag Label Levels

Flag labels can be set at the following levels:

1. By Transform Calculations
2. By Output Matrix
3. General

If there is a combination of custom flag labels defined, then they are handled in the order given above. Custom labels higher in the list take precedence over those further down the list (e.g., any specified in the transform implementation override any defined at both the output matrix and general level).

## XML Configuration

These advanced settings are only available through the XML editor. To access the XML editor, select the XML tab in the Assay Properties panel.

## Custom Flag Label: General

The general custom flag label is the label to use in place of the default Flagged label. Note, when specifying custom flag text, strikethrough is not applied.

You can set the general custom flag label at the Transforms root level. All flagged data positions on any matrix (raw or calculated) will then use this setting. Enter the flag label with the following syntax:
<Transforms FlagLabel="No result" >

With this setting, the flagged data will appear as follows:

| Sample | Positions | Raw | Concentration |
| :--- | :--- | :--- | :--- |
| OUnknown1 | A3 | 4.599 | No result |
|  | A4 | 4.665 |  |

How to: Set General Custom Flag Label

1. In the Assay Properties panel, select the XML tab.

If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Locate the Transforms element.
3. Add the FlagLabel element to the Transforms element.

## Custom Flag Label: By Output Matrix

A custom flag label can be set on any output matrix of any transform. This defines a common flag label to use for all data positions flagged by the transform.

Provide the label with the following syntax (editable label highlighted in yellow):
<Matrix Id="Calc1" Name="Calc1" FlagLabel = "Outlier Removed" />
This setting only applies to data points flagged by the transform.
How to: Set Custom Flag Label for an Output Matrix

1. Select the XML tab in the Assay Properties panel.

If the XML tab is not immediately visible, click the $\ll$ Show All Properties button to expand.
2. Locate the Transforms element.
3. Locate Output element.
4. Add the FlagLabel element to the Matrix element.

## Custom Flag Label: By Transform Calculations

The Standard Curve Fit and General Curve Fit transforms provide custom flag settings by calculation. For more details, see Custom Flag Labels by Fit Transform Calculations.

## Custom Flag Label Reporting

The specified custom flag labels will be used in the report and export; however, the Sample Table Replicates setting of matrices configuration also impacts which labels are displayed. This is explained as follows:

## - List

For any flagged position that has a custom flag label associated with it, that custom flag label is displayed.

## - Average

In this case the flag label is displayed only if all positions in a sample group are flagged; otherwise, average of non-flagged data points is calculated. For the former, the following scenarios apply:

1. If all positions have no custom flag label then the default flagging settings are used.
2. If all positions have the same custom flag label, associated with them, then that custom label is displayed.
3. If positions of a sample group have different flag labels associated with them, then the list of flag labels (custom and/or default) are displayed in the order of the positions. Example: If there are 3 positions in a group and they are all flagged (the first has no custom label, the second has custom label "Label A," and the third has custom label "Label B"), then "Flagged, Label A, Label B" will be displayed.

## - First

Any custom flag label associated with the first position is displayed.

## Editing Protocol Overview

The contents appearing in the Overview tab can be modified to include any useful information about the assay; this might include how to perform or use the assay or a description of the data analysis performed. Also, the assay name appearing in the tab can be modified.

The provided Name applies only to the name appearing in the Overview tab (it does not change the file name of the assay protocol file).

## Style Tags

The bold, italics and sub-script HTML style tags are available for use in the Overview Name and Description. For example:
<b>Your text</b>-displays as: Your text.
<i>Your text</i> - displays as: Your text.
H <sub> Your text</sub>0 - displays as: $\mathrm{H}_{2} \mathrm{O}$.

## Resource References

In addition to specifying the assay name and overview text, use the Resource Reference feature to provide links to related content that might be useful to users of the assay. This might include SOPs or troubleshooting note; in fact links to any internal or external resources can be provided.

## Editing Assay Protocol Name and Description

In the Overview pane, you can edit assay protocol's name and description by:

1. Launch an assay protocol file.
2. Right-click anywhere on Overview pane.
3. Click Edit Overview.

This opens an Edit Overview dialog.
4. Enter or paste Name and Description.
5. To save changes, click OK.

This updates the contents of the Overview pane accordingly.

## Referencing External Resources in Protocol Overview

You can include links to any external resources associated with a protocol in the assay overview. For example, you could use this feature to link the assay-specific standard operating procedure (SOP) directly to the protocol.

The included resource references appear under the Resources section of the Overview tab. The reference can be applied to a single protocol or to all protocols produced by your MyAssays Desktop application. If both reference types are specified for the protocol, then protocol-specific links are displayed first.

If you are using Enhanced Security edition, assign the users of your system to an authorization group where the Analysis | Edit References authorization is enabled. For more details about authorization settings, see Authorizations.

## Link External Resources in the Overview

1. Launch an assay protocol file.
2. Right-click anywhere on Overview pane.
3. Click Edit Resource References.

This opens Resource Reference Manager.
4. Depending on where you want to add links to, select:

- This Assay tab to apply the references to the current assay protocol only.
- All Assays tab to apply the references to all assay protocols.

Only Windows administrator account can make changes that apply to all users.
5. Click Add Resource.
6. For each added resource, configure Text and Link.

To learn how the reference will appear in the Overview, see How References Appear in Overview below.
To remove a reference, click the Remove Reference icon ( $\mathbb{X}$ ) on the right of the Resource link field.
7. To save changes, click Save.

This updates the contents of the Overview pane accordingly.

## What Resources Can Be Referenced?

Using Resource References Manager, in the Link field, you can include references to:

- Websites. Example: www.myassays.com.
- External Folder (Local or Domain). Example: Local: c:\MyInstrumentOutput $\backslash$ Domain: <br>server\MyInstrumentOutput.
- External File (Local or Domain). Example: Local: c:\Conventions\SOP.docx Domain: <br>server\Conventions\SOP.docx.

When the reference is clicked, the contents will be opened in the default associated application.If you reference a domain resource, make sure that users can log in to that resource. Otherwise, they will not be able to see the associated contents.

## How References Appear in Overview

Each reference can be configured with Text, Link, or both. If you specify:

- Both Text and Link, the reference will include named hyperlink:


## Resources

## MyAssays Desktop Home Page

- Only Link, the reference will include exposed hyperlink:


## Resources

https://www.myassays.com/myassays-desktop.html

- Only Text, the reference will include plain text:


## Resources

MyAssays Desktop Home Page

## Viewing Available Matrices and Configuring Their Settings

MyAssays Desktop stores all data sets in matrices. Each matrix can contain one or more samples, and for each matrix relevant transforms can be applied. You can configure the settings for each matrix in your assay through the Matrices Tab.

## View Matrices Tab

1. Launch an assay protocol or open a results file.
2. In the Assay Properties panel, select the Matrices tab.

If you cannot see Assay Properties panel, click Properties to open it.
If the Matrices tab is not immediately visible, click << Show All Properties to expand.

## Selecting a Matrix to Configure

On the left-hand Matrix Selection pane, you can view a tree list with available matrices branched by their type:

Raw
These matrices contain the input data that is stored in the Measurements Tab. The quantity and type of Raw data matrices will depend on the assay type and configuration.

## Calculated

All new matrices created and added by transforms are included in this branch.

## Field

This branch lists all text matrices that are stored in the Measurements Tab. The presence, quantity, and type of Field data matrices will depend on the assay configuration.

Select a matrix for the list to see its configuration and applicable settings.

## Applying Settings to the Matrix

Each input and output matrix of a newly added transform is automatically included in both Sample and Matrix Tables. With the following settings, you can configure what matrices will be displayed and how they will appear.

## General

| Name | A title for a matrix in the Sample Table and Matrix Table headers. <br> If it is not specified, then the default title is used. |
| :--- | :--- |

## Numerical Formatting

| Precision | The number of digits to be displayed for a numerical value. The interpretation <br> of Precision depends on the Type setting. |
| :--- | :--- |
| Type | Specifies how a numerical value is formatted with regard to precision and <br> rounding options: |
| - Significant Figures - The value is reported to the specified number of |  |
| significant figures. This accepts Precision values in range 1 through 14. |  |
| - Decimal Places - The number of digits to display to the right of the |  |
| decimal point. This accepts Precision values in range 0 through 14. |  |

## Report Contents

| Include in <br> Matrix <br> Tables | Defines if the associated matrix will be included in the Matrix Tables. |
| :---: | :---: |
| Include in Sample Tables | Defines if the associated matrix will be included in the Sample Tables. |
| Matrix Table Ids | Defines in which Matrix Tables the associated matrix should be included. |
| Sample Table Ids | Defines in which Sample Tables the associated matrix should be included. |
| Sample <br> Table Rep- <br> licates | Determines how replicates of each sample group are included in the Sample Table: <br> - Average - The average value of all replicates is displayed. This applies to Endpoint data only. <br> - List - All replicate values are included in the table. This does not apply to Field matrices. <br> - First - Only the first value is displayed. This simplifies the view of transforms that result in the same values for all replicates of each sample group (e.g., Average, XY Replicates Average). |

## Additional Settings for Endpoint Matrices

You can also apply the following settings to Raw and Calculated endpoint matrices.

## Sample Labels

If a Label is specified, the Condition is evaluated for each position of the associated matrix and the Label is displayed in place of the value for the position. For additional details on this feature, see Applying Labels to Endpoint Data.

## Value Bars

Within this category you can configure if and how to fill replicate values with colour for any
Raw or Calculated endpoint matrix. The value bars are created using the minimum and maximum data values of the matrix. These are treated as $0 \%$ and $100 \%$, respectively, and filled in accordingly.

| Display <br> Value Bars | Defines whether this feature is used in the Sample Tables. |
| :--- | :--- |
| Fill | Defines how to fill in the value bars: <br> - Solid - Self-coloured with Fill Colour. <br> - Gradient - Gradation from Fill Colour in the left to white on the right. |
| Fill Colour | Click the drop-down to open a colour picker and select the colour to fill the <br> bars with. |
| Border | It defines how to fill in the value bar borders: <br> - Solid - Self-coloured with Fill Colour. <br> - None - Don't display the Border. |

## Value Colour Scales

For more flexibility in formatting your data analysis results, you can apply preset colourscaling schemes for any Raw or Calculated endpoint matrix in the Sample Table, Matrix Table, or both. Using this functionality can simplify comparing value variance and distribution within a matrix.

When the colour scheme is applied, the values are shaded with gradations that correspond to minimum (smaller), midpoint (middle), and maximum (larger) values.

| Value Colour Scales | Click the drop-down to select the colour scale scheme: <br> • None <br> • Green-Yellow-Red <br> - Red-Yellow-Green <br> - Green-White-Red <br> - Red-White-Green |
| :--- | :--- |

## Handling a Large Number of Raw and Field Matrices

When creating a new protocol via New Protocol Wizard, some readings may contain a large amount of input data including both raw matrices of data and field data matrices that accompany the measurements. Including all of these matrices will make the report excessively large and hard to manage. To avoid such complications, if the number of imported matrices exceeds the default matrix limit, MyAssays Desktop will notify you accordingly about their default exclusion.

All other matrices will be available for analysis, processing and reporting. This topic explains how to select and include additional matrices to your reports, and how the limit is applied.

## Including Additional Matrices in the Report

For including any matrix in the report's Sample Table, Matrix Table, or both, follow these steps:

1. Launch an assay protocol or open a results file.
2. In the Assay Properties panel, select the Matrices tab.

If you cannot see Assay Properties panel, click Properties to open it. If the Matrices tab is not immediately visible, click \ll Show All Properties to expand
3. From the Matrix Selection pane, select the matrix you want to include. The measurements and the field data matrices are stored under the Raw and Field branches, respectively.
4. In the Matrix Configuration pane, locate the Report Contents settings and select the Include in Sample Tables or Include in Matrix Tables check boxes to add the matrix to the associated report element.
5. Review or edit other data analysis settings.
6. Click Calculate to perform the calculations with the new settings and to see the results.

Refer to Viewing Available Matrices and Configuring Their Settings to learn more about configuration of the matrices.

## How the Matrix Limit Is Applied

When importing data into a new protocol, the matrix limit restricts reporting the measurements and field data as follows:

- For raw data that contain more than 10 matrices, only the first matrix is included.
- For field data that contain more than 5 matrices, these matrices will all be excluded.

See Adding Field Data Alongside Measurements to learn more about field matrices.

All of the imported data is stored inside the protocol's configuration and you can add the necessary data to your reports in the MAA.

For advanced users, it is possible to change the number of matrices restricted by the limit. See Changing the Default Matrix Limit for Including Raw and Field Matrices for reference.

## Applying Labels to Endpoint Data

Applying labels to data quickly draws attention to particular data analysis results. This section covers how, where, and what data you can apply a label to. For example, you can mark all samples that are close to the asymptotes of the expected range or evaluate the within-run accuracy and highlight it if it crosses the acceptable threshold.

- About Labels
- Applying Sample Labels to Matrices
- Applying Evaluation Labels to Evaluation Results
- Configuring Labels
- Advanced (XML) Labels
- Reporting Matrix and Sample Table with Labels
- Reporting Evaluations Table with Labels
- Examples of Label Conditions


## About Labels

Labels can contain text, numbers, symbols, and icons in place of or alongside the resulting value. Applying label, unlike flagging, does not exclude data from calculations, nor does it change the actual value used in subsequent calculations. It simply replaces the reported values with a specified label and applies any colouring to the text or background. This feature is useful for detecting and reporting samples that are outside expected ranges. For example, you might want to highlight or label samples whose concentration, response, or \%CV is outside a specific range.

Labels are not applicable to $\underline{X Y}$ data matrices. To label XY data, use an XY Reduction transform and label the endpoint result.

The Labels editor is used to configure the labels for your assay. Depending on what you label, the title of the setting expands to:

- Sample Labels - For samples or replicates of raw, calculated, or field data matrices. The results with labels appear in associated matrix's Sample Table and Matrix Table.
- Evaluation Labels - For single evaluation result or all results in an evaluations table. The results with labels appear in Evaluations Table.

It is also possible to mark other data with labels. For more information, see Advanced (XML) Labels.

## Apply Sample Labels to a Matrix

1. Launch an assay protocol or open a results file.
2. In the Assay Properties panel, select the Matrices tab.

If you cannot see Assay Properties panel, click Properties to open it. If the Matrices tab is not immediately visible, click \ll Show All Properties to expand.
3. Select one of the numbered matrices from under Raw or Calculated branch, which contains the data to label.
If your assay contains field data, you can also select a matrix under the Field branch.
4. Click the Sample Labels drop-down of the Matrix Configuration pane.
5. Enter the condition (in terms of $x$ ) along with the label and any highlight or text colouring to apply.
6. Click Add to add further rules. Click outside the editor when you finish.
7. Click Calculate to perform calculations with the new settings and to see the results.

## Apply Evaluation Labels to Evaluations Table or Evaluation

1. Launch an assay protocol or open a results file.
2. In the Assay Properties panel, select the Evaluation tab.

If you cannot see Assay Properties panel, click Properties to open it. If the Evaluation tab is not immediately visible, click $\ll$ Show All Properties to expand.
3. Select Evaluations Table or Evaluation, which contains the data to label, and then click the Evaluation Labels drop-down.
4. Enter the condition (in terms of $x$ ) along with the label and any highlight or text colouring to apply.
5. Click Add to add further rules. Click outside the editor when you finish.
6. Click Calculate to perform calculations with the new settings and to see the results.

## Label Settings

Label, colouring, or both are applied in accordance with the rules that you specify. You can define as many rules as you need. If a value satisfies multiple label conditions, then all corresponding labels are displayed in the report.


You can add and remove any number of conditions and move them up or down with the corresponding arrow buttons. The rules are specified with the following editable text box properties:

| Condition | A Boolean condition to evaluate (typically a conditional expression) in terms of $x$. The $x$ value specified in the expression refers to: <br> - A sample or replicate of the selected raw or calculated matrix <br> - A result of a single evaluation or all results in an evaluations table <br> If the Condition is satisfied, the value is reported with the corresponding <br> Label in place of its value. |
| :---: | :---: |
| Match | For Field data matrices, enter any text, numbers, or both to find and replace with the Label. |
| Label | Enter or paste text, numbers, symbols, or their combination to display if the Condition is satisfied or Match found. |
|  | 1. Use $\{\mathbf{x}\}$ to refer to the original data value that is used in the condition. |
| Symbol | Select a graphical icon to display to the left of the Label: <br> - None - Display no icon <br> - Pass - Display a icon <br> - Fail - Display a $\boldsymbol{\$}$ icon <br> - Question - Display a 8 icon |


| Highlight | Set the background colour for the cell when Condition is satisfied. <br> Where there are multiple Conditions satisfied, the colour of the first <br> satisfied Condition is applied. |
| :--- | :--- |
| Text | Set the text colour for the cell when Condition is satisfied. <br> Where there are multiple Conditions satisfied, the colour of the first <br> satisfied Condition is applied. |

## Additional Settings

Click More>> to expand editor with more configuration settings:

| Sample Types | When configuring the labels for matrices, select the sample types to include when evaluating a Condition. By default, label applies to all sample types. |
| :---: | :---: |
|  | Select this to apply a Highlight to the whole row and not just the cell. |
| Highlight Row | Where there are multiple rules satisfied with Highlight Row, the colour of the first satisfied rule is applied. This applies to rules <br> (1.) satisfied in the associated matrix or evaluation and rules satisfied in other matrices or evaluations (in case a label applies to the evaluations table). |

## Advanced (XML) Labels

In addition to the Sample and Evaluations Labels, you can also label other elements in the XML editor:

- Flagged replicates or sample groups. For more information, see Custom Flag Labels.
- Values flagged by Standard Curve Fit or General Curve Fit transform. For more information, see Custom Flag Labels by Fit Transform Calculations.
- Fit Table results of Dose-Response Curves transform. For more information, see Advanced Dose-Response Curves Evaluations.


## Reporting Data with Sample Labels in Matrix Tables and Sample Tables

 Conditions for sample labels are applied to the corresponding data included in the report:- In the Matrix Table report element, the label or colouring applies to each position.


## Example

Here is a Matrix Table output highlighting all sample measurements $\mathbf{> 1 . 5}$ in yellow.

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | 6 | $\mathbf{7}$ | 8 | 9 | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 1.586 | 1.575 | 1.590 | 1.556 | 1.575 | 1.517 | 1.741 | 1.671 | 1.668 | 1.778 | 1.669 | 1.718 |
| B | 0.179 | 0.186 | 0.174 | 0.176 | 0.181 | 0.175 | 2.153 | 2.067 | 2.239 | 2.084 | 2.177 | 2.054 |
| C | 2.372 | 2.394 | 2.306 | 2.483 | 2.447 | 2.267 | 1.771 | 1.691 | 1.711 | 1.731 | 1.735 | 1.843 |
| D | 1.408 | 1.397 | 1.440 | 1.424 | 1.470 | 1.411 | 0.589 | 0.566 | 0.560 | 0.565 | 0.595 | 0.587 |
| E | 1.019 | 1.047 | 1.005 | 0.974 | 1.014 | 0.997 | 2.347 | 2.336 | 2.423 | 2.344 | 2.315 | 2.426 |
| F | 2.115 | 2.163 | 2.069 | 2.015 | 2.010 | 2.017 | 0.522 | 0.537 | 0.528 | 0.513 | 0.536 | 0.505 |
| G | 1.377 | 1.342 | 1.330 | 1.360 | 1.439 | 1.379 | 0.082 | 0.085 | 0.086 | 0.084 | 0.083 | 0.079 |
| H | 1.734 | 1.760 | 1.753 | 1.800 | 1.705 | 1.793 | 0.209 | 0.201 | 0.200 | 0.218 | 0.215 | 0.204 |

- In the Sample Table report element, you can configure each matrix to display the average of the sample group or list each replicate. (This can be set with the Sample Table Replicates setting in the Matrix configuration.)


## Example

The following Sample Table has been configured with the Raw data listing all replicate values. In this case, the label is applied to each replicate that satisfies the rule.

| Sample | Positions | Raw |
| :--- | :--- | :--- |
| Unknown1 | A1 | $1.586>1.5$ |
|  | A2 | $1.575>1.5$ |
|  | A3 | $1.590>1.5$ |
|  | A4 | $1.556>1.5$ |
|  | A5 | $1.575>1.5$ |
|  | A6 | $1.517>1.5$ |

In the following example, the Sample Table has been configured with the Raw data listing the average of the replicates values. In this case, the label is applied to the computed average.

| Sample | Positions | Raw |
| :--- | :--- | :--- |
| OUnknown1 | A1 | $1.566>1.5$ |
|  | A2 |  |
|  | A3 |  |
|  | A4 |  |
|  | A5 |  |
|  | A6 |  |

## Example with $\{x\}$ Reference

Here the Condition $\mathbf{x}>\mathbf{1}$ and Label $\mathbf{> 1}$ are specified for Concentration matrix. In this case, the calculation of the concentration for positions $\mathbf{A} 3$ and $\mathbf{A} 4$ is reported as $>\mathbf{1}$.

| Sample | Positions | Raw | Concentration |
| :--- | :--- | :--- | :--- |
| Onknown1 | A3 | 1.694 | $>1$ |
|  | A4 | 1.766 | $>1$ |

Where label has been applied on the data and there is a $\{\mathbf{x}\}$ reference associated with the Label, the numeric value appears in the Label. For example, with the Condition $\mathbf{x}>\mathbf{1}$ and Label $\{\mathbf{x}\}>1$, the calculation results are reported as:

| Sample | Positions | Raw | Concentration |
| :--- | :--- | :--- | :--- |
| OUnknown1 | A3 | 1.694 | $2.153>1$ |
|  | A4 | 1.766 | $3.857>1$ |

## Reporting Evaluations Tables with Labels

Depending on what a label applies to, the report displays:

- A specific evaluation result with a label.


## Example

Here the Condition $\boldsymbol{x}$, Label $\mathbf{S S C}=\{\mathbf{x}\}$, Highlight (Orange), and Highlight Row selected for SSC evaluation.

| Description | Expression | Results |
| :--- | :--- | :--- |
| SSR | tov4.ssr | 0.0220295 |
| SSC | tov4.ssc | SSC $=0.00335946$ |
| Residual SumSquares | eval1.SSR+eval1.SSC | 0.025389 |

- All associated evaluation results that satisfy the condition are reported with label.


## Example

Here the Condition $\boldsymbol{x} \mathbf{< 0 . 0 5}$, Label $\{\mathbf{x}\}<\mathbf{0 . 0 5}$ and Highlight (Yellow) are specified for Evaluations Table.

| Description | Expression | Results |
| :--- | :--- | :--- |
| SSR | tov4.ssr | $0.0220295<0.05$ |
| SSC | tov4.ssc | $0.00335946<0.05$ |
| Residual SumSquares | eval1.SSR+eval1.SSC | $0.025389<0.05$ |

- Different labels are applied to both the evaluations table and a specific evaluation, both are reported but the label specified for evaluation takes precedence.


## Example

Here the table is configured with Condition $\boldsymbol{x}<\mathbf{0 . 0 5}$, Label $\{\mathbf{x}\}<\mathbf{0 . 0 5}$ and Highlight (Yellow), and SSC evaluation result contains label with Condition $\boldsymbol{x}$, Label $\mathbf{S S C}=\{\mathbf{x}\}$,

Highlight (Orange), and Highlight Row selected:

| Description | Expression | Results |
| :--- | :--- | :--- |
| SSR | tov4.ssr | $0.0220295<0.05$ |
| SSC | tov4.ssc | SSC $=0.00335946$ |
| Residual SumSquares | eval1.SSR+eval1.SSC | $0.025389<0.05$ |

## Examples of Label Conditions

| Condition | Label Applied If |
| :--- | :--- |
| $\mathbf{x < 1}$ | The value is less than 1. |
| and $(\mathbf{x}>\mathbf{0}$, <br> $\mathbf{x}<\mathbf{1})$ | The value is in the 0-1 range. |
| $\mathbf{x < C o n t r o l \mathbf { 1 }}$ | The value is less than mean of Control1 sample group. |
| $\mathbf{x < e v a l \mathbf { 1 . s s r }}$ | The value is less than the result of the first evaluation (assuming that the $1^{\text {st }}$ is <br> assigned with a ssr name). See $\underline{\text { Named Evaluation Variables for more details of }}$ <br> how to refer to such results. |
| $\mathbf{x}>$ TOV1.d | The value is greater than the d coefficient of the $\underline{4 P L}$ curve fit (assuming that <br> the 1st transform is a Standard Curve Fit transform using the $\underline{4 \mathrm{PL}}$ curve fit). See <br> Transform Output Variables for more details of how to refer to such results. |

## Validation

You can automatically check that measurements and results are within expected ranges by using validations. Any configured validation tests are performed as a part of each assay run with the results (pass or fail) being clearly presented in the report. For example, you can test that the \%CV of the replicates of all sample groups is less than 15.

- About Validations and Validations Tables
- Adding Validation to an Assay
- Changing the Sequence of Validations in a Table
- Deleting Validations Tables and Validations
- Configuring Validation Rules (with Examples)
- Validating Non-Matrix Data
- Validation Results


## About Validations and Validations Tables

A validation is a rule defined using a Boolean condition. This condition is specified as an expression along with a matrix reference and type. If the condition evaluates to true, then the result for the validation is Pass. If the condition evaluates to false, the validation result is Fail. For example, the validation condition expression
$x<15$
when associated with the \%CV matrix and evaluated on all groups, checks that the \%CV of all sample groups is $<15$.

Validation rules are arranged by Validations Tables in the Validation tab. It consists of two panes:

- Tables - For defining the tables that are available for inclusion in the report. You can create as many validations tables as you need and group related validations into tables accordingly. For example, you might want each validations table to appear on a different sheet in the report.
For configuring the order, settings, and inclusion of each Validations Table, see Report contents.
- Condition Configuration - For specifying a condition for a validation rule.



## Add Validations Tables and Validations

1. Launch an assay protocol or open a results file.
2. In the Assay Properties panel, select the Validation tab.

If you cannot see Assay Properties panel, click Properties to open it. If the Validation tab is not immediately visible, click << Show All Properties to expand.
3. Add validation table for your assay with one of the following:

- In the Validations pane, click Add Validation Table.
- Below the pane, click Add Element button, and then select Validation Table.

Each table is given a unique identifier (e.g., ValidationsTable1) and is automatically populated with a single validation element.

Each element is given a description (e.g., Condition \#1). You can change it immediately after adding or do so later, after you configure its settings. To change the description, right-click the Condition and select Edit from the drop-down list or, on the Condition Configuration pane, edit the Description setting.
4. Add more validations to this table with one of the following:

- Under the validation element, click Add Validation.
- Below the pane, click Add Element button, and then select Validation.

You may prefer to include all validations in a single table or arrange them
(1) between multiple tables. To add another table and populate it with validation conditions, repeat the steps 3 and 4 .

## Change the Sequence of Validations

You can set the order in which the validation conditions appear in the selected validations table. To do this, select the evaluation you want to move and click Up or Down arrow button on the right of the Validations pane.

The validations cannot be moved between the tables.

## Delete Validations Tables or Validations

To delete a table or condition from your assay, do one of the following:

- Right-click on the item and select Remove from the drop-down menu.
- Select the item and click Remove button below the pane.

If you delete a Validations Table, all associated conditions are also deleted.

## Configure Validation Rules

The Condition Configuration pane is used to specify the selected validation's condition.

## Condition Configuration Settings

To set up a condition to a validation rule, define the following settings:

| Description | Use this to provide a clear textual description; this is used in the report to <br> convey what has been validated. <br> The other way to change description is to right-click on the condition in the <br> Conditions pane's tree view and select Edit. |
| :--- | :--- |


| Expression | The Expression setting is used to define the validation expression Boolean <br> condition. This is typically an expression with a logical operator and perhaps a <br> conditional function. The interpretation of the expression depends on the <br> Validate setting. <br> For a complete description of available expression functionality, see <br> Expressions. |
| :--- | :--- |
| Select the matrix the Condition applies to from the drop-down list. For <br> example, if you plan to validate measurement data (i.e., a Raw matrix), select <br> the Raw matrix to validate. Any of the raw or calculated matrices that contain <br> endpoint data can be validated. |  |
| If there are no available matrices, then the (NA) label appears. In this <br> case, validation conditions can still be created that do not refer to <br> endpoint matrix data. |  |
| Validation conditions are not applicable to XY data matrices. To <br> validate XY data, use an XY Reduction transform and validate the <br> endpoint result. |  |


| Validate | Click on the drop-down and select how to evaluate the Expression: <br> Expression - The expression is evaluated once on the specified matrix of each container (useful for checking specific sample groups). Example: <br> Standard1 < Standard2 validates that the average of Standard1 replicates is less than those of Standard2 on the specified matrix (for multiple plate assays, this condition is evaluated on every plate). <br> Expression with Every Sample Group (x) - The expression is evaluated in terms of $x$ for every sample group on the matrix of every container. A sample group reference $x$ refers to the average of the replicates that belong to the group that are not flagged. Example: to check that the average value of each group in a matrix is less than 3, use the Expression with Every Sample Group ( $\mathbf{x}$ ) setting with the expression $\mathbf{x}<3$. In this case, the average of every sample group (on the selected matrix) is validated. <br> Expression with Every Sample Replicate (x) - The expression is evaluated in terms of $x$ for every sample replicate on the matrix of every container. The $x$ reference here is expanded to value at each position. <br> Example: to check that the value at every measured position in a matrix is greater than 0.5 , then use the Expression with Every Sample Replicate <br> ( $\mathbf{x}$ ) setting with the expression: $\mathbf{x} \boldsymbol{\mathbf { 0 . 5 }}$. In this case, every data point (on the selected matrix) is validated. |
| :---: | :---: |
| Sample <br> Types | Specify which sample types to evaluate an Expression with Every Sample Replicate (x) or Expression with Every Sample Group (x) expressions on. By default, these validation expressions are evaluated for all sample types. If you only want to test specific sample types, then use this setting. |

## Examples of Condition Expressions

Here are examples of validations along with descriptions of their behavior. They apply the validation on the selected data matrix.

| Type | Condition | Validates |
| :---: | :---: | :---: |
| Expression with Every <br> Sample Replicate (x) | $x<2.5$ | All positions (that are not flagged) have a value less than 2.5. |
| Expression with Every <br> Sample Replicate (x) | and ( $x>0, x<2.5$ ) | All positions (that are not flagged) have a value greater than zero and less than 2.5. |
| Expression with Every <br> Sample Group (x) | $x<2.5$ | The average of the replicates (that are not flagged) of all sample groups is less than 2.5. |


| Type | Condition | Validates |
| :---: | :---: | :---: |
| Expression with Every Sample Group (x) | and ( $x>0, x<2.5$ ) | The average of the replicates (that are not flagged) of all sample groups is greater than 0 and less than 2.5. |
| Expression | Control1<1.5 | The average of Control 1 replicates (that are not flagged) is less than 1.5 . |
| Expression | sd(Control 1 ) < 10 | The standard deviation of the Control 1 replicates (that are not flagged) is less than 10. |
| Expression | pcv(Control 1 ) <15 | The \%CV of the Control 1 replicates (that are not flagged) is less than 15 . |
| Expression | median <br> (Control 1 ) >0.75 | The median of the Control 1 replicates (that are not flagged) is greater than 0.75 . |
| Expression | TOV1.R2>0.9 | The $R^{2}$ value of the curve fit (as used in the first transform) is greater than 0.9. (The $R^{2}$ value is referenced using a Transform Output Variable). |

## Validate Non-Matrix Data

In addition to validating matrix data, it is also possible to validate other types of numerical data. For example, you may want to verify that the $R^{2}$ value of a curve fit is greater than a specific threshold. These types of validations can be defined using the Expression type and referring to the data to validate within the expression. This is typically using a Transform Output Variable (TOV). The TOVs that are available depend on your configured transforms.
For example, the expression TOV1.R2 $\mathbf{>} \mathbf{0 . 9}$ checks that the $R^{2}$ value of the curve fit (as used in the first transform) is greater than 0.9.

## Validation Results

Validation conditions are checked when the data analysis is performed (after all transform calculations). All validations are evaluated irrespective of whether they are included in the report. In any case, the results of each validation test are listed in the Calculation log.
Each validation test verifies the result in terms of the specified Boolean condition.
The results appear in the associated Validations Table in the report and are marked as Pass,
Fail, or Inconclusive accordingly:

## Pass

If the validation condition is true (the numerical result $\neq 0$ ).

# For Expression with Every Sample Replicate (x) and Expression with Every Sample Group (x) validations, the result is Pass only if all positions or groups evaluate to true. 

## * Fail

If the validation condition is false (the numerical result $=0$ ). For Fail, the whole validation table row is highlighted with yellow background.

## 8 Inconclusive

If the validation expression could not be evaluated (e.g., a syntax error in the expression), the result is treated as Inconclusive with the error message shown in Results column (in red text).

If you apply any new or modified settings to validation rules, click Calculate and inspect the report to see the results.

## Evaluation

To calculate and present specific quantities of interest in the report, you can use MyAssays Desktop's Evaluation feature. For example, you can compute and report the limits of detection of an assay using an evaluation. The topics in this section cover how to add, define, label, and format evaluations.

- About Evaluations and Evaluations Tables
- Adding Evaluations to an Assay
- Changing the Sequence of Evaluations in the Table
- Deleting Evaluations Tables or Evaluations
- Configuring Evaluation Settings (with Examples)
- Named Evaluation Variables
- Evaluation Results


## About Evaluations and Evaluations Tables

Evaluations are expression-based calculations that result in a single numerical value and can be referenced in other calculations.

Evaluations are arranged and reported as Evaluations Tables. Any value that can be referenced or computed can be included in an Evaluations table. Each evaluation can be given a description to accompany the result. If there is an error in evaluating the expression, then the error message is reported (in red) as the result.

It is useful to group related evaluations into Evaluations Tables accordingly. You can add as many evaluations as you need to each Evaluations Table. You can create as many tables as you need and configure how and if they are reported.


## Add Evaluations Tables and Evaluations

1. Launch an assay protocol or open a results file.
2. In the Assay Properties panel, select the Evaluation tab.

If you cannot see Assay Properties panel, click Properties to open it.
If the Evaluation tab is not immediately visible, click << Show All Properties to expand.
3. Add evaluation table for your assay with one of the following:

- In the Evaluations pane, click Add Evaluation Table.
- Below the pane, click Add Element button, and then select Evaluation Table.

Each table is given a unique identifier (e.g., EvaluationsTable1) and is automatically populated with a single evaluation element.
4. Add more evaluations to this table with one of the following:

- Under the evaluation element, click Add Evaluation.
- Below the pane, click Add Element button, and then select Evaluation.

Each element is given a description (e.g., Evaluation \#1). You can change it immediately after adding or do so later, after you configure its settings. To change the description, right-
click the Evaluation and select Edit from the drop-down list or, on the Expression Configuration pane, edit the Description setting.

You may prefer to include all evaluations in a single table or arrange evaluations

(1)between multiple tables. To add another table and populate it with evaluations, repeat the steps 3 and 4.

## Change the Sequence of Evaluations

You can set the order in which the evaluations will appear in the selected Evaluation Table. To do this, select the evaluation you want to move and click Up or Down arrow button on the right of the Evaluations pane.

The evaluations cannot be moved between the tables.

## Delete Evaluations Tables or Evaluations

To delete a table or evaluation from your assay, do one of the following:

- Right-click on the item and select Remove from the drop-down menu.
- Select the item and click Remove button below the pane.

If you delete an Evaluations Table, all associated Evaluations are also deleted.

## Configure Evaluations and Evaluations Tables

Depending on what you are configuring, the right pane expands to:

- Table Configuration - For configuring settings of the selected Evaluations Table. This includes filling the results with colour and value bars, and applying labels on all results in this table.


## Evaluations Tables Settings

On this pane you can define how the table will appear. In addition to the settings described here, you can configure the Evaluations Table report element settings.

Each evaluation table is a separate report element and can be configured separately. For example, you might want each evaluation table to appear on a different sheet.

## Evaluation Labels

|  | Use this to replace the results of any evaluations included in this table <br> with a label you specify. Label is applied if the results meet the condition <br> and can contain text, numbers, symbols, and icons in place of or alongside <br> the resulting values. |
| :--- | :--- |
| Evaluation <br> Labels | A label applied here is overriden by any defined for the <br> associated Evaluation. |
|  | To learn more about how to configure and use this feature, see Applying <br> Labels to Endpoint Data. |

## Value Bars

The value bars are created using the minimum and maximum values of the evaluated results in the table. These are treated as $0 \%$ and $100 \%$, respectively, and filled in accordingly.

| Display <br> Value Bars | Select to fill evaluation result values with colour in the report. |
| :--- | :--- |
| Fill | Click the drop-down and choose how to fill in the value bar: <br> - Solid - To fill value bars self-coloured with Fill Colour. <br> the left to - white on the right. |
| Fill Colour | Click the drop-down to open a colour picker. |
| Border | Click the drop-down and choose how to fill in the value bar's border: <br> - Solid - To fill value bars self-coloured with Fill Colour. <br> - None - To not display the Border. |

- Expression Configuration - For setting up the selected Evaluation. Here you specify an expression to evaluate, how to format the result, and assign a name for the result to reference it in further calculations. Formatting settings and naming the result are optional. Also, you can specify a label to report instead of the resulting value.


## Evaluation Expressions and Settings

Each Evaluation is defined with the following settings.

## Evaluation Labels

|  | Use this to replace the evaluation result with a label you specify. Label is <br> applied if the result meets the condition and can contain text, numbers, <br> symbols and icons in place of or alongside the resulting value. |
| :--- | :--- |
| Evaluation | A label applied here overrides any defined for the associated <br> Evaluations Table. |

To learn more about how to configure and use this feature, see Applying Labels to Endpoint Data.

## General

| Expression | Defines the expression to evaluate. This might reference a specific sample group or position on the selected matrix. It may also perform a calculation or function. The evaluation applies on the non-flagged samples of the selected endpoint Matrix or on non-matrix data. <br> For a complete description of available expression functionality, see Expressions. |
| :---: | :---: |
| Description | Use this to provide a clear textual description; this will be used in the report to convey what has been evaluated. <br> The other way to change this is to right-click on the evaluation in the Evaluations pane's tree view and select Edit. |
| Matrix | Select the matrix the Expression applies to from the drop-down list. For example, if you plan to evaluate a quantity using measurement data (i.e., a Raw matrix), select the Raw matrix. Any of the raw or calculated matrices that contain endpoint data can be referenced in an evaluation. Also, it is possible to evaluate non-matrix data (such as Transform Output Variables). |
|  | If there are no available matrices, then the (NA) label appears. In this case, you can still create evaluations that do not refer to endpoint matrix data. |
|  | Evaluations are not applicable to $X Y$ data matrices. To perform an evaluation on XY data, first use an XY Reduction transform and evaluate the endpoint result. |


| Name | Designates a name to the evaluation result. The named evaluation can be <br> referenced in other calculation as a variable. <br> See Named Evaluation Variables for more details. |
| :--- | :--- |

## Numerical Formatting

| Precision | The number of digits to be displayed for a numerical value. The <br> interpretation of Precision depends on the Type setting. |
| :--- | :--- |
| Type | Specifies how a numerical value is formatted with regard to precision and <br> rounding options: |
| - Significant Figures - The value is reported to the specified number |  |
| of significant figures. This accepts Precision values in range 1 |  |
| through 14. |  |
| - Decimal Places - The number of digits to display to the right of the |  |
| decimal point. This accepts Precision values in range 0 through 14. |  |

## Examples of Evaluation Expressions

Here are examples of expressions along with descriptions of their behavior.
For a complete description of the expressions available, see Expressions section.

| Evaluates | Computes |
| :--- | :--- |
| Control1 | The average of all Control1 replicates |
| Control1/Control2 | The ratio of Control1 and Control2 |
| sd(Control1) | The standard deviation of the Control1 replicates |
| pcv(Unknown1) | The percentage coefficient of variation of Unknown1. |
| Blank1+(3*sd(Blank1)) | The average of the sum of Blank1 plus 3 times the <br> standard deviation of Blank1 (this is often used to <br> compute LLD). |
| pcv <br> ([Unknown1,Unknown2]) | The percentage coefficient of variation of all Unknown1 <br> and Unknown2 replicates. |
| median(Unknown.2.AlI) | The median of all Unknowns on the $2^{\text {nd }}$ matrix across all <br> containers. |

## Named Evaluation Variables

When evaluating non-matrix results for an assay, it is useful to refer to the evaluation results from other calculations. A Named Evaluation Variable (NEV) is simply an evaluation result
that has been assigned a name. The NEV can be referenced using expressions in:

- Evaluation expressions (that follow its definition)
- Validation conditions
- Label conditions


## Referencing Named Evaluation Variables

The syntax for referencing NEVs in expressions is as follows:
eval\{N\}.\{Name\}
Where:
\{N\}-1-based Evaluations Table index
\{Name\} - The name assigned to the evaluation
All calculations are computed in the order they are defined in the Report Elementspane, therefore ensure that Evaluations Tables appear earlier in the sequence of the included elements to where they are referenced. (To change the actual report content ordering, use a Report Template.)

For example, to refer to a NEV in the Sample Label configuration, the Evaluations Table must be above the Sample Table and, if included, Matrix Table in the report elements order.

## Evaluation Results

Unlike the Sample Table or Matrix Table report elements (which display the results of a calculation performed on each sample), an Evaluations Table reports calculations that return a single numerical result.

All evaluations are performed irrespective of whether they are included in the report. You can configure how and if the Evaluations Tables are reported. In any case, the results of each evaluation are listed in the Calculation log.

Evaluations are computed when the data analysis is performed (after all transform calculations). To apply any new or modified settings, click Calculate and inspect the report to see the results.


To calculate a result for every sample, use a transform with the results for each sample displayed in the Sample Table or Matrix Table.

## Specifying the Report Content

The report is the primary output of the data analysis process. You can use MyAssays Desktop's comprehensive functionality to specify:

- Content - What elements of the performed data analysis will be featured in your report.
- Presentation - The order and inclusion of elements, and any additional styling specified.
- Export - The format in which your report will be output.

By using these options, you can customize your report to display the data exactly as you require.

Within the MyAssays Desktop Application, the report can be displayed as Sheet View or
Page View; these settings are available in the Report ribbon tab. When getting started, we suggest using the Sheet View so that your outputs are not confined by the limitations of a printed page. Use the Page View only if and when the layout of the printed page is important. You can also define a report template to apply to your MyAssays Desktop output.

## About Report Content

The content available for inclusion in the report depends on the configuration of each of the following settings (these each correspond to a tab in the Assay Properties pane):

- Transforms - Any configured transforms will result in calculated elements (such as numerical results or charts).
- Matrices - The settings of each matrix (raw measurement data and any calculated) can be configured. This includes how numerical data is presented (numerical formatting settings), if it is included, how replicate values are reported, and any optional coloured value bars.
- Evaluation - Any configured evaluation settings will result in one or more evaluations tables, each listing the results of the computed evaluations.
- Validation - Any configured validation settings will result in one or more validations tables, each listing the results (pass or fail) of the computed validations.

Various report elements are available depending on these settings. For example, if a curvefitting transform such as the Standard Curve Fit is configured, you can include the calculated concentrations in a Sample Table and/or Matrix Table, along with the transform-specific elements such as fit chart and equations. Each of the included elements can be configured further.
(1) All MyAssays Desktop reports are generated using Tahoma 10pt font by default.

## Report Element Configuration

The ordering and basic configuration of each report element is defined in the Report tab.

## View Report Tab

1. Launch an assay protocol or open a results file.
2. If you cannot see the Assay Properties panel, click Properties to open it.
3. Select the Report tab.

If the Report tab is not immediately visible, click \ll Show All Properties to expand.
4. Review and edit the report elements as required.

The Report tab consists of two panes:

- Report Elements - This pane manages the report elements and their ordering.
- Report Configuration - This displays the available settings for the selected report element.


## Report Elements Pane

The Report Elements pane lists the elements that will be included in the report. Their ordering reflects the order in which they will be added to the report.

Click Add Element to add a new report element. A list of available report elements will appear. The selected item will be added to the end of the list.

Click Remove to remove the selected report element.
To change the ordering of report elements, select an item and use the Up or Down arrow buttons on the right of this pane.

## Report Configuration Pane

The Report Configuration pane displays the properties of the selected report element. The actual settings depend on the element; however, there are a number of common settings:

## General

| Id Each report element is automatically given a unique identifier (Id). This <br> identifier is used to identify the report element when configuring custom <br> report templates (It is also used for advanced settings such as custom export <br> and XML configuration). <br> Include This check box can be used to exclude the report element (without deleting <br> the settings). <br> The identifier.  |
| :--- | :--- |
|  |

## Sheet

| Sheet | This setting defines the name of a sheet to contain the report elements. This is <br> only applicable to sheet-based content such as Excel output or data in the <br> Sheet View. <br> Name <br> Each report element can be included on its own separate sheet or elements <br> can be added together on the same sheet. Where a common sheet name is <br> used across report elements, the elements are added in the order as defined <br> in the Report Elements pane. <br> This property can refer to report macros (such as \{PlateNum\}, \{PlatelD\} <br> and \{AnalyteName\}) to automatically group associated content into a sheet <br> of the same name. <br> Note, if Sheet Name is defined for one report element, then the report <br> content is exported on one sheet with provided Name. |
| :--- | :--- |

When viewing report content in Sheet View, the sheet names appear in the bottom left of the Report tab (in the Results tab). If there is only one named sheet, then this sheet name will not appear.

## Report Elements and Multiple Plate or Multiplex Analyses

If your assay is comprised of measurements across multiple plates or measurements for multiple analytes (multiplex assay), then the report will contain an element for each plate or analyte.

To help arrange this type of content into an easy-to-navigate report, we recommend using the
Sheet Name setting to arrange content into separate sheets.
For example, with a multiple plate assay, set the Sheet Name setting to:

## Plate \{PlateNum \}

With this setting applied to a Sample Table element, the results for each plate will appear in a separate worksheet named by the plate number (such as Plate 1, Plate 2, Plate 3, etc.).

Using Plate \{PlateID\} for multiple plate and Analyte \{AnalyteName\} for multiplex assays, you can arrange the results from each plate/analyte into separate worksheets titled by their provided IDs (as configured in Measurements | Plate IDs/Analytes).

## Report Macros

The following table lists the macros that are available for use in the Title and Sheet Name properties.

| Macro | Expands To <br> \{PlateNum $\}$ |
| :--- | :--- |
| 1-based plate number |  |
| \{ContainerNum $\}$ | 1 -based container number (for a multiple plate assay, this is the plate <br> number; for multiplex, this is the analyte number) |
| \{PlateID $\}$ | The plate ID as it appears under the Measurements \| Plate IDs |
| \{PlateName $\}$ | The analyte name as it appears under the Measurements $\mid$ Analytes |
| \{AnalyteName $\}$ | The |
| \{ContainerName $\}$ | For a multiple plate assay, this is the plate name; for multiplex, this is the <br> analyte name |

You can combine these macros with other text. For example, Plate $\{$ PlateNum\} will be expanded to Plate 1 for the first plate.

## Report Elements

To simplify configuration, various report elements are automatically added to the report and configured with default settings as you add new steps to your analysis:

- The Sample Table is the most widely used report element, so it is included by default in all configurations. The Sample Table displays the results of each defined sample group (in accordance with the defined layout).
- If any Evaluation or Validations are configured, an Evaluation or Validation report element will be added automatically.
- If any transform generates additional content, a Transform Content element will automatically be added. For example, the Standard Curve Fit transform produces a standards chart, concentrations table, and equations; this content is automatically added to the report as a Transform Content report element.

You will see these elements appear automatically in the Report Elements pane of the
Report tab. You are free to edit or delete this default content as you require. If analysis steps are removed, then the associated report element will also be automatically removed.

Beyond the automatically added report elements, you can further add your own elements to the report:

How to: Add a Report Element to the Report

1. Select the Report tab.

If the Report tab is not immediately visible, click << Show All Properties to expand.
2. In the Report Elements pane, select the required report element from the Add Element drop-down list.
3. Review and edit the element's settings as required.
4. Click Calculate to perform calculations with the new settings.

The following table summarises each available report element. Further details and settings are included in the provided linked topics.

| Report Element | Description <br> Curve Overlaid chart displaying two or more curves for visual <br> comparison. |
| :--- | :--- |
| $\underline{\text { Evaluations Table }}$ | This is an advanced report element that is configured and <br> included in the report through XML. |
| $\underline{\text { Histogram }}$Table. |  |
| The Histogram represents the data by displaying the average of each <br> sample in vertical columns. Each sample is denoted by a bar matching <br> the colour of its sample type. <br> In addition, to indicate the error or uncertainty in a reported <br> measurement or calculated data, the variability of data can be |  |


| Report Element | Description |
| :---: | :---: |
|  | represented with optional error bars. |
| Layout | A graphical representation of the sample layout. |
| Matrix Table | The results displayed in a matrix-table structure. For endpoint data, a numerical value is reported. For XY data, a thumbnail plot is displayed. <br> Each raw or calculated matrix can be marked for inclusion or exclusion in a matrix table using the Report Contents section of the Matrices tab. |
| Matrix View | A layout-based representation of the matrix data. For endpoint data, a numerical value will appear at each position. For XY data, a plot will appear at each position. The colour of each position can be determined using the layout colour or with a heatmap to represent the endpoint value intensity. |
| Measurement Meta <br> Data | A table listing the measurements meta data (reported as name value pairs). This corresponds to the data as it appears under the Measurements \| Meta tab. |
|  | Any notes associated with the assay run. |
| Notes | Notes can be entered using the Notes button in the View section of the Home tab. |
| QC Results | Inter-assay quality control results (as defined by Quality Control settings). This could include Observation Table, Levey-Jennings, or CUSUM charts reporting the latest results along with historical results. |
| Sample Table | A table reporting the results of each defined sample group (in accordance with the defined layout). Each row contains the data for a specific sample group. <br> For endpoint data, a numerical value is reported. For XY data, a thumbnail plot is displayed. <br> Each raw or calculated matrix can be marked for inclusion or exclusion in a sample table using the Report Contents section of the Matrices tab. |
| Text | A textual information element allowing the optional inclusion of macros. |
| Transform Content | Various report elements generated as a consequence of a specific transform. <br> For example, a Standard Curve Fit transform adds a concentration |


| Report Element | Description <br> table, a chart, and equations to the report as Transform Content report <br> elements. |
| :--- | :--- |
| Validations Table | The details and results of any configured validations arranged in a <br> table. Each validation result is reported as a pass or fail. |
| XY Plot | A chart that includes position plots for one or more XY positions. |
| This is an advanced report element that is configured and <br> included in the report through XML. |  |

## Selecting the Data to Include in a Sample Table/Matrix Table

The data to include in a Sample Table or Matrix Table can be configured on a matrix-by-matrix basis. For example, you may want to include only certain matrices in your tables, perhaps excluding the results of intermediary data analysis steps.

The easiest way to specify which data to include in your Sample Tables or Matrix Tables is to use the matrix options: Include in Matrix Tables and Include in Sample Tables. These settings are available for each matrix. To access these settings, select the Matrices tab and select the matrix to edit. These settings apply to all of the Sample Tables or Matrix Tables in your report.

For each matrix, the Sample Table Replicates setting can be used to specify how to report the values for replicates in the Sample Table on a matrix-by-matrix basis.

## Working with Multiple Sample Tables or Multiple Matrix Tables

You may want to include multiple Sample Tables or Matrix Tables in your report with each reporting different aspects of the data.

When working with multiple tables, you might require control over which data to include in which Sample Tables or Matrix Tables. The Sample Table IDs and Matrix Table IDs settings can be used to select which tables each matrix should be included in. The table Ids correspond to the report element Ids of the Sample Table or Matrix Table (as defined in the Report tab).

Each table could be configured to report data for different sample types using the Sample Table's Type setting.

## Report Elements

## Curve Overlay Chart Configuration

This is an advanced feature that can only be configured through the XML editor.

A Curve Overlay Chart can be used to plot curves from different sources to compare them on a single chart. It's possible to include a chart from the current analysis or a chart made on a previous run. The overlaid charts can originate from analyses computed by the Standard Curve Fit, Standard Curve Fit (from Library) or General Curve Fit transforms.

For example:


Here, the Curve Overlay chart is titled Dose-Response. It contains two overlaid curves named Curve 1 and Curve 2. Also, the axes titles and types are defined.

It's also possible to compute values from each curve using the Curve Overlay Evaluations feature:


## Settings and Configuration

You can configure one or more Curve Overlay charts to include in your report. Specify their attributes and syntax in the XML Editor as described below.

## How to: Add Curve Overlay Configuration to Report

1. In the Assay Properties panel, select the XML tab.

If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Locate the Report element and add the CurveOverlay child element and then specify its settings as described below.
3. Add a child element Curves and for each curve add a child element Curve and define its attributes.
4. Click Apply to save changes to the protocol and then Calculate to generate new report.

## CurveOverlay Attributes

On this level, specify general settings that apply to a Curve Overlay chart.

## General

| Id | A unique identifier that is used to identify the report element in the Report <br> Elements pane and when configuring custom report templates. |
| :--- | :--- |
| SheetName | If defined, inserts curve overlay chart to a sheet with the specified SheetName. <br> For more details, see Sheet Name in the Report content section. |


| Title | An optional title for a curve overlay chart that can refer to Report Macros. If <br> not specified, then a default "Curve Overlay" title is displayed. |
| :--- | :--- |
| Include | Boolean setting that defines whether the curve overlay chart is included in the <br> report. |

You can also optionally specify axes settings. If any of them is not specified, then the setting from the first defined curve is used.

## Axes

| XAxisTitle | The name of the x -axis on the chart. |
| :--- | :--- |
| XAxisType | The scale type to be used for the x -axis: <br> • Logarithmic <br> • Linear |
| XAxisPrecision | The precision to plot the $x$-axis with. |
| YAxisTitle | The name of the y-axis on the chart. |
|  | The scale type to be used for the $y$-axis: <br> • Logarithmic |
| YAxisType | When $y$-axis is logged, negative (or zero) $y$ values are not <br> included in the plot. |
| YAxisPrecision | The precision to plot the y-axis with. |

This is specified in the XML editor inside the CurveOverlay element with following syntax:
<CurveOverlay Id="/d" SheetName="Name" XAxisTitle="Title" YAxisType="Type"
YAxisTitle="Title" XAxisType="Type" Title="Title">

## Curve Attributes

Specify each curve to include in the chart with the following attributes:

| TransformNum | This refers to 1-based number of the transform in the sequence. |
| :--- | :--- |


| FilePath | When referencing a Library Curve file: <br> - For files stored in the Curves folder of your MyAssays root folder, <br> enter or paste curve.xml filename without the folder. |
| :--- | :--- |
| - For files stored in other locations, provide a full file path to an existing |  |
| curve.xml file. |  |

The TransformNum and FilePath settings are mutually exclusive.
This is specified in the XML editor as a child of the CurveOverlay element with following syntax:

```
<Curves>
```

<Curve TransformNum="Number" Name="Name"/>
<Curve FilePath="Full path to a Library Curve file" Name="Name"/>
</Curves>

## XML Example Configuration

The following XML snippet shows a complete configuration of the Curve Overlay chart for the example image (without evaluations) above:

```
<CurveOverlay Id="CurveOverlay1" SheetName="Curves" XAxisTitle="Dose
(ng/mL)" YAxisType= "Linear" YAxisTitle="Response (B/B0%)"
XAxisType="Logarithmic" Title="Dose-Response">
    <Curves>
        <Curve TransformNum="2" />
        <Curve FilePath="4PL.curve.xml" />
    </Curves>
</CurveOverlay>
```


## Curve Overlay Evaluations

For each Curve Overlay, you can additionally evaluate expressions that:

- Result in a single numerical value for each curve featured on the chart
- Plot the evaluation result on each curve
- Are included in the report in a table

The Curve Overlay Evaluations are added as a child of CurveOverlay element. These are configured and displayed in the report in the same way as the Standard Curve Fit Evaluations. For example, the Curve Overlay configuration for the example chart image (with evaluations) above:
<Evaluations>
<Evaluation Type="CalcX" Expression="20" Description="ED20" Name="ED20"/>
<Evaluation Type="CalcX" Expression="50" Description="ED50" Name=ED50"/>
<Evaluation Type="CalcX" Expression="80" Description="ED80" Name="ED80"/>
</Evaluations>
In addition to the chart, the computed results are displayed in the report as a simple threecolumn table:

| Curve 1 | ED20 | 162.67 |
| :--- | :--- | :--- |
| Curve 1 | ED50 | 63.3061 |
| Curve 1 | ED80 | 29.1162 |
| Curve 2 | ED20 | 130.595 |
| Curve 2 | ED50 | 1.55167 |
| Curve 2 | ED80 | 0.998171 |

The columns contain curve name in bold, Description if specified or Expression if it was not, and the numerical value of the evaluated result or any syntax or computation error in red (in same way as the Evaluation feature).

## Evaluations Table

The Evaluations Table report element includes in the report details and results of any configured evaluations arranged in a table, which typically consists of three columns. For example,

| Description | Expression | Results |
| :--- | :--- | :--- |
| Evaluation \#1 | Unknown | 0.826 |
| Evaluation \#2 | median(Unknown) | 0.649 |
| Median of all Unknown | median(Unknown) | 0.649 |

Here, the Description column contains Expression \#\{index\} in the first and the second rows and the specified description in the third. The Expression column contains the evaluated expression and the Results display the computed result of each configured Evaluation.

## Settings

Depending on the configuration of the Evaluation settings, the results are arranged in one or more Evaluations Tables. The number of rows in each table corresponds to the number of configured Evaluations.

## Evaluations Table

While the Results are definitely included, you might prefer to hide other columns by clearing corresponding check boxes.

| Include Description | Include the description column. |
| :--- | :--- |
| Include Expression | Include the expression column. |
| Include Header | Determines whether to include the table header. |

The Evaluation Table is also configured with common report settings that are covered in the Report Content section.

## Histogram

The Histogram report element includes in the report a chart that graphically presents the spread of measurements or results. This can be useful for spotting outliers.

For example,


Here the Histogram was generated using a configuration to plot the Average values of Control, Unknowns, Blank and Standards samples with error bars indicating the standard deviation and a custom title.

## Settings

The Histogram is configured with the common report settings covered in the Report Content section plus the Histogram specific settings described below.

## Axes (Advanced)

Use these additional settings to adapt the chart's appearance to your needs.

| X Axis <br> Major Units | The numeric interval between numbers on the $x$-axis. Starting from the minimum value, a major unit is displayed at every increment. |
| :---: | :---: |
| X Axis Minor Units | The numeric interval between supplementary tickmarks on the $x$-axis. The minor units are not displayed unless this attribute is specified. |
| X Axis Numerical Formatting | The formatting for the X Axis Major Units values. <br> To learn about available formatting options, see Custom Format Codes for Numbers of the Chart. |

$\left.\begin{array}{|l|l|}\hline & \begin{array}{l}\text { Select where the x-axis will cross the } y \text {-axis from the drop-down: } \\ \text { - Automatic - Determine the location that makes the most sense for your } \\ \text { data automatically. }\end{array} \\ \text { X Crosses Y } \\ \text { At }\end{array} \quad \begin{array}{l}\text { - Axis Value - Cross y-axis at specified } \mathbf{X} \text { Crosses } \mathbf{Y} \text { at Value. } \\ \text { - Maximum Axis Value - Cross } y \text {-axis at the maximum } y \text { value of the } \\ \text { chart. } \\ \text { - Minimum Axis Value - Cross y-axis at the minimum y value. } \\ \text { If axis type is set to Logarithmic, then it is the lowest positive value. }\end{array}\right\}$

These settings can be also applied to other charts as an advanced configuration. To learn more, see Change the Units and Number Format of the Charts and Customize X and Y Axes Positions for Curve Charts topics.

General

| Matrix | The matrix of data to plot. Select the relevant matrix name from a drop-down <br> list. |
| :--- | :--- |

Each histogram can be configured to plot samples of one or more types (e.g., Standards, Unknowns, etc.). The values of each sample group is plotted as a coloured bar on the x-axis. The colour of each sample bar matches its sample type with a label to indicate the sample group name (or any applied Sample ID).

## Histogram

| Error Bars | Use this setting to select the type of error bars to use (or none). <br> Error bars can display the Standard Error, \%CV, or Standard Deviation <br> of the (non-flagged) replicates of the group. If the Replicates option is <br> selected the error bars reflect the minimum and maximum value of the <br> replicates of the sample. <br> With the None selection, the error bars are not shown. |
| :--- | :--- |
|  | The title for a histogram chart. |
|  | Which sample types to include in the histogram. |

## Layout

The Layout report element includes in the report an image of the sample layout used in the assay. This includes a layout itself and a legend with the sample types that are included in the layout. For example:


Here is the layout for a 96 -well microplate with 6 Standard, 1 Blank, 1 Control, and 40
Unknown samples in duplicate. Under the layout image, a sample types collection of the assay is displayed.

## Settings

Layout report element includes the following configurable setting:

## General

| Size | Small, Medium, or Large (medium size will fit on an A4 page-width). |
| :--- | :--- |

(1)
If multiple layouts are defined in the assay protocol, then all of them will be included in the report.

As with other Report Elements, you can configure how it appears in the report using common report settings.

## Matrix Table

The Matrix Table report element includes in the report the results of the performed analysis in a matrix-table structure. This is a table matching the layout dimensions with the data reported in each cell corresponding to the associated position on the matrix. Flagged data is displayed as defined by the associated transform and matrix configuration. Each cell in the table corresponds to the associated position on the matrix.

For example,

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

Here is the Matrix Table is reported for an endpoint matrix. In each cell, the value for a corresponding position is displayed. For XY data, each position would contain a thumbnail plot.

All matrices could be reported separately (i.e., a separate Matrix Table for each included matrix) or in a combined arrangement.

For example,

|  | 1 | 2 | 3 | 4 | 5 | 6 |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A |  |  |  |  |  |  |  |

Here is a snippet of a Matrix Table for the XY Reduction Average raw and calculated data, where each cell contains the thumbnail plot for corresponding input XY position and the calculated numerical result. The last row features the key explaining the arrangement in each position.

## Matrix Table with Multiple Plate/Multiplex

If the assay defines multiple plates then matrix tables are included for each of them. Each set of matrices for each plate is prefixed with the plate name and number (e.g., "Plate 1 "), then the matrices are included for that matrix.

With Combined, there will be one matrix table for each plate.
With Separate, there might be more than one matrix table for each plate.

## Settings

The Matrix Table is configured with specific to this report element and with general report configuration settings.

## Matrix Table

The matrix table has 2 configurations that define how the results will be displayed.

| Arrangement | Select Combined for each cell in the table to list the values of all included <br> matrices (per plate/analyte). In this case, the final row of the table will <br> contain a key that explains ordering of the matrices. <br> Or select Separate for a separate table for each included matrix. |
| :--- | :--- |
| Field Data | If your assay contains field data, this indicates where it appears in the table: <br> - Before - On rows before the numerical data. <br> - After - On rows after the numerical data. |

For XY data, there are additional settings for configuring how the thumbnail plots are displayed in the report.

## Thumb Plot

For XY data, various options for defining the inclusion and sizing of $X Y$ thumb plot charts are available.

| Include <br> Thumb <br> Plots | Defines whether thumb plots are included in the report. |
| :--- | :--- |
| Thumb Plot <br> Height | Specifies the height for all thumbs in the report. |
| Thumb Plot <br> Width | Specifies the width for all thumbs in the report. |

For XY data, various options are included for defining how XY thumb plot charts are scaled. These can be fixed values or determined for each position. The plot lines will be plotted within the defined scale.

## Thumb Scale

|  | Specifies whether the maximum $x$ scale of each plot is: <br> - Fixed - The scale limit of the thumb plots used in the table is specified by <br> the associated Thumb Scale Max $\mathbf{X}$ value. |
| :--- | :--- |
| Thumb |  |
| Auto Scale |  |
| $\operatorname{Max} \mathbf{X}$ | • Plot - The scale limit of each thumb plot is automatically determined by <br> the maximum $x$ data in each plot (i.e., each plot may have a different <br> maximum $x$ limit). |
| - Table - The scale limit of every thumb plot in the table is the same. This is |  |
| determined from the maximum $x$ data point from all plots in the table. |  |


| Thumb <br> Auto Scale Max Y | Specifies whether the maximum $y$ scale of each plot is: <br> - Fixed - The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Max $\mathbf{Y}$ value; <br> - Plot - The scale limit of each thumb plot is automatically determined by the maximum $y$ data in each plot (i.e., each plot may have a different maximum y limit). <br> - Table - The scale limit of every thumb plot in the table is the same. This is determined from the maximum $y$ data point from all plots in the table. |
| :---: | :---: |
| Thumb <br> Auto Scale <br> Min X | Specifies whether the minimum $x$ scale of each plot is: <br> - Fixed - The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Min X value. <br> - Plot - The scale limit of each thumb plot is automatically determined by the minimum $x$ data in each plot (i.e., each plot may have a different minimum $x$ limit). <br> - Table - The scale limit of every thumb plot in the table is the same. This is determined from the minimum $x$ data point from all the plots in the table. |
| Thumb <br> Auto Scale <br> Min Y | Specifies whether the minimum $y$ scale of each plot is: <br> - Fixed - The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Min $\mathbf{Y}$ value. <br> - Plot - The scale limit of each thumb plot is automatically determined by the minimum $y$ data in each plot (i.e., each plot may have a different minimum y limit). <br> - Table - The scale limit of every thumb plot in the table is the same. This is determined from the minimum $y$ data point from all the plots in the table. |
| Thumb Scale Max X | Determines custom maximum $x$ scale for thumb plots if Thumb Auto Scale Max X is Fixed. |
| Thumb Scale Max Y | Determines custom maximum y scale for thumb plots if Thumb Auto Scale Max $\mathbf{Y}$ is Fixed. |


| Thumb <br> Scale Min X | Determines custom minimum $x$ scale for thumb plots if Thumb Auto Scale <br> Min $\mathbf{X}$ is Fixed. |
| :--- | :--- |
| Thumb <br> Scale Min $\mathbf{Y}$ | Determines custom minimum $y$ scale for thumb plots if Thumb Auto Scale <br> Min $\mathbf{Y}$ is Fixed. |

## Matrix View

The Matrix View report element includes in the report a layout-based graphical representation of the matrix data. All corresponding settings of the associated matrix are applied to the view too. Flagged positions are indicated and displayed with a cross-through it.
Matrix view can be output with colouring of:

- Layout - Each position shows the numeric value in endpoint matrices or a thumb in XY data according to its sample type. Also, the sample type legend is included.

For example,


- Heatmap - Each position displays the numeric value with respect to the default or custom heatmap scale. The scale legend is also included.

This setting is relevant for endpoint data only.

For example,


## Settings

Matrix View includes the following configurable settings:

## General

| Matrix | The matrix of data to plot. Select the relevant matrix name from a drop-down <br> list. |
| :--- | :--- |
| Size | Small, Medium, or Large (medium size will fit on an A4 page-width). |

## Matrix View

| Colouring | Layout or Heatmap (Heatmap setting does not apply to XY data). |
| :--- | :--- |

The Matrix View is also configured with common report settings that are covered in the Report Content section.

## Measurement Meta Data

The Measurement Meta Data report element includes in the report a table listing the measurements metadata (reported as name-value pairs). This corresponds to the data as it appears under the Measurements tab, in Meta tab.

This data is reported in a two-column table with the name of each entry and its value. For example,

| Data Filename | C: \(\ ··· . . <br> ) Documents <br> ance.xlsx |
| :--- | :--- |
| Data Main Filename | ELISA Absorbance |
| Data File Created | $7 / 10 / 2017$ |
| Data File Last Modified | $7 / 10 / 2017$ |
| MEASUREMENT START DATE | Tue Jun 13 2017 13:02:29 GMT+0100 (GMT Summer Time) |
| MEASUREMENT COMMENT | Comment On Multiple Lines |
| User interface program ver- <br> sion | 3.1 .1 .9 |
| Instrument interface version | 0 |
| Instrument serial number | 0 |
| PROTOCOL NAME | ELISA Absorbance |
| PROTOCOL CREATOR DATE | FACTORY PROTOCOL |
| PLATE TYPE | PerkinElmer OptiPlate |
| PLATE FORMAT | 96 wells (8X12) |

Here the metadata table features details about the measurements data file and the details obtained from the VICTOR plate reader.

## Settings

The Measurement Meta Data is configured with common report settings that are covered in the Report Content section.

## Notes

The Notes report element includes in the report any provided notes (e.g., preparation notes, Lot ID, etc.). Notes can be used to reference the peculiarities of this data analysis run.

How to: Add Notes to the Analysis

1. Launch an assay protocol or open a results file.
2. On the Home tab, in the View group, click Notes.
3. The Notes panel will appear down-most of the screen.
4. Enter or paste any Notes as required.

Entered Notes will appear under Notes: title. For example,

## Notes:

An eyelash contaminated well B4.

## Settings

## Notes

Notes element includes the following check box setting:

| Exclude If <br> Empty | Exclude Notes from the results, if it contains no information. |
| :--- | :--- |

The Notes element is also configured with common report settings that are covered in the Report Content section.

## QC Results

The QC Results report element includes in the report any inter-assay quality control results (as defined by Quality Control settings) defined for an assay. This could include Observation Table, Levey-Jennings or CUSUM charts, and QC summary reporting the latest results along with historical results.

For example,


| Observation | Control1 Raw | Date | File |
| :--- | :--- | :--- | :--- |
| $\mathbf{3}$ (Current) | $\mathbf{0 . 8 2 3}$ | $\mathbf{2 0 1 7 - 0 7 - 0 1 ~ / ~}$ <br> $\mathbf{1 6 : 3 4 : 0 7}$ | ELISA (3) |
| 2 | 0.733 | $2017-07-01 /$ <br> $12: 32: 04$ | ELISA (2) |
| 1 | 0.722 | $2017-07-01 /$ <br> $11: 54: 02$ | ELISA (1) |

- All observations are within the $\pm 2 \mathrm{SD}$ range.

Here the Levey-Jennings chart, Observation Table and Observation summary are reported for current and two previous results of an ELISA assay protocol.

## Settings

The QC Results element is configured with common report settings that are covered in the Report Content section.

## Sample Table

The Sample Table report element lists the results of the performed data analysis by sample groups defined on the layout. Flagged data is displayed as defined by the associated transform and matrix configuration. Each row in the table corresponds to a sample.

Here is an example of a Sample Table:

| Sample | Positions | My Raw Data | \%CV |
| :--- | :--- | :--- | :--- |
| Standard1 | A1 | 0.364 | 8.440 |
|  | A2 | 0.323 |  |
| Standard2 | B1 | 0.455 | 2.210 |
|  | B2 | 0.441 |  |
| Standard3 | C1 | 0.587 | 1.460 |
|  | C2 | 0.575 |  |
| Standard4 | D1 | 0.806 | 2.864 |
|  | D2 | 0.774 |  |
| Standard5 | E1 | 1.105 | 2.607 |
|  | E2 | 1.065 |  |

It includes a snippet of five Standard samples and the positions they are assigned to. Also, the raw values (My Raw Data column) for each replicate and the calculated results from the Percentage Coefficient of Variation transform (\%CV column) are included for each sample. Each row contains replicates of the corresponding sample in the Vertical orientation. Use the Sample Data Processing options to further configure the results in the table by sample. Using these settings, you can:

- Order the results by specific values.

For example, report from highest to lowest concentration values.

- Filter.

Include only sample results of interest. It is possible to filter by text, numerical Boolean condition or regular expression. For example, configure a sample table that includes only Positive Sample Label results. Or, include only results that are above or below a threshold.

- Limit the number of included samples.

Show only the first $n$ samples; this is useful when combined with the ordering setting.
For XY data, the Sample Table contains Thumb Plots which are thumbnail graphical representations of each kinetic/spectral data set plotted for each position.


Here the replicates for each sample group, XY Raw data and calculated results for the XY Replicates Average transform are displayed in Horizontal orientation.

## Settings

The Sample Table is configured with general report configuration settings. Element-specific settings are listed in the tables below:

## Sample Table

| Field Data | If your assay contains field data, this indicates where it appears in the table: <br> - Before - On columns to the left of the numerical data. <br> - After - On columns to the right of the numerical data. |
| :--- | :--- |
| Include Pos- <br> itions | Include the Positions column to report the sample positions where the data <br> was measured. For plate assays, this is selected by default (with Include <br> Sample Numbers setting cleared). |
| Include <br> Sample <br> Icons | Include coloured circle icons to the left of each sample. |


| Include <br> Sample <br> Numbers | Include a column titled Number to report the 1-based position number for each sample. For rack assays, this is selected by default (with Include Positions setting cleared). |
| :---: | :---: |
|  | If both Include Positions and Include Sample Numbers are selected, the latter appears to the right of the former. |
| Replicate Orientation | How the replicates appear in the table: <br> - Vertical - Each position is listed in a new line of the column. <br> - Horizontal - All positions are listed in the same line. |
| Sample Types | Which sample types to include in the sample table. |

## Sample Data Processing

Using these settings, you can shape the appearance of the Sample Table to report exactly the data you need. Initially, the editor includes three check box options that expand with associated settings when selected:

- Filter

You can filter your data analysis results to include only samples that satisfy specific conditions. For example, you could filter a sample table so that it only includes samples marked with a specific label (such as "Positive"), or you could include only results that are associated with a specified field data value (such as "Male"). It's also possible to filter by numeric conditions (such as $x<1$ or $x>$ Control 1 ) so that the sample table includes only values of interest.

Use this feature in conjunction with Sample Labels to filter samples by their associated label.

## Sample Filtering Settings

Configure the sample table to include only the values of interest. Use the Filter Rows By Column setting to specify which column is used for filtering. Then define one or more filter rules. Each specified filter rule is used to test each sample's value (in the selected column); only sample values that satisfy any of the filter rule(s) are included.

There are 3 filter rules options:

- By Text
- By Regular Expression
- By Numeric Expression

A sample is included if a filter rule is satisfied for the sample's value. A sample's value comprises of any text, numbers, symbols, or their combination as they appear in the report. If the sample result is reported using Sample Labels, then any applied label will be considered as the sample's value (this makes it easy to filter by sample labels).

The three filter options can be used in conjunction with each other; all samples which satisfy any of the specified filter rules will be included in the sample table.

|  | Select a column to filter the rows by from the drop-down list. In addition to the available matrices, you can also filter the table by Samples and Positions included in Sample Table. |
| :---: | :---: |
| Filter Rows <br> By Column | In general, this feature will be used where there is a single value or result appearing in each row of the sample table (i.e. Average <br> (1.) or First setting of Sample Table Replicates). If the selected column is selected to display List of Sample Table Replicates, the value of the first replicate will be used to filter each row by. |
| Filter By <br> Text | Filter using a simple text match. Any value that contains a case-sensitive match will be included. <br> For example, if Positive and Negative labels are used, use the setting Positive to show only Positive results. To show only flagged results enter Flagged. |
| Filter By <br> Regular <br> Expression | Filter using a text string comparison defined using a regular expression. This offers more flexibility in text matching than the Filter By Text option but does require knowledge of regular expressions to work with. <br> For example, to include values that are either Positive or Negative, use the regular expression: <br> Positive\| Negative <br> To include only values that are not marked as Flagged, use the regular expression: <br> ^(?!Flagged).*\$ |


|  | Filter using a numeric Boolean expression. The expression is defined terms <br> of $x$ and is evaluated for each numeric sample value. Only results that <br> evaluate to true (equal to 1) will be included. (Sample values that are not <br> numeric will not be tested and not included.) <br> The expression should be written in terms of $\mathbf{x}$ where $\mathbf{x}$ refers to the <br> fample value being tested. <br> Numeric <br> Expression |
| :--- | :--- |
| The expression may refer to all operands (such as sample groups), <br> operators and functions available in MyAssays Desktop expressions. <br> For example, to include only values less than 1: <br> $\mathbf{x < 1}$ <br> To include only values greater than the value of Control1: <br> $\mathbf{x}>\mathbf{C o n t r o l \mathbf { 1 }}$ |  |

## Example

In this example, we will create a Sample Table that only reports values with a concentration over 100. To do this, we use the Sample Label feature to assign the Positive label to any sample with a concentration over 100 and then use the filter feature to only include samples assigned with the Positive label. This is achieved as follows:

1. In the Matrices tab, select Concentration matrix and apply Sample Label with Condition $\mathbf{x > = 1 0 0}$ and Label Positive. With this, the Sample Table looks like:

| Sample | Positions | Raw | Blank Corrected | Concentration | \%CV |
| :--- | :--- | :--- | :--- | :--- | :--- |
| OUnknown1 | A3 | 0.347 | 0.3455 | Positive | 0.280776 |
|  | A4 | 0.346 |  |  |  |
| OUnknown2 | B3 | 0.586 | 0.584 | 80.302 | 0.232345 |
|  | B4 | 0.584 |  |  |  |
| Unknown3 | C3 | 0.549 | 0.547 | 85.5316 | 0.250679 |
|  | C4 | 0.547 |  |  |  |
| OUnknown4 | D3 | 0.320 | 0.3215 | Positive | 1.7101 |
|  | D4 | 0.325 |  |  |  |
| OUnknown5 | E3 | 0.486 | 0.487 | 95.9167 | 0.584993 |
|  | E4 | 0.490 |  |  |  |

2. In the Report tab, add an additional Sample Table element, and specify Sheet Name as Positive Results. By default, it will be assigned the SampleTable2 Id.
3. In the SampleTable2 element, from the Sample Data Processing, select Filter. The editor will expand with applicable settings.
4. In Filter Rows By Column, select the Concentration from the drop-down.
5. Enter or paste Filter By Text as Positive.
6. Click Calculate to generate a new report with the updated settings.

On calculation, the results with Positive label are additionally reported on a separate Positive Results spreadsheet (in the Sheet View) as follows:

| Sample | Positions | Raw | Blank Corrected | Concentration | \%CV |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Control1 | H1 | 0.297 | 0.296 | Positive | 0 |
|  | H2 | 0.297 |  |  |  |
| Unknown1 | A3 | 0.347 | 0.3455 | Positive | 0.280776 |
|  | A4 | 0.346 |  |  |  |
| Unknown4 | D3 | 0.320 | 0.3215 | Positive | 1.7101 |
|  | D4 | 0.325 |  |  |  |
| Unknown7 | G3 | 0.356 | 0.353 | Positive | 1.06505 |
|  | G4 | 0.352 |  |  |  |
| Unknown9 | A5 | 0.325 | 0.322 | Positive | 1.36171 |
|  | A6 | 0.321 |  |  |  |

## - Limit

When only the highest or lowest data analysis results matter, you can limit the number of rows to include in the table. With this setting you can review or print only those results that are in the range of interest.

Use this setting to include only the first $n$ rows which satisfy any filtering rules and/or the ordering of any defined sequence.

## Sample Limiting Settings

To limit the number of rows in the Sample Table:
Limit To Maximum Count Enter a 1-based number of rows to include.

## Example

In this example, we'll include only the three highest results of the Average transform to the sample table. We'll also use Sample Ordering Settings to rank the results. Here is a snippet of how the Sample Table looks before setting the row limitation:

| Sample | Positions | My Raw Data | Average |
| :--- | :--- | :--- | :--- |
| Unknown1 | A1 | 0.364 | 0.3435 |
|  | A2 | 0.323 |  |
| Unknown2 | A3 | 2.105 | 2.1145 |
|  | A4 | 2.124 |  |
| Unknown3 | A5 | 1.213 | 1.2195 |
|  | A6 | 1.226 |  |
| Unknown4 | A7 | 1.159 | 1.1745 |
|  | A8 | 1.19 |  |
| Unknown5 | A9 | 0.11 | 0.113 |
|  | A10 | 0.116 |  |

To limit the number of samples included, follow these steps:

1. In the Report tab, select SampleTable element.

If your report features more than one Sample Table, select the element with appropriate ID such as SampleTable2.
2. In the SampleTable element, from the Sample Data Processing, select Limit and Order check boxes.
The editor will expand with applicable settings.
3. In the Order Column, select the Average from the drop-down.
4. In the Order Sequence, select Descending.
5. In Limit To Maximum Count, enter 3.
6. Click Calculate to generate a new report with the updated settings.

Here is the Sample Table that lists the highest three results of the Average transform:

| Sample | Positions | My Raw Data | Average |
| :--- | :--- | :--- | :--- |
| OUnknown2 | A3 | 2.105 | 2.115 |
|  | A4 | 2.124 |  |
| Unknown8 | B3 | 1.869 | 1.879 |
|  | B4 | 1.888 |  |
| UUnknown37 | G1 | 1.791 | 1.797 |
|  | G2 | 1.803 |  |

- Order

By default, samples are arranged by sample type and group number, but you might prefer to display your samples in a different order. For example, it might be useful to present your results in concentration order with the highest first.

## Sample Ordering Settings

The ordering of rows is defined with the following settings:

| Order |  |
| :--- | :--- |
| Column | Select the column to sort the rows by from the drop-down list. In addition <br> to the available matrices, you can also sort the table by Samples and <br> Positions included in your Sample Table. <br> If the specified column contains a List of Sample Table Replicates, then <br> the value of the first replicate is used to sort the rows. |
| Order | Specify the order sequence, based on the selected Order Column <br> setting: <br> Sequence |
| Ascending - For numerical values, the results are listed from the <br> lowest to the highest value. For textual content, the results are listed in <br> alphabetical order. <br> Descending - For numerical values, the results are listed from the <br> highest to the lowest value. For textual content, the results are listed in <br> reverse alphabetical order. |  |

## Example

In this example, we'll sort samples by Standard Curve Fit (Concentration) results from highest to lowest values. By default, the Sample Table presents data consequently for all included Sample Types:

| Sample | Positions | Raw | Concentration |
| :--- | :--- | :--- | :--- |
| OUnknown1 | A3 | 0.347 | 142.103 |
|  | A4 | 0.346 |  |
| Unknown2 | B3 | 0.586 | 80.302 |
|  | B4 | 0.584 |  |
| Unknown3 | C3 | 0.549 | 85.5316 |
|  | C4 | 0.547 |  |
| Unknown4 | D3 | 0.320 | 157.858 |
|  | D4 | 0.325 |  |
| Unknown5 | E3 | 0.486 | 95.9167 |
|  | E4 | 0.490 |  |

To change how samples are ordered, follow these steps:

1. In the Report tab, select SampleTable element.

If your report features more than one Sample Table, select the element with appropriate ID such as SampleTable2.
2. In the SampleTable element, from the Sample Data Processing, select Order. The editor will expand with applicable settings.
3. In the Order Column, select Concentration from the drop-down.
4. In the Order Sequence, select Descending.
5. Click Calculate to generate a new report with the updated settings.

With the example configuration provided above, the samples will be ordered as follows:

| Sample | Positions | Raw | Concentration |
| :--- | :--- | :--- | :--- |
| Unknown33 | A11 | 0.241 | 327.389 |
|  | A12 | 0.246 |  |
| Unknown4 | D3 | 0.320 | 157.858 |
|  | D4 | 0.325 |  |
| Unknown9 | A5 | 0.325 | 157.465 |
|  | A6 | 0.321 |  |
| Unknown1 | A3 | 0.347 | 142.103 |
|  | A4 | 0.346 |  |
| Unknown7 | G3 | 0.356 | 138.052 |
|  | G4 | 0.352 |  |

## Thumb Plot, Scale, and Data Format Settings for XY Data

For XY data, there are additional settings for configuring how the thumbnail plots are displayed in the report.

## Thumb Plot

For XY data, various options for defining the inclusion and sizing of XY thumb plot charts are available.

| Include <br> Thumb <br> Plots | Defines whether thumb plots are included in the report. |
| :--- | :--- |
| Thumb Plot <br> Height | Specifies the height for all thumbs in the report. |
| Thumb Plot <br> Width | Specifies the width for all thumbs in the report. |

For XY data, various options are included for defining how XY thumb plot charts are scaled. These can be fixed values or determined for each position. The plot lines will be plotted within the defined scale.

## Thumb Scale

| Thumb <br> Auto Scale <br> Max X | Specifies whether the maximum $x$ scale of each plot is: <br> - Fixed - The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Max X value. <br> - Plot - The scale limit of each thumb plot is automatically determined by the maximum $x$ data in each plot (i.e., each plot may have a different maximum $x$ limit). <br> - Table - The scale limit of every thumb plot in the table is the same. This is determined from the maximum $x$ data point from all plots in the table. |
| :---: | :---: |
| Thumb <br> Auto Scale <br> Max Y | Specifies whether the maximum $y$ scale of each plot is: <br> - Fixed - The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Max $\mathbf{Y}$ value; <br> - Plot - The scale limit of each thumb plot is automatically determined by the maximum $y$ data in each plot (i.e., each plot may have a different maximum y limit). <br> - Table - The scale limit of every thumb plot in the table is the same. This is determined from the maximum $y$ data point from all plots in the table. |
| Thumb <br> Auto Scale <br> Min X | Specifies whether the minimum $x$ scale of each plot is: <br> - Fixed - The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Min X value. <br> - Plot - The scale limit of each thumb plot is automatically determined by the minimum $x$ data in each plot (i.e., each plot may have a different minimum $x$ limit). <br> - Table - The scale limit of every thumb plot in the table is the same. This is determined from the minimum $x$ data point from all the plots in the table. |


| Thumb Auto Scale Min Y | Specifies whether the minimum $y$ scale of each plot is: <br> - Fixed - The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Min $\mathbf{Y}$ value. <br> - Plot - The scale limit of each thumb plot is automatically determined by the minimum $y$ data in each plot (i.e., each plot may have a different minimum y limit). <br> - Table - The scale limit of every thumb plot in the table is the same. This is determined from the minimum $y$ data point from all the plots in the table. |
| :---: | :---: |
| Thumb Scale Max X | Determines custom maximum $x$ scale for thumb plots if Thumb Auto Scale Max X is Fixed. |
| Thumb Scale Max Y | Determines custom maximum y scale for thumb plots if Thumb Auto Scale Max $\mathbf{Y}$ is Fixed. |
| Thumb Scale Min X | Determines custom minimum $x$ scale for thumb plots if Thumb Auto Scale Min X is Fixed. |
| Thumb Scale Min Y | Determines custom minimum y scale for thumb plots if Thumb Auto Scale Min $\mathbf{Y}$ is Fixed. |

## $\underline{X Y}$

|  | Specifies how XY data is included in the report: |
| :--- | :--- |
| - Thumb - If Include Thumb Plots is selected, a thumb plot is reported |  |
| for each plot. This is the default option. |  |
| Format | • Y Values - A list of $y$ data point values is reported for each plot. <br> - X and Y Values - A list of $x$ and $y$ data point values is reported for each <br> plot. <br> - Index and Y Values - For each plot, this reports a list where $x$ are <br> displayed as 1-based continuous data point numbers and $y$ as data point <br> values. |

## Text

The Text report element includes any specified text and/or the evaluation of any available text macros.

This element is useful to include custom wording in the report. Also, text macros can be used to include data that may change from run-to-run of the assay. Available text macros include:

- Any imported meta-data
- Filenames (protocol, results, imported data file)
- Current date/time
- Calculation version number
- User name
- PC name

For example, with the following Text configuration:
Protocol: <ProtocolName>
Imported Data: <DataMainFilename>
Results: <ResultsName>
Date: <CurrentDateLong>
In the report, the macros will convert to:
Protocol: ELISA (96 well)
Imported Data: 96 wells_20170613_152121
Results: ELISA (96 well) (21)
Date: 21 June 2020

## Settings

## Text

| Text | Click the drop-down to expand the text editor. <br> The editor consists of: |
| :--- | :--- |
| • A multiline edit box where you can enter or paste any required <br> information. <br> - A full list of all available macros and their current value. To insert a macro <br> at current cursor position, double-click it in the list. |  |

The Text element is also configured with common report settings that are covered in the Report Content section.

## Transform Content

The Transform Content report element includes additional report content to illustrate settings or results of a specific transform. If the transform applied to the assay possesses any kind of optional items, they are added to the report automatically. Certain transforms comprise a number of optional content.

The additional report elements are:

- Additional Sample Table Column - This is an optional additional column that can be included in the sample table. For example,

| Sample | Positions | Factor | My Raw Data | Concentration (x Dil. Factor) |
| :--- | :--- | :--- | :--- | :--- |
| OUnknown1 | A3 | 2 | 2.105 | 4.210 |
|  | A4 |  | 2.124 | 4.248 |
| Unknown2 | B3 | 5 | 1.869 | 9.345 |
|  | B4 |  | 1.888 | 9.440 |
| Unknown3 | C3 | 2 | 0.474 | 0.948 |
|  | C4 |  | 0.514 | 1.028 |
| Unknown4 | D3 | 5 | 0.364 | 1.820 |
|  | D4 |  | 0.378 | 1.890 |
| Unknown5 | E3 | 2 | 0.279 | 0.558 |
|  | E4 |  | 0.367 | 0.734 |

Here is the Dilution Factors transform outputs optional report content named Factor Column.

- Fit Chart - The visual representation of a plotted curve that is included in a report by default. E.g.:


Here is a chart for Standard Curve Fit transform example.

- Table - The table containing additional values computed from a transform. For example, this might refer to Goodness Measures Table, Dose-Response Curves Table, Curve Data Table, etc.
- Equation - The formula of a fit method that was used to plot a curve. E.g.:
$y=m x+c$
Here is the formula for Linear Regression.


## Settings

A report can contain one or more Transform Content subelements.
The Transform Content is configured with common report settings that are covered in the Report Content section and the setting described below.

## Content

| Content | Use this drop-down to specify which subelements to include (or exclude) and <br> their ordering. The available subelements depend on the associated <br> transform. <br> Select the subelement, move in to the Included or Excluded pane with right <br> and left arrow buttons, or change its' positioning with the Up and Down <br> icon buttons on the right of the Included pane. <br> To include or exclude all transform content from the report, click the Add All <br> or Remove All buttons, respectively. |
| :--- | :--- |

## Page Settings

| Chart Size | If the transform content includes chart, here you can select Small, Medium, <br> or Large option. |
| :--- | :--- |

How to: Configure Transform Content in the Report

1. In the Assay Properties panel, select the Report tab.

If the Report tab is not immediately visible, click \ll Show All Properties to expand.
2. Select Transform Content element that contains transform name, e.g.,

## TransformContentDoseResponseCurvesTable1.

3. On the Report Configuration pane, click Content drop-down.
4. Review and edit content items as required.
5. Click Calculate to perform calculations with the new settings.

## Curve Fit Transforms Report Contents

Curve-fitting endpoint transforms produce a number of additional report items under their Transform Content report element. This topic covers the items available for Standard Curve Fit, Standard Curve Fit (from Library) and General Curve Fit transforms, their content, and the way they will be included in the report. Some of the elements also contain additional advanced settings can be used to customize the report.

## About Curve Fit Transforms Report Contents

The content for a specific transform is named as TransformContent\{TransformName\}\#, where:

- \{TransformName\} is the title of the transform
- \# indicates the number of the transform

If your assay is comprised of two Standard Curve Fit transforms (for example the unrestricted and the restricted curve fits), the transform content will be titled as
TransformContentStandardCurveFit1 for the first transform in the sequence, and TransformContentStandardCurveFit2 for the second.

When configuring the report using the report template, the Id of each specific item is appended to the general transform content Id. For example,
\{TransformContentStandardCurveFit1GoodnessMeasuresTable1\} will
expand to the goodness of the fit measures calculated for the first defined Standard Curve Fit.

## Best Fit Results Table

This table displays the results of Best Fit search ordered by rank (the fit with the highest score is listed first). It includes the following columns:

- Rank - The ranking of the fit ( 1 is the best).
- Score - The computed result of the Scoring Method for the fit.
- Fit Method - The curve-fitting method used for the search.
- Weight Method - The weighting method applied to the fit.


## Coefficients Table

This table lists the calculated coefficients of the selected Fit Method, their value, and $\pm$ error. For customizing the numerical formatting option for the result and error, see Setting Numerical Formatting for Goodness Measures and Coefficients of the Fit.

## Curve Data Table

This table reports the curve-fitting data used for the fit (for the Best Fit method the results of the best performing fit are displayed).

- For Standard Curve Fit and Standard Curve Fit (from Library), the table lists sample groups of the specified Standard Type, the defined concentrations, the responses, the calculated concentrations, and the computed \% Accuracy and Precision (\%CV).
To clearly indicate whether each calibrator is within the acceptable range or not, each row is highlighted based on whether it satisfies the precision and accuracy rules (rows in lightred fail the test). Any flagged samples are indicated using the same convention as in the Sample Table.
For advanced configuration settings, see Configure Contents of Curve Data Tables.
- For General Curve Fit, the table includes defined and calculated $x$ and $y$ values used in the curve fitting.


## Goodness Measures Table

The table listing each calculated goodness measure.
To define the numerical formatting for the results, see Setting Numerical Formatting for Goodness Measures and Coefficients of the Fit.

Only curve-fit methods with a single equation are reported. For fit methods computed from multiple segments (Point-to-Point and the Cubic Spline methods) equations are not reported.

## Fit Equation

## Id: \{FitEquation\#\}

The equation of the curve fit using mathematical notation.

## Fit Equation with Values

## Id: \{FitEquationWithValues\#\}

The equation with the coefficients replaced by the values determined from the fit. For example, the linear regression equation is:

## $y=m x+c$

With linear regression, a value for $\mathbf{m}$ and $\mathbf{c}$ are computed. For example, if $\mathbf{m}=1.23$ and $\mathbf{c}=4.56$, then the equation would be:
$y=1.23 x+4.56$
Inverse Fit Equation
Id: \{FitEquationInverse\#\}
The equation of the curve fit in terms of $y$ presented in mathematical notation.

## Inverse Fit Equation with Values

Id: \{FitEquationInverseWithValues\#\}
The equation in terms of $y$ with the coefficients replaced by the values determined from the fit. For example, the inverse linear regression equation is:
$x=(y-c) / m$
With linear regression, a value for $\mathbf{m}$ and $\mathbf{c}$ are computed. For example, if $\mathbf{m}=1.23$ and $\mathbf{c}=4.56$, then the equation would be:
$x=(y-4.56) / 1.23$

## Fit Evaluation Table

## Id: \{FitEvaluationTable\#\}

This table lists results of fit evaluations in a table. For adding and configuring custom evaluations, see Advanced Curve Fit Evaluations.

For Standard Curve Fit and Standard Curve Fit (from Library), the first entries are the determined LLOQ and ULOQ that match one of the specified concentration values.

LLOQ is the first concentration value $(x)$ where:

- \% Accuracy value (from the Curve Data Table) is within 100 +/- Accuracy ( $\pm \%)$
- \%CV value (from the Curve Data Table) is less than Precision (\%CV) limit

ULOQ is the highest concentration value (starting from the highest value and going backward) where:

- \% Accuracy value (from the Curve Data Table) is within 100 +/- Accuracy ( $\pm \%)$
- \%CV value (from the Curve Data Table) is less than Precision (\%CV) limit


## Fit Chart

## Id: \{Chart\#\}

The Fit Chart plots the calibrator data points on axes with the response values (typically measured, corrected, or normalized values) versus the specified concentrations. The chart includes the fit line and calibrators. The calibrator replicates are plotted with dots. Flagged points are not included in the chart.

©
When using Standard Curve Fit with Fit To Standard set to Average, then the average values are also plotted (in addition to the replicates) using a triangle (pink).

The chart's axes types are set in accordance with $\mathbf{X}$ Axis Type and $\mathbf{Y}$ Axis Type. The thick black line shows the curve calculated from these standards.

To plot non-calibrator sample types on the fit line in the report, you can use Points on Curve setting.

You can adjust the chart's appearance in the report. Refer to the Change the Units and Number Format of the Charts and Customize X and Y Axes Positions for Curve Charts topics to learn more.

## Validations Table

The Validations Table report element includes the details and results of any configured validations arranged in a table. This table indicates whether each validation has passed or failed and, when validations are defined, is added to the report automatically.

The Validation Table typically consists of three columns. For example,

| Results | Expression | Description |
| :--- | :--- | :--- |
| Fail | $x<2.5$ | All non-flagged positions have a value less than 2.5 <br> (Expression with Every Sample Replicate (x) type) |
| Pass | $x<2.5$ | The average of the replicates (that are not flagged) of all <br> sample groups is less than 2.5 (Expression with Every <br> Sample Group (x) type) |
| Pass | Unknown1 <br> $>1.5$ | Condition \#3 |
| ? Syntax Error | Control1 ! $<$ <br> 1.5 | Condition \#4 |

Here, the Results column displays the evaluated result of each configured Validation. For the Fail and Inconclusive (i.e., Syntax Error), the row is highlighted with yellow and red background colour, respectively. The Expression column contains the verified condition. Note, that the expression for the first and the second validation is the same, but the type of validations is different, as stated in the Description. Finally, the Description column contains the description if specified (e.g., on third row) or Condition\#\{index\} if it is not.

## Settings

Depending on the configuration of the Validation settings, the results are arranged in one or more Validations Tables. By default, the Validation table is displayed as three-column table with the number of rows corresponding to the number of Conditions added.

While the Results column is definitely included to the report, you might prefer to hide former columns by clearing corresponding check boxes.

| Description <br> Header | A title for a Validation Table in the report. |
| :--- | :--- |
| Include <br> Description | When selected, the column contains Description if specified or <br> Condition\#\{index\} if it was not. |
| Include <br> Expression | If included, this outputs the Condition defined for each Validation. |


| Include <br> Header | Determines whether to include the table header. |
| :--- | :--- |
| Results <br> Column | This specifies whether Results column appears left-most or right-most in the <br> table. |
| Results <br> Header | A title for the Results column in the report. |

The Validation Table is also configured with common report settings that are covered in the Report Content section.

## XY Plot

This is an advanced feature that can only be configured through the XML editor.
XY Plot report element can be used to display plots from different positions on a single overlaid chart similar to the Position Mode of Kinetic/Spectral Overlay chart. The y-axis of the chart corresponds to measurement values; the $x$-axis represents (depending on the assay) cycle, time, or wavelength values. The data points for different positions are denoted with different colours. The flagged positions or data points are not included in the chart.

For example:


Here, the XY Plot chart contains three overlaid plots from positions A2, F2, and H2. The position plots can originate from any number of positions of any raw or calculated XY matrix.

## nfiguration

You can configure one or more XY Plot charts to include in your report. Specify their attributes and syntax in the XML Editor as described below.

## How to: Add XY Plot Configuration to Report

1. In the Assay Properties panel, select the XML tab.

If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Locate the Report element and add the XYPlot child element and then specify its attributes as described below.
3. Click Apply to save changes to the protocol and then Calculate to generate new report.

## XY Plot Attributes

## General

| Id | A unique identifier that is used to identify the report element in the Report <br> Elements pane and when configuring custom report templates. |
| :--- | :--- |
| MatrixId | An identifier of the matrix to be plotted on the y-axis. For example, Raw1, <br> Calc1, etc. |
| Container | A 1-based container ID. If not specified, then the first container ID is used. |
| Positions | A list of positions to overlay on the chart separated by commas. For example, <br> A1,A2,A3. |
| SheetName | If defined, inserts the chart to a sheet with the specified SheetName. For more <br> details, see Sheet Name in the Report Content section. |
| Title | An optional title for an XY Plot chart that can refer to Report Macros. If not <br> specified, then no title is displayed. |
| Include | Boolean setting that defines whether XY Plot chart is included in the report. If <br> not specified, then the chart is included. |

You can also optionally specify axes settings. If the settings are not specified, then the setting from the associated matrix is used.

## Axes

| X Axis Title | The name of the $x$-axis on the chart. |
| :--- | :--- |
| X Axis Type | The type of x -axis to plot: |
|  | - Logarithmic |
| - Linear |  |

[^0]<XYPlot Id="/d" MatrixId="/d" Container="Number" Positions="List of positions" Include="Boolean" SheetName="Name" XAxisTitle="Title" YAxisType="Type" YAxisTitle="Title" XAxisType="Type" Title="Title"/>

## XML Example Configuration

The following XML snippet shows a complete configuration of the XY Plot chart for the example image above:
<XYPlot Id="XYPlot1" Positions="F2,H2,A2" MatrixId="Raw1" />

## Configuring Template for Page-Based Report

The report template enables presenting your analysis results tailored to your laboratory's standards and requirements. This feature merges together report elements with a configured template. Use the full power of MS Word or WordPad features to customize page-based reporting of the results.

For example, you can define a custom sequence of selected report elements, insert relevant images or textual descriptions for a printed document. Simply apply styling settings and include the results required.

Report template affects Page View and page-based outputs (DOC, DOCX, PDF) of the analysis results only.

## Add New Report Template for Page-Based Reporting

1. Launch an assay protocol or open a results file.
2. On the Report ribbon tab, in the Views group, select the Page View of the results.
3. Navigate to the Template group and click New.

This launches the Template.DOCX file with a default application (typically MS Word or WordPad).
4. Review and edit the reporting template content as required.
5. After making and saving changes to the report template, click Calculate to generate a report with the new settings.

The application displays a Template.DOCX file for editing - this is the template file. Initially, it contains the MyAssays report element identifiers (Ids) in curly brackets.

Report templates have no restrictions regarding the specific information they contain. You can make any changes to the template and save them. For example, reposition, remove or duplicate Ids, change their font, style, and other properties, add textual information, macros etc. In fact, any change is possible if it is supported in the editor.

## Add Report Element Id to the Report Template

1. Launch an assay protocol or open a results file.
2. Select the Report tab.
3. Select the Report Element from the list.
4. Copy the element's Id and insert it in the appropriate place of the template in curly brackets and press the spacebar to insert a space (e.g., \{SampleTable1\} ).
If there is no space after the element's Id, table elements such as Sample Table will be merged together in the report.
5. Save changes to the report template and click Calculate to generate a report with the new settings.

After modifying the template file, you can switch back to MAA and generate an updated report while keeping the editor open. This way you can review modified report in Page View and make additional changes if needed.

The defined template will be applied to all page-based reports of this assay-results file.

Only one template can be defined for the assay-results file at a time. If the previously defined template is no longer useful, edit or delete it.

## Edit/Remove Existing Template

After you have created New template file, the Template group expands with two new options:

- Edit - Modifies the template file's content according to newly added report elements or updated formatting. This option will launch your default .DOCX editor with the existing template file.
- Delete - Removes the existing template file (a warning message will prompt for confirmation before this action is completed).


## Print Settings and Print Preview

You can print your generated results directly from MAA with MyAssays Desktop printing functions. These include printing the generated report with the default or a selected printer, and previewing results before printing them. You can also configure automatic printing of the first results version if the data analysis is performed from an assay-protocol (this is useful when a protocol is launched automatically, perhaps by Folder Poll or from the command line).

## Before Printing

To make sure that your report meets your specifications, preview your report before sending it to the printer. This can avoid wasting paper and verify that all the information is presented appropriately.

## To Preview Report Before Printing

1. Launch an assay protocol or open a results file.

If you launch a protocol, click Calculate to generate results.
2. Do one of the following:

- On the Report tab of the ribbon, click Print Preview.
- On the File backstage view, select Print tab, and then click Print Preview.

This launches the Preview window, where you can preview your report before printing it. To print your report and check or change the settings for your printer, click the Print icon in the upper-left corner of the window.

## Set Up Report Layout and Content

MyAssays Desktop provides great flexibility in how your report content is presented. For maximum flexibility, consider using a page-based report template; with this feature you can change the font properties, page orientation and margins, and add any additional content needed. To learn about custom page-based layout for your report, see Configuring Template for Page-Based Report.


After you create or edit a template, click Calculate to apply changes and update the report.

## Automatically Print Report on First Calculation

When setting up the data analysis for a protocol, you can configure it to automatically print the report once the first set of results are calculated (using the default printer).

## To Automatically Print Report on First Calculation

1. Launch an assay protocol.
2. On the File backstage view, select Print tab, and then select Print report on first calculation check box.
3. Save the updates to the protocol.
4. Click Calculate.

On calculation, MAA switches to results mode, saves the results to .assay-results file and prints the report automatically. If you launch this protocol again (maybe importing a new set of measurements) and click Calculate, the results will be saved to another .assay-results file and printed automatically.
For more information, see Save Options and Versioned Analysis.

## Print Report with Default Printer

By default, all report content is printed as you see it in the Page View and in the Preview window with the default printer. If there is no printer available, you will be able to preview but not print generated reports.

## To Print Report with Default Printer

1. Launch an assay protocol or open a results file.

If you launch a protocol, click Calculate to generate results.
2. Do one of the following:

- On the Report tab of the ribbon, click Print.
- On the File backstage view, select Print tab, and then click Print.


## Change Printer or Set Up Print Settings

If there are several printers connected to your PC, you can select the one you'd like to print to. You can also set up printing settings such as number of copies, printing preferences and page range.

To Print with Custom Printer or Set Up Print Settings

1. Launch an assay protocol or open a results file.

If you launch a protocol, click Calculate to generate results.
2. On the File backstage view, select Print tab, and then click Custom Print.
3. Review or edit your printer settings, then click Print.

## Avoiding Horizontal Page Splitting in Page-Based Reports

If the report contains content wider than the print area, the elements may be split horizontally between pages in the Page View and the page-based reporting. In this case, MyAssays Desktop will notify you and suggest rearranging the content to avoid undesirable formatting. This topic covers suggestions on how to resolve split tables or cropped images in your reports.

## Why Page Splitting Occurs

MyAssays Desktop presents the calculated results in Sheet View and Page View. The pagebased reporting is configured using the Page View and is limited by A4 page dimensions. Contents are fit to the page width. Therefore, if elements exceeding the width limitation are cropped horizontally and split between pages.

This is typically the case if your report contains a Sample Table with a lot of columns or image elements such as layouts or charts that are set to Large size.

## Tips for Avoiding Horizontal Splitting over Two Pages

Here are some ways to prevent the content of your report from splitting (with the easiest being Fit Sheet on One Page):

## Fit Each Sheet on One Page

The easiest way to eliminate horizontal page splitting is to use the report's Print Scaling setting; this can automatically resize the content to fit each sheet on one page by setting to Fit Sheet on One Page.

Assuming you have the assay to modified already opened, follow these steps:

1. In the Assay Properties panel, select the Report tab. If you cannot see the Assay Properties panel, click Properties to open it. If the Report tab is not immediately visible, click \ll Show All Properties to expand.
2. From the Report header in the Report Tree, select the Sheet | Print Scaling dropdown box.
3. Select from the options the most appropriate setting for your assay:
4. Fit Sheet on One Page - with this setting, all report elements contained on a single sheet will be fit to one page
5. Fit All Columns on One Page - with this setting, all columns in the sample table will be scaled to fit on one page
6. Fit All Rows on One Page - with this setting, all rows in the sample table will be scaled to fit on one page
7. Click Calculate to perform the calculations with the new settings and to view the report.

## Exclude Redundant Columns from the Sample Table

If your Sample Table contains a lot of columns, you can consider excluding some matrices from the table. For example, you could choose to exclude intermediate transform results that are not essential for your experiment. Assuming you have the assay results already launched, follow these steps:

1. In the Assay Properties panel, select the Matrices tab.

If you cannot see Assay Properties panel, click Properties to open it. If the Matrices tab is not immediately visible, click << Show All Properties to expand.
2. From the Matrix Selection pane, select the matrix you want to exclude.
3. In the Matrix Configuration pane, locate the Report Contents settings and select the Include in Sample Tables or Include in Matrix Tables check boxes to add the matrix to the associated report element.
4. Review or edit other data analysis settings.
5. Click Calculate to perform the calculations with the new settings and to view the report.

## Split the Results between Sample Tables

If all the transform results should be reported but the Sample Table is split inconveniently, you can divide the content between several Sample Tables. Assuming you have the assay results already launched, follow these steps:

1. Add additional Sample Table element to the report.

By default, it will be assigned with the SampleTable2 Id.
2. In the Matrices tab, from the Matrix Selection pane, select the matrix for including in the second Sample Table.
3. In the Matrix Configuration pane, under the Report Contents, click Sample Table Ids drop-down, and remove selection from SampleTable1. This will include the matrix in the SampleTable2 only.
In the same way, select the matrices to include in SampleTable1 only.
4. Review or edit other data analysis settings.
5. Click Calculate to perform the calculations with the new settings and to view the report.

## Define Template for Page-Based Reports

Using the report template, you can configure the report to appear as desired. Refer to the Configuring Template for Page-Based Report to learn about setting up the template.
Below are some settings you can use to avoid cropping and splitting the contents between pages:

- Use smaller font for Sample Tables, Matrix Tables, or both
- Narrow the margins
- Change the page orientation to Landscape


## Enhanced Security <br> Introduction

MyAssays Desktop Pro's Enhanced Security edition provides features compatible with working in GxP and 21 CFR Part 11 environments. This includes protected and tracked access to MyAssays Desktop Pro's features and data from inside a secured environment. The system can securely import, process, and output secure and protected content.

This edition is only available to users with a MyAssays Desktop Pro Enhanced Security license.

Key features include:

- Access control - Authentication and authorization are used to permit or deny access to MyAssays Desktop functions.
- Operating-system-level security data storage protection - Only authorized users can make changes to MyAssays Desktop Pro data and configuration files.
- Secure hand-off of data - To ensure integrity of imported measurement data.
- Electronic Signatures - approve and sign protocols and results files electronically in accordance with 21CFR11 rules.
- Secured file protection - To ensure that data files are only accessible by authorized users.
- Audit trail and Login log - All activity is logged with date and time stamp against the logged in user.
- Excel and Word report protection - Changes to all Excel outputs can be protected with a password.
- Autologin account - To automate working with third-party software in an efficient and secure manner.

The Enhanced Security system shares user management and system security policies with Windows to simplify user management and to eliminate redundant administrator tasks. For example, if your users are already configured on a domain, it is easy to provide those domain users with secured access to MyAssays Desktop Pro.

The Enhanced Security system provides a number of configuration options to support different workflows, system configurations, and data handling methods.

Use of the Enhanced Security mode alone does not provide compliancy with 21 CFR

Part 11. Any facility that requires compliance with 21 CFR Part 11 rules must implement the necessary procedures and controls specified by the regulation throughout the workplace.
The US FDA does not preapprove or certify systems with respect to 21 CFR Part 11. (It is misleading for any vendor to claim that a system, whether it be an instrument or standalone software, is Part 11 compliant.)
Full compliancy is always a combination of both technological and procedural controls. The compliancy assessment is always made on-site by the FDA.

## Training Course

The restricted-access online training course has been designed to equip users in regulatory environments with training to configure and operate the secured system. Assessments and participant certification is available to verify that your team are up-to-speed.

The course covers all topics for installation, configuration, usage. The course is arranged into multiple tracks to cater for different user types:

- Security Administrators
- User Managers
- Analysts
- Operators

Features:

- Video content with interactive links
- Assessments
- Tracked Attempts/Score Results
- Multi-track course
- Participation/Results certification

Available at https://www.myassays.com/myassays-desktop-es-training-course.html

## Authentication and Authorization

Throughout this documentation, the terms authentication and authorization are used; it is important that these terms be clearly defined and understood:

Authentication - The process by which users are identified by their username and password

Authorization - The actions that an authenticated user is allowed to perform
For example, when a user logs in to Windows by providing her username and password, she is authenticated. An authenticated user is authorized to perform certain operations. For example, an administrator is authorized to perform administrator operations whereas a standard user is not.

## Authentication Options

With MyAssays Desktop Pro's Enhanced Security, there are two different authentication systems available:

## - Windows Log In

- MyAssays Desktop Log In

The key difference is how the user must log in, either through the standard Windows Log In method or through a MyAssays Desktop Log In.

With Windows Log In, users will log in to Windows as they would do normally. The MyAssays Desktop application can be used without requiring an additional log in prompt.

With MyAssays Desktop Log In, when the MyAssays Desktop application is launched, the user must log in to the application to authenticate.

In both cases, user accounts are managed (e.g., adding/creating/disabling/locking user accounts) by the administrator using the standard Windows user management tools (see General User Administration). The user authorizations that are specific to MyAssays Desktop are managed using the MyAssays Desktop Pro Security Management tool.

## Authentication Context

MyAssays Desktop Pro's Enhanced Security supports these authentication contexts:

## - Local Machine

## - Domain

This means that user accounts can be managed either locally (on the local machine itself) or via a domain controller.

## Which Authentication Method?

Deciding on the correct authentication method depends on how the workstation will be accessed:

- If each user will log in to Windows using their own Windows account, then use Windows

Log In.

- If the workstation is configured to use a single common login that is shared by multiple users for general access, then use MyAssays Desktop Log In.

In many ways, the Windows Log In method is the simplest to work with and administer. End users have a single familiar login method to work with.

However, if you are working with third-party software (such as instrument control software), you may find that it imposes certain requirements on the Windows log in that must be followed. For example, it may be necessary for that software to be operated under a specific restricted Windows login to ensure it is correctly secured. In these cases, it may not be feasible to allow system users' their own Windows log in session; it is for this reason that the MyAssays Desktop Log In authentication option is available.

## Windows Log In <br> MyAssays Desktop Log In

The workstation can only be accessed by a user providing their own personal credentials via the Windows Log In. The credentials provided when the user logs in to Windows are used by MyAssays Desktop. This means that the user does not need to log in to both Windows and MyAssays Desktop separately.

The workstation is routinely used through a single Windows log shared by multiple users. To secure MyAssays Desktop data and features by user, each user has their own user account that MyAssays Desktop uses to authenticate. This means that when MyAssays Desktop is used, the user must log in to the application directly.

## Authorization

Any authenticated user will be able to launch the software; however, the operations that they can perform depend on the authorization settings that have been assigned to them.

For a complete list of available authorization settings, see Authorizations.
Each user is assigned to a single authorization group. The system administrator can assign authorizations to users with the MyAssays Desktop Pro Security Management tool.
For example, a user assigned to the Run and Review Only authorization group would only be able to run protocols and review results (he would not be able edit protocol settings), whereas a user assigned to the Unrestricted authorization would be able to perform any permitted operation.

If a user successfully logs in to MyAssays Desktop Pro Enhanced Security and that user has not yet been added to the system, the user will be treated as a Guest user with no permitted authorizations. (The system advises guest users to contact the administrator to gain access to the software).

With MyAssays Desktop Pro Enhanced Security, any attempt to perform a protected operation is stored in the audit trail (including whether the operation was permitted or denied in accordance with the user's authorization).

## Secure Data Hand-Off

When working with measurement data originating from instrumentation (such as microplate readers or gamma counters), it is important to ensure the integrity of the imported data.

In many systems, the hand-off of data between one system and another provides an opportunity for users to tamper with and modify the data either unscrupulously or in error. Such a vulnerable workflow is unlikely to pass regulatory validation or stand up to scrutiny. For example, after the readings are completed the measurement data is written to a file. This data must then be transferred or imported into the analysis software (MyAssays Desktop Pro). It is at this point that the data could be intentionally (or unintentionally) falsified. MyAssays Desktop Pro Enhanced Security can detect and block such insecure actions.

In addition, in certain scenarios, it is important to ensure that a data file originates from a particular source. For example, in a laboratory with multiple plate readers, it may be important to ensure that measurement data is collected from a specific reader and not transferred to the system from another unexpected or untracked source.

It is for these reasons that MyAssays Desktop Pro Enhanced Security supports restricting the import of measurement in a number of different ways including:

- Requiring a valid data certificate
- Blocking files that were modified after creation
- Restricting data import from only a specific location

These methods can be used alone or in combination to ensure that the data imported into the system meets regulatory requirements for authenticity and traceability.

## Data Certification

The most reliable method to secure the hand-off of data is to configure the system so that a valid data certificate is required for all measurement data to import.

A MyAssays data certificate file is generated by the instrument control software. The certificate file includes the information necessary for MyAssays Desktop to validate the authenticity and source of the measurement data file.

If the certificate file is missing, or if the data file has been modified since the measurement data file was certified, then MyAssays Desktop Pro Enhanced Security will block the import (and log the attempt).

The certificate file also stores the origin of the file; this makes it possible for MyAssays Desktop to reject data files that are transferred from another system.

## Data Certification with PerkinElmer EnVision

PerkinElmer's EnVision microplate reader supports MyAssays data certification.

To export certified measurement from the EnVision:

1. Ensure that you are using v14.xxx or later of the manager software.
2. Configure or perform the export function.
3. Select Export format option as CSV for MyAssays Desktop option:


The generated data certificate files are stored alongside the exported file. Data certificate files are hidden by default (so they would not appear in Windows Explorer unless it is configured to show hidden files).

## Data Certification with PerkinElmer VICTOR Nivo (GxP)

PerkinElmer's VICTOR Nivo GxP microplate reader produces MD5 files. The generated MD5 files are stored alongside the exported file.

MyAssays Desktop can use the MD5 file as a certificate file to validate that the exported file has not been modified since its creation.

To configure MyAssays Desktop Enhanced Security for VICTOR Nivo certification:

1. Log into the MyAssays Desktop Explorer using an Administrator account
2. Open the Security Configuration Wizard under Tools | Security Configuration
3. Proceed through the steps of the Wizard to reach the Data Certification Method step.
4. Select the MD5 Hash Code (Nivo) Setting

A Security Configuration Wizard

Specify the Data Certification Method:
The method by which measurement imports are validated for authenticity.
MyAssays Desktop

- MD5 Hash Code (Nivo)


## Data Certification with Other Instrumentation

If you are working with other instrumentation, please contact support@myassays.com for advice how to secure the hand-off of data.

MyAssays Ltd. offers solutions for retrofitting secure data certification to legacy systems (without requiring any modification to the third-party software).

## Data Protection

One of the advantages of MyAssays Desktop Pro is that it does not require a database to administer. All assay data is stored in standard files, making it easy to manage and transfer data files between users and systems. The Enhanced Security features enable you to configure and restrict access to MyAssays Desktop data files and folders, and to restrict the operations that can be performed on those files.

For a complete list of available authorization settings, see Authorizations.
MyAssays Desktop Pro's Enhanced Security provides various features and options for securely protecting files. The configuration you choose will depend on how your organization prefers to arrange data. For example, you can:

- Partition files by users (Windows Log In authentication only)
- Share files between users


## What Are the Assay Data Files?

The term assay data files refers to all the types of files used by MyAssays Desktop to configure, manage, and store details about the operations that it performs. This includes files for:

- Protocol
- Results
- Layout
- Export method
- Library curves
- Quality control settings
- User preferences
- Export reports (only those exported to the default Reports folder)


## What about Measurement Data Files?

Measurement data files are generated by instrument manager software and therefore cannot be managed or controlled directly by MyAssays Desktop Pro Enhanced Security. It is the function and responsibility of the instrument manager software to ensure the integrity and security of the measurement data files it generates. To ensure that you have a fully secured system, you will need to ensure that the instrument manager software is sufficiently secured to meet your regulatory requirements.

Where MyAssays Desktop Pro Enhanced Security can help is in the secure hand-off of measurement data from the instrument manager through to data analysis in MyAssays Desktop Pro. In this way, a secure workflow from instrument to exported results can be achieved.

If you can configure the location of where your instrument manager software outputs measurement data files, then it is suggested that you use the
Measurements subfolder as the default location of the Assay Data Folder. In this case, the administrator will need to configure appropriate permissions for the instrument manager software to be able to write to this folder.

## Where Are the Assay Data Files Stored?

With MyAssays Desktop, data file management is simplified using the Assay Data Folder
Root. All assay data is stored under this parent folder (data is arranged under subfolders). Using a single parent folder for all assay data simplifies management of users' assets. A typical scenario (and the default scenario when Enhanced Security is not used) is for the Assay Data Folder Root to be the MyAssays folder under each user's My Documents folder. Each user has their own protected folder for storing their own data files. Each user's data is kept separate from one another. In this case, the operating system provides secure functions for restricting access and permissions to resources.
A common requirement is to share all assay data between users of the system. In this case, the Assay Data Folder Root is the same path for all users. This could be a local file path or a network location. This is easy to configure by changing the Assay Data Folder Root. However, securing data shared in this way can be a challenge, and it is for this reason that MyAssays Desktop Pro Enhanced Security provides functions to simplify and manage this process.
Traditionally, managing shared access to common assets is difficult in Windows with various authorization settings being spread across different management tools. MyAssays Desktop Pro Enhanced Security simplifies this file management using easy-to-use authorization groups.

For example, if a user should not be permitted to delete or edit files, he can be assigned to the restricted Run and Review authorization group. This group does not include the permissions necessary to delete data files.

## How Are Files Protected?

Protected data is stored in secure folders protected at the operating-system level. This means that only programs and users with permission to access or modify data can access and make
changes to the protected data. Any attempts to manipulate data using tools outside MyAssays Desktop Pro will be denied by the operating system.

There are two types of data protected by MyAssays Desktop Pro's Enhanced Security mode:

## Security Data

Only the security system itself has access to the Security Data folder (where all audit trail, login, and user details, and all authorization settings, are stored). In all cases, the security data is protected and cannot be accessed or modified by users without administrator privileges.

## Assay Data

Where assay data is shared between more than one user, MyAssays Desktop can provide Secure File Protection. With this feature enabled, MyAssays Desktop Enhanced Security takes control over securing the assay data files between different users.

Only MyAssays Desktop Pro Enhanced Security (and an administrator account) has the ability to make changes to data stored in MyAssays Desktop Pro data folders; in this way, the assay data files are securely protected. Only operations approved by the system (in the form of an authenticated user with the required authorizations granted) can be performed. Also, secured operations on the data are logged in the audit trail.

## Electronic Signatures

MyAssays Desktop Enhanced Security's Electronic Signatures permits authorized users to approve and sign protocols and results files electronically. Electronic signatures are:

- Protected from removal or tampering
- Stored as part of the protocol or results files
- Recorded in the audit-trail

The Electronic Signatures feature of MyAssays Desktop Enhanced Security feature has been designed and implemented to satisfy 21 CFR Part 11 rules.

To sign a file two distinct identification components must be entered (username and password). Each stored signature contains the signer's unique User ID.

Each user can be assigned specific signing rights and actions. For each signing a reason for signing is provided, including:

- Approve and Lock
- Disapprove
- Lock
- Unlock
- Review
- Take Authorship
- Take Responsibility
- (Plus, custom actions...)

For example, users in the analyst role would typically be granted rights to create protocols as well as sign protocols as "approved and locked". Users in the operator role would not be able to create, edit or approve protocols and only be able to use protocols that have been approved.

In this way, electronic signature supports traceability and accountability as well as controlling access to approved functions.

To support typical workflows, MyAssays Desktop Enhanced Security provides a default configuration with authorizations and signing rights. Simply assign users to the appropriate authorization group.

## Configuring Electronic Signatures

Enable Electronic Signatures from the Security Configuration Wizard:

| Q Security Configuration Wizard |  |
| :---: | :---: |
| $\checkmark$ Enable Electronic Signatures |  |
| $V$ Revoke sign actions for disabled accounts |  |
| If a user's account is disabled then all of the sign actions perform user will be revoked. <br> © For example, if User A approves a protocol for use then that pro be used for as long as User A's account is active. If User A's acco then the approved protocol will need to be approved by anothe can be used for further analysis. | that <br> can only disabled before it |
| Back | Next |

There is one sub-option: Revoke signatures for disable accounts. Set this option to revoke any signatures that belong to a disable account. With this setting, in any case where a user has electronically signed a file but that user account is disabled, any actions granted by the user's signing will be revoked. More...

## Electronic Signatures Authorizations

The following electronic signature authorizations can be configured and assigned to each user:

| Authorization | Operation |
| :--- | :--- |
| Protocols |  |
| Requires | Assay protocol files require approval for launch (i.e. a protocol must have |
| Approval for | been electronically signed with reason: approve). |
| Launch |  |


| Authorization | Operation |
| :--- | :--- |
| Results Requires <br> Authorship for <br> Exporting | Assay results files require authorship for exporting (i.e. a results file must <br> have been signed with reason: authorship before it can be exported). |
| Results Requires <br> Authorship for <br> Printing | Assay results files require authorship for printing (i.e. a results file must <br> have been signed with reason: authorship before it can be printed). |
| Results Requires <br> Authorship for <br> Saving | Assay results files require authorship for saving (i.e. a results file must <br> have been signed with reason: authorship before it can be saved). |

For example, if a user is assigned to a user authorization group with the Protocols Require Approval for Launch setting then the user can only launch protocols which have been approved.

The Security Management tool can be used to define authorization groups including authorizations associated with Electronic Signatures:


Add Group...

Review and export operations only (cannot edit or create new files)
Authorizations Electronic Signatures - Signing Rights tait protocol/ results K
Add. edit remove Quality Control configuration of a protocol/results file
Create On-The-Fly QC Report
Generate instant QC report that is not applied to a protocol file
Edit QC Settings File
Edit configuration of a Quality Control File

## Electronic Signatures

Protocols Requires Approval for Launch
Assay protocol files require approval for launch
$\checkmark$ Results Requires Authorship for Exporting
Assay results files require authorship for exporting
/ Results Requires Authorship for Printing
Assay results files require authorship for printing
Results Requires Authorship for Saving
Assay results files require authorship for saving

## Signing Rights

Each authorization group can be assigned electronic signing rights. Each user will be able to sign in accordance with the authorization group assigned to.

Rights can be specified for Protocol and Results file separately:


## Signing

When a user electronically signs a document, the user name and password must be entered. Also, a reason for signing should be specified. The available reasons will depend on the authorization group the user is assigned to.

| \|r. Sign Assay Protocol | - |
| :--- | :--- |
| User Name: |  |
| Password: |  |
| Select the reason for signing: | Approve and Lock |
|  |  |
| $\boxed{V}$ Save Assay Protocol |  |

## Sign Actions

Each electronic signature is associated with a sign action as given by the signer as the reason for signing. The electronic signing of a document will perform the action on the document as described here:

| Reason <br> Approve and <br> Lock | Action |
| :--- | :--- |
| Disapprove | Unlocks protocol for editing, prevents users from opening with "Protocol <br> Requires Approval for Launch" authorization setting. |
| Lock | Locks protocol against further modification. |
| Unlock | Unlocks protocol allowing modification by users with appropriate <br> authorizations. |
| Review | Indicates review of protocol/results. |
| Take Authorship | Marks the user as an author of the file (more than one user can be <br> marked as the author). |
| Take <br> Responsibility | Marks the user as responsible for the file (more than one user can be <br> marked as responsible). |

## Electronic Signatures in Report

To include details of the signings in the report add the Electronic Signature report element to the report:

4 Report
-TransformContentStandardCurveFit1
-SampleTable1
-TransformContentDilutionFactors1
... ElectronicSignature1
This will show details of all signings associated with the report. For example:

Protocol Approved and Locked by taylo- 14:05 20 January 2020

## Revoking Electronic Signatures

When working with electronic signatures that are used to control access to the system, consideration must be given to the validity of signatures if a user's access to the system is removed.

For example, if an analyst's electronic signature is used to approve protocols for use by operators, if that analyst's access to the system is removed - then should those approvals still be valid and should those protocols still be usable?

MyAssays Desktop Enhanced Security provides an option to define what happens if a user's account is disabled. If an account is disabled, any signings by that account can be revoked automatically. This can be useful to prompt a review of the former user's signings.

Use the Security Configuration Wizard to specify if signatures should be revoked for disabled users.


If the Revoke sign actions for disabled accounts is selected then signatures will be revoked automatically for any disabled user account.

Revoked signatures appear with a strike-through. For example, here the Operator's account has been disabled causing this signature to be revoked:

| Electronic Signatures (Protocol) |  |
| :--- | :--- | :--- |
| Date/Time User Name Reason Description <br> 10 February-2020-19:50:49 Operater Appreve-and Leck Operater-signed the-assay-pretecel file; reasen: f <br> 10 February 2020 $19: 55: 48$ Darren Cook Approve and Lock | Darren Cook signed the assay-protocol file; reaso |

If a valid signature is required to permit operations (such as an approval signature is required for a protocol to be launched) then any impacted files will need to be signed by a user with the required signing rights.

## Audit Trail and Login Log

The Audit Trail keeps track of user activity; user operations are logged by user with a date and time stamp. In addition, the Login Log keeps track of log in attempts.

The log data is stored in a protected folder, so it cannot be accessed or modified by standard users.

Log data can be exported to CSV or XML.

## Autologin Account

When working with MyAssays Desktop authentication, the MyAssays Desktop Autologin account is available and provided for:

- Performing automated data analysis in a secured environment, such as under regulatory requirements
- Working with third-party systems, such as reader software

Typically, when you work under regulatory requirements, the third-party data collection or reader software provides their own user-management and security protections. Because users usually have to log in to each secured system separately, automating the workflow can become tedious.

To simplify this integration, MyAssays Desktop Enhanced Security enables performing the permitted data analysis under a protected and tracked Autologin account. This avoids the need to log in the software just to run data analysis.

## The Autologin account is intended to be used solely for automatic operations where user intervention is not required.

With this approach, the users can benefit from a seamless secured solution. The workflow is simply a case of initiating measurements from the reader control software. Meaningful analyzed results and reports can be provided automatically be MyAssays Desktop along with necessary tracking information all securely protected.

The Autologin account is not applicable for working with Windows Log In authentication. This configuration does not require a separate log in because all operations are performed using the logged in Windows user.

For information on how to enable Autologin account, see Configuring Autologin Account. To learn how to set up analysis with it, see Launching Analysis with the Autologin Account.

By default, Autologin is only available for use under the specified shared Windows Log In Account. If Autologin is required for use from other standard Windows accounts then the feature can be enabled as described here.

## Configuring Autologin Account

When working with MyAssays Desktop authentication, normally a log in prompt appears to perform the data analysis process. However, with the Autologin account, you do not need to log in to MyAssays Desktop. Instead, you can run permitted operations (such as data import, analysis, and export) automatically under this account.

Typically, the measurement data is collected from a reader with a separate third-party software. If such software provides their own user-management system to satisfy regulatory requirements, the users need to log in. And, rather than also logging in the MyAssays Desktop Enhanced Security system each time, use the Autologin account to fully automate the process in a secure and traceable manner.

## How Is the Data and Workflow Secured?

The MyAssays Desktop Enhanced Security administrator can define which operations can be performed by the Autologin account. Routine day-to-day operations such as running particular protocols are limited to just that with full traceability.

To perform operations such as creating protocols and changing the analysis settings, a log in to MyAssays Desktop account with the necessary permissions is required.

To satisfy regulatory requirements when working with separate systems, always ensure that there is a clear data trail from measurements to results.

We advise including metadata that describes the origins of the imported data in the exported data or reports generated by MyAssays Desktop. This should include details such as date/time of measurements, instrument serial number, imported filenames and the operator details from the reader software. This content can be added to the MyAssays Desktop report using the report elements: Measurement Meta Data or Text (with macros) or both.
The availability of such data depends on the reader software that is in use and the data that is being imported into MyAssays Desktop. MyAssays Desktop can be configured to import measurement and metadata from many sources and file formats. If you are working with a data format not already supported, please contact support@myassays.com for details of how to import the necessary metadata in a secure and traceable manner.

Setting Up the Autologin Account

1. Launch MADE.
2. On the Tools tab, click Enable Security, and then click On.
3. Select MyAssays Desktop Log In, and then specify the Windows Log In account that must be used by your reader control software (e.g., LabUser).
4. Select the authentication context.

Typically this will be the Local Machine.
5. Enter or accept the User Group Name.
6. Enable the Autologin Account.
7. Select the authorization group that the Autologin account should belong to.

This defines what operations can be performed by the Autologin account. Typically, this should be configured with only the operations necessary to perform the required data analysis.

If you select None, then the Autologin account will not be able to perform any operations. The Run and Review Only option is preconfigured to permit operations typical for running and reviewing data analyses.

> If you're not yet sure which operations should be permitted under the Autologin group, then select None for now. You can define your own authorization group to specify exactly what operations should be permitted under this account from the Security Management tool (available after completing the Security Configuration tool). From here you can assign the MyAssays Desktop Autologin account to the appropriate group.
> For a complete list of available authorization settings, see Authorizations.
8. Complete the setup as required.

Because Autologin account is intended for automated workflow only, users cannot log in with it through the Log In dialog. The only way to use this account is to run
MAA with /autologin command-line argument. See Launching Analysis with the Autologin Account.

## Data Analysis Integration

When you have configured Autologin account and assigned it to an authorization group that satisfies your security requirements, then:

- Integrate a suitable existing protocol (e.g., download from MyAssays.com, use a installed protocol).
- Create assay protocols that perform the required data analysis.
- Copy or move the protocols from another source (e.g., obtained from colleagues) to the Protocols folder.

When you create new protocols or transfer them from another source, log in to MyAssays Desktop Enhanced Security with an account that has the necessary permissions. Then test that the protocols import measurements, run the data analysis, and export the results or report as required. Do not use the Autologin account to test these because the authorization group it belongs to should not have such permissions.

Once the data analysis operations performed by your MyAssays Desktop assay protocols are meeting your requirements, you can integrate the data analysis as part of an automated workflow with:

- Command Line - If your reader software supports launching an application after completing measurements and provides access to the new filename, then configure the reader software to launch MyAssays.Desktop.Analysis.exe using the command line. The Analysis Launcher can help you to build the command-line arguments.
- Folder Poll - If your reader software does not support launching an application when measurements are completed, then use the included Folder Poll tool to set this up. See https://www.myassays.com/folder-poll-with-myassays-desktop.video.


## Autologin with the Shared Windows Log In Account

With MyAssays Desktop Log In authentication, the system will be routinely accessed through a shared single Windows log in. This is a standard Windows log in account that is shared between users of the system. This shared Windows Log In account is used to provide quick and easy access to the system to all users in the lab. This Windows Log In Account is specified in the Security Configuration Wizard, for example, here it is set to LabUser:
A Security Configuration Wizard
Select the authentication method:

Windows Log In
The Windows Log In is used to authenticate and identify the user. (Each user does not need to log in to MyAssays Desktop.)

- MyAssays Desktop Log In

Each user must log in to MyAssays Desktop.
(This is useful if the PC is used on a routine basis with a single Windows $\log$ in. A standard Windows log in is used to access the computer and each user logs in to the MyAssays Desktop application.)

Windows Log In account: LabUser
(1) The Windows Log In Account must be a standard account (i.e. not an administrator account).

## Back

With this configuration, each user will log in to Windows using the LabUser account.
With MyAssays Desktop Log In authentication, each time a user works with MyAssays Desktop, the user will log in to the MyAssays Desktop software by providing their own private credentials.

With the Autologin feature enabled, permitted operations can be performed automatically without requiring authentication. This is useful to automate operations (such as performing data analysis as soon as measurement are completed).

By default, the system is configured to only allow these autologin operations if the current Windows session is either:

- The specified Windows Log In Account (e.g. LabUser)
- A local machine administrator

Or, the process is launched using local machine administrator privileges (e.g. elevated command line).

In cases where autologin function is used from standard Windows sessions, including:

- Local machine accounts
- Domain accounts
access to the autologin feature is blocked by default. This is to ensure that autologin operations are only available under the specified Shared Windows Log In Account.


## Enable Autologin from Any Windows Account

Where autologin functionality is required under standard Windows accounts (in addition to the specified Windows Log In Account), an HKLM registry setting is available to permit autologin operations from standard user accounts. This can be enabled by creating a DWORD key:

AutologinAnyWindowsAccount at:
HKEY_LOCAL_MACHINE\Software\MyAssays\MyAssays Desktop\Security
Set this to $\mathbf{1}$ to enable autologin with any Windows account. Set this to $\mathbf{0}$ or remove the key to disable autologin for users other than those specified above.

## Launching Analysis Using Autologin Account

When you use the command line to launch protocol with MyAssays.Desktop.Analysis.exe, include the /autologin command-line parameter. With this, the analysis will be performed under the Autologin account (without requiring to log in).

When a command line is run with the /autologin parameter, any open MyAssays
Desktop windows (running sessions with another user account) will be automatically closed.

To ensure that the MAA window is closed after calculations are completed and to prevent any user from being able to interact with the analysis application, use the /exit parameter.

If the /exit is not used, then the analysis window will remain open and allow the user to interact with the software anonymously. Please be aware that the user will be able to perform any operation that the Autologin account is authorized to do, although such operations will be logged against the Autologin account. For this reason, it is important that the Autologin account is restricted to only allow operations that are permitted for anonymous use (or use /exit if the user should not be able to interact anonymously).

To help build and configure command line, use the Analysis Launcher.
If you are working with Folder Poll, you can add this setting via Folder Poll Manager:

1. Open Folder Poll Manager to review the changes.
2. Edit the Poll, select Operations and add /autologin to the Parameters.

For example:


Close MyAssays Analysis after Analysis check box here corresponds to the /exit parameter.

If you select Automatically launch this protocol when a new measurements file is detected from the Import Wizard, then you can configure the automated workflow for the protocol on the last step of the wizard.

## Administrator Tasks

This section covers the system administrator tasks necessary to set up and maintain the system:

- Install MyAssays Desktop Pro
- Enable and Configure Enhanced Security
- Manage/Add/Authorize Users
- Monitor Usage


## Installation

Installation tasks must be performed by a Windows user with local machine administrator privileges.
Please refer to release notes for latest installation instructions.

## Enable and Set Up Enhanced Security

After you have installed MyAssays Desktop and activated your license, the Enhanced Security system will not initially be enabled. To enable and initially configure Enhanced Security system with MyAssays Desktop Pro:

1. Launch MyAssays Desktop Explorer.
2. On the Tools tab, select Enable Security.

This will launch the Security Configuration Wizard.
Security Configuration Wizard makes changes to the system and thus requires local administrator privileges. If the standard Windows User Access Control prompt appears, click Yes to start the wizard.
3. Select On to enable the Enhanced Security features.
4. Depending on how the users will log in to the application, select one of the following and follow the substeps:

Each user works under their own Windows account (Windows Log In)
a. Select Windows Log In and click Next.
b. Select to authenticate Local Machine Users, Domain Users, or both.

When selecting Domain Users, enter or paste the AD Domain Name of your domain.

When specifying the AD Domain Name in the Security Configuration Wizard, use the Active Directory (NetBIOS) domain name, e.g., CORP.

Windows refers to two types of domain names: Active Directory (AD) and DNS. Windows will use the AD name when listing users, however the DNS domain is used to specify to the name of the network domain. For example:

AD Domain name: CORP
DNS name: corp.test
c. Specify the path for saving your data files with one of the following:

## - User's Own Folder

Each user's assay data is stored under the logged in Windows users account's
default Documents location. This is useful if you want to partition each user's data by their own account. In this case, Windows manages the partitioning of data (e.g., one standard user cannot access another user's data).

## - Shared Folder

All users of the system share the same folder. Select the Secure File
Protection check box to secure the specified folder. With this, when running under a standard Windows account, authorized users can perform operations on files in that folder.
For more information about this feature, see Secure File Protection.

```
All users of the system share a single Windows account (MyAssays Desktop Log In)
```


## a. Select MyAssays Desktop Log In.

With this, each user will use their login and passwords to log in to MyAssays Desktop.
b. Specify a standard Windows Log In account and click Next.

This is a non-administrator account from which the system will be used routinely, with the account's access being shared between all users of the system. You can use default account name of LabUser or enter a different account name as required. In any case, please ensure that the account name is a valid local machine account.
c. Select authentication context.

When selecting Domain Users, enter or paste the AD Domain Name of your domain.

When specifying the AD Domain Name in the Security Configuration Wizard, use the Active Directory (NetBIOS) domain name, e.g., CORP.

Windows refers to two types of domain names: Active Directory (AD) and DNS. Windows will use the AD name when listing users, however the DNS domain is used to specify to the name of the network domain. For example:

AD Domain name: CORP
DNS name: corp.test
d. Enter or accept the User Group Name.

Only users that belong to the specified User Group (e.g.,
MyAssaysDesktopUsers) will be able to authenticate to access MyAssays Desktop Pro. In most cases, you can accept the default settings here to use the specified User Group Name.
e. Select the Add the Current user to the user group (and set as an System Administrator) check box and click Next.

For Domain Users, the Current User is always added as a system administrator.
f. To allow automating routine operations, select Enable Autologin Account and then select an Autologin group. For more details, see Configuring Autologin Account.

For more information, see Authentication Options.
5. If you want to limit importing files only from the selected folder, keep Define Approved Import Folder check box selected, specify path to this folder and then click Next.

For more information, see Approved Folder for Import.
6. To restrict editing of the reports, keep Use Word/Excel Report Protection check box selected and enter or paste password for allowing to modify the output files.

With Word outputs, you can select to lock the whole document or only the results from editing.

For more information about this feature, see Protecting Word and Excel Reports with Password.
7. Review the summary of your configuration, close any open MyAssays Desktop windows and then click Apply if you are content with the settings.
8. Click Finish to close the wizard. Changes will be applied the next time MyAssays Desktop is launched.

When working with a shared assay data folder, Iaunch MyAssays Desktop Pro at least once with a successful authentication to install the preconfigured protocol and example files under the protected folder.

If you want to make changes to the initial configuration, run the Security Configuration Wizard again.

## What's Next?

After you set up the general configuration of the Enhanced Security system, follow these steps:

- Add users of your Enhanced Security system

For more information about adding, creating, disabling, and locking accounts, see General User Administration.

- Review and configure Groups
- Set up the authorizations for each group
- Configure each user


## Secure File Protection

When all users of the system share the same Windows account or the same assay data folder, it is important to ensure the security and integrity of sensitive data. With Secure File Protection, the files stored in the secured Shared Folder are protected against unauthorized altering, deleting, copying, and moving.

## How Secure File Protection Works

MyAssays Desktop Enhanced Security protects the assay data files stored under the Shared Folder. Only the application itself and a system administrator account have sufficient permission to make changes to the data stored in MyAssays Desktop Pro data folders. This prevents any unauthorized operations on data.

Secure File Protection requires that the system account has permission to take ownership of the specified resource. This is normally the case for the resources of a local machine (such as a local hard drive). If this is not the case (such as with a restricted network resource), then data can still be shared between users, however the protection of such files should be managed and configured by the resource's administrator.

Users can only perform operations approved by the system (in the form of an authenticated user with the required authorizations). Also, secured operations - Copy and Paste, and Delete - are logged in the audit trail.

For more information about how the operations are secured, see File Operations.

## When Secure File Protection Is Applied

When you enable Enhanced Security on your system with:

- MyAssays Desktop Log In authentication, the same Windows account (e.g., LabUser) is typically logged in all day with different users in the lab using it. However, MyAssays Desktop authentication controls the access to MyAssays Desktop Pro and to a folder where all assay data is stored. With this, different users have different levels of access to the data as authorized for the group they belong to. In this case, data is already stored under a protected shared folder.
- Windows Log In Authentication, you can turn on Secure File Protection by selecting the check box if you select Shared Folder as the Assay Data Folder.

To change the initial settings, run Security Configuration wizard again.
A shared data root folder that is protected during Security Configuration remains in the same state even if an administrator selects another folder to protect. The
administrator must acquire ownership and reconfigure the previously protected folder security permissions manually.

## Where the Shared Folder Is

The default location of the shared assay data folder is
C:\ProgramData\MyAssays\MyAssays Desktop ES\Assay Data. For Windows Log In authentication, it can be changed to another location within the computer or on the network.

## Active Directory (AD) Domain Name

When setting up Enhanced Security for domain accounts, you have to provide the AD Domain Name of the domain you are using.

## What Is AD Domain Name?

The AD Domain Name is the short name of your Active Directory domain. It is also referred to as the NetBIOS name. The Active Directory Domain Name is not the full DNS domain name but typically the leftmost part of the DNS name. For example:

AD Domain name: CORP
DNS name: corp.example.org

## How to Determine AD Domain Name?

1. Log in to Windows with a domain account.
2. Launch Command Prompt.
a. Display Run menu by pressing Windows logo key + R.
b. Type cmd and click OK or press Enter key.
3. At the command prompt, type ECHO \%USERDOMAIN\%.
4. Press Enter key.

This displays the AD Domain name (NetBIOS name) of your domain. For example:


Provide this name in the AD Domain Name field when setting up Enhanced Security or reconfiguring the system to include domain accounts.

## Approved Folder for Import

To provide additional control surrounding data import, you can restrict the import of measurement data files to a specified approved folder and its subfolders. This is typically an instrument output folder. For example, the instrument control software might output measurement data to a specific folder. For additional security, the system could be configured so that only the instrument reader software can write to this folder.

## How to Restrict Import to an Approved Folder

First, when enabling or reconfiguring Enhanced Security on your system:

1. In the Security Configuration Wizard, select Define Approved Import Folder check box.
2. Specify the existing folder where data files can be imported from.
3. Complete the wizard.

Second, assign the users of your system to an authorization group where the Data Import | Import from Approved Folder authorization is enabled and where the Data Import | Import from Any Folder is disabled.

For more details about authorization settings, see Authorizations.
If an authorization group has Import from Any Folder permission, then users can import data from any location irrespective of this setting.

Therefore, to restrict which folders users can import from only a specific folder, grant only the Import from Approved Folder permission. Using this setting, files can only be imported from the specified folder (the Approved Import Folder) and its subfolders. If a user tries to import from other resources, the operation will be blocked by the system and corresponding entry will be added in the audit trail.

## Protecting Word and Excel Reports

To ensure the integrity of data contained in exported reports, Word (.doc/.docx) and/or Excel (.xls/.xlsx) outputs can be protected, preventing any further modification after they have been generated. In this way, users will not be able to accidentally or deliberately change or delete data contained in MyAssays Desktop exported reports.

## How the Reports Are Protected?

The administrator can specify to protect the exported outputs with a password. Only users with knowledge of this password will be able to edit the protected documents.

For Excel outputs, the password protects the entire workbook against any further changes.
For Word outputs, there are two additional options:

- Whole Document - Prevent users from making any changes to the document.
- Only Results - Restrict modifying the sections of the document containing results generated by MyAssays Desktop, but, make other parts of the file editable. In this way, you will maintain accuracy and consistency of MyAssays Desktop data analysis results but allow inclusion of additional content such as digital signatures or other additions a user might want to make.


## How to Set Up Password for Outputs?

This is a two-level process:
First, when enabling Enhanced Security on your system, select and configure password protection:

1. In the Security Configuration Wizard, select Use Word/Excel Report Protection check box.
2. Enter or paste the password to apply for document protection.

You can change this password by rerunning the wizard. This will apply to exports created after the change is made.
3. Select how to protect Word outputs from the drop-down list.
4. Complete the wizard.

Second, assign the users of your system to authorization group with the required Data Export operations selected. This is Protect XLS/XLSX for Excel and Protect DOC/DOCX for Word outputs. Users belonging to an authorization group with these authorizations set will only be able to export protected documents.

## Security Configuration

To configure Enhanced Security system with MyAssays Desktop Pro, use Security
Configuration Wizard. When you enable Enhanced Security, it guides you through setting up the initial configuration of the system.

To make changes to an existing configuration, run this wizard again and follow the steps as described below:

1. Launch MyAssays Desktop Explorer.
2. On the Tools tab, select Security Configuration.

When you rerun the Security Configuration Wizard, it features all the configuration settings you initially set up when enabling Enhanced Security on your system. These are covered in the Enable and Set Up Enhanced Security.

However, it also contains an additional step that allows you to keep the existing user
3. management and security data configuration or remove it.
4. Select Use existing security data check box to keep your administration settings, or clear it to delete the configuration from your system. Click Next to apply the changes and finish the wizard.

## What is Security Data?

Security data includes all your configured user administration and security settings:

- Login $\log$ and audit trail
- User details
- Authorization settings
- Security options (for MyAssays Desktop Log In method)


## User Administration

With Enhanced Security enabled and configured, initially the only user account that will be added to the Enhanced Security system is the user account that was used to perform the setup (i.e., the administrator account). This account is automatically added to the
Unrestricted authorization group and assigned to the Enhanced Security account type System Administrator.

To grant other users permission to use MyAssays Desktop Pro, each user must be added to the Enhanced Security system and assigned to an appropriate authorization group and account type.

## Guest Users

When working with Windows Log In authentication, users that are not added to the Enhanced Security system can launch MyAssays Desktop Pro but will be treated as a guest user. Guest users are advised to contact the system administrator for access to the software. Guest users cannot perform any operation.

## User Accounts

The Enhanced Security system uses the Windows user management system for administering user accounts. This means that you do not need to create a whole new set of user accounts; you can use existing accounts that may already be defined on your system (both local or domain accounts).

## Autologin Account

With MyAssays Desktop Log In authentication, if this account is enabled, it is added automatically alongside administrator account. Autologin account is useful if working with third-party software (such as instrument control software or LIMS) where operations should be performed independently of any specific user account with an automatic log in procedure. For more information, see Autologin Account.

## General User Administration

General user management (such as adding, creating, disabling, and locking accounts) can be administered using the standard Windows user management tools.

## Configuring Local Machine Accounts

In Windows 10, if using local machine context:

1. Right-click Start and then click Computer Management.
2. Under System Tools, select Local Users and Groups and then select Users.

Password policy rules (password history, minimum length, etc.) can be set in Windows 10 from Start | Run | secpol.msc| Security Settings | Account Policies | Password Policy and Account Lockout Policy. These should be set in accordance with your 21 CFR Part 11 compliance requirements. For more information on configuring Windows password policy, please see https://docs.microsoft.com/en-us/windows/device-security/security-policy-settings/password-policy.

## Configuring Domain Accounts

If your network is connected to a domain, then your password policy rules and user management will be administered by the domain controller. Contact your domain administrator for more information.

## Domain Users

MyAssays Desktop Enhanced Security can work with your existing domain accounts.
For both Windows Log In Authentication and MyAssays Desktop Log In
Authentication, it is not necessary for a domain administrator to configure the system. The system configuration requires only local administrator privileges. This local administrator can set up the system and assign users (including domain users) access to the software at various authorization levels.

With MyAssays Desktop Log In Authentication, the process of adding a user
to the MyAssays Desktop user group might require authentication of the domain account. In this case, the user account to add can be used.

## Adding Users

To grant a user account permission to use MyAssays Desktop Pro, the account must be added to the Enhanced Security system. The steps to add a user depend on the authentication method in use.

How to Add Users (Windows Log In)
With Windows Log In authentication, the administrator must add each user that requires access using the Security Management tool. Users that attempt to access MyAssays
Desktop Pro but have not been added to the Enhanced Security system will be automatically assigned to the Guest account type.

The administrator can add users to the Enhanced Security system as follows:

1. Launch Security Management and click Add.
2. Under the Username, enter the Windows user name (as the user would enter it when logging in).

If the user is a domain user, then enter the AD domain name followed by backslash ( $\mathbf{V}$ ) and the username (e.g., for a domain "CORP" and username "LabUser" the Username should be: CORP\LabUser).
3. Provide a Full Name for the user.

If a domain user name is specified, Windows may prompt for the name and password of an account with permission to access the domain. The credentials of the user account to add can be used (or a domain administrator account can be used if it is available).

For information about logging in with domain account, see Log In Options and Password Management.

Please now proceed to the Configuring a User section.
How to Add Users (MyAssays Desktop Log In)
With MyAssays Desktop Log In authentication, the administrator must first add each user that requires access to MyAssays Desktop to the defined MyAssays Desktop user group. This is the group that was specified in the Security Configuration Wizard and defaults to

## MyAssaysDesktopUsers:

Users can be added to this local machine group using the standard Windows user management tools:

1. In Windows 10, right-click Start | Computer Management and expand System Tools | Local Users and Groups | Groups.
2. Locate the user group MyAssaysDesktopUsers (or any custom name provided in the configuration) and double-click.
3. Click Add and enter the name (or names, each separated by a semicolon) of the user account to add. Click Check Names to verify the entry is correct, and click OK to confirm.

To add domain users, enter the AD domain name followed by backslash ( $($ ) and the username (e.g., for a domain "CORP" and username "LabUser" the Username should be: CORP\LabUser).

For information about logging in with domain account, see Log In Options and Password Management.

Users added to this user group will now be able to authenticate themselves when launching MyAssays Desktop Pro; however, each user must also be assigned to an appropriate authorization group and account type as described in the following section.

## Configuring a User

After adding users to the Enhanced Security system (using the method appropriate for the authentication method), each user should be configured as required. This involves setting an appropriate:

- Account type
- Authorization group

Each user's settings are specified through the User Details panel, which can be accessed as follows:

1. In MADE, on the Tools ribbon tab, in the Security group, launch the Security Management tool.
2. Double-click a user, or select and click Modify to modify the user's details.
3. Review or edit the settings as required.
4. Click OK to accept changes.

## Account Types

The account type or Level can be one of the following settings:

- User - A standard user of the system. These users can perform operations permitted for their authorization group and cannot access the Security Management or Security Configuration tools.
- User Administrator - A Windows administrator account that has limited access the Security Management. This account can assign users to preexisting authorization groups and assign user levels to either User or User Administrator.
- System Administrator - The highest level administrator. A system administrator has all functions of the user administrator and can also:
- Assign user levels to System Administrator.
- Create and edit authorization groups (using Manage Groups).
- With MyAssays Desktop Log In authentication, can change Security Options for the users.
- Run Security Configuration Wizard to modify initial Enhanced Security configuration.


## User Group

The user group (or Group) is an authorization group that defines which functions of the software the user can perform. MyAssays Desktop Enhanced Security provides various predefined authorization groups including:

- None
- Unrestricted
- Review/Export Only


## - Run and Review

For example, users belonging to the Run and Review groups are restricted to running protocols and reviewing results. Conversely, an Unrestricted user can perform all operations in MyAssays Desktop Pro.

Authorization groups and permissions for each group can be configured by the system administrator as described in the following section.

## Authorization Groups

System administrators can manage authorization groups.
Click Manage Groups in the Security Management tool to display the User Group Management dialog:


The list on the left shows the existing list of authorization groups. The controls allow a system administrator to edit operations and create new authorization groups. The Add Duplicate button can be used to create a new authorization group, which is a duplicate of the currently selected group. This is useful for quickly creating similar groups.

## Adding Custom Authorization Group

To grant specific combination of authorizations, add a custom group and select the relevant permissions as follows:

1. Launch MADE.
2. On the Tools tab, click Security Management.
3. Click Manage Groups.
4. Click Add Group.
5. Specify a Name and, optionally, a Description for your group.
6. Select Authorizations for this group.

## 7. Click OK.

8. Assign users to this group.

## Authorizations

With the Enhanced Security system enabled, users can only perform authorized operations. The tables below list all available authorizations (arranged by category), their operations, and any special preconditions.

When a user attempts to perform any of these operations, an entry is recorded in the audit trail marking whether the operation was authorized or denied (alongside the username and date/time).

## General

General authorizations relate to using MyAssays Desktop protocol and results files and working with sample layouts.

| Authorization | Operation |
| :---: | :---: |
| New Protocol | Create new protocols in the New Protocol Wizard. |
| Launch Protocol | Launch and view existing .assay-protocol files in MAA. |
| Open Results | Open and view existing .assay-results files in MAA. |
| Open Files from Other Locations | Launch and view protocols, results, and layout files stored outside the Assay Data Folder Root. If this is not selected, user will be able to launch protocols, results, and layouts only from Assay Data Folder Root. |
| Download Protocol | Download preconfigured assay-protocol files from Search MyAssays.com. |
| Create Layout | Create a new assay layout in the New Protocol Wizard, and when running protocol or results in MAA. |
| Edit Layout | Change the existing .assay-layout files when running New Protocol Wizard, double-clicking on a file in MADE, or when running protocol or results in MAA. |
| Select Sample <br> Types | Select existing sample types to use for layouts of the current assay. This refers to both the New Protocol Wizard and when running protocol or results in MAA. |
| Manage Sample Types | Create or edit sample types used in the assay when running protocol or results in MAA, and also remove sample types when creating a new protocol with New Protocol Wizard. |

## Parent Directory for Data Files

This authorization is applicable only for Windows Log In authentication method with User's Own Folder data arrangement setting. For more information about assay data, see Data Protection.

| Authorization | Operation |
| :--- | :--- |
| Edit Assay Data | Choose a custom root folder to store all assay data files in. By default, all <br> file types used by MyAssays Desktop are stored in the default location. <br> Folder Root |
| ith this setting, you can allow or restrict changing the default root <br> folder. |  |

## File Operations

For Windows Log In with Shared Folder and Secure File Protection feature enabled, files in the protected folder (Assay Data Folder Root) cannot be duplicated or removed. Authorizations in this category can be granted to allow users making such changes (either in MADE or in Windows Explorer). For more details, see File Operations.

## Authorization Operation

| Delete Data File | Delete protocol, results, or layout files through Enhanced Security. |
| :--- | :--- | :--- |

Copy + Paste Data File

## Analysis

Analysis authorizations relate to operations available when running protocols or viewing results in MAA.

If a user has not been granted permission to perform an operation that is otherwise available through a button, then the button will not be displayed.

| Authorization <br> Edit <br> Measurements | Operation |
| :--- | :--- |
| Edit Container <br> IDs | Change Plate IDs, Analytes or Batch IDs in the Measurements tab of <br> MAA. |
| Edit FieId Data | Change the field data in the Measurements tab of MAA. |
| Edit Overview | Change protocol name and overview via the Overview Editor. |$|$| Configure external references in the current or for all protocols in the |
| :--- | :--- |
| Resource Reference Manager. |.


| Authorization | Operation |
| :---: | :---: |
| Manage Templates | Create new, make changes to or remove the existing report templates for page-based outputs (DOC, DOCX, and PDF). |
| Edit <br> Header/Footer | Insert a custom header or footer for page-based outputs (DOC, DOCX, and PDF). |
| Edit Notes | Add, edit, or remove Notes associated with assay. |
| Save Results | Save changes to the current .assay-results file. |
| Save Protocol | Save changes to the current .assay-protocol file. |
| Results Save As | Save opened .assay-results file to a different location or with different name. |
| Protocol Save As | Save opened assay protocol file to a different location or with different name. This also allows the user to save results as a protocol. |
| Edit Results Naming | Change results file naming conventions and storage location for an assay protocol. |
| Add/Remove Transforms | Add or remove transforms in the data analysis configuration. |
| Edit Transforms | Make changes to the settings of a transform. For example, change the concentrations in Standard Curve Fit transform. |
| Edit Matrices | Edit the settings of a matrix in the Matrices tab of the Assay Properties panel. |
| Edit Evaluations | Add, configure, and remove Evaluations Tables and Evaluations in the Evaluations tab of the Assay Properties panel. |
| Edit Validations | Add or remove Validations Tables, configure Validations in the Validations tab of the Assay Properties panel. |
| Edit Report | Add, configure, and remove report content in the Report tab of the Assay Properties panel. |
| Edit XML | Make changes to the assay configuration in the XML tab of the Assay Properties panel. |
| Flag <br> Sample/Point | Flag positions or data points to mark them as outliers to remove them from further analysis. |

## Data Import

Data Import authorizations relate to importing data when:

- Creating new protocols (requires General New Protocol permission)
- Working with protocols or results in the MAA using the Import wizard (requires General Launch Protocol, Open Results permission, or both)
- Automating data analysis with Analysis Launcher or Folder Poll (including via the command line arguments)

| Authorization | Operation |
| :---: | :---: |
| Import <br> Measurement Data | Import measurements from a single or multiple files. This permission defines whether these files can be imported. Other Import authorizations can be used to restrict which files can be imported and from what locations. |
| Import Requires Certification | Restrict import of measurements only to the files that are validated against their signed certificates. |
| Import Requires Certification from This Computer | Restrict import of measurements only to files that are created on this computer and validated against their signed certificates. |
|  | To enable this restriction, select Import Requires Certification. |
| Import Requires no Modification | Import only from measurement data files that have not been modified. |
| Import Non- <br> Measurement <br> Data | Import data such as sample IDs, container IDs, or any assay-specific nonmeasurement data from a file. |
| Import from Any Folder | Import data from files stored in any location. |
|  | $\stackrel{3}{3}$ Overrides the Import from Approved Folder. |
| Import from Approved Folder | Restricts importing files from an approved folder (and its subfolders) only. |
|  | To use this authorization, first define the approved folder for import in enabling Enhanced Security on your system or to use Security Configuration wizard to make changes to the current configuration. If the Approved Folder is not specified, the import will be denied. |
|  | Do not use in conjunction with Import from Any Folder |

## Data Export

Data Export authorizations relate to export operations including an option to protect the exported content from further modification (available for Excel and Word outputs).

| Authorization | Operation |
| :---: | :---: |
| Export XLS | Export report in the Excel file with XLS extension. |
| Export XLSX | Export report in the Excel file with XLSX extension. |
|  | Restrict making changes to the Excel reports with a password. For more details, see Protecting Word and Excel Reports with Password. |
| Protect XLS/XLSX | To use this authorization, first select Use Word/Excel Report Protection check box and provide a password when enabling Enhanced Security or use Security Configuration wizard to make changes to the current configuration. |
| Export PDF | Export report in the PDF file format. |
| Export DOC | Export report in a Word file with DOC extension. |
| Export DOCX | Export report in a Word file with DOCX extension. |
|  | Restrict making changes to the Word reports with a password. For more details, see Protecting Word and Excel Reports with Password. |
| Protect DOC/DOCX | To use this authorization, you have to select Use Word/Excel Report protection check box and provide a password when enabling Enhanced Security on your system or to use Security Configuration wizard to make changes to the current configuration. |
| Export XML | Export the original XML report file. |
| Export with XSLT | Export the report with specific settings using XSLT transformations. For more details about these transformations, see How to Use XSLT for Custom Export. |
| Export with <br> Plug-In | Export the report with specific export settings using Export Plug-In. |
| Export to Any Folder | Select a folder to export the reports to. |

## Quality Control

Quality Control authorizations are related to QC Add-On.

| Authorization | Operation |
| :--- | :--- |
| Edit | Protocol/Results |
| Add, edit, remove Quality Control configuration of a protocol or results |  |
| QC | file. |
| Create On-The- | Generate instant QC report that is not applied to a protocol file. |


| Authorization | Operation |
| :--- | :--- |
| Fly QC Report |  |
| Edit QC Settings <br> File | Edit configuration of a QC Configuration File. |

## Electronic Signatures

Electronic Signatures authorizations are related to Electronic Signatures. If this feature is enabled then users can be granted or denied the following operations based on whether the document has been electronically signed with the specified reason. For example, if a user is assigned to an authorization group with the Protocols Requires Approval for Launch setting selected, then the suer can only use protocols which have been approved.

| Authorization | Operation |
| :--- | :--- |
| Protocols <br> Requires <br> Approval for <br> Launch | Assay protocol files require approval for launch (i.e. a protocol must have <br> been electronically signed with reason: approve). |
| Results Requires <br> Authorship for <br> Exporting | Assay results files require authorship for exporting (i.e. a results file must <br> have been signed with reason: authorship before it can be exported). |
| Results Requires <br> Authorship for <br> Printing | Assay results files require authorship for printing (i.e. a results file must <br> have been signed with reason: authorship before it can be printed). |
| Results Requires <br> Authorship for <br> Saving | Assay results files require authorship for saving (i.e. a results file must <br> have been signed with reason: authorship before it can be saved). |

## Automatic Logout

The Automatic Logout function suspends any open MyAssays Desktop windows after a defined period of inactivity. In this situation, the existing user must reenter their credentials to resume their session. If the correct credentials are not supplied, then the session is terminated (possibly with data loss).

The Automatic Logout feature is applicable only when working with MyAssays Desktop Log In authentication. With MyAssays Desktop Log In authentication, multiple users share a single Windows session and the operating system cannot provide any automatic logout of the MyAssays Desktop users. (With
Windows Log In authentication, automatic log out can be controlled by the system settings).

## When Is User Logged Out?

System administrators can configure the period of inactivity after which the users need to log in again under Security Options. To do this:

1. Launch MADE.
2. On the Tools tab, click Security Management.
3. Click Security Options.
4. Make sure that Enable check box is selected.
5. Review or edit Log out after (seconds).
6. To accept changes, click OK.

When configuring Security Management settings, log out and then back in for
 changes to take effect. Make sure you save your work and close all MyAssays Desktop windows.

## Monitor Usage

User administrators and system administrators can use the Audit Trail and Login Log buttons to view the corresponding logs of information. For example:

| A Audit Logs |  |  |  |  | $\square \times$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Filter by |  | Date/Time | User Name | Type | Data |
| $\checkmark$ Date |  | November 18, 2017 17:45:35 | Darren Cook | LaunchingMAD | MyAssays Desktop 1^ |
| Start date | 11/11/2017 | November 18, 2017 17:45:56 | Darren Cook | ExitingMAD | MyAssays Desktop I |
|  | 5 | November 18, 2017 17:46:23 | Bob | LaunchingMAD | MyAssays Desktop I |
| End date | 18/11/2017 | November 18, 2017 17:47:01 | Darren Cook | LaunchingMAD | MyAssays Desktop I |
| $\square$ User |  | November 18, 2017 17:47:18 | Darren Cook | CreateUser | UserName=Bob;Ful |
|  |  | November 18, 2017 17:47:31 | Bob | ExitingMAD | MyAssays Desktop I |
| User name | Darren Cook | November 18, 2017 17:47:44 | Bob | LaunchingMAD | MyAssays Desktop I |
|  |  | November 18, 2017 17:47:50 | Bob | OperationApproved | Operation: General |
| $\square$ Description |  | November 18, 2017 17:47:58 | Bob | OperationApproved | Operation: General |
| Description |  | November 18, 2017 17:47:59 | Bob | LoadPackage | Launch Protocol: C: |
|  |  | November 18, 2017 17:48:22 | Bob | SavePackage | Assay Protocol Save |
|  |  | November 18, 2017 17:48:22 | Bob | SavePackage | Assay Results Saved |
|  |  | November 18, 2017 17:48:23 | Bob | SavePackage | Assay Results Saved |
|  |  | November 18, 2017 17:48:34 | Bob | CalculatePackage | Results calculated () |
|  |  | November 18, 2017 17:48:40 | Bob | ReportExported | Report exported to: |
|  |  | November 18, 2017 17:51:46 | Bob | OperationApproved | Operation: General |
|  |  | November 18, 2017 17:51:53 | Bob | OperationApproved | Operation: General |
|  |  | November 18, 2017 17:51:55 | Bob | LoadPackage | Launch Protocol: C: |
|  | Apply | Nnvemher 18 2017 17.5).16 | Roh | SaveDarkane | Accav Drataral Saye |
|  |  | Refresh | Print... | Export... | OK Cancel |

## Filter

Tools are provided to filter the log by date, user, and description. Select the appropriate controls, enter the parameters, and click Apply to filter the log.

## Export

The Export button displays the Export log tool, which can be used to export the selected log information to an XML or a CSV file. Click Export to export the contents.


## Enhanced Security Configuration Backup

The Enhanced Security Configuration Backup tool simplifies the back-up of Enhanced Security configuration data.

This tool does not back-up assay data files; it only backs up the Enhanced Security configuration.

The back-up tool is available for administrators from MyAssays Desktop Explorer | File | Data

## | Enhanced Security:

## Enhanced Security



Configuration Backup
Create an encrypted backup of the Enhanced Security Configuration.

Press Config Backup to create a backup.
This will create an encrypted and password protected backup ZIP of the ES configuration (stored in the Documents folder and named based on the current date and time).

By default, the ZIP file is protected with a default private password (known only to MyAssays Ltd.).

To set a custom password, create a registry string value named: BackupPassword at registry key: HKEY_LOCAL_

MACHINE\SOFTWARE\WOW6432Node\MyAssays\MyAssays
Desktop\Security\}

## Working with MyAssays Desktop Pro and Enhanced Security

This section explains the functional differences when working with Enhanced Security enabled with MyAssays Desktop Pro; this section provides information for all users of the secured system.

## MyAssays Desktop Explorer

The MyAssays Desktop Explorer Welcome screen displays a padlock icon to clearly identify that Enhanced Security is enabled.

Also, the Welcome screen displays the logged in user's Full Name and the details of the last log in attempt (this will include any failed login attempts).

The File | User Accounts section displays further details about the currently logged-in user including authorization group and account type.

Administrators can access the Security group under the Tools tab to find the various Enhanced Security configuration tools.

## Administrator Security Tools

For administrator users, security tools are available from the Security group of the Tools tab. This includes the Security Configuration and Security Management options, i.e.:

| File | Home | Tools | Support |
| :---: | :---: | :---: | :---: |
| Folder Poll Analysis <br> Manager Certificate <br> Launcher <br> Utilities | Security <br> Configuration Management <br> Security |  |  |

These operations are only available for administrator users.
The Security Configuration Wizard can be used to modify the security setup (only available to system administrators).

The Security Management tool provides control over:

- Configuring users
- Assigning users to and setting up authorization groups
- Viewing and editing authorizations assigned to a group
- Viewing and exporting the Audit Trail and Login Log
- Automatic Logout settings

The Security Management tool is available for system administrators and user administrators.

## File Operations

With Enhanced Security activated and the Secure File Protection feature enabled, destructive file operations on MyAssays Desktop Pro data stored inside the protected folders are prohibited. This means that the following Windows functions cannot be performed on protected MyAssays Desktop Pro assay data files:

- Delete
- Rename
- Create
- Move

However, authenticated users can be authorized to perform file deletion and file duplication through Enhanced Security by granting those users permission to:

## - Delete Data File

## - Copy + Paste Data File

in the File Operations section of Authorizations.
These operations are managed by the Enhanced Security system so they cannot be performed using the standard Windows commands (i.e., Windows delete and copy + paste will not work). Instead, the secure version of these operations can be accessed through the context menu (by right-clicking on a selected single file). These secure file operations are indicated with the security padlock icon.
For example, to duplicate an existing MyAssays Desktop Pro protocol file, right-click the file to duplicate and select the Secure Copy + Paste option:

## Protocols



If the authenticated user is authorized to perform the secure file operation, then MyAssays Desktop Pro will prompt for a file name for the new file. Any performed secure file operation is logged in the audit trail alongside the authenticated user.

These secure file operations are available through the right-click context menu in MyAssays Desktop Explorer and in Windows Explorer. Note, the secure file operations can only be
performed on one file at a time (the secure file operations are not available when multiple files are selected).

## Login Options and Password Management

The logging in options depend on the selected authentication method. The specifics of how each of them work are different and are described below.

## Windows Log In Authentication

With Windows Log In authentication, the credentials used to log in to Windows are used to identify the current user; no additional log in step is required when launching any MyAssays Desktop window.

For domain accounts, the user has to enter the AD domain name followed by backslash ( $\backslash$ ) and account name (as configured when adding users to the Enhanced Security system).

For logging out, the File menu includes the Log Out + Exit option. This command will exit MyAssays Desktop Explorer and log out of the current Windows session (closing any other open applications).

## MyAssays Desktop Log In Authentication

With MyAssays Desktop Log In authentication, users will need to log in to the application with a Log $\mathbf{I n}$ dialog:


The Log In to:<context> message shows to which authentication context (local machine or domain name) the user will log in.

## Log In to Local Machine Account

In the Username and Password field, users need to enter or paste their provided credentials (as configured when adding users to the Enhanced Security system) and then click

## Log In.

## Log In to Domain Account

In the Username field, the user has to enter the AD domain name followed by backslash ( ( ) and account name (as configured when adding users to the Enhanced Security system). For example, for a domain "CORP" and account name "LabUser" the Username should be: CORP $\backslash$ LabUser. In the Password field, type in their password, and then click Log In.

To log in to local system administrator account, in the Username field, enter dot (.) instead of the AD domain name. For example, . $\backslash$ Admin.

## Logging Out

With MyAssays Desktop Log In authentication, users should log out when they have finished working with MyAssays Desktop Pro (otherwise other users can perform unauthorized changes on their behalf). Users can log out from the MyAssays Desktop Explorer under File | Log Out + Exit.

## Automatic Logout

If the administrator has enabled the Automatic Logout feature, then the user session is kept active while there is any user activity. If the Automatic Logout period elapses without any user activity, then the user will be logged out of the system with any open windows closed. User can resume the session by entering the password again in the Resume dialog.

If the administrator has disabled Automatic Logout, then the user session is kept active while there are any MyAssays Desktop windows opened.

## Password Management

For both authentication methods, password management is handled using the Windows security policy. If the security policy of the system dictates that the user must change his or her password, then after successful login, the user is advised to change the password. The new password must adhere to the system's password policy options (such as minimum length and password history).

## Quality Control

This section describes the Quality Control (QC) feature of MyAssays Desktop.

This feature is only available to users with the QC feature enabled on their license.

## Overview

The Quality Control features enable the monitoring of measurements and analysis results across assays runs. This is known as "inter-assay analysis" and can be used to track whether assay performance remains in control by comparing current results with historical observations.

For example, a QC test can check whether the value of the Controll in all results files falls in the range of $+/-2 * \mathrm{SD}$ and indicate cases where results fall outside this range.

For intra-assay monitoring you can use the Validation feature.

## QC Observations

The general approach is to define specific observations of interest; these are collected and compared across assay runs.

Each observation results in a single numeric value for each assay run. An observation can be a measurement or calculated result (including TOVs). For example, the measurement or calculated results for the sample Control1 is an observation that is commonly monitored.

The QC process will gather the values of each selected observation across runs of interest. The results can be presented in a table and/or plotted on a QC chart to monitor inter-assay change. If these observations deviate from the specified range, then the assay is "out-ofcontrol" and is highlighted accordingly.

## QC Configuration

QC is configured using the intuitive QC Wizard that can be launched from both MADE and MAA.

QC can be configured:

- On demand - Create a QC report as an when you need it
- As part an assay protocol - With the QC results included in each results' report.

Additionally, you can save the defined configuration to a QC settings file and reuse it for future QC operations.

QC Results

In the simplest case, the result for each observation can be displayed in a table reporting observation result for each included assay. More sophisticated outputs can be generated plotting the observation data over time as CUSUM or Levey-Jennings charts.
The following subsections explain how to add, define, and review inter-assay QC analyses.

## QC Wizard

## Introduction

Inter-assay quality control is configured through the QC Wizard.
The QC features can be used in various scenarios to track inter-assay data for charting, analysis, and validation. The wizard provides a range of features and scenarios for convenient and simple inter-assay analysis tailored to your lab's needs and workflow.

The QC Wizard will walk you through the process of:

1. Selecting the files to consider
2. Specifying the observations to monitor
3. Selecting how the results should be presented

The QC Wizard can be launched in two different ways depending on the type of analysis you require:

## QC On-Demand

QC On-Demand allows you to generate QC reports as and when you need to, selecting the files to consider. This is useful for the production of an occasional QC report.

To perform QC On-Demand, launch the QC Wizard from MADE.

## How to: Launch the QC Wizard from MADE

1. Launch MADE.
2. Select the Results tab.
3. On the Ribbon, hover to the Quality Control group.
4. Click Create to launch QC Wizard or the Edit to launch it with an existing configuration file.

Select two or more results files and then press Create to automatically populate the wizard with these files.

## QC as Part of an Assay

QC can be built into an assay so that each time the assays is used the results are compared with historical results of the same protocol.

To add QC to an assay launch the QC Wizard from MAA.

## How to: Launch the QC Wizard from MAA

1. Launch an assay protocol or open a results file.
2. On the Ribbon, click the Add drop-down button of the Quality Control group.
3. Click New Configuration to launch the QC Wizard or the Existing Configuration to specify the configuration file to launch the wizard with.

## Selecting Files for Analysis

The Selecting Files for Analysis QC Wizard step specifies the results files to include in the inter-assay QC analysis.

The files could be added in the following ways:

## Folder

Select this setting if the results you want to compare are stored in the same location and follow the same name convention.

- Path - The full path to the results folder location. Click the Browse button to select a folder in which your results files are stored.
- Pattern - Defines the pattern to extract results file with.

By default, the MyAssays Desktop organizes results files in accordance with the protocol name. For example, if a protocol is named "My Endpoint $12 \times 8$ Protocol," each run of the protocol will generate a results file that is named after "My Endpoint $12 \times 8$ Protocol" convention with a run number in brackets. For example, if the protocol was ran for three times, the newest results file is named "My Endpoint 12x8 Protocol (3)".

This organization of data simplifies the extraction of observation data from previous runs of a particular protocol. With this in place, the results are specified as:

## Protocol Name*

If the QC Wizard is launched in MAA, then the files to monitor are set up to match the configuration of the results naming setting.

For example, for the My Endpoint 12x8 Protocol results files, it would be My Endpoint 12x8 Protocol*.

With this setting, all results for this protocol will be automatically added to QC analysis. In case you want to analyze only some of them, use the Specific Files setting.

## Specific Files

This setting is useful if you want to track changes between specific results files or the results stored in different folders. For example, you might need to compare the results from first, fifth, and seventh run of an assay.

To add the files to the list, click the Add icon button and select the required results. For adding multiple files, hold the Shift or Ctrl key, if the files are or are not sequential.

To remove a file from the list, select it and click the Remove icon button.
Click Next to continue with the Wizard, and configure the observations to be tracked between the specified files.

To use both file selection patterns at the same time or define several naming patterns, use the XML editor on the Configuring Observations step. See Advanced QC Wizard Settings to learn more.

## Configuring Observations

This QC Wizard step allows you to add and configure the observations necessary to achieve your requirements of inter-assay quality control. Use the Add Observation or Remove Observation buttons to add observations as required.

## Observations

## Name

For each observation, a description can be specified under the Name column. Use this to provide a clear textual description for your QC monitoring. This will be used in the header for the Observation Expression column in the QC Results table to convey what has been monitored.

## Data

For each Matrix-Transform Data Analysis Assays observation, specify which matrix the observation applies to. The numbering is sequential and starts from the index of the first matrix at 1 onward. The list of the available matrices (both raw and calculated) with their indexes is displayed in the Matrices tab. Any of the raw or calculated matrices that contain endpoint data can be referenced in an observation.

## Matrix Name

If all selected assays have the same number of matrices and the same matrix names (e.g., Raw, Concentration, and Average), then a list box with the matrix names will be displayed.

## Expression

An expression is used to define the observation data. The type of expression depends on the assay type:

- Observation (for Matrix-Transform Data Analysis Assays) - Enter an expression to query the data of the specified matrix. This could simply be the name of a sample group, e.g.,
Control1 or a more sophisticated calculation expression. Non-matrix data can be queried by using transform output variables.
- XPath (for other assays types) - An XPath expression that obtains single numerical value; that expression is then queried for each of the specified results.

The type of expression to use will be reported in the column name.

## Output

The inter-assay observation data can be plotted simply as a table of the values or as a CUSUM or Levey-Jennings chart alongside. For all output types, a text summary is included under each Observation Table.

## SD $\pm$ Limit

For each observation, control limits are expressed in terms of standard deviation range and are calculated automatically for the observation data.

You can define required range for the Table and CUSUM outputs and select the control rule for the Levey-Jennings.

The observation results for an assay run fall outside this range, they are highlighted in the resulting table.

## Create QC Report Instantly

You can export a report to an Excel spreadsheet straight-away with the Create Now option. The report will be saved to your MyAssays Reports folder.

## Configuring QC Observations in the XML Editor

After you have selected the files to monitor and configured at least one observation, you can select the XML tab to bring your quality-control setup to a new level. Using the built-in XML editor, you can configure your QC analysis to meet your needs. This includes:

- Monitoring multiple files, folders, or their combinations at the same time
- Modifying the display of resulting charts
- Formatting the results according to your lab's standards


## Monitor Multiple Files, Folders, or Their Combination

The files you've included on the Selecting Files for Analysis step are stored in the Files element (the Folder option) or in its File child elements (the Specific Files option). You can define a combination of files to monitor by adding their associated elements.

## Include Multiple Folders into a Single Observation

If your files are stored in several folders, or in the same folder with different naming patterns, add the following information for each folder:

## <Files Folder="Full Path" Search="Pattern*"/>

Replace Full Path with the actual full path to the folder and Pattern with the common filename component to search for applicable files, followed by an asterisk *. For example,

```
<Files Folder="C:\Users\User1\Documents\MyAssays\Results\"
Search="ELISA*"/>
<Files Folder="C:\Users\User1\Documents\MyAssays\Results\"
Search="NEW*"/>
```

will include all .assay-results files that start with ELISA (e.g., "ELISA(1).assay-results", "ELISA (2).assay-results", etc.) and NEW (e.g., "NEW_ELISA(1).assay-results", "NEW 4PL.assay-results", etc.) into QC monitoring.

## Compare Folder Against File

If you want to monitor the folder and a specific assay-results file in a single observation, for each file, add a File element as a child to Files and specify the full path to the file:

## <File Path="Full Path"/>

For example:

```
<Files Folder="C:\Users\User1\Documents\MyAssays\Results\ELISA\"
Search="*">
    <File Path="C:\Users\User1\Documents\MyAssays\Results\ELISA
    (1).assay-results"/>
```

</Files>

This configuration will include ELISA(1).assay-results stored in the MyAssays\Results and all .assay-results files from its ELISA subfolder.

## Modify the Appearance of the Chart

When selecting the output that includes CUSUM or Levey-Jennings chart, you can change the chart display as needed. The available settings include customizing the major and minor units of both axes and setting the number format for the axes values.

To customize the chart in your QC report, add the ChartCustomization element as a child of the associated Observation and set its attributes. See Change the Units and Number Format of the Charts to see all available attributes.

The editor adds the xmlns="http://www.myassays.com/ChartCustomizations" by default. This attribute is required for the customization.

For example:

```
<Observation Name="Control Values Check" Expression="Control1" Matrix="1"
SDLimits="2" Output="Levey-Jennings" />
    <ChartCustomization
    xmlns="http://www.myassays.com/ChartCustomizations"
    XAxisMajorUnits="10" XAxisMinorUnits="5" YAxisMajorUnits="0.2"
    YAxisMinorUnits="0.1" YAxisNumberFormat="[Red][&lt;1]0.00;
    [Green][&gt;=1]0.00"/>
</Observation>
```


## Set Numerical Formatting of the Results

By default, the results in the QC report are displayed to 6 significant figures. To change the formatting, add the NumericalFormatting element as a child of the Observation and set its attributes. To learn about the numerical formatting settings, see Viewing Available Matrices and Configuring Their Settings.

The Type settings are entered into XML without spaces.

For example:

```
<Observation Name="Observation1" Expression="Control1" Matrix="1"
SDLimits="2" Output="Table" />
    <NumericalFormatting Type="DecimalPlaces" Precision="3"/>
```

</Observation>
Here the numerical formatting is set to 3 decimal places.

## Set the Type of Observation Expression

Inside the Observation element, you can enter and set to true or false an optional Boolean
IsExpressionXPath setting to treat the expression accordingly.

## QC Results

On this step, if the wizard is launched from MAA (with a protocol or results), generated results can be added to a report (and generated on the next calculation). Otherwise, the results can be saved to the QC Configuration file.

Press Finish to exit the wizard.

## Observation Evaluation Issues

If one or more observations is not evaluated, they are listed in a separate table to indicate their exclusion from the inter-assay analysis. In this case, the error message describing the issue is displayed instead of result in Result column.

| Message | lssue |
| :--- | :--- |
| QC not available for this file. | The latest version of results was generated before the |
| Please open file and recalculate |  |
| to enable QC | QC feature was introduced and needs to be recal- <br> culated to enable QC. |
| Failed to evaluate expression: <br> \{expression $\}$ | The specified observation expression is not valid for <br> the file. |
| Failed to parse value:\{value\} | The specified XPath expression returns non-numeric <br> value and is thus not valid. |
| Failed to evaluate XPath <br> expression | The specified XPath expression is not valid for the file. |

## QC Workflows

Here are described the typical workflows when working with the Quality Control. These can be combined to provide accurate and consistent results for all of your research needs.

## Generate Report Instantly

In the simplest case, specify the results you want to compare, launch (in either MADE or MAA) and configure observations through the wizard, and click Create Now. The .XLSX report with your configured observation results is then generated and stored in your Reports folder.

## Schedule QC Monitoring for all Future Runs of the Protocol

Typically, a protocol is created for an assay of a particular type (e.g., quantitative measurement of cortisol). This protocol is then run each time the assay is required. Running a protocol acquires the relevant measurement data and processes it in accordance with the calculations configured in the protocol; this generates a results file. If you set the QC to a protocol, it will be applied for all runs of this protocol in the future (so you don't need to repeat the configuration again next time).

Running this workflow is useful for designing systematic QC procedures. With this, the QC analysis is automatically performed as soon as a new results file is generated. For example, perhaps you are running a routine assay and want to monitor if the \%CV of the measurements between runs does not go beyond a range of $\pm 2 *$ SD.

In this case, open the protocol and launch the QC Wizard. Select the Add QC content to report option if you want to include the QC Results in the report automatically when new results are calculated.

## Compare Results and Add QC Results to Report

Perhaps, you want to determine if the response of control results in today's assay is significantly different from corresponding control's responses in the reference assays. The defined observations you want to include in the report for current assay run only.

In this case, launch today's results file, select the results for comparison, configure required observations in the wizard, and select the Add QC content to report option.

When Add QC content to report option is selected, the results of the current assay are always included as the last observation and indicated with (Current) after the observation number.

QC Configuration File

For example, your lab has multiple analyses running under the same acceptable limits or works under general quality standards. In this case, you can run and configure typical validating observations and reuse them for every assay.

To create QC configuration file, select the Save settings to file check box when finishing working with QC Wizard. This file with all specified settings in place is stored under the QC subfolder in your MyAssays folder or any other specified location.

Note, this configuration may be tweaked depending on the analysis peculiarities (Edit or
Add Existing Configuration commands) and optionally saved to another file, protocol, or results.

## Reference

## Transforms

Transforms can be described as sets of configurable operations that are performed to generate results. The results might be included in a report or used as inputs to other transforms. You can add multiple transforms in any order to perform a required data analysis with the leverage of transforms performed previously.

Most transforms use one input matrix and produce one output matrix (like Factor). However, this is not always the case. Here are the general rules:

- Each transform can have one or more input matrices.
- Each transform can produce one or more output matrices.
- A particular transform will always have the same number of input and output matrices.
- The input and output matrix types of a transform are fixed.


## Matrices and Transforms

## Input Matrices

A transform can accept one or more input matrices. Where an input matrix is used, the transform performs calculations using the data on those matrices. For example, the Factor transform multiplies all points on the input matrix by a specific value.

The input matrices available to a transform include the compatible raw data (i.e., of a particular data type) and compatible data that has been calculated prior to this transform.

For example, assuming all data is endpoint, the first transform can only access data on the Raw matrices, whereas a second transform can access data on the Raw matrices and/or the first Calculated matrix.

## Output Matrices

Each transform will add one or more calculated matrices to the Matrices at each step. As each transform is evaluated, each container in the results will always contain the same number of matrices (raw and calculated) as all other containers in the results.

As you add transforms, you can configure output settings and display in the report for each of them.

## Expressions and Transforms

In addition to the input matrices, any transform that uses MyAssays Analysis Expressions can access data directly from the current transform (including data that is not on the input
matrices). Similar to available input matrices, the data that can be accessed depends on the data that has been calculated up to this point of the evaluation (and data of the compatible type). Thus, the data accessible by an expression includes compatible raw and calculated matrices.

## Transforms Tab

Transforms are added and configured via the Transforms tab.

## How to Add a Transform

1. Launch an assay protocol or open a results file.
2. If you cannot see the Assay Properties panel, click Properties to open it.
3. Click Add.
4. Select a transform and click Create.
5. Review or edit the transform's input matrix and output matrix names as required.
6. Provide your required settings for the transform (see Properties section above).
7. Click Calculate to perform the calculations with the new settings and to see the results.

Each transform has a set of configurations to specify the parameters of the analysis. Some transforms can also add optional content to the report and be configured with specific calculation settings.

## Transform Relevance

A transform may or may not be relevant to the current assay configuration. In this context, the configuration refers to:

- Matrices (both raw and calculated) - Certain transforms require a particular data type to be executed.
- Layout - Some transforms require specific layout rules to be followed.


## General Transform Properties

With regard to flexibility of assay setup, each transform can be configured to fully match assay requirements with a number of settings.

Each transform has zero or more specific settings that allow customization of the transform operations. These are covered under each transform section specifically.

In addition to their specific settings, all transforms share the following common settings:
Advanced

| Included <br> Samples | The sample types to be computed by this transform. |
| :--- | :--- |
|  | Sample types not selected in the Included Samples option will not be <br> computed by this transform; for those cases, this setting specifies what the <br> result will be for those excluded samples: |
| Excluded | - Flagged With Value - The result is flagged with the input value <br> reported. This will appear in the report with the value with strikethrough, <br> Reg., 7.23. <br> - Flagged No Value - The result is flagged with no value reported. This <br> will appear in the report as Flagged. |
| - Equal Input - The result is set to match the value of the input. |  |

Units

| Output | The measurement units to be included in the header of the Sample Table. <br> If the output units of the transform are the same as the input, then use "ditto" <br> Units |
| :--- | :--- |
| units to automatically carry these units through (i.e., apply the same units to <br> the transform's output). This shortcut means that it is not necessary to repeat <br> settings across transforms. Ditto units are specified with two dots, i.e.: <br> . |  |

In addition, the $\mathbf{X Y}$ transforms and $\mathbf{X Y}$ Reduction transforms can be configured with Data and Range settings.

## Endpoint Transforms

Endpoint transforms operate on matrices of data that consist of a single numerical value at each position on the layout. An endpoint transform will compute a single numerical result for each position on the layout.

For example, with a typical ELISA microplate assay, a plate reader will measure and report a single numerical value for each successfully measured well on the plate. Each measurement value is the endpoint of the detection technology for the observed sample. The Standard Curve Fit transform is an endpoint transform that can compute the concentration endpoint from the measurement endpoint.

> For consistency, we refer to all matrices with a single numerical result as an endpoint matrix. Strictly speaking, of course an endpoint matrix might not actually be the final conclusion of the analysis. Using MyAssays Desktop, you can layer as many transforms as you need to perform the various analysis steps that are required (resulting in multiple "intermediary endpoint" matrices).

## Flagged Endpoints

All transforms take into account the flagged status of each input value. Any data point that is flagged is not included in any calculation and ignored in any reference to the sample group that it belongs to.

For example, if a transform subtracts the average of a blank group from all samples, if the blank group is made up of 3 replicates but the $3^{\text {rd }}$ replicate is flagged, then all the samples will be corrected by the average of the 2 non-flagged samples.

In addition, a transform computation for a position might result in a flagged endpoint result. This could be because of a calculation error or because the transform has performed some kind of automatic outlier detection (such as Auto Flag and Replicate Outlier Removal).

## Sample Types to Process

For all endpoint transforms, by default the calculations are performed on all sample types.
For greater flexibility, you can use the Advanced settings of each transform to select to perform the transform's computations on only a subset of the sample types. In this case, it is possible to set what the excluded samples results should be (e.g., flagged or to equal the input endpoint).

For example, it might only make sense to perform a certain calculation on Unknown and Control samples but not with other sample types.

## Endpoint Transforms

| Transform | Operation |
| :---: | :---: |
| Auto Flag | Flags all data points that satisfy the specified Boolean expression. |
| Average | Calculates the average of replicates for each sample group. |
| Blank <br> Correction | Subtracts the specified blank value from each input data point. |
| Dilution <br> Factors | Multiplies sample groups of the selected type(s) by a specified factor (a unique value can be specified for each group). |
| Dose- <br> Response <br> Curves | Creates a dose-response chart, fit selected curve fitting method, and calculates the response for the specified dose from the curve for each group of the specified type. |
| Expression <br> by Matrix <br> (Dual) | Evaluates the expression in terms of $x$ and $y$ for each sample group. The $x$ and $y$ variables refer to the values on the first and on the second input matrix, respectively. |
| Expression by Matrix | Evaluates the expression in terms of $x$. |
| Expression by Position | Evaluates an expression in terms of $x$ for each specified position. |
| Expression <br> by Sample <br> Type | Evaluates an expression in terms of $x$ for each specified sample type. |
| Factor | Multiplies all input data points of the selected sample type(s) by a specified factor. |
| General Curve Fit | Fits selected fit method to the specified calibrators and then calculates either $y$ or $x$ values on the curve from input values for the selected sample types. |
| Matrix <br> Difference | Calculates the difference between values on the first matrix and the second for each sample group. |
| Median | Calculates the median of the replicates for each sample group. |
| Percentage <br> Coefficient of <br> Variation | Calculates the \%CV of the replicates for each sample group. |
| Percentage | Calculates a percentage value for each data point with respect to the specified $0 \%$ and $100 \%$. |
| Replicate <br> Outlier <br> Removal | Flags the specified number of replicates for each sample group, in which \%CV of its replicates is greater than specified threshold. |
| Standard Curve Fit | Constructs a standard curve by plotting the standard input concentration values versus data points. |
| Standard | Calculates concentrations of all included samples using a Library Curve. |


| Transform Operation <br> Curve Fit <br> (from <br> Library)  <br> Standard Calculates the standard deviation of the replicates for each sample group. <br> Deviation  <br> Standard <br> Error of Calculates the standard error of the mean for replicates of each sample group. <br> $\underline{\text { Mean }}$  <br> $\underline{\text { Variance }}$ Calculates the variance of the replicates for each sample group. |
| :--- | :--- |

## Auto Flag

[Input: Endpoint $\Rightarrow$ Output: Endpoint]
This transform flags positions where a Boolean condition is satisfied. This is useful for automatically rejecting and ignoring data points that are outside an expected range.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

## Auto Flag

The expression to evaluate, where a single $x$ variable is set to value of each position of the input matrix.

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to flag all positions with a value greater than 1 . Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

To flag values in this matrix where the measurement is greater than 1 , specify an Auto Flag expression of $\boldsymbol{x}>\mathbf{1}$.

## Calculated

The transform will generate an output matrix named Calculated with the value at each position matching the input data and positions that do not meet the specified condition
flagged. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 0.364 | 0.323 | 2.105 | 2.124 | 7.213 | 7.226 | 7.159 | 1.790 | 0.110 | 0.116 | 7.463 | 7.487 |
| B | 0.455 | 0.441 | 7.869 | 7.888 | 7.203 | 1.218 | 7.180 | 7.206 | 0.132 | 0.130 | 0.786 | 0.7 |
| C | 0.587 | 0.575 | 0.474 | 0.514 | 7.206 | 1.239 | 7.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.6 |
| D | 0.80 | 0.774 | 0.364 | 0.378 | 7.167 | 1.187 | 7.217 | 1.247 | 0.101 | 0.106 | 0.489 | 0.4 |
| E | 7.105 | 1.065 | 0.279 | 0.367 | 7.215 | 1.225 | 7.233 | 1.262 | 0.111 | 0.11 | 7.579 | 1.5 |
| F | 7.425 | 1.445 | 0.378 | 0.411 | 7.196 | 1.206 | 7.226 | 7.199 | 0.109 | 0.108 | 0.792 | 0.77 |
| G | 7.797 | 1.803 | 0.560 | 0.597 | 7.205 | 1.228 | 7.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.65 |
| H | 0.253 | 0.270 | 0.463 | 0.470 | 7.274 | 1.261 | Q. 1 | 0.116 | 0.115 | 0.11 | 0.4 | 0.477 |

## Average

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform calculates the average of the replicates in each sample group (flagged replicates are not included).

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to find the average position values in each sample group.

This transform averages the replicates of the sample group and stores the resulting value to each sample group replicate of the output matrix. Therefore, the result will depend on the layout configuration.

Here is some example raw data displayed to 3 decimal places:
Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

## Layout

The example uses a layout with Unknowns in duplicate (e.g., Unknown1 at A1 and A2,
Unknown2 at B1 and B2, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 1 | 1 | 1 | 2 | 2 | 3 | 3 | 4 | 4 | 5 | 5 | 6 |

## Average

The transform will generate an output matrix named Average with the value at each position being the averaged input data for each sample group.

With the example data provided above, the results are as follows (also displayed to 3 decimal places):

## - Sample Table

Here is a Sample Table snippet, which lists the details of the calculations. For each sample, the following details are reported:

| Sample | Positions | My Raw Data | Average |
| :--- | :--- | :--- | :--- |
| OUnknown1 | A1 | 0.364 | 0.3435 |
|  | A2 | 0.323 |  |
| OUnknown2 | A3 | 2.105 | 2.1145 |
|  | A4 | 2.124 |  |
| Unknown3 | A5 | 1.213 | 1.2195 |
|  | A6 | 1.226 |  |
| OUnknown4 | A7 | 1.159 | 1.1745 |
|  | A8 | 1.19 |  |
| OUnknown5 | A9 | 0.11 | 0.113 |
|  | A10 | 0.116 |  |

## - Matrix Table

Here all calculated data is displayed in a Matrix Table.

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.344 | 0.344 | 2.115 | 2.115 | 1.220 | 1.220 | 1.175 | 1.175 | 0.113 | 0.113 | 1.472 | 1.472 |
| $\mathbf{B}$ | 0.448 | 0.448 | 1.879 | 1.879 | 1.211 | 1.211 | 1.193 | 1.193 | 0.131 | 0.131 | 0.777 | 0.777 |
| $\mathbf{C}$ | 0.581 | 0.581 | 0.494 | 0.494 | 1.223 | 1.223 | 1.207 | 1.207 | 0.115 | 0.115 | 0.620 | 0.620 |
| $\mathbf{D}$ | 0.790 | 0.790 | 0.371 | 0.371 | 1.177 | 1.177 | 1.226 | 1.226 | 0.104 | 0.104 | 0.490 | 0.490 |
| $\mathbf{E}$ | 1.085 | 1.085 | 0.323 | 0.323 | 1.220 | 1.220 | 1.248 | 1.248 | 0.113 | 0.113 | 1.564 | 1.564 |
| $\mathbf{F}$ | 1.435 | 1.435 | 0.395 | 0.395 | 1.201 | 1.201 | 1.213 | 1.213 | 0.109 | 0.109 | 0.784 | 0.784 |
| $\mathbf{G}$ | 1.797 | 1.797 | 0.579 | 0.579 | 1.217 | 1.217 | 1.307 | 1.307 | 0.127 | 0.127 | 0.656 | 0.656 |
| $\mathbf{H}$ | 0.262 | 0.262 | 0.467 | 0.467 | 1.238 | 1.238 | 0.116 | 0.116 | 0.115 | 0.115 | 0.484 | 0.484 |

## Blank Correction

[Input: Endpoint $\Rightarrow$ Output: Endpoint]
This transform subtracts the average of a specified blank group (or groups) from each sample. This is useful for subtracting background noise from measurements.

The blank value is computed from the average of the (non-flagged) blank replicates. In the simplest configuration, a single blank value is subtracted from all samples. More sophisticated correction methods are possible, including:

- 1-1 - Where each sample group is corrected by its associated blank group
- By-row - One blank group on each row
- By-column - One blank group on each column

For more complicated blank correction calculations, an alternative approach is to use the Expression by Position transform.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Blank | The group number of the sample to use for correction. This can be defined as <br> a number or as an expression. The expression can refer to $x$, which is the <br> Group |
| :--- | :--- |
| group number of the sample to be corrected. For example, set this to $x$ to <br> perform a 1-to-1 correction where each group is corrected by the sample of <br> the specified blank type with the same group number. |  |
| Blank Type | The sample type used as the blank. |

## Example: Single Blank

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to perform a blank correction using the average of one blank group. This blank group itself is measured in duplicate. The mean of these replicates will be subtracted from all other samples.

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.192 | 0.199 | 0.347 | 0.346 | 0.325 | 0.321 | 0.803 | 0.797 | 1.589 | 1.593 | 0.241 | 0.246 |
| $\mathbf{B}$ | 0.293 | 0.300 | 0.586 | 0.584 | 0.800 | 0.793 | 0.402 | 0.401 | 0.839 | 0.837 | 0.562 | 0.564 |
| $\mathbf{C}$ | 0.537 | 0.534 | 0.549 | 0.547 | 0.878 | 0.875 | 0.471 | 0.471 | 0.759 | 0.756 | 1.039 | 1.043 |
| $\mathbf{D}$ | 0.858 | 0.855 | 0.320 | 0.325 | 0.531 | 0.530 | 1.552 | 1.551 | 0.429 | 0.427 | 0.424 | 0.424 |
| $\mathbf{E}$ | 1.523 | 1.527 | 0.486 | 0.490 | 1.289 | 1.283 | 1.430 | 1.438 | 1.494 | 1.501 | 1.427 | 1.429 |
| $\mathbf{F}$ | 1.622 | 1.621 | 0.621 | 0.626 | 0.667 | 0.663 | 1.350 | 1.347 | 0.631 | 0.634 | 1.695 | 1.694 |
| $\mathbf{G}$ | 0.001 | 0.001 | 0.356 | 0.352 | 0.418 | 0.412 | 1.172 | 1.174 | 1.572 | 1.570 | 0.595 | 0.590 |
| $\mathbf{H}$ | 0.297 | 0.297 | 0.796 | 0.801 | 1.517 | 1.515 | 0.960 | 0.965 | 0.534 | 0.527 | 0.585 | 0.584 |

This transform requires a layout with a minimum 1 Blank group.

## Layout

The example uses a layout with Standards, Control, Blank, and Unknowns in duplicate (e.g., Standard1 in A1 and A2, Unknown1 at A3 and A4, Unknown2 at B3 and B4, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | 2 | 2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | 3 | 3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | 4 | 4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | 5 | 5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | 6 | 6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | 1 | 1 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | 31 | 39 | 39 |
| H | 1 | 1 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | 40 | 40 |

Standard $\bigcirc$ Blank $\bigcirc$ Control $\bigcirc$ Unknown
To subtract the mean of the blank positions from the measurements, specify Blank Group to 1 and Blank Type to Blank.

## Blank Corrected

The transform will generate an output matrix named Blank Corrected with the value at each position being the subtraction of the blank value from the input data. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.191 | 0.198 | 0.346 | 0.345 | 0.324 | 0.320 | 0.802 | 0.796 | 1.588 | 1.592 | 0.240 | 0.245 |
| $\mathbf{B}$ | 0.292 | 0.299 | 0.585 | 0.583 | 0.799 | 0.792 | 0.401 | 0.400 | 0.838 | 0.836 | 0.561 | 0.563 |
| $\mathbf{C}$ | 0.536 | 0.533 | 0.548 | 0.546 | 0.877 | 0.874 | 0.470 | 0.470 | 0.758 | 0.755 | 1.038 | 1.042 |
| $\mathbf{D}$ | 0.857 | 0.854 | 0.319 | 0.324 | 0.530 | 0.529 | 1.551 | 1.550 | 0.428 | 0.426 | 0.423 | 0.423 |
| $\mathbf{E}$ | 1.522 | 1.526 | 0.485 | 0.489 | 1.288 | 1.282 | 1.429 | 1.437 | 1.493 | 1.500 | 1.426 | 1.428 |
| $\mathbf{F}$ | 1.621 | 1.620 | 0.620 | 0.625 | 0.666 | 0.662 | 1.349 | 1.346 | 0.630 | 0.633 | 1.694 | 1.693 |
| $\mathbf{G}$ | 0.000 | 0.000 | 0.355 | 0.351 | 0.417 | 0.411 | 1.171 | 1.173 | 1.571 | 1.569 | 0.594 | 0.589 |
| $\mathbf{H}$ | 0.296 | 0.296 | 0.795 | 0.800 | 1.516 | 1.514 | 0.959 | 0.964 | 0.533 | 0.526 | 0.584 | 0.583 |

## Example: 1-1 Blank Correction

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to perform a 1-to-1 correction, where each sample group is corrected by the blank that it is associated with. The blank numbering used in the layout defines the associations.

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 1.962 | 1.999 | 2.038 | 1.922 | 1.995 | 0.002 | 1.628 | 1.670 | 1.624 | 1.554 | 1.701 | 0.000 |
| $\mathbf{B}$ | 0.784 | 0.760 | 0.754 | 0.751 | 0.797 | 0.002 | 1.124 | 1.104 | 1.072 | 1.077 | 1.106 | 0.002 |
| $\mathbf{C}$ | 0.586 | 0.575 | 0.582 | 0.590 | 0.576 | 0.001 | 0.724 | 0.737 | 0.690 | 0.711 | 0.717 | 0.002 |
| $\mathbf{D}$ | 0.441 | 0.441 | 0.427 | 0.419 | 0.439 | 0.002 | 0.095 | 0.095 | 0.093 | 0.096 | 0.096 | 0.001 |
| $\mathbf{E}$ | 1.956 | 1.970 | 1.875 | 2.043 | 1.959 | 0.001 | 1.449 | 1.479 | 1.428 | 1.395 | 1.385 | 0.001 |
| $\mathbf{F}$ | 1.416 | 1.358 | 1.407 | 1.352 | 1.452 | 0.001 | 0.242 | 0.230 | 0.249 | 0.235 | 0.251 | 0.000 |
| $\mathbf{G}$ | 1.438 | 1.429 | 1.488 | 1.401 | 1.372 | 0.002 | 0.943 | 0.952 | 0.956 | 0.912 | 0.969 | 0.002 |
| $\mathbf{H}$ | 0.840 | 0.827 | 0.812 | 0.838 | 0.860 | 0.002 | 1.320 | 1.260 | 1.313 | 1.363 | 1.312 | 0.001 |

## Layout

The example uses a layout with 16 Unknowns groups (with Unknown1 in positions from A1 to A5, Unknown2 in positions from B1 to B5, etc.) and 16 Blank singlets:

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 1 | 1 | 1 | 9 | 9 | 9 | 9 | 9 | 9 |
| B | 2 | 2 | 2 | 2 | 2 | 2 | 10 | 10 | 10 | 10 | 10 | 10 |
| C | 3 | 3 | 3 | 3 | 3 | 3 | 11 | $(11$ | 11 | $(11$ | 11 | 11 |
| D | 4 | 4 | 4 | 4 | 4 | 4 | 12 | 12 | 12 | 12 | 12 | 12 |
| E | 5 | 5 | 5 | 5 | 5 | 5 | 13 | 13 | 13 | 13 | 13 | 13 |
| F | 6 | 6 | 6 | 6 | 6 | 6 | 14 | 14 | 14 | 14 | 14 | 14 |
| G | 7 | 7 | 7 | 7 | 7 | 7 | 15 | 15 | 15 | 15 | 15 | 15 |
| H | 8 | 8 | 8 | 8 | 8 | 8 | 16 | 16 | 16 | 16 | 16 | 16 |Blank $\bigcirc$ Unknown

To perform 1-1 correction, specify Blank Group to $x$ and Blank Type to Blank.
With this specified, the value of each blank will be subtracted from every position of associated group. In other words, Blank1 (stored in A6) value will be subtracted from A1, A2, A3, A4, A5 (the members of Unknown1), and from A6 itself. Therefore, the calculated matrix will contain blank corrected Unknown values and all Blanks will be 0.

## Blank Corrected

The transform will generate an output matrix named Blank Corrected with the value at each position being the subtraction of the associated blank value from the input data. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 1.960 | 1.997 | 2.035 | 1.919 | 1.992 | 0.000 | 1.628 | 1.670 | 1.623 | 1.554 | 1.701 | 0.000 |
| $\mathbf{B}$ | 0.783 | 0.758 | 0.752 | 0.750 | 0.796 | 0.000 | 1.122 | 1.102 | 1.070 | 1.075 | 1.104 | 0.000 |
| $\mathbf{C}$ | 0.585 | 0.574 | 0.581 | 0.589 | 0.575 | 0.000 | 0.722 | 0.735 | 0.688 | 0.709 | 0.714 | 0.000 |
| $\mathbf{D}$ | 0.439 | 0.439 | 0.425 | 0.417 | 0.437 | 0.000 | 0.094 | 0.094 | 0.093 | 0.095 | 0.095 | 0.000 |
| $\mathbf{E}$ | 1.955 | 1.969 | 1.873 | 2.042 | 1.958 | 0.000 | 1.448 | 1.478 | 1.427 | 1.394 | 1.384 | 0.000 |
| $\mathbf{F}$ | 1.415 | 1.358 | 1.406 | 1.352 | 1.451 | 0.000 | 0.241 | 0.229 | 0.249 | 0.235 | 0.251 | 0.000 |
| $\mathbf{G}$ | 1.436 | 1.427 | 1.486 | 1.399 | 1.369 | 0.000 | 0.941 | 0.950 | 0.955 | 0.910 | 0.967 | 0.000 |
| $\mathbf{H}$ | 0.839 | 0.825 | 0.810 | 0.837 | 0.858 | 0.000 | 1.319 | 1.260 | 1.312 | 1.362 | 1.311 | 0.000 |

Advanced Example: By-Row Blank Correction
In this example, we would like to configure blank correction on a row-by-row basis to blank correct each sample by the average of the two blank samples of the same row.

For better clearness of the process, this example uses simplified endpoint data for a $12 \times 8$ microplate:

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| $\mathbf{A}$ | 1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 1 |
| $\mathbf{B}$ | 2 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 2 |
| $\mathbf{C}$ | 3 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 3 |
| $\mathbf{D}$ | 4 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 4 |
| $\mathbf{E}$ | 5 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 5 |
| $\mathbf{F}$ | 6 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 6 |
| $\mathbf{G}$ | 7 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 7 |
| $\mathbf{H}$ | 8 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 8 |

Layout
The example uses a layout with 80 singlet Unknowns and 8 Blank duplicates for each row (with Blank1 in positions A1 and A12, Blank2 in positions B1 and B12, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 9 | 17 | 25 | 33 | 41 | $(49$ | 57 | 65 | 73 |
| B | 2 | 2 | 10 | 18 | 26 | 34 | $(42$ | 50 | 58 | 66 | 74 | 2 |
| C | 3 | 3 | 11 | 19 | 27 | 35 | 43 | 51 | 59 | 67 | 75 | 3 |
| D | 4 | 4 | 12 | 20 | 28 | 36 | $(44$ | 52 | 60 | 68 | 76 | 4 |
| E | 5 | 5 | 13 | 21 | 29 | 37 | 45 | 53 | 61 | 69 | 77 | 5 |
| F | 6 | 6 | 14 | 22 | 30 | 38 | 46 | 54 | 62 | 70 | 78 | 6 |
| G | 7 | 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 | 71 | 79 | 7 |
| H | 8 | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 | 80 | 8 |

Blank Unknown
With the Blank Group setting defined as the expression:

## $1+$ floor((x-1)/(LayoutWidth()-2), 1)

This converts the group number of the sample to blank correct $(x)$ to the blank number on its row. In other words, for $\boldsymbol{x}=\mathbf{1}$, this means $\mathbf{1 + f l o o r ( ( 1 - 1 ) / 1 0 ) , 1 ) , ~ f l o o r = 0 ~ a n d ~ t h e ~ B l a n k ~}$
Group is taken as 1 . For the first row, $x$ is in range from 1 to 10 and the expression outputs 1 , thus for Unknowns from 1 to 10 the average of Blank1 is subtracted.

For the second, $x$ is from 11 to 20 , $\mathbf{f l o o r}=\mathbf{1}$, so the Blank2 group is subtracted from
Unknown11-20, etc.
Blank Corrected

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| $\mathbf{A}$ | 0 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 |
| $\mathbf{B}$ | 1 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 1 |
| $\mathbf{C}$ | 2 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 2 |
| $\mathbf{D}$ | 3 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 3 |
| $\mathbf{E}$ | 4 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 4 |
| $\mathbf{F}$ | 5 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 5 |
| $\mathbf{G}$ | 6 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 6 |
| $\mathbf{H}$ | 7 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 7 |

## Dilution Factors

[Input: Endpoint $\Rightarrow$ Output: Endpoint]
This transform multiplies each selected sample group by a specified factor (a factor can be specified for each sample group).

A sample prepared with a dilution of $1: 2$ would use a dilution factor of 2 .

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Factored <br> Samples | The sample types to which the dilution factor should be applied |
| :--- | :--- |
| Factors | The dilution factor to use for each selected Factored Samples |

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to multiply all odd Unknown sample groups by 2 and all even Unknown sample groups by 5 .

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

## Layout

The example uses a layout with Standards, Control, Blank, and Unknowns in duplicate (e.g., Standard1 in A1 and A2, Unknown1 at A3 and A4, Unknown2 at B3 and B4, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 1 | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | 2 | 2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | 3 | 3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | 4 | 4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | 5 | 5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | 6 | 6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | 1 | 1 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | 31 | 39 | 39 |
| H | 1 | 1 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | 40 | 40 |
| O Standard O Blank | O Control | O Unknown |  |  |  |  |  |  |  |  |  |  |

To multiply selected sample types in this matrix by specified factors, set Factored Samples to Unknown and specify Factors as $\mathbf{2}$ for every odd sample group and as $\mathbf{5}$ for every even sample group.

## Concentration (x Dil. Factor)

The transform will generate an output matrix named Concentration (x Dil. Factor) with the value at each position of the selected Factored Samples multiplied by specified Factor and sample groups of other types matching the input data.

With the example data provided above, the results are as follows (also displayed to 3 decimal places):

- Sample Table

Here is a Sample Table snippet, which lists the details of the calculations for Unknown samples and the additional report content. For this transform, it is a Factor column displaying specified Factor for each sample group.

For each sample, the following details are reported:

| Sample | Positions | Factor | My Raw Data | Concentration (x Dil. Factor) |
| :--- | :--- | :--- | :--- | :--- |
| OUnknown1 | A3 | 2 | 2.105 | 4.210 |
|  | A4 |  | 2.124 | 4.248 |
| Unknown2 | B3 | 5 | 1.869 | 9.345 |
|  | B4 |  | 1.888 | 9.440 |
| Unknown3 | C3 | 2 | 0.474 | 0.948 |
|  | C4 |  | 0.514 | 1.028 |
| Unknown4 | D3 | 5 | 0.364 | 1.820 |
|  | D4 |  | 0.378 | 1.890 |
| Unknown5 | E3 | 2 | 0.279 | 0.558 |
|  | E4 |  | 0.367 | 0.734 |

The location of Factor column in the Sample table can be customized in the XML tab.

## How to: Set Factor Column Position in the Report

1. In the Assay Properties panel, select the XML tab.

If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Inside the ReportContent element, locate the Factor element.
3. Add the SampleTableColumnPosition element to the Factor element. Specify the column positioning with one of the following options:

- AfterOutputMatrix - On the right of the output matrix
- LeftMost - Before all matrix data
- RightMost - After all matrix data


## - Matrix Table

Here all calculated data is displayed in a Matrix Table.

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 4.210 | 4.248 | 2.426 | 2.452 | 2.318 | 2.380 | 0.220 | 0.232 | 2.926 | 2.962 |
| $\mathbf{B}$ | 0.455 | 0.441 | 9.345 | 9.440 | 6.015 | 6.090 | 5.900 | 6.030 | 0.660 | 0.650 | 3.930 | 3.840 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.948 | 1.028 | 2.412 | 2.478 | 2.406 | 2.420 | 0.226 | 0.232 | 1.226 | 1.252 |
| $\mathbf{D}$ | 0.806 | 0.774 | 1.820 | 1.890 | 5.835 | 5.935 | 6.055 | 6.205 | 0.505 | 0.530 | 2.445 | 2.450 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.558 | 0.734 | 2.430 | 2.450 | 2.466 | 2.524 | 0.222 | 0.228 | 3.158 | 3.098 |
| $\mathbf{F}$ | 1.425 | 1.445 | 1.890 | 2.055 | 5.980 | 6.030 | 6.130 | 5.995 | 0.545 | 0.540 | 3.960 | 3.875 |
| $\mathbf{G}$ | 1.791 | 1.803 | 1.120 | 1.194 | 2.410 | 2.456 | 2.616 | 2.612 | 0.252 | 0.254 | 1.304 | 1.318 |
| $\mathbf{H}$ | 0.253 | 0.270 | 2.315 | 2.350 | 6.070 | 6.305 | 0.575 | 0.580 | 0.575 | 0.575 | 2.450 | 2.385 |

## Dose-Response Curves

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform fits a dose-response curve for each sample group (with each sample group being dosed in the same way). The resulting dose-response curves are overlaid on a single chart for visual comparison.

The transform can also optionally compute and report values from each sample's curve, including:

- ED50/EC50/IC50, etc.
- ED(n)/EC(n)/IC(n), etc.
- Upper and lower bounds
- The dose at a specified response
- Any mathematical function (via an expression)

To learn about computing additional values from each curve, see Advanced Dose-Response Curves Evaluations.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.
Properties

## Axes

| $\mathbf{X}$ Axis Title | The name of the x -axis on the chart. |
| :--- | :--- |
| X Axis Type | The scale type to be used for the x -axis: <br> • Logarithmic <br> - Linear |
| $\mathbf{Y}$ Axis Title | The name of the y -axis on the chart. |
|  | The scale type to be used for the $y$-axis: <br> - Logarithmic |
| Y Axis Type | When $y$-axis is logged, negative (or zero) $y$ values are not <br> included in the plot. |

## Axes (Advanced)

Use these additional settings to adapt the chart's appearance to your needs.

| X Axis Major Units | The numeric interval between numbers on the $x$-axis. Starting from the minimum value, a major unit is displayed at every increment. |
| :---: | :---: |
| X Axis Minor Units | The numeric interval between supplementary tickmarks on the $x$-axis. The minor units are not displayed unless this attribute is specified. |
| X Axis <br> Numerical Formatting | The formatting for the X Axis Major Units values. <br> To learn about available formatting options, see Custom Format Codes for Numbers of the Chart. |
| X Crosses Y <br> At | Select where the $x$-axis will cross the $y$-axis from the drop-down: <br> - Automatic - Determine the location that makes the most sense for your data automatically. <br> - Axis Value - Cross y-axis at specified $\mathbf{X}$ Crosses $\mathbf{Y}$ at Value. <br> - Maximum Axis Value - Cross y-axis at the maximum $y$ value of the chart. <br> - Minimum Axis Value - Cross y-axis at the minimum y value. If axis type is set to Logarithmic, then it is the lowest positive value. |
| X Crosses Y at Value | The numerical value at which the $x$-axis will cross the $y$-axis. This value must be in the range of calculated results. If axis type is set to logarithmic, the provided value must be greater than 0 . |
| Y Axis Major Units | The numeric interval between the gridlines on the $y$-axis. Starting from the minimum value, a major unit is displayed at every increment. |
| Y Axis Minor Units | The numeric interval between supplementary tickmarks on the $y$-axis. The minor units are not displayed unless this attribute is specified. |
| Y Axis Numerical Formatting | The formatting for the $\mathbf{Y}$ Axis Major Units values. <br> To learn about available formatting options, see Custom Format Codes for Numbers of the Chart. |


| Y Crosses $\mathbf{X}$ | Select where the $y$-axis will cross the $x$-axis from the drop-down: <br> - Automatic - Determine the location that makes the most sense for your <br> data automatically. |
| :--- | :--- |
| • Axis Value - Cross x-axis at specified $\mathbf{Y}$ Crosses $\mathbf{X}$ at Value. |  |
| - Maximum Axis Value - Cross $x$-axis at the maximum $x$ value of the |  |
| chart. |  |
| - Minimum Axis Value - Cross $x$-axis at the minimum $x$ value. |  |
| If axis type is set to Logarithmic, then it is the lowest positive value. |  |

These settings can be also applied to other charts as an advanced configuration. To learn more, see Change the Units and Number Format of the Charts and Customize $X$ and $Y$ Axes Positions for Curve Charts topics.

## Chart

| Overlay <br> Multiple <br> Plate Charts | Select to plot the data from multiple plates on a single chart rather than <br> plotting separate charts for each plate. |
| :--- | :--- |
| Plot <br> Response <br> for Dose <br> Calculation | Select to plot the Response for Dose Calculation (a black line showing <br> the specified response) on the chart. |

## Dose-Response Curves

| Doses | This specifies dose concentration values for each group of the specified Type. Click the drop-down to display the editor and specify doses settings. <br> Dose Replicates <br> Select the number of adjacent positions each dose value applies to. Dose Replicates are determined automatically and include all proper divisors (i.e., all divisors excluding itself) of the number of positions in each sample. <br> For example, for a $12 \times 8$ layout with 8 Unknown sample groups, each group contains 12 positions. Thus, Dose Replicates options include 1, 2, 3, 4, 6 . <br> Dose Concentrations <br> The left-hand Doses grid is used to select and indicate the dose value to edit. Select the dose to edit in a two-column grid. <br> The Sample column is numbered from 1 upward. The number of rows is updated according to the selected Dose Replicates option. This is the number of replicates in a sample divided by the selected Dose Replicates option. <br> In the Dose column, click the dose to edit its value. There are two modes for specifying dose values: <br> - Series - Provide dose for the first sample of the specified Type and common series to apply to all samples. <br> - Non-Serial - Provide a specific dose value for each sample separately. <br> Click the Series button to switch between modes. |
| :---: | :---: |
|  | All sample groups are assumed to have the same number of positions defined on the layout (e.g., if Unknown1 has 10 replicates, then all Unknowns on the layout must also have 10 replicates). |
|  | Paste Option <br> You can copy and paste the clipboard contents to the Dose column. The content is pasted starting from the first row on a line by line basis. This is useful if you have your dose values in another application (such as Excel or notepad). |
|  | When you click Paste, the mode automatically switches to Non- |


|  | Serial. |
| :--- | :--- |
| Response <br> for Dose <br> Calculation | A numerical value or an expression representing the response value to <br> calculate the dose for each sample. |
| Type | The sample type to use for dose calculation. |

## Fit

| Fit Method | The curve-fitting method to construct a dose-response curve for your data based on the specified Type. |
| :---: | :---: |
| Weight Method | Specifies the weight function to use in the curve-fitting method. |
| X Transform | Determines how the $x$ values are interpreted in the transform: <br> - None - The $x$ values remain as they are. <br> - Log10 - Each $x$ point is taken as a common logarithm of $x$. <br> - Log2 - Each $x$ point is taken as a binary logarithm of $x$. <br> - LogN - Each $x$ point is taken as a natural logarithm of $x$. |
| Y <br> Transform | Determines how the $y$ values are interpreted in the transform: <br> - None - The $y$ values remain as they are. <br> - Log10 - Each $y$ point is taken as a common logarithm of $y$. <br> - Log2 - Each $y$ point is taken as a binary logarithm of $y$. <br> - LogN - Each $y$ point is taken as a natural logarithm of $y$. |

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to fit a dose-response curve to each sample group of the Unknown type and calculate a dose that has a response of 1.5 from the curve. Here is some example raw data displayed here to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.000 | 2.682 | 2.345 | 2.612 | 2.732 | 2.675 | 2.664 | 2.574 | 2.580 | 2.361 | 0.871 | 2.839 |
| $\mathbf{B}$ | 0.000 | 2.577 | 1.735 | 2.518 | 2.701 | 2.537 | 2.571 | 2.450 | 2.453 | 2.171 | 0.437 | 2.809 |
| $\mathbf{C}$ | 0.000 | 2.537 | 1.290 | 2.497 | 2.683 | 2.447 | 2.490 | 2.273 | 2.344 | 1.695 | 0.222 | 2.783 |
| $\mathbf{D}$ | 0.000 | 2.358 | 0.793 | 2.318 | 2.585 | 2.195 | 2.205 | 1.856 | 2.069 | 1.070 | 0.094 | 2.658 |
| $\mathbf{E}$ | 0.000 | 1.958 | 0.369 | 1.799 | 2.224 | 1.636 | 1.661 | 1.192 | 1.485 | 0.550 | 0.037 | 2.322 |
| $\mathbf{F}$ | 0.000 | 1.352 | 0.151 | 1.118 | 1.517 | 0.909 | 0.947 | 0.638 | 0.868 | 0.257 | 0.013 | 1.495 |
| $\mathbf{G}$ | 0.000 | 0.745 | 0.053 | 0.540 | 0.872 | 0.425 | 0.485 | 0.282 | 0.417 | 0.094 | 0.002 | 0.862 |
| $\mathbf{H}$ | 0.000 | 0.312 | 0.018 | 0.250 | 0.398 | 0.180 | 0.200 | 0.113 | 0.194 | 0.038 | 0.001 | 0.438 |

## Layout

The example uses a layout with one Unused column (in positions from $\mathbf{A 1}$ to $\mathbf{H 1}$ ) and Unknowns in columns (e.g., Unknown1 at positions from A2 downward to H2,
Unknown2 at A3-H3, etc.).:

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |  |
| B | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |  |
| C | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |  |
| D | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |  |
| E | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |  |
| F | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |  |
| G | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |  |
| H | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |  |

To create a curve and calculate concentrations for this matrix, specify the following settings:

1. Set $\mathbf{X}$ Axis Type to Logarithmic.
2. Set $\mathbf{X}$ Transform to None.
3. Set $\mathbf{Y}$ Axis Type to Linear.
4. Set $\mathbf{Y}$ Transform to None.
5. Select the Plot Response for Dose Calculation check box.
6. In the Doses editor, set Dose for the Sample $\mathbf{1}$ to 0.01 and Series to multiply by 3, and select Dose Replicates of 1 .
7. Set a Response for Dose Calculation to 1.5.
8. Set Type to Unknown.
9. Set Fit Method of 4PL.
10. Select a Weight Method of None.
11. Select an $\mathbf{X}$ Transform of None.
12. Select a $\mathbf{Y}$ Transform of None.

The transform generates a Dose-Response Curves Table and a Dose-Response Curve Chart by default. Other elements such as Matrix table and Notes can be added to report optionally.

## Dose-Response Curves Table

This table lists coefficients calculated from the selected Fit Method and a resulting response value for each sample. With the example data provided above, the results are as follows:

| Sample | $\mathbf{a}$ | $\mathbf{b}$ | $\mathbf{c}$ | $\mathbf{d}$ | $\mathbf{R}^{\mathbf{2}}$ | Result |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| OUnknown1 | 2.67099 | 0.875198 | 2.65478 | -0.0584816 | 0.999497 | 1.91503 |
| OUnknown2 | 3.29931 | 0.606878 | 0.0441357 | -0.0901615 | 0.997736 | 0.0541024 |
| OUnknown3 | 2.59484 | 1.07281 | 1.69869 | 0.103291 | 0.999371 | 1.35375 |
| OUnknown4 | 2.73875 | 1.10415 | 2.7794 | 0.176798 | 0.999281 | 2.61824 |
| OUnknown5 | 2.64392 | 0.998377 | 1.25839 | 0.0355624 | 0.999147 | 0.982572 |
| OUnknown6 | 2.66458 | 0.980399 | 1.28776 | 0.0581275 | 0.999619 | 1.03568 |
| OUnknown7 | 2.60957 | 0.929062 | 0.682855 | 0.0190159 | 0.999764 | 0.500446 |
| OUnknown8 | 2.57555 | 0.936045 | 1.10936 | 0.0468791 | 0.999425 | 0.804401 |
| OUnknown9 | 2.53025 | 0.919968 | 0.194091 | 0.53025 | 0.999769 | 0.13033 |
| OUnknown10 | 6.05263 | 0.681115 | 0.000740073 | -0.00938084 | 0.999738 | 0.00374295 |
| OUnknown11 | 2.83485 | 1.20751 | 2.35471 | 0.298789 | 0.999004 | 2.56967 |

## Dose-Response Curve Chart

The chart contains a curve fit plotted with 4PL for dose and for each sample group.


## Dose-Response Curve Data Tables

For each fitting, these tables list the measurement and calculation details for the calibration doses used in the fit. Here is a snippet of the Dose-Response Curves Data Table for Dose 1 to 4 of Unknown1 (using the data provided above):

| Calibrator | Positions | Dose (Defined) | Raw | Dose (Calc.) | \%CV (Dose Calc.) | \% Accuracy |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| OUnknown1 (Dose <br> $1)$ | A2 | 0.01 | 2.682 | (NA) | 0 | (NA) |
| OUnknown1 (Dose <br> 2) | B2 | 0.03 | 2.577 | 0.0588537 | 0 | 196.179 |
| OUnknown1 (Dose <br> 3) | C2 | 0.09 | 2.537 | 0.089809 | 0 | 99.7878 |
| OUnknown1 (Dose <br> $4)$ | D2 | 0.27 | 2.358 | 0.256915 | 0 | 95.1538 |

## Dose

The output matrix named Dose can be optionally added to the report. It contains the calculated dose that has a response of 1.5 for each data point. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | Flagged | 1.915 | 0.054 | 1.354 | 2.618 | 0.983 | 1.036 | 0.500 | 0.804 | 0.130 | 0.004 | 2.570 |
| $\mathbf{B}$ | Flagged | 1.915 | 0.054 | 1.354 | 2.618 | 0.983 | 1.036 | 0.500 | 0.804 | 0.130 | 0.004 | 2.570 |


|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{C}$ | Flagged | 1.915 | 0.054 | 1.354 | 2.618 | 0.983 | 1.036 | 0.500 | 0.804 | 0.130 | 0.004 | 2.570 |
| $\mathbf{D}$ | Flagged | 1.915 | 0.054 | 1.354 | 2.618 | 0.983 | 1.036 | 0.500 | 0.804 | 0.130 | 0.004 | 2.570 |
| $\mathbf{E}$ | Flagged | 1.915 | 0.054 | 1.354 | 2.618 | 0.983 | 1.036 | 0.500 | 0.804 | 0.130 | 0.004 | 2.570 |
| $\mathbf{F}$ | Flagged | 1.915 | 0.054 | 1.354 | 2.618 | 0.983 | 1.036 | 0.500 | 0.804 | 0.130 | 0.004 | 2.570 |
| $\mathbf{G}$ | Flagged | 1.915 | 0.054 | 1.354 | 2.618 | 0.983 | 1.036 | 0.500 | 0.804 | 0.130 | 0.004 | 2.570 |
| $\mathbf{H}$ | Flagged | 1.915 | 0.054 | 1.354 | 2.618 | 0.983 | 1.036 | 0.500 | 0.804 | 0.130 | 0.004 | 2.570 |

Example: Calculate $\mathrm{EC}(20), \mathrm{EC}(50)$ and $\mathrm{EC}(80)$ from Each Dose-Response

## Curve

Using this transform, you can compute specific results from each dose-response curve and report them in a Dose-Response Fit Table.
Here is an example of how to compute $\mathrm{EC}(20), \mathrm{EC}(50)$ and $\mathrm{EC}(80)$ from each dose-response curve taking the $y$-axis as the \% with $c^{*}(((a-d) /(E C(n)-d))-1)^{\wedge}(1 / b)$ expression, where $E C(n)$ is the percentage value to calculate. This example uses the measurements data and layout from the Dose-Response Curves example to illustrate the results.

Assuming you have already added Dose-Response Curves transform, follow these steps to evaluate effective concentrations:

1. In the Assay Properties panel, select the XML tab.

If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Add the Dose-Response Fit Table.
3. Add evaluations as follows:

Since the coefficient values in all curve fits from the example above are small, we add abs function to the formula to avoid negative results. So, the expression in this case is $c^{\star} \mathbf{a b s}(((a-d) /(E C(n)-d))-1)^{\wedge}(1 / b)$.
<FitTable>
<Evaluation Description="EC(20)" Expression="c*abs(((a-d)/(20-d))-1)^ (1/b)"/>
<Evaluation Description="EC(50)" Expression="c*abs(((a-d)/(50-d))-1)^ (1/b)"/>
<Evaluation Description="EC(80)" Expression="c*abs(((a-d)/(80-d))-1)^ (1/b)"/>

## </FitTable>

You can also optionally label the results in the table and change their
(1) numerical formatting. For details, see Advanced Dose-Response Curves Evaluations.
4. Add Dose-Response Fit Table to report.
5. Click Apply to accept changes and then click Calculate.

Here is a snippet that shows the calculated EC(n) results for Unknown1 curve:

| Sample | EC(20) | EC(50) | EC(80) |
| :--- | :--- | :--- | :--- |
| OUnknown1 | 2.24619 | 2.49004 | 2.55162 |

## Example: Use Area under Curve to Estimate the Difference between Dose-

## Response Curves

You also can assess and compare the areas under the fitted dose-response curves using auc function. This is handy for comparing curves with small number of doses, when determining EC50 (or IC50), slope, and maximum effect (or maximum inhibition) with reasonable precision is not always possible. With this, you can capture additional information about differential sensitivity (response).

To learn more about the auc function and its syntax, see Calculation Functions.
To evaluate area under curve (AUC) for each dose-response curve and assuming that you have already added Dose-Response Curves transform, follow these steps to calculate the areas under plotted curves:

1. In the Assay Properties panel, select the XML tab.

If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Add the Dose-Response Fit Table.
3. Add evaluation:

## <FitTable>

<Evaluation Description="Area Under Curve" Expression="auc ('4PL',dosemin,dosemax,1000,a,b,c,d)"/> </FitTable>
4. Add Dose-Response Fit Table to report.
5. Click Apply to accept changes and then click Calculate to generate new results.

Here is a snippet that shows the calculated area under Unknown1 curve (using the example data from the Dose-Response Curves example above):

| Sample | AUC |
| :--- | :--- |
| OUnknown1 | 16.0385 |

## Expression by Matrix (Dual)

## Input: Dual Endpoint $\Rightarrow$ Output: Endpoint

This transform evaluates an expression defined in terms of $x$ and $y$ for all data points (excluding flagged samples). $\boldsymbol{x}$ refers to the data for the sample on the first input matrix, and $\boldsymbol{y}$ refers to the data for the same sample on the second input matrix.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

Dual Matrix The expression to evaluate, where $x$ and $y$ variables are set to value of each Expression position of the input matrices

If the expression does not contain the $x$ and $y$ variables, the transform will output the

(1)same evaluation result for all positions. For example, if you enter $\left\{2^{\wedge} 3+4\right\}$, the resulting matrix will contain 12 in each position.

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw (1) and Raw (2) matrices. In this example, we would like to summarize position values in these matrices.

Here is some example raw data displayed to 3 decimal places:
Raw (1)

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

Raw (2)

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.204 | 0.201 | 0.213 | 0.209 | 0.206 | 0.208 | 0.216 | 0.217 | 0.216 | 0.213 | 0.205 | 0.218 |
| $\mathbf{B}$ | 0.216 | 0.200 | 0.214 | 0.214 | 0.204 | 0.201 | 0.215 | 0.216 | 0.203 | 0.219 | 0.207 | 0.204 |
| $\mathbf{C}$ | 0.206 | 0.210 | 0.208 | 0.203 | 0.201 | 0.218 | 0.212 | 0.217 | 0.216 | 0.213 | 0.208 | 0.204 |
| $\mathbf{D}$ | 0.215 | 0.215 | 0.214 | 0.203 | 0.214 | 0.213 | 0.219 | 0.219 | 0.213 | 0.201 | 0.202 | 0.219 |
| $\mathbf{E}$ | 0.215 | 0.220 | 0.219 | 0.212 | 0.210 | 0.211 | 0.214 | 0.214 | 0.201 | 0.202 | 0.202 | 0.207 |
| F | 0.214 | 0.212 | 0.202 | 0.200 | 0.201 | 0.217 | 0.213 | 0.209 | 0.218 | 0.207 | 0.216 | 0.216 |
| $\mathbf{G}$ | 0.211 | 0.211 | 0.218 | 0.209 | 0.214 | 0.213 | 0.201 | 0.211 | 0.205 | 0.210 | 0.201 | 0.212 |
| $\mathbf{H}$ | 0.216 | 0.209 | 0.215 | 0.206 | 0.218 | 0.206 | 0.209 | 0.219 | 0.215 | 0.219 | 0.216 | 0.217 |

To add values from Raw(2) to corresponding values of Raw(1), specify an expression of $\boldsymbol{x}+\boldsymbol{y}$.

## Calculated

The transform will generate an output matrix named Calculated with the value at each position being the point by point sum of the Raw (1) and Raw (2) matrices values. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.568 | 0.524 | 2.318 | 2.333 | 1.419 | 1.434 | 1.375 | 1.407 | 0.326 | 0.329 | 1.668 | 1.699 |
| $\mathbf{B}$ | 0.671 | 0.641 | 2.083 | 2.102 | 1.407 | 1.419 | 1.395 | 1.422 | 0.335 | 0.349 | 0.993 | 0.972 |
| $\mathbf{C}$ | 0.793 | 0.785 | 0.682 | 0.717 | 1.407 | 1.457 | 1.415 | 1.427 | 0.329 | 0.329 | 0.821 | 0.830 |
| $\mathbf{D}$ | 1.021 | 0.989 | 0.578 | 0.581 | 1.381 | 1.400 | 1.430 | 1.460 | 0.314 | 0.307 | 0.691 | 0.709 |
| $\mathbf{E}$ | 1.320 | 1.285 | 0.498 | 0.579 | 1.425 | 1.436 | 1.447 | 1.476 | 0.312 | 0.316 | 1.781 | 1.756 |
| F | 1.639 | 1.657 | 0.580 | 0.611 | 1.397 | 1.423 | 1.439 | 1.408 | 0.327 | 0.315 | 1.008 | 0.991 |
| $\mathbf{G}$ | 2.002 | 2.014 | 0.778 | 0.806 | 1.419 | 1.441 | 1.509 | 1.517 | 0.331 | 0.337 | 0.853 | 0.871 |
| $\mathbf{H}$ | 0.469 | 0.479 | 0.678 | 0.676 | 1.432 | 1.467 | 0.324 | 0.335 | 0.330 | 0.334 | 0.706 | 0.694 |

## Expression by Matrix

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform evaluates an expression defined in terms of $x$ for all data points (excluding flagged samples).

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Matrix <br> Expression | The expression to evaluate, where a single $x$ variable is set to value of each <br> position of the input matrix |
| :--- | :--- |

If the expression does not contain the $x$ variable, the transform will output the same

(1)evaluation result for all positions. For example, if you enter $\left\{\mathbf{2}^{\wedge} \mathbf{3 + 4}\right\}$, the resulting matrix will contain 12 in each position.

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to square each measurement.

Here is some example raw data displayed to 3 decimal places:
Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

The Matrix Expression setting is specified with expression of $\boldsymbol{x}^{\boldsymbol{*}} \boldsymbol{x}$.

## Calculated

The transform will generate an output matrix named Calc with the value at each position being the square of the input data. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.132 | 0.104 | 4.431 | 4.511 | 1.471 | 1.503 | 1.343 | 1.416 | 0.012 | 0.013 | 2.140 | 2.193 |
| $\mathbf{B}$ | 0.207 | 0.194 | 3.493 | 3.565 | 1.447 | 1.484 | 1.392 | 1.454 | 0.017 | 0.017 | 0.618 | 0.590 |
| $\mathbf{C}$ | 0.345 | 0.331 | 0.225 | 0.264 | 1.454 | 1.535 | 1.447 | 1.464 | 0.013 | 0.013 | 0.376 | 0.392 |
| $\mathbf{D}$ | 0.650 | 0.599 | 0.132 | 0.143 | 1.362 | 1.409 | 1.467 | 1.540 | 0.010 | 0.011 | 0.239 | 0.240 |
| $\mathbf{E}$ | 1.221 | 1.134 | 0.078 | 0.135 | 1.476 | 1.501 | 1.520 | 1.593 | 0.012 | 0.013 | 2.493 | 2.399 |
| $\mathbf{F}$ | 2.031 | 2.088 | 0.143 | 0.169 | 1.430 | 1.454 | 1.503 | 1.438 | 0.012 | 0.012 | 0.627 | 0.601 |
| $\mathbf{G}$ | 3.208 | 3.251 | 0.314 | 0.356 | 1.452 | 1.508 | 1.711 | 1.706 | 0.016 | 0.016 | 0.425 | 0.434 |
| $\mathbf{H}$ | 0.064 | 0.073 | 0.214 | 0.221 | 1.474 | 1.590 | 0.013 | 0.013 | 0.013 | 0.013 | 0.240 | 0.228 |

## Expression by Position

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform enables an expression to be defined for each position in terms of $x$ (where $x$ refers to the value of position on the input matrix). This is useful for applying various tests to different samples simultaneously.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

This allows different expressions to be configured and calculated on the positions of the same index on different plates. Expressions are stored by container (1-based values are used for each container separately).

Click drop-down to open a Position Expressions Editor with grid-based view of input data. The configuration of the grid depends on the selected layout, thus each position's background is coloured to match its sample type.

|  | If the protocol contains $>1$ plate/analyte, select the one to specify expressions <br> for from a drop-down list in the upper right corner of the Editor. To maximize <br> its view, click the arrow button in the lower left corner of the Editor. <br> Within each grid you can select and specify an expression in terms of $x$ for: <br> - A single position. <br> - Multiple positions (within a single plate) to set to a same expression. <br> Position <br> Expressions |
| :--- | :--- |
| - Dragging the mouse (for rectangular selection) <br> - Using Ctrl to select random positions <br> - Using Shift to select the end position (rectangular selection) |  |
| (1) Paste, Copy, and Clear commands are supported. |  |
| For positions with default (simply $x$ ) or empty expression, the output position <br> is equal to the input position. |  |

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to multiply positions from 1 to 5 by 10 , positions 10 and 96 by 100, and position 50 by 2 .

Here is some example raw data displayed to 3 decimal places:
Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

Provide expressions for positions in Position Expressions Editor:

- A1, A2, A3, A4, A5 with the expression $\boldsymbol{x}^{*} \mathbf{1 0}$
- A10 and $\mathbf{H} 12$ with the expression $\boldsymbol{x}^{*} \mathbf{1 0 0}$
- E2 with the expression $\boldsymbol{x}$ *2


## Calculated

The transform will generate an output matrix named Calculated with the resulting values being the product of input data and specified expressions. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 3.640 | 3.230 | 21.050 | 21.240 | 12.130 | 1.226 | 1.159 | 1.190 | 0.110 | 11.600 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 2.130 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 47.700 |

## Expression by Sample Group

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform enables an expression to be defined for each sample group of one of more sample types in terms of $x$ (where $x$ refers to the value of position on the input matrix). This is useful for applying different calculations to each sample group.

The Expression by Sample Group transform is an Import Target. This means that it in addition to configuration through the Property Editor, it supports configuration through the Import Wizard, command line arguments or section data. For example, values or expressions to associate with each sample group can be automatically imported from a file. This is useful if your analysis depends on data values stored in a data file which vary from run-to-run; MyAssays Desktop can automatically import those values and use them as part of your data analysis.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

|  | Specify an expression to calculate for each sample group on the selected <br> layout. Click the drop-down to display the editor, where you can enter an <br> expression in terms of $x$ for each sample group. |
| :--- | :--- |
| Expression <br> by Sample <br> Group | Paste, Copy, and Cut commands are supported. |
| For types with simply $x$ (or empty expression), the output value is equal to the <br> input value of the group. |  |
| Sample | Select which sample type or types an expression can be entered for. An <br> expression can be provided for each group of the selected types. |

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to multiply Unknown1 by 2 and Unknown2 by 5.

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

## Layout

The example uses a layout with Standards, Control, Blank, and Unknowns in duplicate (e.g., Standard1 in A1 and A2, Unknown1 at A3 and A4, Unknown2 at B3 and B4, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | 2 | 2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | 3 | 3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | 4 | 4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | 5 | 5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | 6 | 6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | 1 | 1 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | $(31$ | 39 | 39 |
| H | 1 | 1 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | 40 | 40 |

Standard $\bigcirc$ Blank $\bigcirc$ Control $\bigcirc$ Unknown

Select Unknown as the Sample Types
In Expressions by Sample Group Editor, provide:

- Unknown1 type with the expression $\boldsymbol{x}^{*} \mathbf{2}$
- Unknown2 with the expression $\boldsymbol{\boldsymbol { * } ^ { * } \mathbf { 5 }}$


## Calculated

The transform will generate an output matrix named Calculated with the resulting values of each specified type evaluated with provided expressions. With the example data provided above, the results are as follows (also displayed here to 3 decimal places). Here it can be seen that the values for Unknown1 (A3+A4) and Unknown2 (B3+B4) have been computed using the expressions provided.

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 4.210 | 4.248 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 9.345 | 9.440 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

## Advanced: Section Data

To add a section control for editing Expression by Sample Group expressions, add a section to the assay.xml as follows:

```
<section id="Parameters" type="analysis" customName="Values">
<ExpressionBySampleGroup TransNum="1" DataTitle="Custom Title"
Description="Custom Description" />
```


## </section>

Set TransNum to the 1-based transform number of the transform to associate the control with.

The optional ExpressionBySampleGroup attribute ShowSampleTypesSelector can be used to show or hide the Sample Types selector control.

## Expression by Sample Replicates

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform enables an expression to be defined in terms of each replicate within each sample group (where $a$ refers to the first replicate's value and $b$ refers to the second, etc.). This is useful for applying calculations within each sample group.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Replicates <br> Expression | Specify an expression to calculate for each sample in terms of the replicates of <br> each sample. <br> $a=1$ st replicate <br> $b=2$ nd replicate <br> $c=3$ rd replicate <br> etc. <br> Replicate ordering follows row-wise ordering. For example, if a <br> sample group is replicated at positions A1, A2, B1 and B2, then the <br> expression variables correspond as follows: <br> $a=\mathrm{A} 1, b=\mathrm{A} 2, \mathrm{C}=\mathrm{B} 1, d=\mathrm{B} 2$ |
| :--- | :--- |

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. All samples are arranged in duplicate. In this example, we would like to compute \%D of each sample - that is the absolute difference between the replicates divided by the sum of the replicates.

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

## Layout

The example uses a layout with Standards, Control, Blank, and Unknowns in duplicate (e.g., Standard1 in A1 and A2, Unknown1 at A3 and A4, Unknown2 at B3 and B4, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | 2 | 2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | 3 | 3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | 4 | 4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | 5 | 5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | 6 | 6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | 1 | 1 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | $(31$ | 39 | 39 |
| H | 1 | 1 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | 40 | 40 |

Standard $\bigcirc$ Blank $\bigcirc$ Control $\bigcirc$ Unknown

In Replicates Expression enter the \%D expression which is: 100*abs(a-b)/(a+b)

## Calculated

The transform will generate an output matrix named Calculated with the resulting values of each specified type evaluated with provided expressions. With the example data provided above, the results are as follows displayed here to 2 decimal places). Here it can be seen that the values for all samples have been computed in accordance with the defined \%D calculation:

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 5.97 | 5.97 | 0.45 | 0.45 | 0.53 | 0.53 | 1.32 | 1.32 | 2.65 | 2.65 | 0.61 | 0.61 |
| $\mathbf{B}$ | 1.56 | 1.56 | 0.51 | 0.51 | 0.62 | 0.62 | 1.09 | 1.09 | 0.76 | 0.76 | 1.16 | 1.16 |
| $\mathbf{C}$ | 1.03 | 1.03 | 4.05 | 4.05 | 1.35 | 1.35 | 0.29 | 0.29 | 1.31 | 1.31 | 1.05 | 1.05 |
| $\mathbf{D}$ | 2.03 | 2.03 | 1.89 | 1.89 | 0.85 | 0.85 | 1.22 | 1.22 | 2.42 | 2.42 | 0.10 | 0.10 |
| $\mathbf{E}$ | 1.84 | 1.84 | 13.62 | 13.62 | 0.41 | 0.41 | 1.16 | 1.16 | 1.33 | 1.33 | 0.96 | 0.96 |
| $\mathbf{F}$ | 0.70 | 0.70 | 4.18 | 4.18 | 0.42 | 0.42 | 1.11 | 1.11 | 0.46 | 0.46 | 1.08 | 1.08 |
| $\mathbf{G}$ | 0.33 | 0.33 | 3.20 | 3.20 | 0.95 | 0.95 | 0.08 | 0.08 | 0.40 | 0.40 | 0.53 | 0.53 |
| $\mathbf{H}$ | 3.25 | 3.25 | 0.75 | 0.75 | 1.90 | 1.90 | 0.43 | 0.43 | 0.00 | 0.00 | 1.34 | 1.34 |

## Expression by Sample Type

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform enables an expression to be defined for each sample type in terms of $x$ (where $x$ refers to the value of position on the input matrix). This is useful for applying different calculations to each sample type.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.
Properties

## Settings

|  | Specify an expression to calculate for each sample type on the selected layout. <br> Click the drop-down to display the editor, where you can enter an expression <br> in terms of $x$ for the selected type. |
| :--- | :--- |
| Expression |  |
| by Sample |  |
| Type |  |$\quad$| Paste, Copy, and Cut commands are supported. |
| :--- |

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to multiply standards by 2 and unknowns by 5.

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

## Layout

The example uses a layout with Standards, Control, Blank, and Unknowns in duplicate (e.g., Standard1 in A1 and A2, Unknown1 at A3 and A4, Unknown2 at B3 and B4, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | 2 | 2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | 3 | 3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | 4 | 4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | 5 | 5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | 6 | 6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | 1 | 1 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | $(31$ | 39 | 39 |
| H | 1 | 1 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | 40 | 40 |

Standard $\bigcirc$ Blank $\bigcirc$ Control $\bigcirc$ Unknown
In Expressions by Sample Type Editor, provide:

- Standard type with the expression $\boldsymbol{x}^{\boldsymbol{*}} \mathbf{2}$
- Unknown with the expression $\boldsymbol{X}^{*} \mathbf{5}$


## Calculated

The transform will generate an output matrix named Calculated with the resulting values of each specified type evaluated with provided expressions. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.728 | 0.646 | 10.525 | 10.620 | 6.065 | 6.130 | 5.795 | 5.950 | 0.550 | 0.580 | 7.315 | 7.405 |
| $\mathbf{B}$ | 0.910 | 0.882 | 9.345 | 9.440 | 6.015 | 6.090 | 5.900 | 6.030 | 0.660 | 0.650 | 3.930 | 3.840 |
| $\mathbf{C}$ | 1.174 | 1.150 | 2.370 | 2.570 | 6.030 | 6.195 | 6.015 | 6.050 | 0.565 | 0.580 | 3.065 | 3.130 |
| $\mathbf{D}$ | 1.612 | 1.548 | 1.820 | 1.890 | 5.385 | 5.935 | 6.055 | 6.205 | 0.505 | 0.530 | 2.445 | 2.450 |
| $\mathbf{E}$ | 2.210 | 2.130 | 1.395 | 1.385 | 6.075 | 6.125 | 6.165 | 6.310 | 0.555 | 0.570 | 7.895 | 7.745 |
| $\mathbf{F}$ | 2.850 | 2.890 | 1.890 | 2.055 | 5.980 | 6.030 | 6.130 | 5.995 | 0.545 | 0.540 | 3.960 | 3.875 |
| $\mathbf{G}$ | 1.791 | 1.803 | 2.800 | 2.985 | 6.025 | 6.140 | 6.540 | 6.530 | 0.630 | 0.635 | 3.260 | 3.295 |
| $\mathbf{H}$ | 0.253 | 0.270 | 2.315 | 2.350 | 6.070 | 6.305 | 0.575 | 0.580 | 0.575 | 0.575 | 2.450 | 2.385 |

## Factor

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform multiplies data points by a common factor. The factor can be specified as a numeric value or as an expression to evaluate.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Factor | The value to multiply all data points by. This can be specified as a number or <br> an expression to evaluate. |
| :--- | :--- |

## Example:

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to multiply each measurement by a common factor of 2.

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

The Factor setting for this example is specified as 2.

## Factored

The transform will generate an output matrix named Factored with the value at each position being the product of the input data and the factor. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.728 | 0.646 | 4.210 | 4.248 | 2.426 | 2.452 | 2.318 | 2.380 | 0.220 | 0.232 | 2.926 | 2.962 |
| $\mathbf{B}$ | 0.910 | 0.882 | 3.738 | 3.776 | 2.406 | 2.436 | 2.360 | 2.412 | 0.264 | 0.260 | 1.572 | 1.536 |
| $\mathbf{C}$ | 1.174 | 1.150 | 0.948 | 1.028 | 2.412 | 2.478 | 2.406 | 2.420 | 0.226 | 0.232 | 1.226 | 1.252 |
| $\mathbf{D}$ | 1.612 | 1.548 | 0.728 | 0.756 | 2.334 | 2.374 | 2.422 | 2.482 | 0.202 | 0.212 | 0.978 | 0.980 |
| $\mathbf{E}$ | 2.210 | 2.130 | 0.558 | 0.734 | 2.430 | 2.450 | 2.466 | 2.524 | 0.222 | 0.228 | 3.158 | 3.098 |
| $\mathbf{F}$ | 2.850 | 2.890 | 0.756 | 0.822 | 2.392 | 2.412 | 2.452 | 2.398 | 0.218 | 0.216 | 1.584 | 1.550 |
| $\mathbf{G}$ | 3.582 | 3.606 | 1.120 | 1.194 | 2.410 | 2.456 | 2.616 | 2.612 | 0.252 | 0.254 | 1.304 | 1.318 |
| $\mathbf{H}$ | 0.506 | 0.540 | 0.926 | 0.940 | 2.428 | 2.522 | 0.230 | 0.232 | 0.230 | 0.230 | 0.980 | 0.954 |

## General Curve Fit

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

The General Curve Fit transform is suitable for performing a curve fit on any set of data with the calibrator points being defined as values, expressions or references to sample groups.
For calculating concentrations from a standards curve, use the Standard Curve Fit transform.

This transform:

1. Plots specified calibration data ( $x$ and $y$ values). These might be numeric values or expressions that can refer to samples on specific matrices or containers.
2. Fits a curve to the calibration data (optionally using weight methods).
3. Uses the resulting curve to predict either $x$ or $y$ value on the curve at each $y$ or $x$ input value for selected sample types.

For multiple plate and multiplex assays, a single curve is plotted, used to compute values for all containers, and included in the report.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Axes

| X Axis Title | The name of the x -axis on the chart. |
| :--- | :--- |
|  | The scale type to be used for the x -axis: |
| X Axis Type | - Logarithmic <br> - Linear |
| $\mathbf{Y}$ Axis Title | The name of the y -axis on the chart. |


| Y Axis Type | The scale type to be used for the y-axis: <br> - Logarithmic |
| :--- | :--- |
|  | When y-axis is logged, negative (or zero) $y$ values are not <br> included in the plot. |

## Axes (Advanced)

Use these additional settings to adapt the chart's appearance to your needs.

| X Axis Major Units | The numeric interval between numbers on the $x$-axis. Starting from the minimum value, a major unit is displayed at every increment. |
| :---: | :---: |
| $X$ Axis Minor Units | The numeric interval between supplementary tickmarks on the $x$-axis. The minor units are not displayed unless this attribute is specified. |
| X Axis <br> Numerical <br> Formatting | The formatting for the X Axis Major Units values. <br> To learn about available formatting options, see Custom Format Codes for Numbers of the Chart. |
| X Crosses Y <br> At | Select where the $x$-axis will cross the $y$-axis from the drop-down: <br> - Automatic - Determine the location that makes the most sense for your data automatically. <br> - Axis Value - Cross y-axis at specified $\mathbf{X}$ Crosses $\mathbf{Y}$ at Value. <br> - Maximum Axis Value - Cross y-axis at the maximum $y$ value of the chart. <br> - Minimum Axis Value - Cross y-axis at the minimum $y$ value. If axis type is set to Logarithmic, then it is the lowest positive value. |
| X Crosses Y at Value | The numerical value at which the $x$-axis will cross the $y$-axis. This value must be in the range of calculated results. If axis type is set to logarithmic, the provided value must be greater than 0. |
| Y Axis Major Units | The numeric interval between the gridlines on the $y$-axis. Starting from the minimum value, a major unit is displayed at every increment. |
| Y Axis Minor Units | The numeric interval between supplementary tickmarks on the $y$-axis. The minor units are not displayed unless this attribute is specified. |


| Y Axis Numerical Formatting | The formatting for the $\mathbf{Y}$ Axis Major Units values. <br> To learn about available formatting options, see Custom Format Codes for Numbers of the Chart. |
| :---: | :---: |
| $\begin{aligned} & \text { Y Crosses X } \\ & \text { At } \end{aligned}$ | Select where the $y$-axis will cross the $x$-axis from the drop-down: <br> - Automatic - Determine the location that makes the most sense for your data automatically. <br> - Axis Value - Cross $x$-axis at specified $\mathbf{Y}$ Crosses $\mathbf{X}$ at Value. <br> - Maximum Axis Value - Cross $x$-axis at the maximum $x$ value of the chart. <br> - Minimum Axis Value - Cross $x$-axis at the minimum $x$ value. If axis type is set to Logarithmic, then it is the lowest positive value. |
| Y Crosses X at Value | The numerical value at which the $y$-axis will cross the $x$-axis. This value must be in the range of calculated results. If axis type is set to logarithmic, the provided value must be greater than 0 . |

These settings can be also applied to other charts as an advanced configuration. To learn more, see Change the Units and Number Format of the Charts and Customize $X$ and $Y$ Axes Positions for Curve Charts topics.

## Calibration Data



## Chart

| Points on | In the drop-down editor, select any sample types to plot on the curve; this is <br> useful to show where samples fall on the curve. The computed results of each <br> selected sample will be plotted. In this case, all successfully computed non- <br> flagged sample groups of the selected types will be displayed on the chart <br> and in the report. |
| :--- | :--- |

## Fit

| Calculate From | Defines how to calculate the output matrix results from the computed curve for each sample (i.e., is a result calculated from the curve for each replicate or for the average of the replicates?). Select either: <br> - Replicates - Every position will contain the result calculated from value of every Predict Input replicate. <br> - Average - Replicates from the same sample contain the result calculated from averaged value of Predict Input replicates. |
| :---: | :---: |
|  | (1) Any flagged positions on the input are also flagged in the output. |
| Fit <br> Expression | When using the Custom Fit, you can enter or paste the equation to create your curve fit. |
| Fit Method | The curve-fitting method to construct a curve for your data. You can also use the Best Fit to plot the most appropriate fit for your data automatically or the Custom Fit to supply your own equation or model to fit the data. |
| Fit Methods | When using the Best Fit, this editor specifies the Fit Methods and Weight Methods to process your data with. You can select any combination of items in corresponding check box lists. |
|  | At least 1 check box must be selected in each list. |


| Fit Score Method | When the Best Fit feature is selected, to construct the curve, you can additionally specify the scoring method to be used in evaluating the goodness of applied fit methods: <br> - $R^{2}$ <br> - $R^{2}$ with Low-End Accuracy <br> - $R^{2}$ with High-End Accuracy <br> - $R^{2}$ with Low-End and High-End Accuracy <br> - 1/Standard-Error <br> - 1/P-Value <br> - 1/AIC <br> - 1/AICc <br> - 1/BIC |
| :---: | :---: |
| Weight Method | Specifies the weight function for the transform. |
| $x$ <br> Transform | Determines how the $x$ values are interpreted in the transform: <br> - None - The $x$ values remain as they are. <br> - Log10 - Each $x$ point is taken as a common logarithm of $x$. <br> - Log2 - Each $x$ point is taken as a binary logarithm of $x$. <br> - LogN - Each $x$ point is taken as a natural logarithm of $x$. |
| Y <br> Transform | Determines how the $y$ values are interpreted in the transform: <br> - None - The y values remain as they are. <br> - Log10 - Each $y$ point is taken as a common logarithm of $y$. <br> - Log2 - Each $y$ point is taken as a binary logarithm of $y$. <br> - LogN - Each $y$ point is taken as a natural logarithm of $y$. |

## General

| Predict | The resulting curve is used to make predictions of data by either calculating $x$ <br> or $y$ values for the input matrix. The output matrix contains the results of <br> these predictions. Select: |
| :--- | :--- |
| Input | -Y - To compute $x$ values from the resulting curve at each $y$ value in the <br> input matrix <br> - $\mathbf{X}$ - To compute $y$ values from the resulting curve at each $x$ value in the <br> input matrix |

## Example: Using General Curve Fit for a Standard Curve

Let's assume that we have endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to fit 4PL to the specified calibrators and calculate $x$ values for the resulting matrix.
The Standard Curve Fit transform could be used to perform this analysis more conveniently, however, this example demonstrates performing the same analysis using the General Curve Fit to illustrate how to refer to samples defined on the layout along with values.

## Raw

Here is some example raw data displayed to 3 decimal places:

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.192 | 0.199 | 0.347 | 0.346 | 0.325 | 0.321 | 0.803 | 0.797 | 1.589 | 1.593 | 0.241 | 0.246 |
| $\mathbf{B}$ | 0.293 | 0.300 | 0.586 | 0.584 | 0.800 | 0.793 | 0.402 | 0.401 | 0.839 | 0.837 | 0.562 | 0.564 |
| $\mathbf{C}$ | 0.537 | 0.534 | 0.549 | 0.547 | 0.878 | 0.875 | 0.471 | 0.471 | 0.759 | 0.756 | 1.039 | 1.043 |
| $\mathbf{D}$ | 0.858 | 0.855 | 0.320 | 0.325 | 0.531 | 0.530 | 1.552 | 1.551 | 0.429 | 0.427 | 0.424 | 0.424 |
| $\mathbf{E}$ | 1.523 | 1.527 | 0.486 | 0.490 | 1.289 | 1.283 | 1.430 | 1.438 | 1.494 | 1.501 | 1.427 | 1.429 |
| $\mathbf{F}$ | 1.622 | 1.621 | 0.621 | 0.626 | 0.667 | 0.663 | 1.350 | 1.347 | 0.631 | 0.634 | 1.695 | 1.694 |
| $\mathbf{G}$ | 0.001 | 0.001 | 0.356 | 0.352 | 0.418 | 0.412 | 1.172 | 1.174 | 1.572 | 1.570 | 0.595 | 0.590 |
| $\mathbf{H}$ | 0.297 | 0.297 | 0.796 | 0.801 | 1.517 | 1.515 | 0.960 | 0.965 | 0.534 | 0.527 | 0.585 | 0.584 |

## Layout

The example uses a layout with Standards, Control, Blank, and Unknowns in duplicate (e.g., Standard1 in A1 and A2, Unknown1 at A3 and A4, Unknown2 at B3 and B4, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | 2 | 2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | 3 | 3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | 4 | 4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | 5 | 5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | 6 | 6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | 1 | 1 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | 31 | 39 | 39 |
| H | 1 | 1 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | 40 | 40 |

Standard $\bigcirc$ Blank $\bigcirc$ Control $\bigcirc$ Unknown
To configure a General Curve Fit for this matrix, follow these steps:

1. Specify Calibration Data:

- $\mathbf{X = 4 0 0} \mathbf{Y}=$ Standard 1
- $\mathbf{X = 2 0 0} \mathbf{Y}=$ Standard2
- $\mathbf{X = 1 0 0} \mathbf{Y}=$ Standard3
- $\mathbf{X = 5 0 ~ Y = S t a n d a r d 4 ~}$
- $\mathbf{X = 2 5 ~ Y = S t a n d a r d 5 ~}$
- $\mathbf{X = 1 2 . 5} \mathbf{Y}=$ Standard6

2. Set Calculate From to Replicates.
3. Select a Fit Method of 4PL.
4. Select a Weight Method of None.
5. Set the $\mathbf{X}$ Transform to None.
6. Set the $\mathbf{Y}$ Transform to None.
7. Set Predict Input to $\mathbf{Y}$.

## Calculated

The transform will generate an output matrix named Calculated with $x$ values from the calibration curve for each $y$ value in the input matrix. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | Flagged | Flagged | 141.821 | 142.385 | 155.949 | 158.981 | 58.993 | 59.443 | 17.416 | 17.180 | 345.017 | 309.761 |
| $\mathbf{B}$ | 186.523 | 178.336 | 80.170 | 80.434 | 59.217 | 59.747 | 118.015 | 113.354 | 56.393 | 56.533 | 83.466 | 83.180 |
| $\mathbf{C}$ | 87.237 | 87.715 | 85.380 | 85.683 | 53.755 | 53.952 | 99.439 | 99.439 | 62.426 | 62.672 | 44.297 | 44.084 |
| $\mathbf{D}$ | 55.086 | 55.290 | 159.767 | 155.949 | 88.200 | 88.363 | 19.495 | 19.549 | 109.743 | 110.304 | 111.160 | 111.160 |
| $\mathbf{E}$ | 21.026 | 20.819 | 96.313 | 95.520 | 32.112 | 32.388 | 25.589 | 25.210 | 22.494 | 22.145 | 25.731 | 25.636 |
| $\mathbf{F}$ | 15.385 | 15.450 | 75.832 | 75.251 | 70.807 | 71.217 | 29.307 | 29.445 | 74.680 | 74.341 | 9.612 | 9.715 |
| $\mathbf{G}$ | Flagged | Flagged | 137.012 | 139.091 | 112.924 | 114.764 | 37.594 | 37.498 | 18.392 | 18.505 | 79.005 | 79.648 |
| $\mathbf{H}$ | 181.711 | 181.711 | 59.519 | 59.142 | 21.334 | 21.436 | 48.695 | 48.405 | 87.715 | 88.856 | 80.302 | 80.434 |

## Example: Variation Between Controls on 3 Plates of Data

In this example, we will show the variation of control values on different plates of data. To do so, we will fit the linear regression to the calibrators. For this example, we will use the control values on each plate $(y)$ and plate numbers $(x)$ the calibrators.

For conciseness, a small $3 \times 4$ layout with 3 plates of endpoint measurements is used.

## Raw

Here is some example raw data displayed to 3 decimal places:

| Plate 1 |  |  |  | Plate 2 |  |  |  |  | Plate 3 |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ |  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ |  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ |  |
| $\mathbf{A}$ | 1.503 | 1.490 | 1.499 | $\mathbf{A}$ | 1.521 | 1.521 | 1.523 | $\mathbf{A}$ | 1.501 | 1.500 | 1.497 |  |
| $\mathbf{B}$ | 1.801 | 1.849 | 1.838 | $\mathbf{B}$ | 0.927 | 0.893 | 0.959 | $\mathbf{B}$ | 1.094 | 1.076 | 1.074 |  |
| $\mathbf{C}$ | 1.824 | 1.871 | 1.781 | $\mathbf{C}$ | 1.866 | 1.917 | 1.958 | $\mathbf{C}$ | 1.028 | 1.031 | 1.072 |  |
| $\mathbf{D}$ | 0.509 | 0.519 | 0.494 | $\mathbf{D}$ | 1.731 | 1.677 | 1.693 | $\mathbf{D}$ | 2.046 | 2.105 | 2.070 |  |

## Layout

All plates use the same layout with 1 Control and 3 Unknown sample groups in triplicates (i.e., Control1 at A1,A2, A3; Unknown1 at B1, B2, B3, etc.).
Control ○ Unknown

To determine the variation between controls, follow these steps:

1. Specify Calibration Data:

- $\mathbf{X}=1 \mathbf{Y}=$ Control $1 . .1$
- $\mathbf{X}=2 \mathbf{Y}=$ Control $1 . .2$
- $\mathbf{X}=3 \mathbf{Y}=$ Control $1 . .3$

2. Set the Calculate From to Replicates.
3. Select a Fit Method of Linear Regression.
4. Select a Weight Method of None.
5. Set the $\mathbf{X}$ Transform to None.
6. Set the $\mathbf{Y}$ Transform to None.
7. Set the Predict Input to $\mathbf{Y}$.

## Chart

In this example, we are interested in seeing the variation of control values across plates rather than predicting values. With General Curve Fit, it is easy to plot such values on the chart and visually assess the stability of the samples. With the example data provided above, the chart is as follows:


## General Curve Fit TOVs:

This transform produces the following TOVs.
Here the $\{\mathbf{n}\}$ specifies the position of General Curve Fit in the sequence of transforms configured via Transform tab.

## TOV $\mid$ Refers to

## Coefficients of the Fit Method

The calculated values of the coefficients used in the selected fit method's equation.
The actual coefficients available will depend on the fit method, please refer to Curve Fitting Methods for more details. For example, with Linear Regression, the coefficients $\mathbf{m}$ and $\mathbf{c}$ will be available.

| For example, the Four Parameter Logistic Regression: |  |
| :--- | :--- |
| TOV $\{\mathbf{n}\} . \mathbf{a}$ | The $\mathbf{a}$ coefficient value of 4PL |
| $\mathbf{T O V}\{\mathbf{n}\} . \mathbf{b}$ | The $\mathbf{b}$ coefficient value of 4PL |
| $\mathbf{T O V}\{\mathbf{n}\} . \mathbf{c}$ | The $\mathbf{c}$ coefficient value of 4PL |
| $\mathbf{T O V}\{\mathbf{n}\} . \mathbf{d}$ | The $\mathbf{d}$ coefficient value of 4 PL |

## Goodness of Fit Measures

The values of calculated goodness measures determined from the curve fit.

| TOV $\{\mathbf{n}\} . R 2$ | The $R^{2}$ value |
| :--- | :--- |


| TOV | Refers to |
| :---: | :---: |
| TOV $n$ n\}.AR2 | The adjusted $\mathrm{R}^{2}$ value |
| TOV\{n\}.SE | The Standard Error value |
| TOV\{n\}.F | The F-Statistic value |
| TOV\{n\}.P | The P-value |
| TOV\{n\}.AIC | The Akaike's Information Criterion value |
| TOV \{n\}.AICc | The Akaike's Information Criterion corrected for small sizes value |
| TOV\{n\}.BIC | The Bayesian Information Criterion value |
| TOV\{n\}.DWS | The Durbin-Watson Statistic value |
|  | The number of Degrees of Freedom |
| TOV \{n\}.DoF | $\stackrel{0}{2}$ If there are not enough Degrees of Freedom, then values for $\mathbf{P}, \mathbf{F}, \mathbf{S E}$ and $\mathbf{a R}^{\mathbf{2}}$ cannot be computed. |
| TOV \{n\}.RMS | The Root Mean Square of the residuals value (reported for Auto Smoothed Spline only) |
| Named Evaluations <br> If there are any Named Evaluations defined for the General Curve Fit transform, they can also be referenced in the TOVs. For example: |  |
| TOV \{n\}.LLD | The determined value of Lower Limit of Detection for the curve fit |

## Matrix Difference

Input: Dual Endpoint $\Rightarrow$ Output: Endpoint
This transform calculates the difference between two input matrices sample-by-sample.

If either of the matrix inputs are flagged, the result will be set to Flagged.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw (1) and Raw (2) matrices. In this example, we would like to find the difference between values in these matrices.

Here is some example raw data displayed to 3 decimal places:
Raw (1)

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

[^1]|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.204 | 0.201 | 0.213 | 0.209 | 0.206 | 0.208 | 0.216 | 0.217 | 0.216 | 0.213 | 0.205 | 0.218 |
| $\mathbf{B}$ | 0.216 | 0.200 | 0.214 | 0.214 | 0.204 | 0.201 | 0.215 | 0.216 | 0.203 | 0.219 | 0.207 | 0.204 |
| $\mathbf{C}$ | 0.206 | 0.210 | 0.208 | 0.203 | 0.201 | 0.218 | 0.212 | 0.217 | 0.216 | 0.213 | 0.208 | 0.204 |
| $\mathbf{D}$ | 0.215 | 0.215 | 0.214 | 0.203 | 0.214 | 0.213 | 0.219 | 0.219 | 0.213 | 0.201 | 0.202 | 0.219 |
| $\mathbf{E}$ | 0.215 | 0.220 | 0.219 | 0.212 | 0.210 | 0.211 | 0.214 | 0.214 | 0.201 | 0.202 | 0.202 | 0.207 |
| $\mathbf{F}$ | 0.214 | 0.212 | 0.202 | 0.200 | 0.201 | 0.217 | 0.213 | 0.209 | 0.218 | 0.207 | 0.216 | 0.216 |
| $\mathbf{G}$ | 0.211 | 0.211 | 0.218 | 0.209 | 0.214 | 0.213 | 0.201 | 0.211 | 0.205 | 0.210 | 0.201 | 0.212 |
| $\mathbf{H}$ | 0.216 | 0.209 | 0.215 | 0.206 | 0.218 | 0.206 | 0.209 | 0.219 | 0.215 | 0.219 | 0.216 | 0.217 |

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If the data point values of the Raw (2) are greater than the values of Raw (1), the resulting matrix will contain negative values.

## Difference

The transform will generate an output matrix named Difference with the value at each position being the subtraction between values of the Raw (1) and Raw (2) matrices. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.160 | 0.122 | 1.892 | 1.915 | 1.007 | 1.018 | 0.944 | 0.973 | -0.106 | -0.097 | 1.258 | 1.263 |
| $\mathbf{B}$ | 0.239 | 0.241 | 1.656 | 1.674 | 0.999 | 1.017 | 0.965 | 0.990 | -0.071 | -0.089 | 0.579 | 0.564 |
| $\mathbf{C}$ | 0.381 | 0.365 | 0.266 | 0.311 | 1.005 | 1.021 | 0.991 | 0.993 | -0.103 | -0.097 | 0.405 | 0.422 |
| $\mathbf{D}$ | 0.591 | 0.559 | 0.150 | 0.175 | 0.953 | 0.974 | 0.992 | 1.023 | -0.112 | -0.095 | 0.287 | 0.271 |
| $\mathbf{E}$ | 0.890 | 0.845 | 0.060 | 0.155 | 1.005 | 1.014 | 1.019 | 1.048 | -0.090 | -0.088 | 1.377 | 1.342 |
| $\mathbf{F}$ | 1.211 | 1.234 | 0.176 | 0.211 | 0.995 | 0.989 | 1.013 | 0.990 | -0.109 | -0.099 | 0.576 | 0.559 |
| $\mathbf{G}$ | 1.580 | 1.592 | 0.342 | 0.389 | 0.991 | 1.016 | 1.107 | 1.095 | -0.079 | -0.083 | 0.451 | 0.447 |
| $\mathbf{H}$ | 0.037 | 0.061 | 0.248 | 0.264 | 0.996 | 1.055 | -0.094 | -0.103 | -0.100 | -0.104 | 0.274 | 0.260 |

## Median

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform calculates the median of the replicates in each sample group (flagged replicates are not included).

(1)
The median is the middle value of the values sorted into order. If there is an even number of values, the result is the average between the two middle values.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to find median value for each measurement sample group.

Here is some example raw data displayed to 3 decimal places:
Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

Layout
The example uses a layout with Unknowns in triplets (e.g., Unknown1 in positions from A1 to A3, Unknown2 in from A4 to A6, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 |
| B | 5 | 5 | 5 | 6 | 6 | 6 | 7 | 7 | 7 | 8 | 8 | 8 |
| C | 9 | 9 | 9 | 10 | 10 | 10 | 11 | 11 | 11 | 12 | 12 | 12 |
| D | 13 | 13 | 13 | 14 | 14 | 14 | 15 | 15 | 15 | 16 | 16 | 16 |
| E | 17 | 17 | 17 | 18 | 18 | 18 | 19 | 19 | 19 | 20 | 20 | 20 |
| F | 21 | 21 | 21 | 22 | 22 | 22 | 23 | 23 | 23 | 24 | 24 | 24 |
| G | 25 | 25 | 25 | 26 | 26 | 26 | 27 | 27 | 27 | 28 | 28 | 28 |
| H | 29 | 29 | 29 | 30 | 30 | 30 | 31 | 31 | 31 | 32 | 32 | 32 |

O Unknown

## Median

The transform will generate an output matrix named Median with the value at each position being the median of replicates of each sample group. With the example data provided above, the results are as follows (also displayed to 3 decimal places):

## - Sample Table

Here is a Sample Table snippet, which lists the details of the calculations. For each sample, the following details are reported:

| Sample | Positions | My Raw Data | Median |
| :--- | :--- | :--- | :--- |
| OUnknown1 | A1 | 0.364 | 0.364 |
|  | A2 | 0.323 |  |
|  | A3 | 2.105 |  |
| OUnknown2 | A4 | 2.124 | 1.226 |
|  | A5 | 1.213 |  |
|  | A6 | 1.226 |  |
| Unknown3 | A7 | 1.159 | 1.159 |
|  | A8 | 1.190 |  |
|  | A9 | 0.110 |  |
| Unknown4 | A10 | 0.116 | 1.463 |
|  | A11 | 1.463 |  |
|  | A12 | 1.481 |  |
| Unknown5 | B1 | 0.455 | 0.455 |
|  | B2 | 0.441 |  |
|  | B3 | 1.869 |  |

## - Matrix Table

Here all calculated data is displayed in a Matrix Table.

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.364 | 0.364 | 1.226 | 1.226 | 1.226 | 1.159 | 1.159 | 1.159 | 1.463 | 1.463 | 1.463 |
| $\mathbf{B}$ | 0.455 | 0.455 | 0.455 | 1.218 | 1.218 | 1.218 | 1.180 | 1.180 | 1.180 | 0.768 | 0.768 | 0.768 |
| $\mathbf{C}$ | 0.575 | 0.575 | 0.575 | 1.206 | 1.206 | 1.206 | 1.203 | 1.203 | 1.203 | 0.613 | 0.613 | 0.613 |
| $\mathbf{D}$ | 0.774 | 0.774 | 0.774 | 1.167 | 1.167 | 1.167 | 1.211 | 1.211 | 1.211 | 0.489 | 0.489 | 0.489 |
| $\mathbf{E}$ | 1.065 | 1.065 | 1.065 | 1.215 | 1.215 | 1.215 | 1.233 | 1.233 | 1.233 | 1.549 | 1.549 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.425 | 1.425 | 1.196 | 1.196 | 1.196 | 1.199 | 1.199 | 1.199 | 0.775 | 0.775 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.791 | 1.791 | 1.205 | 1.205 | 1.205 | 1.306 | 1.306 | 1.306 | 0.652 | 0.652 | 0.652 |
| $\mathbf{H}$ | 0.270 | 0.270 | 0.270 | 1.214 | 1.214 | 1.214 | 0.115 | 0.115 | 0.115 | 0.477 | 0.477 | 0.477 |

## Percentage Coefficient of Variation

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform calculates the \%CV of the replicates in each sample group (flagged replicates are not included).

The \%CV is the Standard Deviation divided by the sample group mean and multiplied by 100. (If the mean or the Standard Deviation is 0 , then the result is forced to 0 .)

For groups with less than 2 non-flagged replicates, the result for the group will be set to Flagged.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to find the percentage coefficient of variation for each sample group of the input matrix.

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

## Layout

The example uses a layout with Standards, Control, Blank, and Unknowns in duplicate (e.g., Standard1 in A1 and A2, Unknown1 at A3 and A4, Unknown2 at B3 and B4, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 1 | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | 2 | 2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | 3 | 3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | 4 | 4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | 5 | 5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | 6 | 6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | 1 | 1 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | 31 | 39 | 39 |
| H | 1 | 1 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | $(40$ | 40 |

## \%CV

The transform will generate an output matrix named $\% \mathbf{C V}$ with the value at each position being the percentage coefficient of variation in each sample group. The \%CV is useful for measuring the deviation between the replicates. The \%CV is calculated for each sample group as the \% of the standard deviation of the replicate measurements divided by their mean. Typically, for replicates one might expect a $\%$ CV value to be $<15$. A value greater than this might indicate a preparation or layout error. Acceptable limits depend on the application.

With the example data provided above, the results are as follows (also displayed to 3 decimal places):

## - Sample Table

Here is a Sample Table snippet, which lists the details of the calculations. For each sample, the following details are reported:

| Sample | Positions | My Raw Data | \%CV |
| :--- | :--- | :--- | :--- |
| Standard1 | A1 | 0.364 | 8.440 |
|  | A2 | 0.323 |  |
| Standard2 | B1 | 0.455 | 2.210 |
|  | B2 | 0.441 |  |
| Standard3 | C1 | 0.587 | 1.460 |
|  | C2 | 0.575 |  |
| Standard4 | D1 | 0.806 | 2.864 |
|  | D2 | 0.774 |  |
| Standard5 | E1 | 1.105 | 2.607 |
|  | E2 | 1.065 |  |

## - Matrix Table

Here all calculated \%CV data is displayed in a Matrix Table.

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 8.440 | 8.440 | 0.635 | 0.635 | 0.754 | 0.754 | 1.866 | 1.866 | 3.755 | 3.755 | 0.865 | 0.865 |
| $\mathbf{B}$ | 2.210 | 2.210 | 0.715 | 0.715 | 0.876 | 0.876 | 1.541 | 1.541 | 1.080 | 1.080 | 1.638 | 1.638 |
| $\mathbf{C}$ | 1.460 | 1.460 | 5.726 | 5.726 | 1.909 | 1.909 | 0.410 | 0.410 | 1.853 | 1.853 | 1.484 | 1.484 |
| $\mathbf{D}$ | 2.864 | 2.864 | 2.668 | 2.668 | 1.202 | 1.202 | 1.730 | 1.730 | 3.416 | 3.416 | 0.144 | 0.144 |
| $\mathbf{E}$ | 2.607 | 2.607 | 19.265 | 19.265 | 0.580 | 0.580 | 1.644 | 1.644 | 1.886 | 1.886 | 1.356 | 1.356 |
| $\mathbf{F}$ | 0.986 | 0.986 | 5.915 | 5.915 | 0.589 | 0.589 | 1.575 | 1.575 | 0.652 | 0.652 | 1.534 | 1.534 |
| $\mathbf{G}$ | 0.472 | 0.472 | 4.523 | 4.523 | 1.337 | 1.337 | 0.108 | 0.108 | 0.559 | 0.559 | 0.755 | 0.755 |
| $\mathbf{H}$ | 4.597 | 4.597 | 1.061 | 1.061 | 2.686 | 2.686 | 0.612 | 0.612 | 0.000 | 0.000 | 1.901 | 1.901 |

## Percentage

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform calculates a percentage value for each sample with respect to the specified 0\% and $100 \%$ values.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

$\mathbf{P 0}$ and $\mathbf{P 1 0 0}$ can be defined as sample groups, evaluated in the context of the container. For example, if a multiple container layout contains 2 containers of data, both container 1 and container 2 include 2 Control groups (but in different places on each container). If $\mathbf{P 0}$ is defined as Control1 and P100 is Control2, then when the analysis is performed, the \%s for container 1 will be calculated using Control1 and Control2 on container 1, whereas the \%s for container 2 will calculate \%s using the Control1 and Control2 from container 2.

| P0 | The value treated as "0\%." This can be defined as a number or as an <br> expression. All values less than P0 will have negative value in resulting <br> matrix. <br> $\mathbf{P 1 0 0}$ |
| :--- | :--- |
| The value treated as "100\%." This can be defined as a number or as an <br> expression. All values greater than $\mathbf{P 1 0 0}$ will have values greater than 100 <br> resulting matrix. |  |

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to calculate each measurement percentage value with respect to 0-3 range.

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

To calculate all percentage values in this matrix, specify a P0 of 0 and $\mathbf{P 1 0 0}$ of 3 .

## \%

The transform will generate an output matrix named $\%$. The value at each position will equal its percent rank with respect to the specified $0 \%$ and $100 \%$. With the example data provided above, the results are as follows (displayed here to 2 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 12.13 | 10.77 | 70.17 | 70.80 | 40.43 | 40.87 | 38.63 | 39.67 | 3.67 | 3.87 | 48.77 | 49.37 |
| $\mathbf{B}$ | 15.17 | 14.70 | 62.30 | 62.93 | 40.10 | 40.60 | 39.33 | 40.20 | 4.40 | 4.33 | 26.20 | 25.60 |
| $\mathbf{C}$ | 19.57 | 19.17 | 15.80 | 17.13 | 40.20 | 41.30 | 40.10 | 40.33 | 3.77 | 3.87 | 20.43 | 20.87 |
| $\mathbf{D}$ | 26.87 | 25.80 | 12.13 | 12.60 | 38.90 | 39.57 | 40.37 | 41.37 | 3.37 | 3.53 | 16.30 | 16.33 |
| $\mathbf{E}$ | 36.83 | 35.50 | 9.30 | 12.23 | 40.50 | 40.83 | 41.10 | 42.07 | 3.70 | 3.80 | 52.63 | 51.63 |
| $\mathbf{F}$ | 47.50 | 48.17 | 12.60 | 13.70 | 39.87 | 40.20 | 40.87 | 39.97 | 3.63 | 3.60 | 26.40 | 25.83 |
| $\mathbf{G}$ | 59.70 | 60.10 | 18.67 | 19.90 | 40.17 | 40.93 | 43.60 | 43.53 | 4.20 | 4.23 | 21.73 | 21.97 |
| $\mathbf{H}$ | 8.43 | 9.00 | 15.43 | 15.67 | 40.47 | 42.03 | 3.83 | 3.87 | 3.83 | 3.83 | 16.33 | 15.90 |

## Replicate Outlier Removal

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform flags up to a specified number of outlier data points in each sample group. The output matrix values match the input matrix values with any outlier(s) flagged. Outliers are determined and removed as follows:

For each sample group, the transform:

1. Calculates the \%CV of its replicates (including only the replicates not already flagged).
2. If the \%CV is greater than the specified $\% \mathbf{C V}$ Threshold, then the replicate furthest from the mean is removed.

These steps are repeated until either the new \%CV of the replicates that are not flagged is below the threshold, or until the specified number of outliers to remove has been removed.

This transform can be used to perform automatic outlier rejection (useful when samples are measured in replicate groups of 3 or more).

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Outliers To <br> Remove | Specifies the maximum number of outliers to remove. The minimum and <br> default value for this setting is 1. |
| :--- | :--- |
| \%CV | Defines threshold percentage coefficient of variance. Data point with superior <br> Threshold <br> or inferior values will be flagged. The default value is 15\%. |

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to remove 1 outlier from each sample group.

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

## Layout

The example uses a layout with Unknowns in columns (e.g., Unknown1 vertically at positions from $\mathbf{A} 1$ to $\mathbf{H} 1$, Unknown2 from $\mathbf{B 2}$ to $\mathbf{H} 2$, etc.). I.e.:

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| B | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| C | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| D | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| E | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| F | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| G | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| H | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |

Unknown
To flag 1 outlier data point for each sample group, specify an Outliers To Remove as 1 and \%CV Threshold as $15 \%$.

## Calculated

The transform will generate an output matrix named Calculated with the value at each position matching the input data and outlier data points flagged. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | $z .105$ | $z .124$ | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 4.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

If there are only 2 remaining replicates (before the flag), both of them will be flagged and a message will be added to the calculation log.

## Standard Curve Fit

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform:

1. Plots the specified concentration values $(x)$ against the measurement data $(y)$ for each standard
2. Fits a curve to the standard data points (optionally using weight methods)
3. Calculates concentration values from the curve
4. Determines LLOQ and ULOQ using the specified quantification limits
5. Optionally computes any additional values from the curve (such as LLD)

To use Standard Curve Fit transform, select or create a layout with a minimum of 2 Standard sample groups.

You can use Library Curve to save the curve created by this transform for use in other assays (perhaps in assays where standards are not measured).

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

Axes

| X Axis Title | The name of the x -axis on the chart. |
| :--- | :--- |
|  | The scale type to be used for the x -axis: |
| X Axis Type | - Logarithmic <br> - Linear |
| Y Axis Title | The name of the y-axis on the chart. |


| Y Axis Type | The scale type to be used for the y-axis: <br> - Logarithmic |
| :--- | :--- |
|  | When y-axis is logged, negative (or zero) $y$ values are not <br> included in the plot. |

## Axes (Advanced)

Use these additional settings to adapt the chart's appearance to your needs.

| X Axis Major Units | The numeric interval between numbers on the $x$-axis. Starting from the minimum value, a major unit is displayed at every increment. |
| :---: | :---: |
| $X$ Axis Minor Units | The numeric interval between supplementary tickmarks on the $x$-axis. The minor units are not displayed unless this attribute is specified. |
| X Axis <br> Numerical <br> Formatting | The formatting for the X Axis Major Units values. <br> To learn about available formatting options, see Custom Format Codes for Numbers of the Chart. |
| X Crosses Y <br> At | Select where the $x$-axis will cross the $y$-axis from the drop-down: <br> - Automatic - Determine the location that makes the most sense for your data automatically. <br> - Axis Value - Cross y-axis at specified $\mathbf{X}$ Crosses $\mathbf{Y}$ at Value. <br> - Maximum Axis Value - Cross y-axis at the maximum $y$ value of the chart. <br> - Minimum Axis Value - Cross y-axis at the minimum $y$ value. If axis type is set to Logarithmic, then it is the lowest positive value. |
| X Crosses Y at Value | The numerical value at which the $x$-axis will cross the $y$-axis. This value must be in the range of calculated results. If axis type is set to logarithmic, the provided value must be greater than 0. |
| Y Axis Major Units | The numeric interval between the gridlines on the $y$-axis. Starting from the minimum value, a major unit is displayed at every increment. |
| Y Axis Minor Units | The numeric interval between supplementary tickmarks on the $y$-axis. The minor units are not displayed unless this attribute is specified. |


| Y Axis <br> Numerical <br> Formatting | The formatting for the $\mathbf{Y}$ Axis Major Units values. <br> To learn about available formatting options, see Custom Format Codes for Numbers of the Chart. |
| :---: | :---: |
| $\begin{aligned} & \text { Y Crosses X } \\ & \text { At } \end{aligned}$ | Select where the $y$-axis will cross the $x$-axis from the drop-down: <br> - Automatic - Determine the location that makes the most sense for your data automatically. <br> - Axis Value - Cross $x$-axis at specified $\mathbf{Y}$ Crosses $\mathbf{X}$ at Value. <br> - Maximum Axis Value - Cross $x$-axis at the maximum $x$ value of the chart. <br> - Minimum Axis Value - Cross $x$-axis at the minimum $x$ value. If axis type is set to Logarithmic, then it is the lowest positive value. |
| Y Crosses X at Value | The numerical value at which the $y$-axis will cross the $x$-axis. This value must be in the range of calculated results. If axis type is set to logarithmic, the provided value must be greater than 0 . |

These settings can be also applied to other charts as an advanced configuration. To learn more, see Change the Units and Number Format of the Charts and Customize $X$ and $Y$ Axes Positions for Curve Charts topics.

## Calibrators

|  | Defines the $x$ value (the concentration) of each calibration point. <br> Click the Concentrations setting drop-down to display the editor. <br> Concentration values can be specified using two different modes: |
| :--- | :--- |
| - Series - Provide the concentration value of the first calibrator and |  |
| define a series to compute the remaining calibrators. This is useful if |  |
| your samples are in a serial dilution. |  |
| - Non-Serial - Provide concentrations values for every calibrator. |  |
| Concentrations |  |

## Chart

|  | In the drop-down editor, select any sample types to plot on the curve; this is <br> useful to show where samples fall on the curve. The computed results of each <br> Pelected sample will be plotted. In this case, all successfully computed non- <br> Curve <br> flagged sample groups of the selected types will be displayed on the Fit Chart <br> and in the report. (Only non-calibrator sample types are applicable e.g., <br> Unknown, Control, Blank.) |
| :--- | :--- |

## Fit

| Calculate From | Defines how the output matrix results are calculated from the computed curve for each sample (i.e., is a result calculated from the curve for each replicate or for the average of the replicates?). Select either: <br> - Replicates - Every position will contain the result of $x$, calculated from value of every replicate $(y)$. <br> - Average - Replicates from the same sample contain the result of $x$, calculated from averaged value of replicates ( $y$ ). |
| :---: | :---: |
|  | 1.) Any flagged positions on the input are also flagged in the output. |
| Fit <br> Expression | When using the Custom Fit, you can enter or paste the equation for your curve fit. |
| Fit Method | The curve-fitting method to construct a curve for your data based on the specified Standard Type. <br> If your license is Pro, you can also use the Best Fit feature to plot the most appropriate fit for your data automatically or the Custom Fit feature to supply your own equation/model to fit the data with. |
| Fit Methods | When using the Best Fit feature, this editor specifies the Fit Methods and Weight Methods to process your data with. You can select any combination of items in corresponding check box lists. |
|  | At least 1 check box must be selected in each list. |


| Fit Score <br> Method | When the Best Fit feature is selected, to construct the curve, you can additionally specify the scoring method to be used in evaluating the goodness of applied fit methods: <br> - $R^{2}$ <br> - $\mathrm{R}^{2}$ with Low-End Accuracy <br> - $R^{2}$ with High-End Accuracy <br> - $R^{2}$ with Low-End and High-End Accuracy <br> - 1/Standard-Error <br> - 1/P-Value <br> - 1/AIC <br> - 1/AICc <br> - 1/BIC |
| :---: | :---: |
| Fit To Standard | Defines which points are used as the calibrators to fit the curve to: <br> - Replicates - All replicate points from each Standard sample group are used as calibrators. <br> - Average - Average of the replicates from each Standard sample group are used as the calibration points for the curve. |
| Weight Method | Specifies the weight function for the transform. |
| X <br> Transform | Determines how the $x$ values are interpreted in the transform: <br> - None - The $x$ values remain as they are. <br> - Log10 - Each $x$ point is taken as a common logarithm of $x$. <br> - Log2 - Each $x$ point is taken as a binary logarithm of $x$. <br> - LogN - Each $x$ point is taken as a natural logarithm of $x$. |
| Y <br> Transform | Determines how the $y$ values are interpreted in the transform: <br> - None - The $y$ values remain as they are. <br> - Log10 - Each $y$ point is taken as a common logarithm of $y$. <br> - Log2 - Each $y$ point is taken as a binary logarithm of $y$. <br> - LogN - Each $y$ point is taken as a natural logarithm of $y$. |

## Quantification Limits

| Accuracy <br> $( \pm \%)$ | Defines a percentage limit of accuracy for Standard Type sample groups. <br> This is used to verify whether the accuracy of the calibrators' fits in required <br> limits. The default value is 20\%. |
| :--- | :--- |
| Precision <br> $(\% C V)$ | Defines a \%CV precision limit. This is used to verify whether \%CV for each <br> Standard Type sample group is lower than required limit. The default value <br> is 20\%. |

The Quantification limits properties are used to determine the Lower Limit of Quantification (LLOQ) and Upper Limit of Quantification (ULOQ) from the fit. The LLOQ and ULOQ are computed and added to the report automatically.

The method used to calculate LLOQ and ULOQ can be overridden by setting named evaluations with corresponding Name settings. In this case, the custom calculated values will replace the default values.

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to construct a curve fit and calculate concentrations for input matrix.

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.192 | 0.199 | 0.347 | 0.346 | 0.325 | 0.321 | 0.803 | 0.797 | 1.589 | 1.593 | 0.241 | 0.246 |
| $\mathbf{B}$ | 0.293 | 0.300 | 0.586 | 0.584 | 0.800 | 0.793 | 0.402 | 0.401 | 0.839 | 0.837 | 0.562 | 0.564 |
| $\mathbf{C}$ | 0.537 | 0.534 | 0.549 | 0.547 | 0.878 | 0.875 | 0.471 | 0.471 | 0.759 | 0.756 | 1.039 | 1.043 |
| $\mathbf{D}$ | 0.858 | 0.855 | 0.320 | 0.325 | 0.531 | 0.530 | 1.552 | 1.551 | 0.429 | 0.427 | 0.424 | 0.424 |
| $\mathbf{E}$ | 1.523 | 1.527 | 0.486 | 0.490 | 1.289 | 1.283 | 1.430 | 1.438 | 1.494 | 1.501 | 1.427 | 1.429 |
| $\mathbf{F}$ | 1.622 | 1.621 | 0.621 | 0.626 | 0.667 | 0.663 | 1.350 | 1.347 | 0.631 | 0.634 | 1.695 | 1.694 |
| $\mathbf{G}$ | 0.001 | 0.001 | 0.356 | 0.352 | 0.418 | 0.412 | 1.172 | 1.174 | 1.572 | 1.570 | 0.595 | 0.590 |
| $\mathbf{H}$ | 0.297 | 0.297 | 0.796 | 0.801 | 1.517 | 1.515 | 0.960 | 0.965 | 0.534 | 0.527 | 0.585 | 0.584 |

## Layout

The example uses a layout with Standards, Control, Blank, and Unknowns in duplicate (e.g., Standard1 in A1 and A2, Unknown1 at A3 and A4, Unknown2 at B3 and B4, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 1 | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | 2 | 2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | 3 | 3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | 4 | 4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | 5 | 5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | 6 | 6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | 1 | 1 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | 31 | 39 | 39 |
| H | 1 | 1 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | 40 | 40 |

Standard $\bigcirc$ Blank $\bigcirc$ Control $\bigcirc$ Unknown
To configure a Standard Curve Fit for this matrix, provide following settings:

1. Set $\mathbf{X}$ Axis Title to Concentration.
2. Set $\mathbf{X}$ Axis Type to Logarithmic.
3. Set $\mathbf{Y}$ Axis Title to Raw.
4. Set Y Axis Type to Linear.
5. Set Concentrations in Standard 1 to 0.125 and a Series to multiply by 2.
6. Set Standard Type to Standard.
7. Select Show Points on Curve to None.
8. Set the Calculate From to Average.
9. Select a Fit Method of 4PL.
10. Set Fit To Standard to Average.
11. Select a Weight Method of None.
12. Set the $\mathbf{X}$ Transform to None.
13. Set the $\mathbf{Y}$ Transform to None.
14. Set Accuracy ( $\pm \%$ ) to $20 \%$.
15. Set Precision (\%CV) to 20\%.

## Concentration

The transform will generate an output matrix named Concentration with concentrations ( $x$ ) calculation results for all samples using the curve fit. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | Flagged | Flagged | 0.352 | 0.352 | 0.318 | 0.318 | 0.844 | 0.844 | 2.890 | 2.890 | 0.153 | 0.153 |
| $\mathbf{B}$ | 0.274 | 0.274 | 0.623 | 0.623 | 0.841 | 0.841 | 0.423 | 0.423 | 0.886 | 0.886 | 0.600 | 0.600 |
| $\mathbf{C}$ | 0.572 | 0.572 | 0.585 | 0.585 | 0.928 | 0.928 | 0.503 | 0.503 | 0.799 | 0.799 | 1.131 | 1.131 |
| $\mathbf{D}$ | 0.906 | 0.906 | 0.317 | 0.317 | 0.566 | 0.566 | 2.561 | 2.561 | 0.454 | 0.454 | 0.450 | 0.450 |
| $\mathbf{E}$ | 2.390 | 2.390 | 0.521 | 0.521 | 1.550 | 1.550 | 1.969 | 1.969 | 2.240 | 2.240 | 1.947 | 1.947 |
| $\mathbf{F}$ | 3.243 | 3.243 | 0.662 | 0.662 | 0.704 | 0.704 | 1.702 | 1.702 | 0.671 | 0.671 | 5.174 | 5.174 |
| $\mathbf{G}$ | Flagged | Flagged | 0.362 | 0.362 | 0.439 | 0.439 | 1.332 | 1.332 | 2.710 | 2.710 | 0.630 | 0.630 |
| $\mathbf{H}$ | 0.275 | 0.275 | 0.843 | 0.843 | 2.338 | 2.338 | 1.030 | 1.030 | 0.566 | 0.566 | 0.622 | 0.622 |

To learn how to configure this transform for an assay measured on more than one plate, see Multiple Plate Standard Curve Fit Analysis.

## Example Additional XML Evaluation and Flagging Configuration

In addition to plotting a curve fit and calculating concentrations, additional values can be obtained with Advanced Curve Fit Evaluations. Moreover, with Custom Flag Labels by Fit Transform Calculations, you can exclude from analysis and mark with a custom label samples that are, for example, outside the range of the standard calibrator.

In this example (using the data provided above):

- Lower Limit of Detection (LLD) is computed from the curve by calculating the concentration at the average of the blank measurements +3 * standard deviation of the blank positions.
- The Name attribute for LLD is specified and is used as a variable to flag condition $x<$ LLD.


## XML Configuration

The set attributes are highlighted in yellow.
<Settings>
<StandardCurveFit StandardType="2" FitMethod="4PL" Concentrations="0.25,0.5,1,2,4,8" XAxisTitle="Concentration" YAxisTitle="OD" FitToStandard="Average"
CalculateFrom="Average">
<Evaluations>
<Evaluation Type="CalcY" Expression="Blank+(3*sd(Blank))"
Description="My LLD"Name="LLD" />
</Evaluations>
<Flags>
< Flag Type="Input"Condition=" $x$ \< LLD"Label="Below LLD"
/>
</Flags>
</StandardCurveFit>
</Settings>
Upon calculating data with the aforementioned XML configuration defined, the LLD evaluation is computed and added to the report below an interactive chart. It is displayed in the table below LLOQ and ULOQ, which are calculated from the curve automatically.

| LLOQ | 0.25 |
| :--- | :--- |
| ULOQ | 2 |
| My LLD | 0.222063 |

With the example data provided above, the results are computed as follows:

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | Below <br> LLD | Below <br> LLD | 0.352 | 0.352 | 0.318 | 0.318 | 0.844 | 0.844 | 2.890 | 2.890 | 0.153 | 0.153 |
| $\mathbf{B}$ | 0.274 | 0.274 | 0.623 | 0.623 | 0.841 | 0.841 | 0.423 | 0.423 | 0.886 | 0.886 | 0.600 | 0.600 |
| $\mathbf{C}$ | 0.572 | 0.572 | 0.585 | 0.585 | 0.928 | 0.928 | 0.503 | 0.503 | 0.799 | 0.799 | 1.131 | 1.131 |
| $\mathbf{D}$ | 0.906 | 0.906 | 0.317 | 0.317 | 0.566 | 0.566 | 2.561 | 2.561 | 0.454 | 0.454 | 0.450 | 0.450 |
| $\mathbf{E}$ | 2.390 | 2.390 | 0.521 | 0.521 | 1.550 | 1.550 | 1.969 | 1.969 | 2.240 | 2.240 | 1.947 | 1.947 |
| $\mathbf{F}$ | 3.243 | 3.243 | 0.662 | 0.662 | 0.704 | 0.704 | 1.702 | 1.702 | 0.671 | 0.671 | 5.174 | 5.174 |
| $\mathbf{G}$ | Below <br> LLD | Below <br> LLD | 0.362 | 0.362 | 0.439 | 0.439 | 1.332 | 1.332 | 2.710 | 2.710 | 0.630 | 0.630 |
| $\mathbf{H}$ | 0.275 | 0.275 | 0.843 | 0.843 | 2.338 | 2.338 | 1.030 | 1.030 | 0.566 | 0.566 | 0.622 | 0.622 |

## Standard Curve Fit TOVs:

This transform produces the following TOVs.
Here the $\mathbf{\{} \mathbf{n}\}$ specifies the position of Standard Curve Fit in the sequence of transforms configured via Transform tab.

## TOV $\quad$ Refers to <br> Coefficients of the Fit Method

The calculated values of the coefficients used in the selected fit method's equation.

The actual coefficients available will depend on the fit method, please refer to Curve Fitting Methods for more details. For example, with Linear Regression, the coefficients $\mathbf{m}$ and $\mathbf{c}$ will be available.

For example, the Four Parameter Logistic Regression:

| TOV $\{\mathbf{n}\} . \mathbf{a}$ | The $\mathbf{a}$ coefficient value of 4PL |
| :--- | :--- |
| TOV $\{\mathbf{n}\} . \mathbf{b}$ | The $\mathbf{b}$ coefficient value of 4PL |
| TOV $\{\mathbf{n}\} . \mathbf{c}$ | The $\mathbf{c}$ coefficient value of 4PL |
| TOV $\{\mathbf{n}\} . \mathbf{d}$ | The $\mathbf{d}$ coefficient value of 4PL |

## Goodness of Fit Measures

The values of calculated goodness measures determined from the curve fit.

| TOV \{n\}.R2 | The $\mathrm{R}^{2}$ value |
| :---: | :---: |
| TOV \{n\}.AR2 | The adjusted $\mathrm{R}^{2}$ value |
| TOV\{n\}.SE | The Standard Error value |
| TOV\{n\}.F | The F-Statistic value |
| TOV\{n\}.P | The P-value |
| TOV\{n\}.AIC | The Akaike's Information Criterion value |
| TOV \{n\}.AICc | The Akaike's Information Criterion corrected for small sizes value |
| TOV\{n\}.BIC | The Bayesian Information Criterion value |
| TOV\{n\}.DWS | The Durbin-Watson Statistic value |
|  | The number of Degrees of Freedom |
| TOV \{n\}.DoF | If there are not enough Degrees of Freedom, then values for $\mathbf{P}, \mathbf{F}, \mathbf{S E}$ and $\mathbf{a R}^{\mathbf{2}}$ cannot be computed. |
| TOV \{n\}.RMS | The Root Mean Square of the residuals value (reported for Auto Smoothed Spline only) |


| TOV | Refers to |
| :---: | :---: |
|  | Quantification Limits |
| The lower and upper concentration values determined from the curve. |  |
| TOV\{n\}.ULOQ | The determined ULOQ value of the curve fit |
| TOV\{n\}.LLOQ | The determined LLOQ value of the curve fit |
|  | Sum of Squares |
| The sum of squared distances from the fit to the data. |  |
| TOV\{n\}.SSR | The sum of squares computed from input $y$ replicates and $y$ average (flagged points not included) |
| TOV\{n\}.SSC | The sum of squares computed from input $y$ average and $y^{\prime}$ average (flagged points not included) |
|  | (1) The $y$ ' is computed from curve at $x$ |
| Named Evaluations <br> If there are any Named Evaluations defined for the Standard Curve Fit transform, they can also be referenced in the TOVs. For example: |  |
|  |  |
| TOV\{n\}.LLD | The determined value of Lower Limit of Detection for the curve fit |

## Standard Curve Fit (from Library)

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform uses a Library Curve to calculate concentration values.
This transform will compute concentrations values using a standards curve that was measured and constructed in a previous assay run. In this way, concentrations can be computed from a library curve without needing to measure new standards as part of this protocol.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

Library Curve File $\mid$ Specifies the full file path to the Library Curve file.

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to use a curve created from a previous assay run that used the Standard Curve Fit transform. This library curve has been saved with the name "My Library Curve".

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 1.529 | 1.540 | 1.379 | 1.261 | 0.940 | 1.040 | 0.846 | 0.845 | 1.739 | 1.755 | 0.552 | 0.557 |
| $\mathbf{B}$ | 1.130 | 1.136 | 0.719 | 0.728 | 1.123 | 1.074 | 1.641 | 1.648 | 1.274 | 1.232 | 2.429 | 2.322 |
| $\mathbf{C}$ | 1.771 | 1.730 | 2.169 | 2.151 | 0.954 | 0.926 | 0.472 | 0.463 | 1.368 | 1.319 | 1.055 | 1.049 |
| $\mathbf{D}$ | 1.140 | 1.151 | 2.351 | 2.304 | 0.287 | 0.279 | 0.228 | 0.235 | 0.435 | 0.425 | 1.999 | 2.006 |
| $\mathbf{E}$ | 1.550 | 1.553 | 0.333 | 0.320 | 1.275 | 1.325 | 0.127 | 0.126 | 1.489 | 1.547 | 0.740 | 0.758 |
| $\mathbf{F}$ | 1.600 | 1.617 | 1.976 | 1.973 | 1.006 | 1.050 | 1.013 | 1.073 | 1.404 | 1.388 | 1.136 | 1.109 |
| $\mathbf{G}$ | 0.830 | 0.817 | 1.070 | 1.046 | 0.198 | 0.192 | 1.218 | 1.200 | 1.174 | 1.154 | 1.188 | 1.060 |
| $\mathbf{H}$ | 0.192 | 0.184 | 1.952 | 2.030 | 0.510 | 0.502 | 0.975 | 0.982 | 0.734 | 0.711 | 1.656 | 1.658 |

[^2]The example uses a layout with Unknowns in duplicate (e.g., Unknown1 in A1 and A2,
Unknown2 at B1 and B2, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 | 41 |
|  | 41 |  |  |  |  |  |  |  |  |  |  |  |
| B | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 | 42 | 42 |
| C | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 | 43 | 43 |
| D | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 | 44 | 44 |
| E | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 | 45 | 45 |
| F | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 | 46 | 46 |
| G | 7 | 7 | 15 | 15 | 23 | 23 | 31 | 31 | 39 | 39 | 47 | 47 |
| H | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | 40 | 40 | 48 | 48 |

To reuse an existing curve, select "My Library Curve" file from Curve Library and apply it to calculate results for an assay.

## Concentration

The transform will generate an output matrix named Concentration with concentrations (X) calculation results for all samples using the curve from the specified library curve file. With the example data provided above, the results are as follows (also displayed here to 3 decimal places):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 2.448 | 2.448 | 1.629 | 1.629 | 1.064 | 1.064 | 0.894 | 0.894 | $\begin{aligned} & \text { Flagge } \\ & \left.\begin{array}{l} d \end{array}\right) \end{aligned}$ | $\begin{aligned} & \text { Flagge } \\ & \left.\begin{array}{l} d \end{array}\right) . \end{aligned}$ | 0.591 | 0.591 |
| B | 1.266 | 1.266 | 0.764 | 0.764 | 1.213 | 1.213 | 3.608 | 3.608 | 1.480 | 1.480 | Flagge $d$ | Flagge $d$ |
| C | Flagge <br> d | Flagge <br> d | Flagge <br> d | Flagge d | 1.002 | 1.002 | 0.499 | 0.499 | 1.689 | 1.689 | 1.147 | 1.147 |
| D | 1.286 | 1.286 | Flagge <br> d | Flagge <br> d | 0.249 | 0.249 | 0.105 | 0.105 | 0.457 | 0.457 | $\begin{aligned} & \text { Flagge } \\ & \left\|\begin{array}{l} \text { ang } \end{array}\right\| \end{aligned}$ | $\begin{aligned} & \text { Flagge } \\ & \left\|\begin{array}{l} \text { ang } \end{array}\right\| \end{aligned}$ |
| E | 2.561 | 2.561 | 0.323 | 0.323 | 1.582 | 1.582 | Flage <br> a | Flagge d | 2.349 | 2.349 | 0.790 | 0.790 |
| F | 3.079 | 3.079 | $\begin{aligned} & \text { Flagge } \\ & \text { \& } \end{aligned}$ | Flagge <br> d | 1.114 | 1.114 | 1.134 | 1.134 | 1.839 | 1.839 | 1.250 | 1.250 |
| G | 0.870 | 0.870 | 1.155 | 1.155 | Flagge <br> * | Flagge <br> * | 1.395 | 1.395 | 1.317 | 1.317 | 1.252 | 1.252 |
| H | Flagge | Flagge | Flagge $d$ | Flagge d | 0.541 | 0.541 | 1.050 | 1.050 | 0.763 | 0.763 | 3.865 | 3.865 |

All TOVs, produced by the original Standard Curve Fit transform can be referenced from where the Standard Curve Fit (from Library) transforms is used.

## Standard Deviation

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform calculates the standard deviation of the replicates in each sample group (flagged replicates are not included). The sample of population method is used.The standard deviation is a measure of how widely the replicate values are dispersed from the average of the group.

For groups with less than 2 non-flagged replicates, the result for the group will be set to Flagged.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to find the deviation between replicates in each sample group.

Here is some example raw data displayed here to 3 decimal places:
Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

Layout
The example uses a layout with Standards, Control, Blank, and Unknowns in duplicate (e.g., Standard1 in A1 and A2, Unknown1 at A3 and A4, Unknown2 at B3 and B4, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 1 | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | 2 | 2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | 3 | 3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | 4 | 4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | 5 | 5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | 6 | 6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | 1 | 1 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | 31 | 39 | 39 |
| H | 1 | 1 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | $(40$ | 40 |

## SD

The transform will generate an output matrix named SD with the value at each position being the product of the input data and deviation between positions of each sample group. With the example data provided above, the results are as follows (also displayed to 3 decimal places):

## - Sample Table

Here is a Sample Table snippet, which lists the details of the calculations. For each sample, the following details are reported:

| Sample | Positions | My Raw Data | SD |
| :--- | :--- | :--- | :--- |
| Standard1 | A1 | 0.364 | 0.029 |
|  | A2 | 0.323 |  |
| Standard2 | B1 | 0.455 | 0.010 |
|  | B2 | 0.441 |  |
| Standard3 | C1 | 0.587 | 0.008 |
|  | C2 | 0.575 |  |
| Standard4 | D1 | 0.806 | 0.023 |
|  | D2 | 0.774 |  |
| Standard5 | E1 | 1.105 | 0.028 |
|  | E2 | 1.065 |  |

## - Matrix Table

Here all calculated data is displayed in a Matrix Table.

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.029 | 0.029 | 0.013 | 0.013 | 0.009 | 0.009 | 0.022 | 0.022 | 0.004 | 0.004 | 0.013 | 0.013 |
| $\mathbf{B}$ | 0.010 | 0.010 | 0.013 | 0.013 | 0.011 | 0.011 | 0.018 | 0.018 | 0.001 | 0.001 | 0.013 | 0.013 |
| $\mathbf{C}$ | 0.008 | 0.008 | 0.028 | 0.028 | 0.023 | 0.023 | 0.005 | 0.005 | 0.002 | 0.002 | 0.009 | 0.009 |
| $\mathbf{D}$ | 0.023 | 0.023 | 0.010 | 0.010 | 0.014 | 0.014 | 0.021 | 0.021 | 0.004 | 0.004 | 0.001 | 0.001 |
| $\mathbf{E}$ | 0.028 | 0.028 | 0.062 | 0.062 | 0.007 | 0.007 | 0.021 | 0.021 | 0.002 | 0.002 | 0.021 | 0.021 |
| $\mathbf{F}$ | 0.014 | 0.014 | 0.023 | 0.023 | 0.007 | 0.007 | 0.019 | 0.019 | 0.001 | 0.001 | 0.012 | 0.012 |
| $\mathbf{G}$ | 0.008 | 0.008 | 0.026 | 0.026 | 0.016 | 0.016 | 0.001 | 0.001 | 0.001 | 0.001 | 0.005 | 0.005 |
| $\mathbf{H}$ | 0.012 | 0.012 | 0.005 | 0.005 | 0.033 | 0.033 | 0.001 | 0.001 | 0.000 | 0.000 | 0.009 | 0.009 |

## Standard Error of Mean

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform calculates the standard error of the mean (SEM) of the replicates in each sample group (flagged replicates are not included). The sample of population method is used.


#### Abstract

The standard error of the mean quantifies how precisely the average of the replicates estimates the true mean of the population. Lower values of SEM indicate more precise estimates of the population mean. Typically, increasing the number of replicates will result in a smaller SEM value and a more precise estimate.


For groups with less than 2 non-flagged replicates, the result for the group will be set to Flagged.

For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to find the SEM of each sample group.

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

## Layout

The example uses a layout with Unknowns in triplets (e.g., Unknown1 in positions from $\mathbf{A} 1$ to $\mathbf{A 3}$, Unknown2 in from $\mathbf{A} 4$ to $\mathbf{A 6}$, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 |
| B | 5 | 5 | 5 | 6 | 6 | 6 | 7 | 7 | 7 | 8 | 8 | 8 |
| C | 9 | 9 | 9 | 10 | 10 | 10 | 11 | 11 | 11 | 12 | 12 | 12 |
| D | 13 | 13 | 13 | 14 | 14 | 14 | 15 | 15 | 15 | 16 | 16 | 16 |
| E | 17 | 17 | 17 | 18 | 18 | 18 | 19 | 19 | 19 | 20 | 20 | 20 |
| F | 21 | 21 | 21 | 22 | 22 | 22 | 23 | 23 | 23 | 24 | 24 | 24 |
| G | 25 | 25 | 25 | 26 | 26 | 26 | 27 | 27 | 27 | 28 | 28 | 28 |
| H | 29 | 29 | 29 | 30 | 30 | 30 | 31 | 31 | 31 | 32 | 32 | 32 |

O Unknown

## SEM

The transform will generate an output matrix named SEM with the value at each position being the product of the input data and the standard deviation divided by the square root of the number of non-flagged replicates in each group. With the example data provided above, the results are as follows (also displayed to 3 decimal places):

## - Sample Table

Here is a Sample Table snippet, which lists the details of the calculations. For each sample, the following details are reported:

| Sample | Positions | My Raw Data | SEM |
| :--- | :--- | :--- | :--- |
| Unknown1 | A1 | 0.364 | 0.587 |
|  | A2 | 0.323 |  |
|  | A3 | 2.105 |  |
| Unknown2 | A4 | 2.124 | 0.302 |
|  | A5 | 1.213 |  |
|  | A6 | 1.226 |  |
| Unknown3 | A7 | 1.159 | 0.355 |
|  | A8 | 1.190 |  |
|  | A9 | 0.110 |  |
| Unknown4 | A10 | 0.116 | 0.452 |
|  | A11 | 1.463 |  |
|  | A12 | 1.481 |  |
| Unknown5 | B1 | 0.455 | 0.474 |
|  | B2 | 0.441 |  |
|  | B3 | 1.869 |  |

## - Matrix Table

Here all calculated data is displayed in a Matrix Table.

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.587 | 0.587 | 0.587 | 0.302 | 0.302 | 0.302 | 0.355 | 0.355 | 0.355 | 0.452 | 0.452 | 0.452 |
| $\mathbf{B}$ | 0.474 | 0.474 | 0.474 | 0.226 | 0.226 | 0.226 | 0.354 | 0.354 | 0.354 | 0.216 | 0.216 | 0.216 |
| $\mathbf{C}$ | 0.036 | 0.036 | 0.036 | 0.236 | 0.236 | 0.236 | 0.365 | 0.365 | 0.365 | 0.168 | 0.168 | 0.168 |
| $\mathbf{D}$ | 0.142 | 0.142 | 0.142 | 0.266 | 0.266 | 0.266 | 0.375 | 0.375 | 0.375 | 0.128 | 0.128 | 0.128 |
| $\mathbf{E}$ | 0.269 | 0.269 | 0.269 | 0.284 | 0.284 | 0.284 | 0.379 | 0.379 | 0.379 | 0.483 | 0.483 | 0.483 |
| $\mathbf{F}$ | 0.352 | 0.352 | 0.352 | 0.263 | 0.263 | 0.263 | 0.368 | 0.368 | 0.368 | 0.225 | 0.225 | 0.225 |
| $\mathbf{G}$ | 0.412 | 0.412 | 0.412 | 0.207 | 0.207 | 0.207 | 0.394 | 0.394 | 0.394 | 0.176 | 0.176 | 0.176 |
| $\mathbf{H}$ | 0.067 | 0.067 | 0.067 | 0.256 | 0.256 | 0.256 | 0.000 | 0.000 | 0.000 | 0.123 | 0.123 | 0.123 |

## Variance

## [Input: Endpoint $\Rightarrow$ Output: Endpoint]

This transform calculates the variance of the replicates in each sample group (flagged replicates are not included). The sample of population method is used.

The variance is the sum of the squares of the difference of each replicate and the mean divided by $\mathbf{n - 1}$ (where $\mathbf{n}$ is the number of the replicates).

For groups with less than 2 non-flagged replicates, the result for the group will be set to Flagged.

For viewing common transform settings, see General Transform Properties. To learn about adding this transform to your assay run, see Transforms Tab.

## Example

Let's assume that we have some endpoint measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to find the variance between measurements in each sample group.

Here is some example raw data displayed to 3 decimal places:

## Raw

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.364 | 0.323 | 2.105 | 2.124 | 1.213 | 1.226 | 1.159 | 1.190 | 0.110 | 0.116 | 1.463 | 1.481 |
| $\mathbf{B}$ | 0.455 | 0.441 | 1.869 | 1.888 | 1.203 | 1.218 | 1.180 | 1.206 | 0.132 | 0.130 | 0.786 | 0.768 |
| $\mathbf{C}$ | 0.587 | 0.575 | 0.474 | 0.514 | 1.206 | 1.239 | 1.203 | 1.210 | 0.113 | 0.116 | 0.613 | 0.626 |
| $\mathbf{D}$ | 0.806 | 0.774 | 0.364 | 0.378 | 1.167 | 1.187 | 1.211 | 1.241 | 0.101 | 0.106 | 0.489 | 0.490 |
| $\mathbf{E}$ | 1.105 | 1.065 | 0.279 | 0.367 | 1.215 | 1.225 | 1.233 | 1.262 | 0.111 | 0.114 | 1.579 | 1.549 |
| $\mathbf{F}$ | 1.425 | 1.445 | 0.378 | 0.411 | 1.196 | 1.206 | 1.226 | 1.199 | 0.109 | 0.108 | 0.792 | 0.775 |
| $\mathbf{G}$ | 1.791 | 1.803 | 0.560 | 0.597 | 1.205 | 1.228 | 1.308 | 1.306 | 0.126 | 0.127 | 0.652 | 0.659 |
| $\mathbf{H}$ | 0.253 | 0.270 | 0.463 | 0.470 | 1.214 | 1.261 | 0.115 | 0.116 | 0.115 | 0.115 | 0.490 | 0.477 |

## Layout

The example uses a layout with Standards, Control, Blank, and Unknowns in duplicate (e.g., Standard1 in A1 and A2, Unknown1 at A3 and A4, Unknown2 at B3 and B4, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 1 | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | 2 | 2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | 3 | 3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | 4 | 4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | 5 | 5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | 6 | 6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | 1 | 1 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | 31 | 39 | 39 |
| H | 1 | 1 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | $(40$ | 40 |

## Variance

The transform will generate an output matrix named Variance with the values at each sample group being the product of the input data variance. With the example data provided above, the results are as follows (also displayed to 3 decimal places):

## - Sample Table

Here is a Sample Table snippet, which lists the details of the calculations. For each sample, the following details are reported:

| Sample | Positions | My Raw Data | Variance |
| :--- | :--- | :--- | :--- |
| Standard1 | A1 | 0.364 | 0.001 |
|  | A2 | 0.323 |  |
| Standard2 | B1 | 0.455 | 0.000 |
|  | B2 | 0.441 |  |
| Standard3 | C1 | 0.587 | 0.000 |
|  | C2 | 0.575 |  |
| Standard4 | D1 | 0.806 | 0.001 |
|  | D2 | 0.774 |  |
| Standard5 | E1 | 1.105 | 0.001 |
|  | E2 | 1.065 |  |

## - Matrix Table

Here all calculated data is displayed in a Matrix Table.

|  | $\mathbf{1}$ | $\mathbf{2}$ | $\mathbf{3}$ | $\mathbf{4}$ | $\mathbf{5}$ | $\mathbf{6}$ | $\mathbf{7}$ | $\mathbf{8}$ | $\mathbf{9}$ | $\mathbf{1 0}$ | $\mathbf{1 1}$ | $\mathbf{1 2}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{A}$ | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| $\mathbf{B}$ | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| $\mathbf{C}$ | 0.000 | 0.000 | 0.001 | 0.001 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| $\mathbf{D}$ | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| $\mathbf{E}$ | 0.001 | 0.001 | 0.004 | 0.004 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| $\mathbf{F}$ | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| $\mathbf{G}$ | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| $\mathbf{H}$ | 0.000 | 0.000 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

## General Transform Properties for XY Data

The transforms that operate on the XY Data are configured with:

- General settings for all transforms
- General settings for XY and XY Reduction transforms (described below)
- Specific settings (if available)


## General Settings for XY and XY Reduction Transforms

## Data

| X Axis | This setting determines which values to use in the $x$-axis on the chart and for evaluations: <br> - Index - The 1-based position number of the data point in the collection. <br> - X - Depending on the measurements data type, this option is displayed as: <br> - X (Cycle) - For kinetic data with no time values, $\mathbf{X}$ equals the Index. <br> - X (Time) - For kinetic data, if time values are stored, then this is the time stamp associated with the data point. <br> - X (Wavelength) - For spectral data, if wavelength values are stored, then this is the wavelength of the data point. <br> - $\mathbf{Y}$ - Each value is set to a $y$ value of the corresponding input matrix's data point. |
| :---: | :---: |


| Y Axis | This setting determines which values to consider in the $y$-axis on the chart and for evaluations: <br> - Index - The 1-based position number of the data point in the collection. <br> - X-Depending on the measurements data type, this option is displayed as: <br> - X (Cycle) - For kinetic data with no time values, $\mathbf{X}$ equals the Index. <br> - X (Time) - For kinetic data, if time values are stored, then this is the time stamp associated with the data point. <br> - X (Wavelength) - For spectral data, if wavelength values are stored, then this is the wavelength of the data point. <br> - $\mathbf{Y}$ - Each value is set to a $y$ value of the corresponding input matrix's data point. |
| :---: | :---: |

## Range

The range settings enable the specification of a subset of the data points to consider; only data points within the specified range will be processed. When a range is set, data points outside the specified range are excluded from the calculation. Thus, only the data points inside the specified minimum and maximum range (non-flagged) are processed by the transform.

The range can be defined by setting the $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum properties to a value or expression:

| $\mathbf{X}$ Minimum | A numerical value or expression that defines the minimum $x$ data point to <br> include within the subset. If this is not specified, then all data points less than <br> $\mathbf{X}$ Maximum are included. |
| :--- | :--- |
| $\mathbf{X}$ | A numerical value or expression that defines the maximum $x$ data point to <br> include within the subset. If this is not specified, then all data points greater <br> (han $\mathbf{X}$ Minimum are included. |

To refer to the position's corresponding data on an endpoint matrix, use a Matrix Position Reference (e.g., \#2) to refer to the data on the second matrix. This is useful to set the range of calculations for each sample to be based on the results of previous calculation steps.

In addition, the range can be adjusted interactively by positioning range bars. The range bars are displayed as vertical dashed lines on the chart that you can either:

- Drag with mouse to correct range values
- Double-click to reset minimum or maximum value


## XY Transforms

XY transforms operate on matrices of data that consist of XY data and result in a new XY data matrix.

For example, the XY Baseline Correction transform can be used to remove the inherent baseline from each sample to result in a new XY plot with the measurements shifted down the $y$-axis.

## XY Data

Throughout MyAssays Desktop, we refer to measurements that yield a 2-dimensional data sets for each sample in general terms as "XY Data". This encompasses:

- Kinetic data with measurements made over time (with or without time values).
- Spectral data measured at different wavelengths.
- Scan data measured as a single line (with a distance from an origin).


## XY Axes

With certain measurement types, there might be different data available to plot on the $x$ - and $y$-axis. For example, with kinetic data measured over time, you might want to use the time values as the x-axis or the cycle number instead. The axes settings specify what data should be processed by the transform. For additional details, see General Transform Properties for XY Data subsection.

## Flagged XY Data Points

Any data point that is part of an XY data set can be flagged. Flagged data points are excluded from the calculation.

For example, with the XY Baseline Correction transform, if the baseline is computed using the average of the 4 lowest measurements but the lowest measurement is flagged, then the baseline will be computed using the next 4 lowest data points.

In addition to each data point, each position can also be flagged to completely exclude the sample from any further analysis. This follows the same convention as Flagged Endpoint.

## XY Range

By default, XY data transform calculations are performed on all XY data points that are not flagged. However, it is also possible to define a range of points to process. This is useful if the area of interest is only a subset of the measurements. The range minimum and maximum values can be set as an absolute value or as an expression (that can in turn refer to other computed results).

For example, the XY Expression transform could be configured to work with input XY data that has 100 data points and to use only points $>=20$ and $<=80$. In this case, the resulting output XY matrix would contain only this subset.

Refer to the General Transform Properties for XY Data subsection for more details.

## XY Transforms

| Transform | Operation |
| :---: | :---: |
| Baseline Correction | This transform removes the inherent variable background, or baseline, from each input position. |
| Blank <br> Correction | Subtracts the average of specified Blank group(s) from each input position. |
| Expression <br> (Dual) | Evaluates an expression in terms of $x$ and $y$, where variables are set to the value of each position of the first $(x)$ and second $(y)$ input matrices. |
| Expression | Evaluates the specified expression for each input position. |
| Fit | Fits a curve to each used XY plot. |
| Join | Creates new combined XY plot from two input XY matrices for each position by appending data points from the second matrix to those of the first. |
| Outlier <br> Removal | Flags data points that are considered to be outliers. The values of the output matrix match the input matrix (the only difference being that outlier(s) might be flagged). |
| Outlier <br> Removal (for <br> Horizontal) | Flags the specified number of data points for each plot, in which \%CV of its points is greater than specified threshold. |
| Replicates <br> Average | Calculates the XY average plot for each sample group. |
| Replicates <br> Function | Calculates the new plot for each sample group by using specified function on its replicates. |

## XY Baseline Correction

[Input: $\mathbf{X Y} \Rightarrow$ Output: XY]
This transform removes the inherent variable background (or baseline) from each XY position. Flagged positions or points are not included in the calculations. The operation is only performed on data within specified range.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Method | The method for a baseline correction: <br> - Average Between X1 And X2 - Correct points by the average $y$ of the non-flagged points between and including points $\mathbf{X}$ Index $\mathbf{1}$ and $\mathbf{X}$ Index 2. <br> - Average Of N Lowest - Correct points by the average $y$ of the lowest non-flagged $\mathbf{N}$. <br> - Proportional N1 Lowest and N2 Highest - Proportional percentage between average of the $\mathbf{N} \mathbf{1}$ lowest and $\mathbf{N} \mathbf{2}$ highest non-flagged points. |
| :---: | :---: |
| The visibility of the following settings depend on the selected Method. For example, if you choose the Average Of $\mathbf{N}$ Lowest, then only the $\mathbf{N}$ setting will be displayed. |  |


| $\mathbf{X}$ Index $\mathbf{1}$ | These settings are used in the Average Between X1 And X2 method. <br> These are 1-based index numbers and apply to the data sets with or without <br> time values. Only non-flagged points that are <=X Index $\mathbf{1}$ and $>=\mathbf{X}$ |
| :--- | :--- |
| Index $\mathbf{2}$ are used. $\mathbf{X}$ Index $\mathbf{1}$ and $\mathbf{X}$ Index $\mathbf{2}$ must be $>=\mathbf{1}$ and $\mathbf{X}$ Index $\mathbf{2}$ |  |
| must be >=X Index $\mathbf{1}$. |  |
| If there are no included points between $\mathbf{X}$ Index $\mathbf{1}$ and $\mathbf{X}$ Index 2, then no |  |
| correction will be made and the output position will be flagged. |  |


| N | This setting is used in the Average Of N Lowest method. <br> It is the number of lowest non-flagged data points within a subset. If the number of included points $<\mathbf{N}$, then the output position will be flagged. If the number of included points $=\mathbf{N}$, then the result is that all points are corrected by the average of all points. |
| :---: | :---: |
| N1 N2 | These settings are used in the Proportional N1 Lowest and N2 Highest method. <br> $\mathbf{N} \mathbf{1}$ is the number of the lowest points, $\mathbf{N} \mathbf{2}$ is the number of the highest points used for correction. $\mathbf{N} 1$ and $\mathbf{N} \mathbf{2}$ must be $>=\mathbf{1}$. The average of each of these is computed. Then each $y$ value is scaled between these points (e.g., if a point equals the average of the lowest, then it is set to 0 ; or if a point equals the average of the highest, it is set to 100). <br> Also, note results here are clipped. In other words, all results will fall within the range 0 and 100 , so values which are calculated to $<0$ or $>100$ will be set to 0 and 100 , respectively. <br> If the average of the lowest values is equal to the average of the highest values, then this cannot be calculated and the output position will be flagged. If there are less than $\mathbf{N} 1$ or $\mathbf{N} \mathbf{2}$ valid readings, then the output position will be flagged. |

## Example

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to remove the inherent variable background from each point. Here this background is measured as the average of five lowest points for each XY plot and is then subtracted from each point of this plot. The calculations for the $\mathbf{A} 1$ position are shown for illustration of the transform.

## Raw

Here is an XY matrix with each position containing 80 XY data points. Position data plots of the matrix are displayed in Plate Scale.


The raw data is displayed here to 3 decimal places:
A1 317.780;0.235 377.710;0.230 437.630;0.231 497.530;0.232 557.440;0.229 617.360;0.232 677.280;0.234 737.180;0.242 797.080;0.360 856.980;0.567 916.900;0.705 976.840;0.766 1036.760;0.791 1096.680;0.798 1156.580;0.800 1216.480;0.797 1276.410;0.786 1336.330;0.769 1396.230;0.746 1456.150;0.721 1516.050;0.696 1575.980;0.666 1635.900;0.634 1695.850;0.599 1755.750;0.560 1815.670;0.518 1875.590;0.470 1935.510;0.416 1995.410;0.364 2055.320;0.319 2115.220;0.277 2175.120;0.261 2235.020;0.255 2294.970;0.251 2354.890;0.233 2414.790;0.231 2474.690;0.228 2534.620;0.227 2594.520;0.229 2654.420;0.226 2714.340;0.226 2774.280;0.225 2834.180;0.226 2894.100;0.225 2954.030;0.226 3013.970;0.225 3073.900;0.224 $3133.800 ; 0.2273193 .700 ; 0.2253253 .600 ; 0.2243313 .500 ; 0.2253373 .420 ; 0.225$ $3433.340 ; 0.2243493 .270 ; 0.2243553 .210 ; 0.2233613 .130 ; 0.2233673 .030 ; 0.224$ $3732.950 ; 0.2233792 .860 ; 0.2243852 .760 ; 0.2233912 .660 ; 0.2233972 .580 ; 0.223$ 4032.510;0.223 4092.460;0.222 4152.390;0.222 4212.330;0.225 4272.230;0.223 4332.130;0.223 4392.030;0.223 4451.930;0.223 4511.830;0.223 4571.730;0.222 4631.650;0.221 4691.560;0.220 4751.500;0.224 4811.420;0.222 4871.350;0.222 4931.280;0.222 4991.180;0.222 5051.080;0.222

To correct each point with the specified baseline:

1. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
2. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.

## 3. Specify Settings: Method as Average Of N Lowest, $\mathbf{N}$ as 5.

4. Click Calculate.

## Corrected

The transform will generate an output XY matrix named Corrected where each position will contain the plot with the specified baseline subtracted from each input point.

With the example data provided above, the result for the $\mathbf{A 1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows:


## - Sample Table

Here is a Sample Table snippet, which shows the difference between input and output plot at the A1 position. For each sample, the following details are reported:

| Sample | Positions | Raw | Corrected |
| :--- | :--- | :--- | :--- |
| OUnknown1 | A1 | $\Omega$ | $\Omega$ |

## XY Blank Correction

## [Input: XY $\Rightarrow$ Output: XY]

This transform subtracts the average of a specified blank group (or groups) from each sample on a point-by-point basis. This is useful for subtracting background noise from measurements.

The background plot is first determined by creating an average XY plot of all replicates of the blank group. This background plot is then subtracted from each associated sample on a point-by-point basis.

Flagged positions or points are not included in the calculations. The operation is only performed on data within specified range.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

This transform requires a layout with at least 1 Blank group.

| Blank | The group number of the sample to use for correction. This can be defined as <br> a number or as an expression. The expression can refer to $x$ which is the <br> Group number of the sample to be corrected. |
| :--- | :--- |
| Blank Type | For example, set this to $x$ to perform a 1-to-1 correction where each group is <br> corrected by the sample of the specified blank type with the same group <br> number. |

## Example: Single Blank

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to perform a blank correction using the average of one blank group. This blank group itself is measured in duplicate. The mean of these replicates will be subtracted from all other samples.

## Layout

The example uses a layout with Standards, Control, Blank, and Unknowns in duplicate (e.g., Standard1 in A1 and A2, Unknown1 at A3 and A4, Unknown2 at B3 and B4, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A | 1 | 1 | 1 | 1 | 9 | 9 | 17 | 17 | 25 | 25 | 33 | 33 |
| B | 2 | 2 | 2 | 2 | 10 | 10 | 18 | 18 | 26 | 26 | 34 | 34 |
| C | 3 | 3 | 3 | 3 | 11 | 11 | 19 | 19 | 27 | 27 | 35 | 35 |
| D | 4 | 4 | 4 | 4 | 12 | 12 | 20 | 20 | 28 | 28 | 36 | 36 |
| E | 5 | 5 | 5 | 5 | 13 | 13 | 21 | 21 | 29 | 29 | 37 | 37 |
| F | 6 | 6 | 6 | 6 | 14 | 14 | 22 | 22 | 30 | 30 | 38 | 38 |
| G | 1 | 1 | 7 | 7 | 15 | 15 | 23 | 23 | 31 | 31 | 39 | 39 |
| H | 1 | 1 | 8 | 8 | 16 | 16 | 24 | 24 | 32 | 32 | $(40$ | 40 |

To subtract the mean of the blank positions from the measurements, specify Blank Group to 1 and Blank Type to Blank.

## Blank Corrected

The transform will generate an output matrix named Blank Corrected with the value at each position being the subtraction of the average of Blank1 sample group from the input data.

## Example: 1-1 Blank Correction

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to perform a 1-to-1 correction, where each sample group is corrected by the blank that it is associated with. The blank numbering used in the layout defines the associations.

## Layout

The example uses a layout with 16 Unknowns groups (with Unknown1 in positions from A1 to A5, Unknown2 in positions from B1 to B5, etc.) and 16 Blank singlets:

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 1 | 1 | 1 | 9 | 9 | 9 | 9 | 9 | 9 |
| B | 2 | 2 | 2 | 2 | 2 | 2 | 10 | 10 | 10 | 10 | 10 | 10 |
| C | 3 | 3 | 3 | 3 | 3 | 3 | 11 | 11 | 11 | $(11$ | 11 | $(11$ |
| D | 4 | 4 | 4 | 4 | 4 | 4 | 12 | 12 | 12 | 12 | 12 | 12 |
| E | 5 | 5 | 5 | 5 | 5 | 5 | 13 | 13 | 13 | 13 | 13 | 13 |
| F | 6 | 6 | 6 | 6 | 6 | 6 | 14 | 14 | 14 | 14 | 14 | 14 |
| G | 7 | 7 | 7 | 7 | 7 | 7 | 15 | 15 | 15 | 15 | 15 | 15 |
| H | 8 | 8 | 8 | 8 | 8 | 8 | 16 | 16 | 16 | 16 | 16 | 16 |

Blank Unknown
Specify Blank Group to $x$ and Blank Type to Blank to perform 1-1 correction.
With this specified, the value of each blank will be subtracted from every replicate of associated sample group. In other words, Blank1 (stored in A6) value will be subtracted from A1, A2, A3, A4, A5 (the members of Unknown1), and from A6 itself.

## Blank Corrected

The transform will generate an output matrix named Blank Corrected with the value at each position being the subtraction of the associated blank value from the input data. Therefore, calculated matrix will contain blank corrected Unknown values and all Blanks will be 0 .

## Advanced Example: By-Row Blank Correction

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to configure blank correction on a row-by-row basis to blank correct each sample by the average of the two blank samples of the same row.

## Layout

The example uses a layout with 80 singlet Unknowns and 8 Blank duplicates for each row (with Blank1 in positions A1 and A12, Blank2 in positions B1 and B12, etc.):

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 9 | 17 | 25 | 33 | 41 | $(49$ | 57 | 65 | 73 |
| B | 2 | 2 | 10 | 18 | 26 | 34 | $(42$ | 50 | 58 | 66 | 74 | 2 |
| C | 3 | 3 | 11 | 19 | 27 | 35 | 43 | 51 | 59 | 67 | 75 | 3 |
| D | 4 | 4 | 12 | 20 | 28 | 36 | $(44$ | 52 | 60 | 68 | 76 | 4 |
| E | 5 | 5 | 13 | 21 | 29 | 37 | 45 | 53 | 61 | 69 | 77 | 5 |
| F | 6 | 6 | 14 | 22 | 30 | 38 | 46 | 54 | 62 | 70 | 78 | 6 |
| G | 7 | 7 | 15 | 23 | 31 | 39 | 47 | 55 | 63 | 71 | 79 | 7 |
| H | 8 | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 | 80 | 8 |

- Blank $\bigcirc$ Unknown

The Blank Group setting uses the expression:

## 1+floor((x-1)/(LayoutWidth()-2), 1)

This converts the group number of the sample to blank correct ( $\boldsymbol{x}$ ) to the blank number on its row. In other words, for $\boldsymbol{x}=\mathbf{1}$ this means $\mathbf{1 + f l o o r ( ( 1 - 1 ) / 1 0 ) , 1 ) , ~ f l o o r = 0 ~ a n d ~ t h e ~ B l a n k ~}$

Group is taken as 1 . For the first row, $x$ is in range from 1 to 10 and the expression outputs 1 , thus for Unknowns from 1 to 10 the average of Blank1 is subtracted.

For the second, $x$ is from 11 to 20, floor=1, so the Blank2 group is subtracted from
Unknowns11-20, etc.

## Blank Corrected

The transform will generate an output matrix named Blank Corrected with the value at each position being the subtraction of the average of the associated Blank group from the input data.

## XY Expression (Dual)

## [Input: Dual XY $\Rightarrow$ Output: XY]

This transform evaluates an expression defined in terms of $x$ and $y$ for each non-flagged input XY data point within the specified range. In this case $x$ refers to the data for the sample on the first input matrix and $y$ refers to the data for the same sample on the second input matrix.

This transform is useful when it is necessary to perform a common operation on two sets of XY data. For example, this transform could be used to create a plot for each sample, that is the difference or ratio of two sets of $X Y$ measurements.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Expression The expression to evaluate, where variables are set to the value of each <br> position of the first $(x)$ and second $(y)$ input matrices. Default expression value <br> is $\boldsymbol{x}-\boldsymbol{y}$. <br>  To refer to the position's corresponding data on an endpoint matrix, <br> use a Matrix Position Reference (e.g., \#2) to refer to the data on the <br> second matrix. <br> Plot Input <br> on Chart When the check box is selected, the chart includes both the input and the <br> calculated plot. If it is cleared, the chart shows the calculated plot only. |
| :--- | :--- |

## Example

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the
Raw and Corrected matrices. In this example, we would like to calculate a new plot by summarizing input plot values in these matrices point-by-point. The calculations for the A1 position are shown for illustration of the transform.

Here are XY matrices with each position containing 80 XY data points. Position data plots of the matrices are displayed in Plate Scale.

## Raw



Corrected


To add plot values of the input matrices and calculate a new plot for each position:

1. Specify output matrix name as Raw+Corrected.
2. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
3. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
4. Specify Settings: Expression as $\boldsymbol{x}+\boldsymbol{y}$, select the Plot Inputs on Chart check box.
5. Click Calculate.

## Raw+Corrected

The transform will generate an output XY matrix named Raw +Corrected where each position will contain the plot with each data point being point by point sum of the Raw and Corrected matrices plots.

With the example data provided above, the result for the $\mathbf{A 1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows:

Dual Expression


## - Sample Table

Here is a Sample Table snippet, which shows the input plots and calculated resulting plot at the A1 position. For each sample, the following details are reported:

| Sample | Positions | Raw | Corrected | Raw+Corrected |
| :--- | :--- | :--- | :--- | :--- |
| OUnknown1 | A1 | $\sim$ |  |  |

## XY Expression

## [Input: XY $\Rightarrow$ Output: XY]

This transform evaluates a specified expression for each input XY data point (only points within the specified range that are not flagged are processed). The resulting XY plot is generated from this expression.

This transform is useful when it is necessary to perform an operation on all input

(1)points to calculate a new plot for each sample. For example, this transform could be used to subtract, correct, or multiply all plots by a specified value.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Expression | The expression to evaluate, where the $y$ variable is set to the value of each <br> position of the input matrix. |
| :--- | :--- |
|  |  |
|  | When the check box is selected, the chart includes both the input and the <br> calculated plot. If it is cleared, the chart shows the calculated plot only. |

## Example

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to double data points in each input plot. The calculations for the $\mathbf{A} 1$ position are shown for illustration of the transform.

## Raw

Here is an XY matrix with each position containing 80 XY data points. Position data plots of the matrix are displayed in Plate Scale.


To double each data point in each plot:

1. Specify output matrix name as Raw *2.
2. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
3. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
4. Specify Settings: Expression as $\boldsymbol{y}^{* 2}$, select the Plot Input on Chart check box.
5. Click Calculate.

## Raw *2

The transform will generate an output XY matrix named Raw*2 where each position will contain the plot with each input point multiplied by 2 .
With the example data provided above, the result for the $\mathbf{A 1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows:


## - Sample Table

Here is a Sample Table snippet, which shows the input plot and calculated resulting plot at the A1 position. For each sample, the following details are reported:

| Sample | Positions | Raw | Raw *2 |
| :--- | :--- | :--- | :--- |
| OUnknown1 | A1 |  | $\Omega$ |

## XY Fit

## [Input: XY $\Rightarrow$ Output: XY]

This transform fits a curve to each XY plot and generates a new XY plot from the calculated curve.

The resulting plot is computed by calculating the $y$ value of each $x$ point using the fitted curve. (Only points within the specified range that are not flagged are processed.)

This transform can be used to smooth each XY plot using a particular curve fitting model. If you are interested in a fitting parameter of the curve fit (such as the slope of linear regression or midpoint of $\underline{4 \mathrm{PL}) \text {, then use the XY Reduction Fit Parameter }}$ transform.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

## Fit Method

 The curve-fitting method to construct the curve (from each XY plot).
## Example

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to fit a 4PL curve for each input plot. The calculations for the $\mathbf{A} \mathbf{1}$ position are shown for illustration of the transform.

## Raw

Here is an XY matrix with each position containing 80 XY data points. Position data plots of the matrix are displayed in Plate Scale.


To fit a $\underline{4 \mathrm{PL}}$ curve to each input data plot:

1. Specify output matrix name as Smoothed.
2. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
3. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
4. Specify Settings: Fit Method as 4PL.

## 5. Click Calculate.

## Smoothed

The transform will generate an output XY matrix named Smoothed where each position will contain the 4PL curve fitted to each input plot.

With the example data provided above, the result for the $\mathbf{A 1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows:
Fit


| $y=0.0663775+\frac{0.334767-0.0663775}{1+\left(\frac{x}{18.0566}\right)^{3.56957}}$ | Coefficient | Value | +/- Error | Goodness Measure | Value |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | a | 0.334767 | 0.00396867 | $\mathrm{R}^{2}$ | 0.988655 |
|  | b | 3.56957 | 0.223465 | $\mathrm{aR}^{2}$ | 0.987844 |
|  | c | 18.0646 | 0.333216 | P | 0 |
|  | d | 0.0663775 | 0.00352412 | SE | 0.0109585 |
|  |  |  |  | F | 1219.99 |

## - Sample Table

Here is a Sample Table snippet, which shows the input plot and calculated resulting plot at the A1 position. For each sample, the following details are reported:

| Sample | Positions | My Raw Data | Smoothed |
| :--- | :--- | :--- | :--- |
| OUnknown1 | A1 |  |  |

## XY Join

## [Input: Dual XY $\Rightarrow$ Output: XY]

This transform accepts two XY input matrices and creates new combined XY plot for each position by appending data points from the second matrix to those of the first (Any flagged data points are not included in the output matrix).

This transform can be used to combine two separate sets of XY measurements to a single plot (for each sample) for further analysis or reporting.

When using this transform, consider the $\mathbf{X}$ Axis setting of the Data group. The joined data will use the data from both input matrices. To make sure that the resulting joined contents has sequential $x$ values, set the $\mathbf{X}$ Axis setting to Index.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

Advanced and Range settings do not apply to this transform.

Data

If $\mathbf{X}$ is set as Index, then the first index of the joined matrix is 1 plus the last used index.
If $\mathbf{X}$ is set as $\mathbf{X}$, then the $x$ values specified in each input matrix are used (unchanged) in the output matrix.

Units

| Output | The measurement units to be included in the header of the Sample Table. <br> If the output units of the transform are the same as the input, then use "ditto" <br> Units <br> units to automatically carry these units through (i.e., apply the same units to <br> the transform's output). This shortcut means that it is not necessary to repeat <br> settings across transforms. Ditto units are specified with two dots, i.e.: <br> . |
| :--- | :--- |

## Second Matrix X Range Correction

This is an advanced feature that can only be configured through the XML editor.

| CorrectSecondMatrixX | This setting defines how the $x$ values used in the second matrix <br> are corrected if $\mathbf{X}$ Axis is not Index. This can be used to ensure <br> that the $x$ values of the joined data are contiguous with a regular <br> interval. <br> - None - The results are merged on a point-by-point basis (i.e., <br> first data point from the second matrix is appended to the first <br> point of the first matrix, etc.) <br> - GaplsAveragelnterval - For each plot, the average interval <br> of the data points in the first input matrix is computed. The <br> first $x$ value of the second input matrix is set to equal the $x$ <br> value of the last data point plus the computed average interval. <br> The subsequent data points are first normalized by subtracting <br> the original value of the first data point. Then, the computed $x$ <br> of the first data point is added to ensure a contiguous range of <br> $x$ values |
| :---: | :--- |
| In this case, flagged data points are considered and <br> used in these $x$ correction calculations (however, any |  |
| flagged data points in the input matrices will also be |  |
| flagged in the output). |  |

## Example

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to create a new combined plot for each position. The calculations for the $\mathbf{A 1}$ position are shown for illustration of the transform.

Here are XY matrices with each position containing 60 XY data points. Position data plots of the matrices are displayed in Plate Scale.

## Read 1



Read 2


To append the data points from the second matrix to those of the first for each plot:

1. Specify output matrix name as Joined.
2. Specify Data: X Axis as Index, Y Axis as Y.
3. Click Calculate.

## Joined

The transform will generate an output XY matrix named Joined where each position will contain a new XY plot which is simply the plot from the first matrix with the plot from the second matrix appended to it.

With the example data provided above, the result for the $\mathbf{A 1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows:


- Sample Table

Here is a Sample Table snippet, which shows the input plots and output appended plot at the A1 position. For each sample, the following details are reported:

| Sample | Positions | Read 1 | Read 2 | Joined |
| :--- | :--- | :--- | :--- | :--- |
| OUnknown1 | A1 |  |  | $\ddots$ |

## XY Outlier Removal

## [Input: XY $\Rightarrow$ Output: XY]

This transform flags data points that are considered to be outliers. The values of the output matrix match those of the input matrix (the only difference being that any specified outlier(s) are flagged).

For each XY plot, the transform:

1. Calculates the linear regression of the specified number of adjacent points (segment)
2. Uses the calculated line to calculate $y$ from each $x$ in the segment
3. Compares each calculated $y$ with the original $y$ and flags the points where the $\%$ difference is > than the specified threshold

This process is repeated across each sequential segment in the plot for adjacent point sets.
This transform can be used to automatically remove outlier points in XY data.
For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Num <br> Regression <br> Points | The number of regression points used for each linear regression segment <br> when determining the maximum slope. The default and minimum value is 2. |
| :--- | :--- |
| Threshold | It calculates absolute \% difference between back fit value and the original $y$ <br> value. The back fit is computed from slope and intercept for each $x$ value in <br> the regression set. If this \% difference is $>$ the specified Threshold, then the <br> data point is flagged on the output matrix. |
| Points that are flagged in one regression set are not included in <br> calculations of following regression sets. |  |

## Example

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the

Raw matrix. In this example, we would like to remove up to 3 outliers from each input plot. The calculations for the $\mathbf{A 1}$ position are shown for illustration of the transform.

## Raw

Here is an XY matrix with each position containing 80 XY data points. Position data plots of the matrix are displayed in Plate Scale.


To flag outliers for each plot:

1. Specify output matrix name as Outliers Removal.
2. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
3. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
4. Specify Settings: Number of Regression Points as $\mathbf{6}$ and the Threshold as $\mathbf{4 5}$.
5. Click Calculate.

## Outliers Removal

The transform will generate an output XY matrix named Outliers Removal where each position will contain the plot matching the input plot and outlier data points flagged.

With the example data provided above, the result for the $\mathbf{A 1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows:

Outlier Removal


- Sample Table

Here is a Sample Table snippet, which shows the input plot and calculated resulting plot at the A1 position. For each sample, the following details are reported:

| Sample | Positions | Raw | Outliers Removal |
| :--- | :--- | :--- | :--- |
| OUnknown1 | A1 | $\Omega$, | $\Omega$ |

## XY Outlier Removal (for Horizontal)

## [Input: XY $\Rightarrow$ Output: XY]

This transform flags data points that are considered to be outliers. The values of the output matrix match those of the input matrix (the only difference being that any specified outlier(s) are flagged).

For each XY plot, the transform:

1. Calculates the \%CV of each set of its $X Y$ points (including only the points not already flagged).
2. If the \%CV is greater than the specified \%CV Threshold, then the point furthest from the mean is removed.

These steps are repeated until either the new \%CV of the data points that are not flagged is below the threshold, or until the specified number of outliers to remove has been removed. Calculations are performed on the non-flagged replicates within the specified range of each XY plot.

This transform can be used to automatically remove outlier points in XY data.
For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| \% CV | Defines threshold percentage coefficient of variance. Data points with values <br> Thresulting in a \%CV greater than the specified threshold will be flagged. The <br> default value is $15 \%$. |
| :--- | :--- |
| Outliers To <br> Remove | Specifies the maximum number of outliers to remove. The minimum and <br> default value for this setting is 1. |

## Example

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the
Raw matrix. In this example, we would like to remove 1 outlier from each input plot. The calculations for the $\mathbf{A} 1$ position are shown for illustration of the transform.

## Raw

Here is an XY matrix with each position containing 33 XY data points. Position data plots of the matrix are displayed in Plate Scale.


To flag 1 outlier for each plot:

1. Specify output matrix name as Outlier Removal (Horizontal).
2. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Cycle), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
3. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
4. Specify Settings: \%CV Threshold as $\mathbf{5}$ and the Outliers To Remove as $\mathbf{1}$.
5. Click Calculate.

## Outlier Removal (Horizontal)

The transform will generate an output XY matrix named Outliers Removal where each position will contain the plot matching the input plot and outlier data points flagged.

With the example data provided above, the result for the $\mathbf{A 1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows:

Outlier Removal for Horizontal


## - Sample Table

Here is a Sample Table snippet, which shows the input plot and calculated resulting plot at the A1 position. For each sample, the following details are reported:

| Sample | Positions | Raw | Outliers Removal |
| :--- | :--- | :--- | :--- |
| OUnknown1 | A1 |  |  |

## XY Replicates Average

## [Input: XY $\Rightarrow$ Output: XY]

This transform calculates the average XY (kinetic/spectral) plot for each sample group. Both the $x$ and $y$ values of the replicates in each group are averaged.

If your XY data features unique $x$ values for each point (such as ascending time values that are unique for each sample), you may prefer to set the $x$-axis type to Index so that the resulting plot is sequential with an increment of 1 . Alternatively, if another x -axis setting is used, please note that the resulting XY plot will contain the average of the input $x$ values.

Any flagged data points are not included in the analysis.
As with other XY transforms, the calculations are performed on data within the specified ranges only.

(1)
To use this transform, select a layout with at least one sample group that contains more than one replicate.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Example

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like calculate the average plot of the replicate plots for each sample group. The calculations for the Unknown1 group are shown for illustration of the transform.

## Raw

Here is an $\mathbf{X Y}$ matrix with each position containing 80 XY data points. Position data plots of the matrix are displayed in Plate Scale.


## Layout

The example uses a layout with 32 Unknowns groups in triplicates (with Unknown1 in positions from $\mathbf{A} \mathbf{1}$ to $\mathbf{A} \mathbf{3}$, Unknown2 in positions from $\mathbf{A} 4$ to $\mathbf{A 6}$, etc.). I.e.:

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 4 |
| B | 5 | 5 | 5 | 6 | 6 | 6 | 7 | 7 | 7 | 8 | 8 | 8 |
| C | 9 | 9 | 9 | 10 | 10 | 10 | 11 | $(11$ | 11 | 12 | 12 | 12 |
| D | 13 | 13 | 13 | 14 | 14 | 14 | 15 | 15 | 15 | 16 | 16 | 16 |
| E | 17 | 17 | 17 | 18 | 18 | 18 | 19 | 19 | 19 | 20 | 20 | 20 |
| F 21 | 21 | 21 | 22 | 22 | 22 | 23 | 23 | 23 | 24 | 24 | 24 |  |
| G | 25 | 25 | 25 | 26 | 26 | 26 | 27 | 27 | 27 | 28 | 28 | 28 |
| H 29 | 29 | 29 | 30 | 30 | 30 | 31 | 31 | 31 | 32 | 32 | 32 |  |

To calculate averaged data plot for each sample group:

1. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
2. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
3. Click Calculate.

## Average

The transform will generate an output XY matrix named Average where each position will contain the averaged data plot for each sample group.

With the example data provided above, the result for the Unknown1 averaged plot is displayed on the Kinetic/Spectral Analysis Chart as follows:


## - Sample Table

Here is a Sample Table snippet, which shows the input plots and averaged output plot at the Unknown1 group. For each sample, the following details are reported:

| Sample | Positions | Raw | Average |
| :--- | :--- | :--- | :--- |
| OUnknown1 | A1 | $\ddots$ | $\Omega$ |
|  | A2 | $\ddots$ |  |
|  | A3 | $\ddots$ |  |
|  |  |  |  |

## XY Replicates Function

## [Input: XY $\Rightarrow$ Output: XY]

This transform computes a new plot for each XY (kinetic/spectral) replicate group where the $y$ values of the resulting plot are computed from the selected function applied to the corresponding replicate values of the group.

(1)
Selecting the Average function will yield the same results at the XY Replicates
Average transform, i.e., an average XY plot of the replicates.
The $y$ value of each point in the plot is the result of the selected function applied to each $y$ value of the point for the replicate. The $x$ value of each point is the average of the $x$ values of the replicates.

If your XY data features unique $x$ values for each point (such as ascending time values that are unique for each sample), you may prefer to set the $x$-axis type to Index so that the resulting plot is sequential with an increment of 1 . Alternatively, if another x -axis setting is used, please note that the resulting XY plot will contain the average of the input $x$ values.

Any flagged data points are not included in the analysis.
As with other XY transforms, the calculations are performed on data within the specified ranges only.

(1)
To use this transform, select a layout with at least one sample group that contains more than one replicate.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| For each sample group, the $y$ values are plotted as: |
| :---: | :---: |
| - Average - The point-to-point average of the replicates $y$ values |
| - Median - The point-to-point median of the replicates $y$ values |
| - Standard Deviation - The point-to-point standard deviation between |
| the replicates $y$ values |
| - \%CV - The point-to-point percentage coefficient of variation between the |
| replicates $y$ values |
| - Sum - The point-to-point sum of the replicate plots |
| - Minimum - For each sample, the point-to-point minimum $y$ value of |
| each point is used to create the resulting plot |
| - Maximum - For each sample, the maximum $y$ value of each point is |
| used to create the resulting plot |

## Example

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like calculate the standard deviation of the replicate plots for each sample group. The calculations for the Unknown1 group are shown for illustration of the transform.

## Raw

Here is an $\mathbf{X Y}$ matrix with each position containing 80 XY data points. Position data plots of the matrix are displayed in Plate Scale.


## Layout

The example uses a layout with 32 Unknowns groups in triplicates (with Unknown1 in positions from $\mathbf{A} \mathbf{1}$ to $\mathbf{A} \mathbf{3}$, Unknown2 in positions from $\mathbf{A} 4$ to $\mathbf{A 6}$, etc.). I.e.:

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 3 | 4 | 4 | 4 |
| B | 5 | 5 | 5 | 6 | 6 | 6 | 7 | 7 | 7 | 8 | 8 | 8 |
| C | 9 | 9 | 9 | 10 | 10 | 10 | 11 | 11 | 11 | 12 | 12 | 12 |
| D | 13 | 13 | 13 | 14 | 14 | 14 | 15 | 15 | 15 | 16 | 16 | 16 |
| E | 17 | 17 | 17 | 18 | 18 | 18 | 19 | 19 | 19 | 20 | 20 | 20 |
| F | 21 | 21 | 21 | 22 | 22 | 22 | 23 | 23 | 23 | 24 | 24 | 24 |
| G | 25 | 25 | 25 | 26 | 26 | 26 | 27 | 27 | 27 | 28 | 28 | 28 |
| H 29 | 29 | 29 | 30 | 30 | 30 | 31 | 31 | 31 | 32 | 32 | 32 |  |

To find the standard deviation between input replicate plots for each sample group:

1. Specify output matrix name as Replicates SD.
2. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
3. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
4. Specify Settings: Function as Standard Deviation.
5. Click Calculate.

## Replicates SD

The transform will generate an output XY matrix named Replicates SD where each position will contain the calculated plot with deviation between input plots of each sample group. With the example data provided above, the results for the Unknown1 averaged plot are displayed on the Kinetic/Spectral Analysis Chart as follows:


## - Sample Table

Here is a Sample Table snippet, which shows the input plots and output plot at the Unknown1 group. For each sample, the following details are reported:

| Sample | Positions | Raw | Replicates SD |
| :--- | :--- | :--- | :--- |
| OUnknown1 | A1 | $\Omega$ |  |
|  | A2 | $\ddots$ |  |
|  |  | $\Omega$ |  |
|  | $A 3$ | $\Omega$ |  |

## XY Reduction Transforms

XY transforms operate on matrices of data that consist of XY data and result in a new endpoint data matrix.

For example, the XY Reduction Peak Point transform will compute the peak value of each XY plot.

## XY Data

Throughout MyAssays Desktop, we refer to measurements that yield a 2-dimensional data sets for each sample in general terms as "XY Data". This encompasses:

- Kinetic data with measurements made over time (with or without time values).
- Spectral data measured at different wavelengths.
- Scan data measured as a single line (with a distance from an origin).


## XY Axes

With certain measurement types, there might be different data available to plot on the $x$ - and $y$-axis. For example, with kinetic data measured over time, you might want to use the time values as the x-axis or the cycle number instead. The axes settings specify what data should be processed by the transform. For further details, see General Transform Properties for XY Data subsection.

## Flagged XY Data Points

Any data point that is part of an XY data set can be flagged. Flagged data points are excluded from the calculation.

For example, with the XY Reduction Peak Point transform, if the peak is set to minimum and is computed using the lowest measurement but the lowest measurement is flagged, then the peak will be computed using the next lowest data point.

In addition to each data point, each position can also be flagged to completely exclude the sample from any further analysis. This follows the same convention as for Flagged Endpoint.

## XY Range

By default, XY data transform calculations are performed on all XY data points that are not flagged. However, it is also possible to define a range of points to process. This is useful if the area of interest is only a subset of the measurements. The range minimum and maximum values can be set as an absolute value or as an expression (that can in turn refer to other computed results).

For example, the XY Reduction Average transform could be configured to work with input XY data that has 100 data points and to use only points >=20 and <= 80. In this case, the resulting output endpoint matrix would contain only mean value from this subset at each position.

Refer to the General Transform Properties for XY Data subsection for more details.

## XY Reduction

| Transform | Reduces Data To |
| :---: | :---: |
| Area Under Curve | The area under the curve computed using either the trapezia or summation method. |
| Average | The average of non-flagged data points within a subset at each position. |
| Fit Parameter | The specified parameter for non-flagged data points within specified range at each position. |
| Maximum <br> Slope | Determined maximum slope between two adjacent points within specified range at each position. |
| Peak from <br> Fit | Determined peak (minimum or maximum) value interpolated from the curve fitted to the of non-flagged points within specified range at each position. |
| Peak Point | Determined peak (minimum or maximum) data point within the specified range of the non-flagged points at each position. |
| Total | The sum of non-flagged data points within specified range at each position. |
| $X$ at $Y$ | The $x$ value at which the specified $y$ value crosses the evaluated plot of nonflagged points within the specified range at each position. |
| $X$ between $Y$ | The difference between the first two $x$ points that equal or cross specified $y$ value at each position. |
| Y at X | Determined $y$ value for a specified $x$ value within the specified range of the non-flagged points at each position. |

## XY Reduction Area Under Curve

## [Input: XY data $\Rightarrow$ Output: Endpoint]

This reduction transform computes the area under the curve of each XY plot using either the trapezia or summation method (within the specified range of points to consider). Flagged positions or points are not included in the calculations.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Example

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to reduce data in each position to the area under data points and store resulting value to corresponding position of an endpoint matrix.

## Raw

Here is an XY matrix with each position containing 10 XY data points. Position data plots of the matrix are displayed in Plate Scale.


Here the reduction of data in the $\mathbf{A} 1$ position is shown for illustration of the transform. The raw data is displayed here to 3 decimal places:

A1 0.000;0.555 0.100;0.988 0.200;1.613 0.300;1.938 0.400;2.608 0.500;2.889 0.600;3.773 0.700;3.816 0.800;4.898 0.900;5.491

To reduce $\mathbf{X Y}$ data to an average endpoint value:

1. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
2. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
3. Click Calculate.

## Area Under Curve

The transform will generate an endpoint matrix named Area with the value at each position being the area under the curve. With the example data provided above, the resulting computed area using the sum of trapezia method for the $\mathbf{A 1}$ plot is (displayed here to 3 decimal places): $\mathbf{2 . 5 5 5}$

## XY Reduction Average

## [Input: XY data $\Rightarrow$ Output: Endpoint]

This reduction transform computes the average $y$ value of each XY plot (within the specified range of points to consider). Flagged positions or points are not included in the calculations.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Example

Let's assume that we have some $\mathbf{X Y}$ measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to reduce data in each position to average of its data points and store resulting value to corresponding position of an endpoint matrix.

## Raw

Here is an XY matrix with each position containing 10 XY data points. Position data plots of the matrix are displayed in Plate Scale.


Here the reduction of data in the $\mathbf{A 1}$ position is shown for illustration of the transform. The raw data is displayed here to 3 decimal places:

A1 0.000;0.555 0.100;0.988 0.200;1.613 0.300;1.938 0.400;2.608 0.500;2.889 0.600;3.773 0.700;3.816 0.800;4.898 0.900;5.491

To reduce $\mathbf{X Y}$ data to an average endpoint value:

1. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
2. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.

## 3. Click Calculate.

## Average

The transform will generate an endpoint matrix named Average with the value at each position being the averaged value of the $Y$ input data points. With the example data provided above, the resulting averaged value for the $\mathbf{A 1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows (also displayed here to 3 decimal places):

### 2.857



Average is 2.857

## XY Reduction Function

## [Input: XY data $\Rightarrow$ Output: Endpoint]

This reduction transform performs the selected mathematical function on all of the (notflagged) $y$ values of each XY plot (within the specified range of points to consider). Flagged positions or points are not included in the calculations.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Example

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to reduce data in each position to the average of its data points and store resulting value to corresponding position of an endpoint matrix.

## Raw

Here is an XY matrix with each position containing 10 XY data points. Position data plots of the matrix are displayed in Plate Scale.


Here the reduction of data in the $\mathbf{A} 1$ position is shown for illustration of the transform. The raw data is displayed here to 3 decimal places:

A1 0.000;0.555 0.100;0.988 0.200;1.613 0.300;1.938 0.400;2.608 0.500;2.889 0.600;3.773 0.700;3.816 0.800;4.898 0.900;5.491

To reduce $\mathbf{X Y}$ data to an average endpoint value:

1. Select Average as the Function.
2. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
3. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.

## 4. Click Calculate.

## Function

The transform will generate an endpoint matrix named with the value at each position being the averaged value of the $Y$ input data points. With the example data provided above, the resulting averaged value for the $\mathbf{A 1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows (also displayed here to 3 decimal places):
2.857


Average is 2.857

## XY Reduction Fit Parameter

## [Input: XY data $\Rightarrow$ Output: Endpoint]

This reduction transform performs the selected curve fit on each XY plot and sets the result to the value of a specified fit parameter. Points outside the specified range and flagged positions/points are excluded from calculations.

(1)This transform can be used to obtain the specific fitting parameter of a curve fit to each plot (such as the slope of linear regression or midpoint of $\underline{4 \mathrm{PL}}$ ).

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Fit Method | The curve-fitting method to construct a curve for your data. |
| :--- | :--- |
| Parameter | Represents the Fit Transform Context Parameter to fit data points by. |

## Example

Let's assume that we have some kinetic measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to fit the Linear Regression and calculate the $\mathbf{R}^{\mathbf{2}}$ parameter for each position plot.

## Raw

Here is an XY matrix with each position containing 10 XY data points. Position data plots of the matrix are displayed in Plate Scale.


Here the reduction of data in the $\mathbf{A} 1$ position is shown for illustration of the transform. The raw data is displayed here to 3 decimal places:

A1 0.000;0.555 0.100;0.988 0.200;1.613 0.300;1.938 0.400;2.608 0.500;2.889 0.600;3.773 0.700;3.816 0.800;4.898 0.900;5.491

To reduce the plot at each position to its $R^{2}$ parameter:

1. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
2. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
3. Select Linear Regression Fit Method and specify Parameter as R2 (with the syntax, provided in Fit Transform Context Parameters).
4. Click Calculate.

## Calculated

The transform will generate an output endpoint matrix named Calculated with the value at each position $R^{2}$ measure for each input plot. With the example data provided above, the resulting averaged value for the $\mathbf{A 1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows (also displayed here to 3 decimal places):
0.987

Fit Parameter


## XY Reduction Maximum Slope

## [Input: XY data $\Rightarrow$ Output: Endpoint]

This reduction transform finds the maximum slope of each XY plot. The result can be set to the slope, or to the $x$ or $y$ value at the maximum slope (absolute or relative to the start $x$ ).

This transform can be used in kinetic applications to calculate the time to reach a maximum rate or to find the maximum rate in a reaction.

The maximum slope is determined by computing the linear regressions of adjacent points (using the specified number of linear regressions points) and determining the slope with the maximum (upward or downward) value.

Where the result is set to be the $x$ or $y$ value of the maximum slope:

- The $x$ value is calculated as the centre of the points with the maximum slope.

This may not exactly equal an $x$ data point.

- The $y$ value is computed from the linear regression of the calculated center $x$.

Flagged positions or points or those outside specified ranges are not included in the calculations.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Max is | Determines the type of slope to be found by this transform. The settings <br> are: <br> - Auto - The maximum slope is determined from the general direction <br> of each plot by using the linear regression. If the slope of the plot is <br> positive, the Fastest Increase is used; if negative, the Fastest <br> Decrease is used. |
| :--- | :--- |
| - Fastest Increase - The maximum slope is the slope with the highest |  |
| positive value. |  |
| - Fastest decrease - The maximum slope is the slope with the greatest |  |
| negative value. |  |


| Result is | Specifies the result of the analysis. The settings are: <br> - Slope - The result is the slope of the determined maximum slope. <br> - X At Centre Absolute - The result is the $x$ at the center of the determined maximum slope. (The center is the midpoint of the data points with the maximum linear regression slope). <br> - Y At Centre Absolute - The result is the $y$ at the center of the determined maximum slope. (The center is the $y$ value computed from the $x$ midpoint of the data points with the maximum linear regression slope). <br> - X At Centre Relative - The result is the $x$ at the center of the determined maximum slope relative to the first non-flagged data point that is greater than $\mathbf{X}$ Minimum. (The center is the midpoint of the data points with the maximum linear regression slope). <br> - Y At Centre Relative - The result is the $y$ at the center of the determined maximum slope relative to the first non-flagged data point that is greater than $\mathbf{X}$ Minimum. (The center is the $y$ value computed from the $x$ midpoint of the data points with the maximum linear regression slope). <br> - Extrapolated $\mathbf{Y}$ - A value of $y$ is calculated from the determined maximum slope using the $x$ value specified with $\mathbf{X}$ For Extrapolation. |
| :---: | :---: |
| X for <br> Extrapolation | When Extrapolated $\mathbf{Y}$ option in the Result Is setting is chosen, then this can be used to specify an expression used to determine the $x$ value to calculate $y$ for. |

## Example

Let's assume that we have some $\mathbf{X Y}$ measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to reduce data for each replicate to maximum slope between its data points and store resulting value to corresponding position of an endpoint matrix.

## Raw

Here is an XY matrix with each position containing 10 XY data points. Position data plots of the matrix are displayed in Plate Scale.


Here the reduction of data in the $\mathbf{A} 1$ position is shown for illustration of the transform. The raw data is displayed here to 3 decimal places:

A1 0.000;0.555 0.100;0.988 0.200;1.613 0.300;1.938 0.400;2.608 0.500;2.889 0.600;3.773 0.700;3.816 0.800;4.898 0.900;5.491

To reduce $\mathbf{X Y}$ plot at a position to a maximum slope to an endpoint value:

1. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
2. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
3. Define Max is as Auto, Num Regression Points as 2, and Result is as Slope.
4. Click Calculate.

## Maximum Slope

The transform generates an endpoint matrix named Maximum Slope with the value at each position being the maximum slope between points at each kinetic/spectral plot. With the example data provided above, the result for the $\mathbf{A 1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows (also displayed here to 3 decimal places):
10.815


## XY Reduction Peak from Fit

## [Input: XY data $\Rightarrow$ Output: Endpoint]

This reduction transform:

1. Fits a specified curve to each $X Y$ plot.

Flagged positions or points and points outside the specified range are not included in the calculations.
2. Interpolates $y$ for each $x$ value at every specified increment between the lowest and highest $x$ values of included points.
3. Determines the peak - maximum or minimum - from interpolated $y$ values
4. Reports specified value ( $x$ or $y$ ) of the determined peak.

This transform is useful for finding a peak that might fall between points by using

(1)interpolation. To reduce each position to the value of its highest or lowest data point, use XY Reduction Peak Point.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

\(\left.\begin{array}{|l|l|}\hline \& Select a curve-fitting method to construct a curve for your data: <br>
- Cubic Regression <br>
- Cubic Spline Akima <br>
- Cubic Spline Natural <br>

- Cubic Spline Smoothed\end{array}\right]\)| - Cubic Spline Smoothing |
| :--- |
| - Polynomial Order 4 Regression |
| - Polynomial Order 4 Regression |

## Example

Let's assume that we have some spectral measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we will reduce each input plot to the $x$ of the peak (maximum) value. We will determine the peak by fitting Polynomial Order 4 Regression, interpolating the curve at 0.1 increments in the 430-450 wavelength range.

## Raw

Here is an XY matrix with each position containing 61 XY data points. Position data plots of the matrix are displayed in Plate Scale.


Here the reduction of data in the $\mathbf{A} \mathbf{1}$ position is shown for illustration of the transform:
A1 420;0.142 421;0.143 422;0.144 423;0.145 424;0.147 425;0.148 426;0.149 427;0.15 428;0.152 429;0.153 430;0.155 431;0.156 432;0.158 433;0.16 434;0.161 435;0.162 436;0.163 $437 ; 0.164438 ; 0.165439 ; 0.166440 ; 0.166441 ; 0.166442 ; 0.165443 ; 0.165444 ; 0.165$ 445;0.165 446;0.164 447;0.163 448;0.162 449;0.162 450;0.161 451;0.161 452;0.161 453;0.16 454;0.16 455;0.159 456;0.159 457;0.159 458;0.159 459;0.159 460;0.158 461;0.158 462;0.158 463;0.158 464;0.159 465;0.159 466;0.159 467;0.159 468;0.159 469;0.159 470;0.16 471;0.16 472;0.16 473;0.16 474;0.16 475;0.16 476;0.16 477;0.161 478;0.161 479;0.161 480;0.161

To reduce spectral plot in each position to its fitted maximum endpoint value:

1. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Wavelength), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
2. Provide $\mathbf{X}$ Minimum of 430 and $\mathbf{X}$ Maximum of 450 .

Only data points within this range will be considered when evaluating the transform.
3. Select Fit Method of Polynomial Order 4 Regression.
4. Enter Interpolation Increment of 0.1.
5. Define Peak as Max and Result as X.
6. Click Calculate.

## Peak Fit

The transform will generate an output endpoint matrix named Peak Fit with the value at each position being the $x$ projection of the interpolated maximum $y$ value of each input replicate plot. With the example data provided above, the result for the $\mathbf{A} \mathbf{1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows:

441


## XY Reduction Peak Point

## [Input: XY data $\Rightarrow$ Output: Endpoint]

This reduction transform computes the peak of all values for each XY plot (within the specified range of points to consider). Flagged positions or points are not included in the calculations.

The result can be set to the $x$ or $y$ value at the peak, with the peak being defined as either the minimum or maximum value.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Peak | This setting determines which data point is considered as the peak: <br> • Max - The point with the maximum $y$ value <br> • Min - The point with the minimum $y$ value |
| :--- | :--- |
| Result | This setting determines which axis's coordinate is stored in resulting matrix: <br> • $\mathbf{X}$ - Projection of Peak value on $x$-axis <br> • $\mathbf{Y}$ - Projection of Peak value on $y$-axis |

## Example

Let's assume that we have some kinetic measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to reduce each input plot to the value at its peak point (maximum).

## Raw

Here is an XY matrix with each position containing 10 XY data points. Position data plots of the matrix are displayed in Plate Scale.


Here the reduction of data in the $\mathbf{A} 1$ position is shown for illustration of the transform. The raw data is displayed here to 3 decimal places:

A1 300.000;0.002 350.000;0.021 400.000;0.106 450.000;0.279 500.000;0.384 550.000;0.279 600.000;0.106 650.000;0.021 700.000;0.002

To reduce kinetic plot in each position to its maximum endpoint value:

1. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
2. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
3. Define Peak as Max and Result as X.
4. Click Calculate.

## Peak

The transform will generate an output endpoint matrix named Peak with the value at each position being the maximum $y$ point of each input replicate plot. With the example data provided above, the result for the A1 plot is displayed on the Kinetic/Spectral Analysis Chart as follows (also displayed here to 3 decimal places):
500.000


Maximum point is $(500,0.384)$, result is 500.000

## XY Reduction Total

## [Input: XY data $\Rightarrow$ Output: Endpoint]

This reduction transform computes the sum of all $y$ values for each $X Y$ plot (within the specified range of points to consider). Flagged positions or points are not included in the calculations.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Example

Let's assume that we have some kinetic measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to reduce data in each position to sum of its data points and store the resulting value to the corresponding position of an endpoint matrix.

## Raw

Here is an XY matrix with each position containing 10 XY data points. Position data plots of the matrix are displayed in Plate Scale.


Here the reduction of data in the $\mathbf{A} 1$ position is shown for illustration of the transform. The raw data is displayed here to 3 decimal places:

A1 0.000;0.555 0.100;0.988 0.200;1.613 0.300;1.938 0.400;2.608 0.500;2.889 0.600;3.773 0.700;3.816 0.800;4.898 0.900;5.491

To reduce $\mathbf{X Y}$ data to a sum of its data points:

1. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
2. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.

## 3. Click Calculate.

## Total

The transform will generate an endpoint matrix named Total with the value at each position being the sum of $y$ input data point values. With the example data provided above, the result for the $\mathbf{A 1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows (also displayed here to 3 decimal places):
28.569


Total is 28.569

## XY Reduction X at Y

## [Input: XY data $\Rightarrow$ Output: Endpoint]

This reduction transform finds the $x$ value at which the specified $y$ value (Cursor) crosses the plot (within the specified range). If the $y$ position is between two adjacent data points, then an interpolated value is calculated from the bounding points.
$X$ and $y$ values can be specified as a relative to the first point or as an absolute value. Flagged positions and points are not included in calculations.

(1)
This transform can be used to determine the $x$ value at the point where the horizontal (defined by $y$ ) value crosses each plot.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Cursor | The expression evaluated for each position that represents the target in $Y$ value. The default value is 1 . |
| :---: | :---: |
|  | To refer to the position's corresponding data on an endpoint matrix, use a Matrix Position Reference (e.g., \#2) to refer to the data on the second matrix. |
| Cursor <br> Type | This defines how the Cursor is treated for each position. The settings are: <br> - Absolute - The value specified by Cursor is the value to use in the calculation of $x$. <br> - Relative From First Measured - The value of Cursor is added to the $y$ value of the first point for the position (even if the first point is Flagged or outside the range). <br> - Relative From First In Range - The value of Cursor is added to the $y$ value of the first non-flagged data point within the range. |


| Result is | Specifies the result of the analysis. The settings are: <br> - Absolute - The computed $x$ value. <br> - Relative From First Measured - The computed $x$ value minus the $x$ <br> value of the first point for the position (even if the first point is Flagged <br> or out of range). <br> - Relative From First In Range - The computed $x$ value minus the $x$ <br> value of the first non-flagged data point within the range. |
| :--- | :--- |

## Example

Let's assume that we have some kinetic measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to reduce data in each position to the value of $x$ at which the specified $y$ value crosses the plot.

## Raw

Here is an XY matrix with each position containing 10 XY data points. Position data plots of the matrix are displayed in Plate Scale.


Here the reduction of data in the A1 position is shown for illustration of the transform. The raw data is displayed here to 3 decimal places:

A1 0.000;0.555 0.100;0.988 0.200;1.613 0.300;1.938 0.400;2.608 0.500;2.889 0.600;3.773 0.700;3.816 0.800;4.898 0.900;5.491

To reduce the plot at each position to the value of $x$ when crossing/equaling specified

## Cursor:

1. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
2. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
3. Specify Settings: Cursor as 1, Cursor Type and Result is as Absolute.
4. Click Calculate.

## X

The transform will generate an endpoint matrix named $\mathbf{X}$ with the value at each position being kinetic/spectral plot reduced to resulting numeric value (as defined in specified settings). With the example data provided above, the result for the $\mathbf{A} \mathbf{1}$ plot is displayed on the Kinetic/Spectral Analysis Chart as follows (also displayed here to 3 decimal places):
0.102


## XY Reduction X between $\mathbf{Y}$

## [Input: XY data $\Rightarrow$ Output: Endpoint]

This reduction transform computes the difference between the first two $x$ points at which the specified $y$ value (Cursor) crosses or equals the plot.
$Y$ value can be specified as relative to the first point or as an absolute value. Flagged positions and points outside the specified range are not included in calculations.

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

| Cursor | The expression evaluated for each position that represents the target in $y$ value. The default value is 1 . |
| :---: | :---: |
|  | To refer to the position's corresponding data on an endpoint matrix, use a Matrix Position Reference (e.g., \#2) to refer to the data on the second matrix. |
| Cursor Type | This defines how the Cursor is treated for each position. The settings are: <br> - Absolute - The value specified by Cursor is the value to use in the calculation of $x$. <br> - Relative From First Measured - The value of Cursor is added to the $y$ value of the first point for the position (even if the first point is Flagged or outside the range). <br> - Relative From First In Range - The value of Cursor is added to the $y$ value of the first non-flagged data point within the range. |

If the plot is not crossed or only crossed once, the result will be flagged.

## Example

Let's assume that we have some XY measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to calculate the difference between the first two $x$ points that equal or cross the specified Cursor $(y)$ and then reduce data to the difference
between these $x$ points in each position．

## Raw

Here is an XY matrix with each position containing 40 XY data points．Position data plots of the matrix are displayed in Plate Scale．

Raw data kindly provided by Andreas Engel Krag，Aarhus Universitetshospital．

|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | ： | ， | 10 | ＂ 1 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A | ， | 几 | 几 | 几 | 0 | N | N | $\Omega$ | 几 | 几 | N1 |
| － |  |  |  |  |  |  |  |  |  |  |  |
| c |  |  | r | N | － | r | N | N |  |  |  |
| － | ת | ก | $\bigcirc$ |  | N | N | $\square$ |  | N | N | 几 |
| E | n | त |  |  | N | N | N | N |  |  | ก1 |
| F | $\Omega$ | $\Lambda$ |  |  | N | $\Omega$ | n | 几 |  |  |  |
| － |  | $\sim$ |  |  | $\sim$ |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |

The reduction of data in the $\mathbf{A 1}$ position is shown for illustration of the transform．The raw data is displayed here to 3 decimal places：

A1 317．780；0．235 377．710；0．230 437．630；0．231 497．530；0．232 557．440；0．229 617．360；0．232 677．280；0．234 737．180；0．242 797．080；0．360 856．980；0．567 916．900；0．705 976．840；0．766 1036．760；0．791 1096．680；0．798 1156．580；0．800 1216．480；0．797 1276．410；0．786 1336．330；0．769 1396．230；0．746 1456．150；0．721 1516．050；0．696 1575．980；0．666 1635．900；0．634 1695．850；0．599 1755．750；0．560 1815．670；0．518 1875．590；0．470 1935．510；0．416 1995．410；0．364 2055．320；0．319 2115．220；0．277 2175．120；0．261 2235．020；0．255 2294．970；0．251 2354．890；0．233 2414．790；0．231 2474．690；0．228 2534．620；0．227 2594．520；0．229 2654．420；0．226

To reduce the plot at each position to the difference between the first and the second $x$ value when crossing／equaling specified Cursor：

1．Specify Data： $\mathbf{X}$ Axis as $\mathbf{X}$（Time）， $\mathbf{Y}$ Axis as $\mathbf{Y}$ ．
2．Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank．

## 3. Specify Settings: Cursor as 0.7, Cursor Type as Absolute.

## 4. Click Calculate.

## Difference

The transform will generate an endpoint matrix named Difference with the value at each position being the XY plot reduced to resulting numeric value (as defined in specified settings). With the example data provided above, the result for the $\mathbf{A 1}$ plot is as displayed on the Kinetic/Spectral Analysis Chart follows (also displayed here to 3 decimal places):

### 591.737



[^3]
## XY Reduction Y at X

## [Input: XY data $\Rightarrow$ Output: Endpoint]

This reduction transform finds the $y$ value at which the specified $x$ value (Cursor) crosses the plot (within the specified range). If the $x$ position is in between two adjacent data points, then an interpolated value is calculated from the bounding points.

The $x$ value can be specified as a relative to the first point or as an absolute value. Flagged positions and points are not included in calculations.

(1)
This transform can be used to determine the $y$ value at the point where the vertical (defined by $x$ ) value crosses each plot (or more simply the $y$ at a given $x$ ).

For viewing XY data common settings, see General Transform Properties for XY Data.
For viewing common transform settings, see General Transform Properties.
To learn about adding this transform to your assay run, see Transforms Tab.

## Properties

## Settings

|  | The expression evaluated for $x$ value of each position. |
| :---: | :---: |
| Cursor | To refer to the position's corresponding data on an endpoint matrix, use a Matrix Position Reference (e.g., \#2) to refer to the data on the second matrix. |
| Cursor <br> Type | This defines how the Cursor is treated for each position. These settings are used to handle scenarios where $x$ values for each position may be out of sync by adding an offset to the cursor. The settings are: <br> - Absolute - The value specified by the Cursor is the value to use in the calculation of $y$. <br> - Relative From First Measured - The Cursor value is added to the $x$ value of the first point for the position (even if the first point is Flaggect or out of range). <br> - Relative From First In Range - The Cursor value is added to the $x$ value of the first non-flagged data point within the range |

## Example

Let's assume that we have some kinetic measurement data for a $12 \times 8$ microplate stored in the Raw matrix. In this example, we would like to reduce data in each position to the $y$ value at which the specified Cursor ( $x$ ) crosses the plot. The resulting value is then stored to corresponding position of an endpoint matrix.

## Raw

Here is an XY matrix with each position containing 10 XY data points. Position data plots of the matrix are displayed in Plate Scale.


Here the reduction of data in the $\mathbf{A 1}$ position is shown for illustration of the transform. The raw data is displayed here to 3 decimal places:

A1 0.000;0.555 0.100;0.988 0.200;1.613 0.300;1.938 0.400;2.608 0.500;2.889 0.600;3.773 0.700;3.816 0.800;4.898 0.900;5.491

To reduce $\mathbf{X Y}$ data to the defined by the Cursor data points in each position:

1. Specify Data: $\mathbf{X}$ Axis as $\mathbf{X}$ (Time), $\mathbf{Y}$ Axis as $\mathbf{Y}$.
2. Leave $\mathbf{X}$ Minimum and $\mathbf{X}$ Maximum blank.
3. Specify Settings: Cursor as 0.2, Result is as Absolute.
4. Click Calculate.

## Y

The transform will generate an endpoint matrix named $\mathbf{Y}$ with the value at each position being the $x$ value defined by the specified Cursor. With the example data provided above, the result for the A1 plot is displayed on the Kinetic/Spectral Analysis Chart as follows (also displayed here to 3 decimal places):

### 1.613



## General Assay Advice

## Common ELISA Problems and Solutions

Enzyme-linked immunosorbent assays (ELISA) are widely used tests to detect and quantify biological molecules. They are relatively simple and inexpensive to perform. However, one needs to fully understand the principles of an ELISA to properly interpret the results. As with any biological assay, things occasionally don't go as planned. In this article, we will describe useful controls to include with each assay run and how to interpret results, and suggest solutions to try when the controls indicate a problem with the assay.
ELISAs come in many forms. For the purpose of this article, we will refer to a relatively straightforward and common ELISA format: the antigen capture.

## Prototype Antigen Capture Assay



Each reagent is made in optimized buffers. One starts by adsorption of the anti-target capture monoclonal (MAB) to the microtiter plate. Excess MAB is washed out of the wells, and a blocking buffer is added to prevent further binding of any reagents to the plate itself. Typically there is a wash step between all reagent addition steps of the ELISA. A sample containing the target antigen is added and bound by the capture antibody. A second anti-target MAB, known as the detector, is added. This also binds to the target antigen. The detector MAB may be labeled in some way, or a third anti-mouse isotype-specific MAB which binds to the detector $M A B$ is added to the wells. This third antibody will be labeled. Typically the label is an enzyme, but it may be any easily detectable entity. If an enzyme is used, then a substrate for that enzyme is added to the wells. The substrate is designed such that the action of the enzyme will produce a colorimetric change in the well, which may easily be measured at the proper wavelength of light by spectrographic instrumentation. This colorimetric change is usually
reported as an optical density (OD, the log of the absorbance at the chosen wavelength). The optical density is then proportional to the amount of captured antigen in the sample.

Of course, controls should be performed along with the sample to ensure proper interpretation of the results for a given assay run. It is these controls that allow one to ensure that the assay is performed according to expectations. There is a short list of common controls typically included with each ELISA. Below we will define those controls and discuss what they can tell us about the performance of a given ELISA run.

## The Blank (B)

The B control consists of a microtiter well or wells that are coated with the capture antibody and blocked with blocking buffer. These wells do not receive any sample or detector antibodies. The blank wells control for any variation, or contribution, of the plate itself to the measured OD. Expected values for the blank are quite low, approaching zero. Each assay typically has a few wells designated for the blank control. These wells may be placed adjacent to each other, or randomly across the plate to make the statisticians happy. If all goes well, then one can assume that the plates were manufactured properly and the blocking agent is performing as expected. However, if the blank well OD is higher than normally seen, the assay may have a problem. High ODs in the blank well may indicate a plate-washer problem. Further investigation of the plate-washing procedure, buffers, and instrumentation are warranted. The problem may be as simple as a clogged tube on the washer, especially if the problem is isolated to a single well. If the problem occurs in more than one well, a more thorough investigation of the wash process is needed. Once the washer checks out, then consideration of the substrate preparation should be investigated. Too much substrate can push the blank well ODs up as well.

## Zero Concentration (ZC)

This ZC control is similar to the blank but contains all buffers and reagents from each step of the assay. The sample should contain only sample buffer with no target antigen. This is sometimes difficult to achieve, especially if one is measuring a common antigen that is found at measurable concentrations in the likely sample matrix. Sometimes one has to be creative to obtain or create a true zero concentration control. Once created or obtained, a ZC control allows one to determine the contribution of all reagents and buffers to our assay signal, absent any target antigen. Thus we have determined the true "background" of the assay.

If all goes well with the assay, one should expect to see OD values in the ZC wells that are only slightly higher than our blank wells. The actual values seen will likely vary among different assays for different target antigens. For each given assay, the control values should be consistent across runs. This control is also necessary if one wants to calculate a true limit of detection for an assay. Once the zero concentration is established, it serves as a benchmark for the sum of all reagents and buffers in the assay. Changes to the signal, either up or down,
indicate a change to the assay that requires investigation. As with most ELISA performance issues, double-check the plate washer as described before. It cannot be stressed enough that proper care and maintenance of your plate washer will prevent many assay problems. However, once you have ruled out a plate-washer problem, it will be necessary to doublecheck reagent preparation, storage, and delivery. One needs to understand the stability profiles of all ELISA reagents. Adhering to the commercial kit guidelines usually prevents problems, but not always, so be aware.

## Non-Specific Binding (NSB)

This is a variation on the blank and zero concentration wells. The goal is to further isolate the performance of assay reagents to ensure their proper function in the assay. For the NSB control, the wells are blocked as usual, but then blocking or wash buffer is added in place of the reagents at each step of the assay. The final addition of the labeled detector antibody is performed in the usual manner. Subsequent substrate development then occurs. This control allows one to assess the contribution of the labeled detector antibody to the overall OD signal of the assay. Desired OD results for NSB wells are slightly over the B control wells, but not over the ZC wells. Any differences in signal, up or down, can be attributed to the performance of the labeled detector antibody and may need to be investigated. Look carefully at reagent preparation and delivery. Improperly prepared detector antibody or substrate will easily affect this control and of course the rest of the assay.

## Maximum Binding (B0)

We have been focusing on controls that look at the low end, or background, of the assay signal. Of equal importance is a good estimate of the maximum signal possible in the assay. This can be achieved by adding saturating amounts of sample to the capture assay, followed by saturating amounts of the labeled detector. After addition of substrate, a maximum colorimetric response will be determined.

A maximum binding control is necessary if one wants to subsequently calculate a \% bound of sample. This calculation is performed when utilizing a competitive format antigen capture assay. In this case, the labeled target antigen would compete with unlabeled from the sample. However, even without using the competitive format, it is a good idea to perform a maximum binding control. By using this control, one will know the upper limit of the signal generated by the assay. Depending on how the assay has been developed, values for such a control may actually exceed the ability of the instrument to measure OD. If this is the case, it may be necessary to cut back on the target antigen concentration until the OD is reduced to measurable levels. If the maximum binding OD values then drop, or even rise, one knows that there has been a change in the assay performance. As always, give the washer and the wash procedure a good review. After determining that the washer is working well, examine assay procedures and reagent preparation and delivery. Typically this control will pick up sample,
substrate, or detector antibody issues. If a problem occurs, it is likely the B0 control will come in lower than expected, which may indicate a preparation or delivery problem in one or more of the three key reagents.

## Summary

The use of the controls described above will help in determining the probable cause and hopefully lead to solutions to any problems encountered in the performance of an ELISA. In summary, there are really only a few things that can go wrong with an ELISA. As stressed above, the plate-wash instrumentation, buffers, and procedure are always likely causes of problems. Plate-wash problems frequently mimic reagent issues, so it is always wise to ensure that the washer is not the source of problems before pursuing other ends. Assuming the washer is not the problem, one typically can categorize the problems as follows: low or no signal, high background, inconsistent results between replicate samples or controls, or samples out of range (high or low).

If the entire plate is blank or has minimal signal, one can conclude that a procedural error has occurred or a key reagent was bad. Since the low signal is seen across the plate, it is likely that the labeled detector antibody was not added to the assay system. It is also possible that the enzyme is non-functional, but this is rare. A quick test for enzyme functionality is to add some correctly prepared enzyme labeled MAB directly to the substrate. The expected colorimetric change should occur rapidly. Another possibility is that the substrate is bad or was improperly prepared. Double-check procedures to make sure that the appropriate substrate buffer was used, as this may severely inhibit the expected colour change. If you have an old lot of substrate, you can compare this to the new lot to ensure activity is relatively similar.

A high background is likely a reagent preparation problem. Too much labeled detector may cause this type of response in an assay. One has to double-check that the blocking step was also performed correctly. Was the right amount of block used in the right buffer?

Inconsistent results across replicate samples or controls are one of the most difficult issues to deal with. Assuming we have ruled out the plate washer, then one might have to consider a bad lot of plates or manufacturing issues. To rule out manufacturer problems, it may be necessary to test different kit lots. Be sure to track the lot numbers of the individual components of each test kit. Frequently, kit lots share the lot numbers of one or more components. In addition to bad plates, one has to look at the ability of the operator to pipette accurately. Small variations in pipetting across multiple reagents can produce results that will not pass quality control limits of variability.

Sometimes the controls all pass quality control criteria, but the samples themselves appear to be problematic. On occasion one may notice that the samples are exhibiting higher or lower than normal OD signals. Depending on the expected variability of samples, this can be difficult to notice. It is likely due to procedural issues such preparing the wrong sample dilution, using
the wrong buffer, or pipetting the incorrect amount. One should also be aware of sample handling. While it is relatively easy to document sample handling in one's own laboratory, it can be quite difficult to determine if problems have occurred somewhere else in the sample's chain of custody. Be aware that sample handling is of the utmost importance for reliable results, both within one's own laboratory and outside the laboratory.

Should your assay not behave as expected, the solutions discussed above will provide good starting points to quickly resolve your problems.

Written by James E. Drummond Ph.D

## How to Choose and Optimize ELISA Reagents and Procedures

ELISAs have been used for detection of biological molecules since the 1970s. Over time, scientists have developed and utilized many variations of this assay format. The various types of ELISAs (Antibody detection, Antigen capture, Sandwich, Competitive, etc.) are really variations on a theme. The overarching themes of ELISAs are to specifically and sensitively detect a biological molecule (antibody, cell receptor, cytokine, etc.) in a biological matrix. Virtually any type of molecule (protein, lipid, carbohydrate, nucleic acid, etc.) can be detected by an appropriately developed ELISA.

Briefly, the ELISA works by attaching a capture moiety to a microtiter plate, allowing a sample to bind, and detecting the sample via another binding moiety coupled to a signal generating molecule. The capture and detector components are usually added at saturating concentrations and allowed to come to binding equilibrium. Excess unbound material is washed out of the wells in between each assay step. The sample concentration will, of course, be variable. This article will describe general concepts to keep in mind as you choose and optimize ELISA reagents and procedures.

In this day and age it is usually possible to find many commercially available ELISA reagents. These include a wide variety of antibodies to many different biologically important molecules, multiple buffers, and a wide assortment of detection and signal generation molecules. However, sometimes appropriate reagents are not available and will need to be developed. The rest of this article will assume the development of an antigen capture ELISA for detection of a serum cytokine. In general, try to maximize desired binding events while minimizing the unintended binding events. Optimization of reagents is accomplished by maximizing "signal to noise" ratios between positive and negative samples. Using the example assay, key areas that need attention in the development of every ELISA assay will be addressed.

## The Target

First and foremost one must understand the target of the ELISA. By this I mean more than just what the target is, although that is definitely an important first step. One must understand the biological and chemical characteristics of the target molecule. Both aspects of a target cytokine can affect the ELISA. Understanding the chemical nature of the target molecule is essential to choosing appropriate sample buffers to obtain, store, and test the target molecule. Obtaining some purified target material will assist with preliminary stability testing in likely buffers and matrices. On the biological front, some easy questions, which might not have easy answers, need to be asked. First, how much target cytokine is likely to be present in the desired sample? If there is very little target present, such as picograms or less, then one will need to spend time and effort to find or develop sensitive capture and detector reagents. If
the target is present at milligram quantities or greater, then it will probably be easier to find or develop the capture and detection reagents.

Secondly, how does the target cytokine exist in its normal matrix? Does the target have any interactions with other biological molecules and to what extent? Does it exist as whole molecules or as various metabolic fragments? Cytokines frequently exist in serum bound to other molecules. These other binding partner molecules may confound the binding of capture and/or detection reagents to the cytokine. Capture and detector reagents that work in the presence of these target binding partners need to be carefully chosen or developed to accurately detect the target cytokine. If the target can exist in the sample matrix as a whole molecule along with some number of metabolic fragments, then internal competition of fragments with whole molecules may occur within the ELISA. One fragment may bind nicely to the capture reagents, but not present a suitable epitope for the detector reagents. Many variations of this problem will impact the accuracy of the ELISA. In essence, one needs to develop and optimize the capture and detector reagents as a pair, not as stand-alone molecules.

## The Capture

Assuming one has the target identified and well characterized; it is time for a first go at the cytokine detection assay. It is necessary to line up the anticipated capture antibody, samples, detector antibody, signal generator, and all the appropriate buffers to go along with them. Choose an assay format that can fit with the available laboratory tools and instrumentation (i.e., pipetters, plate washers, plate readers, etc.). The 96 well polystyrene microtiter plate is generally a great place to start. There are many variations of microtiter plate with different binding characteristics. For initial attempts, a generic ELISA plate is recommended. Once the assay is established, one can decide if the more expensive high binding plates will improve the assay or not.

The cytokine antigen assay will utilize an anti-cytokine polyclonal antibody (PAB) to capture the target cytokine. The choice of capture reagent will change depending on the needs of each assay. It is important to optimally attach the capture PAB to the plate. This can usually be accomplished via passive adsorption. A fairly minimalist buffer such as phosphate buffered saline (PBS pH 7.4), or carbonate/bicarbonate buffer ( pH 9.5 ), is typically used to dissolve the capture PAB. Adjusting the pH is an easy way to alter the charge on the capture PAB or other protein that one may be adsorbing to a plate. Try a few different concentrations of capture PAB because the buffer/antibody/plate combination may vary in binding capacity. A good PAB concentration to bracket around is $1 \mu \mathrm{~g} / \mathrm{ml}$. This may vary for other proteins. It is recommended that you test the binding of the PAB to the plate by completing the assay. Since the rest of the assay has yet to be optimized you need to use reasonable reagent concentrations and chemistries for choosing the remaining reagents. These will subsequently be optimized. Initial capture PAB binding to the microtiter plate should incubate for at least 1
hour. Incubation times may need further optimization to ensure complete binding has occurred to the microtiter plate.

## The Wash

After the capture PAB incubation one needs to utilize a wash buffer. The purpose of the wash buffer is to remove assay components that are not bound to the plate in an intended fashion. Wash buffers are usually formulated at physiologic pH and salt concentrations (e.g., PBS). The goal is to be gentle and prevent denaturation of any assay components while at the same time removing excess material not specifically and tightly bound to the well. To accomplish this, it may be necessary to add some mild detergents to the wash buffer. A good place to start is with Tween-20, or something similar, at $0.5 \%$ or thereabouts.

The type of detergent and its concentration must be compatible with all assay reagents. Too much detergent, or the wrong one, may wash capture PAB off the plate or inhibit binding of assay components. Sometimes additional proteins may be added to help stabilize assay components. It will be necessary to do some empirical testing to make sure of complete assay compatibility. For ease of use a single wash buffer is generally used for a given ELISA. Rarely certain assay reagents may require different wash buffers. Understanding the chemistry and biology is a must. The proper wash will greatly stabilize the assay leading to sensitive and accurate results.

The number of washes is arrived at empirically. In essence, washing is just diluting the soluble components of the well. Think about how much of a dilution is needed at each step in the ELISA. One needs to balance washing out the undesired components with reducing the amount of the desired components in the assay. Stability of the wash fluid is often overlooked, especially in laboratories with low throughput. Wash fluids can begin to grow unwanted "things" if left sitting. Adding a preservative or ensuring the wash is made fresh for each assay will likely prevent any stability problems. Don't forget plate washer maintenance to prevent clogging of tubes. Nothing creates false positives faster than a faulty plate washer.

## The Block

After the first wash to remove excess capture molecules, it is necessary to block the well. The purpose of the blocking step is to prevent any non-specific (i.e., unintended) binding of other assay components to the well. The blocking agent usually consists of some assay neutral protein that is unlikely to interfere with any of the other binding events in the assay. Try readily available solutions first, such as 1-5\% albumin or casein, before investigating more exotic blocking mixes. There are many commercial blocking mixes that work very well and can sometimes serve as a starting point for further modification to achieve optimal blocking. A good blocking buffer may also contain detergents. The detergents may be similar to the wash buffer or may vary. Understanding the interactions of a detergent with the chosen microtiter plate and other assay reagents is necessary to help in the selection. Be careful with detergents
as they sometimes have a tendency to remove desired components from the well, thereby decreasing sensitivity. All components of a blocking solution should be tested to ensure they are not inhibiting any of the subsequent binding events in the assay. The last step of the block is to wash out the excess blocking solution in preparation for sample addition.

## The Sample

The sample is, of course, the key component to any ELISA. Take care in acquiring, transporting, and storing samples. These are critical components of any assay. It is essential that everything under your control happens as you planned. Things out of your control need to be documented and the impact on the sample well understood. For instance, one should be able to tell that the samples sat under the equatorial sun for three days and therefore may not be suitable for the assay. But, let's get back to the things happening in the lab that are under your control

When the sample arrives in the lab, it should be stored in a manner that will stabilize the target molecules and minimize any other degradation that could interfere with the assay. Many samples are not assay ready as collected. They will need to be further processed to alter the concentration and sample matrix/buffer. This buffer should be well thought out to prevent any degradation to the target molecule in the sample. In addition, the sample buffer must be compatible with all other components in the assay. A good first choice of sample buffer is, in fact, the blocking buffer. Depending on the sample, the buffer may be adjusted (i.e., pH, salts, detergent, stabilizers) based on the biologic and chemical constraints of the target molecule. The sample incubation must achieve binding equilibrium with the capture molecules. The timing of this will vary depending on sample concentration and affinity for the capture reagents. As always the microtiter plates are washed prior to the next step.

## The Detector

There are many different detection systems employed by ELISAs. The goal of each is sensitive and specific detection of the target molecule. The cytokine detection assay will employ a standard monoclonal antitarget Monoclonal antibody (MAB). There are many fine commercially available antibodies to many biologic molecules. Of course, you will have to test a given MAB in the assay to ensure that it meets your needs. The detector MAB is usually unlabeled. This ensures that the MAB has not been altered in any way and will provide the desired sensitivity and specificity. The detection antibody is usually diluted in the blocking buffer, or perhaps in a manufacturers recommended buffer. Either buffer is a great place to start, but always keep in mind that modifications may be necessary based on the chemical needs of the target and other assay components. The proper dilution is usually arrived at empirically by titering the antibody and comparing relative sample to background signal strength between known positive and negative samples. The goal of the assay to measure very low concentration or perhaps high concentration samples may drive the final choice for
detector MAB concentration and buffer. After suitable sample incubation which allows for binding equilibrium, excess non-bound detector is washed from the wells.

## The Signal

At this point the signal generation components are added. For the cytokine assay an antispecies antibody directed against our detector MAB is utilized. The anti-species antibody is conjugated to an enzyme. Some popular enzymes are alkaline phosphatase and horseradish peroxidase. There are many commercially available antibody-enzyme signal generation combinations for a multitude of species. However, they are not all created equal, and it is usually prudent to test several. In the end, price and convenience may also drive the choice of signal system. Test the signal system by bracketing test dilutions around the manufacturer's recommendation. Frequently you can choose lower concentrations and save some money, but sometimes you may need to have a higher concentration to get the desired assay signal strength. If the manufacturer has a recommended buffer compatible with your assay, then start with that. If you need to use your own buffer, a good place to start is with the blocking buffer. As with all buffer decisions, ensure compatibility with all other assay components.

After signal reagent incubation and wash, an appropriate substrate is added. There are many commercially available stable substrates. It is recommended to go with the commercially available substrates because most of the stability issues have already been solved by the manufacturer. The substrate incubation time will need to be arrived at empirically depending on the amount of target molecule in the samples and the absolute sensitivity of the assay. The substrates will undergo a distinct colorimetric change in the presence of the enzyme. The substrate should show little to no conversion in blank or negative wells. Some substrate systems require a stop solution to be added to the wells after the incubation period. This has the advantage of stopping the reaction across multiple plates at approximately the same time, thereby preventing the last plates read from incubating longer than the first plates read. The plate is now read using any one of many suitable microtiter plate spectrophotometers to measure the optical density of the wells. The optical density is proportional to the amount of target captured in the wells.

There are many different molecules besides enzymes that can generate signals in ELISAs. Some of these include fluorescent molecules, chemiluminescent molecules, and even colloidal gold. Discussion of the relative merits of the different signal generation molecules is best left to another article.

## The Incubations

The term incubation has been mentioned several times above. Clearly each step of the ELISA has an incubation component. For an ELISA to work, the various components of the capture, sample, detector, and signal generation must be allowed to come together. Incubation time and temperature are two additional components that greatly affect the outcome of the ELISA.

Sufficient time for binding equilibrium to occur must be achieved at each step. The higher the affinity between components the faster this equilibrium can be achieved. Typical ELISA steps require a minimum 30 minutes of incubation and may easily require more. Sufficient time to achieve equilibrium between surface bound and soluble components at each step of the ELISA must be achieved.

It is much better to use some sort of controlled temperature incubator rather than to trust the building maintenance man to keep the room air at a constant temperature. Even 2-3â C differences will affect ELISA results, especially if one is trying to quantitate or measure samples near the lower cutoff of the assay. Time and temperature optimization and consistency will greatly add to assay stability over time or between labs.

In addition, gentle mixing of the microtiter plate is sometimes recommended. If mixing is needed, it can best be addressed with an optimized assay. Remember that the conditions the assay has been optimized under need to be replicated whenever and wherever the assay is performed. Will the likely users of the assay have all the necessary equipment and reagents to successfully execute the ELISA? Keep this in mind if your assay users will be in clinically regulated laboratories or out in the bush.

## The Analysis

During the reagent optimization the assay signal from known positive samples is continuously compared with the signal from known negative samples. Assay reagent concentrations, buffers, and incubations are adjusted to maximize this "signal to noise" ratio. Now one has an optimized assay format along with all key reagents and buffers. The assay is ready to be put to the test. Can the ELISA distinguish between true positive and negative samples? What are the upper and lower limits of the assay? How reproducible is the assay? If necessary, work with your organization's internal review board to ensure that appropriate consent has been obtained if you are planning to use human samples.

It is essential to obtain or create a panel of samples consisting of positives, negatives, and blanks. The panels should be 10 or more samples from populations likely to serve as actual sample sources. If the ELISA will be used on many different populations, then these populations should be represented in the panels. The positives need to cover the expected range of target molecules one is likely to acquire under real use conditions. In the case of the cytokine we are seeking, it may not be possible to obtain strong enough positives unless we acquire samples from ill individuals or "create" them by spiking known amounts of purified cytokine into negative samples. On the other hand, it may not be possible to obtain negative samples if the cytokine is normally at detectable levels in all people. In that case some thought may be needed on how to create a negative that is as similar to the real sample matrix as possible. Depleting samples of the target may be a viable option to create negative samples. This needs to be done in a manner that is not harmful to the sample matrix. Substituting with
other species samples can be done but should be approached with caution due to specificity concerns.

However one obtains or creates the appropriate positive and negative samples, it is now time to test the system. Consulting with a statistician trained in the art of assay development would be wise at this juncture. The statistician can help with plans to show the limits of the assay and how to set a cutoff. Repeat assay runs can easily document the true reproducibility of the assay. That being said, a good place to start with a cutoff is $2-3$ standard deviations above the average response of your negative samples. Multiple runs will be necessary to accurately determine the reproducibility of the ELISA. The goal is to set a cutoff that minimizes false negatives and positives.

With all that has been discussed, one should be well equipped to develop and optimize a reasonable ELISA. Remember that every target/Ab combination is unique. If one is ready for surprises, they can usually be addressed.

Written by James E. Drummond Ph.D

## Curve Fitting

## Is My Fit a Good Fit?

The term "good fit" can cover a number of factors. To evaluate how good your curve fit is, you can assess your curve and verify if it fits with the following suggested evaluations.

First, it is always important to view each curve critically to check for sample preparation errors or an erratic fit and to verify that the curve's general direction matches the expectations for the assay type.
One common goodness measure is $R^{2}$. If this metric is $>0.9$, then generally speaking, the model is considered to be a good fit to the data (the closer to 1 the better). Each laboratory will likely define an acceptable threshold for the goodness measure used to validate the assay. Other goodness measures are also available.

In addition, it is important to consider how good the fit is across the standards by considering the precision and accuracy of the fit at each point. This is why the software computes LLOQ and ULOQ and includes the calibrator table to clearly indicate where the precision (\%CV) and accuracy (\% Accuracy) are within range.

It is quite common to see poorer accuracy at the upper or lower ranges of the standards. In this case, you may want to consider reassaying those samples that fall outside of this range (by diluting or concentrating and applying a suitable dilution factor).

Where precision is poor an outlier might be the cause. In this case, the software can be used to flag and ignore the outlier, and the results can be recalculated excluding the outliers.

To automate the determination of a good fit, controls can be included. These will typically have a known concentration (or an acceptable concentration range) with validations used to automatically check that the computed concentrations match these expected quantities.

## Goodness of Fit Measures

The goodness of fit measures are calculated when each fit method is used. These measures indicate how well the curve model fits the data.

The following table describes these measures and how they can be used to assess the fit.

| Name | Description |
| :---: | :---: |
| $\mathrm{R}^{\mathbf{2}}$ | $R^{2}$ is 1 minus the ratio of the sum of the squares of the residuals divided by the sum of the squares of the differences between $y$ fit and the mean $y$ value). This will equal 1 for a perfect fit and tend toward 0 for a bad fit. <br> In other words, $R^{2}$ is the ratio of variation that is explained by the curve-fitting model to the total variation in the model. <br> In most situations, irreducible errors in measurement will prevent the model from explaining all the variation. Models using a larger set of factors may produce an $\mathrm{R}^{2}$ value that is closer to 1 . However, it may be that the additional factors are essentially modeling noise. |
| $a R^{2}$ | $a R^{2}$ is the adjusted $R^{2}$ value. <br> $a R^{2}$ is $R^{2}$ adjusted downward to compensate for overfitting. The larger the number of independent variables is (compared to the number of observations), the lower the adjusted $R^{2}$ value will be. When using curve fitting models with a larger number of independent variables, the additional variables may be simply modeling noise. |
| P | P is the P -value. P is the significance of the model as a probability. It is the P value of $F$ for the curve-fitting model. This compares the variance attributed to the model with the variance of the residual. |
| SE | SE is the standard error. The standard error is the root mean square of the residuals. |
| F | $F$ is the $F$-statistic. $F$ is the ratio of the variance explained by the curve fitting model to the residual variance. |
| AIC | AIC is Akaike's Information Criterion; this provides an estimate of the quality of the curve-fitting model for comparison against other models. AIC can be used to compare curve-fitting models and determine which is the best. The model with the lowest AIC value is most likely to be correct. AIC will favour simpler curve-fitting models over those with more parameters (it achieves this by applying a penalty proportional to the number of parameters). |
| AICc | AICc is Akaike's Information Criterion corrected for small sample sizes. Use this method instead of AIC when comparing curve-fitting models for smaller sample sizes (number of calibrators). |


| Name | Description |
| :--- | :--- |
| BIC | BIC is Bayesian Information Criterion; this is very similar to AIC. The key <br> difference is that BIC imposes a higher penalty for the number of parameters <br> in the model. |
| DWS | DWS is the Durbin-Watson Statistic; this is used to detect the presence of only applicable when the number of calibrators is at <br> autocorrelation (at lag 1) in the residuals of the regression. DWS values range <br> between 0 and 4. A value close to zero indicates a positive autocorrelation <br> between the residuals. A value close to 4 indicates a negative autocorrelation <br> between the residuals. A value around 2 indicates there is no significant <br> autocorrelation. |
| DoF | DoF is Degrees of Freedom; this is computed as the number of calibrators <br> minus the number of parameters in the fit. The number of calibrators <br> depends on the selected Fit To Standard option; for Replicates (if there <br> are replicates) will be more degrees of freedom than for Average. |
|  | If there are not enough Degrees of Freedom, then values for $\mathbf{P}, F_{,}$ <br> SE and aR ${ }^{2}$ cannot be computed. |
| RMS | RMS is the Root Mean Square of the residuals (reported for Auto Smoothed <br> Spline only). RMS is the square root of the residual mean squared. |

## Accuracy and Precision

This section describes how the accuracy and precision of the Standard Curve Fit are defined, used, and computed in a quantitative analysis.

## Definition

Accuracy is a measure of how close a measurement matches its known value (standard).
Precision is a measure of how close replicated measurements are to each other.

## Limits

You will typically want to define acceptable limits of accuracy and precision for your assays. The acceptable limits depend on the application of the assay. MyAssays Desktop default limits are as follows:

- Accuracy $\pm 20 \%$
- Precision $\leq 20 \%$

These values can be adjusted through the Accuracy and Precision Quantification Limits of the Standard Curve Fit.

With a Standard Curve Fit, the accuracy and precision are likely to vary across the standards. Because of this, it is useful to determine the upper and lower limits of quantification (ULOQ and LLOQ) and only use the curve to compute values within that range.

These quantification limits are determined by computing the accuracy and precision of each standard measurement and computed concentration from the curve.

## Computation

## Accuracy

To measure the accuracy of the curve at a particular standard, the $x$ value of the known concentration (expected) is compared against the $x$ value at this point computed from the curve (actual).

So \% Recovery or \% Accuracy is simply:

## $=100$ * Actual / Expected

= 100 * (calculated concentration) / (defined concentration)

## Precision

To measure the precision of the curve for a particular calibrator (Standard Type sample), the \%CV is calculated as the \% of the standard deviation (SD) of the standard's replicate measurements divided by their mean.

This way \% CV Precision is calculated as:

## 100 * SD / Average

= 100* (standard deviation of calibrator)/(average value of calibrator's replicates)

## Evaluation and Results

Accuracy and precision are calculated from the curve with the LLOQ and ULOQ determined from these values; the results for each standard are included in right-most columns of Curve Data Table.

To clearly indicate whether each standard is within the acceptable range or not, each row is highlighted based on whether it satisfies the specified precision and accuracy rules (rows in light-red fail the test).

## Fit Methods

## Which Fit Method Should I Use?

A single fit method that works perfectly for all assays sadly does not exist. Any curve fitting model is a compromise and simplification of reality in some way or another.

As a first step of evaluating any applied fit, it is important to critically view the curve to check for sample preparation errors or an erratic fit.

In general, the 4PL or 5PL are the best models for dose-response curves; these model the sigmoidal nature of saturation curves very well. These methods are widely used, cited, and recommended. However, where samples fall outside these saturation limits (the upper and lower asymptotes) no result can be computed and this can be frustrating. Where this occurs, the common advice is to dilute/concentrate the sample and reassay. Alternatively, with weighting applied to these models, the fit can be biased to go through specific points (such as those nearer the $x$ or $y$ axis). This is useful, if it is important to have good curve accuracy at one end of the curve and to ensure the fit has an expected range of usage.

For certain assays (and especially when working with validated kits), a statistician or manufacturer may recommend a specific 4PL or 5PL model with a particular weighting. In those cases, it is important to use the same method for each run of the assay (and, of course, MyAssays Desktop supports that).

However, without this guidance, it can become tedious to try out different methods and models for each assay run. It is for this reason that MyAssays Desktop Pro includes the Best Fit feature. With this option, MyAssays Desktop will perform multiple fits with different weighting options and automatically pick and apply the "best". What is deemed to be the best is open to interpretation and it is for this reason that the software allows you to select different scoring methods. The fit with the highest score is considered to be the best.

After selecting the Best Fit fit method, select which fits and weighting methods to consider. For example, with:

| Fits: | Weighting: |
| :---: | :---: |
| ( $\mathrm{V}^{\text {PL }}$ | ( None |
| ( 5PL | $\square 1 / \mathrm{x}$ |
| $\square$ Linear Regression | $\square 1 / \mathrm{y}$ |
| $\square$ Quadratic Regression | (1) $1 / x^{2}$ |
| $\square$ Cubic Regression | (1/ $1 / y^{2}$ |
| $\square$ Quartic Regression | ( $\mathrm{x}^{2}$ |
| $\square$ Quintic Regression | (1) $\mathrm{y}^{2}$ |
| $\square$ Michaelis-Menten |  |
| $\square$ Exponential |  |
| $\square$ Power |  |

10 methods will be compared.
Next, specify a fit score method from:

- $R^{2}$
- $R^{2}$ with Low-End Accuracy
- $R^{2}$ with High-End Accuracy
- $\mathrm{R}^{2}$ with Low-End and High-End Accuracy
- 1/Standard-Error
- 1/P-Value
- 1/AIC
- 1/AICc
- 1/BIC
$R^{2}$ is a common method for evaluating a fit, however, care should be taken when using this to compare different fit models (it does not take into account the differences between models). $1 /$ Standard-Error is a good choice for finding the fit with the lowest standard error. The AIC, AICC, and BIC methods are useful for evaluating different models and these penalize more complicated models (i.e., those with more terms).

For even further flexibility, it is also possible to define your own fit score methods. To learn more about this feature, see Custom Fit.

Alternatively, MyAssays Desktop includes legacy fits like the Auto Smoothed Spline method in an attempt to provide a "set-it and forget-it" solution. This may be preferable in some situations (like RIAs) where a good fit is acceptable, as opposed to identifying a more accurate
fit that requires operator intervention. Auto Smoothed Spline is based on the cubic spline fit, so the accuracy can be poor and can lead to unnatural looking curves and erratic behaviour. The pure Cubic Spline method will exactly trace through every data point so the Auto Smoothed method attempts to smooth the fit automatically. The Cubic Spline Smoothed applies a fixed smoothing parameter.

## Linear Regression

It is a basic and easy regression model that fits data linearly.
Linear regression sets the linear relationship between the dependent variable ( $y$ ) and a single independent variable ( $x$ ). This model provides starting approximation and, although is seldom used for biological systems, is often used to visualize data relationship at a glance.

The equation for the model is:
$y=m x+c$

## Variables

In this model we have the following:
$y=$ the dependent variable (i.e., what you measure as the signal)
$x=$ the independent variable (i.e., what you control, such as dose, concentration, etc.)

## Coefficients of the Fit

$\mathbf{m}=$ the slope of the fitted line
$\mathbf{c}=$ the intercept of the dependent axis
The rearranged equation to solve $x$ is:
$x=\frac{y-c}{m}$
The goal is to determine values of $\mathbf{m}$ and $\mathbf{c}$ that minimize the sum of squares (SS) of the residuals between the observed values (i.e., your data) and the predicted values (i.e., the fitted curve). As some observed values will likely be above the fitted curve and some below, you will get positive and negative residuals. SS is used to square each of the residuals, which render all the values positive, then sum them. The smaller the SS, the closer the observed values are to the predicted, the better the model predicts your data.

The fitted plot may show the data to be nonlinear. In this case, it is advised to consider using different methods.

## Four Parameter Logistic Regression (4PL)

This nonlinear symmetrical sigmoidal model is known as 4-parameter logistic regression (4PL).

It is quite useful for dose-response and/or receptor-ligand binding assays, or other similar types of assays. As the name implies, it has 4 parameters that need to be determined in order to "fit the curve." The model fits data that makes a sort of S-shaped curve that is symmetrical around its inflection point.

The equation for the model is:
$y=d+\frac{a-d}{1+\left(\frac{x}{c}\right)^{b}}$

## Variables

In this model we have the following:
$y=$ the dependent variable (i.e., what you measure as the signal)
$x=$ the independent variable (i.e., what you control, such as dose, concentration, etc.)

## Coefficients of the Fit

The 4 parameters consist of the following:

- $\mathbf{a}=$ the minimum value that can be obtained (i.e., what happens at 0 dose).
- $\mathbf{d}=$ the maximum value that can be obtained (i.e., what happens at infinite dose).
- $\mathbf{c}=$ the point of inflection (i.e., the value of $x$ at the point on the S-shaped curve halfway between a and d).
- $\mathbf{b}=$ Hill's slope of the curve (i.e., this is related to the steepness of the curve at point $\mathbf{c}$ ). It could either be positive or negative.

The rearranged equation to solve $x$ is:
$x=c\left(\frac{a-d}{y-d}-1\right)^{\frac{1}{b}}$

The $\mathbf{a}$ and $\mathbf{d}$ values might be flipped; however, $\mathbf{a}$ and $\mathbf{d}$ will always define the upper and lower asymptotes (horizontals) of the curve. $\mathbf{a}$ and $\mathbf{d}$ are the same units at $y$. The curve can only be used to calculate concentrations for signals within a and $\mathbf{d}$. Samples outside the range of the determined $\mathbf{a}$ and $\mathbf{d}$ cannot be calculated.

A common requirement is to calculate IC50 from the fit. The IC50 is the concentration of an inhibitor where the response (or binding) is reduced by half. There are actually two ways to do this depending on what you consider IC50 to be:

- Firstly, the midpoint of the sigmoid of the 4 PL is equal to the $\mathbf{c}$ coefficient of the 4PL. In this case you can simply look at the calculated coefficient. Use this method if you consider the midpoint of the sigmoid to be equal to IC50. Mathematically, this is the case as it is the $x$ point at exactly half way between the two horizontal asymptotes.
- Alternatively, if the response is measured between 0 and $100 \%$ and you consider IC50 to be where $y=50$, then you can calculate where $y=50$ using the equation to solve $x$ (above), substituting in the calculated coefficients.


## Best Fit

The Best Fit feature will automatically find the most appropriate fit for your data. The feature processes your data using multiple curve fits and weighting methods and automatically chooses and applies the best performing fit.

When working with multiplex data and best fit, a best fit search is performed on each analyte. Thus, the results for each analyte in an assay may use different fit methods

- the best for each analyte.


## Which Fits?

The default configuration will compare the 4PL and 5PL fit methods each with unweighted and various weighting models. These fit methods are the most widely used fit methods for most assays.

Beyond this default configuration, you can select which fit methods and which weighting models to compare. It is also possible to define your own curve fit equation.

## What Is Best?

The method used to evaluate which fit is best can be customized. For example, you might consider the fit with the highest $\mathbf{R}^{2}$ to be the best. You also might want to consider the accuracy of the curve at certain regions of the fit (e.g., at the low or high end of your standards data).

To achieve this flexibility, the system uses a fit score method to quantify how good each fit is. The fit with the highest score is considered to be the best fit.

## Fit Score Methods

On completion of each fit, the fit is scored using a fit score method equation. The scores of each fit are compared, and the fit with the highest score is considered to be the best. Thus, the score method equation should quantify how good the fit is.

For convenience, the following preset fit score methods are available for selection:

- $R^{2}$
- $R^{2}$ with Low-End Accuracy
- $R^{2}$ with High-End Accuracy
- $\mathrm{R}^{2}$ with Low-End and High-End Accuracy
- $1 /$ Standard-Error
- 1/P-Value
- 1/AIC
- 1/AICc
- 1/BIC

Beyond these presets, you can define your own fit score methods to evaluate and compare each attempted fit.

## Fit Score Method Expressions

This section describes how to define your own fit score method expressions to evaluate a fit. The fit score method equation can reference the goodness measures determined from the fit:

## Goodness Measures

To learn more about available measures and how they can be used to assess the fit, see Goodness of Fit Measures.

## Accuracy Functions

In addition to the Goodness Measures, various accuracy functions are available to assess the accuracy of the fit at various points. These accuracy functions enable a comparison to be made between the expected value (the known $x$ of the calibrator) and its actual value calculated from the curve.

The calibration data set is the known concentration values of the standards ( $x$ ) plotted against their measurements $(y)$. Each curve is fitted to the calibration data set.

To simplify combining accuracy functions together and with other measures (such as $\mathbf{R}^{\mathbf{2}}$ ), the accuracy functions return a value between 0 and 1 (where 1 is perfectly accurate).

| Function | Description <br> AccuracyRatio |
| :--- | :--- |
| Calculates the accuracy ratio of the expected value and its actual <br> value. This is similar to \% recovery; however, here a value <br> between 0 and 1 where 1 is perfect accuracy (i.e., 100\% recov- <br> ery). A \% Recovery of 120\% or 80\% both equal an Accuracy <br> Ratio of 0.8. With AccuracyRatio, if the actual value differs from <br> the expected by a factor of 2 or more, the accuracy ratio is 0. |  |
| AccuracyFactor | Calculates an accuracy factor of the expected value and its actual <br> value. This is computed using \% Recovery and a Gaussian <br> function such that: |
| \% Recovery | Accuracy Factor |
| 100 | 1 |


| Function | Description | 0.5 |
| :--- | :--- | :--- |
| 0 0.5 <br> 200 Value between 0.5 and 0 <br> $<0 \%$ Value between 0.5 and 0 <br> $>200 \%$ This function avoids reporting a value of 0 for scenarios where <br> the \% Recovery is $<0 \%$ and $>200 \%$. <br> Diff The absolute difference between the expected value, and is the <br> actual value. |  |  |

## Accuracy Function Forms

Each accuracy function comes in three forms:

- At Specified X Point
- From Low end (ends with Low)
- From High end (ends with High)

The following table provides example expressions calling accuracy functions and their result:

| Expression | Result |
| :--- | :--- |
| AccuracyRatio (0.125) | The accuracy ratio for calibrator where $x=0.125$ |
| AccuracyRatioLow (1) | The accuracy ratio for the calibrator with the lowest $x$ value |
| AccuracyRatioLow (2) | The accuracy ratio for the calibrator with the second-lowest $x$ <br> value |
| AccuracyRatioHigh (1) | The accuracy ratio for the calibrator with the highest $x$ value |
| AccuracyRatioHigh (2) | The accuracy ratio for the calibrator with the second-highest $x$ <br> value |

## Combining Measures

A fit score method can reference various goodness measures and accuracy calculations. Remember that the fit with the highest score is considered to be the best. Therefore, if you plan to consider multiple measures, it is recommended that each measure is a value between 0 and 1 and that the various measures are multiplied together.

## Score Method Preset Expressions

To illustrate example scoring methods, the following table lists the expressions used for each of the preset scoring methods:

| Preset | Expression |
| :--- | :--- |
| $\mathbf{R}^{\mathbf{2}}$ | $\mathrm{R}^{2}$ |
| $\mathbf{R}^{\mathbf{2}}$ with Low-End Accuracy | $\mathrm{R}^{2 *}$ AccuracyFactorLow(1) |
| $\mathbf{R}^{\mathbf{2}}$ with High-End Accuracy | $\mathrm{R}^{2 *}$ AccuracyFactorHigh(1) |
| $\mathbf{R}^{\mathbf{2}}$ with Low-End and High-End Accur- <br> acy | $R^{2 *}$ AccuracyFactorLow(1)*AccuracyFactorHigh <br> $(1)$ |
| 1/Standard-Error | $1 / \mathrm{SE}$ |
| 1/P-Value | $1 / \mathrm{P}$ |
| 1/Akaike's Information Criterion | $1 / \mathrm{AIC}$ |
| 1/Akaike's Information Criterion <br> corrected | $1 / \mathrm{AICc}$ |
| 1/Bayesian Information Criterion | $1 / \mathrm{BIC}$ |

## Custom Fit Method

With this feature, you can define your own curve equation to use when fitting a curve to your calibration data. The software will automatically determine the best set of values for each unknown coefficient in your equation. In this way you can model your data using your own method.

## Writing a Custom Fit Expression

Your curve equation must be defined as an expression as follows:

- Write the equation in terms of $x$ (i.e., there must be at least one $x$ reference)
- Use mathematical operators
- Use any single letter (excluding $x$ and $y$ ) for each coefficient to compute. For longer coefficient names, enclose the name in square brackets (e.g., [intercept])

At least 1 unknown coefficient must be determined by the fit.

Also, you can optionally:

- Refer to TOVs of previous transforms (e.g., calculated coefficients, goodness measures)
- Insert constants (numerical values)
- Specify a custom name, weighting, inverse equation and define heuristic rules to improve the curve-fitting accuracy (see Advanced topic)


## Examples of Custom Fit Expressions

For example, here are the alternatives for custom fit equation of 4PL with r (from expression):


- tov1.d+((tov2.a-tov1.d)/(1+(r*x/tov2.c)^tov1.b)) - The expression uses TOVs from previous transforms as coefficients
- $\mathbf{1 . 2 3 + ( ( \mathbf { a } - 1 . 2 3 ) / ( 1 + ( \mathbf { r } ^ { * } \mathbf { x } / \mathbf { c } ) ^ { \wedge } \mathbf { b } ) ) - \text { Here the } \mathbf { d } \text { coefficient is a constant (1.23) } ) ~ ( 1 )}$
- $\mathbf{d + ( ( a - d ) / ( 1 + ( \mathbf { r } ^ { * } \mathbf { x } / [ \text { Slope] } ) ^ { \wedge } \mathbf { b } ) ) ~ - ~ H e r e ~ t h e ~ c o e f f i c i e n t ~ i s ~ n a m e d ~ S l o p e ~}$

In addition to defining the expression, you can configure Custom Fit Method further by using advanced custom fit method settings.

## Library Curve

The Library Curve feature can be used to save a curve that was measured and computed in one assay for reuse in other assays (perhaps where no standards are measured).

## Storing a Curve to the Library

Any curve created with the Standard Curve Fit or General Curve Fit transform can be extracted and saved to a Curve Library file.

The Curve Library file contains all of the data necessary to reproduce the curve and the chart in future analyses. This includes:

- Curve Configuration - All the transform settings (e.g., Fit Method, Calculate From, etc.) specified for the original curve
- Curve Plot Data - Calibration data (and average points) used in the curve fit
- Transform Output Content - Transform-specific report content (as specified in the assay-results file from which it was created)
- Origin - The full path to the assay-results file from which it was created


## Save a Curve to the Library

1. Add the Standard Curve Fit or General Curve Fit transform.
2. In the Results tab, select the Standard Curve Fit or General Curve Fit subtab. If your assay comprises multiple plates, select the one that contains the analysis view for your data.
3. Click the Save to Library button. If there are changes to the Standard Curve Fit or General Curve Fit settings that have not yet been calculated, then a message box will appear with the prompt to choose which results version the curve should be saved.
4. Review and edit a name and (optionally) description for your curve library file as required.
5. Click the Finish button.


Curve Library files are stored in the Curves folder of your MyAssays root folder.

## Calculating Results with a Curve from the Library

After the curve is saved, you can apply it by adding a Standard Curve Fit (from Library) transform. The selected curve file is used to recreate the curve for computing the concentrations of all samples measured in the referring assay.

When using the Standard Curve Fit (from Library) transform on an assay that also includes Standards defined on the layout, concentrations for these standards will be computed from the original curve. These standards will not be used to recompute the curve. It can be useful to include controls or a subset of the original standards in subsequent assay runs and use the Validation feature to define expected results to detect sample preparation issues.

## Library Curve Details

When reviewing the curve used by the Standard Curve Fit (from Library) transform, click the Library Curve Details button on the Standards Chart to review the name, description, and assay results file, from which the Curve Library file is created. Click the results hyperlink to open the containing folder and open then original results file from where the curve was created.

4PL Library Standard Library Average Unknown
Library Curve Details...


## Curve-Fitting Transforms Settings and Configuration Standard Curve Fit Settings <br> Multiplex Concentration Editor for Standard Curve Fit <br> This editor is used to specify concentration values and units of each

 analyte for the Standard Curve Fit transform when working with a multiplex assay.The editor is displayed by clicking on the Concentrations setting drop-down.
Multiplex concentrations data can be specified in a number of different ways including:

- A start concentration for each analyte with a common dilution/concentration series
- Unique concentration values for each calibrator (standard) of each analyte
- Common concentration units for all multiplexed analytes
- Unique concentration units for each analyte

The display and behaviour of the editor depends on the way in which the data will be specified.

For example, here the editor is displaying a start concentration and dilution series for each analyte with the same concentration units applying to all analytes:

| Analyte | Start Conc. | Series |  | Calibrator |
| ---: | ---: | :---: | :---: | ---: |
| Kim1 Conc. ( $\mathrm{nm} / \mathrm{ml}$ ) |  |  |  |  |
| Kim1 | 700 | $\mathbf{x}$ | $\div$ |  |
| Standard1 | 700 |  |  |  |
| Clusterin | 1000 |  |  | Standard2 |
| OPN | 10 | 2 |  | Standard3 |

## Selecting the Analyte to Edit

The Analyte column of the left-hand Analyte grid is used to select and indicate the analyte to edit. Click an analyte name to select to edit its concentration settings.

## Concentration Modes

There are two modes for specifying concentrations:

- Series - Provide a Start Conc. and common series to apply to all analytes. Use this if all of the analytes share a common concentration/dilution series.
- Non-Serial - Provide a specific concentrations value for each calibrator of each analyte. Use this if your analytes do not share a common concentration/dilution series or if your concentration values are not in a simple mathematical series.

Click Series to switch between the modes.

## Series Mode

This mode allows you to define a series to calculate the concentrations of each analyte (from a specified starting concentration). The series calculations are specified using the selection of the multiplication or division operator along with a specified factor or divisor.

With the Series turned on, enter or paste the starting calibrator concentration values for each Analyte in the Start Conc. column of the left-hand Analyte grid. The right-hand grid shows the computed concentrations of the selected analyte.

For example, for the selected Kim1 analyte with a Start Conc. specified as 700 and Series mode on, with multiplication selected and a factor of 2 specified, the concentrations of the analyte are computed and displayed in the right-hand grid as shown here:

| Analyte | Start Conc. | Series | Calibrator | Kim1 Conc. ( $\mathrm{nm} / \mathrm{ml}$ ) |
| :---: | :---: | :---: | :---: | :---: |
| Kim1 | 700 | $\div$ | Standard1 | 700 |
| Clusterin | 1000 |  | Standard2 | 1400 |
| OPN | 10 | 2 | Standard3 | 2800 |
| IL8 | 50 | $\equiv$ Units | Standard4 | 5600 |
| CystatinC | 10 |  | Standard5 | 11200 |
| NGAL | 20 | $\mathrm{nm} / \mathrm{ml}$ | Standard6 | 22400 |
| 50 | 10 |  | Standard7 | 44800 |
|  | 同 |  |  |  |

## Non-Serial Mode

Non-Serial mode allows the specification of the concentration of each calibrator separately.
With the Series turned off, select an analyte in the left-hand Analyte grid and enter or paste concentration values on the right-hand Calibrators grid.

For example, here Kim1 analyte concentration values are specified in non-serial mode.

| Analyte | Series | Calibrator | Kim1 Conc. ( $\mathrm{nm} / \mathrm{ml}$ ) |
| :---: | :---: | :---: | :---: |
| Kim1 |  | Standard1 | 700 |
| Clusterin | 三 Units | Standard2 | 1400 |
| OPN | $\mathrm{nm} / \mathrm{ml}$ | Standard3 | 2800 |
| IL8 |  | Standard4 | 5600 |
| CystatinC |  | Standard5 | 11200 |
| NGAL |  | Standard6 | 22400 |
| 50 |  | Standard7 | 44800 |
|  |  |  | [1] |

## Units Mode

There are two modes for specifying the concentration units of the analytes:

- The same concentration units applies to all analytes
- Concentration units specified for each analyte

Click $\equiv$ Units to switch the concentration mode state.

## Identical Units for All Analytes

When $\equiv$ Units is turned on, the single specified units of concentrations applies to all analytes. In this case, enter the units below the $\equiv$ Units button.

## Different Units per Analyte

When $\equiv$ Units is turned off, you can enter or paste different output units for each analyte in the Units column of the left-hand Analytes grid.

| Analyte | Start Conc. | Units | Series | Calibrator | Clusterin Conc. ( $\mathrm{nm} / \mathrm{ml}$ ) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| Kim1 | 700 | pmol/uL |  | Standard1 | 1000 |
| Clusterin | 1000 | $\mathrm{nm} / \mathrm{ml}$ |  | Standard2 | 2000 |
| OPN | 10 | $\mathrm{ng} / \mathrm{ml}$ | 2 | Standard3 | 4000 |
| IL8 | 50 | $\mathrm{nm} / \mathrm{\mu l}$ | Units | Standard4 | 8000 |
| CystatinC | 10 | $\mathrm{nm} / \mathrm{ml}$ |  | Standard5 | 16000 |
| NGAL | 20 | $\mathrm{ng} / \mathrm{ml}$ |  | Standard6 | 32000 |
| 50 | 10 | $n \mathrm{~m} / \mathrm{\mu l}$ |  | Standard7 | 64000 |
|  | 同 | [1] |  |  |  |

## Paste Option

For all columns with a paste icon at the bottom, you can copy and paste the clipboard contents to the corresponding column. The content is pasted starting from the first row on a
line by line basis. This is useful if you have your concentration data in another application (such as Excel or notepad).

## Standard Curve Fit Analysis for Multiple Plates

For quantitative assays, you may arrange standards on every plate in the batch or you may decide to measure standards only on some plates in the batch. Also, you can specify how the standard curve is generated and which curve to use to calculate concentrations from for each plate.

## Common Scenarios

- Measure standards on every plate in the batch. You may select to calculate concentrations using the standard curve on the same plate as the samples. Example: 3 plates are read, each uses the same layout, a curve is created for each plate, and concentrations are calculated using the standard curve on the same plate as the samples being calculated.
- Measure standards on some or all plates and generate a single standard curve using standard replicates from across plates. In this case sample concentrations are calculated using a common curve. Example: 3 plates are read, each uses the same layout, and a single curve is created using the standard measurements from each plate. The common standard curve is then used to calculate concentrations for all samples on all plates.
- Using a different layout on each plate read. Example: 3 plates are read, each uses a different layout, and a single curve is created using the standard measurements from each plate.
- Combine these options and configure a layout that uses a specific curve or common curve for each plate.


## Standard Curve Fit Evaluation with Multiple Plate

The additional complexity with evaluating the Standard Curve Fit transform across multiple plates is that for each plate the concentrations must be calculated using the correct curve as specified in the Layout Editor (and taking into account if there are more plates of data than defined in the layout).

For the details of the general procedure, see Multiple Plate Support.
For performance reasons, curves should not be unnecessarily recalculated, thus for evaluating the Standard Curve Fit analysis with a multiple plate assay:

1. Identify the curve to use for each plate.
2. Prepare the calibration data for each required curve.
3. Fit each curve once.

Then, after the curve data has been prepared, go through each plate and use the associated fitted curve to calculate the concentration values.
(1) There may be more than one curve generated.

## Preparing Calibration Data

1. Where the Curve selection for a plate refers to the curve on a specific plate (and not the average), the calibration data is prepared using the calibrator data from the specified plate number.
2. If the curve selection is an Average Across Plate selection, then the calibration data for the curve is obtained from all layouts with this setting; to be clear, all plates with this selection will use the same common curve that is computed from points across separate plates.

As with all other analyses, flagged points are not included in the fit.
If the Standard Curve Fit transform's FitToStandard is Replicates, then this is the data that will be used for the fit. If FitToStandard is Average, then the replicates are averaged (i.e., for each $x$ value, there is only $1 y$ value and that $y$ value is the average of the non-flagged replicates).

## Different Concentrations per Container/Plate

When the data for the curve is obtained from the same plate that is being evaluated, you can provide different concentration values per plate/container. With this configuration, the standard concentrations used for the fit are as defined in the Plates section in the XML Editor.

## How to: Specify Different Concentrations per Container/Plate

```
<StandardCurveFit StandardType="2" FitMethod="4PL"
Concentrations="0.125,0.25,0.5,1,2,4" XAxisTitle="Concentration" YAxisTitle="Raw"
FitToStandard="Average" CalculateFrom="Replicates">
<Plates>
<Container Concentrations="1,2,4"/>
<Container Concentrations="10,20,40"/>
<Container Concentrations="100,200,400"/>
</Plates>
</StandardCurveFit>
```

If there are more data containers than Container elements defined, then those additional containers will use the primary Concentrations attribute of the Standard Curve Fit properties.

If there are less containers in the data containers than Container elements defined, then those additional Container elements are simply ignored.

## Zero Concentration Calibrator

Using a theoretical zero concentration as a standard curve calibrator can be the source of many mathematical problems when fitting standard curves. Firstly, a zero standard can skew the fitting process with certain fit methods leading to an unexpected curve. Also, with any fit method, a zero value cannot be plotted on a logarithmic $x$-axis (as is typically used to plot a standard curve). Another issue is that a zero concentration will break fit methods with weighting models such as $1 / x^{2}$. A common workaround is to use a suitably small specified concentration value instead (such as 0.001).

If using a suitably small value is not ideal there are some other options.

## Measure Zero Without Using It as a Calibrator

In this scenario the zero standard is measured but not included as a calibrator for the fit; the zero standard is used as a control (and not as a calibrator). To achieve this, add the Zero sample type to your layout configuration to denote the location of the zero concentration sample(s).

## Including a Zero Standard Calibrator

In this case standards are added to the layout as normal, however, the first standard (Standard1) is given a known concentration of 0 . In some cases, this might not map directly to your actual standard numberings and you might prefer for Standard1 to map to the first non-zero standard. In this case, please try the following scenario.

## Including a Zero Calibrator

With this scenario, you can specify a Zero sample group to be used as a calibrator along with the Standard groups. If included, the curve fit will include this sample (i.e., all of its replicates) as a part of the calibrators (with a zero concentration value). The values will be added in the same way as for the standards (i.e., Average or Replicates using Calculate From setting). The $x$ value of these points will be zero.

To achieve this, first add the Zero sample type to your layout along with your standards. Next, to include the Zero group in the calibrators of the curve, an additional configuration step is required:

## Add Zero Concentration to a Standard Curve Fit

1. In the Assay Properties panel, select the XML tab.

If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Locate the StandardCurveFit element.

## 3. Add a ZeroType and optionally ZeroGroup attributes (inside the StandardCurveFit).

4. Click Apply to save changes to the protocol.

## Attributes and Syntax

The Zero concentration is calibrated via XML with the following attributes:

| ZeroType | The sample type index for a zero sample. |
| :--- | :--- |
| ZeroGroup | The sample group number for a zero sample. If Zero Type is specified but <br> this value is omitted, then a default value of 1 is applied. |

For example:
<Settings>
<StandardCurveFit StandardType="2" FitMethod="4PL" Concentrations="0.25,0.5, 1,2,4,8" XAxisTitle="Concentration" YAxisTitle="OD" FitToStandard="Average" CalculateFrom="Average" ZeroType="27" ZeroGroup="1">

This will set the zero type to Zero sample type and zero group to 1 .

The zero data points are included in Fit Chart in the same way as standards (i.e., withpink average triangle when relevant) but only if the $\mathbf{X} \mathbf{A x i s}$ Type is Linear. If it is
Logarithmic, then these values do not appear on the chart as a calibrator.

## Weighting Infinity Resolution

Applying weighting on calibration data points can lower the overall error of the method and therefore improve the quality of the results. However, weighting could result in the weight for a zero point to be infinite and thus skew the curve. An infinite weight can occur if the weight method is one of the forms: $1 / \mathrm{y}$ or $1 / \mathrm{x}$ (or $1 / \mathrm{y}^{2}$, etc.) and the calibration point has a 0 value in the axis of the denominator (e.g., $(0,0)$ ).

The notification will advise you if this issue has occurred. In this case, to avoid the infinite weights, you can use the following XML attributes:
\(\left.$$
\begin{array}{|l|l|}\hline \text { WeightInfinityResolution } & \begin{array}{l}\text { Specifies how to resolve infinite weights for the } \\
\text { curve: }\end{array}
$$ <br>
- None - Any infinite weights are used. <br>
- MaxFiniteMultipliedByFactor - Any <br>
infinite weight will be replaced by a finite <br>
weight which is computed as the maximum <br>
finite weight in the calibrators multiplied by <br>
the specified factor ( <br>
WeightInfinityReplacementFiniteMax <br>

Factor).\end{array}\right\}\)| When |
| :--- |
| WeightInfinityResolutionWeighting is |
| Wactor |
| MaxFiniteMultipliedByFactor, this is the |
| factor to multiply the maximum finite weight by, |
| the result is used in place of any infinite |
| weights. |

For example:
<Settings>
<StandardCurveFit StandardType="2" FitMethod="4PL" Concentrations="0.25,0.5,1,2,4,8"
XAxisTitle="Concentration" YAxisTitle="OD" FitToStandard="Average"
CalculateFrom="Average" WeightInfinityResolution="MaxFiniteMultipliedByFactor"
WeightInfinityReplacementFiniteMaxFactor="1">
With this configuration, the infinite weight will be replaced with the maximum finite weight multiplied by 1 .

## Advanced Fit Charts Configuration

## Customize X and Y Axes Positions for Curve Charts

You can define at which point the vertical axis (y-axis) crosses the horizontal axis (x-axis) and vice versa. You can specify axes crossing settings for:

- Charts generated by endpoint curve-fitting transforms (Standard Curve Fit, General Curve Fit, and Dose-Response Curves)
- Curve Overlay, Histogram, and XY Plot report element charts
- QC Output Levey-Jennings and CUSUM charts

To learn about customizing chart appearance for QC results, see Configuring QC Observations in the XML Editor.

You can also use Axes (Advanced) settings in the transform properties and Histogram settings.

## About Axes Crossing

By default, the axes cross each other at the minimum data point values. Using axis crossing XML attributes, you can set custom crossing points such as a specific value, maximum point on either axis, or allow the software to determine the best position automatically. For example, the default chart for the ELISA protocol is displayed:


With the VerticalAxisCrosses="MaximumAxisValue" attribute added to the StandardCurveFit element in the XML editor, the chart will be displayed:


## Specify How the Axes Cross in the XML

1. In the Assay Properties panel, select the XML tab.

If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Depending on which chart you would like to customize, locate:

- For Standard Curve Fit, the StandardCurveFit element.
- For General Curve Fit, the GeneralCurveFit element.
- For Dose-Response Curves, the DoseResponseCurves element.
- For Curve Overlay, under the Report element, the Curve child element.
- For Histogram, under the Report element, the Histogram child element.
- For XY Plot, under the Report element, the XYPlot child element.

3. Add the child element ChartCustomization to the appropriate element.
4. Inside the ChartCustomization element, add axis crossing attributes and specify their syntax.
The editor adds the xmlns="http://www.myassays.com/ChartCustomizations" by default. This attribute is required for the customization.
5. Click Apply to save changes and then Calculate to generate new results.

## Axis Crossing Attributes and Syntax

With these attributes, you can choose where you want the $x$-axis to cross the $y$-axis and vice versa. By default, the axes cross each other at the minimum axes values. For axes of logarithmic type, that is the lowest positive value.
$\left.\left.\begin{array}{|l|l|}\hline \text { VerticalAxisCrosses } & \begin{array}{l}\text { Specify where the vertical axis (y-axis) should cross the } \\ \text { horizontal axis (x-axis): }\end{array} \\ \text { - Automatic - Automatically determines the location that } \\ \text { makes the most sense for your data. } \\ \text { - AxisValue - Cross horizontal axis at specified } \\ \text { VerticalAxisCrossesValue. } \\ \text { - MaximumAxisValue - Cross horizontal axis at the } \\ \text { maximum horizontal value of the chart. }\end{array}\right\} \begin{array}{l}\text { - MinimumAxisValue - Cross horizontal axis at the } \\ \text { minimum horizontal value. If axis type is set to } \\ \text { logarithmic, then it is the lowest positive value. }\end{array}\right\}$

## Example in the XML:

For example, with Dose-Response Curves the configuration will look like:
<DoseResponseCurves Type="Type" Doses="List of Doses" FitMethod="Method"

PlotResponseForDoseCalculation="true"
ResponseForDoseCalculation="Response" >
<ChartCustomization
xmlns="http://www.myassays.com/ChartCustomizations" VerticalAxisCrosses="AxisValue" VerticalAxisCrossesValue="5" HorizontalAxisCrosses="MinimumAxisValue"/>
</DoseResponseCurves>

## Change the Units and Number Format of the Charts

To make the chart data easier to interpret, you can adjust the major and minor units displayed on the chart's axes. You can also define custom formatting for values shown on the axes.

You can apply custom settings to:

- Fit charts generated by endpoint curve-fitting transforms (Standard Curve Fit, General Curve Fit, and Dose-Response Curves)
- Curve Overlay, Histogram, and XY Plot report element charts
- QC Output Levey-Jennings and CUSUM charts

To learn about customizing chart appearance for QC results, see Configuring QC Observations in the XML Editor.

You can also use Axes (Advanced) settings in the transform properties and Histogram settings.

## About Units and Number Format

By default, MyAssays Desktop determines the chart's major units automatically based on your data. Changing these units will affect the intervals and values displayed on the axes as well as the gridlines of the $y$-axis. Minor units are not displayed unless specified, but they can help you estimate the data point values at a glance.

Also, for Excel outputs, you can specify the convention for formatting the numbers displayed on either axis. The options include setting the required number of decimal places or significant figures, changing font colour, rounding options, and much more.

For example, this is how the chart for the ELISA protocol can appear:


## Specify the Axes Display Settings in the XML Tab

1. In the Assay Properties panel, select the XML tab.

If the XML tab is not immediately visible, click \ll Show All Properties to expand.
2. Depending on which chart you would like to customize, locate:

- For Standard Curve Fit, the StandardCurveFit element.
- For General Curve Fit, the GeneralCurveFit element.
- For Dose-Response Curves, the DoseResponseCurves element.
- For Curve Overlay, under the Report element, the Curve child element.
- For Histogram, under the Report element, the Histogram child element.
- For XY Plot, under the Report element, the XYPlot child element.

3. Add the child element ChartCustomization to the appropriate element.
4. Inside the ChartCustomization element, aadd display units, number format attributes, or both and specify their syntax.
The editor adds the xmlns="http://www.myassays.com/ChartCustomizations" by default. This attribute is required for the customization.
5. Click Apply to save changes and then Calculate to generate new results.

## Attributes and Syntax

With these attributes, you can set the chart's appearance according to your needs.

| XAxisMajorUnits | The major unit to use as the numeric interval between numbers on <br> the x-axis. Starting from the minimum value, a major unit is <br> displayed at every increment. |
| :--- | :--- |
| XAxisMinorUnits | The minor unit that defines the numeric interval between <br> supplementary tickmarks on the x-axis. The minor units are not <br> displayed unless this attribute is specified. |
| XAxisNumberFormat | Specify the number formatting for the values displayed on the x- <br> axis. <br> To learn about available formatting options, see Custom Format <br> Codes for Numbers of the Chart. |
| YAxisMajorUnits | The major unit to use as the numeric interval between the gridlines <br> on the y-axis. Starting from the minimum value, a major unit is <br> displayed at every increment. |
| YAxisMinorUnits | The minor unit that defines the numeric interval between <br> supplementary tickmarks on the y-axis. The minor units are not <br> displayed unless this attribute is specified. |
| YAxisNumberFormat | Specify the number formatting for the major unit values of the y- <br> axis. <br> To learn about available formatting options, see Custom Format <br> Codes for Numbers of the Chart. |

## Example in XML:

Here is the XML snippet that shows the configuration of the Fit Chart from the example image above:

```
<StandardCurveFit StandardType="2" FitMethod="4PL"
Concentrations="400,200,100,50,25,12.5" XAxisTitle="Concentration"
YAxisTitle="Measurement" FitToStandard="Average"
CalculateFrom="Replicates">
    <ChartCustomization
    xmlns="http://www.myassays.com/ChartCustomizations"
    XAxisMajorUnits="10" XAxisMinorUnits="5" YAxisMajorUnits="0.2"
    YAxisMinorUnits="0.1" YAxisNumberFormat="[Red][\&lt;1]0.00;
    [Green][\&gt;=1]0.00"/>
</StandardCurveFit>
```


## Advanced Settings for Curve-Fitting Transforms

## Advanced Curve Fit Evaluations

When using the Standard Curve Fit and General Curve Fit transform, you can evaluate expressions that compute values from the determined curve. This topic describes how to configure, report, and optionally use evaluations as variables in other calculations.

This is an advanced feature that can only be configured through the XML editor.

## About Curve Fit Evaluations

In addition to performing curve fit and computing values from the curve, you can evaluate expressions that:

- Calculate $x$ or $y$ values on the curve (using interpolation or extrapolation)
- Reference the coefficients and goodness measures of the curve
- Can be referred to as a variables in other evaluations or Curve Fit Custom Flag Labels
- Can be referenced as the Transform Output Variables

This capability is useful for a range of scenarios, including calculating and reporting limits of detection and quantification.

## Add Evaluations to the Transform Configuration

1. In the Assay Properties panel, select the XML tab.

If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Depending on the transform you are using, locate:

- For Standard Curve Fit, the StandardCurveFit element.
- For General Curve Fit, the GeneralCurveFit element.

3. Add an Evaluations child element.
4. Add an Evaluation child element for each required evaluation. Set the evaluation attributes in accordance with the table below.
5. Click Apply to save changes and then Calculate to generate new results.

## Evaluation Attributes and Syntax

| Expression | The expression to evaluate. It can refer to: <br> - The Fit Transform Context Parameters and TOVs <br> - Sample values of the input matrix <br> - Any existing Curve Fit Evaluation result using its Name attribute (the referenced evaluation must be defined prior to where it is referenced) |
| :---: | :---: |
|  | A curve fit evaluation expression should not refer to $x$ or $y$ variables. |
| Type | This setting describes how the expression is treated in report: <br> - CalcX - Calculates the $x$ value from the fit line using Expression as $y$ value. The resulting values are reported using numerical formatting of input matrix. <br> - CalcY - Calculates the $y$ value from the fit line using Expression as $x$ value. The resulting values are reported using standard numerical formatting. <br> - Parameter - Outputs the computed Expression value. |
| Plot | Optional setting with Boolean type values: <br> - true - Plots the Expression result for CalcX or CalcY Type on the curve. The calculated point $(x, y)$ is connected to $x$-axis and $y$-axis with solid blue lines. <br> - false - An Expression result is not plotted on the curve. |
| Description | Optional setting to define a textual description of the expression. If this is not specified, the Expression itself is used in the report. |
| Name | Optional setting to define a name for evaluation. If it is specified, the Name can be used as a variable for other XML configurations (as described below). |

## Named Curve Fit Evaluations

If the Name attribute is set, then the evaluation can be referred to by this Name as a variable in:

- Context variables in evaluations that are defined in the XML editor below it
- Curve Flag Label conditions
- Transform Output Variables

The LLOQ and ULOQ are computed automatically and can be used as an evaluation and flag variables too.

## Example XML Configuration

In this example, we will configure evaluation of the Lower Limit of Detection (LLD). The LLD is often computed as the concentration $(x)$ at the average of the blank replicates +3 * standard deviation of the blank replicates. This can be computed by calculating the $x$ value on the curve where $\boldsymbol{y}=$ Blank+3*sd(Blank):
<Evaluations>
<EvaluationType="CalcX" Expression="Blank1 + (3* sd(Blank1))"
Description="LLD"Name="LLD" />
</Evaluations>
In this XML snippet, the expression defines the $y$ value to compute, the expression's type CalcX indicates that the $x$ value on the curve which equals the Expression should be computed.

## Customizing LLOQ and ULOQ in Standard Curve Fit

For Standard Curve Fit, the LLOQ and ULOQ calculations are included by default and equal a defined concentration value. If you create a named evaluation using LLOQ or ULOQ, it will replace the default LLOQ and ULOQ calculation:
<Evaluations>
<Evaluation Type="CalcY" Expression="Control1 + (10 * sd(Control1))"
Description="My LLOQ"Name="LLOQ" />
</Evaluations>

## Results and Report

The evaluation results are displayed below the interactive Fit Chart and in the report as a simple two-column Fit Evaluations table. The first column contains Description if specified or Expression if it was not. The second column contains the numerical value of the evaluated result or any syntax/computation error in red (in same way as the Evaluation feature).

For Standard Curve Fit, the LLOQ and ULOQ are the first entries; any further evaluations follow them.

## Advanced Dose-Response Curves Evaluations

Using the Dose-Response Curves transform, you can assess and report many more analytical values from each sample's curve. The additional evaluations are arranged and reported in a Dose-Response Fit Table. The below topics cover how to add, configure, and report the fit table, including an example configuration of common estimation factors.

## About Dose-Response Fit Table

Expression-based calculations from curves, computed by the Dose-Response Curves transform, result in a single numerical value for each curve. They are arranged and reported in a Dose-Response Fit Table. If there is an error in evaluating the expression, then the error message is reported (in red) as the result.

You can also specify the numerical formatting and label for each evaluation in the report.
When configuring expressions directly in the XML editor, it is necessary to follow XML conventions. To learn about XML syntax and settings, see XML Editor.

## Add Dose-Response Fit Table and Configure Evaluations

Assuming you have already added Dose-Response Curves transform, configure custom evaluations as follows:

1. In the Assay Properties panel, select the XML tab.

If the $\mathbf{X M L}$ tab is not immediately visible, click << Show All Properties to expand.
2. Locate the DoseResponseCurves element and add a FitTable child element.
3. For each required evaluation, add a child element Evaluation and set the attributes:

| Expression | An expression to evaluate for each sample. The expression can <br> refer to any matrix and/or non-matrix data (i.e., contains no $x$ <br> references) and results in a single numerical value. |
| :--- | :--- |
| Description | A header for column with the results of the Expression in the <br> report. |

Also, for each defined evaluation, you can optionally specify numerical formatting settings under the NumericalFormatting child element.

The Type settings are entered into XML without spaces.

## Example in the XML:

<FitTable>
```
<Evaluation Description="Description" Expression="Expression"/>
<Evaluation Description="Description" Expression="Expression">
    <NumericalFormatting Type="DecimalPlaces" Precision="3"/>
```
</Evaluation>
</FitTable>

## Add Dose-Response Fit Table to Report

Because this is an advanced configuration, add the following XML elements to include the Dose-Response Fit Table in the report:

1. Locate the ReportContent element and add a child element Item with the following attributes:

| Id | A unique identifier that is used to identify the report element in <br> the Report Elements pane and when configuring custom report <br> templates. For example, DoseResponseCurvesFitTable1. |
| :--- | :--- |
| Name | Enter or paste DoseResponseCurvesFitTable. |
| Type | Select Table from a drop-down list. |

Example in the XML:
<Item Id="DoseResponseCurvesFitTable1" Name="DoseResponseCurvesFitTable" Type="Table" />
2. Locate the TransformContent Id="TransformContentDoseResponseCurves1" (inside the Report element) and, for each defined Fit Table, add a child element Content with the following attributes:

| ItemId | Enter or paste the Id from Item element. |
| :--- | :--- |
| Include | Select $\mathbf{1}$ or true to include the table in the report. |
| SheetName | Optionally include the table on a separate sheet. For more details, <br> see Sheet Name. |

## Example in the XML:

<Content ItemId="DoseResponseCurvesFitTable1" Include="true" SheetName="Fit Table" />
Click Apply to save changes to the protocol and Calculate to inspect the results with the new settings.

## Label Dose-Response Fit Table Evaluation Result in the Report

You can also highlight the Fit Table results with sample labels.
Assuming you have already added Dose-Response Curves Fit Table and specified needed evaluations, configure the conditions in the XML as follows:

1. Locate the Evaluation element for which you want to set the label.
2. Add a child element SampleReportLabel.
3. For each required evaluation, add a child element Rule and set the attributes:
$\left.\begin{array}{|l|l|}\hline \text { Condition } & \begin{array}{l}\text { Boolean condition to evaluate (typically a conditional expression) } \\ \text { in terms of } x . \text { The } x \text { value specified in the expression refers to the } \\ \text { sample. }\end{array} \\ \hline \text { Label } & \begin{array}{l}\text { The label to be displayed in the Fit Table if the Condition is } \\ \text { satisfied. Use }\{\mathbf{x}\} \\ \text { condition refer to the data value that was used in the original data point). }\end{array} \\ \hline \text { Symbol } & \begin{array}{l}\text { A graphical icon to display to the left of the Label if the } \\ \text { Condition is satisfied: }\end{array} \\ \text { - None - Display no icon } \\ \text { - Pass - Display a icon } \\ \text { - Fail- Display a icon } \\ \text { - Question - Display a B icon }\end{array}\right\}$

## Example XML Configuration

With this XML snippet, all calculated $\mathrm{R}^{2}$ values that are greater than 0.999 are displayed as $\boldsymbol{x}$ HIGH in red and with a yellow background.

## <FitTable>

<Evaluation Description="R2" Expression="R2">
```
        <SampleReportLabel>
            <Rule Condition="x &gt; 0.999" Label="{x} HIGH"
        Symbol="None" BackColour="#FFFFFFO0"
        ForeColour="#FFFF0000" />
        </SampleReportLabel>
    </Evaluation>
</FitTable>

```

With the measurements data and layout from the Dose-Response Curves example, the results for Unknown1 are as follows:
\begin{tabular}{|l|l|}
\hline Sample & \(\mathbf{R}^{\mathbf{2}}\) \\
\hline OUnknown1 & 0.999497 HIGH \\
\hline
\end{tabular}

\section*{Dose-Response Curves Context Parameters}

When configuring evaluations in Dose-Response Fit Table, you can use Fit Transform Context Parameters.
\begin{tabular}{|c|c|c|}
\hline Parameter & Description & Example in XML \\
\hline groupnum & Refers to the group number of the dose-response curve. & \begin{tabular}{l}
GroupValue(\&quot;Unknown\&quot; (groupnum*2)-1) \\
Returns the value of each Unknown1 on a dynamic rack layout.
\end{tabular} \\
\hline dosemin & Refers to the minimum numerical value from the Doses drop-down. & \begin{tabular}{l}
auc \\
('4PL',dosemin,dosemax,1000,a,b,c,d)
\end{tabular} \\
\hline dosemax & Refers to the maximum numerical value from the Doses drop-down. & Calculates the area under each 4PL curve. \\
\hline
\end{tabular}

For information about functions you can use when configuring expressions, see Functions. To learn how to apply auc to your assay run, see Use Area under Curve to Estimate the Difference between Dose-Response Curves.

\section*{Configure Contents of Curve Data Tables}

Curve Data Tables list the measurement and calculation details for the calibrators or doses used in the Standard Curve Fit or the Dose-Response Curves transforms. You can customize the content included in Curve Data Tables by including additional or excluding existing columns. For example, you may prefer to collect more statistical data about the fitting, or limit the table to the input or calculated data only.

This is an advanced feature that can only be configured through the XML editor.

\section*{Configure Curve Data Table in XML}

To configure how the Curve Data Table appears in the report, follow these steps:
1. In the Assay Properties panel, select the XML tab.

This opens the built-in XML editor. If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Inside the Report element, locate the TransformContent element with the Id of the transform you are using:
- For Standard Curve Fit, the TransformContentStandardCurveFit, and then locate Content child element with CurveDataTable ItemId.
- For Dose-Response Curves, the TransformContentDoseResponseCurves, and then locate Content child element with DoseResponseCurveDataTables ItemId.
3. Add child element Setting for each column you'd like to modify and specify their attributes:
\begin{tabular}{|l|l|}
\hline Property & \begin{tabular}{l} 
Enter or paste the Column name from the Available Curve Data \\
Table Contents table below.
\end{tabular} \\
\hline Value & \begin{tabular}{l} 
Boolean setting that determines how to treat the columns. Enter: \\
- true to include the column in the report. \\
- false to exclude the column from the report.
\end{tabular} \\
\hline
\end{tabular}

Example in the XML:
<Setting Property="Column" Value="Boolean"/>
4. Click Apply to save changes, and then Calculate to generate report with new settings.

\section*{Available Curve Data Table Contents}

Here the columns that you can include in or exclude from the Curve Data Tables are listed in the order they will appear. The columns that are included by default are marked with asterisk (*).
\begin{tabular}{|l|l|}
\hline Column & Content \\
\hline IncludeRaw & The raw measurements — the first raw matrix. \\
\hline IncludeRawAverage & \begin{tabular}{l} 
The \%CV of the replicates of each group of the raw \\
measurements.
\end{tabular} \\
\hline IncludeRawPCV & \begin{tabular}{l} 
The standard deviation calculation of the raw \\
measurements.
\end{tabular} \\
\hline IncludeRawSD & \begin{tabular}{l} 
The transform input data.
\end{tabular} \\
\hline IncludeTransformInput* & \begin{tabular}{l} 
The concentrations or calibration doses calculated from \\
the curve.
\end{tabular} \\
\hline IncludeCaIculated* & \begin{tabular}{l} 
Depending on the selected Calculate From \\
setting of the Standard Curve Fit, a value will \\
be displayed for each non-flagged replicate \\
(Replicates) or per sample group (Average).
\end{tabular} \\
\hline IncludeCalculatedPCV* & \begin{tabular}{l} 
The percentage coefficient of variation of the calculated \\
concentrations or doses.
\end{tabular} \\
\hline IncludePercentAccuracy* & \begin{tabular}{l} 
This column is included only if the calibrators \\
are replicated.
\end{tabular} \\
\hline IncludeCalculatedStandardError & \begin{tabular}{l} 
The \% Accuracy column ratio of the average of the cal- \\
culated value against the original specified con- \\
centration or dose.
\end{tabular} \\
\hline IncludeSampleNumbers & \begin{tabular}{l} 
Includes the Sample Numbers in a column directly to \\
the right of the Calibrators column.
\end{tabular} \\
\hline The Standard Error (SEM) of the raw data. \\
\hline The Standard Error (SEM) of the concentrations or doses \\
are replicated.
\end{tabular}

\section*{Example XML Configuration}

With this XML snippet, the Curve Data Table of Standard Curve Fit will feature two additional columns in the report. These columns will present the standard deviation between replicates of calibrator samples and the standard error for calculated concentrations.
<ContentItemId="CurveDataTable1" Include="true">
<SettingProperty="IncludeRawSD" Value="true"/>
<SettingProperty="IncludeCalculatedStandardError" Value="true"/>
<Content/>

\section*{Constrain Coefficients of the Fit}

For certain types of curve-fitting applications, it is useful to be able to constrain one or more coefficients of the fit to specific values or ranges. With coefficient constraints imposed, the resulting curve fit equation will follow these constraints.

For example, when using the 4 PL or 5 PL model, it might be useful to constrain the upper and/or lower asymptotes of the curve to equal known saturation points (such as NSB or BO). These constraints could be based on measurements from specific samples in the assay and used to set limits on the computed curve.

Also, where the resulting fit is expected to fall within certain ranges, you can define constraints to guide the curve fitting process as required. For example, you may be using a curve-fitting equation that uses a coefficient that must be within a certain range (such as positive), a range constraint defined with a bound can be applied to direct the curve fit accordingly.

\section*{Where and How to Apply Coefficient Constraints}

You may constrain one or more of the coefficients of the selected fit method when working with the following endpoint transforms:
- Dose-Response Curves
- General Curve Fit
- Standard Curve Fit

This is an advanced feature that can only be configured through the XML editor.
Each coefficient can be constrained using either:
- A fixed value (specified using an expression that can refer to samples or mathematical operations)
- Bound (upper, lower or both)

The values of these constraints can be specified using an expression and may refer to a TOV.

\section*{Coefficient Constraints and Parallel Line Analysis}

Coefficient Constraints are useful when performing Parallel Line Analysis. In this case, an unconstrained fit can be performed followed by constrained fits that use limits defined from the unconstrained fit. The resulting constrained fits can then be compared and considered for parallelism.

> When working with Standard Curve Fit or General Curve Fit transforms, you can define a Custom Fit expression with a constant value instead of using a fixed coefficient constraint.

How to: Add Coefficient Constraints to a Curve-Fitting Transform Configuration
1. In the Assay Properties panel, select the XML tab.

If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Depending on the transform you're using, locate the:
- For Standard Curve Fit, the StandardCurveFit element.
- For General Curve Fit, the GeneralCurveFit element.
- For Dose-Response Curves, the DoseResponseCurves element.
3. Add a FitConstraints child element.
4. Add a child element Constraint for each required operation. Set the attributes in accordance with the syntax below.
5. Click Apply to save changes and then Calculate to generate new results.

\section*{Attributes and Syntax}

For each constraint, use the Coeff attribute to specify the coefficient to constrain.
The value of these constraints can be specified using an expression (that results in a single numerical value) and may refer to a TOV. The interpretation of the constraint depends on the type of restriction setting:
- Fixed - Sets a coefficient to a fixed value
- LowerBound - Limits the search range for the coefficient to be greater than specified constraint
- UpperBound - Limits the search range for the coefficient to be less than specified constraint

The UpperBound and LowerBound can be used separately or in a conjunction.
However, Fixed type overrides any UpperBound or LowerBound setting.

\section*{Example Configuration}

Here is an XML snippet that shows a complete configuration of the StandardCurveFit element with Constraints for the 4 PL fit coefficients.
<StandardCurveFit StandardType="2" FitMethod="4PL" Concentrations="0.25,0.5, , , 2,4,8" XAxisTitle="Concentration" YAxisTitle="OD" FitToStandard="Average" CalculateFrom="Replicates">
<FitConstraints>
<Constraint Coeff="a" Fixed="Blank1" />
<Constraint Coeff="b" LowerBound="0" />
<Constraint Coeff="c" UpperBound="100" />
<Constraint Coeff="d" Fixed="average(tov2.d, tov1.d)" />
</FitConstraints>
With this configuration the coefficients will be constrained as follows:
- a will equal the average of Blank1 sample replicates
- b will be greater than 0
- c will be less than 100
- d will equal the average of the d coefficients as computed from two previous Standard Curve Fit transforms (referenced using TOVs )

\section*{Custom Fit Advanced Settings}

When using the Standard Curve Fit's or General Curve Fit's Custom curve fit option, it is possible to define further fit settings via the XML editor including:
- Name - A name for your custom fit to be displayed on chart and in the report
- Weighting - A weighting model to apply
- Inverse Equation - How to compute \(x\) values from \(y\) (i.e., provide an equation in terms of \(y\) )
- Coefficient Estimate Heuristics - Rules for estimating each coefficient's starting value
- Coefficient Constraints/Ranges - Constraints or acceptable ranges for each coefficient

This is an advanced feature that can only be configured through the XML editor.
To learn about Custom Fit feature, see Custom Fit.

\section*{How to: Add Attributes to a Custom Fit Configuration}
1. In the Assay Properties panel, select the XML tab.

If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Depending on the transform you would like to create a custom fit, locate:
- For Standard Curve Fit, the StandardCurveFit element.
- For General Curve Fit, the GeneralCurveFit element.
3. Locate the child element Method.
4. Inside the Method element, define custom fit attributes and specify their syntax.
5. Click Apply to save changes and then Calculate to generate new results.

\section*{Attributes and Syntax}

You can specify Custom Fit settings with the following optional attributes:
\begin{tabular}{|l|l|}
\hline DisplayName & A title of the fit for chart and Calculation Log. \\
\hline Inverse & \begin{tabular}{l} 
The inverse equation of the fit in terms of \(y\). This equation is used to \\
compute each \(x\) value from the curve (so make sure this is correct). If the \\
equation is not provided, then results will be computed using an automated \\
but slower method.
\end{tabular} \\
\hline
\end{tabular}
\begin{tabular}{|l|l} 
Weight & The weighting method to use.
\end{tabular}

\section*{Configuring Coefficient Estimate Heuristics}

Defining a good starting point for calculating initial coefficient estimates improves the accuracy and speed of the fit. For a custom fit, you can provide the starting point estimates for any or all of the coefficients.

In addition, a range can be specified to limit or constrain the fit.
To specify an estimate, add an Estimate element (as a child of the Method) for each required estimate and set the attributes in accordance with the table below.
\begin{tabular}{|l|l|}
\hline Expression & A numeric value or a heuristic expression to calculate. \\
\hline Name & A name of a coefficient for which the estimate is for. \\
\hline Min & \begin{tabular}{l} 
The minimum value to limit the search range for the coefficient value. If not \\
specified, then the range is -Infinity.
\end{tabular} \\
\hline Max & \begin{tabular}{l} 
The maximum value to limit the search range for the coefficient value. If not \\
specified, then the range is + Infinity.
\end{tabular} \\
\hline
\end{tabular}

Heuristics expressions are used to compute starting estimates. An expression can reference aspects of the calibration data. For example, with 4PL model, the expected result for a coefficient might be the \(y\) value at the peak of the curve. In this case, you can reference maxy variable to define a starting estimate for an upper asymptote (a). It will refer to the maximum \(y\) value of your calibration data.

The following variables are available for reference.
\begin{tabular}{|l|l|}
\hline Variable & Value \\
\hline averagex & Average of all \(x\) values \\
\hline averagey & Average of all \(y\) values \\
\hline minx & Minimum \(x\) \\
\hline miny & Minimum \(y\) \\
\hline maxx & Maximum \(x\) \\
\hline maxy & Maximum \(y\) \\
\hline yatminx & \(y\) at the minimum \(x\) \\
\hline yatmaxx & \(y\) at the maximum \(x\) \\
\hline xatminy & \(x\) at minimum \(y\) \\
\hline xatmaxy & \(x\) at maximum \(y\) \\
\hline medianx & Median of all \(x\) values \\
\hline mediany & Median of all \(y\) values \\
\hline \hline
\end{tabular}
\begin{tabular}{|l|l|}
\hline Variable & Value \\
\hline slope & Slope of the data set \\
\hline intercept & Intercept of the data set \\
\hline
\end{tabular}

For more sophisticated estimates, you can use conditional function. For example, if the coefficient value depends on whether the \(y\) increases or decreases with \(x\), you can compare yatmaxx and yatminx values and adjust the estimate value accordingly.

\section*{Example Configuration}

The following XML snippet shows a complete configuration of the Method element with:
- Custom Display Name
- Inverse Expression
- Estimate Heuristics Expressions for each coefficient
<Method DisplayName="4PL (from expression)" Expression="d+((a-d)/(1+(x/c)^b))" Inverse="c*pow(((a-d)/(y-d))-1,1/b)" xmlns="http://www.myassays.com/bestfitsearch">
<Estimate Name="a" Expression="maxx" />
<Estimate Name="d" Expression="minx" />
<Estimate Name="b" Expression="if(yatminx \&lt; yatmaxx,3,-3)" />
<Estimate Name="c" Expression="medianx" />
</Method>

\section*{Custom Flag Labels by Fit Transform Calculations}

Using Custom Flag Labels by Fit Transform Calculations, you can specify when a result of Standard Curve Fit or General Curve Fit transform should be flagged and reported with a custom label rather than as a result or error.

> Custom Flag Labels by Fit Transform Calculations have higher precedence and override any other defined labels. To learn more about specific labels for flagged results, see Custom Flag Label.

The feature only applies to data points specifically flagged by the transform itself. If an input point is already flagged, then any existing custom label (or the absence of the custom label) is copied to the output.

\section*{About Custom Flag Labels by Fit Transform Calculations}

You can use this advanced flagging feature to label results for fit transforms where the results cannot or should not be computed from the curve. For example, if a sample is outside the usable range of the curve, then it might be useful to flag and label the result accordingly. What is considered as the usable range of the curve is open to your own interpretation. For example, the use of the curve could be limited by:
- The defined or determined ULOQ and LLOQ values
- The mathematical limits of the curve
- Your own defined limits (defined as absolute values or using expressions with limits computed from the curve fit)

When using Standard Curve Fit or General Curve Fit transform, you can apply:
- General CurveError label to use if a value cannot be computed from the curve.

You can use optional CurveError setting to specify a default custom flag label to use if the result is flagged on the input matrix or while calculating results. It simply replaces the default flagged result in the report with specified CurveError label.
- Custom flagging conditions-the expressions written in terms of \(x\)-based on the transform's input or output data (calculated from the curve).

You can define any number of conditions in any order. If any of these conditions are met, then result is flagged with a specified label applied to it. If multiple labels are configured, only the first label condition to be evaluated as true is used (in the order they appear in the XML editor). These conditions take precedence over the default flagging settings and

CurveError. So, if any condition with Type="Input" is evaluated as true, then these will be used in place of any curve error.

\section*{Add Custom Flag Label to the Fit Transform}

Assuming you have already added Standard Curve Fit or General Curve Fit transform, configure custom flag labels as follows:

In the Assay Properties panel, select the XML tab.
If the XML tab is not immediately visible, click << Show All Properties to expand.
1. Depending on the transform you're using, locate the:
- For Standard Curve Fit, the StandardCurveFit element.
- For General Curve Fit, the GeneralCurveFit element.

Add the Flags child element.
Inside the Flags element, optionally add a CurveError attribute with the following syntax:
\begin{tabular}{|l|l|}
\hline \multirow{3}{*}{ CurveError } & \begin{tabular}{l} 
The custom flag label to use in place of the default Flagged label. \\
\end{tabular} \\
& When specifying custom flag text, strikethrough is not applied. \\
\hline
\end{tabular}

\section*{Example in the XML:}
<<Flags CurveError="My Custom Label" >
Add a child element Flag for each required flagging condition and specify its attributes:
\begin{tabular}{|l|l|}
\hline Type & The value of \(x\) depends on the expression type: \\
•For Type="Input", \(x\) is the value of the input position. \\
•For Type="Output", \(x\) is the value computed for the output position.
\end{tabular}
\begin{tabular}{|c|c|}
\hline Condition & \begin{tabular}{l}
The condition to evaluate (typically a conditional expression). \\
The flag condition expression can refer to: \\
- the value of each position using the reference \(x\) \\
- the computed coefficients of the fit and goodness measures \\
- Advanced Curve Fit Evaluations \\
- sample values of the input matrix
\end{tabular} \\
\hline Label & If a flag Condition is satisfied (evaluated to true), then the result of the transform for the position is flagged with the associated Label. If you insert \(\{\mathbf{x}\}\) in the Label, it inserts the value of \(x\) (e.g., \(\{\mathbf{x}\}\) is Below LLD might be reported as \(\mathbf{0 . 2 5}\) is Below LLD). \\
\hline
\end{tabular}

\section*{Example in the XML:}
<Flag Type="Type" Condition="Expression" Label="My Custom Label" />
Click Apply to save changes and then Calculate to generate new results.

\section*{Specifying Conditions in XML}

When entering conditions in XML, there are certain symbols that must be encoded using correct XML syntax. These are listed in the table below.
\begin{tabular}{|l|l|l|}
\hline Operator & Description & XML Encoding \\
\hline\(<\) & Less Than & \&lt; \\
\hline\(>\) & Greater Than & \&gt; \\
\hline \(\boldsymbol{\&}\) & Ampersand & \&amp; \\
\hline\("\) & Double Quote & \&quot; \\
\hline, & \begin{tabular}{l} 
Apostrophe/Single \\
Quote
\end{tabular} & \&apos; \\
\hline
\end{tabular}

For example, if the condition is \(\boldsymbol{x}<\mathbf{a}\) then this would be encoded in XML using \(\boldsymbol{x} \boldsymbol{\&} \mid \mathbf{t}\); \(\mathbf{a}\).

\section*{Example Configuration}

With this XML snippet configuration of the StandardCurveFit element, any sample whose measurement response is above or below the computed asymptotes of the 4 PL (the \(\mathbf{a}\) and \(\mathbf{d}\) coefficients of the fit) will have a label associated with it accordingly.
<StandardCurveFit StandardType="2" FitMethod="4PL"
Concentrations=" \(0.125,0.25,0.5,1,2,4\) " XAxisTitle= "Concentration" YAxisTitle="Raw" FitToStandard="Average" CalculateFrom="Average">
<Flags CurveError="Could not calculate from curve" >
<Flag Type="Input" Condition="x \& It; min(a,d)" Label="\{x\} Below Lower Asymptote" />
<Flag Type="Input" Condition="x \&gt; max(a,d)" Label="\{x\} Above Upper Asymptote" />
</Flags>
</StandardCurveFit>

\section*{Example Flag Conditions}
\begin{tabular}{|c|c|}
\hline XML Condition & Description \\
\hline ```
<Flag Type="Input" Condition="x &/t;
Blank1+3*sd(Blank1)" Label="{x} Below LLD"
/>
``` & If the measurement response is less than Blank1 + 3SD, then the result is flagged and reported with the Label. \\
\hline \begin{tabular}{l}
<Flag Type="Input" Condition="x \& It; min \\
(a,d)" Label="Below Lower Asymptote" />
\end{tabular} & If the measurement response is less than the lower value of the a and d coefficients (as computed from the curve), then the result is flagged and reported with the Label. \\
\hline ```
<Flag Type="Input" Condition="x &lt; 0.125"
Label= "OD &|t; 0.125" />
``` & If the measurement response is less than 0.125 , then the result is flagged and reported with the Label. \\
\hline \begin{tabular}{l}
<Flag Type= "Output" Condition="x \&lt; \\
0.125" Label ="Conc. \&lt; 0.125" />
\end{tabular} & If the computed concentration is less than 0.125 , then the result is flagged and reported with the Label. \\
\hline
\end{tabular}

\section*{Setting Numerical Formatting for Goodness Measures and Fit Coefficients}

To customize how the goodness measures and fit coefficients are formatted in the report, you can set their numerical formatting in the XML editor. This topic covers the attributes to specify for configuring the format of:
- Goodness Measures and Fit Coefficient Results
- Fit Coefficient \(\pm\) Errors

This is an advanced feature that can only be configured through the XML editor.
Locating in the XML
To configure the formatting, follow these steps:
1. In the Assay Properties panel, select the XML tab.

This opens the built-in XML editor. If the XML tab is not immediately visible, click << Show All Properties to expand.
2. Inside the Report element, locate the TransformContent Id="TransformContentStandardCurveFit1" and:
- For Fit Coefficients table, under the "CoefficientsTable1" item
- For Goodness Measures table, under the "GoodnessMeasuresTable1" item

Add child element Setting for each formatting setting and specify their attributes as described below.
3. Click Apply to save changes, and then Calculate to generate report with new settings.

\section*{Setting Up Format of Goodness Measures and Fit Coefficient Results}

To set up the numerical formatting of these report elements, add and define numerical formatting settings according to the following example:
<Setting Property="ValueFormattingType" Value="Type"/>
<Setting Property="ValueFormattingPrecision" Value="Value"/>
Where Type specifies how a numerical value is formatted with regard to precision and rounding options:
- SignificantFigures - The value is reported to the specified number of significant figures. This accepts integer Value in range 1 through 14.
- DecimalPlaces - The number of digits to display to the right of the decimal point. This accepts integer Value in range 0 through 14.

For example:
<Content ItemId="GoodnessMeasuresTable1" Include="true">
<Setting Property="ValueFormattingType" Value="DecimalPlaces"/>
<Setting Property= "ValueFormattingPrecision" Value="3"/>
<Content/>
With this configuration, the determined goodness measures will be presented to 3 decimal places.

\section*{Setting Up Format of Fit Coefficient \(\pm\) Errors}

For Coefficients table, you can additionally define how the \(\pm\) errors are reported with
ErrorFormattingType and ErrorFormattingPrecision properties. Thus, the complete configuration for this element looks like as follows:
```

<Content ItemId="CoefficientsTable1" Include="true">
<Setting Property="ValueFormattingType" Value="SignificantFigures"/>
<Setting Property="ValueFormattingPrecision" Value="4"/>
<Setting Property="ErrorFormattingType" Value="SignificantFigures"/>
<Setting Property="ErrorFormattingPrecision" Value="4"/>

```
<Content/>

This will report calculated coefficients and their \(\pm\) errors to 4 significant figures.

\section*{Troubleshooting Results}

\section*{What a High Blank (or Background) Means, and How to Deal with It}

A high blank or background reading normally indicates a preparation error.
First, check that the plate was washed correctly and that the washer is not the source of problem. Second, consider the possibility of a reagent preparation issue. Too much labeled detector may cause this type of response in an assay. Check that the blocking step was also performed correctly, was the right amount of block used in the right buffer?

\section*{Curve Fitting with a High Blank}

With data analyses that perform a Blank Correction and Standard Curve Fit, if the blank value is higher than some of the standards (e.g., the lowest standard), then a part or all of the resulting curve will be negative and fall below the \(x\)-axis.


In this scenario, one point to understand is that the blank correction does not actually impact the calculated concentrations. Because all samples (standards and unknowns) are blank corrected in the same way, the whole curve shifts down the \(y\)-axis but the computed concentrations will be the same. Thus, the results will be the same if the blank is 0 , small, or high. It seems counterintuitive, but this is the case. You might then wonder what is the point of performing blank correction; a blank is used in this way to simplify comparing curves across assay runs (and in the perfect situation generating a nice-looking curve through \(y=0\) although that is rarely the case). The blank can also be used to identify problems with the assay run, for example a high blank may indicate preparation errors as discussed above.

With MyAssays Desktop, negative \(y\) values (i.e., where blank is high) are included in the curve fit, the result is that part of the curve will appear below the \(x\)-axis, but all points are included in the fit. In this case you'll get the same results, but it's clear from the chart that the blank correction is too high (causing negative standards).

\section*{Expressions}

Expressions are available for use throughout MyAssays Desktop to provide flexibility in dataanalysis design and configuration.

An expression is simply an operation to be computed. In the simplest case, an expression can refer to a specific numerical value, measurement, or result. For example, the expression Control1 refers to the average of the Control 1 sample replicates (that are not flagged).

When writing expressions that refer to specific samples, in general, it is preferable to refer to a sample group, such as Control1, rather than specific layout positions (such as A1). With this approach, if your sample layout changes (perhaps you run the assay with a different sample layout), then you will not need to update your expressions.

An expression might perform mathematical or statistical operations on these values. For example, the expression pcv(Control1) computes the \%CV of the Control1 replicates.

An expression can be applied at various levels of the data analysis to define and compute various results. For example, matrix calculations, quantification limits, validation rules, values from fitted curves, and best fit score methods (to decide on the best fit) can all be defined using expressions.

Wherever a numerical value can be entered as a settings parameter, an expression can usually be provided to compute that parameter from other sources.

The Expressions system has been designed to:

\section*{Provide custom analysis using a familiar mathematical syntax (similar to Excel) - Provide an easy way to reference sample data \\ Take into account flagged data}

Expressions can refer to measurements or calculated results from specific layout positions (e.g., A1), sample groups (e.g., Control1), variables, and also the results of other expressions.

\section*{Culture Settings}

When providing numerical values or expressions through the user interface, enter numerical values and lists using your system's regional settings. There are two settings that can vary between culture settings:
- Decimal separator - Typically a period (.) on non-European systems and a comma (.) on typical European system
- List separator - Typically a comma (,) on non-European systems and a semi-colon (;) on typical European system

For example, depending on your system's culture settings, an example expression could be entered in two forms:

\section*{mean(1.5,2.5)}

\section*{mean(1,5;2,5)}

> When configuring expressions directly in the XML editor, it is necessary to follow XML conventions and provide data using invariant settings; that is period decimal separator for numeric values and comma list separator.
> To learn about XML syntax and settings, see XML Editor.

\section*{Syntax and Context}

The syntax of expressions is consistent throughout MyAssays Desktop. However, the application of an expression depends on the context it is being used in. For example, expressions are used in the configuration of transforms, validations, and evaluations. In each case, the application of the expression is slightly different.

Where an expression is used as a parameter to another setting, you can provide an expression that can be evaluated to a numerical result, such as Control 1*10.

There are several situations where an expression is entered in terms of \(x\) (where \(x\) refers to an input value that will be substituted with a value for each evaluation of the expression). For example, the Expression by Matrix transform expects an expression in terms of \(x\). In this context, you can provide an expression such as \(\boldsymbol{x}\)-Blank1 to subtract the mean of Blank1 from all samples.

Where an expression is entered in terms of \(x\), the expression is evaluated multiple times with \(x\) being substituted with different values. In the case of the Expression by Matrix transform, the expression is applied to every position on the input matrix to calculate the result of the output matrix.

There are also scenarios where a Boolean expression is expected. Validations are defined using a Boolean expression to define rules that must be satisfied for the assay to be considered valid.
For example, a validation rule can be defined with the expression Standard1 <Standard2 to check that the standard measurements are in the expected order. Similarly, a validation expression \(\mathbf{x}<\mathbf{1 5}\) could be used along with \%CV data to validate that all samples have a \%CV of less than 15 .

A typical expression comprises of:
- operations (e.g., \(+,{ }^{*}, /, \wedge\) )
- operands (e.g., A1, 1.23, x, Control1, TOV1.a)
- functions (e.g. pcv, sd, median, floor)

The following table lists some examples of expressions you may use in your assay.

\section*{Example Expressions}

All expression examples provided here use a period decimal separator(.) and a comma list separator(,). Use local settings of your system when entering expressions on your own system.
\begin{tabular}{|c|c|}
\hline Expression & Evaluated to \\
\hline Unknown1 & The average of the replicates of Unknown1 (that are not flagged). \\
\hline Unknown & The average of all Unknown samples (that are not flagged). \\
\hline A1 & The data for position A1. \\
\hline A1+A2 & The sum of the data at positions A1 and A2. \\
\hline mean(A1,A2,A3) & The average of the data at positions A1, A2, and A3. \\
\hline pcv(Unknown1) & The percentage coefficient of variation of Unknown1. \\
\hline Blank1+(3*sd(Blank1)) & The average of the sum of Blank1 plus 3 times the standard deviation of Blank1 (this is often used to compute LLD). \\
\hline \begin{tabular}{l}
pcv \\
([Unknown1,Unknown2])
\end{tabular} & The percentage coefficient of variation of all Unknown1 and Unknown2 replicates. \\
\hline median(Standard1) & The median of the replicates of Standard1 (that are not flagged). \\
\hline x-Blank1 & The value of variable \(x\) minus the average of Blank1 position. \\
\hline \(x>15\) & A Boolean expression that would evaluate to 1 (true) if the value of variable \(x\) is greater than 15 ; otherwise it evaluates to 0 (false). \\
\hline Ifthenelse( \(\mathrm{x}>15,15, \mathrm{x}\) ) & If the value of \(x\) is greater than 15 , then the result is 15 ; otherwise the result is \(x\). \\
\hline \(x-y\) & The difference between \(x\) and \(y\). Here \(x\) and \(y\) are the values of corresponding positions from two matrices of data (as used in the Expression by Matrix (Dual) transform). \\
\hline median(Unknown.2.All) & The median of all Unknowns on the \(2^{\text {nd }}\) matrix across all \\
\hline
\end{tabular}
\begin{tabular}{|c|c|}
\hline Expression & Evaluated to \\
\hline & containers. \\
\hline Unknown1.2.3 & The average of Unknown1 sample group on the \(2^{\text {nd }}\) matrix of \(3^{\text {rd }}\) plate. \\
\hline TOV1.R2 & The \(R^{2}\) value of the curve fit as used in the first transform (referenced using a Transform Output Variable). \\
\hline GroupValue("Blank",1) & The value of the group that is Blank1. (This is equivalent to the reference Blank1, however this method can be useful for applying calculations on group number references.) \\
\hline \begin{tabular}{l}
x-GroupValue \\
("Blank",PosGroup())
\end{tabular} & The value of variable \(x\) minus the average of the Blank sample which has a group number that matches the group number of the sample at the position where the same is being evaluated. This technique can be used to write expressions that apply a calculation in accordance to a layout, such as subtracting Blanks values on 1-to-1 basis. With a layout where each Unknown group has its own Blank group (e.g., Unknown1, Blank1, Unknown2, Blank2, etc.) this expression will subtract the blank group associated with each sample based on the layout. \\
\hline
\end{tabular}

\section*{Operators}

\section*{Unary Arithmetic Operator}
\begin{tabular}{|l|l|l|}
\hline Operator & Operation & Examples \\
& Minus Sign & -1.23 \\
& & \(-x\) \\
\hline
\end{tabular}

\section*{Binary Arithmetic Operators}
\begin{tabular}{|c|c|c|}
\hline Operator & Operation & Example \\
\hline + & Addition & \[
\left\lvert\, \begin{gathered}
2+1 \\
x+y \\
x+1
\end{gathered}\right.
\] \\
\hline - & Subtraction & \[
\begin{aligned}
& 2-1 \\
& x-y \\
& x-1
\end{aligned}
\] \\
\hline * & Multiplication & \[
\begin{array}{|l}
\hline 2 * 3 \\
x^{*} y \\
y^{*} 2
\end{array}
\] \\
\hline / & Division & \[
\begin{aligned}
& 2 / 2 \\
& x / y \\
& x / 2
\end{aligned}
\] \\
\hline \(\wedge\) & Exponentiation & \[
\begin{aligned}
& 2^{\wedge} 3 \\
& x^{\wedge} y \\
& x^{\wedge} 2
\end{aligned}
\] \\
\hline
\end{tabular}

\section*{Binary Logical Operators}

The numerical evaluation of 1 for true and 0 for false. Thus, \(1>0=1\).
\begin{tabular}{|l|l|l|}
\hline Operator & Operation & Examples \\
\hline \multirow{4}{*}{} & Less Than & \(1<2\) \\
& & \(x<y\) \\
\(x<2\) \\
\hline & Greater Than & \(2>1\) \\
& & \(x>y\) \\
\(x>2\) \\
\hline \hline
\end{tabular}
\begin{tabular}{|c|c|c|}
\hline Operator & Operation & Examples \\
\hline = & Equal To & \[
\begin{aligned}
& 1=1 \\
& x=y \\
& x=2
\end{aligned}
\] \\
\hline <= & Less Than Or Equal & \[
\begin{aligned}
& 3<=9 \\
& 3<=3 \\
& x<=y \\
& x<=x \\
& x<=3
\end{aligned}
\] \\
\hline >= & Greater Than Or Equal & \[
\begin{aligned}
& 9>=3 \\
& 3>=3 \\
& y>=x \\
& x>=x \\
& x>=9
\end{aligned}
\] \\
\hline <> & Not Equal & \[
\begin{aligned}
& 2<>1 \\
& x<>y \\
& x<>3
\end{aligned}
\] \\
\hline
\end{tabular}

\section*{Boolean Operations}

The Boolean operations (and, or, not) are implemented as Conditional Functions.

\section*{Operands}

\section*{Numbers}

Number operands are simply numbers (e.g., 1.23, \(-100,14\) ).
Make sure that all numerical values entered into the user interface are provided using the local settings of your system.

\section*{Variables}

The \(x\) and \(y\) variables refer to the value of each position on the associated matrix. All references to these variables in expressions are equated to the associated values.

For example, in the Expression by Matrix transform, you may enter the expression \(\boldsymbol{x}^{\mathbf{*}} \mathbf{1 0 0}\), where the \(x\) variable will refer to the positions of the input matrix. In the resulting matrix, all positions will be multiplied by 100. In Expression by Matrix (Dual), the \(x\) and \(y\) variables are set to the position values of the first and second matrices. Thus, when you enter an expression \(\boldsymbol{x}+\boldsymbol{y}\), each position on the resulting matrix will be the sum of input matrices values.
\begin{tabular}{|c|c|}
\hline Variable & Relevant For \\
\hline None & Installed Protocols, Certain Endpoint: Auto Flag, Average, General Curve Fit, Matrix Difference, Median, Percentage, Percentage Coefficient of Variation, Replicate Outlier Removal, Standard Curve Fit, Standard Deviation, Variance \\
\hline \(x\) & Certain Endpoint: Blank Correction, Dose-Response Curves, Dilution Factors, Expression by Matrix, Expression by Position, Expression by Sample Type, Factor, XY Blank Correction \\
\hline \(x, y\) & Expression by Matrix (Dual), XY Expression (Dual) \\
\hline \(y\) & XY Expression \\
\hline
\end{tabular}

\section*{Fit Transform Context Parameters}

Fit Transform Context Parameters apply only within transforms that perform curve fitting as settings or in the intra-transform advanced calculations. Some of the parameters can be referenced in the general configuration of the assay (e.g., validation or evaluation tables) or in other transforms as Transform Output Variables (see subsection below).

\section*{General Fit Transform Context Parameters}

These parameters can be used in configuration settings (for XY Reduction Fit Parameter, General Curve Fit, and Standard Curve Fit when using Custom Fit), flagging conditions, constraining the fit, and additional evaluations:
- Goodness of Fit Measures calculated from the fit.
\begin{tabular}{|l|l|}
\hline Name & Expression Syntax \\
\hline \(\mathbf{R}^{\mathbf{2}}\) & r2 \\
\hline \(\mathbf{a R}^{\mathbf{2}}\) & ar2 \\
\hline \(\mathbf{F}\) & f \\
\hline \(\mathbf{P}\) & p \\
\hline \(\mathbf{S E}\) & se \\
\hline \(\mathbf{A I C}\) & aic \\
\hline \(\mathbf{A I C c}\) & aicc \\
\hline BIC & bic \\
\hline DWS & dws \\
\hline DoF & dof \\
\hline RMS & rms \\
\hline
\end{tabular}
- Coefficients of the Fit - These vary depending on the selected fit method and are specified within documentation of these methods.

\section*{Specific Fit Transform Context Parameters}

When using General Curve Fit and Standard Curve Fit, any defined named evaluations can be used as context parameters. You can use them in further evaluations, and custom flagging within the transform.

Also, the Dose-Response Curves transform features its own context parameters groupnum, dosemin, and dosemax. To learn more about their usage, see Advanced Dose-Response Curves Evaluations.

\section*{Named Evaluation Variables}

Named Evaluation Variables can be referenced in any evaluation (including validation and label conditions) computed after the named evaluation. For detailed configuration and usage, see Named Evaluation Variables.

\section*{Transform Output Variables}

Transform Output Variables (TOVs) can be referenced in any expression that is evaluated after the transform that produced the TOV. To learn more about their configuration and usage, see Transform Output Variables.

\section*{Layout References}

When an evaluation depends on and is associated with certain layout, the expression can include references to positions, sample groups, and/or types defined on the layout. These are
defined with case-insensitive input (i.e., uppercase and lowercase letters in expressions are treated the same).

For example, Unknown1 is the same as unknown1, UNKNOWN1, and UnKnOWN1.
General information on referencing the sample layout and its components is provided in the Layout References in Expressions.

\section*{Position References}

A position ID refers to a specific position on the layout (e.g., A1, H12). To learn more, see Positioning on the Layout.

The available dimensions depend on the layout in use. A position on a particular matrix and/or plate can be referenced as described in sample references in multiple plate assays.

> When writing expressions, it is preferable to refer to sample groups such as Blank 1 rather than specific positions. If you have to modify your layout (perhaps because of pipetting error), you do not need to update your expressions as the group reference will refer to the correct sample on the modified layout.

\section*{Row and Column References}

The Row and Column operands refer to data on a particular layout row or column (on the selected matrix). The data here refers to all non-flagged data positions with a type that is not Unused. Refer to these operands with the following syntax:

\section*{RowN}

\section*{ColN}

Where \(\mathbf{N}\) is a positive integer to represent the row or column number. In microplate layouts, Row 1 refers to Row A on the microplate. Example: average(Row1) will compute the mean of all non-flagged positions on Row1 for sample positions that are used (i.e., sample type is not Unused).

\section*{Sample References}

Sample operand can contain a reference to:
- Sample group - It can refer to any group of any type defined on the layout. To use this operand in the expression, type in the sample type name (e.g., Unknown, Blank,
Standard) and sample group number. Example: Unknown1 refers to all non-flagged Unknown1 sample group positions on the layout.
- Sample type - If no group number is specified, then it refers to all positions of that type on the layout (e.g., all Unknowns, Blanks, Standards). Example: Unknown refers to all non-flagged data points of Unknown type on the layout.

The simple sample reference usually refers to the average of non-flagged positions of the type/group. Only for group-enabled functions does it refer to the list of values of each non-flagged position that belong to the sample reference. Example: median
(Unknown1) calculates the median of all non-flagged Unknown1 position values defined on the layout.

If a sample reference is enclosed in square brackets (e.g., [Unknown1]), it refers to the list of values of each non-flagged position. So [Unknown1] is actually expanded to the list of results for the non-flagged members of Unknown1.

\section*{Sample References in Multiple Plate Assays}

To simplify evaluation and avoid the need to write a different expression for each container, expressions use local group numbering. Thus, the Unknown1 reference always refers to the first Unknown sample group on the plate being evaluated. However, the sample group, type, or position from a particular matrix or plate can be referenced.
To learn more about referencing samples, see Sample References in Multiple Container Assays topic.
\begin{tabular}{|l|l|}
\hline Reference & Refers To \\
Unknown1 & \begin{tabular}{l} 
The average of the first Unknown sample group on a matrix and plate \\
being evaluated.
\end{tabular} \\
\hline A1.2 & A1 position for \(2^{\text {nd }}\) matrix. \\
\hline A1.2.3 & A1 position for the \(2^{\text {nd }}\) matrix of \(3^{\text {rd }}\) plate. \\
\hline A1..3 & A1 position for \(3^{\text {rd }}\) plate. \\
\hline A1.2.All & The average of all \(\mathbf{A 1}\) positions on the \(2^{\text {nd }}\) matrix across all plates. \\
\hline Unknown1.2 & Unknown1 sample group for 2 \({ }^{\text {nd }}\) matrix of data. \\
\hline Unknown.2.All & The average of all Unknown samples on the \(2^{\text {nd }}\) matrix across all plates. \\
\hline Unknown1.2.All & \begin{tabular}{l} 
The average of all Unknown1 replicates on the \(2^{\text {nd }}\) matrix across all \\
plates.
\end{tabular} \\
\hline Unknown1.2.3 & Unknown1 sample for the \(2^{\text {nd }}\) matrix of \(3^{\text {rd }}\) plate. \\
\hline Unknown1..3 & Unknown1 sample on the \(3^{\text {rd }}\) plate. \\
\hline
\end{tabular}

For more information about configuring data analysis for multiple plate assays, see Multiple Plate Analysis.

\section*{Numerical Field Data Reference}

Numerical Field Data values can be referenced in expressions by specifying the field data matrix letter as part of the reference. The field data matrix lettering starts from A. View the Matrices tab to see the lettering of any field data matrices. The following table provides examples:
\begin{tabular}{|l|l|}
\hline Reference & \begin{tabular}{l} 
Refers To \\
Unknown1.A
\end{tabular} \\
\begin{tabular}{l} 
The average of the first Unknown sample group on the first field matrix \\
(lettered A) and
\end{tabular} \\
\hline A1.A & A1 position for the first field matrix (lettered A). \\
\hline A1.B & A1 position for the second field matrix (lettered B). \\
\hline \begin{tabular}{l} 
Unknown1.A \\
\(\mathbf{. 2}\)
\end{tabular} & \begin{tabular}{l} 
The average of the first Unknown sample group on the first field matrix \\
(lettered A) on the second container.
\end{tabular} \\
\hline H12.C.4 & H12 position for the third field matrix (lettered C) on the fourth container. \\
\hline
\end{tabular}

\section*{Matrix Position References}

This operand comes in handy for accessing the data on the specified matrix at the position the expression is being evaluated for. It is relevant for endpoint data only; however, it is useful for configuring how the XY (kinetic or spectral) data is treated in analysis.

For additional details and configuration examples, see the Matrix Position Reference subsection.

\section*{Matrix Position Reference}

A Matrix Position Reference is a convenient way to refer to data in a specific matrix. For example, the expression:

\section*{\#1-\#2}
can be used to compute the differences between the values on the first and second matrix (for all positions on the layout).

A Matrix Position Reference refers to data at the associated position on a specific matrix (and optionally on a specific container/plate/analyte).

\section*{When is a Matrix Position Reference Available?}

Matrix Position References are available where the expression being evaluated is associated with a position. Examples of transforms with position associated expressions are:
- Expression by Matrix
- Expression by Matrix (Dual)
- XY Expression
- XY Expression (Dual)
- XY Reduction \(X\) at \(Y\)
- XY Reduction Y at X

Also, the Range parameters ( \(\mathbf{X}\) Minimum and \(\mathbf{X}\) Maximum) of \(\underline{X Y \text { transforms are position }}\) associated.

In these cases, the expression is evaluated at each and every position. The matrix position reference provides a convenient method for accessing the data on the specified matrix at the position the expression is being evaluated for.

\section*{Syntax}

A Matrix Position Reference is available in two forms:
- With Matrix Number
- With Matrix Number and Container Number (e.g., plate/multiplex analyte)

The matrix number is the 1-based number of the matrix. These are listed in the Matrices tab.

\section*{Example References}
\begin{tabular}{|l|l|}
\hline Matrix Position Reference & Refers To \\
\hline\(\# 1\) & Matrix 1 \\
\hline\(\# 1.2\) & Matrix 1 Container 2 \\
\hline
\end{tabular}

Matrix Position References are only applicable to endpoint matrices. If you mistakenly refer to a matrix that is not endpoint (e.g., XY, kinetic, or spectral) there will be an error evaluating the expression.

\section*{XY Data and Matrix Position Reference Examples}

When working with XY data (e.g., kinetic or spectral), it is useful to be able to reference calculation results from the previous matrices for further analyses.

\section*{Example 1}

For a kinetic data analysis, it might be useful to perform calculations only on data after a peak point has occurred. To achieve this, the first step would be to add an XY Reduction Peak Point transform that will compute the peak for each plot. To do this, use the default settings of this transform and set the result to be the \(x\) value at the peak for each position.

At this point we have two matrices, one Raw and one Calculated. The matrices (and their numbers) can be found under the Assay Properties | Matrices tab, e.g.,


The next step would be to add another transform that uses the computed value of each sample. For example, add the XY Reduction Total transform. This simply calculates the total of the measurements. To configure the transform to only consider measurements from the peak set the \(\mathbf{X}\) Minimum range option to:

\section*{\#2}
I.e.:


With this setting, the transforms will compute the total of the measurements for each sample but only from the peak point, e.g.:

Total


\section*{Example 2}

In another kinetic example, the time taken to reach half peak can be computed. In this case:
1. The XY Reduction Peak Point can be used to compute the peak, this time with the result being set to the \(y\) value of the peak.
2. The Expression by Matrix transform is used to compute the \(y\) value at half of the peak.
3. The \(X Y\) Reduction \(X\) at \(Y\) transform can be used to calculate the time \((x)\) when the halfpeak ( \(y\) ) is reached. In this case the Matrix Position Reference \#3 is used to reference matrix 3 (the half-peak) as the Cursor for the calculation.
I.e.:


\section*{\(X\) at \(Y\)}


\section*{Transform Output Variables}

The Transform Output Variable (TOV) feature enables a transform to expose certain calculated numerical results of the analysis as variables. TOVs can be used to reference specific quantities of interest for further analysis.
For example, if you wanted to validate that the calculated \(R^{2}\) value from a Standard Curve Fit transform was greater than a specific expected value, you could create a Validation condition that references the \(R^{2}\) value of the Standard Curve Fit transform. Using this feature you could validate that the \(R^{2}\) value is \(>0.9\) with the user being alerted if the fit is below an acceptable threshold.

\section*{TOV Definition}

Each TOV is a named variable with a numerical value that can be referenced in an expression. The following evaluated values are produced as TOVs by the Standard Curve Fit transform:
- All valid calculated coefficients
- All non-matrix data (quantification limits and sum of squares)
- All valid calculated goodness measures
- All valid calculated Standard Curve Fit Named Evaluations

Here "valid" means that result was computed successfully and has a value.

\section*{Referencing a TOV}

Transform Output Variables can be referenced using expressions for:
- Any transform evaluated after the transform that produced the output variable (only where the later transform supports parameters with expression)
- The Validation expression
- The Evaluation expression
- The Label expression
- The Fit Constraint expression
- The Custom fit expression

\section*{Identifying Available TOVs}

The available TOVs depend on your configured transforms. For each transform that produces TOVs, the available TOVs are added to the calculation log.

How to: Identify Available TOVs
1. Launch an assay protocol or open a results file.
2. Add and configure the transforms as required.
3. After the results were calculated, open Calculation log and scroll down to the end.
4. If the transform produces TOVs, review or copy the available TOVs from the list.

The syntax of the TOV as it appears in the Calculation log is as follows:

\section*{TOV added: TOV\{N\}.\{Name\}=\{Value\}}

Where:
\(\{\mathbf{N}\}\) - The number of transform in the sequence configured via Transform tab
\{Name\} - The name of a specific evaluated value
\{Value\} - The evaluated result of the TOV
When referenced in expression, a syntax is TOV\{N\}.\{Name\}, e.g., TOV1.SE.
You can only reference TOVs that originate from transforms that are defined and evaluated prior to where the reference is being made. For example, the TOV2.R2 could be referenced from the third transform onward, but not from the first. For validations, evaluations and labels, any TOV can be referenced as these elements are prepared after all transforms are computed.

\section*{TOVs and Multiple Plate or Multiplex Analyses}

For multiple container assays (such as multiple plate or multiplex) the reference to the TOV refers to the TOV output on the container it is associated with.

For example, TOV1.SE would refer to the SE value of the \(1^{\text {st }}\) transform on the container being evaluated.

\section*{Examples:}

Here are examples of TOVs along with descriptions of their behaviour.
\begin{tabular}{|l|l|}
\hline TOV & Refers to \\
\hline TOV1.ULOQ & \begin{tabular}{l} 
The ULOQ value of the curve-fit as calculated in the first trans- \\
form.
\end{tabular} \\
\hline TOV3.m & \begin{tabular}{l} 
The \(\mathbf{m}\) coefficient value of Linear Regression curve fit method as \\
calculated in the third transform.
\end{tabular} \\
\hline TOV1.SE & \begin{tabular}{l} 
The Standard Error value of the curve-fit as calculated in the first \\
transform.
\end{tabular} \\
\hline TOV1.SSC & The sum of squares computed from input \(y\) average and \(y^{\prime}\) \\
\hline \hline
\end{tabular}
\begin{tabular}{|l|l|}
\hline TOV & Refers to \\
& average (flagged points not included) \\
\hline TOV2.R2 & \begin{tabular}{l} 
The R \\
value of the curve-fit as calculated in the second \\
transform.
\end{tabular} \\
\hline
\end{tabular}

\section*{Sample References in Multiple Plate Assays}

This section describes how to refer to samples when defining calculations that will be performed across multiple plate assays.

MyAssays Desktop's expression system has been designed to make it easy to define calculations that apply to both single and multiple plates. Expressions can include references to sample groups.

For example, with the Expression by Matrix transform, the expression:

\section*{x-Blank 1}
can be used to subtract the average of the Blank1 samples from all other samples on the plate.

This is easy to follow for a single plate assay. However, it might not be clear how to define such a calculation when running a multiple plate assay with a blank group on each plate. In this case, it might seem necessary to define an expression for each plate - this would become quite tedious if your assay is ran across many plates. Thankfully, this is not the case.

\section*{Local-Group Sample Numbering}

For simplicity, MyAssays Desktop layouts and expressions use local-group numbering. This means that when you define the layout or refer to groups in an expression, the group numbers start at 1 for each plate.

Following previous example for a multiple plate assay with a single blank group on each plate, the layout definition would include Blank1 for each plate. Thus we can continue to use the expression:

\section*{\(x\)-Blank1}
to perform the blank correction on each and every plate. The software will subtract the average of the first blank group from all other samples on the same plate.

Regardless of how many plates you read, the same single expression can be used. If you are using more or less plates or need to change their order, there is no need to change your expression. This is sure to save your time, especially when compared to the equations you would need to use to configure this in Excel.

\section*{Global-Group Sample Numbering}

Once you've calculated your results, MyAssays Desktop uses global-group numbering. Results displayed on screen or in the report will be contiguous across plates. Referring back to the same example, with global-group numbering Blank1 is on the first plate and Blank2 is on the second plate, etc.

This distinction between using local-group numbering for layout definition and expressions and global-group numbering for results display is the key to simplifying how to define common mathematical operations that can be applied across multiple plates.

©
To learn more about group numbering in multiple plate assays, see Multiple Plate Group Numbering.

\section*{Referring to a Specific Sample on a Specific Plate}

To refer to a group on a specific plate, append the group reference with two dots followed by the plate number. For example, to refer to the Blank1 group on the first plate, use:

\section*{x-Blank1.. 1}

With this expression applied to the Expression by Matrix transform, every sample on every plate would be corrected by the Blank1 group measured on the first plate. In this case, it would only be necessary to perform a blank measurement on the first plate.
Referencing Samples on the Specific Plate and/or Matrix
You can also reference samples on the specific matrix and plate as follows:

\section*{[Reference].[Matrix].[Plate]}

Where:
- Reference - Sample type, group, or a position you want to refer to, e.g., Blank, Unknown1, A5.
- Matrix - The 1 -based sequential number of the matrix. Matrix 1 will always be the first set of measurement data. Depending on your assay, subsequent matrices might be further measurements or the results of any transforms.
To learn more about the matrix numbering, see Viewing Available Matrices and Configuring Their Settings.
- Plate - The plate number on the Measurements tab, in the Plate IDs subtab. Add All instead of a plate number to reference samples across all plates.

If you omit [Plate], then this will reference sample on the specific matrix of corresponding plate. For example, Blank1.2 refers to the first blank group on the second matrix of the plate being evaluated.


If your assay contains one matrix of data, and you configured the Expression by Matrix transform, then Blank1.2 refers to transform result for Blank1 sample.

The reference of transform results can be included in the expressions for any transform downward to the referenced one (only where the later transform supports parameters with expression), validation, evaluation, and labels.

To reference samples from a specific plate, append the [Reference] with .. and plate number, e.g., Blank1..1 refers to the first blank group of the selected matrix on the first plate.

\section*{Functions}

This section provides reference information for various functions available to perform calculations on data.

\section*{Syntax}

These take the form of a function name followed by a comma-separated list of values in brackets. The function data can refer to any operand type.
1. If the function refers to a single operand of any type:

\section*{function(operand)}
2. If the function refers to a list of operands:
- If operands do NOT include sample references: function(operand1,operand2,...,operandN)
- If operands include ONLY sample references: function([operand1,operand2,...,operandN])
- If operands include any operand type AND sample references:

\section*{function}
([samplereference1,samplereference2,...,samplereferenceN],operand1,opera nd2,...,operandN)
3. If the function requires vector operands:
- function(\{list of \(y s\},\{l i s t\) of \(x s\})\)
- function(\{(samplereference1),(samplereference2),...,(samplereferenceN)\}, \{operand1,operand2,...,operandN\})

\section*{Calculation Functions}

This section describes functions that calculate the result of mathematical operations on an argument or a list of arguments. Calculation functions use general syntax for functions and perform an operation on a list of 1 or more operands. The evaluation of these functions results in a single numeric value.
\begin{tabular}{|c|c|c|}
\hline Name & Description & Example \\
\hline abs & Returns the absolute value of argument. The absolute value of a non-negative argument is the argument itself. The absolute value of a negative argument is -1 times the argument. & \[
\begin{array}{r}
\text { abs }(-1.23) \\
=1.23 \\
\operatorname{abs}(1.23) \\
=1.23
\end{array}
\] \\
\hline average & Described in group-enabled functions. & \[
\begin{gathered}
\text { average(1,2) } \\
=1.5 \\
\text { average }(-3,0.25,5) \\
=0.75
\end{gathered}
\] \\
\hline auc & \begin{tabular}{l}
Syntax: auc \\
(method,xmin,xmax,number,coefficients...) \\
Calculates the area under the plotted 4 PL or 5PL curve using the sum of trapezia method. The area under the curve is the area between the curve and the \(x\)-axis. Areas below the \(x\)-axis are considered negative. \\
Input parameters: \\
- method - This is either 4PL or 5PL fit method. Specify within single or double quotes: "4PL" or '4PL'; "5PL" or '5PL'. \\
- xmin - The minimum numerical value of the area on the \(x\)-axis. \\
- xmax - The maximum numerical value of the area on the \(x\)-axis. \\
- number - The number of trapezium intervals for computing the area under the curve. Higher number of intervals gives more accurate results. We recommend to use value of 1000 .
\end{tabular} & ```
auc
('4PL',0.023,
22.727,1000,
12.648,2.499,0.151,
73.746)
    =1663.5
auc
("5PL",0.125,4,100,
0.226,1.956,2538,
1.648,2439570)
=1663.5
``` \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|}
\hline Name & Description & Example \\
\hline & \begin{tabular}{l}
- coefficients - The coefficients of the selected method that must be specified in the following order: \\
For 4PL: a, b, c, d \\
For 5PL: a, b, c, d, m \\
When calculating area under curve (AUC) for Dose-Response Curves, replace xmin with dosemin and xmax with dosemax context variables. These are set to the minimum and maximum numerical values in the Doses. For more information, see Advanced DoseResponse Curves Evaluations.
\end{tabular} & \\
\hline ceiling & \begin{tabular}{l}
Syntax: ceiling(argument,multiple) \\
Returns argument rounded up, away from zero, to the nearest multiple of significance. If it is an exact multiple, no rounding occurs. \\
Input parameters: \\
- argument - The value you want to round off. \\
- multiple - The multiple to which you want to round. \\
If both parameters are negative, the value is
rounded down, away from zero. If argument is negative, and multiple is positive, the value is rounded up towards zero.
\end{tabular} & \[
\begin{gathered}
\text { ceiling(1.23,0.05) } \\
=1.25 \\
\text { ceiling(-1.23,0.05) } \\
=-1.2
\end{gathered}
\] \\
\hline count & Described in group-enabled functions. & \[
\begin{aligned}
& \text { count(1,2,3) } \\
& =3 \\
& \text { count(- } \\
& \begin{array}{c}
2.44,2,5,98,6) \\
=5
\end{array} \\
& \hline
\end{aligned}
\] \\
\hline exp & \begin{tabular}{l}
Syntax: exp(argument) \\
Returns e raised to the power of the given number. It
\end{tabular} & \[
\exp (2)
\]
\[
\text { = } 7.389056 \ldots
\] \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|}
\hline Name & Description & Example \\
\hline & \begin{tabular}{l}
accepts only a single argument. For example, exp (A1+A2) but not \(\exp (\mathbf{A 1}, \mathbf{A} 2)\). \\
Input parameter: \\
- argument - The exponent applied to the base \(\mathbf{e}\).
\end{tabular} & \[
\begin{aligned}
& \exp (-1) \\
& \quad=0.367879 \ldots
\end{aligned}
\] \\
\hline floor & \begin{tabular}{l}
Syntax: floor(argument,multiple) \\
Returns argument rounded down, toward zero, to the nearest multiple of significance. \\
Input parameters: \\
- argument - The value you want to round off. \\
- multiple - The multiple to which you want to round. \\
With a positive argument, the value is rounded down toward zero. With a negative \\
(1) argument, the value is rounded down away from zero. If the argument is an exact multiple of multiple, no rounding occurs.
\end{tabular} & \[
\begin{array}{r}
\text { floor(1.23,0.05) } \\
=1.2 \\
\text { floor(-1.23,0.05) } \\
=-1.25
\end{array}
\] \\
\hline intercept & \begin{tabular}{l}
Calculates the point at which the least square's fit line will intersect the \(y\)-axis by using existing \(x\) values and \(y\) values. The intercept point is based on a best fit regression line plotted through the known \(x\) values (independent set of arguments) and known \(y\) values (dependent set of arguments). \\
Requires a vector of arguments as parameters. \\
The number of \(y\) and \(x\) operands is required to be the same and greater than 2 . In other words, \(\{y 1, y 2, y 3\}\) and \(\{x 1, x 2, x 3\} ;\{y 1, y 2, \ldots y N\}\) and \(\{x 1, x 2, \ldots x N\}\).
\end{tabular} & \[
\begin{gathered}
\text { intercept }(\{1,2\},\{3,2\}) \\
=4 \\
\text { intercept }(\{- \\
2.44,2,5,98,6\}, \\
\{0.1,0.2,0.3,0.4,0.5\}) \\
=-12.152
\end{gathered}
\] \\
\hline In & \begin{tabular}{l}
Returns the natural logarithm of an argument. Natural logarithms are based on the constant \(\mathbf{e}\) (2.718281828459...). The argument is supposed to be positive. \\
In is the inverse of the exp function.
\end{tabular} & \[
\begin{aligned}
& \ln (10) \\
& \quad=2.302585 \ldots \\
& \ln (1.23) \\
& \quad=0.207014 \ldots
\end{aligned}
\] \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|}
\hline Name & Description & Example \\
\hline \(\log\) & \begin{tabular}{l}
Syntax: log(argument,base) \\
Calculates the logarithm of an argument to the base that you specify. \\
Input parameters: \\
- argument - Positive real numerical value or expression for which you want the logarithm. \\
- base- Base of the logarithm. If the base is omitted, it defaults to 10 .
\end{tabular} & \[
\begin{aligned}
& \log (2,4) \\
& =0.5 \\
& \log (1.23,2) \\
& =0.298658 \ldots .
\end{aligned}
\] \\
\hline \(\log 10\) & Returns the base-10 logarithm of a positive argument. & \[
\begin{gathered}
\log 10(1.23) \\
=0.089905 \ldots \\
\log 10(0.1) \\
=-1
\end{gathered}
\] \\
\hline max & \begin{tabular}{l}
Returns the largest value in a set of values. \\
To determine the maximum of positions in a sample group or sample type, type in the argument it square brackets (i.e., max ([Unknown1])). Otherwise the reference is treated as the average of positions.
\end{tabular} & \[
\begin{gathered}
\max (1,2,3) \\
=3 \\
\max (-2.44,2,5,98,6) \\
=98
\end{gathered}
\] \\
\hline median & Described in group-enabled functions. & \[
\begin{gathered}
\text { median }(1,2,3,4) \\
=2.5 \\
\text { median(- } \\
2.44,2,5,98,6) \\
=5
\end{gathered}
\] \\
\hline min & Inversely to max function, returns the smallest number in a set of values. All requirements of the max apply to min. & \[
\begin{gathered}
\min (1,2,3) \\
=1 \\
\min (-2.44,2,5,98,6) \\
=-2.44
\end{gathered}
\] \\
\hline mod & \begin{tabular}{l}
Syntax: mod (number, divisor) \\
Returns the remainder after the number is divided by a divisor. The result has the same sign as the divisor.
\end{tabular} & \[
\begin{array}{r}
\bmod (\mathbf{2}, \mathbf{0 . 3}) \\
=0.2 \\
\bmod (\mathbf{1 . 2 3 , 5})
\end{array}
\] \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|}
\hline Name & Description & Example \\
\hline & \begin{tabular}{l}
Input parameters: \\
- number - The argument for which you want to find the remainder. \\
- divisor - The value to divide the number with.
\end{tabular} & \(=1.23\) \\
\hline pow & \begin{tabular}{l}
Syntax: power(argument, power) \\
Raises the argument to the given power. \\
Input parameters: \\
- argument - Numeric value or an expression to raise to a power. \\
- power - Exponent to raise power to.
\end{tabular} & \[
\begin{array}{r}
\text { power(2,3) } \\
=8 \\
\text { power(1.23,5) } \\
=2.815305 \ldots .
\end{array}
\] \\
\hline \multirow[t]{3}{*}{slope} & Returns the slope of the linear regression line through data points in \(x\) s and \(y\) s. The slope is the vertical distance divided by the horizontal distance between any two points on the line, which is the rate of change along the regression line. & \multirow[t]{2}{*}{\[
\]} \\
\hline & (1) Requires a vector of arguments as parameters. & \\
\hline & The number of \(y\) and \(x\) operands is required to be the same and greater than 2 (i.e., \(\{y 1, y 2, y 3\}\) and \(\{x 1, x 2, x 3\}\); \(\{y 1, y 2, \ldots y N\}\) and \(\{x 1, x 2, \ldots x N\})\). & \\
\hline \multirow{3}{*}{stdev} & \multirow{3}{*}{The same as sdin group-enabled functions.} & stdev(1,2,3) \\
\hline & & \[
\] \\
\hline & & \\
\hline \multirow{3}{*}{sum} & \multirow{3}{*}{Described in group-enabled functions.} & sum (1,2,3) \\
\hline & & \[
\begin{gathered}
=6 \\
\text { sum }(-2.44,2,5,98,6)
\end{gathered}
\] \\
\hline & & \\
\hline \multirow[b]{2}{*}{var} & \multirow[b]{2}{*}{Described in group-enabled functions.} & \(\operatorname{var}(1,2,6)\) \\
\hline & & \[
=7
\] \\
\hline
\end{tabular}
\begin{tabular}{|l|l|l|}
\hline Name & Description & Example \\
& & \(\operatorname{var}(-2.44,2,5,98,6)\) \\
& & \(=1829.47472\) \\
\hline
\end{tabular}

\section*{Conditional Functions}

This section describes Boolean logical and conditional operations.

\begin{abstract}
As with all expression elements, these functions can be used in conjunction with other expression operands, operators and functions. Where a sample reference is used as an input parameter to a conditional function, it refers to the average of the non-flagged data points.
\end{abstract}

\section*{Boolean Logical Functions}

These are particularly useful for defining validation, flagging, and label conditions.
\begin{tabular}{|c|c|c|}
\hline Name & Description & Example \\
\hline and & \begin{tabular}{l}
Syntax: and ( \(\mathbf{a}, \mathbf{b}\) ) \\
Tests a number of input conditions and returns 1 if all of the conditions evaluate to true, or 0 otherwise.
\end{tabular} & \begin{tabular}{l}
and \\
(Unknown>0,Unknown<10) \\
Tests if the mean of the Unknowns is within the range of 0 to 10 .
\end{tabular} \\
\hline or & \begin{tabular}{l}
Syntax: or(a,b) \\
Tests a number of input conditions and returns 1 if any of the conditions evaluate to true, or 0 otherwise.
\end{tabular} & \begin{tabular}{l}
\[
\operatorname{or}(x<5, x>10)
\] \\
Tests if the value of \(x\) is less than 5 or greater than 10.
\end{tabular} \\
\hline not & \begin{tabular}{l}
Syntax: not(a) \\
Returns a logical value that is the opposite of an input logical value or expression (i.e., returns 0 if the supplied argument is true and returns 1 if the supplied argument is false).
\end{tabular} & \begin{tabular}{l}
not(Blank > 1) \\
Tests if the mean of the Blanks is less than or equal to 1 .
\end{tabular} \\
\hline
\end{tabular}

\section*{Conditional Function}
\begin{tabular}{|c|c|c|}
\hline Name & Description & Example \\
\hline \multirow{3}{*}{if ifthenelse} & Syntax: ifthenelse(1,2,3) or if(1,2,3) & \multirow[b]{3}{*}{\begin{tabular}{l}
ifthenelse \\
(Unknown1>10,10,Unknown1) \\
if(Unknown \(1>10,10\), Unknown1) \\
If the value of Unknown1 is greater than 10 then the result is 10, otherwise the value equals Unknown1's value.
\end{tabular}} \\
\hline & (1) The if and ifthenelse perform the
same function. & \\
\hline & \begin{tabular}{l}
Requires 3 parameters: \\
1. The Boolean condition to evaluate (which will evaluate to true or false). \\
2. The result if the condition is true.
\end{tabular} & \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|}
\hline Name & Description & Example \\
\hline \multirow[t]{2}{*}{} & 3. The result if the condition is false. If the value of the first condition is true, then the result is the second argument otherwise the third. & ifthenelse(PosReplicate
\[
\left(0=1, x^{*} 0.5, x^{*} 0.125\right)
\] \\
\hline & When using the Expression by Matrix transform to define a calculation to perform on all samples, this can be used to concisely define different calculations to perform on different sample types or replicates by Position Functions to query the sample at each position. & \begin{tabular}{l}
if(PosReplicate
\[
\left(0=1, x^{*} 0.5, x^{*} 0.125\right)
\] \\
Where the sample is the first replicate the value is multiplied by a factor of \(1 / 2\) and other replicates by \(1 / 8\). (Here the position function PosReplicate() is used to reference the replicate number.)
\end{tabular} \\
\hline
\end{tabular}

\section*{Group-Enabled Functions}

This section describes functions that operate on list of values with an open-ended length.
Where a sample reference is used as a parameter to a group-enabled function, any sample group/type reference inside a Group-Enabled Function's parameter list refers to the nonflagged values of that group/type.

\section*{Syntax}

These functions are specified with the following syntaxes:
- function(Sample type or group reference) - If there is 1 input sample group/type reference.
- function([Sample type or group reference, Sample type or group reference, ... , Sample type or group reference]) - If there are 2 or more input sample group/type references.

When there are 2 or more sample group/type references inside a parameter list, insert each group reference inside square brackets (e.g., pcv
([Standard1,Unknown2]) to access the values of non-flagged data points.

The following table lists group-enabled functions in alphabetical order:
\begin{tabular}{|l|l|l|l|}
\hline Name & Description & \begin{tabular}{l} 
Minimum \\
number of \\
parameters
\end{tabular} & Example \\
average & The same as mean. & \begin{tabular}{l} 
Unknown1 \\
Returns the mean of all \\
replicates in the \\
Unknown1 sample. \\
average([Unknown, \\
Blank]) \\
Returns the mean of the \\
Unknowns and Blanks.
\end{tabular} \\
\hline choose & \begin{tabular}{l} 
Reference the value at the specified \\
position in a list. This can be used to \\
access a specific replicate within a \\
sample group.
\end{tabular} & 2 & choose(1,[Unknown1]) \\
\hline count & \begin{tabular}{l} 
Counts the number of non-flagged \\
values.
\end{tabular} & 1 & count(Unknown) \\
\hline \hline
\end{tabular}
\begin{tabular}{|c|c|c|c|}
\hline Name & Description & Minimum number of parameters & Example \\
\hline mad & Calculates the median absolute deviation of the non-flagged positions. The median absolute deviation is the median of the absolute values of the deviation of each observation from the mean of the variable. & 1 & \begin{tabular}{l}
mad(Control1) \\
Returns the median absolute deviation of all replicates in the Control1 sample. \\
mean([Unknown, Blank]) \\
Returns the median absolute deviation of the Unknowns and Blanks.
\end{tabular} \\
\hline mean & Calculates the mean of the non-flagged positions. & 1 & \begin{tabular}{l}
mean(Unknown1) \\
Returns the mean of all replicates in the Unknown1 sample. mean([Unknown, Blank]) \\
Returns the mean of the Unknowns and Blanks.
\end{tabular} \\
\hline median & Calculates the median of a finite list of non-flagged positions. If there is an even number of arguments in the set, then median calculates the average of the two positions in the middle. & 1 & \begin{tabular}{l}
median(Unknown1) \\
Returns the median of the Unknown1 replicates. \\
median([Standard2, \\
Unknown4]) \\
Returns the median replicate of Standard2 and Unknown4 samples.
\end{tabular} \\
\hline pcv & Calculates the percentage coefficient of variance (\%CV) of a finite list of nonflagged positions. & 2 & \begin{tabular}{l}
pcv(Unknown) \\
Calculates the \%CV of all Unknowns. \\
pcv([Unknown1, Unknown1.2]) \\
Calculates the \%CV of the
\end{tabular} \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|}
\hline Name & Description & Minimum number of parameters & Example \\
\hline & & & Unknown1 replicates in the first and the second matrix of data. \\
\hline rsd & Calculates the robust standard deviation of a finite list of non-flagged positions. The robust standard deviation is the median average deviation (mad) * 1.4826. & 2 & \begin{tabular}{l}
rsd(Standard2) \\
Calculates the robust standard deviation of the Standard2 sample replicates. \\
rsd([Unknown1, Unknown3]) \\
Calculates the robust standard deviation of the Unknown1 and Unknown3 sample replicates.
\end{tabular} \\
\hline sd & Calculates the standard deviation of a finite list of non-flagged positions. The standard deviation is a measure of how widely values are dispersed from the average value (the mean). & 2 & \begin{tabular}{l}
sd(Standard2) \\
Calculates the standard deviation of the Standard2 sample replicates. \\
sd([Unknown1, Unknown3]) \\
Calculates the standard deviation of the Unknown1 and Unknown3 sample replicates.
\end{tabular} \\
\hline se & Calculates the standard error of a finite list of non-flagged positions. & 2 & \begin{tabular}{l}
se(Unknown1) \\
Calculates the standard error of the Unknown1 sample replicates. \\
se([Unknown, Control]) \\
Calculates the standard
\end{tabular} \\
\hline
\end{tabular}
\begin{tabular}{|c|c|c|c|}
\hline Name & Description & Minimum number of parameters & Example \\
\hline & & & error of allUnknown and Control sample replicates. \\
\hline sum & Returns the total of all items in the finite list of non-flagged positions. & 1 & \begin{tabular}{l}
sum(Unknown) \\
Returns the sum of all samples of the Unknown type. \\
sum([Unknown1, \\
Unknown1.2, \\
Unknown1.3]) \\
Returns the sum of all replicates of the \\
Unknown1 samples on the containers from 1 to 3.
\end{tabular} \\
\hline var & Calculates the variance of a finite list of non-flagged positions. Variance is a measurement of the spread between positions in a data set. The variance measures how far each number in the set is from the mean. & 2 & \begin{tabular}{l}
var(Unknown4) \\
Calculates the variance of the replicates in the Unknown4 sample. var([Unknown1, Unknown1.2]) \\
Calculates the variance of the Unknown1 sample replicates in the first and the second container of data.
\end{tabular} \\
\hline
\end{tabular}

\section*{Example}

Let's assume that we have some endpoint measurement data for a \(12 \times 8\) microplate stored in the Raw matrix. In this example we would like to find medium value for Unknown1 and Unknown2 positions.

The example uses a layout with 16 Unknown sample groups, where Unknown1 at A1, A2 A3, A4, A5, and A6, Unknown2 at A7, A8, A9, A10, A11, A12, etc.). I.e.:


Here is some example raw data displayed to 3 decimal places:
Raw
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|c|c|c|}
\hline & \(\mathbf{1}\) & \(\mathbf{2}\) & \(\mathbf{3}\) & \(\mathbf{4}\) & \(\mathbf{5}\) & \(\mathbf{6}\) & \(\mathbf{7}\) & \(\mathbf{8}\) & \(\mathbf{9}\) & \(\mathbf{1 0}\) & \(\mathbf{1 1}\) & \(\mathbf{1 2}\) \\
\hline \(\mathbf{A}\) & 0.364 & 0.323 & 2.105 & 2.124 & 1.213 & 1.226 & 1.159 & 1.190 & 0.110 & 0.116 & 1.463 & 1.481 \\
\hline \(\mathbf{B}\) & 0.455 & 0.441 & 1.869 & 1.888 & 1.203 & 1.218 & 1.180 & 1.206 & 0.132 & 0.130 & 0.786 & 0.768 \\
\hline \(\mathbf{C}\) & 0.587 & 0.575 & 0.474 & 0.514 & 1.206 & 1.239 & 1.203 & 1.210 & 0.113 & 0.116 & 0.613 & 0.626 \\
\hline \(\mathbf{D}\) & 0.806 & 0.774 & 0.364 & 0.378 & 1.167 & 1.187 & 1.211 & 1.241 & 0.101 & 0.106 & 0.489 & 0.490 \\
\hline \(\mathbf{E}\) & 1.105 & 1.065 & 0.279 & 0.367 & 1.215 & 1.225 & 1.233 & 1.262 & 0.111 & 0.114 & 1.579 & 1.549 \\
\hline \(\mathbf{F}\) & 1.425 & 1.445 & 0.378 & 0.411 & 1.196 & 1.206 & 1.226 & 1.199 & 0.109 & 0.108 & 0.792 & 0.775 \\
\hline \(\mathbf{G}\) & 1.791 & 1.803 & 0.560 & 0.597 & 1.205 & 1.228 & 1.308 & 1.306 & 0.126 & 0.127 & 0.652 & 0.659 \\
\hline \(\mathbf{H}\) & 0.253 & 0.270 & 0.463 & 0.470 & 1.214 & 1.261 & 0.115 & 0.116 & 0.115 & 0.115 & 0.490 & 0.477 \\
\hline
\end{tabular}

We use the expression:

\section*{median([Unknown1,Unknown2])}

Unknown1 and Unknown2 refer to the lists of non-flagged data points for the Unknown1 and Unknown2 sample groups. From this list the median is calculated.

The result will be (also displayed here to 3 decimal places):

\section*{Layout Analysis Functions}

Layout Analysis Functions are available only if the evaluation is associated with a layout. Otherwise an error message will be displayed.

\section*{Position Functions}

Position Functions are only available when there is a position associated with the expression being evaluated. They do not use input parameters.

Position functions can be used in conjunction with conditional function in the
Expression by Matrix transform to perform different calculations on different sample types, positions, or replicates.

The following table lists the position functions:
\begin{tabular}{|c|c|c|}
\hline Name & Description & Example \\
\hline PosValueByIndex & \begin{tabular}{l}
Syntax: PosValueByIndex \\
(PositionNum) \\
Returns the value at the specified (1-based) position number (in accordance with position numbering). \\
The valid range of PositionNum depends on the layout. For a \(12 \times 8\) plate this will be 1-96. For rack layouts, this depends on the number of samples measured.
\end{tabular} & \begin{tabular}{l}
PosValueByIndex (1) \\
Value of position A1 \\
PosValueByIndex (13) \\
Value of position B1 (on a 12x8 layout) \\
PosValueByIndex (PosNum()+2) Value at the position two to the right of the current position.
\end{tabular} \\
\hline PosNum & \begin{tabular}{l}
Syntax: PosNum() \\
Returns the 1-based position number of each position, which is assigned in accordance with position numbering.
\end{tabular} & \begin{tabular}{l}
ifthenelse(mod (PosNum(),2)=0,x,0) \\
Sets the values of odd positions to zero.
\end{tabular} \\
\hline PosGroup & \begin{tabular}{l}
Syntax: PosGroup() \\
Returns the 1-based group number of the sample group at the position. For example, if the position contains Unknown3, then the group is 3 .
\end{tabular} & \begin{tabular}{l}
ifthenelse(PosGroup
\[
\left(0=1,2^{*} x, x\right)
\] \\
Doubles the values of the first replicates in each sample group.
\end{tabular} \\
\hline PosType & \begin{tabular}{l}
Syntax: PosType() \\
Returns the sample type number associated with the position.
\end{tabular} & \begin{tabular}{l}
ifthenelse(PosType \\
()=LayoutType \\
("Blank"),0,x) \\
Sets the values of all
\end{tabular} \\
\hline
\end{tabular}
\begin{tabular}{|l|l|l|l|}
\hline Name & \begin{tabular}{l} 
Description \\
This can be used in conjunction \\
with the LayoutType function to \\
compare the associated position \\
with a particular named sample \\
type.
\end{tabular} & Blank positions to 0.
\end{tabular}

\section*{Layout Functions}

These functions require an associated layout.

\section*{Layout Functions Parameters}

\section*{PositionNum}

To simplify performing analyses based on sample positions, numerical position references are used in various functions. Positions are numbered from 1, left-to-right row-wise. For example, on a \(12 \times 8\) microplate layout, position number 1 is well A 1 , position 2 is well A 2 and B 1 is position number 13.

For rack-based layouts the same numbering convention is used with the upper limit being dependant on the number of samples measured.

\section*{Type}

Type can be specified as a sample type number or case-insensitive sample type name in quotes:
- LayoutNumGroups(3)
- LayoutNumGroups("Blank")
- LayoutNumGroups("blank")
- LayoutNumGroups("bLaNK")
(1) "Unused" type is not supported.

\section*{ContainerNum}

Where a layout function accepts the ContainerNum argument:
- If ContainerNum is 0 (or not specified), then the function will be evaluated for all containers in the layout.
- If ContainerNum is specified (as a 1-based container number), then only the layout for that container is considered.

\section*{GroupNum}

This is the 1-based group number of a sample group.

\section*{Layout Evaluation Functions}

Layout Evaluation functions enable sample group references to be more flexible (e.g., for applying calculations on group number references).
\begin{tabular}{|c|l|l|}
\hline Name & Description & Example \\
GroupValue & \begin{tabular}{l} 
Syntax: GroupValue(Type, GroupNum) \\
Returns the value of the sample group with \\
the specified type and group number.
\end{tabular} & \begin{tabular}{l} 
GroupValue \\
("Unknown",1) \\
The value of the group \\
that is Unknown1.
\end{tabular} \\
\hline
\end{tabular}

The GroupValue function can be used in conjunction with PosGroup() to obtain the group of a specified type that matches the group number of the position being evaluated. For example, if you want to reference the Unknown group that has the same number as the current position, use: GroupValue("Unknown", PosGroup ())

The following table lists the layout functions in alphabetical order:
\begin{tabular}{|l|l|}
\hline Name & Description \\
\hline LayoutCommonNumReplicatesType: LayoutCommonNumReplicatesType & \begin{tabular}{l} 
Syntax: \\
(Type) \\
If all groups of the specified Type on all containers \\
have the same number of replicates, then it returns \\
that number. Otherwise -1 is returned. \\
For example:
\end{tabular} \\
\hline \hline
\end{tabular}
\begin{tabular}{|c|c|}
\hline Name & Description \\
\hline & \begin{tabular}{l}
LayoutCommonNumReplicatesType ("Unknown") \\
Tests if all Unknown sample groups on all containers have the same number of replicates. Returns this number if it is the same; otherwise, returns -1 .
\end{tabular} \\
\hline LayoutHeight & \begin{tabular}{l}
Syntax: LayoutHeight() \\
The number of rows in the layout (note, this is same value for all containers). \\
It is useful for specifying column-based expressions.
\end{tabular} \\
\hline LayoutNumContainers & \begin{tabular}{l}
Syntax: LayoutNumContainers() \\
The number of containers in the layout. \\
It is useful for specifying expressions for a certain container of data.
\end{tabular} \\
\hline LayoutNumGroups & \begin{tabular}{l}
Syntax: LayoutNumGroups \\
(Type, ContainerNum) \\
The number of groups with the specified type. \\
For example: \\
LayoutNumGroups("Blank", 1) \\
Returns the number of Blank sample group on the \(1{ }^{\text {st }}\) container.
\end{tabular} \\
\hline LayoutNumGroupsAcrossContainers & \begin{tabular}{l}
Syntax: LayoutNumGroupsAcrossContainers (Type) \\
Determines the number of groups of the specified Type parameter on each of the containers. If this is the same number on every container (including 0 ), then this value is returned. Otherwise - 1 is returned. \\
For example: \\
LayoutNumGroupsAcrossContainers ("Unknown") \\
Tests if there is the same number of Unknown
\end{tabular} \\
\hline
\end{tabular}
\begin{tabular}{|c|c|}
\hline Name & Description \\
\hline & sample groups on all containers. \\
\hline LayoutNumPositions & \begin{tabular}{l}
Syntax: LayoutNumPositions \\
(Type, GroupNum, ContainerNum) \\
The number of positions with the specified type and group number. \\
For example: \\
LayoutNumPositions("Standard", 3, 1) \\
Returns the number of Standard3 replicates on the \(1^{\text {st }}\) container.
\end{tabular} \\
\hline LayoutNumUsedPositions & \begin{tabular}{l}
Syntax: LayoutNumUsedPositions (ContainerNum) \\
The number of positions that have a type that is not Unused (i.e., Type \(\neq 1\) ). \\
For example: \\
LayoutNumUsedPositions(2) \\
For the 2nd container, returns the number of positions that are not Unused.
\end{tabular} \\
\hline LayoutPositions & \begin{tabular}{l}
Syntax: LayoutPositions() \\
The total number of positions (note, this is same value for all containers). \\
It is useful for specifying position-based expressions.
\end{tabular} \\
\hline \multirow{4}{*}{LayoutType} & \begin{tabular}{l}
Syntax: LayoutType(Type) \\
The sample type number that represents this type.
\end{tabular} \\
\hline & Each sample type is identified by a unique integer value. \\
\hline & For example: \\
\hline & \begin{tabular}{l}
LayoutType("Blank") \\
Is useful for writing expression-based conditions for a particular type.
\end{tabular} \\
\hline LayoutWidth & Syntax: LayoutWidth() \\
\hline
\end{tabular}
\begin{tabular}{|l|l|}
\hline Name & Description \\
& The number of columns in the layout (note, this is \\
same value for all containers). \\
It is useful for specifying row-based expressions \\
(e.g., By-Row Blank Correction).
\end{tabular}

\section*{Probability Functions}

This section describes syntax and usage of probability functions in MyAssays Desktop.

\section*{Overview}

The probability functions can be used to examine variability between two sets of data.

\section*{Syntax and Parameters}
\begin{tabular}{|c|c|c|}
\hline Name & Description & Example \\
\hline f.dist & \begin{tabular}{l}
Syntax:f.dist(x,df1,df2,Cumulative) \\
Evaluates the value of F probability distribution (degrees of diversity) for two data sets. \\
Requires the following parameters: \\
- \(\boldsymbol{x}\) - Positive numerical value at which to evaluate the function \\
- df1 - An integer numerical value or an expression (with integer numeric result), that specifies numerator degrees of freedom. \\
- df2 - An integer numerical value or an expression (with integer numeric result), that specifies denominator degrees of freedom. \\
- Cumulative - A logical value that determines the form of the function. If set to true, then f.dist returns the cumulative distribution ; if false, it returns the probability density function.
\end{tabular} & \begin{tabular}{l}
f.dist \\
(5.7,5,10,true) \\
Calculates a cumulative F probability for 5.7 with degrees of freedom specified as 5 and 10 .
\end{tabular} \\
\hline f.inv & \begin{tabular}{l}
Syntax:f.inv(p,df1,df2) \\
Returns the inverse of the F probability distribution (the inverse of the \(\mathbf{f d i s t}\) ) for a given probability. \\
Requires 3 parameters: \\
- \(\mathbf{p}\) - A probability (between 0 and 1 ) at which to evaluate the inverse of the fdist. \\
- df1 - An integer numerical value or an expression (with integer numeric result), that specifies numerator degrees of freedom. \\
- df2 - An integer numerical value or an expression (with
\end{tabular} & \begin{tabular}{l}
f.inv(0.9,6,96) \\
Finds a numerical value for which the F probability equals 0.9 with degrees of freedom specified as 6 and 96 .
\end{tabular} \\
\hline
\end{tabular}
\begin{tabular}{|l|l|l|}
\hline Name & \begin{tabular}{l} 
Description \\
\begin{tabular}{l} 
integer numeric result), that specifies denominator degrees \\
of freedom.
\end{tabular}
\end{tabular} & Example \\
\hline
\end{tabular}

Typically, when specifying degrees of freedom with an expression, you may find the Layout Analysis Functions in conjunction with count function useful to refer to the number of nonflagged positions of a certain sample type.

\section*{Example Expressions}
\begin{tabular}{|l|l|}
\hline Expression & \begin{tabular}{l} 
Evaluated to \\
f.inv(0.95,2,10)
\end{tabular} \\
\hline \begin{tabular}{l} 
A numerical value for which the F probability \\
equals 0.95 and degrees of freedom specified as 2 \\
and 10.
\end{tabular} \\
\hline \begin{tabular}{l} 
f.inv(0.95,LayoutNumGroups \\
([Standard"),count
\end{tabular} & \begin{tabular}{l} 
A numerical value for which the F probability \\
equals 0.95 and degrees of freedom specified as \\
the number of Standards and the number of all \\
non-flagged Standard and Unknown samples' \\
replicates.
\end{tabular} \\
\hline f.dist(0.5667,2,10,false) & \begin{tabular}{l} 
The F probability density value for 0.5667 and \\
degrees of freedom specified as 2 and 10.
\end{tabular} \\
\hline \begin{tabular}{l} 
f.dist(0.5667,count([Stand- \\
ard,Test]),LayoutNumGroups("Stand- \\
ard"),true)
\end{tabular} & \begin{tabular}{l} 
The cumulative F distribution value for 0.5667. The \\
degrees of freedom here are specified as the num- \\
ber of all non-flagged Standard and Unknown \\
samples' replicates and the number of \\
Standards.
\end{tabular} \\
\hline f.dist(Lay- \\
outCommonNumReplicatesType \\
("Standard")* \\
(tov1.ssr),LayoutNumGroups("Stand- \\
ard"),count([Stand- \\
ard,Unknown]),true) & \begin{tabular}{l} 
The cumulative F distribution value for the num- \\
ber of Standard replicates multiplied by the Sum \\
of Squares TOV from the first configured trans- \\
form. The degrees of freedom are specified as \\
number of Standards and the number of all non- \\
flagged Standard and Unknown samples' rep- \\
licates.
\end{tabular} \\
\hline
\end{tabular}

\section*{Evaluating Z Factor/Z Prime (Z')}

Z Factor/Z Prime (Z') (as defined by: Zhang JH, Chung TD, Oldenburg
KR, A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. J Biomol Screen. 1999;4(2):67-73) is used to assess quality of:
- HTS assay
- Population of tested sample compounds

The commonly used equation is
\(Z=1-\frac{3 S D \text { of sample }+3 S D \text { of control }}{\mid \text { mean of sample }- \text { mean of control } \mid}\)
It represents how well separated the positive and negative controls are, and indicates whether an assay is likely to contain false positives/negatives.

This statistic can be added to an assay using the Evaluation option with the expression:
1-((3*sd(Neg Control) +3*sd(Pos Control))/abs(Neg Control-Pos Control))
Where Neg Control and Pos Control are the sample/control groups to test.

\section*{Integration with Readers}

\section*{EnSight (Kaleido) Integration}

The latest documentation relating to working with MyAssays Desktop Pro and EnSight (via Kaleido software):

\section*{Kaleido 3.0}

MyAssays Desktop Pro for Kaleido 3.0 (Installation Notes).pdf
Using MyAssays Desktop with Kaleido 3.0.pdf
Video tutorials for using MyAssays Desktop and Folder Poll with Kaleido 3.0:
https://www.myassays.com/introduction-to-kaleido-3-0-and-myassays-desktop.video https://www.myassays.com/kaleido-3-0-data-export-and-import-into-myassaysdesktop.video
https://www.myassays.com/create-a-new-protocol-with-kaleido-3-0-xml-data-in-myassaysdesktop.video
https://www.myassays.com/kaleido-3-0-data-analysis-automation-with-myassaysdesktop.video

\section*{Kaleido 2.0}

MAD R3.2 and Folder Poll Configuration for Kaleido 2.0.pdf
Video tutorials for using MyAssays Desktop and Folder Poll with Kaleido 2.0:
https://www.myassays.com/folder-poll-with-myassays-desktop-pro-and-kaleido-2-0-(1-of2).video
https://www.myassays.com/folder-poll-with-myassays-desktop-pro-and-kaleido-2-0-(2-of2).video

\section*{EnVision Integration}

The EnVision manager software can output data in many possible export configurations. If using EnVision Manager v14.xxx or later, then select Export format option as CSV for MyAssays Desktop option:


To configure earlier EnVision Manager versions to output data in a format compatible with MyAssays Desktop EnVision import scripts, use the default CSV Export format: Plate 2 (with well headers) option, e.g.:


\section*{VICTOR Manager Integration with MyAssays Desktop}

\section*{Overview}

This topic describes how to configure the PerkinElmer VICTOR manager software to automatically launch a MyAssays Desktop protocol for analysis of the measured data.

If you are working with the newer VICTOR Nivo reader, see the VICTOR Nivo Integration topic.

With this configuration in place, data analysis can be performed automatically as soon as VICTOR measurements are completed with little or no user intervention is required. In addition, results can be automatically saved/printed/exported as required to provide a seamless integrated measurement and analysis solution.

\section*{Terminology}

Between the separate VICTOR manager software and MyAssays Desktop software, there is some overlap in terms used. Both systems use the term "protocol", for clarity in this section we refer to a VICTOR protocol as the VICTOR measurements protocol and a MyAssays Desktop protocol as the MyAssays Desktop protocol.

\section*{Integration Workflow}

The general workflow is to:
1. Use the VICTOR manager software to create and configure the required measurement settings (the VICTOR measurements protocol).
2. Export the measurement data to a TXT file.
3. Create a MyAssays Desktop protocol to perform the required analysis.
4. Configure the VICTOR measurements protocol to launch the MyAssays Desktop along with the MyAssays Desktop protocol when the measurements are completed and provide the path to the exported TXT file (using the command line).

The creation of a VICTOR measurement protocol and a MyAssays Desktop protocol are not covered here. It is assumed that you already have these in place and are ready to integrate them.

\section*{MyAssays Analysis Launcher}

MyAssays Desktop data analysis can be initiated from the command line. MyAssays Desktop offers many options and settings for complete control over the data analysis process.

Writing command line arguments is typically a complicated error-prone process. To simplify this task, MyAssays Desktop includes the MyAssays Analysis Launcher tool. This provides a point-and-click user interface for building a command line to launch MyAssays Desktop analysis. The resulting command line argument is displayed within the tool and can be easily copied and pasted into other applications.
To get started, we recommend familiarising yourself with the MyAssays Analysis Launcher tool. This can be found in MyAssays Desktop Explorer under the Tools tab. Select the

\section*{Analysis Launcher option to launch it.}

\section*{Using Analysis Launcher}

To use Analysis Launcher, select a MyAssays Desktop protocol file to launch and use the user interface to configure the operations required.

If you have a compatible VICTOR TXT file to hand, use the Import Measurements option and select the file.

If you would like the results to be calculated automatically, select the Calculate option.
Notice, in the lower part of the Analysis Launcher the tool that as settings are made the command line argument is updated accordingly.

To launch the analysis, click Launch.

\section*{Example Integration}

This section describes how to modify a VICTOR measurement protocol to launch MyAssays Desktop:
1. Launch the PerkinElmer VICTOR Manager Explorer.
2. Select and open the VICTOR measurements protocol to edit.

You should see a window displaying the measurement configuration settings, similar to this:

3. Select the Outputs and Events tab
4. Select the File output check box

\section*{5. Select File type as Text}
6. Specify the Filename as required (this could be made up of the various macros)

For example, in this configuration the file is output to the Documents folder with a name created from the Protocol Name and Run ID:


With this setting in place, the results are exported to a text file when measurements are completed. You can try this now (perhaps with an empty plate) to check that the settings are expected.

You can then test importing this data into a MyAssays Desktop protocol "manually" by launching the MyAssays Desktop protocol and using the Import Wizard to import the text file.

The final part is to configure the VICTOR measurement protocol's Assay end system command setting. This is used to issue the command line.
1. Return to the VICTOR measurements protocol editor.
2. In the first edit box, enter the full path to the MyAssays Desktop Analysis exe, this is typically:

\section*{C:\Program Files\MyAssays \(\backslash M y\) Assays Desktop\MyAssays.Desktop.Analysis.exe}

If you have installed MyAssays Desktop to another location, then you will need to enter the correct path. If you are not, use the MyAssays Analysis Launcher application and inspect the command line:

\footnotetext{
"C:\Program Files (x86)\MyAssays\MyAssays Desktop\MyAssays.Desktop.Analysis.exe" "C:\Users\Darren Cook\Documents\MyAssays\Protocols\My Endpoint 12x8 Protocol (1).assay-protocol"
}
3. In the second edit box, enter the MyAssays Desktop protocol filename to use followed by:

\section*{/import-raw:<OutputFileName>}

This setting passes the filename generated by the VICTOR manager software to MyAssays Desktop so that the new measurement data file can be imported. The <OutputFileName> is a macro used by VICTOR manager software to refer to the new measurement data file.

For example, to use the MyAssays Desktop protocol: My Endpoint 12x8
Protocol (1) in my documents folder I use:
"C:\Users\Darren Cook\Documents\MyAssays\Protocols\My Endpoint 12x8 Protocol (1).assay-protocol" /importraw:<OutputFileName>

This would appear in the VICTOR measurements protocol editor as follows:
-Assay end system command

C:\Program Files \MyAssays\MyAssays Desktop\MyAssays.Desktop.Analysis.exe
Protocols\My Endpoint 12×8 Protocol (1).assay-protocol" /import-raw:<OutputFileName>
This command will be pefformed when the instrument has finished measuring the assay using the edited protocol.
4. Save these changes to the VICTOR measurements protocol.

Similarly, if you are not sure, use the MyAssays Analysis Launcher application and inspect the command line.

With this setting in place, the results will be exported to a text file when measurements are completed and then imported into MyAssays Desktop. You can try this now (perhaps with an empty plate) to check that the integration is working.

With these basic settings, the calculations are automatically performed. This gives the user an opportunity to review the settings and perhaps make some changes (such as a different layout or marking outliers to exclude) before pressing the Calculate button.

If you would like calculations to be performed automatically, add:

\section*{/calc}

To the end of the command line argument in the VICTOR Assay end system command, e.g.:
-Assay end system command

C:\Program Files\MyAssays\MyAssays Desktop\MyAssays.Desktop.Analysis.exe ... गls\My Endpoint 12x8 Protocol (1).assay-protocol" /import-raw:<OutputFileName> |calc

This command will be pefformed when the instrument has finished measuring the assay using the edited protocol.

You can also add further command line arguments to automatically print, export, etc. Use the Analysis Launcher tool or refer to the Command Line Arguments section for more information.

\section*{VICTOR Nivo Integration}

To process the readings produced by the Victor Nivo reader, we suggest exporting data in the .XLSX format. Since VICTOR Nivo reader control software is a browser-based application, use your browser to configure readings.

MyAssays Desktop supports automatic import of VICTOR Nivo 2.5.0 XLSX files (and most files from v2.0.2). The import includes:

\section*{- Measurements data}

The readings output by the VICTOR Nivo reader control software. This also includes two technologies combined in a single protocol and data read from partial plates.

\section*{- Metadata}

Any information included in the Parameters worksheet of your Victor Nivo XLSX file. You can include this information in the report via Measurement Meta Data report element.
```

If you are working with the older VICTOR Manager software, see VICTOR Manager
Integration with MyAssays Desktop.

```

\section*{Importing Data from VICTOR Nivo 2.5.0 XLSX Files}

The integration includes automatic processing of:
- New <INTEGER><SPACE>OPERATION naming convention.
- List and plate formats for kinetic measurements.
- Export of two technologies, such as Abs Kinetics and FI Endpoint, combined in one protocol in a single Excel sheet one below the other.
The previous version (v2.0.2) exported each of them to a different sheet.
- Data measured on partial plates.

If the imported VICTOR Nivo data contains positions that were not measured, then these values are imported as excluded and will not be included in the data analysis. Endpoint empty cells are indicated and reported with a dash "-"; not measured XY cells are simply not included.

\section*{Automating Import of VICTOR Nivo Files}

You can use Folder Poll to automatically launch MyAssays Desktop protocol as soon as a new .XLSX file has been saved to a folder. The easiest way to configure the Folder Poll integration is using MyAssays Desktop; MyAssays Desktop can automatically configure folder polls
directly from your MyAssays Desktop protocol. For a video walk-through, see Folder Poll with MyAssays Desktop.

A folder poll can be created either using MyAssays Desktop when creating new protocol, importing data into existing protocol, or via the Folder Poll Manager. Refer to Folder Poll User's Guide to learn more.

If you are using the Enhanced Security edition, you can automate the integration using the Autologin account. For a detailed description of this feature, see Configuring Autologin Account.

\section*{Unsupported Imports}
- Scan well area measurements are not currently supported.
- The unit values are not automatically imported.

You can add the output units later when configuring the transform settings.

\section*{WIZARD \({ }^{2}\) Integration}

MyAssays Desktop Pro provides first class data analysis tools that can be integrated directly with the WIZARD² gamma counter. MyAssays Desktop Pro can be installed and used on the WIZARD²'s own PC for a seamless integration with the counting process. In this way, data analysis can be performed automatically each time counting is completed. Data analysis results can be printed, exported and archived automatically with no user-intervention required after counting.
Also, MyAssays Desktop Pro can be installed and used on a PC separate from the WIZARD². Perhaps at a PC in your office and away from the lab. In this way you can build data analysis protocols and process results at the comfort of your own desk, freeing up access to the WIZARD \({ }^{2}\).

If you are working in a 21CFR11 or GxP environment the Enhanced Security functions provide a secure and tracked integration when working with the WIZARD².

For a walk-through of working with WIZARD \({ }^{2}\) please review these video resources:
- WIZARD \({ }^{2}\) Gamma Counter Integration
- WIZARD \({ }^{2}\) Gamma Counter Integration with Enhanced Security
- WIZARD \({ }^{2}\) Gamma Counter Data Import Support (CSV and XML)

\section*{WIZARD \({ }^{2}\) Data Import}

The WIZARD \({ }^{2}\) manager software can output measurement data (including as CPM) in either CSV or XML format. The XML format is recommended because it provides a richer set of data including:

\section*{- Metadata}

Any metadata associated with the imported measurements. It can then be included in the report via Measurement Meta Data report element.

\section*{- Field Data}

All associated textual or numerical data is automatically imported and stored alongside measurements. See Adding Field Data Alongside Measurements for more details.

\section*{- Spectrum Channel Counts}
- Sample Bar Code

\section*{Importing XML Data from WIZARD \({ }^{2}\)}

You can select which XML data to import into MyAssays Desktop:
```

Custom
WIZARD 2
WIZARD }\mp@subsup{}{}{2}\mathrm{ (CPM)
WIZARD }\mp@subsup{}{}{2}\mathrm{ (Spectral and CPM)

```

The resulting protocol will import only the selected data items.
If you select the All Data option for the measurement files that contain more than 10 matrices of data, MyAssays Desktop will include only the first matrix of the imported data in the report's Sample Table. In this case, MyAssays Desktop will notify you accordingly:


\section*{Including Additional Matrices in the Sample Table}

All of the imported data will be available for analyses and processing. Using Matrices tab of the Assay Properties panel, you can select the matrices of data to include in the Sample Tables. Choose the matrix to include and set the Include in Sample Tables check box:


To learn more about the applicable settings, see Viewing Available Matrices and Configuring Their Settings.

\section*{Unsupported Data Imports}

MyAssays Desktop WIZARD \({ }^{2}\) XML Import does not support importing XML files that contain measurements with:
- Repeat values > 1 .

Only measurements where Repeat="1" will be imported.
- Combination of rack sizes.

\section*{XSLT}

\section*{How to Use XSLT for Custom Export}

In most cases, you will probably export your reports in one of the provided formats. For special circumstances (such as a specific output for a LIMS system), you may need to save the results of your data analysis in another file format, with specific column or decimal separators, or both. For these purposes, you can use XSLT export settings.

\section*{XSLT and Report Files}

When you apply XSLT to transform the report, it converts the report.xml file. This XML file is used to define how to generate the actual report file with the exported data.

For example, the Txt (tab column separator, period decimal separator).xslt transform will convert the report to a text file format as described, whereas the same report could be transformed to HTML using the Html Tables.xslt format.

For more information about XSLT, see https://www.w3.org/TR/xslt-10.
MyAssays Desktop currently only supports XSLT \(\mathbf{1 . 0}\) format.

\section*{Applying XSLT File to Report}

MyAssays Desktop provides some example XSLT files at MyAssays \XSLT. These are already used for some of the predefined export options but you can apply them using the Export Wizard. By using these files, you can specify both the XSLT transformation and the file format your laboratory works with.

\section*{How to Apply XSLT File to Report}
1. Open a results file.
2. On the Home ribbon tab, in the Data group, select Export.

This launches Export Wizard.
3. Select Custom export format from the list.
4. Select XML Transform (via XSLT 1.0).
5. Select a destination to export the report to (optionally using macros).
6. Select a file extension from the drop-down or type another file extension to export your file with.
XSLT does not imply any particular file extension because the output file may be of any type.
7. Specify the XSLT file to transform your report with.

For example, select a predefined file from MyAssays \(\backslash\) XSLT.
If you select Copy XSLT file to protocol, then the selected XSLT file will bestored inside the protocol. It will be available as Protocol Content option on the next runs of Export Wizard even if you will transfer the assay to another source.

Optionally select how to preview the output file from the Preview Transformed Export drop-down.
8. To apply the selected export settings on the future runs, select Configure protocol to automatically export.
To launch the results with the default associated application after each calculation, select Open with default viewer.
9. Click Finish.

The Export Wizard is available only in the results mode. If you want to save the export configuration to an assay-protocol, then after you configured the export apply Save command for this operation.

\section*{Creating Custom XSLT Files}

XSLT converts the report.xml to generate the final output. So, if you want to develop your own XSLT, note that actual content of the report.xml depends on the assay protocol configuration.

We recommend making sure that the protocol you want to transform is complete and you do not plan to change it significantly (e.g., change order or add transforms, add other report elements, etc.). Major changes to the protocol can impact the report.xml, which, in turn, may impact the XSLT transform.

To get started with building your own XSLT file, first generate the report.xml file as follows:
1. Launch an assay protocol, review or edit its settings, and then click Calculate to generate results.
2. On the Home ribbon tab, in the Data group, select Export. This launches Export Wizard.
3. Select Custom export format from the list and then select XML.
4. Complete the wizard to generate the report.xml content for the data.

It is possible to use the existing XSLT files stored in your MyAssays/XSLT folder as a starting point. Copy and paste their content to a new file to experiment and tweak the provided examples. Then you also can apply your XSLT via Export Wizard to test your XSLT files.

To develop XSLT solutions for more demanding requirements, contact support@myassays.com.

\section*{Using Other Scripting Tools}

If you prefer to use other scripting tools instead of XSLT, then:
1. Export a report.

Generate a report.xml file or one of the other formats such as TXT.
2. Launch a scripting tool and convert the exported report with it.

You can automate this by launching MAA from command line with the export parameter and launching the report with your scripting tool.

For further automation of this process, use Folder Poll.

\section*{XSLT Encoding}

When exporting via XSLT, the output encoding can be changed within each XSLT file or using a global setting to apply to all export files. Values set in XSLT will override the global value.
- XSLT encoding can be specified by adding an encoding attribute of the XSLT file itself, e.g. <xsl:output encoding="ISO-8859-1"/>
- To apply globally, use DefaultEncoding attribute in ews.config.xml file (under Settings), e.g. < ExportWizardSettings xmlns:xsi = "http://www.w3.org/2001/XMLSchema-instance" xmlns:xsd="http://www.w3.org/2001/XMLSchema" DefaultEncoding="Windows-1252">

Common encoding values include:
- ISO-8859-1
- UTF-8
- UTF-16
- UTF-32
- ASCII
- US-ASCII
- Windows-1252

\section*{Calculation Log}

The Calculation Log stores all details of the calculations performed.

\section*{How to: Open Calculation Log}
1. Launch an assay protocol or open a results file.
2. Press the Logs button to open Logs panel with Calculation Log tab.
3. Review as required.

\section*{Logged Calculations}

The content included in the Calculation Log depends on the configuration of each of the following settings:
- Transforms - Run and results of each transform (in the sequence they are configured).
- Evaluation - Run and results of the computed evaluations.
- Validation - Run and results (pass or fail) of the computed validations.
- Transform Output Variables - Non-matrix calculations produced by the transforms

Additionally, it might also produce fit transform context parameters and a variety of transform-specific calculations. For example, if a Standard Curve Fit transform is configured, then the log also contains coefficient constraints, weighting infinity resolution, custom flag label for Standard Curve Fit and more.

\section*{Level of Details}

The Calculation Log can be viewed in the following modes:
- Detailed - Lists all logged calculations in great depth (e.g., transform calculations for each position)
- Summary - Contains overview of performed calculations
- None - No data is displayed in the log

By default, the log is displayed in Detailed mode.

(1)
To change the level of details, in the XML editor, add the CalculationLog element as a child of the MatrixTransformAnalysis and select preferable view mode.

\section*{Saving Log to a File}

Press the Save Log button to save the log for the current assay run to your preferred destination in a .TXT file.

\section*{Error Log}

If any errors occurred during an assay run, they are stored and described in the Error log in similar to the Calculation Log level of details. To view the log, select Error Log subpane of Logs pane.

\section*{Command Line Arguments}

MAA can be launched via MyAssays.Desktop.Analysis.exe (the executable responsible for data analysis) along with command line arguments to automate common tasks. This technique can be used to initiate protocol execution from a third-party application. This includes:
- Launching a protocol
- Importing data
- Calculating results
- Exporting data
- Printing report
- Creating new protocol (requires Pro version)
(1) To assist with creating the command line, use the Analysis Launcher tool.

MyAssays Desktop Analysis with Command Line Arguments

\section*{Access MyAssays Desktop Analysis Executable}

The MyAssays.Desktop.Analysis.exe executable can be found in MyAssays Desktop's installation folder. This is typically:
- "C:\Program Files (x86) \MyAssays \MyAssays

Desktop \MyAssays.Desktop.Analysis.exe" - For 32-bit version of Windows
- "C: \Program Files \(\backslash\) MyAssays \(\backslash\) MyAssays

Desktop\MyAssays.Desktop.Analysis.exe" - For 64-bit version of Windows
How to: Access Installation Folder from Registry
To simplify integration with \(3^{\text {rd }}\) party applications, the installation folder of MyAssays Desktop can also be obtained from the registry:
- Computer \(\backslash H K E Y \_L O C A L \_M A C H I N E \backslash S O F T W A R E \backslash M y A s s a y s ~ \ M y A s s a y s\) Desktop \@InstallLocation - For 32-bit version of Windows
- Computer\HKEY_LOCAL_

MACHINE \SOFTWARE \WOW6432Node \MyAssays \(\backslash\) MyAssays
Desktop \@InstallLocation - For 64-bit version of Windows

\section*{Launch Protocol or Results}

In the simplest case, MyAssays.Desktop.Analysis.exe can be launched with an assayprotocol or assay-results file:
```

"C:\Program Files\MyAssays\MyAssays
Desktop\MyAssays.Desktop.Analysis.exe"
"C:\Users\UserName\Documents\MyAssays\Protocols\ELISA
(96 well).assay-protocol"
"C:\Program Files\MyAssays\MyAssays
Desktop\MyAssays.Desktop.Analysis.exe"
"C:\Users\UserName\Documents\MyAssays\Results\ELISA (96
well) (1).assay-results"

```

\section*{Importing Measurements}

When launching an existing assay-protocol file, or creating a new one, you can specify the raw data file to launch it with. For example, the MyAssays.Desktop.Analysis.exe can be launched with:

\section*{/import-raw:"\{filename\}"}

Where \{filename\} is the full path to the data file to import. For example:
```

/import-
raw:"C:\Users\UserName\Documents\MyAssays\Measurements\E
xamples\12x8 Endpoint.txt"

```

In this case, the MyAssays Desktop will launch its New Protocol Wizard with the data file automatically imported (assuming it is a file that can be imported) and ready for the user to build a new protocol for their measurement data.

In addition, a protocol file can also be specified:

\section*{"\{protocol\}" /import-raw:"\{filename\}"}

Where \{protocol\} is either a MyAssays Desktop assay-protocol file (e.g., ELISA (96 well).assayprotocol) or a name to create an assay-protocol file to.

In case the protocol does not yet exist, the MyAssays Desktop will launch the New Protocol Wizard as above and save the new protocol to the specified name. For example:
```

"C:\Program Files\MyAssays\MyAssays
Desktop\MyAssays.Desktop.Analysis.exe"
"C:\Users\UserName\Documents\MyAssays\Protocols \NEW
ELISA.assay-protocol" /import-
raw:"C:\Users\UserName\Documents\MyAssays\Measurements\E
xamples\12x8 Endpoint.txt"

```

If the protocol already exists, then MyAssays Desktop will launch the protocol and import the data. In addition, there are further command-line options that will perform the analysis and export the report (to XLSX, PDF, DOCX). For example, with:

\section*{"\{protocol\}" /import-raw:"\{filename\}" /calc /export-report:xlsx /exit}

MyAssays Desktop will launch the protocol, import the data, calculate the results, and export the results to Excel then exit.

\section*{Command Line Arguments}

When the MyAssays.Desktop.Analysis.exe is launched with a protocol file (with .assayprotocol extension), a number of arguments can be included to automate various functions:

Arguments with values that include spaces must be put in quotes.
\begin{tabular}{|c|c|}
\hline Argument & Use to \\
\hline /autologin & Perform operations permitted under MyAssays Desktop Enhanced Security Autologin account. \\
\hline \multirow[b]{2}{*}{/calc} & Compute data analysis operations configured in the protocol automatically. \\
\hline & 11) Use this option in conjunction with /import-raw to perform calculations on the imported raw data. \\
\hline \[
\begin{aligned}
& \text { /calc-log:" } \\
& \text { \{log\}" }
\end{aligned}
\] & \begin{tabular}{l}
Save calculation log in a file. The \(\{\log \}\) is the full path to the file, including its name and extension. For example: \\
/calc- \\
log:"C:\Users\UserName\Documents\MyAssays\Logs\calculation .log"
\end{tabular} \\
\hline /error-log:" \{filename\}" & \begin{tabular}{l}
Save error \(\log\) in a file. The \(\{\log \}\) is the full path to the file, including its name and extension. For example: \\
/error- \\
log:"C:\Users\UserName\Documents\MyAssays\Logs\error.log"
\end{tabular} \\
\hline /exit & Exit the program automatically after calculations are completed or printed. \\
\hline \multirow[t]{2}{*}{/exportreport: \{filetype\}} & \begin{tabular}{l}
Export the report in XLSX, PDF, or XML \{filetype\}format and then save in \MyAssays\Reports\ folder. For example: \\
/export-report:pdf
\end{tabular} \\
\hline & (1) Add :nolaunch at the end to prevent the report from launching immediately after export. \\
\hline /exportreport:xslt1 \{filetype\}:" & Convert the report using the specified XSLT template where \{filename \(\}\) is the full path to the template file in the \MyAssays\XSLT folder. This report is then exported and saved in HTML, TXT, CSV or XML \{filetype\}format \\
\hline
\end{tabular}
\begin{tabular}{|c|c|}
\hline Argument & Use to \\
\hline \multirow{3}{*}{\{filename\}"} & \begin{tabular}{l}
(\MyAssays\Reports \(\backslash\) folder). For example: \\
/export- \\
report:xslt1csv:"C:\Users\UserName\Documents\MyAssays\XSLT\Csv \\
Tables (by Data).xslt"
\end{tabular} \\
\hline & \begin{tabular}{l}
(1) \\
Add :nolaunch at the end to prevent the report from launching immediately after export.
\end{tabular} \\
\hline & Configure the protocol to use Custom (including text/XML/CSV/LIMS compatible, etc.) Export Wizard's option (requires Pro license) and save the export settings to the protocol (rather than using /export-report). \\
\hline \multirow[t]{2}{*}{/importcontainerid s:\{filename\}} & Import a file with container (plate, analyte, or batch) identifiers from the \{filename\} full path. \\
\hline & Overrides Import Wizard's previously configured setting. \\
\hline \multirow[b]{2}{*}{\begin{tabular}{l}
/import- \\
raw: \\
\{filename\}
\end{tabular}} & Import the raw data file to the protocol; \{filename\} is the full path to the data file to import. \\
\hline & If \(\{\) protocol \(\}\) is not specified, then New Protocol Wizard is launched automatically to create assay-protocol with imported measurements. In this case, all other import options are not supported. \\
\hline \multirow[t]{2}{*}{/importsampleids: \{filename\}} & Import a table-based Sample IDs data contained in text file of any format from the \{filename\} full path. \\
\hline & Overrides Import Wizard's previously configured setting. \\
\hline \multirow{3}{*}{/importtweaks: \{filename\}} & Specify a protocol tweaks file that defines changes to the protocol configuration. \\
\hline & (1) Add :saveprotocol at the end to save the protocol file with tweaks automatically. \\
\hline & If the Save changes to protocol option is selected (under File | Save in the MAA), then changes are saved to the protocol \\
\hline
\end{tabular}
\begin{tabular}{|c|c|}
\hline Argument & Use to \\
\hline & \(\cong\) irrespective of this option (e.g., when calculations are performed). \\
\hline /noappwin & Do not display the application window. \\
\hline /notopmost & By default, the application window is brought to the top when it is loaded. Use this setting to override this default behaviour and to disable bringing the window to the top. \\
\hline /print & Print the report (if the calculations succeeded) with the default printer (if available). \\
\hline "\{protocol\}" & \begin{tabular}{l}
Launch an existing file or create new assay-protocol via New Protocol Wizard (requires Pro version). The "\{protocol\}" argument can be specified as: \\
- Full path to an assay-protocol file to create or launch: \\
"C:\MyAssayData\Protocols\NEW ELISA.assay-protocol" \\
"C:\Users\Username\Documents\MyAssays\Protocols\ELISA (96 well).assay-protocol" \\
- Protocol name without specifying its folder. In this case, the protocol is created in or launched from the default Protocols folder (i.e., as configured by Assay Data Folder Root setting): \\
"NEW ELISA.assay-protocol" \\
"ELISA (96 well).assay-protocol"
\end{tabular} \\
\hline /tab:\{tab\} & Select the tab to launch MAA with; \(\{t a b\}\) is zero-based tab number: Overview \(=0 ;\) Measurements \(=1\); Microplate \(=2\); Sample IDs \(=3\); Results \(=\) 4. \\
\hline
\end{tabular}

How to Use Long Command Line Argument
The maximum number of characters in the command line is \(>255\). If the command line argument exceeds this length, you can either:
- Use shorter file and folder names where possible
- Store the argument list in a text file and execute it with @ syntax. For example:
```

"C:\Program Files\MyAssays\MyAssays
Desktop\MyAssays.Desktop.Analysis.exe" @
"C:\Users\UserName\Documents\MyAssays\arguments.txt"

```

To easily build and configure command line, use the Analysis Launcher.

\section*{Examples of Command Line Usage}
```

"C:<br>Program Files\MyAssays\MyAssays
Desktop\MyAssays.Desktop.Analysis.exe"
"C:\Users\UserName\Documents\MyAssays\Protocols\ELISA
(96 well).assay-protocol" /import-
raw:"C:\Users\UserName\Documents\MyAssays\Measurements\E
lisa-Absorbance.txt" /import-
sampleids:"C:\Users\UserName\SampleID\DemoScanFile.csv"
/calc /export-report:xlsx:nolaunch

```

In this example, the ELISA ( 96 well).assay-protocol is launched and executed in MAA with Elisa-Absorbance.txt measurements and DemoScanFile.csv file with Sample IDs ; calculated results are exported in XLSX report (and not automatically launched) under \(\backslash M y A s s a y s \backslash R e p o r t s \backslash\) folder.
```

"C:<br>Program Files\MyAssays\MyAssays
Desktop\MyAssays.Desktop.Analysis.exe"
"C:\Users\UserName\Documents\MyAssays\Protocols\ELISA
(96 well).assay-protocol" /import-
raw:"C:\Users\UserName\Documents\MyAssays\Measurements\E
lisa-Absorbance.txt" /import-
sampleids:"C:\Users\UserName\SampleID\DemoScanFile.csv"
/calc /export-report:xlsx:nolaunch /print /noappwin

```

As with previous example, the protocol with specified measurements and Sample IDs is computed and the results are exported to XLSX file, but without displaying MAA window. Additionally, the report is printed with default printer.
```

"C:<br>Program Files\MyAssays\MyAssays
Desktop\MyAssays.Desktop.Analysis.exe" "NEW ELISA.assay-
protocol" /import-
raw:"C:\Users\UserName\Documents\MyAssays\Measurements\E
lisa-Absorbance.txt"

```

In this case, New Protocol Wizard launches with Elisa-Absorbance.txt measurements imported to create a new protocol with the specified name (NEW ELISA).

\section*{Tweak Files}

This section provides information about the protocol tweaks feature. This feature enables an external application to change and apply specific settings of the data analysis.

This is an advanced feature and misuse of it could cause problems.

\section*{Overview}

Protocol tweaks are used to define changes to the data analysis parameters of an existing protocol. Any of the assay protocol parameters can be tweaked. This is useful to control the analysis process by other software such as instrument-control software or LIMS.

For example, a LIMS could initiate execution of data analysis and specify customizations (such as standard concentrations or layout); the customisations are merged with the existing protocol, the analysis performed, and results generated with data exported as required.

Protocol tweaks are defined in an XML file and can be applied by launching the protocol and specifying the tweak file to apply.

Any data analysis parameter can be modified.

\section*{Tweak Syntax}

A protocol-tweak.xml file is an XML with the following syntax:
<?xml version="1.0" encoding="utf-8"?>
<ProtocolTweaks>
<Tweak XPath=" Full assay setting to tweak path">Substitute data</Tweak>
</ProtocolTweaks>

You can insert any number of tweaks under <ProtocolTweaks> section.

\section*{Using the Feature}

To use the protocol tweak feature, it is recommend to developing a protocol-tweak file as follows:
1. Build or obtain an assay-protocol file that meets your data analysis requirements.
2. Identify which analysis parameters should be tweaked.
3. Develop a protocol-tweak.xml file.
4. Test the protocol-tweak.xml file.

With your working protocol-tweak.xml file, use your instrument control or LIMS software to insert relevant content into the protocol-tweak.xml file.

\section*{How to: Apply Protocol Tweaks}

The easiest way to apply a protocol tweak file is to use the Analysis Launcher. Use this tool to select the protocol and tweak file and launch the application.

The Analysis Launcher tool will also generate and display the command line argument to use. You can use this command line setting to launch the operation yourself (perhaps calling it from another software application that prepares the tweak file's contents). More information about command-line arguments is available here.

When the protocol is launched and tweak file applied, the system will perform various checks on the incorporated tweaks to detect any report any possible configuration errors in common usage scenarios.

If the protocol is successfully tweaked the analysis operations will continue as specified. The actual behaviour will depend on any further command line settings (such as automatic calculation, export, etc.)

Tweak changes are not automatically saved to the incorporated assay file. A tweaked protocol appears as modified and not saved (with * in title). If you save the protocol, the tweaks will be saved to the protocol file.

\section*{Changing the Default Matrix Limit for Including Raw and Field Matrices}

When creating a new protocol for a large amount of data, MyAssays limits the number of matrices included in the report. This topic explains how to change the default matrix limit settings for both measurements and field data.

This is an advanced configuration setting that can only be changed via XML.

For general principles of working with a lot of readings, see Handling a Large Number of Raw and Field Matrices. To learn more about accompanying the measurements with related information, see Adding Field Data Alongside Measurements.

\section*{Where the Matrix Limit Settings Are Stored}

The common settings for handling large amount of measurements are stored in the pws.config.xml file. This file is located in your Precanned \Settings subfolder in your MyAssays Desktop's installation folder. This is typically:
- "C: \Program Files (x86) \MyAssays MyAssays

Desktop \(\backslash\) Precanned \(\backslash\) Settings \(\backslash\) pws.config.xml" - For 32-bit version of Windows
- "C: \Program Files \(\backslash\) MyAssays \(\backslash M y A s s a y s\)

Desktop \Precanned\Settings \pws.config.xml" - For 64-bit version of Windows

Using this file, you can edit the default matrix limit settings for raw data, field data, or both.

\section*{Changing Matrix Limit}

To change the default limit settings for measurements, follow these steps:
1. Launch the pws.config.xml file with your default XML editor.
2. Locate the Report element.
3. Depending on the data you're changing the limit for, edit:
- MatrixLimit value to alter the default setting for raw measurements data.
- FieldLimit value to alter the default limit for field data matrices.
4. Save the changes to the file.

This change will not affect the application of the limit. Refer to How the Matrix Limit Is Applied to learn more.

\section*{Installing MyAssays Desktop Alongside WorkOut}

MyAssays Desktop can be installed and used alongside an existing WorkOut installation (including WorkOut versions 1.x, 2.x, Plus, and Plus MMD). This is referred to as a Side-By-Side installation.

Certain modules (relating to data import and analysis) are shared between the applications. If you plan to use both WorkOut and MyAssays Desktop on the same system, then please follow these points to avoid known conflict issues:
- Always install MyAssays Desktop after installing WorkOut (i.e., do not install, update, patch, or repair a WorkOut installation after installing MyAssays Desktop).
- If WorkOut is uninstalled leaving a MyAssays Desktop installation, then it will be necessary to also uninstall MyAssays Desktop and then reinstall MyAssays Desktop.

If MyAssays Desktop has been installed with a Side-By-Side WorkOut installation, then this will be reported in the File | Information tab of MyAssays Desktop Explorer.

\section*{Known Issues}

If the points above are not followed, then you may see the following problems with MyAssays Desktop:
- Unable to import data
- Unable to produce a valid report (on data calculation)

To resolve these issues, please uninstall MyAssays Desktop and then reinstall MyAssays Desktop to update the shared component files to the newest version.

\section*{Referencing MyAssays Desktop}

You can refer to MyAssays Desktop in your paper, thesis or publication.
To refer to the data analysis tool include the text:
"MyAssays Desktop Product Version from MyAssays Ltd., https://www.myassays.com"
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[^0]:    This is specified in the XML editor inside the XYPlot element with following syntax:

[^1]:    Raw (2)

[^2]:    Layout

[^3]:    Plot crosses $\mathrm{y}=0.7$ at $\mathrm{X} 1=914.729$ and $\mathrm{X} 2=1506.466$, difference $=591.737$

