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MyAssays Desktop User's Guide



www.myassays.com

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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 <u>myassays.com</u>. 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 <u>Quick Start</u> 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 665nm and 620nm 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 meta data (data accompanying the read such as measurement date/time, barcode, etc.) are imported automatically. (For the latest list of currently support format, see: <u>https://www.myassays.com/supported-file-formats.html</u>.)
- 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 meta data (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 color
 - By graphical value bars

Data Analysis

Beyond the online database of ready-to-use preconfigured protocol, 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 <u>Transforms</u>.

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 R², aR², SE, F-statistic, P-value (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 preinstalled 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.

Product Editions

MyAssays Desktop is available in various configurations to suit different budgets and needs:

Basic: Operates with preconfigured protocols (either those included in the installation package or as downloaded from MyAssays.com). The Basic edition allows tweaking limited protocol parameters (such as fit method, sample concentrations, layout, etc.). **Pro:** Includes all of the features of the Basic edition plus functionality for creating new custom protocols. This edition provides full access to the Matrix-Transform data analysis model, offering a wealth of easy-to-use tools for building data analysis solutions tailored to your lab.

Pro Add-Ons:

Optional add-on functionality is available for the Pro edition:

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® xPONENT and Meso Scale Discovery Workbench® 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 R² 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.

Enhanced Security Add-On

For those users working under GxP or 21CFR11, the Enhanced Security edition offers the features 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/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 log-in 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 outputs).

Licensing

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

Edition Comparison

Feature	Basic	Pro
Downloadable Protocols (from myassays.com)	✓	\checkmark
Preconfigured Protocols (96/384 versions):	✓	\checkmark
• ELISA	✓	\checkmark
DNA/Protein Quantification	✓	\checkmark
ATP lite protocol	×	\checkmark
Generic DELFIA	✓	\checkmark
Generic LANCE	×	\checkmark
Generic Fluorescence Polarization protocol	×	\checkmark
Monochromator Spectral	×	\checkmark
Kinetic for Ca2+	×	\checkmark
Bacterial growth	-	\checkmark
Import Script Support	×	\checkmark
Excel Import	×	\checkmark
Worklist Import	×	<
Data Visualization (3D View/Heatmap/Kinetic		
Overlay/Spectral Plot)		•
Versioned Analysis	✓	\checkmark
Export to XLSX/DOCX/PDF	✓	\checkmark
Edit Protocols	Limited	\checkmark
Report Customization	Limited	\checkmark
Custom Export (e.g., for LIS/LIMS)		\checkmark
Multiple Plate Support	_	\checkmark
Create New Protocols (New Protocol Wizard)	_	\checkmark
Matrix-Transform Data Analysis	-	\checkmark
Custom Validation Rules	-	<
Custom Evaluation Expressions	-	<
Best Fit	-	<
Folder Poll	-	<
PLA	-	<

Getting Started

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:	Intel/AMD 2 GHz or faster processor (32- bit or 64-bit)
Memory:	2GB RAM
Available disk space:	300MB
Operating systems:	Windows 10, Windows 8.x, Windows 7, Windows Vista, Windows XP SP3
Other software:	Microsoft .NET 4.0.3 or later
Display:	1024 x 768 or higher resolution

Installation Process

The latest version of MyAssays Desktop can be downloaded here.

Upon downloading the installation package:

- 1. Double-click on the installation package file to launch MyAssays Desktop Setup Wizard and click **Next** to continue.
- To proceed with installation on your computer, you must accept the terms of End-User License Agreement. Please read the license agreement carefully and then check I accept the terms of the license agreement box and press Next to continue. If you do not agree with terms of the license agreement, click Cancel to exit the setup wizard.
- If you are content with the default path, press Next to continue.
 To pick another destination folder, press Change... and set preferable root.
- 4. Check the choices you made and press Install. The process will take a few moments.
- 5. Click **Finish** to complete the installation. Check **Launch MyAssays Explorer on exit** to start working with the software immediately.
- 6. <u>Activate MyAssays Desktop</u> 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. Please refer to <u>Installing Alongside WorkOut</u> for further details.

Launching

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

On Windows 10:

Choose **Start**, then scroll down to and click on 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 on the desktop icon and select **Pin to Start** or **Pin to Taskbar**.

Product Licensing

To use MyAssays Desktop, you must provide and activate a valid license.

You can obtain a trial, temporary, or permanent license from MyAssays Ltd.

A serial code represents your license or licenses (for multi-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 the I have a serial code to activate button and press Next.

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

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

Your email address is required for administrating 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. Check the **Use internet connection for activation** checkbox and press **Continue** to proceed.
- 2. Fill in your personal details and press 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. Press 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. Uncheck **Use internet connection for activation** checkbox and press **Continue** to proceed.
- 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. Press Close to exit License Activation Wizard and start the application.

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. Select the **File** tab and select the **Information** option:

The availability of licensed features is marked with the following icons:

- for active features.
- × 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 on 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. Set **File** tab and select **Information** section.
- 3. Click on the **Deactivate License** button.

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** checkbox checked and press **Continue** to proceed with deactivation.
- 2. Check the checkbox to confirm deactivation and press 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. Press 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. Uncheck the **Use internet connection for deactivation** checkbox and press **Continue** to proceed with deactivation.
- 2. Check the checkbox to confirm deactivation and press **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 press **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

If you have been provided with a new serial code to replace an existing license (perhaps as the result of a purchased upgrade or support extension), then it will be necessary to activate your new license to replace the existing one.

- 1. Launch MyAssays Desktop Explorer.
- 2. Select the **File** tab and select the **Information** option.
- 3. Select Activate New License.
- 4. Press 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. Select the **File** tab and select the **Information** option.
- 3. Select 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 <u>License Activation</u>).
- 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:

- <u>MyAssays Desktop Explorer (MADE)</u> 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 short-cut 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-hand 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 preinstalled 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 the launched assay name and any description. It might also include a link to online content (such as kit manufacturer's product insert).

Along the left-hand 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 meta-data). 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 <u>Standard Curve Fit</u>).

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.

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 press the **Calculate** button 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.

Pressing the **Calculate** button:

- performs the calculations
- generates the report
- and 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 select 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 <u>reporting template</u> 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 meta-data 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

Press 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 pressed 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. Press the **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

Online videos are provided to help you get started and walk through features in detail:

Getting Started

- http://www.myassays.com/myassays-desktop-fundamentals.video
- http://www.myassays.com/download-and-use-protocols-from-myassays.video

Importing Data

- <u>http://www.myassays.com/import-measurement-data-from-excel-files-with-myassays-desktop.video</u>
- <u>http://www.myassays.com/import-measurement-data-from-multiple-excel-files-with-myassays-desktop.video</u>

ELISA

- http://www.myassays.com/elisa-data-analysis-with-myassays-desktop.video
- <u>http://www.myassays.com/multiple-plate-elisa-with-myassays-desktop.video</u>

Excluding Outliers (Flagging)

• https://www.myassays.com/myassays-desktop-flagging-outliers.video

Worklists

• https://www.myassays.com/myassays-desktop-sample-ids-and-worklists.video

Export

• https://www.myassays.com/myassays-desktop-custom-export-for-lims.video

Multiplex

- <u>https://www.myassays.com/myassays-desktop-introducing-the-multiplex-protocol.video</u>
- <u>https://www.myassays.com/myassays-desktop-automatic-launching-of-multiplex-analysis.video</u>

Folder Poll

• <u>https://www.myassays.com/folder-poll-with-myassays-desktop.video</u>

Inter-Assay Quality Control

• <u>https://www.myassays.com/myassays-desktop-inter-assay-quality-control.video</u>

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 results between systems.

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.

An assay will contain different types of samples such as:

• Unknown – Samples to quantify or qualify

-

- **Standard** Samples with a known quantity used for preparing a calibration or standard curve
- Control Samples used for control purposes such as inter-assay or inter-assay quality control
- Blank A sample without any expected response, used to remove background noise

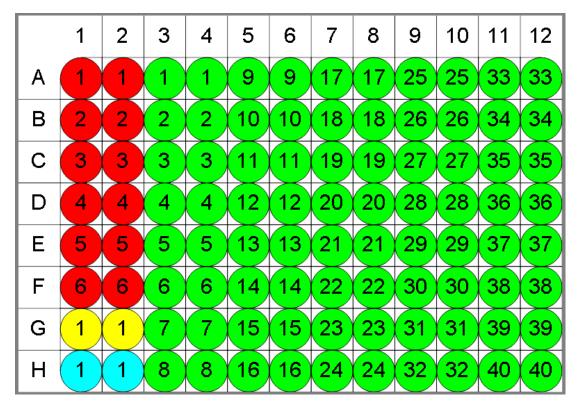
Other sample types are possible; you can create and name your own sample types as you require.

With MyAssays Desktop, we use a color to indicate sample type:

Standard O Blank O Control O Unknown

Typically assay samples will be replicated. To identify replication, each sample is given a number. Thus a sample type and number uniquely identifies a sample. All positions on a layout with the same type and number are replicates of the same sample.

For example, 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.). I.e.:



The use of a sample layout introduces a convenient layer of abstraction over the measurement data. 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. Also, MyAssays Desktop calculations are based around the sample layout, therefore you can easily change the sample layout (perhaps because you need to use a different positioning of your samples on a different run of your assay) without needing to make any changes to your calculation settings. If you have ever had to change an Excel spreadsheet because of a change in layout, then you will appreciate that this abstraction is a valuable time saver.

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 <u>New Protocol Wizard</u>, 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 textbox** 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 <u>Request Protocol</u> 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 <u>Product Licensing</u> describes further details for these options.

- **View** defines whether the <u>Measurements Tab</u> is displayed on the Folder Side Bar and the <u>Launch Assay Protocol with Options</u> setting is displayed on <u>preview pane</u> in the <u>Protocols</u> <u>tab</u>.
- **Data** displays the <u>storage path</u> for persisting your assay data files and <u>Welcome Screen's</u> **Recent** feature <u>options</u>.
- 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 <u>Customize the Work Area</u> to fully adjust it to your needs.

Application Button

Click on 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 on **Show Quick Access Toolbar Below/Above the Ribbon** (depending on its current location), **Minimize the Ribbon**, and change the Quick Access Toolbars view by adding or excluding some options in **More Commands** pop-up menu.

Folder Side Bar

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 subdirectories 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.

Folder Side Bar Content	
-------------------------	--

Tab	Contains Files
Search Results	Assay protocol titles that match search query
<u>Protocols</u>	Assay protocol files with .assay-protocol extension
Measurements	Measurement data files in supported file formats (*.TXT, *.CSV, *.XML, *.DAT, *.XLS, *.XLSX, and others)
Results	Assay results files with .assay-results extension
Layouts	Layout files with .assay-samplelayout extension
Reports	Report files with supported report extension (*.DOC, *PPT, *.XLS, *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 <u>MADE Ribbon</u> options are available when a single file is selected.

Sort and View

The default view of content is **Details**. To change it, right-click on 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 on 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 on 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 in 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.

Folder Tab	Preview Pane Contents
Search Results	A summary of assay protocols, including the name, the instrument settings, the position used and layout, and any configured transformations
<u>Protocols</u>	
Measurements	Pre-canned and custom raw-data files
<u>Results</u>	Two assets:
	 Overview with the assay protocol summary Latest Report with the latest report of the results
	Latest Report with the latest report of the results
<u>Layouts</u>	A microplate control showing each plate layout in the layout
<u>Reports</u>	The preview of the PDF reports

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

Welcome Screen

This is the screen that displays as you launch <u>MADE</u>. 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 <u>New Protocol...</u>, <u>Launch</u>
 <u>Protocol...</u>, and <u>Open Results...</u>
- **Recent.** A specified number of assay protocols and results you have worked with last (the particular title will be launched in <u>MAA</u> upon selection)

You can customize a number of entries in the **Recent** list or clear the file history in <u>Data options</u>.

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:
 - <u>Product Videos</u> 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 <u>Request an Assay</u> <u>Protocol</u> 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 Side Bar 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 MyAssays.com

How to: Find an Assay Protocol from MyAssays.com Knowledge Base

First, in MADE, enter an assay title or keyword (i.e., Cortisol, Human, ELISA):

• In the MADE Menu Bar, Search MyAssays.com panel

OR

• Use a keyboard shortcut (**CTRL + E**), which automatically redirects you to a search textbox

Next, press the **Search** button or **Enter** on keyboard.

When you have performed a search, the <u>Search Results tab</u> is displayed on <u>Folder Side Bar</u>.

Using the Search Results

Upon performing a <u>search</u>, you will see the list of assay protocols that contain a keyword you have entered.

To specify the search results and ensure finding the protocol you're looking for, each protocol contains a detailed summary. To see further information on a particular position, single-click on its title. Some of the assays contain Kit Booklet from the manufacturer. To read it, click on **View Assay PDF** link under the assay summary.

For every assay protocol in the list, the following actions are available on the Preview pane:

 Download and Launch – Persists a single assay protocol to your <u>Protocols tab</u> and automatically launches it in <u>MAA</u>.

You can also double-click on a file from the list to download it to your PC and launch it in MAA automatically.

• Download – Stores a single or multiple assay protocols to your Protocols tab.

If you select multiple items from the list, only this option is available and displayed. To select multiple protocols from the search results, use the **(Ctrl+Shift+Arrow Keys)** keyboard shortcut and **Ctrl+A** to select all search results.

- Launch Assay Protocol Appears if you have downloaded a particular assay protocol previously. The above actions are then not displayed. To enable re-downloading, click on 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.

No Results Found

If the assay you are looking for is not yet in the MyAssays database, an **Information** dialog box appears.

Click **Yes** to proceed to a <u>Request Protocol</u> form.

Search + Download from MyAssays.com

Download and use preconfigured assay protocols from the MyAssays.com knowledge base on your PC.

Submit a <u>Search</u> and explore <u>search results options</u> of the constantly evolving protocols database.

Protocols Tab

The **Protocols Tab** displays a <u>File List and Preview</u> 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:

How to: Create New Assay Protocol

In the MADE Welcome Screen, click on New Protocol....

OR

In the <u>Folder Side Bar</u>, select the **Protocols** tab and click on the **New** button on the <u>MADE</u> Ribbon. When this option is selected, a New Protocol Wizard will be displayed.

How to: Launch Assay Protocol File

Navigate to the <u>Protocols tab</u>, select a single assay result file to open, and do one of the following:

- double-click on its title
- right-click on the title and choose Run with MyAssays Desktop Analysis
- click on the Launch Assay Protocol button in the lower right corner of the <u>Preview pane</u>.

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

You can also launch an assay protocol directly from <u>Search Results</u>.

Launch Assay Protocol with Options

Launch an assay protocol with command-line options for more control over the analysis. This feature will open the <u>Launcher</u> tool with the selected protocol.

If you press the **Launch Assay Protocol with Options** button, the dialog window with a visual representation of <u>supported command-line options</u> will appear.

How to: Launch Assay Protocol with Command Line Options

- 1. Launch MADE.
- 2. Select the **File** tab of <u>MADE Ribbon</u>.
- 3. Select the **View** screen.
- 4. Check the Show Launch Assay Protocol with Options button checkbox.

The **Show Launch Assay Protocol with Options button** state (checked/unchecked) persists between sessions.

If it is checked, a corresponding option appears on the <u>preview pane</u> of the <u>Protocols tab</u> upon protocol file selection.

How to: Request Assay Protocol

Launch MADE and do one of the following:

 Click on the Request an Assay protocol on a Support subpane of the <u>Welcome Screen</u>.

OR

• On the <u>MADE Ribbon</u>, select the **Support** tab and press the **Request an Assay protocol** button.

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, press 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 <u>MADE Ribbon</u>.
- 3. Select the **View** screen.
- 4. Check the **View Measurements** checkbox.

1 The **View Measurements** state (checked/unchecked) persists between sessions.

If it is checked, an additional tab is displayed on the Folder Side Bar.

The measurements tab contains a default **Examples** folder with a 12x8 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 <u>MyAssays Folder Root</u>.

The <u>New protocol wizard</u> 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 (<u>http://www.myassays.com/supported-file-formats.html</u>) to see list of supported measurements data formats.

Results Tab

The **Results Tab** displays a <u>File List and Preview</u> 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.).

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

Navigate to the <u>Results tab</u>, select a single assay result file to open, and do one of the following:

- double-click on its title
- check the **Open** icon on the <u>MADE Ribbons</u> File group
- check the Open icon in Latest Report inset of the Preview pane

By doing this you will launch the file in MAA.

Layouts Tab

The Layouts tab displays a <u>File List and Preview</u> 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 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 on a 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 on 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, press the **Add to Quick Access Toolbar** command. 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 on **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 on **Customize Quick Access Toolbar** on the context menu.

• Click on the **Advanced** option on the Quick Access Toolbar and click on 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, press the **OK** button to save changes or **Cancel** to undo them.

To return the toolbar to its default configuration, click on **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 on any spot on the Ribbon and check **Minimize Ribbon** in the drop-down list. To undo minimizing, repeat these actions and uncheck the command.

Layout Editor

Layout Editor

Overview

Most assays allow you to specify your own layout for processing and distinguishing various types of measurement data.

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. Create new or modify existing layouts after analysis and easily recalculate results to correct for preparation errors.

Using the Layout Editor

The Layout Editor is displayed when you press the **Create** button to create a new layout or **Edit** to edit an existing layout on the **Layout Selection** pane of <u>MAA</u> **Microplate** tab. You can also create a new layout as a step of the <u>New Protocol Wizard</u>.

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 <u>setup the layout as you prefer</u> 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 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. Layout Editor allows you to specify for each plate:

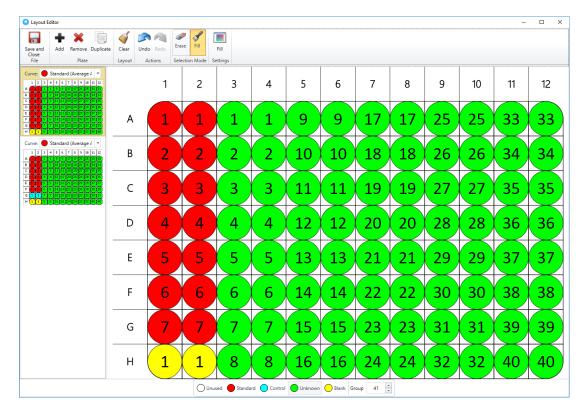
- 1. How samples are arranged
- 2. Which standards measurements should be used to construct a standard curve
- 3. Which curve to use to calculate concentrations from

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, press the **Fill** button. 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
- Snake 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.

J 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, press the **Save + Close** button. This will save the new layout to your <u>Layouts tab</u> 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 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).

Multiplex Options

For multiplex assays, only single layouts can be applied.

Layout Configuration

The <u>Layout Editor</u> 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)

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 Group Numbering

When defining the layout (in the <u>Layout Editor</u>), the numbering for each sample group on each and 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.

When the analysis is performed, the software will apply the group numbers across plates contiguously. 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 **Unknowns11-20**.

Analysis

Versioned Analysis

Overview

When you launch an assay protocol and press the **Calculate** button, 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.

Each time the **Calculate** button is pressed, 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. Hover to a Versions Group on the Home tab of the MAA Ribbon.
- 3. Select the Version you need from Current version drop-down list.

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.

Pressing **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 press **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

8

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.

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 pressed again, the **Version {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 <u>MAA</u>, 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.

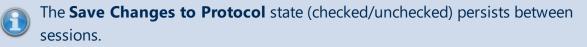
Save Options

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 checkbox appears if you have launched <u>MAA</u> 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.



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
- File | Save BSA

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** checkbox is checked.

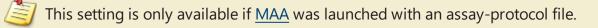
Save Results As

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

Naming Results



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 the **Results Naming** button is pressed, a dialog window with the following options appears:

1. **Default** (automatically named and stored in <u>Results</u> 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 textboxes 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 textbox (manually or from clipboard).
- Click the **Browse...** button to provide the full path to the folder in **Browse for Folder** tree view. Press **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. Press **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. Press **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.

If the **Test settings when OK is clicked** checkbox is enabled, MAA checks the accessibility of specified target path and verifies if the file can be created.

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

New Protocol Wizard

How to: Launch New Protocol Wizard

- 1. Launch the MADE and do one of the following:
- 2. Select the New Protocol... option on MADE Welcome Screen.
- 3. Select the **Protocols** tab and click on the **New** button of **File** group.

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** buttons to configure the following assay parameters:

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

At any step, you can click on 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 textbox 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:

- Press the Analysis Launcher button on the Tools tab in the Utilities group of the MADE <u>Ribbon</u>.
- Enable the <u>Launch Assay Protocol with Options</u> feature and press the Launch Assay Protocol with Options button.
- 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.

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

In the <u>MADE Ribbon</u>, select **Backstage File Screen** | **Data**. Press the **Select Folder Root** button 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.

Data Import Visualizing Data

Heatmap Plate View

The **Heatmap View** displays a plate layout colored 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** tab. Check
- 2. Check the Heatmap View option in the lower left corner of the right pane.



Structure

Heatmap View comprises:

• Plate preview with the color of each position based on its numerical value converted to a color 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 <u>minimum and maximum</u> <u>controls</u>
- 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 color scheme range displayed on the heatmap plate preview. Each position on the layout is colored according to its numerical value and the color scale (using the color range min/max values). Values outside the range are rounded to the nearest min or max color.

How to: Change the Minimum Value

- 1. Hover and click on the minimum value on the left side of the color bar to edit the heatmap minimum value.
- 2. Type in a minimum value for the heatmap (e.g., 0.05).
- 3. Click on the check icon to save your changes or on 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 and click on the maximum value (right side of the color bar) to edit the max value.
- 2. Type in a maximum value for the heatmap (e.g., 2.00).
- 3. Click on the check icon to save your changes or on the cross icon to cancel them.

For multiple plate multiplex assays, consider all used positions before you type in min and max values for the heatmap.

Scale Function

With the <u>Heatmap View</u>, 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 <u>Heatmap View</u> 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 on the Log10 scale button (right one). The heatmap is refreshed to display a Log10 color scale.
 - Linear scale Click on the Linear scale button (left one). The heatmap is refreshed to display a linear color 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 min/max, then the heatmap scale is updated accordingly.

How to: Access the Auto Scale

- 1. With the <u>Heatmap View</u> turned on, click on the **Auto Scale** button (on the right side of the heatmap maximum control).
- 2. Click on the **Auto Scale** button.

You can toggle the Auto Scale setting on and off by clicking on 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 Measurements or Microplate tab.
- 2. Check the Heatmap View option in the lower left corner of right pane.
- 3. Click the **3D** button.



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

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 <u>position</u> 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 **Standard1** comprises of 3 replicates measured at position **A1**, **A2**, and **A3**, if position **A1** is flagged, **Standard1** would refer to only the not-flagged values (i.e., the average of the data at positions **A2** and **A3**).

For kinetic or spectral data, any specific data <u>point</u> 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 **A1** 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 <u>XY Reduction Average</u>) 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:

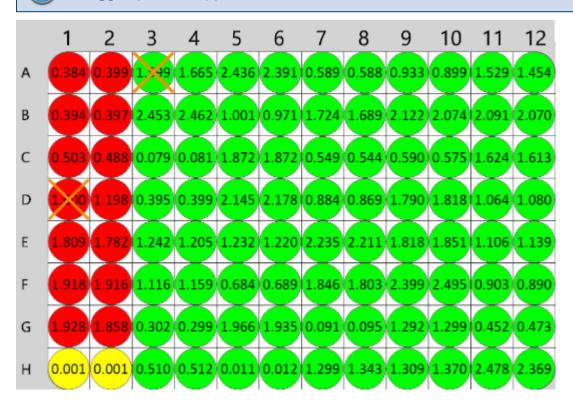
- Measurements View (in the **Measurements** tab)
- Standards Chart (in Results tab when working with Standard Curve Fit transform)

- 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.

A flagged position appears on the Measurements View with a cross-through it.

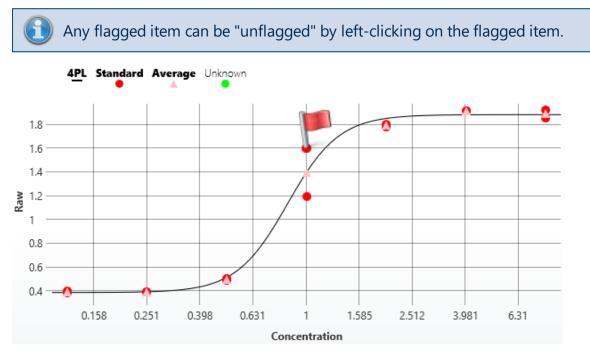


How to: Flag Data Position with Standards Chart

1. Add the Standard Curve Fit Transform:

- 1. Launch an assay protocol or open a results file.
- 2. If you cannot see the Assay Properties panel, press the **Properties** button to open it.

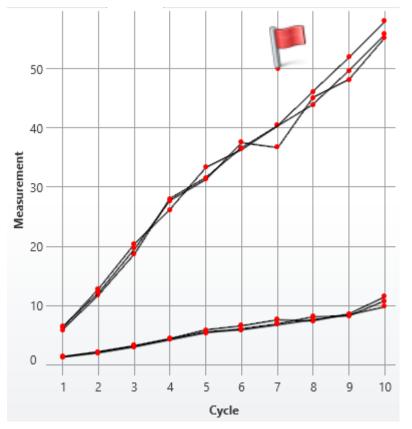
- 3. Press the **Add** button.
- 4. Select the Standard Curve Fit transform.
- 5. Press the **Create** button.
- 6. Review or edit the transform's input matrix and output matrix name as required.
- 7. Provide your required settings for the transform (see the **Properties** section above).
- 8. Press the Calculate button.
- 2. 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.
- 3. Select the sample type on the legend to view how the points are spread on the curve.
- 4. Position the mouse pointer over the point to flag and left-click.
 - 1. Press the **Calculate** button to perform the calculations with the new settings and to see the results.



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.

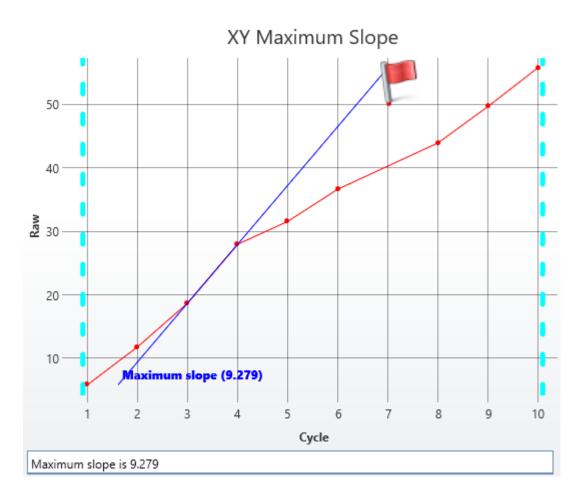


How to: Flag Kinetic/Spectral Data Point with Kinetic/Spectral Analysis Chart

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.



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).

F	lags					×
Γ	Status	Туре	Position	Point	Plate	Matrix
	1	Point	A5	7	Plate 1	Raw
	1	Point	A5	6	Plate 1	Raw
	1	Point	A5	5	Plate 1	Raw
	1	Position	A1	-	Plate 1	Raw
	1	Position	A2	-	Plate 1	Raw
	1	Position	A3	-	Plate 1	Raw

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

When working with kinetic or spectral data, the <u>XY Outlier Removal</u> 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 **1.23** 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 **A4** 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 <u>Custom Flag Label configuration</u>.

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 **1.23** 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 <u>Standard Curve Fit transform</u> 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 <u>Standard Curve Fit Custom Flag Labels</u>.

To label sample results that satisfy specified conditions without flagging them, please refer to the <u>Sample Labels</u> topic.

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
Unknown1	A3	1.599	No result
-	A4	1.665	

How to: Set General Custom Flag Label

- 1. Select the **XML** tab in the **Assay Properties** panel (If the **XML** tab is not immediately visible, press the **<< Show All Properties** button 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, press the **<< 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 transform is the only transform that provides custom flag settings by calculation. For more details, see <u>Standard Curve Fit Custom Flag Labels</u>.

Custom Flag Label Reporting

The specified custom flag labels will be used in the report and export; however, the **Sample Table Replicates** setting of <u>matrices configuration</u> 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.

Matrices

Generally, all data in MyAssays is stored in matrices. Each matrix can contain one or more replicates, and for each matrix certain relevant transforms can be applied. In the **Matrices Tab**, the settings for each of these matrices can be configured.

How to: View Matrices Tab

- 1. Launch an assay protocol or open a results file.
- 2. If you cannot see the Assay Properties panel, press the **Properties** button to open it.
- Select the Matrices tab (If the Matrices tab is not immediately visible, press the << Show All Properties button to expand).

Matrix Selection Pane

This pane includes available matrices in a tree list. This list contains two basic branches: **Raw** and **Calculated**.

Raw

This is input data that is stored in the **Measurements Tab**. The quantity and type of **Raw** matrices depends on the assay type and configuration.

Click here to learn more about measurement data.

Calculated

This consists of all new matrices that are created and added to output by transforms.

Matrix Configuration Pane

Each input and each output matrix of a newly added transform is automatically included in both Sample and Matrix Tables. With the following settings, you can configure whether and how they are displayed.

General

Nam		A title to be used for a matrix in the headers of the Sample Table and Matrix
Name	le	Table. 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
Frecision	of Precision depends on the Type setting.

	Specifies how a numerical value is formatted with regard to precision and rounding options:
Туре	 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 Matrix Tables	Defines whether the associated matrix should be included in the Matrix Tables						
Include in Sample Tables	Defines whether associated matrix should be included in the Sample Tables						
Matrix Table Ids	Defines in which Matrix Table(s) the associated matrix should be included.						
Sample Table Ids	Defines in which Sample Table(s) the associated matrix should be included.						
	Determines how replicates of each sample group are included in the Sample table:						
Sample Table Rep-	 Average – The average value of all replicates is displayed. This applies to Endpoint data only. 						
licates	• List – All replicate values are included in the table.						
	• 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., <u>Average</u> , <u>XY Replicates Average</u>).						

Configuration of Endpoint Matrices

For endpoint data you can additionally configure the following settings to apply on the report.

Sample Labels

If this is specified, the **Condition** is evaluated for each position of the associated matrix and **Label** is displayed in place of the value for the position. For further details on this feature see <u>Sample Labels</u> topic.

Value Bars

Within this category you can configure whether and how to fill replicate values with color for any **Raw** or **Calculated** endpoint matrix.

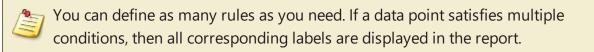
The value bars are created using the minimum and maximum replicate values of the matrix. These are treated as 0% and 100%, respectively, and filled in accordingly.

Display Value Bars	Defines whether this feature is used in the <u>Sample Tables</u> .						
	Defines how to fill in the value bar. Select one of the following types:						
Fill	Solid – To fill value bars self-colored with Fill Colour.						
	Gradient – To fill value bars with the gradation from Fill Color in the left to white on the right.						
Fill Colour	Click on the drop-down to open a color picker.						
Border	It defines how to fill in the value bar's border. Select one of the following types: Solid – To fill value bars self-colored with Fill Colour . None – To not display the Border .						

Sample Labels

The Sample Labels feature can be used add your own text in-place or alongside measurement or computed numerical values. Also, your data or labels can be highlighted to visually enhance the report output; this is useful to draw the reader's eye to specific areas of interest or to color code your report content.

Samples are reported with label and/or colour in accordance with the rules that you specify.



The rules for sample labels are applied to the corresponding data included in the report. In the <u>Matrix Table report element</u>, the label or coloring would apply to each position. In the <u>Sample</u> <u>Table report element</u>, this might be each individual replicate value or each sample's average; it depends on how the data is configured in the report output.

For example, here is a Matrix Table output highlighting all sample measurements > 1.5 in yellow.

	1	2	3	4	5	6	7	8	9	10	11	12
А	1.586	1.575	1.590	1.556	1.575	1.517	1.741	1.671	1.668	1.778	1.669	1.718
В	0.179	0.186	0.174	0.176	0.181	0.175	2.153	2.067	2.239	2.084	2.177	2.054
С	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
Н	1.734	1.760	1.753	1.800	1.705	1.793	0.209	0.201	0.200	0.218	0.215	0.204

With the Sample Table report element, each matrix can be configured to display the average of the sample group or list each replicate. (This can be set with the <u>Sample Table Replicates</u> setting in the Matrix configuration.) In either case the sample label will be applied to the selected data.

For example, the following Sample Table has been configured with the Raw data <u>listing all</u> 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 <u>average</u> of the replicates values. In this case the label is applied to the computed average.

Sample	Positions	Raw
Unknown1	A1	1.566 > 1.5
	A2	
	A3	
	A4	
	A5	
	A6	

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.

Applying label, unlike <u>flagging</u>, does not exclude data from calculations, nor does it change the actual value used in subsequent calculations. It simply replaces their reported values with a specified **Label** and applies any colouring.

How to: Apply a Sample Label

- 1. Launch an assay protocol or open a results file.
- 2. If you cannot see the Assay Properties panel, press the **Properties** button to open it.
- Select the Matrices tab (If the Matrices tab is not immediately visible, press the << Show All Properties button to expand).
- 4. Select one of the numbered matrices from under either the **Raw** or **Calculated** branch, which contains the data to label.
- 5. Click on the drop-down **Label** property of the <u>Matrix Configuration pane</u> to view **Matrix Sample Label Editor**.

- 6. Enter the condition (in terms of x) along with the label and any highlight or text coloring to apply.
- 7. Press **Add** to add further rules. Click outside the Editor when you have finished.
- 8. Press the **Calculate** button to perform calculations with the new settings and to see the results.

In the **Matrix Sample Label Editor**, you can add and remove any number of rules 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 <u>conditional</u> <u>expression</u>) in terms of x. The x value specified in the expression refers to the sample.
Label	 Label – The label to be displayed in the <u>Sample Table</u> and <u>Matrix Table</u>, if the Condition is satisfied. Use {x} to refer to the data value that was used in the condition (i.e., the original data point).
	 Highlight – This sets the background color for the cell when Condition is satisfied. Where there are multiple Conditions satisfied, the color of the first satisfied Condition is applied.
	 Text – This sets the text color for the cell when Condition is satisfied. Where there are multiple Conditions satisfied, the color of the first satisfied Condition is applied.

If the **Condition** is satisfied, the replicate will be reported with the corresponding **Label** in place of its value.

Reporting Data with Labels

The results with labels appear in associated matrix's <u>Sample Table</u> and <u>Matrix Table</u>.

For example,

Sample	Positions	Raw	Concentration
🔵 Unknown1	A3	1.694	>1
	A4	1.766	>1

Here the Condition **x**>**1** and Label >**1** are specified for **Concentration** matrix. In this case the calculation of the concentration for positions **A3** and **A4** is reported as >**1**.

Where label has been applied on the data and there is a **{x}** reference associated with the Label, the numeric value appears in the Label, i.e.:

Sample	Positions	Raw	Concentration
🔵 Unknown1	A3	1.694	2.153>1
	A4	1.766	3.857>1

Sample Labels Examples

Condition	Label Applied If	
x<1	The value is greater than 1.	
and(x>0, x<1)	The value is in the 0–1 range.	
x <control1< th=""><th colspan="2">The value is greater than mean of Control1 sample group.</th></control1<>	The value is greater than mean of Control1 sample group.	
x>TOV1.d	The value is greater than the d coefficient of the 4PL curve-fit (assuming that the 1st transform is a Standard Curve Fit transform using the 4PL curve-fit). See Transform Output Variables for more details of how to refer to such results.	

Validation

MyAssays Desktop's Validation features can automatically check that measurements and results are within expected ranges. Any configured validation tests are performed as part of each assay run with the results (pass or fail) being clearly presented as part of the output report.

For example, a validation can test that the %CV of the replicates of all sample groups is less than 15.

Validation Definition

A validation is defined using a Boolean validation condition. If the condition evaluates to true, then the validation rule is considered to be satisfied and the result for the validation is **Pass**. If the condition evaluates to false, the validation test result is **Fail**.

The validation condition is specified using <u>an expression</u> along with a matrix reference and type. For example, the validation condition expression:

x < 15

When associated with the %CV matrix and evaluated on all groups will check that the %CV of all sample groups is < 15.

Validation rules are arranged by <u>validations tables</u>. You can add as many validations rules as you need to each validation table. You can create as many validations tables as you need. It is useful to group related validations into validations table accordingly.

Each validation table is a separate report element so can be configured separately. For example, you might want each validation table to appear on a different sheet in the report.

Working with Validations

The following sections explain how to add, define, and review validation rules. Also, example validation expressions are provided:

How to: Add Validation to an Assay

- 1. Launch an assay protocol or open a results file.
- 2. If you cannot see the Assay Properties panel, press the Properties button to open it.

- Select the Validation tab (If the Validation tab is not immediately visible, press the << Show All Properties button to expand.)
- 4. Press the **Add** button on **Tables** pane.
- 5. Review and edit the validation's properties as required.

Validation Configuration

The Validation tab consists of three panes:

- **Tables** For managing the validation tables.
- **Conditions** For managing the validation conditions.
- Condition Configuration For specifying a validation condition.

Tables

The Tables pane defines the validation tables that are available for inclusion in the report.

Each validation table is given a unique identifier (e.g., ValidationsTable1).

Use the **Add** and **Remove** buttons to define the number of tables required.

You may prefer to include all conditions in one single table or arrange conditions between multiple tables.

The ordering, settings, and inclusion of each Validation Table is controlled via the **Report** contents.

Conditions

The **Conditions** pane defines each validation condition for the selected validations table.

Use the **Add** or **Remove** buttons to add conditions as required. Use the Up and Down buttons to set the order in which the conditions will appear in the table.

For each condition, a description can be specified under the **Description** column. Use this to provide a clear textual description; this will be used in the report to convey what has been validated.

For each condition, select which Matrix the condition applies to.

For example, if you plan to validate measurement data (i.e., a Raw matrix), select the Raw matrix to validate. Any of the raw or calculated matrices that contain endpoint data can be validated. Also, it is possible to validate non-matrix data (such as transform output variables).

If there are no available matrices, then the (NA) label appears. In this case validation conditions can still be created that do not refer to endpoint matrix data.

Validation conditions are not applicable to XY data matrices. To validate XY data, use an <u>XY reduction transform</u> and validate the endpoint result.

Conditions Configuration

The **Conditions Configuration** pane is used to specify the selected validation's condition.

The **Expression** setting is used to define the validation expression Boolean condition. This will typically be an expression with a <u>logical operator</u> and perhaps a <u>conditional function</u>.

The interpretation of the expression depends on the **Type** setting:

- **Position** The expression is evaluated for every position on the matrix of every container.
- **Group** The expression is evaluated for every sample group on the matrix of every container. (A sample group refers to the average of the replicates that belong to the group that are not flagged).
- **Single** the expression is evaluated once (useful for checking specific sample groups).

Single type expressions are the simplest; they define a single expression that is evaluated once on the specified matrix of each container.

For example, **Standard1 < Standard2** is an example of a **Single** type expression that validates that the value of Standard1 is less than Standard2 on the specified matrix. (For multiple plate assays, this condition will be evaluated on every plate.)

For **Position** or **Group** expressions, the expression will include an **x** reference, and in these cases the <u>x variable</u> refers to each position or each group. For example, if you wanted to check that the value at every measured position in a matrix is greater than 0.5, then use the **Position** setting with the expression: **x** > **0.5**. In this case every data point (on the selected matrix) is validated.

Similarly, if you wanted to check that the average value of each group in a matrix is less than 3, use the **Group** setting with the expression x < 3. In this case the average of every sample group (on the selected matrix) is validated.

The **Types** setting is used to specify which sample types a **Position** or **Group** expression will be evaluated 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 to select which types to validate.

Validating Non-Matrix Data

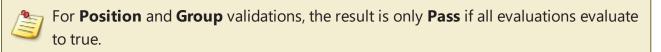
In addition to validating matrix data, it is also possible to validate other types of numerical data. For example, you may want to verify that the R² value of a curve fit is greater than a specific threshold. These types of validations can be defined using the **Single** type and referring to the data to validate within the expression. This will typically be using a Transform Output Variable (TOV). The TOVs that are available will depend on your configured transforms.

For example, the expression **TOV1.R2** > **0.9** will check 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).

To apply any new or modified settings, press the **Calculate** button and inspect the report to see the results. Each validation test verifies the result in terms of the specified Boolean condition.



The results will appear in the associated <u>validation table</u> in the report and will be marked as **Pass**, **Fail**, or **Inconclusive** accordingly:

Pass

If the validation condition is evaluated as true (the numerical result not equal 0).

Fail

If the validation condition is evaluated as false (the numerical result equals 0). For **Fail**, the whole validation table row is highlighted with yellow background.

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).



All validations are evaluated (irrespective of whether they are included in the report). You may <u>configure how and if the Validation Tables are reported</u>. In any case, the results of each validation test are listed in the Calculation log.

Examples

Here are examples of validation rules along with descriptions of their behavior. They apply the validation on the selected data matrix.

Туре	Condition	Validates
Position	x < 2.5	All positions (that are not flagged) have a value less than 2.5.
Position	and (x > 0, x < 2.5)	All positions (that are not flagged) have a value less than 2.5.
Group	x < 2.5	The average of the replicates (that are not flagged) of all sample groups is less than 2.5.
Position	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.
Single	Control1 < 1.5	The average of Control1 replicates (that are not flagged) is less than 1.5.
Single	sd(Control1)<10	The standard deviation of the Control1 replicates (that are not flagged) is less than 10.
Single	pcv(Control1)<15	The %CV of the Control1 replicates (that are not flagged) is less than 15.
Single	median(Control1)>0.75	The median of the Control1 replicates (that are not flagged) is greater than 1.5.
Single	TOV1.R2 > 0.9	The R ² value of the curve fit (as used in the first

Туре	Condition	Validates
		transform) is greater than 0.9. (The R ² value is
		referenced using a Transform Output Variable).

Evaluation

MyAssays Desktop's Evaluation feature can be used to calculate and present specific quantities of interest in the report. For example, the limits of detection of an assay could be computed and reported using an evaluation.

Evaluations are expression-based calculations arranged as <u>Evaluation Tables</u> for inclusion in the report. Any value that can be referenced or computed can be included in an Evaluation table.

Unlike the <u>Sample Table</u> or <u>Matrix Table</u> report elements (which display the results of a calculation performed on each sample), an Evaluation Table reports calculations that are not computed for every sample.

If you want to perform a calculation with a result for every sample, instead of using an evaluation, use a transform with the results for each sample displayed in the Sample Table or Matrix Table.

Evaluation Definition

A single evaluation is defined using <u>an expression</u> and can be given a description to accompany its result.

Evaluations are arranged by Evaluation Tables. You can add as many evaluations as you need to each Evaluation Table. You can create as many tables as you need. It is useful to group related evaluations into Evaluation Tables accordingly.

Working with Evaluations

The following sections explain how to add, define, and review evaluations. Also, example evaluations expressions are provided.

How to: Add an Evaluation to an Assay

- 1. Launch an assay protocol or open a results file.
- 2. If you cannot see the Assay Properties panel, press the Properties button to open it.
- Select the Evaluation tab (If the Evaluation tab is not immediately visible, press the << Show All Properties button to expand).
- 4. Press the **Add** button on **Tables** pane.
- 5. Review and edit the evaluation's properties as required.

Evaluation Configuration

The **Evaluation** tab consists of three panes:

- Tables For managing the evaluation tables
- Evaluations For managing the evaluations
- Expression Configuration For specifying the evaluation expression

Tables

The **Tables** pane defines the Evaluation Tables that are available for inclusion in the report.

Each table is given a unique identifier (e.g., EvaluationsTable1).

Use the Add and Remove buttons to define the number of tables required.

You may prefer to include all evaluations in a single table or arrange evaluations between multiple tables.

The ordering, settings, and inclusion of each Evaluation Table is controlled via the **<u>Report contents</u>**.

Evaluations

The **Evaluations** pane defines each evaluation for the selected evaluations table.

Use the **Add** or **Remove** buttons to add evaluations as required. Use the Up and Down buttons to set the order in which the evaluations will appear in the table.

For each evaluation, a description can be specified under the **Description** column. Use this to provide a clear textual description; this will be used in the report to convey what has been evaluated.

For each condition, select which **Matrix** the condition applies to. 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 validate non-matrix data (such as transform output variables).



If there are no available matrices, then the (NA) label appears. In this case evaluations can still be created that do not refer to endpoint matrix data.

Evaluations are not applicable to XY data matrices. To perform an evaluation on XY data, first use an <u>XY reduction transform</u> and evaluate the endpoint result.

Expression Configuration

The **Expression Configuration** pane is used to specify the selected evaluation's expression.

The **Expression** setting is used to define the expression to evaluate. This might reference a specific sample group or position on the selected matrix. It might also perform a calculation or function. Refer to the <u>expression</u> section for a complete description of available expression functionality.

The **Numerical Formatting** options are used to specify how the computed value is presented:

Numerical Formatting

Precision	The number of digits to be displayed for a numerical value. The interpretation of Precision depends on the Type setting.	
	Specifies how a numerical value is formatted with regard to precision and rounding options:	
Туре	 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. 	

Evaluation Results

Evaluations are computed when the data analysis is performed (after all transform calculations). To apply any new or modified settings, press the **Calculate** button and inspect the report to see the results.

Each evaluated expression results in a single numeric value. If there is an error in evaluating the expression, then the error message is reported (in red) as the result.

All evaluations are performed (irrespective of whether they are included in the report). You may <u>configure how and if the Evaluation Tables are reported.</u> In any case, the results of each evaluation test are listed in the Calculation log.

Examples

Here are examples of evaluations along with descriptions of their behavior. They apply the evaluation on the selected data matrix.

Evaluates	Computes
Control1	The average of all Control1 replicates (that are not flagged)
Control1/Control2	The ratio of Control1 and Control2
sd(Control1)	The standard deviation of the Control1 replicates (that are not flagged)
pcv(Control1)	The %CV of the Control1 replicates (that are not flagged)
Unknown	The average of all replicates of all Unknown samples (that are not flagged)
median	The median of all Unknown sample measurement (that are not
(Unknown)	flagged)
TOV1.R2	The R ² value of the curve fit as used in the first transform (referenced using a Transform Output Variable)

Report Content

Overview

The primary output of the data analysis process is the report. MyAssays Desktop provides comprehensive functionality to configure the report's:

- content
- presentation
- export

This section describes how to configure the content that is included in the report.

Within the MyAssays Desktop Application, the report can be displayed as **Sheet View** or **Page View**; these settings are available in the **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 <u>reporting</u> <u>template</u> to apply to your MyAssays Desktop output).

Report Content Definition

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):

- <u>Transforms</u> Any configured transforms will result in calculated elements (such as numerical results or charts).
- <u>Matrices</u> 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 colored value bars.
- **Evaluation** Any configured evaluation settings will result in one or more evaluation tables, each listing the results of the computed evaluations.
- <u>Validation</u> Any configured validation settings will result in one or more validation 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 Standard Curve Fit transform is configured, then the calculated concentrations will be available for

inclusion in a Sample Table and/or Matrix Table, along with details of the fit (including standards chart and equations), each of which can be further configured.

The configuration and ordering of each report element is defined in the **Report** tab.

How to: View Report Tab

- 1. Launch an assay protocol or open a results file.
- 2. If you cannot see the Assay Properties panel, press the Properties button to open it.
- Select the **Report** tab (If the **Report** tab is not immediately visible, press the << **Show** All **Properties** button to expand).
- 4. Review and edit the report's elements as required.

Report Element Configuration

The **Report** tab consists of two panes:

- **Reports** This pane manages the report elements and their ordering.
- **Report Configuration** This displays the available settings for the selected report element.

Reports Pane

The **Reports** pane lists the report elements that will be included in the report. Their ordering reflects the order in which they will be added to the report.

Use the **Add Element** button 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.

Use the **Remove** button 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 elements:

General

	Id	Each report element is automatically given a unique identifier (ld). This
Ια	ia	identifier is used to identify the report element when configuring custom

	report templates (It is also used for advanced settings such as custom export and XML configuration).
	For general use, it should not be necessary to change the name of the identifier.
Include	This checkbox can be used to exclude the report element (without deleting the settings).
	Optionally provided title for the report element. When specified, the title appears immediately before the element.
Title	This property can refer to report macros (such as {PlateNum} and {AnalyteName}) to automatically refer to the data that it is associated with.
	If a title is not specified, then the element is displayed without any title.

<u>Sheet</u>

	This setting defines the name of a sheet to insert the contents into. This is only applicable to sheet-based content such as Excel output or data in the Sheet View .
Sheet Name	Each report element can be included on its own separate sheet or elements can be added together on the same sheet. Where a common sheet name is used across report elements, the elements are added in the order as defined in the Reports pane.
	This property can refer to report macros (such as {PlateNum} and {AnalyteName}) to automatically group associated content into a sheet of the same name.
	Note, if Sheet Name is defined for one report element, then the report 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 comprises 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, it is recommended to use the **Sheet Name** setting; this can be used 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 sheet tab named on by the plate number (i.e., Plate 1, Plate 2, Plate 3, etc.).

Report Macros

The following table lists the macros that are available for use in the **Title** and **Sheet Name** properties.

Macro	Expands To
{PlateNum}	1-based plate number
{ContainerNum}	1-based container number (for a multiple plate assay, this is the plate number; for multiplex, this is the analyte number)
{PlateName}	The plate name (as it appears under the Measurements Plate IDs)
{AnalyteName}	The analyte name (as it appears under the Measurements Analytes)
{ContainerName}	For a multiple plate assay, this is the plate name; for multiplex, this is the analyte name

These macros can be used in conjunction with other text (e.g., **Plate {PlateNum}** would 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 <u>Sample Table</u> 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 <u>Evaluation</u> or <u>Validations</u> are configured, an Evaluation or Validation report element will be added automatically.
- If any transform generates additional content, a <u>Transform Content</u> 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 **Reports** 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 be automatically removed too.

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

- Open the **Report** tab (If the **Report** tab is not immediately visible, press the << **Show** All **Properties** button to expand).
- 2. Select the required report element from the **Add Element** drop-down list in the **Reports** pane.
- 3. Review and edit the element's settings as required.
- 4. Press the **Calculate** button to perform calculations with the new settings.

The following table describes each report element in more detail along with any applicable settings.

Report Element	Description	Settings
<u>Evaluations</u> <u>Table</u>	The details and results of any configured evaluations arranged in a table.	Include Description – Include the description column. Include Expression – Include the expression column. Include Header – Include the table header.
<u>Histogram</u>	The Histogram represents the data by displaying the average of each sample in	Matrix – The matrix of data to plot.

Report Element	Description	Settings
	vertical columns. Each sample is denoted by a bar of the color of its sample type. In addition, to indicate the error or uncertainty in a reported measurement or calculated data, the variability of data can be represented with optional error bars.	 Error Bars – Use this setting to convey accurate range of +/- data error. Title – The title for a histogram chart. Types – Which sample types to include in the histogram.
<u>Layout</u>	A graphical representation of the sample layout.	Size – Small , Medium , or Large (medium size will fit on a page-width).
	The results displayed 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.	Arrangement – Select Combined for each cell in the table to list the values of all included matrices. Or select Separate for a separate table for each included matrix.
<u>Matrix Table</u>	For endpoint data, a numerical value is reported. For XY data, a thumbnail plot is displayed.	Thumb Plot – For XY data, various options for defining the inclusion and sizing of XY thumb plot charts.
	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 .	Thumb Scale – For XY data, various options for defining how XY thumb plot charts are scaled.
	A layout-based graphical representation of the matrix data.	Matrix – The matrix of data to plot.
Matrix View	For endpoint data, a numerical value will appear at each position. For XY data, a plot will appear at each position.	Size – Small , Medium , or Large (medium size will fit on an A4 page-width).
	The color of each position can be colored using the layout color or with a <u>heatmap</u> to represent the endpoint value intensity.	Coloring – Layout or Heatmap (Heatmap setting does not apply to XY data).

Report Element	Description	Settings
<u>Measurement</u> <u>Meta Data</u>	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.	(No additional settings.)
<u>Notes</u>	Any notes associated with the assay run. Notes can be entered using the Notes button in the View section	Exclude if Empty – If this option is set, then this element is only included if notes are
	of the Home tab. Inter-assay quality control results (as	entered.
QC Results	defined by Quality Control settings). This could include Observation Table, Levey- Jennings, or CUSUM charts reporting the latest results along with historical results.	(No additional settings.)
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. For endpoint data, a numerical value is reported. For XY data, a thumbnail plot is displayed. 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 .	 Include Positions – Include the Positions column to report the sample positions where the sample was measured. Replicate Orientation – How the replicates appear in the table. Types – Which sample types to include in the sample table. Thumb Plot – For XY data, various options for defining the inclusion and sizing of XY thumb plot charts. Thumb Scale – For XY data, various options for defining how XY thumb plot charts are scaled.
Transform	Various report elements generated as a	Content – Use this setting to

Report Element	Description	Settings
<u>Content</u>	consequence of a specific transform. For example, a <u>Standard Curve Fit</u> <u>transform</u> adds a concentration table, a chart, and equations to the report as Transform Content report elements.	specify which sub-elements to include (or exclude) and their ordering. The available sub- elements depend on the associated transform.
<u>Validations</u> <u>Table</u>	The details and results of any configured validations arranged in a table. Each validation result is reported as a pass or fail.	Include Description – Include the description column. Include Expression – Include the expression column.

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.

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 lds correspond to the report element lds of the corresponding Sample Table or Matrix Id (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.

Evaluations Table

How to: Add Evaluations Table Element to the Report

- Open the **Report** tab (If the **Report** tab is not immediately visible, press the << **Show** All **Properties** button to expand).
- 2. Select the Evaluations Table element from the **Add Element** drop-down list in the **Reports** pane.
- 3. Review and edit the settings as required.
- 4. Press the **Calculate** button to perform calculations with the new settings.

Depending on the <u>configuration of the Evaluation settings</u>, 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 unchecking corresponding checkboxes.

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 <u>Report Content</u> section.

The Evaluation Table 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

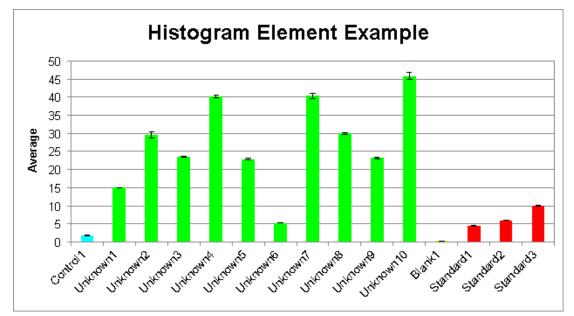
Here, the **Description** column contains the description if specified (e.g., on the third row in this example) or **Expression#{index}** if it is not. The **Expression** column contains the evaluated expression and the **Results** display the computed result of each configured Evaluation.

Histogram

A histogram can be use to graphically present the spread of measurements or results. This can be useful for spotting outliers.

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).

For example, the following Histogram was generated using a configuration to plot the Average values of Control, Unknown and Standards with error-bars indicating the standard deviation and a custom title.



How to: Add Histogram Element to the Report

- Open the **Report** tab (If the **Report** tab is not immediately visible, press the << **Show** All **Properties** button to expand).
- 2. Select the Histogram element from the **Add Element** drop-down list in the **Reports** pane.
- 3. Review and edit the settings as required.
- 4. Press the **Calculate** button to perform calculations with the new settings.

The Histogram is configured with the common report settings as covered in the <u>Report</u> <u>Content</u> section plus the Histogram specific settings described below.

General

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<u>Histogram</u>

Error Bars	Use this setting to select the type of error bars to use (or none). Error bars can display the Standard Error , %CV , or Standard Deviation of the (not-flagged) replicates of the group. If the Replicates option is selected the error bars reflect the minimum and maximum value of the replicates of the sample. With the None selection, the error bars are not shown.
Title	The title for a histogram chart.
Types	Which sample types to include in the histogram.

Layout

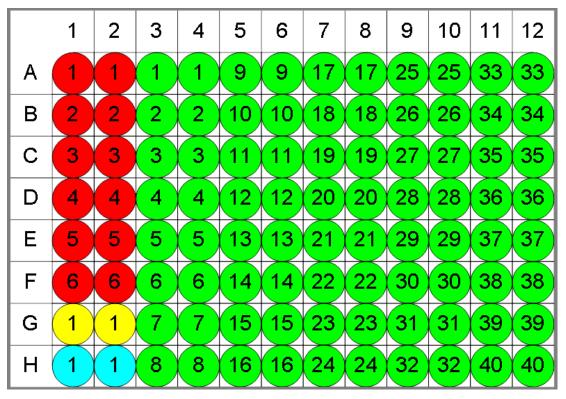
How to: Add Layout Element to the Report

- Open the **Report** tab (If the **Report** tab is not immediately visible, press the << **Show** All **Properties** button to expand).
- 2. Select the Layout element from the **Add Element** drop-down list in the **Reports** pane.
- 3. Review and edit the settings as required.
- 4. Press the **Calculate** button to perform calculations with the new settings.

As with other Report Elements, you can configure how it appears in the report using <u>common</u> <u>report settings</u>.

In addition, Layout has a configurable **Size** setting. You can select **Small**, **Medium**, or **Large** option (medium size will fit on a page-width). If multiple layouts are defined in the assay protocol, then all of them will be included in the report.

The Layout report element adds an image of the sample layout used in the assay to the report. This includes a layout itself and a legend with the sample types that are included in the layout. I.e.:





Matrix Table

How to: Add Matrix Table Element to the Report

- Open the **Report** tab (If the **Report** tab is not immediately visible, press the << **Show** All **Properties** button to expand).
- 2. Select the Matrix Table element from the **Add Element** drop-down list in the **Reports** pane.
- 3. Review and edit the settings as required.
- 4. Press the **Calculate** button to perform calculations with the new settings.

Matrix Table lists the results of the performed analysis in a matrix-table structure. Flagged data is displayed as defined by the associated <u>transform</u> and <u>matrix configuration</u>. Each cell in the table corresponds to the associated position on the matrix.

The Matrix Table is configured with specific to this report element and with <u>general report</u> <u>configuration settings</u>.

Matrix Table

The matrix table(s) has 2 configurations that define how the results will be displayed.

Arrangement matrices (per plate/analyte). In this case, the final row of the contain a key that explains ordering of the matrices.	Select Combined for each cell in the table to list the values of all included
	matrices (per plate/analyte). In this case, the final row of the table will
	contain a key that explains ordering of the matrices.
	Or select Separate for a separate table for each included matrix.

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.

Include Thumb	Defines whether thumb plots are included in the report.
Plots	
Thumb Plot Height	This specifies the height to use for all thumbs in the report.
Thumb Plot	This specifies the width to use for all thumbs in the report.

Width

For XY data, various options for defining how XY thumb plot charts are scaled. These can be fixed values (or determined for each position). The plot lines are plotted within this scan.

Thumb Scale

	Specifies whether the maximum X scale of each plot is:
T L	• Fixed – The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Max X value.
Thumb Auto Scale Max X	 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).
	• Table – The scale limit of every thumb plot in the table is the same. This is determined from the maximum X of all plots data points in the table.
	Specifies whether the maximum \mathbf{Y} scale of each plot is:
Thumb	• Fixed – The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Max Y value;
Auto Scale Max Y	 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).
	• Table – The scale limit of every thumb plot in the table is the same. This is determined from the maximum Y of all plots data points in the table.
	Specifies whether the minimum X scale of each plot is:
Thumb	• Fixed – The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Min X value.
Thumb Auto Scale Min X	 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).
	• Table – The scale limit of every thumb plot in the table is the same. This is determined from the minimum X of all plots data points in the table.
Thumb	Specifies whether the minimum Y scale of each plot is:

Auto Scale Min Y	 Fixed – The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Min Y value. 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). 			
	 Table – The scale limit of every thumb plot in the table is the same. This is determined from the minimum Y of all plots data points 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 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 Y is Fixed .			

For example,

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
Ε	1.105	1.065	0.279	0.367	1.215	1.225	1.233	1.262	0.111	0.114	1.579	1.549
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
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
н	0.253	0.270	0.463	0.470	1.214	1.261	0.115	0.116	0.115	0.115	0.490	0.477

This is how 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 Matrix Table for each included matrix) or in a combined arrangement. For example, here is a snippet of a Matrix Table for the <u>XY Reduction</u> <u>Average</u> raw and calculated data:

	1	2	3	4	5	6
A						\square
	4.318	4.460	15.053	15.003	29.063	30.118
В						
						\leq
	6.045	6.003	23.532	23.636	40.364	39.902
С						
	9.923	10.092	22.744	23.132	5.246	5.273
D			\square	\square		
			Ľ			
	1.809	1.840	40.937	39.919	29.764	30.177
E					\square	\square
						\leq
	0.057	0.057	23.440	22.989	45.226	46.526
Key:	Raw					
	Average	e				

Each cell contains the thumbnail plot for corresponding input XY position and the calculated numerical result.

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.

Matrix View

How to: Add Matrix View Element to the Report

- Open the **Report** tab (If the **Report** tab is not immediately visible, press the << **Show** All **Properties** button to expand).
- 2. Select the Matrix View element from the **Add Element** drop-down list in the **Reports** pane.
- 3. Review and edit the settings as required.
- 4. Press the **Calculate** button to perform calculations with the new settings.

Matrix View displays the data of a specified matrix, using coloring as either Layout or <u>Heatmap</u>. All corresponding settings of the associated matrix are applied to the view too. <u>Flagged positions</u> are indicated and displayed with a cross-through it.

Matrix View includes the following configurable settings:

General

Matrix	The matrix of data to plot. Select the relevant matrix name from a drop-down list.
Size	Small, Medium, or Large (medium size will fit on an A4 page-width).

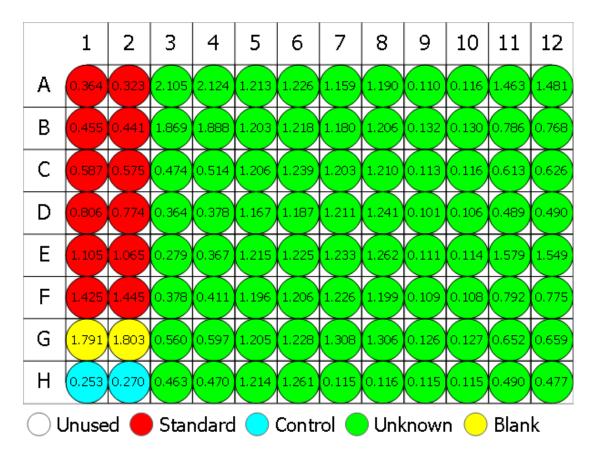
Matrix View

Colouring	Layout or Heatmap (Heatmap setting does not apply to XY data).
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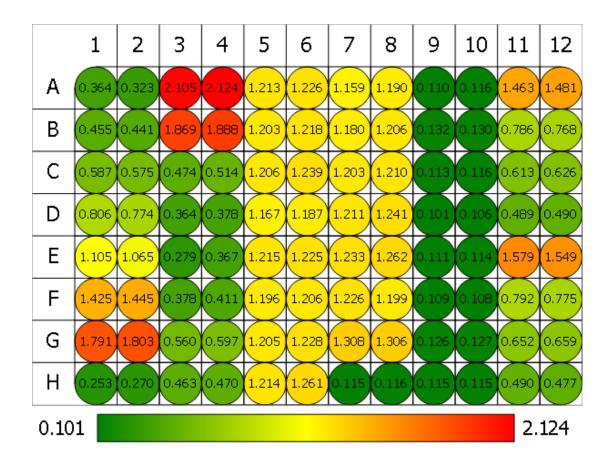
The Matrix View is also configured with common report settings that are covered in the <u>Report Content</u> section.

Matrix view can be configured with coloring 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. E.g.:



• **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. E.g.:



Measurement Meta Data

How to: Add Measurement Meta Data Element to the Report

- Open the **Report** tab (If the **Report** tab is not immediately visible, press the << **Show** All **Properties** button to expand).
- 2. Select the Measurement Meta Data element from the **Add Element** drop-down list in the **Reports** pane.
- 3. Review and edit the settings as required.
- 4. Press the **Calculate** button to perform calculations with the new settings.

The Measurement Meta Data is configured with common report settings that are covered in the Report Content section.

If there is any meta data associated with your measurement data, then it can also be added to the generated report. This data is reported in a two-column table with the name of each entry and its value. For example,

Data Filename	C:\\Documents\MyAssays\Measurements\ELISA Absorb- 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- 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 meta data table features details about the measurements data file and the details obtained from the VICTOR plate reader.

Notes

How to: Add Notes to the Analysis

- 1. Launch an assay protocol or open a results file.
- 2. In the **MAA Ribbon** click on the **Notes** button of the **Home** tab's **View** group.
- 3. The Notes panel will appear down-most of the screen.
- 4. Enter or paste any Notes as required.

For a particular assay run you may optionally provide notes to store alongside results in the report (e.g., preparation notes, Lot ID, etc.). This can be used to reference the peculiarities of this data analysis.

How to: Add Notes Element to the Report

- Open the **Report** tab (If the **Report** tab is not immediately visible, press the << **Show** All **Properties** button to expand).
- 2. Select the Notes element from the **Add Element** drop-down list in the **Reports** pane.
- 3. Review and edit the settings as required.
- 4. Press the **Calculate** button to perform calculations with the new settings.

Entered Notes will appear under Notes: title.

Notes

Notes element includes the following checkbox setting:

Exclude IfThis setting automatically excludes Notes from the results, if it contains noEmptyinformation.

The Notes element is also configured with common report settings that are covered in the <u>Report Content</u> section.

Sample Table

How to: Add Sample Table Element to the Report

- Open the **Report** tab (If the **Report** tab is not immediately visible, press the << **Show** All **Properties** button to expand).
- 2. Select the Sample Table element from the **Add Element** drop-down list in the **Reports** pane.
- 3. Review and edit the settings as required.
- 4. Press the **Calculate** button to perform calculations with the new settings.

Sample Table lists the results of the performed data analysis by sample groups. Flagged data is displayed as defined by the associated <u>transform</u> and <u>matrix configuration</u>. Each row in the table corresponds to a sample.

The Sample Table is configured with general report and specific to this element settings. The former are described in <u>general report configuration settings</u> and the latter are covered below.

Sample Table

Include Pos- itions	Include the Positions column to report the sample positions where the sample was measured.
Replicate Orientation	 How the replicates appear in the table: Vertical – Each position is listed in a new line of the column. Horizontal – All positions are listed in the same line.
Types	Which sample types to include in the sample table.

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.

Include	
Thumb	Defines whether thumb plots are included in the report.
Plots	
Thumb Plot	This specifies the height to use for all thumbs in the report.

Height	
Thumb Plot Width	This specifies the width to use for all thumbs in the report.

For XY data, various options for defining how XY thumb plot charts are scaled. These can be fixed values (or determined for each position). The plot lines are plotted within this scan.

Thumb Scale

	Specifies whether the maximum X scale of each plot is:
Thumb	• Fixed – The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Max X value.
Auto Scale Max X	 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).
	• Table – The scale limit of every thumb plot in the table is the same. This is determined from the maximum X of all plots data points in the table.
	Specifies whether the maximum ${f Y}$ scale of each plot is:
Thumb	• Fixed – The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Max Y value;
Auto Scale Max Y	 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).
	• Table – The scale limit of every thumb plot in the table is the same. This is determined from the maximum Y of all plots data points in the table.
	Specifies whether the minimum X scale of each plot is:
Thumb	• Fixed – The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Min X value.
Auto Scale Min X	 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).
	• Table – The scale limit of every thumb plot in the table is the same. This is

	determined from the minimum ${f X}$ of all plots data points in the table.			
	Specifies whether the minimum Y scale of each plot is:			
Thumb	 Fixed – The scale limit of the thumb plots used in the table is specified by the associated Thumb Scale Min Y value. 			
Auto Scale Min Y	 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). 			
	• Table – The scale limit of every thumb plot in the table is the same. This is determined from the minimum Y of all plots data points 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 Y is Fixed .			
Thumb	Determines custom minimum X scale for thumb plots if Thumb Auto Scale			
Scale Min X	Min X is Fixed.			
Thumb Scale Min Y	Determines custom minimum Y scale for thumb plots if Thumb Auto Scale Min Y is Fixed .			

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 snippet of five Standard samples and the positions they are designated to. Also, the raw values (**My Raw Data** column) for each replicate and the calculated results per sample

for the <u>Percentage Coefficient of Variation</u> transform (**%CV** column) are included. Each row contains replicates of the corresponding sample in the **Vertical** orientation.

For XY data, the Sample Table contains **Thumb Plots** that is simply a thumbnail graphical representation of each kinetic/spectral data plot at a position.

Sample	Positions						Raw	Average
Unknown1	A1	A2	A3	A4	A5	A6		\geq
Unknown2	C1	C2	C3	C4	C5	C6		
Unknown3	E1	E2	E3	E4	E5	E6		\square
Unknown4	G1	G2	G3	G4	G5	G6		
 Blank1 	B1	B2	B3	B4	B5	B6		
Blank2	D1	D2	D3	D4	D5	D6		
OBlank3	F1	F2	F3	F4	F5	F6		
OBlank4	H1	H2	H3	H4	H5	H6		

Here the replicates for each sample group, XY Raw data and calculated results for <u>XY</u> <u>Replicates Average</u> transform are displayed in **Horizontal** orientation.

Transform Content

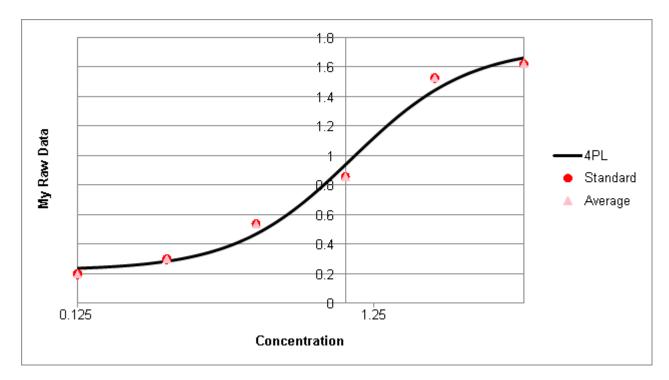
Some transforms can output additional items in the report. If the transform applied to the assay possesses any kind of optional items, they are added to the report automatically. Certain transforms comprise a number of optional content.

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, the <u>Dilution Factors</u> transform outputs optional report content named Factor Column.

Sample	Positions	Factor	My Raw Data	Concentration (x Dil. Factor)
Unknown1	A3	2	2.105	4.210
	A4		2.124	4.248
Unknown2	B3	5	1.869	9.345
	В4		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

Chart – The visual representation of a plotted curve that is included in a report by default.
 E.g., here is a chart for <u>Standard Curve Fit</u> transform example.



- **Table** The table containing additional values computed from a transform. For example, this might refer to Goodness Measures Table, <u>Dilutions Table</u>, Curve Data Table, etc.
- **Equation** The formula of a fit method that was used to plot a curve. E.g., the formula for <u>Linear Regression</u>:

y = mx + c

A report can contain one or more **Transform Content** sub-elements.

How to: Configure Transform Content in the Report

- 1. Open the **Report** tab.
- 2. Select Transform Content element, which contains transform name in the title, e.g. **TransformContentDilutionCurves1**.
- 3. Click on a drop-down of **Content** option of the **Report Configuration** pane.
- 4. Review and edit content items as required.
- 5. Press **Calculate** button to perform calculations with the new settings.

The Histogram is configured with common report settings that are covered in the <u>Report</u> <u>Content</u> section and the setting described below.

	Use this setting to specify which sub-elements to include (or exclude) and their ordering. The available sub-elements depend on the associated
	transform.

You can configure which optional items are actually included in report with the **Content Editor**. Select the sub-element, move in to the Included/Excluded pane with right/left arrow buttons, or change its' positioning with the Up and Down buttons on the right of the Included pane.

To include or exclude all transform content from the report, use the **Add All** or **Remove All** buttons, respectively.

Validation Table

How to: Add Validations Table Element to the Report

- Open the **Report** tab (If the **Report** tab is not immediately visible, press the << **Show** All **Properties** button to expand).
- 2. Select the Validations Table element from the **Add Element** drop-down list in the **Reports** pane.
- 3. Review and edit the settings as required.
- 4. Press the **Calculate** button to perform calculations with the new settings.

Validations Table indicates whether each validation has passed or failed and, when <u>Validations</u> are defined, is added to the report automatically.

By default Validation table in Report is displayed as three-column table with the number of rows corresponding to the number of **Conditions** added. The columns are **Results**, **Expression** and **Description**.

While the **Results** are definitely included to the report, you might prefer to hide former properties by unticking corresponding checkboxes.

Include	When checked, the column contains Description if specified or Condition#			
Description	{index} if it was not.			
Include	If included, this outputs the Condition defined for each Validation .			
Expression	In included, this outputs the Condition defined for each validation .			

The Validation Table is also configured with common report settings that are covered in the <u>Report Content</u> section.

Results	Expression	Description
🔀 Fail	x < 2.5	All non-flagged positions have a value less than 2.5 (Pos- ition type)
✔ Pass	x < 2.5	The average of the replicates (that are not flagged) of all sample groups is less than 2.5 (Group type)
✓ Pass	Unknown1 > 1.5	Condition #3

The Validation Table typically consists of three columns. For example,

Results	Expression	Description
? Syntax Error	Control1 !< 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 color, 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.

Report Template

Overview

The report template enables presenting your analysis results tailored to your laboratory's standards and requirements. This feature merges together <u>report elements</u> with a preconfigured template. Full power of MS Word or WordPad features is available to customise page-based reporting of the results.

For example, you can define custom sequence and/or inclusion of report elements, insert relevant images or textual descriptions for a printed document. Moreover, simply apply styling settings and include the results you require for your current purpose.

Report template affects Page View and page-based outputs (DOC, DOCX, PDF) of the analysis results only.

Report Template Configuration

How to: Add New Report Template for Page-Based Reporting

- 1. Launch an assay protocol or open a results file.
- Select the Ribbon's **Report** tab and switch to the **Page View** of the results (If the **Report** tab is not immediately visible press the << **Show All Properties** button to expand).
- 3. Hover to **Template** group and press **New** button. This launches the Template.DOCX file with 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 press **Calculate** to generate a new report with the new settings.

The application displays a Template.DOCX file for editing – this is the template file. Initially, it contains the MyAssays <u>report element identifiers</u> (**Ids**) in curly braces.

Report templates have no restrictions with regard to the specific information they contain. You can make any changes to the template and save them. For example, reposition, remove or duplicate lds, change their font/style/etc., add textual information, macros etc. In fact, any change is possible provided it is supported in the editor.

How to: Add Report Element Id to the Report Template

- 1. Launch an assay protocol or open a results file.
- 2. Open **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 (e.g., **{SampleTable1}**).
- 5. Save changes to the report template and press **Calculate** to generate a new report with the new settings.

When you have made changes to the template file and saved them, you can switch back to MAA and press **Calculate** to generate a new report with the new settings while keeping the editor open. This way you can review modified report in Page View and make further changes if needed.

The defined template will be applied for all page-based reports for this assay results.

7 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:

- Press **Edit** button to modify you template file's content according to freshly added report elements or updated requirements. It will launch your default .DOCX editor with the existing template file.
- Press **Delete** button to remove the existing template file (a warning message will prompt for confirmation before this action is completed).

Reference

Transforms

Transforms can be described as a set 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 <u>Factor</u>). 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 <u>Factor</u> <u>transform</u> 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 <u>MyAssays Analysis Expressions</u> 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.

Transform Manager

Transforms are added and configured via the Transform Manager. The Transform Manager occupies the full width and height available for the transform tab's contents.

How to: Add the Transform

- 1. Launch an assay protocol or open a results file.
- 2. If you cannot see the Assay Properties panel, press the **Properties** button to open it.
- 3. Press the **Add** button.
- 4. Select the transform.
- 5. Press the **Create** button.
- 6. Review or edit the transform's input matrix and output matrix name as required.
- 7. Provide your required settings for the transform (see **Properties** section above).
- 8. Press the **Calculate** button 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 are required to be executed for a particular <u>data type</u>.

• 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.

On top of that, all transforms share the following common settings:

Advanced

Included Samples	The sample types to be computed by this transform.
	 Sample types not selected in the Included Samples option will not be computed by this transform; for those cases, this setting specifies what the result will be for those excluded samples: Flagged With Value – The result is flagged with the input value reported.
Excluded Results	This will appear in the report with the value with strikethrough, e.g., 1.23 .
	 Flagged No Value – The result is flagged with no value reported. This will appear in the report as Flagged.
	• Equal Input – The result is set to match the value of the input.

<u>Units</u>

	The measurement units to be included in the header of the <u>Sample Table</u> .
Output	If the output units of the transform are the same as the input, then use "ditto" units to automatically carry these units through (i.e., apply the same units to
Units	the transform's output). This shortcut means that it is not necessary to repeat settings across transforms. Ditto units are specified with two dots, i.e.:

In addition, the <u>XY transforms</u> and <u>XY Reduction transforms</u> 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 <u>Standard</u> <u>Curve Fit</u> 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. With MyAssays Desktop, you can layer as many transforms as you require to perform the various analysis steps that are required (resulting in multiple "intermediary endpoint" matrices).

Flagged Endpoints

All transforms take into account the <u>flagged status</u> 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 3rd replicate is flagged, then all the samples will be corrected by the average of the 2 not-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 <u>Auto Flag</u> and <u>Replicate Outlier Removal</u>).

Sample Types to Process

For all endpoint transforms, by default the calculations are performed on all sample types.

For greater flexibility, the **Advanced** settings of each transform can be used 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.

•	
Transform	Operation
<u>Auto Flag</u>	Flag all data points that satisfy the specified Boolean expression.
<u>Average</u>	Calculate the average of replicates for each sample group.
Blank	Subtract the specified blank value from each input data point.
<u>Correction</u>	
<u>Dilution</u>	Create a dilutions chart for each group of the specified sample type, fit selec-
Curves	ted curve-fitting method, and calculate the specified titer from the curve.
<u>Dilution</u>	Multiply sample groups of the selected type(s) by a specified factor (a unique
<u>Factors</u>	value can be specified for each group).
Expression	Evaluate the expression in terms of ${f x}$ and ${f y}$ for each sample group. The ${f x}$ and
<u>by Matrix</u>	${f y}$ variables refer to the values on the first and on the second input matrix,
(Dual)	respectively.
Expression	Evaluate the expression in terms of x .
by Matrix	
Expression	Evaluates an expression in terms of x for each specified position.
by Position	
Factor	Multiply all input data points of the specified sample type(s) by a specified factor.
Matrix	Calculate the difference between values on the first matrix and the second for
Difference	each sample group.
Median	Calculate the median of the replicates for each sample group.
Percentage	
Coefficient of	Calculate the %CV of the replicates for each sample group.
<u>Variation</u>	
<u>Percentage</u>	Calculates a percentage value for each data point with respect to the specified 0% and 100%.
Replicate	Elags the specified number of replicator for each sample aroun in which $\%$ CV
<u>Outlier</u>	Flags the specified number of replicates for each sample group, in which %CV of its replicates is greater than specified threshold.
Removal	
Standard	Constructs a standard curve by plotting the standard input concentration
<u>Curve Fit</u>	values vs data points.

Endpoint Transforms

Transform	Operation
Standard	Calculate the standard doviation of the replicates for each sample group
Deviation	Calculate the standard deviation of the replicates for each sample group.
Standard	
Error of	Calculate the standard error of the mean for replicates of each sample group.
Mean	
Variance	Calculate the variance of the replicates for each sample group.

Auto Flag

[Input: Endpoint Impoint [Input: Endpoint]

This transform flags positions where a <u>Boolean condition</u> is satisfied. This is useful for automatically rejecting and ignoring data points that are outside an expected range.

Properties

Settings

Auto Flag	The expression to evaluate, where a single \mathbf{x} variable is set to value of each position of the input matrix
	position of the input matrix

Example

Let's assume that we have some endpoint measurement data for a 12x8 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

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
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
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
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
н	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 $\mathbf{x} > \mathbf{1}$.

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
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
Е	1.105	1.065	0.279	0.367	1.215	1.225	1.233	1.262	0.111	0.114	1.579	1.549
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
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
н	0.253	0.270	0.463	0.470	1.214	1.261	0.115	0.116	0.115	0.115	0.490	0.477

Average

[Input: Endpoint I Output: Endpoint]

This transform calculates the average of the replicates in each sample group (<u>flagged</u> replicates are not included).

Example

Let's assume that we have some endpoint measurement data for a 12x8 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.

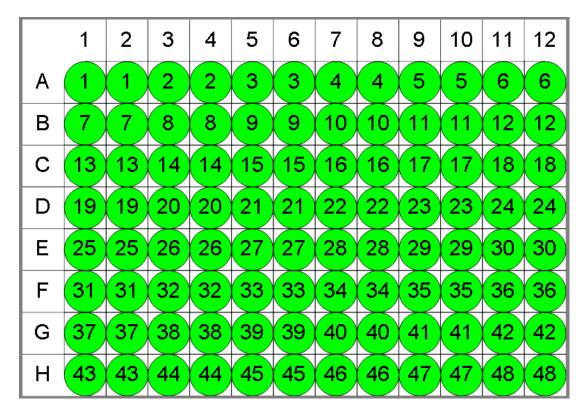
Here is some example raw data displayed to 3 decimal places:

Raw

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
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
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
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
н	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.). I.e.:



💛 Unknown

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 <u>Sample Table</u> 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	0.344
-	A2	0.323	
Unknown2	A3	2.105	2.115
-	A4	2.124	
Unknown3	A5	1.213	1.220
	A6	1.226	
OUnknown4	A7	1.159	1.175
	A8	1.190	
OUnknown5	A9	0.110	0.113
	A10	0.116	

• Matrix Table

Here all calculated data is displayed in a Matrix Table.

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.344	0.344	2.115	2.115	1.220	1.220	1.175	1.175	0.113	0.113	1.472	1.472
В	0.448	0.448	1.879	1.879	1.211	1.211	1.193	1.193	0.131	0.131	0.777	0.777
С	0.581	0.581	0.494	0.494	1.223	1.223	1.207	1.207	0.115	0.115	0.620	0.620
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
Е	1.085	1.085	0.323	0.323	1.220	1.220	1.248	1.248	0.113	0.113	1.564	1.564
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
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
н	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 I 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 (not-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 <u>Expression by Position transform</u>.

Properties

Settings

Blank Group	The group number of the sample to use for correction. This can be defined as a number or as <u>an expression</u> . The expression can refer to \mathbf{x} , which is the group number of the sample to be corrected. For example, set this to \mathbf{x} to perform a 1-to-1 correction where each group is corrected by the sample of 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 12x8 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:

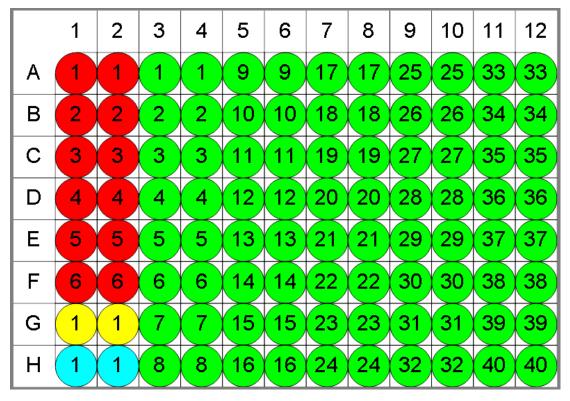
Raw

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.192	0.199	0.347	0.346	0.325	0.321	0.803	0.797	1.589	1.593	0.241	0.246
В	0.293	0.300	0.586	0.584	0.800	0.793	0.402	0.401	0.839	0.837	0.562	0.564
С	0.537	0.534	0.549	0.547	0.878	0.875	0.471	0.471	0.759	0.756	1.039	1.043
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
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
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
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
н	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.). I.e.:



Standard O Blank O Control O 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):

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.191	0.198	0.346	0.345	0.324	0.320	0.802	0.796	1.588	1.592	0.240	0.245
В	0.292	0.299	0.585	0.583	0.799	0.792	0.401	0.400	0.838	0.836	0.561	0.563
С	0.536	0.533	0.548	0.546	0.877	0.874	0.470	0.470	0.758	0.755	1.038	1.042
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
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
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
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
н	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 12x8 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.

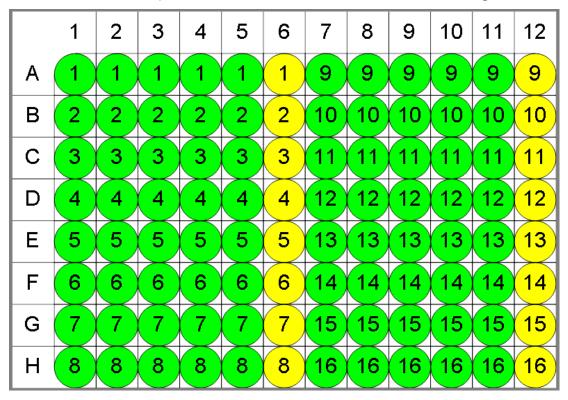
Here is some example raw data displayed to 3 decimal places:

Raw

	1	2	3	4	5	6	7	8	9	10	11	12
Α	1.962	1.999	2.038	1.922	1.995	0.002	1.628	1.670	1.624	1.554	1.701	0.000
В	0.784	0.760	0.754	0.751	0.797	0.002	1.124	1.104	1.072	1.077	1.106	0.002
С	0.586	0.575	0.582	0.590	0.576	0.001	0.724	0.737	0.690	0.711	0.717	0.002
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
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
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
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
н	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 l.e.:



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):

	1	2	3	4	5	6	7	8	9	10	11	12
Α	1.960	1.997	2.035	1.919	1.992	0.000	1.628	1.670	1.623	1.554	1.701	0.000
В	0.783	0.758	0.752	0.750	0.796	0.000	1.122	1.102	1.070	1.075	1.104	0.000
С	0.585	0.574	0.581	0.589	0.575	0.000	0.722	0.735	0.688	0.709	0.714	0.000
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
Е	1.955	1.969	1.873	2.042	1.958	0.000	1.448	1.478	1.427	1.394	1.384	0.000
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
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
н	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

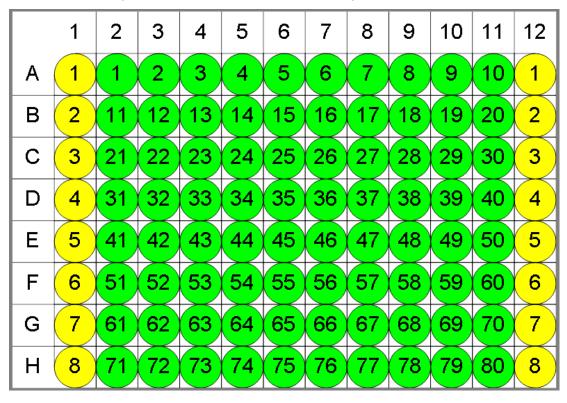
In this example we would like to configure blank correction on a row-by-row basis to blank correct each sample by the average of the two blank samples of the same row.

For better clearness of the process, this example uses simplified endpoint data for a 12x8 microplate:

	1	2	3	4	5	6	7	8	9	10	11	12
Α	1	1	2	3	4	5	6	7	8	9	10	1
В	2	11	12	13	14	15	16	17	18	19	20	2
С	3	21	22	23	24	25	26	27	28	29	30	3
D	4	31	32	33	34	35	36	37	38	39	40	4
E	5	41	42	43	44	45	46	47	48	49	50	5
F	6	51	52	53	54	55	56	57	58	59	60	6
G	7	61	62	63	64	65	66	67	68	69	70	7
н	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.) I.e.:



😑 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 x=1, this means 1+floor((1-1)/10),1), floor=0 and the **Blank 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.

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0	0	1	2	3	4	5	6	7	8	9	0
В	1	9	10	11	12	13	14	15	16	17	18	1
С	2	18	19	20	21	22	23	24	25	26	27	2
D	3	27	28	29	30	31	32	33	34	35	36	3
Ε	4	36	37	38	39	40	41	42	43	44	45	4
F	5	45	46	47	48	49	50	51	52	53	54	5
G	6	54	55	56	57	58	59	60	61	62	63	6
н	7	63	64	65	66	67	68	69	70	71	72	7

Blank Corrected

Dilution Curves

[Input: Endpoint Impoint (Input: Endpoint]

This transform creates a curve fit and calculates, in respect of this curve, a titer value for each sample group of the specified dilution type.

Properties

<u>Settings</u>

Dilution Type	The sample type to use for the dilution.								
Dilutions	This specifies standard concentration values. You can Enter or Paste the values into the edit box. If the values are in a sequence, select the Series button to define the series. The series is calculated from the value specified in the first row and the sequence settings with arithmetic operators and a factor (either even or fractional). Also, you can press the r button to set a number to repeat sequence with in the edit box.								
	All sample groups are assumed to have the same number of positions defined on the layout (e.g., if Unknown1 has 10 samples, then all Unknowns on the layout must also have 10 samples).								
Fit Method	The curve fitting method to construct a curve for your data based on the specified Dilution Type .								
Titer To Calculate	An <u>expression</u> representing the titer value to calculate.								
Weight Method	Specifies the weight function for the transform.								
	A type to plot an X axis in a chart:								
X Axis Type	LogarithmicLinear								
X Transform	Function for X values transform.								
Y Axis Type	A type to plot an Y axis in a chart:								

	LogarithmicLinear
Y Transform	Function for Y values transform.

Example

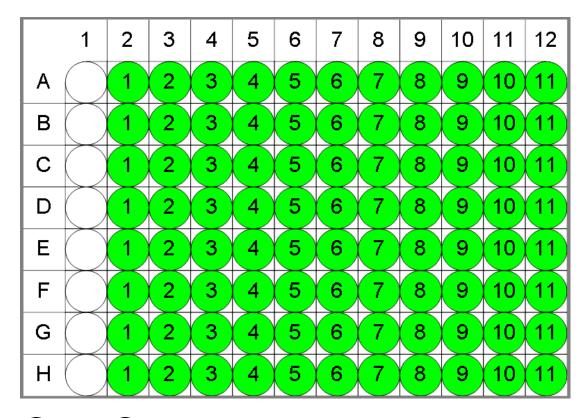
Let's assume that we have some endpoint measurement data for a 12x8 microplate stored in the **Raw** matrix. In this example we would like to create a curve fit for each sample group of the **Unknown** type.

Here is some example raw data displayed here to 3 decimal places:

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.000	2.682	2.345	2.612	2.732	2.675	2.664	2.574	2.580	2.361	0.871	2.839
В	0.000	2.577	1.735	2.518	2.701	2.537	2.571	2.450	2.453	2.171	0.437	2.809
С	0.000	2.537	1.290	2.497	2.683	2.447	2.490	2.273	2.344	1.695	0.222	2.783
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
Ε	0.000	1.958	0.369	1.799	2.224	1.636	1.661	1.192	1.485	0.550	0.037	2.322
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
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
н	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 **A1** to **H1**) and **Unknowns** in columns (e.g. **Unknown1** at positions from **A2** downward to **H2**, **Unknown2** at **A3-H3**, etc.). I.e.:



🔘 Unused 🔵 Unknown

To create a curve and calculate concentrations for this matrix, specify following settings:

- 1. Dilution Type to Unknown.
- 2. **Dilutions** count is equal to maximum number (8) of replicates in sample group of the **Unknown**. Set this parameter to 0.01 and series to divide by 3.
- 3. Set Fit Method of 4PL.
- 4. Set a **Titer To Calculate** to 1.5.
- 5. Select a Weight Method of None.
- 6. Set an **X Axis Type** to **Logarithmic**.
- 7. Set a **X Transform** to **None**.
- 8. Set an X Axis Type to Linear.
- 9. Set a X Transform to None.

The transform generates a Dilutions Table and a Chart by default. Other elements such as <u>Matrix table</u> and Notes can be <u>added to report optionally</u>.

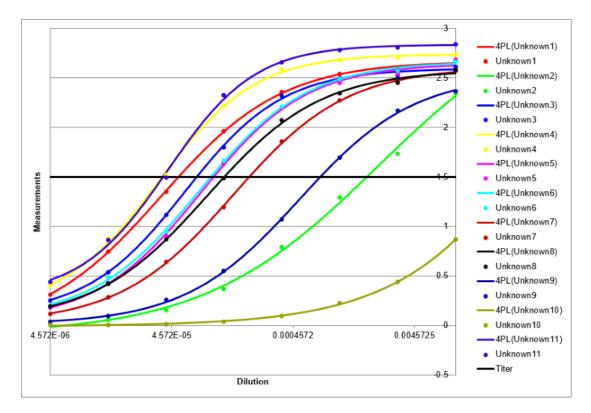
Dilutions Table

This table lists coefficients calculated from the selected Fit Method and a resulting value for each sample. With the example data provided above, the results are as follows:

Sample	а	b	с	d	R ²	Result
Unknown1	-0.0584816	0.875198	0.0000376679	2.67099	0.999497	0.0000522186
Unknown2	-0.0901615	0.606878	0.00226574	3.29931	0.997736	0.00184835
Unknown3	0.103291	1.07281	0.0000588689	2.59484	0.999371	0.0000738691
Unknown4	0.176798	1.10415	0.000035979	2.73875	0.999281	0.0000381935
Unknown5	0.0355624	0.998377	0.0000794665	2.64392	0.999147	0.000101774
Unknown6	0.0581275	0.980399	0.000077654	2.66458	0.999619	0.0000965551
Unknown7	0.0190159	0.929062	0.000146444	2.60957	0.999764	0.000199822
Unknown8	0.0468791	0.936045	0.000090142	2.57555	0.999425	0.000124316
Unknown9	0.0138517	0.919968	0.000515222	2.53025	0.999769	0.000767282
OUnknown10	-0.00938083	0.681115	0.135122	6.05263	0.999738	0.0267169
OUnknown11	0.298789	1.20751	0.0000424681	2.83485	0.999004	0.0000389155

Chart

The chart contains a curve fit plotted with 4PL for titer and for each sample group.



Titer

The output matrix named **Titer** <u>can be optionally added to the report</u>. It contains the calculated titer value for each data point. 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
Α	Flagged	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.027	0.000
В	Flagged	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.027	0.000
С	Flagged	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.027	0.000
D	Flagged	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.027	0.000
Ε	Flagged	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.027	0.000
F	Flagged	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.027	0.000
G	Flagged	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.027	0.000
н	Flagged	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.027	0.000

Dilution Factors

[Input: Endpoint Impoint (Input: 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.

Properties

Settings

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Factored Samples	The sample types to which the dilution factor should be applied
Factors	The dilution factor to use for each selected Factored Samples

Example

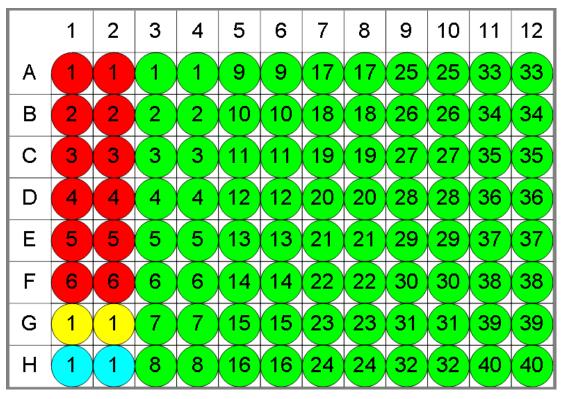
Let's assume that we have some endpoint measurement data for a 12x8 microplate stored in the **Raw** matrix. In this example we would like to multiply all odd **Unknown** sample groups by 2 and all even **Unknown** sample groups by 5.

Here is some example raw data displayed to 3 decimal places:

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
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
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
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
н	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.). I.e.:



🛑 Standard 💛 Blank 🗢 Control 🗢 Unknown

To multiply selected sample types in this matrix by specified factors, set **Factored Samples** to **Unknown** and specify **Factors** as **2** for every odd sample group and as **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 <u>Sample Table</u> 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.

Sample	Positions	Factor	My Raw Data	Concentration (x Dil. Factor)
Unknown1	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

For each sample, the following details are reported:

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. Select the **XML** tab in the **Assay Properties** panel (If the **XML** tab is not immediately visible, press the **<< Show All Properties** button 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.

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	4.210	4.248	2.426	2.452	2.318	2.380	0.220	0.232	2.926	2.962
В	0.455	0.441	9.345	9.440	6.015	6.090	5.900	6.030	0.660	0.650	3.930	3.840
С	0.587	0.575	0.948	1.028	2.412	2.478	2.406	2.420	0.226	0.232	1.226	1.252
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
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
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
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
н	0.253	0.270	2.315	2.350	6.070	6.305	0.575	0.580	0.575	0.575	2.450	2.385

Expression by Matrix (Dual)

Input: Dual Endpoint 🔿 Output: Endpoint

This transform <u>evaluates an expression</u> defined in terms of **x** and **y** for all data points (excluding flagged samples). **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.

Properties

Settings

Dual MatrixThe expression to evaluate, where x and y variables are set to value of eachExpressionposition of the input matrices

If the expression does not contain the **x** and **y** variables, the transform will output the same evaluation result for all positions. For example, if you enter **{2^3+4}**, the resulting matrix will contain 12 in each position.

Example

Let's assume that we have some endpoint measurement data for a 12x8 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)	
--	-----	-----	--

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
Ε	1.105	1.065	0.279	0.367	1.215	1.225	1.233	1.262	0.111	0.114	1.579	1.549
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
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
н	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)

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.204	0.201	0.213	0.209	0.206	0.208	0.216	0.217	0.216	0.213	0.205	0.218
В	0.216	0.200	0.214	0.214	0.204	0.201	0.215	0.216	0.203	0.219	0.207	0.204
С	0.206	0.210	0.208	0.203	0.201	0.218	0.212	0.217	0.216	0.213	0.208	0.204
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
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
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
н	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 **x** + **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):

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.568	0.524	2.318	2.333	1.419	1.434	1.375	1.407	0.326	0.329	1.668	1.699
В	0.671	0.641	2.083	2.102	1.407	1.419	1.395	1.422	0.335	0.349	0.993	0.972
С	0.793	0.785	0.682	0.717	1.407	1.457	1.415	1.427	0.329	0.329	0.821	0.830
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
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
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
н	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 I Output: Endpoint]

This transform <u>evaluates an expression</u> defined in terms of \mathbf{x} for all data points (excluding flagged samples).

Properties

Settings

Matrix	The expression to evaluate, where a single \mathbf{x} variable is set to value of each
Expression	position of the input matrix

If the expression does not contain the **x** variable, the transform will output the same evaluation result for all positions. For example, if you enter **{2^3+4}**, the resulting matrix will contain 12 in each position.

Example

Let's assume that we have some endpoint measurement data for a 12x8 microplate stored in the **Raw** matrix. In this example we would like to square each measurement.

Here is some example raw data displayed here to 3 decimal places:

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
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
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
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
н	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

The **Matrix Expression** setting is specified with expression of **x*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):

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.132	0.104	4.431	4.511	1.471	1.503	1.343	1.416	0.012	0.013	2.140	2.193
В	0.207	0.194	3.493	3.565	1.447	1.484	1.392	1.454	0.017	0.017	0.618	0.590
С	0.345	0.331	0.225	0.264	1.454	1.535	1.447	1.464	0.013	0.013	0.376	0.392
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
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
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
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
н	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 Import: Endpoint]

This transform enables an <u>expression</u> to be defined for each position in terms of \mathbf{x} (where \mathbf{x} refers to the value of position on the input matrix). This is useful for applying various tests to different samples simultaneously.

Properties

Settings

This allows different <u>expressions</u> 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 on a 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 colored to match its sample type.

Position Expressions	 If the protocol contains >1 plate/analyte, select the one to specify expressions for from a drop-down list in the upper right corner of the Editor. To maximize its view, click on the arrow button in the lower left corner of the Editor. Within each grid you can select and specify <u>an expression</u> in terms of x for: a single position. multiple positions (within a single plate) to set to a same expression. 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).
	For positions with default (simply \mathbf{x}) or empty expression, the output position is equal to the input position.

Example

Let's assume that we have some endpoint measurement data for a 12x8 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:

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
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
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
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
н	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

Provide expressions for positions in Position Expressions Editor:

- A1, A2, A3, A4, A5 with the expression x*10
- A10 and H12 with the expression x*100
- E2 with the expression 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):

	1	2	3	4	5	6	7	8	9	10	11	12
Α	3.640	3.230	21.050	21.240	12.130	1.226	1.159	1.190	0.110	11.600	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
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
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
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
н	0.253	0.270	0.463	0.470	1.214	1.261	0.115	0.116	0.115	0.115	0.490	47.700

Factor

[Input: Endpoint Input: Endpoint]

This transform multiplies data points by a common factor. The factor can be specified as a numeric value or as an <u>expression to evaluate</u>.

Properties

Settings

Factor	The value to multiply all data points by. This can be specified as a number or
ractor	an <u>expression to evaluate</u> .

Example:

Let's assume that we have some endpoint measurement data for a 12x8 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 here to 3 decimal places:

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
Ε	1.105	1.065	0.279	0.367	1.215	1.225	1.233	1.262	0.111	0.114	1.579	1.549
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
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
н	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

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):

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.728	0.646	4.210	4.248	2.426	2.452	2.318	2.380	0.220	0.232	2.926	2.962
В	0.910	0.882	3.738	3.776	2.406	2.436	2.360	2.412	0.264	0.260	1.572	1.536
С	1.174	1.150	0.948	1.028	2.412	2.478	2.406	2.420	0.226	0.232	1.226	1.252
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
Ε	2.210	2.130	0.558	0.734	2.430	2.450	2.466	2.524	0.222	0.228	3.158	3.098
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
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
н	0.506	0.540	0.926	0.940	2.428	2.522	0.230	0.232	0.230	0.230	0.980	0.954

Matrix Difference

Input: Dual Endpoint 🔿 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.

Example

Let's assume that we have some endpoint measurement data for a 12x8 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 here to 3 decimal places:

Raw (1)

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
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
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
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
н	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)

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.204	0.201	0.213	0.209	0.206	0.208	0.216	0.217	0.216	0.213	0.205	0.218
В	0.216	0.200	0.214	0.214	0.204	0.201	0.215	0.216	0.203	0.219	0.207	0.204
С	0.206	0.210	0.208	0.203	0.201	0.218	0.212	0.217	0.216	0.213	0.208	0.204
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
Ε	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
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
н	0.216	0.209	0.215	0.206	0.218	0.206	0.209	0.219	0.215	0.219	0.216	0.217

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):

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.160	0.122	1.892	1.915	1.007	1.018	0.944	0.973	-0.106	-0.097	1.258	1.263
В	0.239	0.241	1.656	1.674	0.999	1.017	0.965	0.990	-0.071	-0.089	0.579	0.564
С	0.381	0.365	0.266	0.311	1.005	1.021	0.991	0.993	-0.103	-0.097	0.405	0.422
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
Ε	0.890	0.845	0.060	0.155	1.005	1.014	1.019	1.048	-0.090	-0.088	1.377	1.342
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
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
н	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 Input: Endpoint]

This transform calculates the median of the replicates in each sample group (flagged replicates are not included).

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.

Example

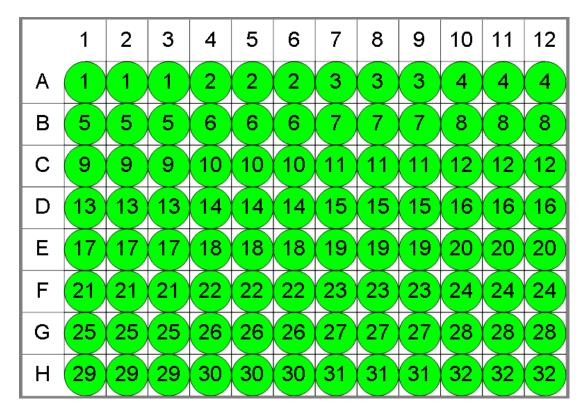
Let's assume that we have some endpoint measurement data for a 12x8 microplate stored in the **Raw** matrix. In this example we would like to find median value for each measurement sample group.

Here is some example raw data displayed here to 3 decimal places:

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
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
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
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
н	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.). I.e.:



💛 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 <u>Sample Table</u> 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	
Unknown2	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.

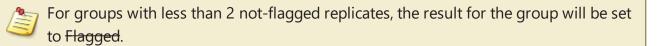
	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.364	0.364	1.226	1.226	1.226	1.159	1.159	1.159	1.463	1.463	1.463
В	0.455	0.455	0.455	1.218	1.218	1.218	1.180	1.180	1.180	0.768	0.768	0.768
С	0.575	0.575	0.575	1.206	1.206	1.206	1.203	1.203	1.203	0.613	0.613	0.613
D	0.774	0.774	0.774	1.167	1.167	1.167	1.211	1.211	1.211	0.489	0.489	0.489
Ε	1.065	1.065	1.065	1.215	1.215	1.215	1.233	1.233	1.233	1.549	1.549	1.549
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
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
н	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 Impoint [Input: Endpoint]

This transform calculates the %CV of the replicates in each sample group (<u>flagged replicates</u> are not included).

The **%CV** is the <u>Standard Deviation</u> divided by the sample group mean and multiplied by 100. (If the mean or the <u>Standard Deviation</u> is 0, then the result is forced to 0.)



Example

Let's assume that we have some endpoint measurement data for a 12x8 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.

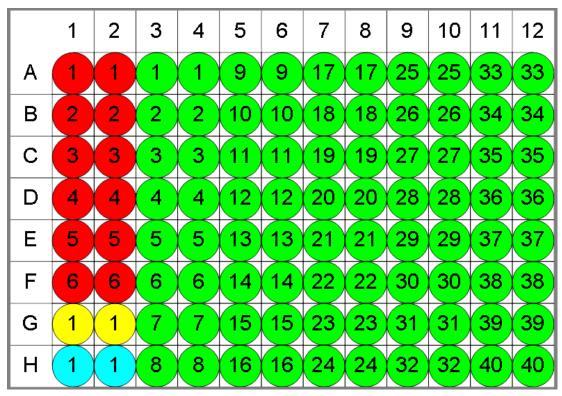
Here is some example raw data displayed here to 3 decimal places:

Raw

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
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
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
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
н	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.). I.e.:



🛑 Standard 💛 Blank 🗢 Control 🗢 Unknown

%CV

The transform will generate an output matrix named **%CV** 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 <u>Sample Table</u> 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.

	1	2	3	4	5	6	7	8	9	10	11	12
Α	8.440	8.440	0.635	0.635	0.754	0.754	1.866	1.866	3.755	3.755	0.865	0.865
В	2.210	2.210	0.715	0.715	0.876	0.876	1.541	1.541	1.080	1.080	1.638	1.638
С	1.460	1.460	5.726	5.726	1.909	1.909	0.410	0.410	1.853	1.853	1.484	1.484
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
Е	2.607	2.607	19.265	19.265	0.580	0.580	1.644	1.644	1.886	1.886	1.356	1.356
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
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
н	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 Input: Endpoint]

This transform calculates a percentage value for each sample with respect to the specified 0% and 100% values.

Properties

<u>Settings</u>

P0 and **P100** 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 **P0** 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 <u>an</u> <u>expression</u> . All values less than P0 will have negative value in resulting matrix.
P100	The value treated as "100%." This can be defined as a number or as <u>an</u> <u>expression</u> . All values greater than P100 will have values greater than 100 in resulting matrix.

Example

Let's assume that we have some endpoint measurement data for a 12x8 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 here to 3 decimal places:

Raw

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
Ε	1.105	1.065	0.279	0.367	1.215	1.225	1.233	1.262	0.111	0.114	1.579	1.549
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
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
н	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 **P100** 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):

	1	2	3	4	5	6	7	8	9	10	11	12
Α	12.13	10.77	70.17	70.80	40.43	40.87	38.63	39.67	3.67	3.87	48.77	49.37
В	15.17	14.70	62.30	62.93	40.10	40.60	39.33	40.20	4.40	4.33	26.20	25.60
С	19.57	19.17	15.80	17.13	40.20	41.30	40.10	40.33	3.77	3.87	20.43	20.87
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
Ε	36.83	35.50	9.30	12.23	40.50	40.83	41.10	42.07	3.70	3.80	52.63	51.63
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
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
н	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 Imput: 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 <u>outlier(s) flagged</u>. 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 **%CV 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).

Properties

Settings

Outliers To	Specifies the maximum number of outliers to remove. The minimum and
Remove	default value for this setting is 1.
%CV	Defines threshold percentage coefficient of variance. Data point with superior
Threshold	or inferior values will be flagged. The default value is 15%.

Example

Let's assume that we have some endpoint measurement data for a 12x8 microplate stored in the **Raw** matrix. In this example we would like to remove 1 outlier from each sample group.

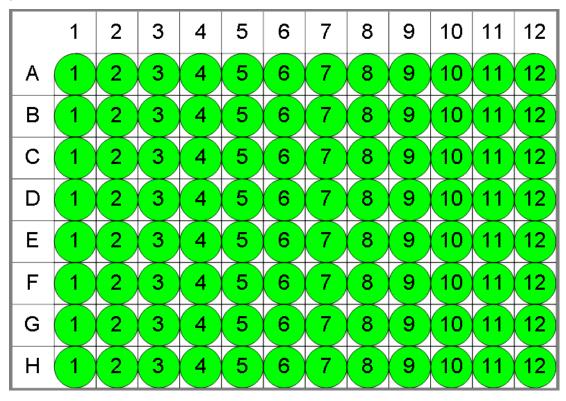
Here is some example raw data displayed to 3 decimal places:

Raw

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
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
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
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
н	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 **A1**to **H1**, **Unknown2** from **B2** to **H2**, etc.). I.e.:



💛 Unknown

To flag 1 outlier data point for each sample group, specify an **Outliers To Remove** as 1 and **%CV Threshold** as 15%.

Calculated

9

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):

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
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
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
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
н	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 Impoint [Input: 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.

Properties

Axes

X Axis Title	A name of the X axis on the chart
	A type to plot an X axis in a chart:
X Axis Type	LogarithmicLinear
Y Axis Title	A name of the Y axis on the chart
Y Axis Type	A type to plot an Y axis in a chart: • Logarithmic • Linear

Calibrators

Concentrations	Defines the X value for each point of the chart.
Standard Type	Defines a sample type to treat as a standard.

<u>Fit</u>

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?). The options are: Replicates – Every position will contain the result of X, calculated from value of every replicate (Y). Average – Replicates from the same sample contain the result of X, calculated from value of replicates from the same sample contain the result of X, calculated from value of every averaged value of replicates (Y). 								
	Any flagged positions on the input are also flagged in the output.								
Fit Method	The curve-fitting method to construct a curve for your data based on the specified Standard Type .								
	You can also use the Best Fit feature to plot the most appropriate fit for your data automatically.								
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. I.e.: R² R² with Low-End Accuracy R² with High-End Accuracy R² with Low-End and High-End Accuracy 1/Standard-Error 1/P-Value 								
Fit To Standard	 Defines which points are used as the calibrators to fit the curve to: Replicates – All replicate points from each Standard sample group are used as calibrators. 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.								

	Determines how the \mathbf{X} values are interpreted in the transform:							
X	• None – The X values remain as they are.							
X Transform	• Log10 – Each X point is taken as a common logarithm of X.							
indisioni	• Log2 – Each X point is taken as a binary logarithm of X.							
	• LogN – Each X point is taken as a natural logarithm of X.							
	Determines how the \mathbf{Y} values are interpreted in the transform:							
	• None – The Y values remain as they are.							
Y Transform	 Log10 – Each Y point is taken as a common logarithm of Y. 							
	• Log2 – Each Y point is taken as a binary logarithm of Y.							
	• LogN – Each Y point is taken as a natural logarithm of Y.							

Quantification Limits

Accuracy (±%)	Defines a percentage limit of accuracy for Standard Type sample groups. This is used to verify whether the accuracy of the calibrators' fits in required limits. The default value is 20%.
Precision (%CV)	Defines a %CV precision limit. This is used to verify whether %CV for each Standard Type sample group is lower than required limit. The default value 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 <u>named</u> <u>evaluations</u> 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 12x8 microplate stored in the **Raw** matrix. In this example we would like to construct a curve fit and calculate concentrations for input matrix.

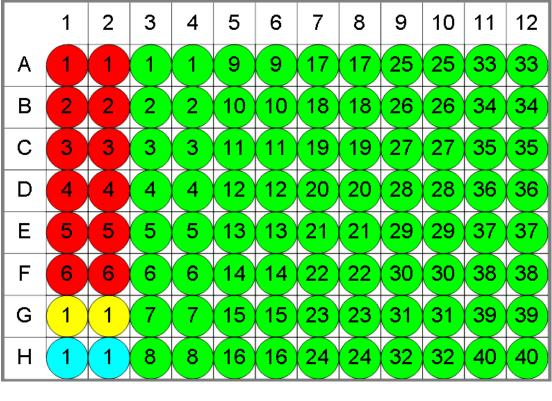
Here is some example raw data displayed to 3 decimal places:

Raw

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.192	0.199	0.347	0.346	0.325	0.321	0.803	0.797	1.589	1.593	0.241	0.246
В	0.293	0.300	0.586	0.584	0.800	0.793	0.402	0.401	0.839	0.837	0.562	0.564
С	0.537	0.534	0.549	0.547	0.878	0.875	0.471	0.471	0.759	0.756	1.039	1.043
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
Е	1.523	1.527	0.486	0.490	1.289	1.283	1.430	1.438	1.494	1.501	1.427	1.429
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
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
н	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.). I.e.:



Standard O Blank O Control O Unknown

To configure a **Standard Curve Fit** for this matrix, provide following settings:

- 1. Set **X Axis Title** to Concentration.
- 2. Set **X Axis Type** to Logarithmic.
- 3. Set **Y Axis Title** to Raw.
- 4. Set **X Axis Type** to Linear.
- 5. Set **Concentrations** in **Standard1** to 0.125 and a **Series** to multiply by 2.
- 6. Set Standard Type to Standard.
- 7. Set Calculate From to Average.
- 8. Select a Fit Method of 4PL.
- 9. Set Fit To Standard to Average.
- 10. Select a Weight Method of None.
- 11. Set a **X Transform** to **None**.
- 12. Set a **X Transform** to **None**.
- 13. Set **Accuracy (±%)** to 20%.
- 14. 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):

	1	2	3	4	5	6	7	8	9	10	11	12
Α	Flagged	Flagged	0.352	0.352	0.318	0.318	0.844	0.844	2.890	2.890	0.153	0.153
В	0.274	0.274	0.623	0.623	0.841	0.841	0.423	0.423	0.886	0.886	0.600	0.600
С	0.572	0.572	0.585	0.585	0.928	0.928	0.503	0.503	0.799	0.799	1.131	1.131
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
Е	2.390	2.390	0.521	0.521	1.550	1.550	1.969	1.969	2.240	2.240	1.947	1.947
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
G	Flagged	Flagged	0.362	0.362	0.439	0.439	1.332	1.332	2.710	2.710	0.630	0.630
н	0.275	0.275	0.843	0.843	2.338	2.338	1.030	1.030	0.566	0.566	0.622	0.622

Click here to learn about 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 <u>Standard Curve Evaluations</u>. Moreover, with <u>Standard Curve Custom Flag</u> <u>Labels</u>, 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>

```
<StandardCurveFitStandardType="2" FitMethod="4PL" Concentrations="0.25,0.5,1,2,4,8"
XAxisTitle="Concentration" YAxisTitle="OD" FitToStandard="Average"
CalculateFrom="Average">
```

```
<Evaluations>

<EvaluationType="CalcY" Expression="Blank + (3 * sd(Blank))"

Description="My LLD"Name="LLD" />

</Evaluations>

<Flags>

<FlagType="Input"Condition="x &It; 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 downward to LLOQ and ULOQ, which are calculated from the curve automatically.

LLOQ	0.25
ULOQ	2
My LLD	0.222063

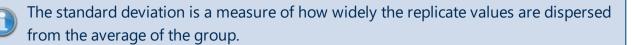
	1	2	3	4	5	6	7	8	9	10	11	12
Α	Below LLD	Below LLD	0.352	0.352	0.318	0.318	0.844	0.844	2.890	2.890	0.153	0.153
В	0.274	0.274	0.623	0.623	0.841	0.841	0.423	0.423	0.886	0.886	0.600	0.600
С	0.572	0.572	0.585	0.585	0.928	0.928	0.503	0.503	0.799	0.799	1.131	1.131
D	0.906	0.906	0.317	0.317	0.566	0.566	2.561	2.561	0.454	0.454	0.450	0.450
Е	2.390	2.390	0.521	0.521	1.550	1.550	1.969	1.969	2.240	2.240	1.947	1.947
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
G	Below LLD	Below LLD	0.362	0.362	0.439	0.439	1.332	1.332	2.710	2.710	0.630	0.630
н	0.275	0.275	0.843	0.843	2.338	2.338	1.030	1.030	0.566	0.566	0.622	0.622

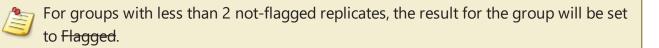
With the example data provided above, the results are computed as follows:

Standard Deviation

[Input: Endpoint Imput: Endpoint]

This transform calculates the standard deviation of the replicates in each sample group (<u>flagged</u> replicates are not included). The sample of population method is used.





Example

Let's assume that we have some endpoint measurement data for a 12x8 microplate stored in the **Raw** matrix. In this example we would like to find the deviation between replicates in each sample group.

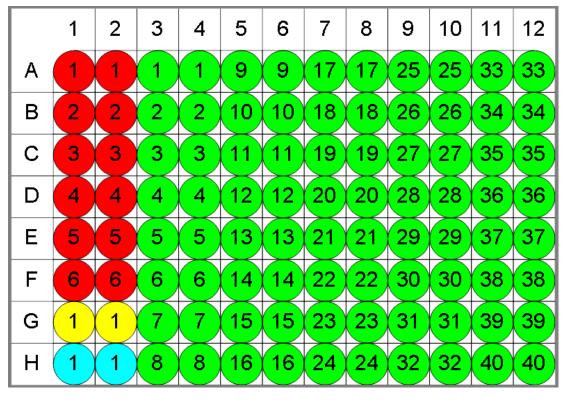
Here is some example raw data displayed here to 3 decimal places:

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
Е	1.105	1.065	0.279	0.367	1.215	1.225	1.233	1.262	0.111	0.114	1.579	1.549
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
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
н	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

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.). I.e.:



Standard O Blank O Control O Unknown

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 <u>Sample Table</u> 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.

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.029	0.029	0.013	0.013	0.009	0.009	0.022	0.022	0.004	0.004	0.013	0.013
В	0.010	0.010	0.013	0.013	0.011	0.011	0.018	0.018	0.001	0.001	0.013	0.013
С	0.008	0.008	0.028	0.028	0.023	0.023	0.005	0.005	0.002	0.002	0.009	0.009
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
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
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
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
н	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 => Output: Endpoint]

This transform calculates the standard error of the mean (SEM) of the replicates in each sample group (<u>flagged</u> replicates are not included). The sample of population method is used.

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 not-flagged replicates, the result for the group will be set to Flagged.

Example

Let's assume that we have some endpoint measurement data for a 12x8 microplate stored in the **Raw** matrix. In this example we would like to find the SEM of each sample group.

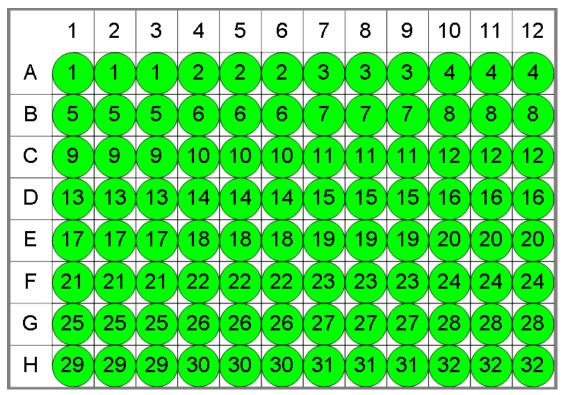
Here is some example raw data displayed to 3 decimal places:

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
Е	1.105	1.065	0.279	0.367	1.215	1.225	1.233	1.262	0.111	0.114	1.579	1.549
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
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
н	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

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.). I.e.:



💛 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 <u>Sample Table</u> 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	
	В3	1.869	

• Matrix Table

Here all calculated data is displayed in a Matrix Table.

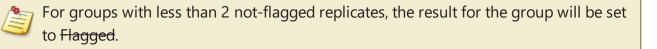
	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.587	0.587	0.587	0.302	0.302	0.302	0.355	0.355	0.355	0.452	0.452	0.452
В	0.474	0.474	0.474	0.226	0.226	0.226	0.354	0.354	0.354	0.216	0.216	0.216
С	0.036	0.036	0.036	0.236	0.236	0.236	0.365	0.365	0.365	0.168	0.168	0.168
D	0.142	0.142	0.142	0.266	0.266	0.266	0.375	0.375	0.375	0.128	0.128	0.128
Ε	0.269	0.269	0.269	0.284	0.284	0.284	0.379	0.379	0.379	0.483	0.483	0.483
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
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
н	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 Impoint (Input: Endpoint)]

This transform calculates the variance of the replicates in each sample group (<u>flagged</u> 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 **n-1** (where **n** is the number of the replicates).



Example

Let's assume that we have some endpoint measurement data for a 12x8 microplate stored in the **Raw** matrix. In this example we would like to find the variance between measurements in each sample group.

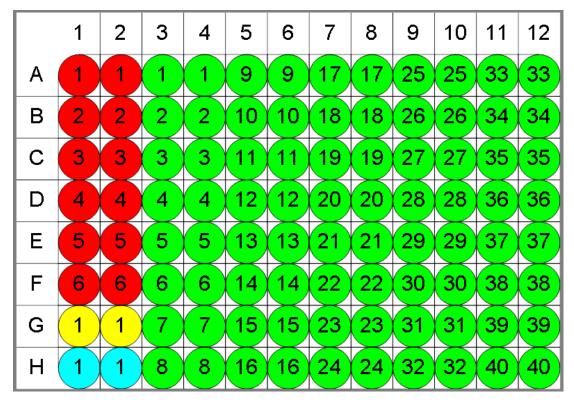
Here is some example raw data displayed to 3 decimal places:

	_		_
Κ	a	W	1

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
Ε	1.105	1.065	0.279	0.367	1.215	1.225	1.233	1.262	0.111	0.114	1.579	1.549
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
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
н	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.). I.e.:



🗧 Standard 💛 Blank 🗢 Control 🗢 Unknown

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 <u>Sample Table</u> 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

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
В	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
С	0.000	0.000	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000
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
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
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
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
н	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000

Here all calculated data is displayed in a Matrix Table.

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

	This setting determines which values to use in the X chart axis and for evaluations:
	• Index – The 1-based position number of the data point in the collection.
	• X – Depending on the measurements data type, this option is displayed as:
	• X (Cycle) – For kinetic data with no time values, X equals the Index.
X Axis	 X (Time) – For kinetic data, if time values are stored, then this is the time stamp associated with the data point.
	 X (Wavelength) – For spectral data, if wavelength values are stored, then this is the wavelength of the data point.
	• Y – Each value is set to a Y value of the corresponding input matrix's data point.
	This setting determines which values to consider in the ${f Y}$ chart axis and for evaluations:
	• Index – The 1-based position number of the data point in the collection.
	• X – Depending on the measurements data type, this option is displayed as:
Y Axis	• X (Cycle) – For kinetic data with no time values, X equals the Index.
	 X (Time) – For kinetic data, if time values are stored, then this is the time stamp associated with the data point.
	 X (Wavelength) – For spectral data, if wavelength values are stored, then this is the wavelength of the data point.

• Y – Eeach value is set to a Y value of the corresponding input matrix's data point.

<u>Range</u>

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 (that are also not flagged) are processed by the transform.

The range can be defined by setting the **X Minimum** and **X Maximum** properties to a value or expression:

X Minimum	A numerical value or <u>expression</u> that defines the minimum X data point to include within the subset. If this is not specified, then all data points less than X Maximum are included.
X Maximum	A numerical value or <u>expression</u> that defines the maximum X data point to include within the subset. If this is not specified, then all data points greater than X Minimum are included.

To refer to the position's corresponding data on an endpoint matrix, use a <u>Matrix</u> <u>Position Reference</u> (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 on 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 <u>XY Baseline Correction</u> 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 axes. 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 axis settings specify what data should be processed by the transform. For further details you can refer to the <u>General Transform</u> <u>Properties for XY Data</u> subsection.

Flagged XY Data Points

Any data point that is part of an XY data set can be flagged. <u>Flagged data points</u> are excluded from the calculation.

For example, with the <u>XY Baseline Correction</u> 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 for <u>Flagged Endpoint</u>.

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 <u>expression</u> (that can in turn refer to other computed results).

For example, the <u>XY Expression</u> 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	This transform removes the inherent variable background, or baseline, from
<u>Correction</u>	each input position.
Blank	Subtracts the average of credified Plank group(s) from each input position
<u>Correction</u>	Subtracts the average of specified Blank group(s) from each input position.
Expression	Evaluates an expression in terms of ${f x}$ and ${f y}$, where variables are set to the
(Dual)	value of each position of the first (\mathbf{x}) and second (\mathbf{y}) input matrices.
Expression	Evaluates the specified expression for each input position.
<u>Fit</u>	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	Flags data points that are considered to be outliers. The values of the output
Removal	matrix match the input matrix (the only difference being that outlier(s) might
	be flagged).
Replicates	Calculates the XY average plot for each sample group.
Average	Calculates the AT average plot for each sample group.

XY Baseline Correction

[Input: XY 🔿 Output: XY]

This transform removes the inherent variable background (or baseline) from each XY position.

<u>Flagged positions or points</u> are not included in the calculations. The operation is only performed on data within specified range.

Properties

<u>Settings</u>

	The method for a baseline correction:						
Method	 Average Between X1 And X2 – Correct points by the average Y of the not-flagged points between and including points X Index 1 and X Index 2. 						
Method	 Average Of N Lowest – Correct points by the average Y of the lowest not-flagged N. 						
	 Proportional N1 Lowest and N2 Highest – Proportional percentage between average of the N1 lowest and N2 highest not-flagged points. 						
19	sibility of the following settings depend on the selected Method . For ole, if you choose the Average Of N Lowest , then only the N setting will be yed.						
	These settings are used in the Average Between X1 And X2 method.						
X Index 1 X Index 2	These are 1-based index numbers and apply to the data sets with or without time values. Only not-flagged points that are <= X Index 1 and >= X Index 2 are used. X Index 1 and X Index 2 must be >= 1 and X Index 2 must be >= X Index 1.						
	If there are no included points between X Index 1 and X Index 2 , then no correction will be made and the output position will be flagged.						
	This setting is used in the Average Of N Lowest method.						
N	It is the number of lowest not-flagged data points within a subset. If the number of included points $< N$, then the output position will be flagged. If the number of included points $= N$, then the result is that all points are corrected						

	by the average of all points.
	These settings are used in the Proportional N1 Lowest and N2 Highest method.
N1	N1 is the number of the lowest points, N2 is the number of the highest points to used for correction. N1 and N2 must be $>= 1$. 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).
N2	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.
	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 N1 or N2 valid readings, then the output position will be flagged.

XY Blank Correction

[Input: XY 🔿 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.

<u>Flagged positions or points</u> are not included in the calculations. The operation is only performed on data within specified range.

Properties

Settings

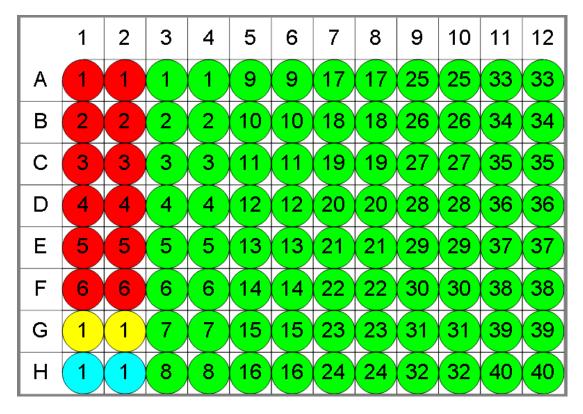
This transform requires a layout with at least 1 Blank group.	
Blank	The group number of the sample to use for correction. This can be defined as a number or as <u>an expression</u> . The expression can refer to \mathbf{x} which is the group number of the sample to be corrected.
Group	For example, set this to \mathbf{x} to perform a 1-to-1 correction where each group is corrected by the sample of 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 **XY** measurement data for a 12x8 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.). I.e.:



Standard O Blank O Control O Unknown

To subtract the mean of the blank positions from the measurements, specify **Blank Group** to **1** and **Blank Type** to **Blank**.

Blank Corrected

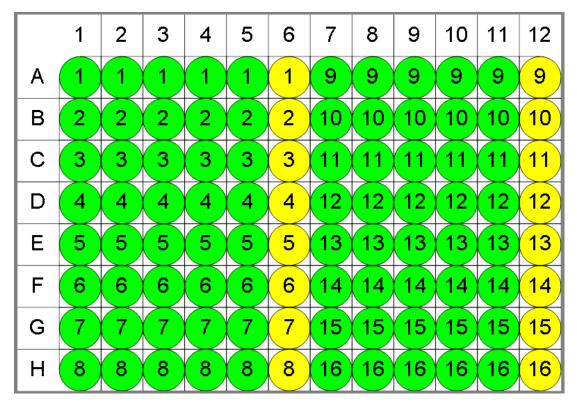
The transform will generate an output matrix named **Blank Corrected** with the value at each position being the subtraction of the average of **Blank1** sample group from the input data.

Example: 1-1 Blank Correction

Let's assume that we have some **XY** measurement data for a 12x8 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. I.e.:



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

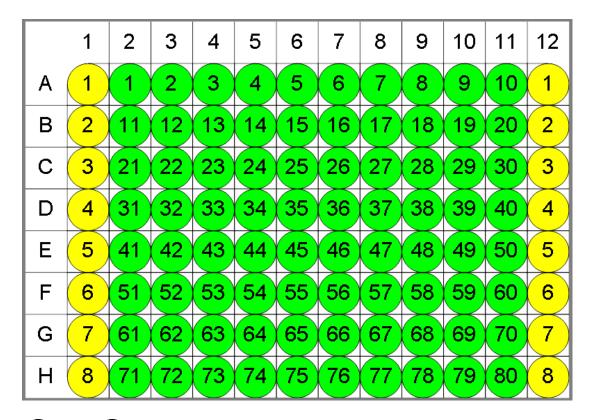
The transform will generate an output matrix named **Blank Corrected** with the value at each position being the subtraction of the associated blank value from the input data. Therefore, calculated matrix will contain blank corrected **Unknown** values and all **Blanks** will be 0.

Advanced Example: By-Row Blank Correction

Let's assume that we have some **XY** measurement data for a 12x8 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.) I.e.:



💛 Blank 🔵 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 (x) to the blank number on its row. In other words, for x=1 this means 1+floor((1-1)/10),1), floor=0 and the **Blank 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** \Longrightarrow Output: **XY**]

This transform <u>evaluates an expression</u> defined in terms of \mathbf{x} and \mathbf{y} for each not-flagged input XY data point within the specified range. In this case \mathbf{x} refers to the data for the sample on the first input matrix and \mathbf{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 XY measurements.

Properties

<u>Settings</u>

	The expression to evaluate, where variables are set to the value of each position of the first (\mathbf{x}) and second (\mathbf{y}) input matrices. Default expression value is $\mathbf{x} - \mathbf{y}$.
Expression	To refer to the position's corresponding data on an endpoint matrix, use a <u>Matrix Position Reference</u> (e.g., #2) to refer to the data on the second matrix.
Plot Input on Chart	When the checkbox is checked, the chart includes both the input and the calculated plot. If it is unchecked, the chart shows the calculated plot only.

XY Expression

[Input: XY 🔿 Output: XY]

This transform evaluates a specified expression for each input XY data point (only points within the specified range that are not <u>flagged</u> 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 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.

Properties

<u>Settings</u>

1

	The expression to evaluate, where the y variable is set to the value of each position of the input matrix.
Expression	To refer to the position's corresponding data on an endpoint matrix, use a <u>Matrix Position Reference</u> (e.g., #2) to refer to the data on the second matrix.
Plot Input on Chart	When the checkbox is ticked, the chart includes both the input and the calculated plot. If it is unchecked, the chart shows the calculated plot only.

XY Fit

[Input: XY 🔿 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 4PL), then use the <u>XY Reduction Fit Parameter</u> transform.

Properties

Settings

Fit Method The curve fitting method to construct the curve (from each XY provided in the curve of the curve	olot)
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XY Join

[Input: Dual XY 🔿 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 <u>flagged</u> <u>data points</u> 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.

Properties

Advanced, **Units** and **Range** settings do not apply to this transform.



If **X** is set as **Index**, then the first index of the joined matrix is 1 plus the last used index.

If **X** is set as **X**, then the **X** values specified in each input matrix are used (unchanged) in the output matrix.



When using this transform, consider the **X Axis Data** setting. The joined data will use the data from both input matrices. To ensure that the resulting joined contents has sequential x values, set the **X Axis Data** setting to **Index**.

XY Outlier Removal

[Input: XY 🔿 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 <u>flagged</u>).

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.

Properties

Settings

Num Regression Points	The number of regression points used for each linear regression segment when determining the maximum slope. The default and minimum value is 2.
Threshold	Defines the threshold % accuracy to flag outliers. It calculates absolute % difference between back fit value and the original y value. The back fit is computed from slope and intercept for each x value in the regression set. If this % difference is > the specified Threshold , then the data point is flagged on the output matrix.
	Points that are flagged in one regression set are not included in calculations of following regression sets.

XY Replicates Average

[Input: XY 🔿 Output: XY]

This transform calculates the average XY (kinetic/spectral) plot for each sample group. Any <u>flagged data points</u> are not included in the analysis.

As with other XY transforms, the calculations are performed on data within the specified ranges only.



To use this transform, select a layout with at least one sample group that contains more than one replicate.

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 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 axes. 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 axis settings specify what data should be processed by the transform. For further details you can refer to the <u>General Transform</u> <u>Properties for XY Data</u> subsection.

Flagged XY Data Points

Any data point that is part of an XY data set can be flagged. <u>Flagged data points</u> are excluded from the calculation.

For example, with the <u>XY Reduction Peak</u> 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 <u>Flagged Endpoint</u>.

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 <u>expression</u> (that can in turn refer to other computed results).

For example, the <u>XY Reduction Average</u> 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
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.
<u>Maximum</u>	Determined maximum slope between two adjacent points within specified
<u>Slope</u>	range at each position.
Peak	Determined peak (minimum or maximum) data point within the specified
<u>reak</u>	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 non-
<u>A ALT</u>	flagged points within the specified range at each position.
X Between Y	The difference between the first two ${f X}$ points that equal or cross specified ${f Y}$
	value at each position.
Y At X	Determined Y value for a specified X value within the specified range of the
	not-flagged points at each position.

XY Reduction Average

[Input: XY data 🔿 Output: Endpoint]

This reduction transform computes the average Y value of each XY plot (within the specified range of points to consider). <u>Flagged positions or points</u> are not included in the calculations.

Example

Let's assume that we have some **XY** measurement data for a 12x8 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 **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

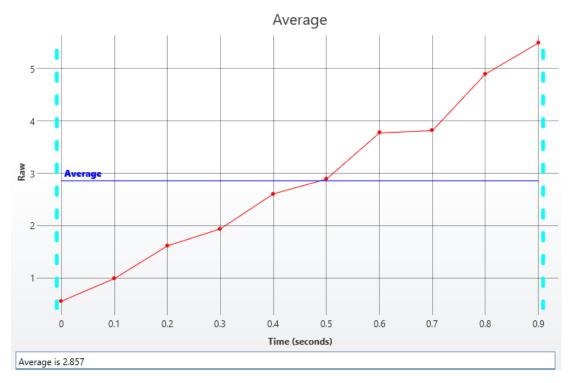
To reduce **XY** data to an average endpoint value:

- 1. Specify Data: X Axis as X (Time), Y Axis as Y.
- 2. Leave **X Minimum** and **X Maximum** blank.
- 3. Press 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 **A1** plot is as follows (also displayed here to 3 decimal places):

2.857



XY Reduction Fit Parameter

[Input: XY data 🔿 Output: Endpoint]

This reduction transform performs the selected curve fit on each XY plot and sets the result to the value of a specified <u>fit parameter</u>. Points outside the specified range and <u>flagged</u> <u>positions/points</u> are excluded from calculations.

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 4PL).

Properties

Settings

9

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

XY Reduction Maximum Slope

[Input: XY data 🔿 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 regression of adjacent points (using the specified number of linear regressions points) and determining the slope with the maximum (upward or downward) value.

<u>Flagged positions or points</u> or those outside specified ranges are not included in the calculations.

Properties

Settings

	Determines the type of slope to be found by this transform. The settings are:
Max is	• Auto – The maximum slope is determined from the general direction of each plot by using the linear regression. If the slope of the plot is positive, the Fastest Increase is used; if negative, the Fastest 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.
Num Regression Points	The number of regression points used for each linear regression segment when determining the maximum slope. The default and minimum value is 2.
Result is	Specifies the result of the analysis. The settings are:
	• Slope – The result is the slope of the determined maximum slope.

	• X At Centre Absolute – The result is the X at the center of the determined maximum slope.
	 Y At Centre Absolute – The result is the Y at the center of the determined maximum slope.
	• X At Centre Relative – The result is the X at the center of the determined maximum slope relative to the first not-flagged data point that is greater than X Minimum.
	• Y At Centre Relative – The result is the Y at the center of the determined maximum slope relative to the first not-flagged data point that is greater than X Minimum .
	 Extrapolated Y – A value of Y is calculated from the determined maximum slope using the X value specified with X For Extrapolation.
X for Extrapolation	When Extrapolated Y option in the Result Is setting is chosen, then this can be used to <u>specify an expression</u> used to determine the X value to calculate Y for.

XY Reduction Peak

[Input: XY data 🔿 Output: Endpoint]

This reduction transform computes the peak of all values for each XY plot (within the specified range of points to consider). <u>Flagged positions or points</u> 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.

Properties

Settings

	This setting determines which data point is considered as the peak:
Peak	• Max – The point with the maximum Y value
	• Min – The point with the minimum Y value
	This setting determines which axis's coordinate is stored in resulting matrix:
Result	• X – Projection of Peak value on X axis
	 Y – Projection of Peak value on Y axis

XY Reduction Total

[Input: XY data 🔿 Output: Endpoint]

This reduction transform computes the sum of all Y values for each XY plot (within the specified range of points to consider). <u>Flagged positions or points</u> are not included in the calculations.

XY Reduction X At Y

[Input: XY data 🔿 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 in 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. <u>Flagged</u> <u>positions and points</u> are not included in calculations.

This transform can be used to determine the X value at the point where the horizontal (defined by Y) value crosses each plot.

Properties

<u>Settings</u>

1

Cursor	The <u>expression</u> 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 <u>Matrix Position Reference</u> (e.g., #2) to refer to the data on the second matrix.
	This defines how the Cursor is treated for each position. The settings are:
Cursor Type	• Absolute – The value specified by Cursor is the value to use in the calculation of X .
	• 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).
	 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:
	Absolute – The computed X value.
	Relative From First Measured – The computed X value minus the X

value of the first point for the position (even if the first point is Flagged or out of range).
Relative From First In Range – The computed X value minus the X value

of the first non-flagged data point within the range.

XY Reduction X Between Y

[Input: XY data 🔿 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. <u>Flagged positions</u> and points outside the specified range are not included in calculations.

Properties

Settings

Cursor	The <u>expression</u> 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 <u>Matrix Position Reference</u> (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:
	 Absolute – The value specified by Cursor is the value to use in the calculation of X.
	• 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).
	• 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.

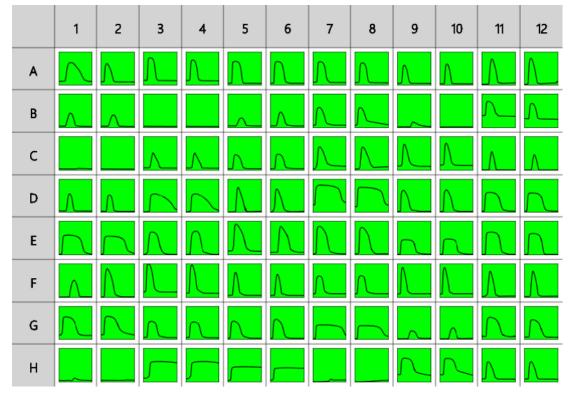
l If the plot is not crossed or only crossed once, the result will be flagged.

Example

Let's assume that we have some XY measurement data for a 12x8 microplate stored in the **Raw** matrix. In this example we would like to calculate the difference between first two **X** points that equal or cross the specified **Cursor** and then reduce data to the difference between these 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.

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 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

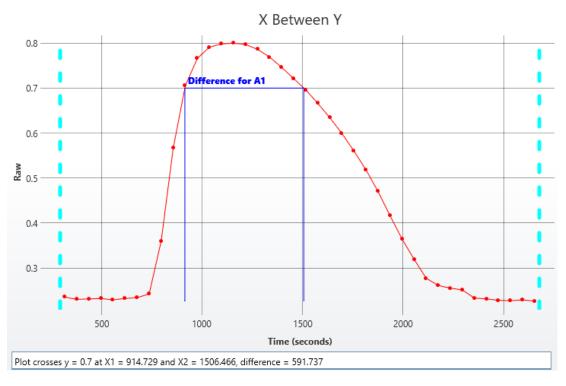
To reduce the plot at each position to the difference between the first and the second **X** value when crossing/equaling specified **Cursor**:

- 1. Specify Data: X Axis as X (Time), Y Axis as Y.
- 2. Leave **X Minimum** and **X Maximum** blank.
- 3. Specify Settings: Cursor as 0.7, Cursor Type as Absolute.
- 4. Press 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 **A1** plot is as follows (also displayed here to 3 decimal places):





XY Reduction Y At X

[Input: XY data 🔿 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. <u>Flagged</u> positions and points are not included in calculations.

D 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).

Properties

<u>Settings</u>

Cursor	The $expression$ evaluated for X value of each position.
	To refer to the position's corresponding data on an endpoint matrix, use a <u>Matrix Position Reference</u> (e.g., #2) to refer to the data on the second matrix.
Cursor	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:
	 Absolute – The value specified by the Cursor is the value to use in the calculation of Y.
Туре	 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 Flagged or out of range).
	 Relative From First In Range – The Cursor value is added to the X value of the first non-flagged data point within the range

Curve Fitting

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:

$$y=d+rac{a-d}{1+\left(rac{x}{c}
ight)^b}$$

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:

- **a** = the minimum value that can be obtained (i.e., what happens at 0 dose).
- **d** = the maximum value that can be obtained (i.e., what happens at infinite dose).
- **c** = the point of inflection (i.e., the value of x at the point on the S-shaped curve halfway between a and d).
- **b** = Hill's slope of the curve (i.e., this is related to the steepness of the curve at point c). It could either be positive or negative.

The rearranged equation to solve x is:

$$x=c\left(rac{a-d}{y-d}-1
ight)^{rac{1}{b}}$$

The **a** and **d** values might be flipped; however, **a** and **d** will always define the upper and lower asymptotes (horizontals) of the curve. **a** and **d** are the same units at **y**. The curve can only be used to calculate concentrations for signals within **a** and **d**. Samples outside the range of the determined **a** and **d** cannot be calculated.

4PL and IC50

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 4PL is equal to the c coefficient of the 4PL. In this case you can simply look at the calculated c 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.

Linear Regression

It is a basic and easy regression model that fits data in a linear way.

Linear regression sets the linear relationship between the dependent variable (\mathbf{y}) and a single independent variable (\mathbf{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 = mx + 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

m = the slope of the fitted line

c = the intercept of the dependent axis

The rearranged equation to solve x is:

$$x = rac{y-c}{m}$$

The goal is to determine values of **m** and **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.

Fitted plot may show the nonlinear character of data. In this case you may and are advised to consider setting other methods.

Goodness of Fit Measures

R², **aR²**, **P**, **SE**, **F** are calculated when each fit method is used. These measures are indicators as to how position the data fits the curve fit model.

The following table describes these measures and how they can be used to assess the fit.

Name	Description
	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.
R ²	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.
	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 R ² value that is closer to 1. However, it may be that the additional factors are essentially modeling noise.
	aR ² is the adjusted R ² value.
aR ²	aR ² is R ² adjusted downward to compensate for over-fitting. 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.
F	F is the F-statistic. F is the ratio of the variance explained by the curve-fitting model to the residual variance.
Р	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.

Best Fit

The Best Fit feature will automatically find the most appropriate fit for your data. The feature processes your data with multiple curve fits and multiple weighting methods and automatically picks 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 dose-response curves.

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?

What is deemed to be the best fit can be customized. For example, you might consider the fit with the highest **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 scoring method to quantify how good each fit is. The fit with the highest score is considered to be the best fit.

For convenience, the following preset scoring methods are available for selection:

- R²
- R² with Low-End Accuracy
- R² with High-End Accuracy
- R² with Low-End and High-End Accuracy
- 1/Standard-Error
- 1/P-Value

Beyond these presets, you can define your own scoring methods to evaluate and compare each attempted fit.

Scoring Method Expressions

This section describes how to define your own scoring method expressions to evaluate a fit:

On completion of each fit, the fit is scored using a scoring 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.

The scoring method equation can reference the goodness measures determined from the fit:

Goodness Measures

Name	Description
	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.
R ²	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.
	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 R ² value that is closer to 1. However, it may be that the additional factors are essentially modeling noise.
	aR ² is the adjusted R ² value.
aR ²	aR ² is R ² adjusted downward to compensate for over-fitting. 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.
F	F is the F-statistic. F is the ratio of the variance explained by the curve-fitting model to the residual variance.
Р	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.

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 (\mathbf{x}) plotted against their measurements (\mathbf{y}). Each curve is fitted to the calibration data set.

To measure the accuracy of the curve at a particular data point, the **x** value of the known concentration (expected) is compared against the **x** value at this point computed from the curve (actual).

% Recovery or % Accuracy is simply:

100 * Actual / Expected

To simplify combining accuracy functions together and with other measures (such as **R**²), the accuracy functions return a value between 0 and 1 (where 1 is perfectly accurate).

Function Name	Description		
AccuracyRatio	value. This is similar to % recov between 0 and 1 where 1 is per ery). A % Recovery of 120% or	fect accuracy (i.e., 100% recov- 80% both equal an Accuracy o, if the actual value differs from	
	Calculates an accuracy factor of the expected value and its actual value. This is computed using % Recovery and a Gaussian function such that:		
	100	Accuracy Factor	
AccuracyFactor	0	0.5	
	200	0.5	
	<0%	Value between 0.5 and 0	
	>200%	Value between 0.5 and 0	
	This function avoids reporting a value of 0 for scenarios where the % Recovery is <0% and >200%.		

Function Name	Description
Diff	The absolute difference between the expected value, and is the
וווע	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 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 value

Combining Measures

A scoring 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
R ²	R ²
R ² with Low-End Accuracy	R ² *AccuracyFactorLow(1)
R ² with High-End Accuracy	R ² *AccuracyFactorHigh(1)

Preset	Expression
R ² with Low-End and High-End Accuracy	R ² *AccuracyFactorLow(1)*AccuracyFactorHigh(1)
1/Standard-Error	1/SE
1/P-Value	1/P

Standard Curve Fit Advanced Configuration

Standard Curve Fit Custom Flag Labels

When computing concentration values from a curve, there might be samples outside certain ranges that should be flagged and reported with a custom label rather than as a result or error.

The Standard Curve Fit Custom Flag Labels feature enables rules to be defined to specify when a result should be flagged and reported using a label.

Example Uses

A typical use of this feature is to label results for standards 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 determined ULoQ and LLoQ values
- the mathematical limits of the curve
- your own defined limits (defined as absolute values or using <u>expressions</u> with limits computed from the curve fit)

The Standard Curve Fit Custom Flag Label is an advanced option only available through XML configuration. This feature is related to the <u>Custom Flag Label</u> feature, in this case Standard Curve Fit Custom Flag Labels have higher precedence and override any other defined labels.

You may configure custom flagging rules in the <u>Standard Curve Fit transform</u> based on the transform's input or output data (calculated from the curve). If any of these rules are satisfied then result is flagged with a specified label applied to it.

The rules are defined using $\underline{expressions}$ written in terms of **x** to define when a result should be flagged.

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.

Evaluation and Results

You can define any number of Custom Flag Label rules in any order. 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). These specified rules take precedence over the default flagging settings or optionally specified CurveError. Therefore, if any of the rules with **Type="Input"** are evaluated as true, then these will be used in place of any curve error.

For multiple plate assays, the associated curve for the position's plate is used.

If a flag condition is satisfied and a Custom Flag Label is used, then an entry is added to the Calculation Log detailing this application. If there is any error in evaluating a Condition, the details of this is added to the error log.

How to: Add Custom Flag Label Rules

Standard Curve Fit Custom Flag Labels are configured using XML. To add flagging rules to a Standard Curve Fit transform configuration:

- 1. Select the **XML** tab in the **Assay Properties** panel (If the **XML** tab is not immediately visible, press the **<< Show All Properties** button to expand).
- 2. Locate the StandardCurveFit element.
- 3. Add a **Flags** element (as a child of the **StandardCurveFit**) and, optionally, a **CurveError** element.
- 4. Add a child element **Flag** for each required flagging rule. Set the attributes (in accordance with the following table).
- 5. Press **Apply** to save changes to the protocol.

CurveError Optional Setting

The optional CurveError setting is used to specify a default custom flag label to use if a value cannot be computed from the curve (i.e., 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.

This is specified in the XML inside the **Flags** element with following syntax:

<Flags CurveError="My Custom Error Label" >

Flag Element Attributes

The following table lists the attributes of the Flag element that must be specified to define the flag rule:

	The value of \mathbf{x} depends on the expression type:
Туре	 For Type="Input" x is the value of the input position.
	• For Type="Output" x is the value computed for the output position.
	The condition to evaluate (typically a <u>conditional expression</u>).
	The flag condition expression can refer to:
	• the value of each position using the reference x .
Condition	 the computed <u>coefficients of the curve and goodness measures</u>.
	<u>Standard Curve Named Evaluations</u> .
	 sample values of the input matrix.
Label	If a flag rule is satisfied (i.e., the Condition is evaluated to true), then the result of the transform for the position is flagged with the associated Label . If you insert {x} in the Label , it inserts the value of x (e.g., {x} is Below LLD
	might be reported as 0.25 is Below LLD).

Specifying Rules with XML

When entering conditions as XML there are certain symbols that must be encoded using correct XML syntax. These are listed in the table below.

Operator	Description	XML Encoding
<	Less Than	<
>	Greater Than	>
&	Ampersand	&
н	Double Quote	"
,	Apostrophe/Single	9 anos
	Quote	'

For example, if the condition is **x** < **a** then this would be encoded in XML using **x &It; a**

Example Configuration

The following XML snippet shows a complete configuration of the StandardCurveFit element with Flag rules. With this configuration, any sample whose measurement response is above or below the computed asymptotes of the 4PL (the a and 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="Below Lower Asymptote" />

<Flag Type="Input" Condition="x > max(a,d)" Label="Above Lower Asymptote" />

</Flags>

</StandardCurveFit>

Example Flag Conditions

The following table lists further example flag conditions.

XML Condition	Description
<flag <="" condition="x &It;</td><td>If the measurement response is less than</td></tr><tr><td>Blank1+3*sd(Blank1)" label="{x} Below LLD" td="" type="Input"><td>Blank1 + 3SD, then the result is flagged and</td></flag>	Blank1 + 3SD, then the result is flagged and
/>	reported with the Label.
	If the measurement response is less than the
<flag condition="x &It; min</td><td>lower value of the a and d coefficients (as</td></tr><tr><td>(a,d)" label="Below Lower Asymptote" type="Input"></flag>	computed from the curve), then the result is
	flagged and reported with the Label.
Clear Type-"Input" Condition-"y Sult 0.125"	If the measurement response is less than
<flag <br="" condition="x < 0.125" type="Input">Label="OD < 0.125" /></flag>	0.125, then the result is flagged and reported
	with the Label.
	If the computed concentration is less than
<flag condition="x <</td><td>0.125, then the result is flagged and reported</td></tr><tr><td>0.125" label="Conc. < 0.125" type="Output"></flag>	with the Label.

Standard Curve Fit Evaluations

In addition to computing concentrations, ULOQ, and LLOQ from the curve, you can evaluate expressions that:

- Calculate **X** or **Y** values on the curve (using interpolation/extrapolation)
- Reference the coefficients and goodness measures of the curve
- Can be referred to as a variable in other evaluations or <u>Standard Curve Fit custom flag</u> <u>labels</u>
- 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.

How to: Add Evaluations to a Standard Curve Fit Transform Configuration

- 1. Select the **XML** tab in the **Assay Properties** panel (If the **XML** tab is not immediately visible, press the **<< Show All Properties** button to expand).
- 2. Locate the **StandardCurveFit** element.
- 3. Add an Evaluations element (as a child of the StandardCurveFit).
- 4. Add a child element **Evaluation** element for each required evaluation. Set the evaluation attributes in accordance with the table below.
- 5. Press **Apply** to save changes to the protocol.

Evaluation Attributes and Syntax

	The <u>expression</u> to evaluate. It can refer to:
	 the computed coefficients of the curve, Transform Output Variables and goodness measures.
Expression	 any defined above in the XML Standard Curve Fit Evaluation with Name attribute specified.
	 sample values of the input matrix.
	Since the result is a single numerical value, the expression cannot refer to x or y variables.

Туре	 This setting describes how the expression is treated in report: 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. CalcY – Calculates the Y value from the fit line using Expression as X value. The resulting values are reported using standard numerical formatting. Parameter – Outputs the computed Expression value. 	
Description	Optionally added textual description of the expression. If this is not specified, the Expression itself is used in report.	
Name	Optional element that defines a name for evaluation. If it is specified, the Name can be used as a variable for other XML configurations (as described below).	

<Settings>

<StandardCurveFitStandardType="2" FitMethod="4PL" Concentrations="0.25,0.5,1,2,4,8" XAxisTitle="Concentration" YAxisTitle="OD" FitToStandard="Average" CalculateFrom="Average">

<Evaluations>

<EvaluationType="Type" Expression="Expression"
Description="Description"Name="Name"/>

</Evaluations>

</StandardCurveFit>

```
</Settings>
```

Named Evaluations

If the Name attribute is set, then the evaluation can be referred by this Name as a variable in:

- Context variables in evaluations that are defined in XML downward to it
- Standard 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.

Configuring Standard Curve Evaluations

Evaluations are configured using XML and are evaluated using the plate for the associated container. This means that **Blank1** always refers to the first blank sample group on the container. Further details are described in the Multiple Plate Standard Curve Fit Analysis topic.

For example, 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 as an expression that calculates **X** from the curve where **Y** = **Blank** + **3** * **sd(Blank)**.

The LLOQ and ULOQ calculations are determined automatically and equal a concentration value (i.e., one of the values defined in Concentrations).

If you create named evaluation LLOQ or ULOQ, then it replaces the default LLOQ and ULOQ calculation, e.g.:

<Evaluations>

```
<EvaluationType="CalcY" Expression="Control1 + (10 * sd(Control1))"
Description="My LLOQ"Name="LLOQ" />
```

</Evaluations>

Results and Report

The LLOQ and ULOQ are the first entries, and any further evaluations are added below them in the table.

The **Evaluation** data is displayed below the interactive chart and in the report as a simple two-column 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 other words, report syntax errors in a table (in same way as the <u>Evaluation feature</u>).

Expressions

Overview

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 Control1 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 scoring 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:

- **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.

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:

mean(1.5,2.5)

mean(1,5;2,5)

For advanced users working with data entered directly into 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 a comma list separator).

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, <u>validations</u>, and <u>evaluations</u>. 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 **Control1 * 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 **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 <u>Expression by Matrix</u> 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. <u>Validations</u> 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 x < 15 could be used along with %CV data to validate that all samples have a %CV of less than 15.

A typical expression comprises of:

- <u>operations</u>, (e.g., +, *, /, ^)
- 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.

Example Expressions

All expression examples provided here use a period decimal separator(.) and a comma list separator(,). Use <u>local settings of your system</u> when entering expressions on your own system.

Expression	Evaluated to	
Unknown1	The average of the replicates of Unknown1 (that are not flagged).	
Unknown	The average of all Unknown samples (that are not flagged).	
A1	The data for position A1 .	
A1+A2	The sum of the data at positions A1 and A2 .	
mean (A1,A2,A3)	The average of the data at positions A1 , A2 , and A3 .	
pcv(Unknown1)	The percentage coefficient of variation of Unknown1 .	
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).	
рсv	The percentage coefficient of variation of all Unknown1 and	
([Unknown1,Unknown2]) Unknown2 replicates.		
median (Standard1)	The median of the replicates of Standard1 (that are not flagged).	
x-Blank1	The value of variable x minus the average of Blank1 position.	
x > 15	A Boolean expression that would evaluate to 1 (true) if the value of variable ${f x}$ is greater than 15; otherwise it evaluates to	

Expression	Evaluated to		
	0 (false).		
If the value of x is greater than 15, then the result is 15; otherwise the result is x .			
x-yThe difference between x and y. Here x and y are corresponding positions from two matrices of dat the Expression by Matrix (Dual) transform).			
median(Unknown.2.All)	The median of all Unknown replicates on matrix 2 across all containers.		
Unknown1.2.3	The average of Unknown1 sample group on the 2nd matrix of 3rd container.		
TOV1.R2	The R ² value of the curve fit as used in the first transform (referenced using a Transform Output Variable).		

Operators

Unary Arithmetic Operator

Operator	Operation	Examples
-	Minus Sign	-1.23 -x

Binary Arithmetic Operators

Operator	Operation	Example
		2+1
+	Addition	x+y
		x+1
		2-1
-	Subtraction	х-у
		x-1
		2*3
*	Multiplication	x*y
		y*2
/	Division	2/2

Operator	Operation	Example
		x/y
		x/2
		2^3
^	Raising to a power	x^y
		x^2

Binary Logical Operators

The numerical evaluation of 1 for true and 0 for false. Thus, 1 > 0 = 1.

Operator	Operation	Examples
		1<2
<	Less Than	x <y< td=""></y<>
		x<2
		2>1
>	Greater Than	x>y
		x>2
		1=1
=	Equal To	x=y
		x=2
		3<=9
		3<=3
<=	Less Than Or Equal	x<=y
		x<=x
		x<=3
		9>=3
		3>=3
>=	Greater Than Or Equal	y>=x
		x>=x
		x>=9
<>	Not Equal	2<>1

Operator	Operation	Examples
		x<>y
		x<>3

Boolean Operations

The Boolean operations (and, or, not) are implemented as Conditional Functions.

Operands

Numbers

Number operands are simply numbers (e.g., 1.23, -100, 14).

Ensure that all number values entered into the user interface are provided using the local settings of your system.

Variables

The \mathbf{x} and \mathbf{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 x*100 expression, 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 x+y, each position on the resulting matrix will be the sum of input matrices values.

Variable	Relevant For	
None	Preconfigured Protocols, Certain Endpoint: Auto Flag, Average, Matrix Dif-	
	ference, Median, Percentage, Percentage Coefficient of Variation, Replicate	
	Outlier Removal, Standard Curve Fit, Standard Deviation, Variance	
x	Certain Endpoint : <u>Blank Correction</u> , <u>Dilution Curves</u> , <u>Dilution Factors</u> , <u>Expres</u> -	
	sion by Matrix, Expression by Position, Factor, XY Blank Correction	
х, у	Expression by Matrix (Dual), XY Expression (Dual)	
у	XY Expression	

Fit Transforms Context Parameters

<u>XY Reduction Fit Parameter</u> and <u>Standard Curve Fit</u> transforms calculate the context parameters of the fit. The former refers to the parameters in configuration settings, and the

latter uses them in flagging conditions and/or additional evaluations. These fit transform context parameters are:

Name	Expression Syntax
R ²	r2
aR ²	ar2
F	f
Р	p
SE	se

• Goodness of Fit Measures calculated from the fit.

• **Coefficients of the Fit** – These vary depending on the selected fit method and are specified within documentation of these methods.

Standard Curve Fit also produces additional values (such as quantification limits) and any defined <u>named evaluations</u>. These can be optionally used for further evaluations of custom flagging within this transform.

Fit Transforms Context Parameters apply within fit transforms only and cannot be used in general configuration of the assay (e.g., <u>validation</u> or <u>evaluation</u> tables) or other transforms.

Transform Output Variables

Any transform evaluated after the transform that produces the output variable can refer to it. This applies only where the later transform supports parameters with expression.

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 as being 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.

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 read. A position on a particular matrix and plate can be specified with a period, as described in <u>sample references in multiple container</u> <u>assays</u>.



When writing expressions, it is better to refer to sample groups such as **Blank1** 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 modified layout.

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 defined on the layout non-flagged Unknown1 position values.*

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**.

Sample References in Multiple Container Assays

To simplify evaluation and avoid the need to write a different expression for each container, expressions use local group numbering. Thus, the simple **Unknown1** reference always refers to the first **Unknown** sample group on the container being evaluated.

You can refer to a specific position, group, or type defined on specific layout plate with the syntax described below.

Reference	Refers To		
A1.2	A1 position for 2nd container of data		
A1.2.3	A1 position for the 2nd matrix of 3rd container of data		
A13	A1 position for 3rd container of data		
A1.2.All	The average of all A1 positions on matrix 2 across all containers		
Unknown1.2	Unknown1 sample group for 2nd container of data		
Unknown.2.All	The average of all Unknown replicates on matrix 2 across all containers		
Unknown1.2.All	The average of all Unknown1 replicates on matrix 2 across all containers		
Unknown1.2.3	Unknown1 sample group for the 2nd matrix of 3rd container of data		
Unknown13	Unknown1 sample data for 3rd container of data		

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 (e.g., kinetic or spectral) data is treated in analysis. To see further detains and configuration examples, please refer to the <u>Matrix Position Reference</u> subsection.

Matrix Position Reference

A Matrix Position Reference is a convenient way to refer to data in a specific matrix. For example, the expression:

#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).

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 (X Minimum and X Maximum) of XY 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.

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.

Example References

Matrix Position Reference	Refers To
#1	Matrix 1
#1.2	Matrix 1 Container 2

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.

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.

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 <u>XY Reduction Peak</u>

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.,

Assay Properties		
Transforms	Matrices	
 ✓—Raw (1) ↓—.1. Raw ✓—Calculated (1) ↓—.2. Peak 		

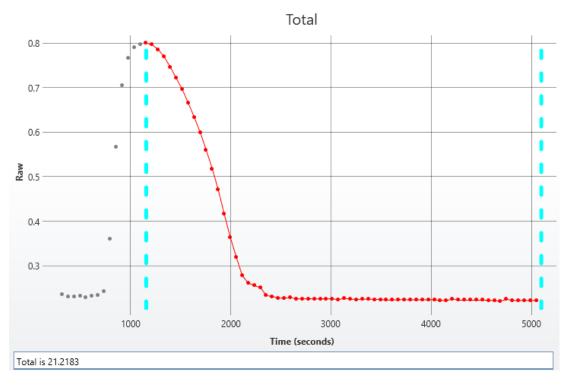
The next step would be to add another transform that uses the computed value of each sample. For example, add the <u>XY Reduction Total</u> transform. This simply calculates the total of the measurements. To configure the transform to only consider measurements from the peak set the **X Minimum** range option to:

#2

l.e.:

ssay Properties						:
Transforms	Matrices	Evaluation	Validation	Report	XML	
Input	0	utput	٦	ransform Configuration		
For each plot, find the	1. XY Reduction Peak maximum value.		Advanced Data			
Raw	🔹 📫 Peak		X Axis	X (Time)		v
For each plot, calculate	2. XY Reduction Total	۲	Y Axis	Υ		~
			- Range			
Raw	🔻 📫 Total		X Minimum	#2		
			X Maximum			
			- Units			
			Output Units			

With this setting, the transforms will compute the total of the measurements for each sample but only from the peak point, e.g.:

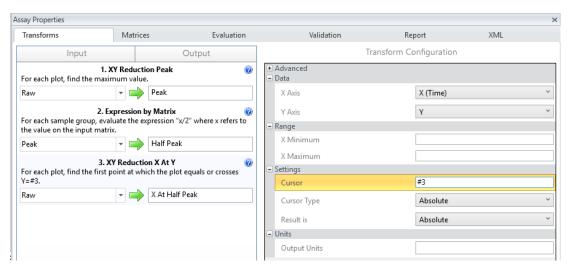


Example 2

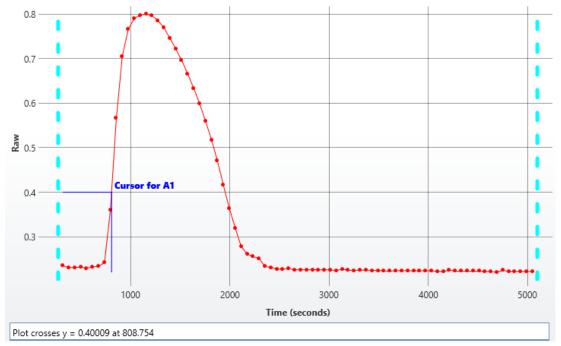
In another kinetic example, the time taken to reach half peak can be computed. In this case:

- 1. The <u>XY Reduction Peak</u> 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 <u>XY Reduction X at Y</u> 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.

l.e.:







Functions

This section provides reference information for various functions available to perform calculations on data.

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 <u>operand type</u>.

1. If the function refers to a single operand of any type:

function (operand)

- 2. If the function refers to a list of operands:
 - If operands do NOT include <u>sample references</u>:
 - function (operand1,operand2, ..., operandN)
 - If operands include ONLY sample references:

function ([operand1,operand2, ..., operandN])

• If operands include any operand type AND sample references:

function ([samplereference1,samplereference2, ..., samplereferenceN], operand1, operand2, ..., operandN)

- 3. If the function requires vector operands:
 - function ({list of Ys}, {list of Xs})
 - function({(samplereference1),(samplereference2), ..., (samplereferenceN)}, {operand1,operand2, ..., operandN})

Group-Enabled Functions

This section describes functions that operate on list of values with an open-ended length.

Where a <u>sample reference</u> 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 non-flagged values of that group/type.

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:

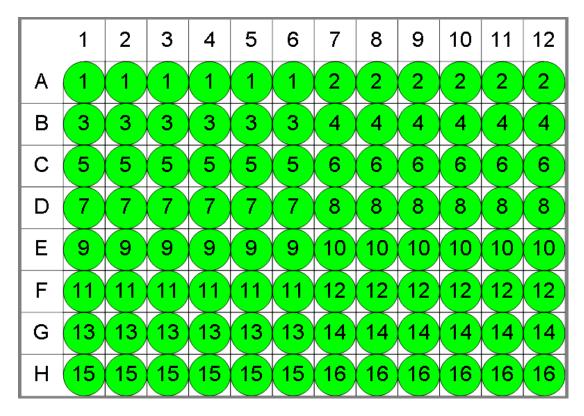
Name	Description	Minimum number of parameters	Examples
average	The same as mean .	1	Unknown1 average ([Unknown, Blank])
count	Counts the number of non-flagged values.	1	count (Unknown)
mean	Calculates the mean of the non-flagged positions.	1	mean (Unknown1) mean ([Unknown, Blank])
median	Calculates the 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	median (Unknown1) median ([Standard2, Unknown4])
рсv	Calculates the percentage coefficient of variance (%CV) of a finite list of non-flagged positions.	2	pcv (Unknown) pcv ([Unknown1, Unknown1.2])
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	sd (Standard2) sd ([Unknown1,

Name	Description	Minimum number of parameters	Examples
			Unknown3])
se	Calculates the standard error of a finite list of non- flagged positions.	2	se (Unknown1) se ([Unknown, Control])
sum	Returns the total of all items in the finite list of non- flagged positions.	1	sum (Unknown) sum ([Unknown1, Unknown1.2, Unknown1.3])
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	var (Unknown4) var ([Unknown1, Unknown1.2])

Example

Let's assume that we have some endpoint measurement data for a 12x8 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.:



Unknown

Here is some example raw data displayed to 3 decimal places:

	1	2	3	4	5	6	7	8	9	10	11	12
Α	0.364	0.323	2.105	2.124	1.213	1.226	1.159	1.190	0.110	0.116	1.463	1.481
В	0.455	0.441	1.869	1.888	1.203	1.218	1.180	1.206	0.132	0.130	0.786	0.768
С	0.587	0.575	0.474	0.514	1.206	1.239	1.203	1.210	0.113	0.116	0.613	0.626
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
Ε	1.105	1.065	0.279	0.367	1.215	1.225	1.233	1.262	0.111	0.114	1.579	1.549
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
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
н	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

We use the expression:

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):

1.202

Conditional Functions

These functions require a specified number of input parameters. Where a <u>sample reference</u> is used as an input parameter to a conditional function, it refers to the average of the non-flagged data points.

Boolean Logical Functions

Name	Description	Examples
and	Tests a number of input conditions and returns 1 if all of the con- ditions evaluate to true, or 0 otherwise.	and(1,1)
or	Tests a number of input conditions and returns 1 if any of the conditions evaluate to true, or 0 otherwise.	or (1,1) or (x<5, x>10)
not	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).	not(x>1)

Conditional Function

Name	Description	Examples
ifthenelse	Requires 3 parameters: 1. The Boolean condition to evaluate (which will evaluate to true or false).	ifthenelse (1=1,2,3) result is 2 ifthenelse (1=0,2,3) result
	If the value of the first condition is true, then the result is the second argument otherwise the third.	3

For example, with the reference:

IfThenElse(Unknown1 > 10, 10, Unknown1)

Unknown1 refers to the average of **Unknown1** non-flagged positions. Thus, the expression evaluation result is 10, if **Unknown1** is greater than 10; otherwise the result is **Unknown1**.

More functions with fixed parameter length are described in Layout Analysis Functions.

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 <u>syntax for functions</u> and perform an operation on a list of 1 or more <u>operands</u>. The evaluation of these results in a single numeric value.

The following table lists calculation functions.

Name	Description	Examples
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.	abs (-1.23)=1.23 abs (1.23)=1.23
average	Described in group-enabled functions.	average(1,2)=1.5 average(-3,0.25,5)=0.75
ceiling	 Returns argument ({0}) rounded up, away from zero, to the nearest multiple of significance ({1}). It requires two input parameters with the following syntax ceiling({0}, {1}), where: {0} is the value you want to round off. {1} is the multiple to which you want to round. Regardless of the sign of a {0}, a value is rounded up when adjusted away from zero. If the {0} is an exact multiple of {1}, no rounding occurs. 	ceiling (1.23, 0.05)=1.25 ceiling (-1.23, 0.05)=-1.2
count	Described in group-enabled functions.	count (1,2,3)=3 count (-2.44,2,5,98,6)=5
exp	Returns e raised to the power of the given number. Syntax: exp({0}) where: {0} is the exponent applied to the base e . It accepts only a single argument. For example, the system accepts exp(A1+A2) and rejects exp(A1,A2).	exp (2)=7.389056 exp (-1)=0.367879
floor	Returns argument ({0}) rounded down, toward zero, to the nearest multiple of significance ({1}). It	floor (1.23, 0.05)=1.2

Name	Description	Examples
	 requires two input parameters with the following syntax floor({0}, {1}), where: {0} is the value you want to round off. {1} is the multiple to which you want to round. Regardless of the sign of a {0}, a value is rounded down when adjusted away from zero. If the {0} is an exact multiple of {1}, no rounding occurs. 	floor (-1.23, 0.05)=-1.25
intercept	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, {y1,y2,y3} and {x1,x2,x3}; {y1,y2, yN} and {x1, x2, xN}.	<pre>intercept({1,2},{3,2})=4 intercept({-2.44,2,5,98,6}, {0.1,0.2,0.3,0.4,0.5})=- 12.152</pre>
In	Returns the natural logarithm of an argument. Natural logarithms are based on the constant e (2.718281828459). The argument is supposed to be positive. In is the inverse of the exp function.	In (10)=2.302585 In (1.23)=0.207014
log	Calculates the logarithm of an argument to the base that you specify. This uses the following syntax with two input parameters: log({0}, {1}) where: {0} is the positive real numerical value or <u>expression</u> for which you want the logarithm.	log (2,4)=0.5 log (1.23,2)=0.298658

Name	Description	Examples
	{1} is the base of the logarithm. If the base is omitted, it is assumed to be 10.	
log10	Returns the base-10 logarithm of a positive argument.	log10(1.23)=0.089905 log10(0.1)=-1
max	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.	max (1,2,3)=3 max (-2.44,2,5,98,6)=98
median	Described in group-enabled functions.	median (1,2,3,4)=2.5 median (-2.44,2,5,98,6)=5
min	Inversely to max function, returns the smallest num- ber in a set of values. All requirements of the max apply to min too.	min (1,2,3)=1 min (-2.44,2,5,98,6)=-2.44
mod	Returns the remainder after the argument is divided by a divisor. The result has the same sign as the divisor. Syntax: mod({0}, {1}) where: {0} is the argument for which you want to find the remainder. {1} is the value by which you want to divide the {0 }.	mod (2,0.3)=0.2 mod (1.23,5)=1.23
pow	Returns the result of a number raised to a power. Syntax: power({0}, {1}) where: {0} is the base argument. {1} is the exponent to which the {0} is raised.	power (2,3)=8 power (1.23,5)=2.815305
slope	Returns the slope of the linear regression line through data points in Xs and Ys. 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.	slope ({1,2},{3,2})=-1 slope ({-2.44,2,5,98,6}, {0.1,0.2,0.3,0.4,0.5})=112.88

Name	Description	Examples
	Requires a vector of arguments as parameters.	
	The number of Y and X operands is required to be the same and greater than 2 (i.e., {y1,y2,y3} and {x1,x2,x3}; {y1,y2, yN} and {x1, x2, xN}).	
stdev	The same as sd in group-enabled functions.	stdev (1,2,3)=1 stdev (- 2.44,2,5,98,6)=42.772359
sum	Described in group-enabled functions.	sum (1,2,3)=6 sum (- 2.44,2,5,98,6)=108.56
var	Described in group-enabled functions.	var (1,2,6)=7 var (- 2.44,2,5,98,6)=1829.47472

Layout Analysis Functions

Layout Analysis Functions are available only if the evaluation is associated with a layout. Otherwise an error message displays.

Layout Evaluation Functions

Layout Evaluation functions are provided to enable sample group references to be more flexibile.

The following table lists the layout evaluation functions:

Name	Description	Example
	Ispecified type and droup number (The type name	GroupValue ("Unknown",1)

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 wanted to reference the Unknown group that has the same number as the current position, use: **GroupValue("Unknown", PosGroup())**

Position Functions

Position Functions are only available when there is a position associated with the expression being evaluated. They take no input parameters.

The following table lists the position functions:

Name	Description	Example
PosNum()	Returns the 1-based position number of each position, which is assigned in accordance with position numbering.	PosNum()
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.	PosGroup()
PosType()	Returns the sample type number associated with the position.	PosType()
PosReplicate()	Returns the 1-based replicate number of the specified in the argument position. Replicate numbers are assigned in accordance with position numbering. This function is based purely on the layout and does not take flagging into account.	PosReplicate ()

Layout Functions

These functions require an associated layout.

Layout Function Parameters

Туре

Type can be specified as a sample type number or case-insensitive sample type name in quotes, e.g.:

- LayoutNumGroups (3)
- LayoutNumGroups ("Blank")

- LayoutNumGroups ("blank")
- LayoutNumGroups ("bLaNK")



Unused" type is not supported.

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.

The following table lists the layout functions:

Name	Description	Example
	The number	
	of columns in	
	the layout	
LayoutWidth()	(note, this is	LayoutWidth()
	same value	
	for all con-	
	tainers).	
	The number	
	of rows in the	
LovoutHoight()	layout (note,	LovoutHoight()
LayoutHeight()	this is same	LayoutHeight()
	value for all	
	containers).	
	The total	
	number of	
	positions	
LayoutPositions()	(note, this is	LayoutPositions()
	same value	
	for all	
	containers).	

Name	Description	Example
LayoutNumContainers()	The number	
	of containers	LayoutNumContainers()
	in the layout.	
	The number	
	of groups	
	with the	
	specified type.	
LayoutNumGroups(Type, ContainerNum)	Input	LayoutNumGroups(3, 1)
containernum)	parameters:	
	<u>Type</u> ,	
	<u>ContainerNu</u>	
	<u>m</u> .	
	The number	
	of positions	
	with the	
	specified type	
	and 1-based	
	group	
LayoutNumPositions(Type,	number	LayoutNumPositions("standard",
GroupNum, ContainerNum)	(GroupNum).	3, 1)
	Input	
	parameters:	
	GroupNum,	
	<u>Type</u> ,	
	<u>ContainerNu</u>	
	<u>m</u> .	
	The number	
	of positions	
	that have a	
LayoutNumUsedPositions	type that is	LayoutNumUsedPositions(2)
(ContainerNum)	not unused	
	(i.e., <u>Type</u> !=	
	1).	
Lay-	Determines	Lay-

Name	Description	Example
	the number of	
	groups of the	
	specified <u>Type</u>	
	parameter on	
	each of the	
	containers. If	
	this is the	
outNumGroupsAcrossContainers	same number	outNumGroupsAcrossContainers
(Туре)	on every	("Unknown")
	container	
	(including 0),	
	then this	
	value is	
	returned.	
	Otherwise -1	
	is returned.	

Integration with Readers

VICTOR Manager Integration with MyAssays Desktop

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.

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.

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*.

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.

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 <u>command line arguments</u> is typically a complicated error-prone process. To simplify this task, MyAssays Desktop includes the <u>MyAssays Analysis Launcher</u> 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 **Analysis Launcher** option to launch it.

Using Analysis Launcher

To use <u>Analysis Launcher</u>, 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, check 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 press **Launch**.

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:

My Protocol - Protocol editor
<u>F</u> ile <u>H</u> elp
ID Samples Measurement Outputs and Events
Number of plates and mapping of each plate:
I I I I I I I I Plate 1 of 1
1 2 3 4 5 6 7 8 9 10 11 12
Plate parameters: <u>M</u> easure each plate: 1 (1 - 300 times) Plate type:
Delay between repeats: 0 (0 - 3600 sec.) Generic &x12 size plate
Measurement height:
Standard (approx. 8 mm.)
C User defined: mm from bottom (3.0 - 18.0 mm)
Temperature level checking: ☐ Wam if plate holder temperature differs from 25 °C
leady

- 3. Select the Outputs and Events tab
- 4. Check the File output setting
- 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:

File outpu	đ	
File output	options:	
File type:	Text 💌	[
Filename:	C:\Users\Darren Cook\Documents\ <protocolname><runid></runid></protocolname>	•
Include in output:	 ✓ List ✓ Plate ✓ Protocol description ✓ Error description ✓ Notes 	

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:

C:\Program Files\MyAssays\MyAssays 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 <u>MyAssays Analysis</u> <u>Launcher</u> application and inspect the command line:

"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:

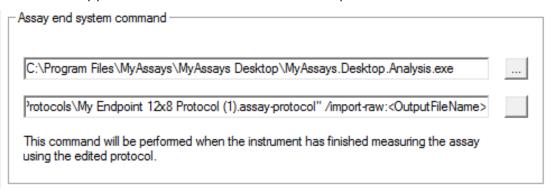
/import-raw:<OutputFileName>

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:



4. Save these changes to the VICTOR measurements protocol.

Similarly, if you are not sure, use the <u>MyAssays Analysis Launcher</u> 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:

/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	
ols\My Endpoint 12x8 Protocol (1).assay-protocol" /import-raw: <outputfilename> /calc</outputfilename>	
This command will be performed 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 <u>Analysis Launcher</u> tool or refer to the <u>Command Line Arguments</u> section for more information.

EnVision Integration

The EnVision manager software can output data in many possible export configurations.

To configure the EnVision manager software 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.:

File Print Event	
File output	
Open file in Excel after assay	
File name:	
<defaultdatafolder>\<date>\<protocolname>_<assayid>.csv</assayid></protocolname></date></defaultdatafolder>	
Export format:	
Plate 2 (with well headers)	-
Assay information to include Plates	
Basic assay information	
V Notifications	
Full protocol information	
Place at end of output	
Plate information to include	
V Plate information	
Background information	
Place at beginning of plate	
Other options	
Show picture	
Each plate to a separate file	
Don't add plate number to the file name	
Use system list separator 🔹 ,	

Command Line Arguments

MAA can be launched directly from the command line with optional specific arguments.

Syntax

>"C:\MyAssays Installation Root path\MyAssays Desktop\MyAssays.Desktop.Analysis.exe" "C:\MyAssays Folder Root path\MyAssays\Protocols\Protocol File Name.assay-protocol" /argument1 /argument2 etc.

Command line arguments are only relevant when the MAA is opened with an .assayprotocol file and can be specified in any order.

The following table lists optional command line arguments:

Argument	Description	Example
/calc	Performs specified calculations automatically (if /import-raw is used, then calculations are performed using the imported raw data).	/calc
/calc-log:{0}	Specifies a file to save calculation progress log.	/calc-log:"Destination file path"
/error-log:{0}	Specifies a file path to save calculation errors log.	/error-log:"Destination file path"
/exit	Closes MAA after calculations are completed/printed.	/exit
/export-report:{0}: {1}	 Specifies the export options. {0} is either: XLSX, PDF, XML (file export type). XSLT. Requires additional arguments to specify output filename extension and path to XSLT 1.0 file. 	/export-report:pdf /export- report:xslt1:csv:"XSLT file path"
	The exported file will be saved	

Argument	Description	Example
	under \MyAssays\Reports\ folder.	
	The optional {1} specifies whether to launch the exported file with its default associated application. If it is specified, the file will not launch.	:nolaunch /export- report:xml:nolaunch
	Specifies a Plate IDs file to use in data analysis.	
/import- containerids:{0}	This command-line argument overrides previously configured by Import Wizard method.	/import- containerids:"Data file path"
/import-raw:{0}	Specifies the raw data file to be used for analysis.	/import-raw:"Raw data file path"
/import- sampleids:{0}	Specifies a Sample IDs file to use in data analysis. This command-line argument overrides previously configured by Import Wizard method.	/import-con- tainerids:"Sample IDs file path"
/import-tweaks:	 Specifies a protocol tweaks file that defines changes to the protocol configuration. {0) - full path to the protocol tweaks file. 	/import- tweaks:"Protocol tweak file path"
{0}:{1)	The optional {1} specifies the save setting. If it is specified, the protocol file will be saved with tweaks automatically.	/import- tweaks:"Protocol tweak file path":saveprotocol

Argument	Description	Example
	protocoloption is selected(under File Save in theAnalysis application),then the changes will besaved to the protocolirrespective of this optionsetting (e.g., whencalculations areperformed).	
/noappwin	If this is specified, then the application window is NOT displayed.	/noappwin
/print	Automatically prints the report (if the calculations succeeded) with the default printer (if available).	/print
/tab:{0}	Allows you to select the tab MAA starts on. {0} is zero-based tab number : Overview = 0; Measurements = 1; Microplate = 2; Sample IDs = 3; Results = 4. If this option is not specified, then the first tab will open.	/tab:1

The maximum command line argument is 255 characters. If the command line argument exceeds this length, then a warning message is displayed and the program is exited.

To use long command line arguments, store the command-line argument list in a text file and launch MyAssays.Desktop.Analysis.exe, passing the text file containing the command-line arguments:

MyAssays.Desktop.Analysis.exe @<CommandTxtFile> Where <CommandTxtFile> is the name of the text file to read the command line from.

Examples

>"C:\MyAssays Installation Root path\MyAssays Desktop\MyAssays.Desktop.Analysis.exe" "C:\MyAssays Folder Root path\MyAssays\Protocols\Protocol File Name.assay-protocol" /import-raw:"Raw data file path" /tab:4 /calc /export-report:xlsx:nolaunch

The selected assay protocol with specified raw data file will be computed. The MAA will start with the **Results tab** and the XLSX report will be saved (but not automatically launched) under \MyAssays\Reports\ folder.

>"C:\MyAssays Installation Root path\MyAssays Desktop\MyAssays.Desktop.Analysis.exe" "C:\MyAssays Folder Root path\MyAssays\Protocols\Protocol File Name.assay-protocol" /calc /print /noappwin

The selected assay protocol with specified raw data file will be computed and the results will be printed out.

These settings can be configured using the Analysis Launcher.

Tweak Files

This section provides information about the protocol tweaks feature. The protocol tweak files are specified as <u>optional command-line arguments</u>.

This is an advanced feature and misuse of it could cause problems.

Overview

You may use tweak settings to define the data analysis parameters of a protocol customization. This enables any of the assay protocol parameters to be specified by other existing 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.

Tweak Syntax

A protocol-tweak.xml file is a 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>

Vou can insert any number of tweaks under <<u>ProtocolTweaks</u>> section.

Using the Feature

With your working protocol-tweak.xml file, use your instrument control or LIMS software to insert relevant protocol-tweak.xml file's data and launch the protocol from the command line when required. These tweak changes are not automatically saved to the assay file, and a tweaked protocol appears as modified and not saved (with * in title).



It is possible to launch, execute (generate reports), and exit <u>MyAssays Desktop Pro</u> analysis without any user intervention required.

To achieve this, we recommend developing the protocol-tweak file as follows:

- 1. <u>Build</u> or <u>obtain</u> an assay-protocol file that meets your data analysis requirements.
- 2. Identify which analysis parameters can be specified by your existing software.
- 3. Develop a protocol-tweak.xml file.
- 4. Test the protocol-tweak.xml file.

Any data analysis parameter can be modified.

How to: Add Protocol Tweaks

- 1. Launch the Command Prompt.
- 2. Enter the file path to the protocol tweak file as a parameter of the <u>MAA command-line</u> <u>arguments.</u>

OR

Add a protocol tweaks file in the Analysis Launcher.

The system will perform certain checks on the incorporated tweaks to detect possible configuration errors in common usage scenarios.

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 **<u>after</u>** 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.

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.

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"

To obtain current *Product Version* open **Information** screen of **File** tab in the <u>MADE</u>. You can either click on the **Copy** icon or enter it manually to your publication.

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Version 2.0, January 2004

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