User Guide

Belysa® Immunoassay Curve Fitting Software

Catalog # 40-122

Version 1.2

MilliporeSigma is the U.S. and
Canada Life Science business of
Merck KGaA, Darmstadt, Germany

Millipore®

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Welcome

Welcome to the Belysa® Immunoassay Curve Fitting Software User Guide.

Technical Support

For technical support and product updates, please visit www.sigmaaldrich.com/belysa

Notices

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Getting Started

Here are some steps to get started using Belysa® immunoassay curve fitting software:

Step 1: Install Software

If you haven't already done so, you should start by running one of the install programs located on the included USB flash drive. See **Install Software** for steps on how to run the installer program.

Step 2: Connect License Dongle

Next you should connect the included license dongle to your computer via an available USB port. The license dongle requires no additional driver installation.

Step 3: Open a File

Belysa® software can analyze data from a variety of different sources. To learn how to open a data file in Belysa® software, see **Open Files**.

Step 4: Analyze Results

Once you have opened your data, you can use the wide variety of analysis tools provided by Belysa® software to evaluate the performance of your assay. You can start by familiarizing yourself with the application **Interface**, or by reading about some of the application's key **Features**.

Step 5: Produce Reports

See **Reports** to learn about the different ways to export data from Belysa® software.

Step 6: Save Your Work

When you are finished analyzing your data, save your work to a Results file by following the steps in **Save Results File**.

Install Software

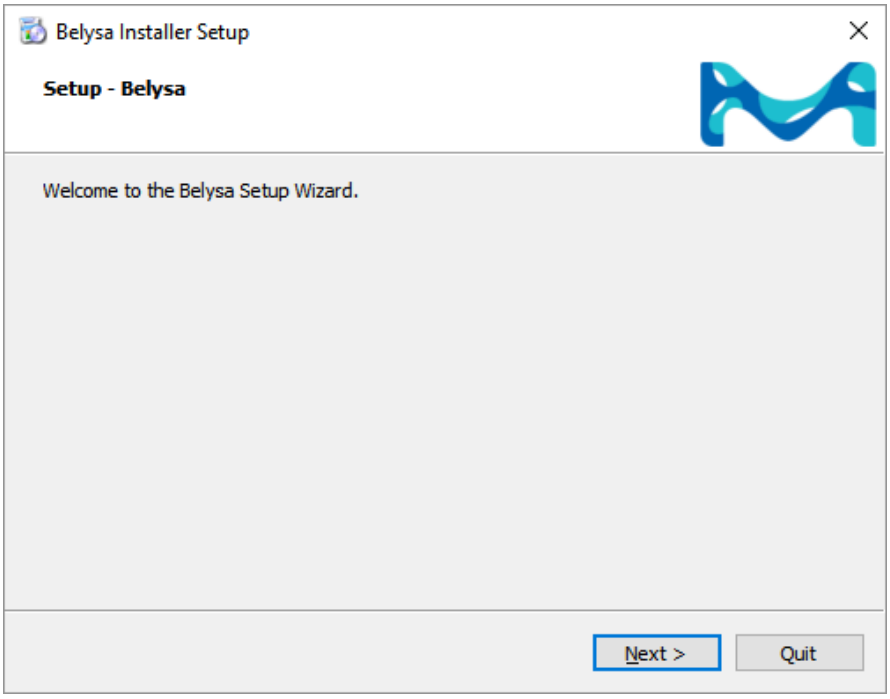
System Requirements

Belysa® immunoassay curve fitting software requires a 64-bit Windows 10 operating system. One available USB-A port is required to attach the provided license dongle.

Installation Instructions

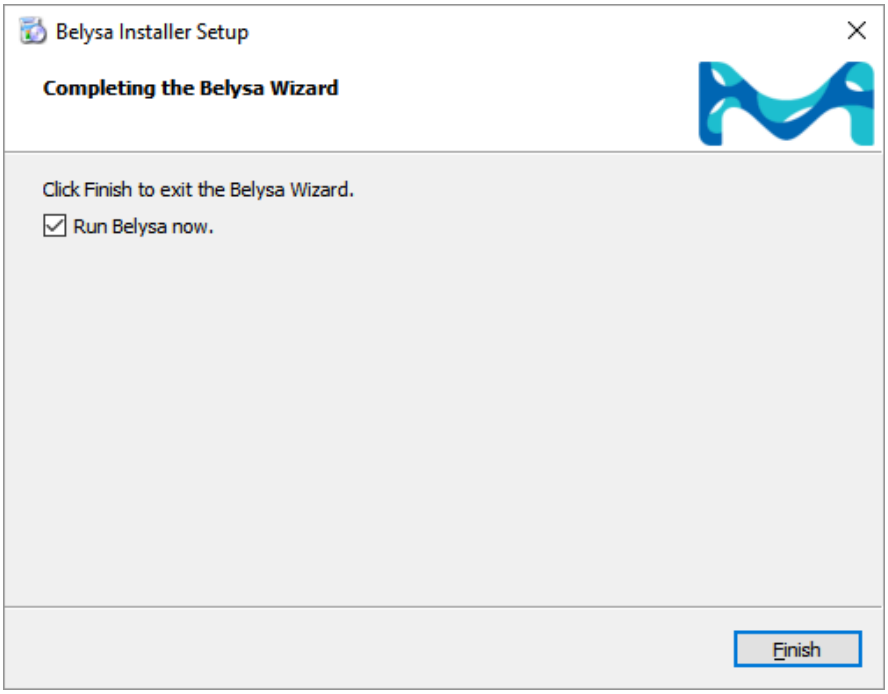
Follow these steps to install Belysa® software on your computer:

1. Identify the correct installer file for your system. If you are running a 64-bit version of Windows, choose **Install-Belysa-1.X.X-x64.exe** (where 1.X.X is a variable version number).
2. Start the installer program by double-clicking on the executable identified in Step 1. The installer program will start:



3. Follow the on-screen instructions to install the software, entering an administrator password if prompted.
4. When complete, select **Finish** to exit the installer. Select **Run Belysa now** to open Belysa®

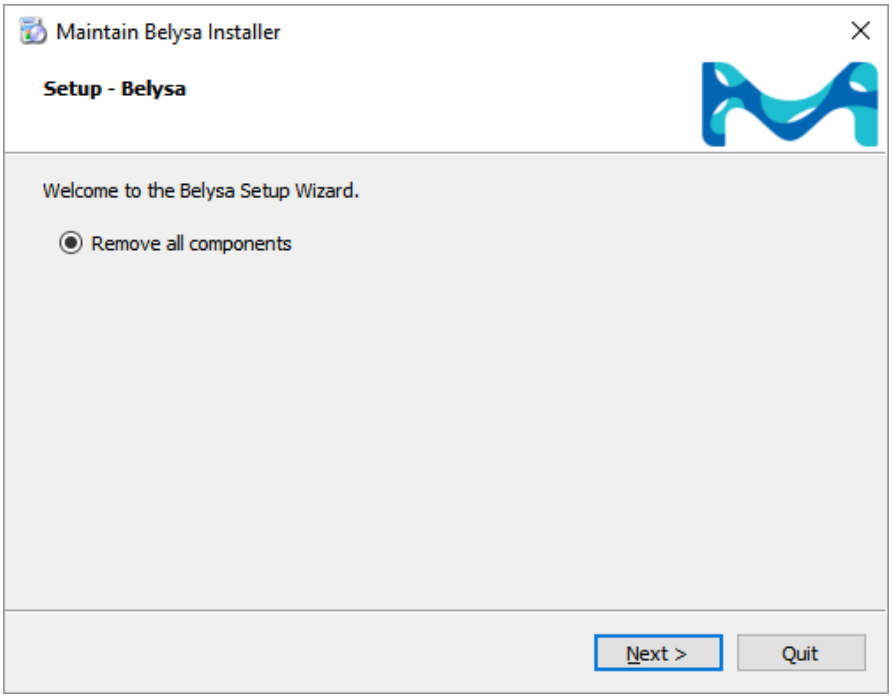
software after the installer exits:



Uninstall Software

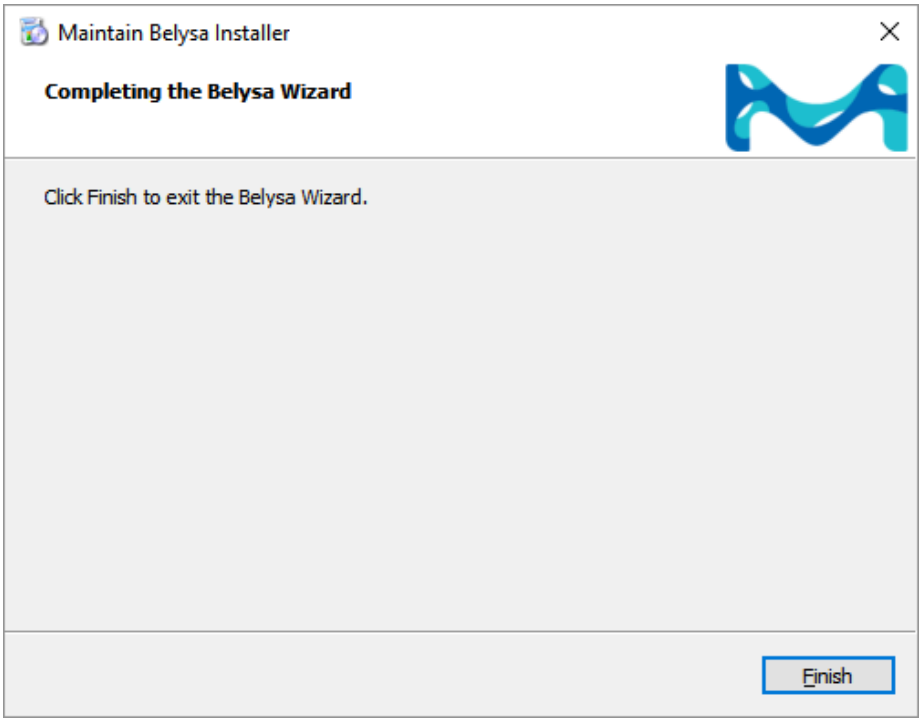
Follow these steps to uninstall Belysa® immunoassay curve fitting software from your computer:

1. Navigate to the Belysa® software install directory (e.g. **C:\Program Files\Belysa**) and double-click on the file **Uninstall-Belysa.exe**. Alternatively, you may uninstall the software via the Windows Control Panel Add/Remove Programs feature.
2. The uninstall program will start:



3. Follow the on-screen prompts to complete the uninstall process. When complete, select **Finish** to

exit the uninstaller:



Features

Here are the key features and concepts in Belysa® immunoassay curve fitting software you are most likely to use when analyzing your data:

- **Opening Files**
    - You can open files from multiple data sources, including Luminex® xPONENT® and SMCxPRO® platforms. See **Open Files**.
- **Single and Multi-Analyte Views**
    - You can view your data in different ways, focusing on a single analyte or a single value across all analytes. See **Single and Multi-Analyte Views**.
- **Plates**
    - Belysa® software supports 96-well and 384-well formatted plate data. See **Plates**.
- **Experiments**
    - Belysa® software organizes results into workspaces called experiments. See **Experiments**.
- **Auto-Flagging**
    - Belysa® software's auto-flagging features make it easy to quickly identify potential issues with your data. See **Auto-Flagging**.
- **Excluding Wells**
    - Wells can be excluded from result calculations by well and by individual analyte. See **Exclude and Include Wells**.
- **Show and Hide Table Columns**
    - To learn how to customize your result views, see **Show and Hide Table Columns**.
- **Produce Reports**
    - Export your data to a variety of report formats. See **Reports**.

Open Files

Belysa® immunoassay curve fitting software supports opening the following types of files:

- SMCxPRO® Data and Results files (*.xpd, *.xpr). See **Opening SMCxPRO® Files**
- xPONENT® files (*.csv). See **Opening xPONENT® Files**
- Bio-Plex Manager™ Multi-Analyte Export files (*.xlsx, *.xls). See **Open Bio-Plex Manager™ Files**.
- Generic ELISA data using the supplied Excel template (*.xlsx). See **Open Generic Data Files**.
- Belysa® software Results files (*.belysa). See **Opening Results Files**.

Opening SMCxPRO® Files

How to open SMCxPRO® Files

You can open XPD and XPR files containing SMCxPRO® instrument data by one of the following methods

- On the menu bar select **File** > **Open...** to display the Open File dialog. Navigate to the file you want to open, select it, and then click **Open**.
- Use the keyboard shortcut **Ctrl+O**. The Open File dialog displays. Navigate to the file you want to open, select it, and then click **Open**.
- Drag the file from your computer's file system onto the center of the Belysa® software application window

Data extracted from file

Belysa® software can recognize and import the following information from SMCxPRO® files

- Plate maps (one or more plates)
- Replicate groups
- Expected concentrations for Standard and Control wells
- Unknown dilution factors
- Response and other raw data values
- Experiments, including standard curves and curve fit selections
- Test information

Opening xPONENT® Files

How to open xPONENT® CSV files

You can open an CSV file generated by xPONENT® software in the following ways:

- Drag the file from your computer's file system onto the center of the Belysa® software application window

- On the menu bar select **File** > **Open...** to display the Open File dialog. Navigate to the file you want to open, select it, and then click **Open**.
- Use the keyboard shortcut **Ctrl+O**. The Open File dialog displays. Navigate to the file you want to open, select it, and then click **Open**.

Data extracted from file

Belysa® software can recognize and import the following information from xPONENT® Version 3.1 and 4.2 CSV files if it is present:

- Plate map
- Replicate groups
- Expected concentrations for Standard and Control wells
- Unknown dilution factors
- Analyte names, bead regions, and units
- Median and Count values
- Header information

Open Bio-Plex Manager™ Files

Belysa® immunoassay curve fitting software can open Excel reports generated by Bio-Plex Manager™ as long as the following conditions are met:

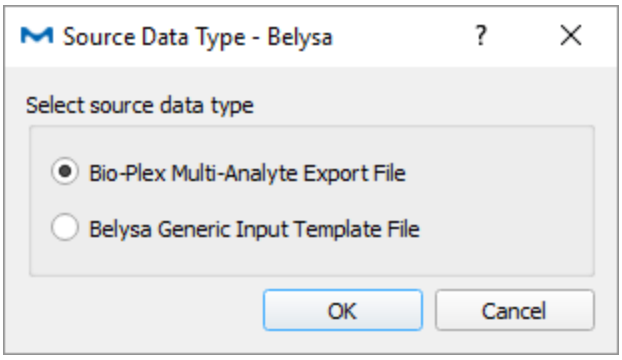
- The report must use the Multiple Analyte Layout
- Show Replicates must be selected
- The FI column must be visible

How to open a Bio-Plex Manager™ Excel File

To open a Bio-Plex Manager™ Excel report file, use one of the following methods

- On the menu bar select **File** > **Open...** to display the Open File dialog. Navigate to the file you want to open, select it, and then click **Open**.
- Use the keyboard shortcut **Ctrl+O**. The Open File dialog displays. Navigate to the file you want to open, select it, and then click **Open**.
- Drag the file from your computer's file system onto the center of the Belysa® software application window

Before opening the file, the Source Data Type dialog will appear asking you to select the source data type. In this case, choose **Bio-Plex Manager Multi-Analyte Export File**



Data extracted from file

Belysa® software can recognize and import the following information from Bio-Plex Manager™ files

- Plate map
- Replicate groups
- FI (MFI) values

Open Generic Data Files

If you have plate-formatted data you'd like to analyze using Belysa® software, you can import it using a provided Excel input template.

How to enter data into the provided Excel template

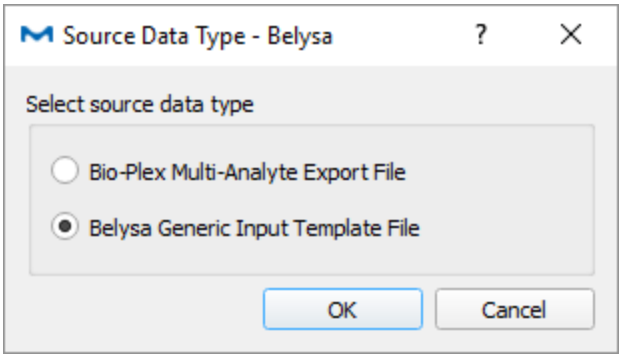
1. Navigate to the Belysa® software installation directory and open the **Templates** folder
2. Open the **Belysa Input Template.xslx**. The template included with the software is not editable, so in the next step we will make a copy to use for your data
3. Go to **File > Save As** and save a copy of this template on your desktop or other writeable location. You may give the copied template a different name.
4. If not already opened, open the copy of the template you made in the above step
5. Follow the directions on the **Instructions** tab to enter your data into the template
6. When you are finished, save the template as an Excel Workbook (.xlsx) file

How to open an Excel template containing data

To open an Excel template workbook containing generic data, use one of the following methods

- On the menu bar select **File** > **Open...** to display the Open File dialog. Navigate to the file you want to open, select it, and then click **Open**.
- Use the keyboard shortcut **Ctrl+O**. The Open File dialog displays. Navigate to the file you want to open, select it, and then click **Open**.
- Drag the file from your computer's file system onto the center of the application window

Before opening the file, the Source Data Type dialog will appear asking you to select the source data type. In this case, choose **Belysa Generic Input Template File**



Data extracted from file

Belysa® immunoassay curve fitting software can recognize and import the following information from generic input template files:

- FI (MFI) values
- Analyte names
- Header information

Opening Results Files

You can open a Results file generated by Belysa® software in the following ways:

- On the menu bar select **File** > **Open...** to display the Open File dialog. Navigate to the file you want to open, select it, and then click **Open**.
- Use the keyboard shortcut **Ctrl+O**. The Open File dialog displays. Navigate to the file you want to open, select it, and then click **Open**.
- Drag the file from your computer's file system onto the center of the Belysa® software application window

Single and Multi-Analyte Views

Belysa® immunoassay curve fitting software provides you with two ways to view and report your multiplex assay data. In *single-analyte* mode, all information is displayed for one analyte at a time. Each column in a table represents a different statistic, and all rows represent the same analyte. In *multi-analyte* mode, information about all analytes is shown for a single statistic (i.e. Result, Recovery, etc.) at a time. In this mode, each column in a table represents a different analyte, and all rows represent the same statistic. To switch between single and multi-analyte views of your data, use the **Analyte View Toolbar**.

Here is an example of a table in **single-analyte** format, showing all statistics for a single analyte:

Plate	Group	Location	Well ID	Analyte	Expected	Unk. Dilution	Sample ID	Result	Result SD	Result CV
Plate 1	S Standard Curve Group 1	A1 B1	1	IFN-gamma	0.00		Background0	-	-	-
Plate 1	S Standard Curve Group 1	C1 D1	2	IFN-gamma	3.20		Standard1	3.53	0.69	19.7%
Plate 1	S Standard Curve Group 1	E1 F1	3	IFN-gamma	16.00		Standard2	15.82	0.36	2.3%
Plate 1	S Standard Curve Group 1	G1 H1	4	IFN-gamma	80.00		Standard3	79.87	0.37	0.5%
Plate 1	S Standard Curve Group 1	A2 B2	5	IFN-gamma	400.00		Standard4	397.31	45.64	11.5%
Plate 1	S Standard Curve Group 1	C2 D2	6	IFN-gamma	2000.00		Standard5	2048.51	18.53	0.9%
Plate 1	S Standard Curve Group 1	E2 F2	7	IFN-gamma	10000.00		Standard6	9953.06	787.23	7.9% 1
Plate 1	U Unknown Group 1	G2 H2	8	IFN-gamma		1.00	Unknown1	177.91	6.51	3.7%
Plate 1	U Unknown Group 1	A3 B3	9	IFN-gamma		1.00	Unknown2	961.43	26.69	2.8%
Plate 1	U Unknown Group 1	C3 D3	10	IFN-gamma		1.00	Unknown3	1530.70	24.63	1.6%
Plate 1	U Unknown Group 1	E3 F3	11	IFN-gamma		1.00	Unknown4	303.83	43.35	14.3%

Here is an example of a table in **multi-analyte** layout, showing a single statistic for multiple analytes:

Plate	Group	Location	Well ID	Sample ID	Standard	IFN-gamma	IL-10	IL-17a	IL-4
Plate 1	S Standard Curve Group 1	C1 D1	2	Standard1	Standard Curve Group 1	3.20 pg/ml	3.20 pg/ml	3.20 pg/ml	3.20 pg/ml
Plate 1	S Standard Curve Group 1	E1 F1	3	Standard2	Standard Curve Group 1	16.00 pg/ml	16.00 pg/ml	16.00 pg/ml	16.00 pg/ml
Plate 1	S Standard Curve Group 1	G1 H1	4	Standard3	Standard Curve Group 1	80.00 pg/ml	80.00 pg/ml	80.00 pg/ml	80.00 pg/ml
Plate 1	S Standard Curve Group 1	A2 B2	5	Standard4	Standard Curve Group 1	400.00 pg/ml	400.00 pg/ml	400.00 pg/ml	400.00 pg/ml
Plate 1	S Standard Curve Group 1	C2 D2	6	Standard5	Standard Curve Group 1	2000.00 pg/ml	2000.00 pg/ml	2000.00 pg/ml	2000.00 pg/ml 2
Plate 1	S Standard Curve Group 1	E2 F2	7	Standard6	Standard Curve Group 1	10000.00 pg/ml	10000.00 pg/ml	10000.00 pg/ml	10000.00 pg/ml 1(
Plate 1	U Unknown Group 1	G2 H2	8	Unknown1	Standard Curve Group 1				
Plate 1	U Unknown Group 1	A3 B3	9	Unknown2	Standard Curve Group 1				
Plate 1	U Unknown Group 1	C3 D3	10	Unknown3	Standard Curve Group 1				
Plate 1	U Unknown Group 1	E3 F3	11	Unknown4	Standard Curve Group 1				
Plate 1	U Unknown Group 1	G3 H3	12	Unknown5	Standard Curve Group 1				

The following report output formats support both single-analyte and multi-analyte formats:

- TXT
- CSV
- Excel

The PDF report currently only supports a single-analye layout.

Experiments

An *experiment* contains the data being used for analysis. An experiment can contain one or more standard curves, each of which may be associated with one or more sample groups. Each experiment is reported separately into its own text, Excel, or PDF files. There are two types of experiments: *quantitative* experiments, which display interpolated concentration values for sample groups associated with a standard curve, and *relative potency* experiments, which display measures of similarity between a control curve and one or more comparison curves.

For more information about working with experiments, see:

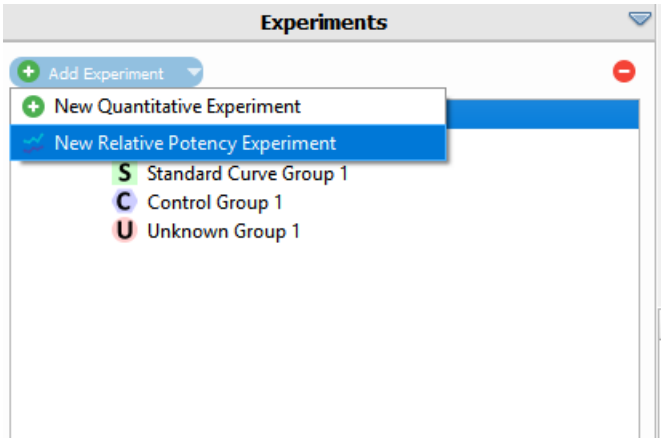
- **Create an Experiment**
- **Add Standard Curves to an Experiment**
- **Edit Experiment Name and Details**
- **Experiment Results**

Create an Experiment

A Results file can contain one or more experiments. To create a new experiments, follow these steps:

How to Create an Experiment

1. To add a new experiment, select the **Add Experiment** button to display the shortcut menu, then select either **New Quantitative Experiment** or **New Relative Potency Experiment** depending on your experiment type:



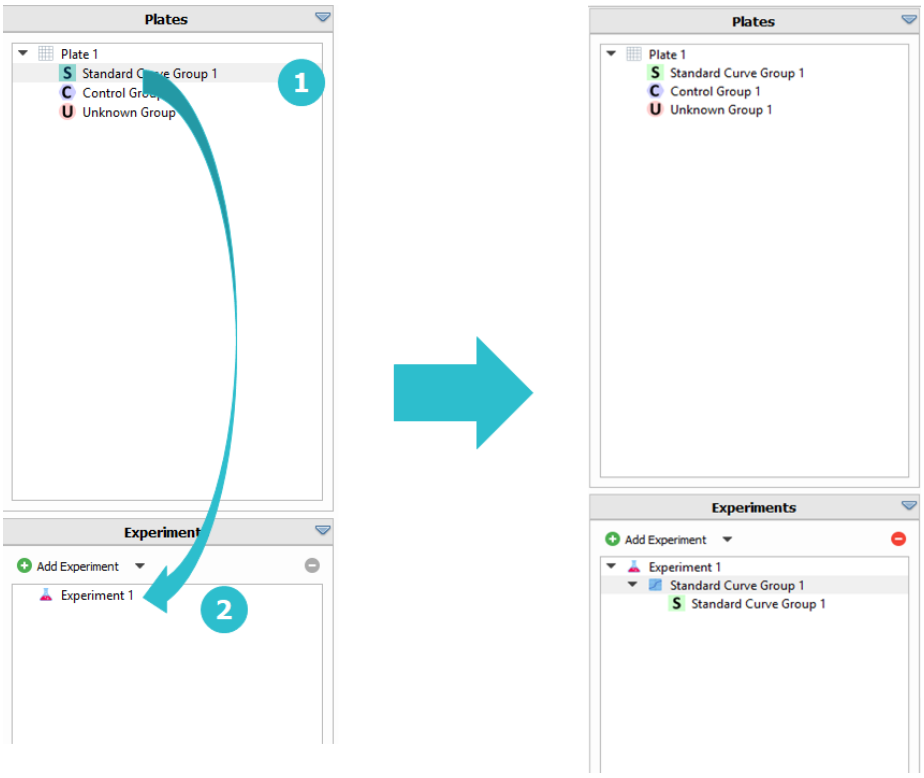
Add Standard Curves to an Experiment

To add a standard curve to an experiment, first make sure you have created a standard group on your plate (see **Create Groups**), and that you have added an experiment to your Results file (see **Create an Experiment**). There are two ways to add a standard curve to an experiment:

Method 1: Drag and Drop from Plate to Experiment

To add a standard curve to an experiment by dragging and dropping, perform the following steps:

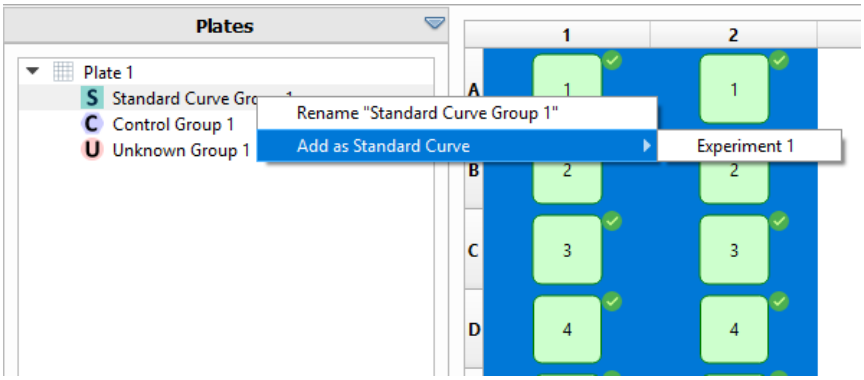
1. In the Plates tree, left-click the standard group you wish to add
2. Drag the standard group to the Experiments tree, dropping it on an experiment



Method 2: Use Shortcut Menu

You may also add standard curves to experiments by right-clicking on a group in the Plates tree, and using the context (pop-up) menu commands:

1. In the Plates tree, right-click the standard group you wish to add to open the shortcut menu
2. Hover over the Add as Standard Curve option, and then select the name of the experiment



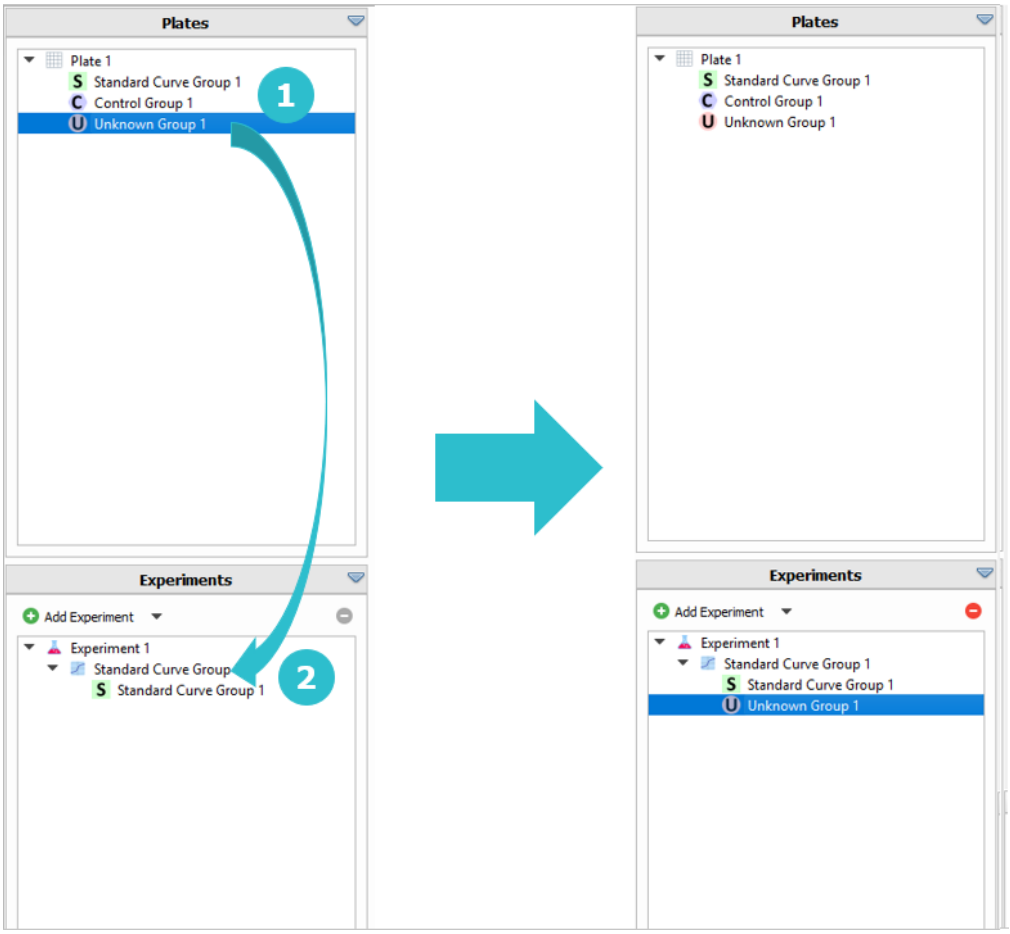
Add Sample Groups to a Standard Curve

To obtain quantitative results for a sample group, first make sure you have created one or more groups on your plate (see **Create Groups**), and that you have added an experiment to your Results file (see **Create an Experiment**). Your experiment should have at least one standard curve (see **Add Standard Curves to an Experiment**). There are two ways to add a sample group to a standard curve:

Method 1: Drag and Drop from Plate to Standard Curve

To add a sample group to a standard curve by dragging and dropping, perform the following steps:

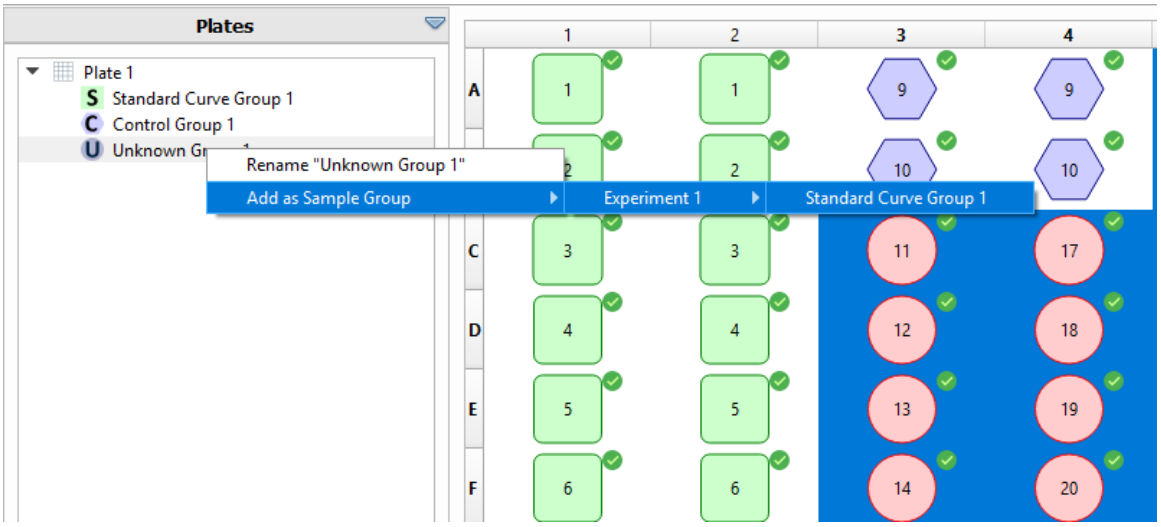
1. In the Plates tree, click the sample group you wish quantify.
2. Drag the standard group to the Experiments tree, dropping it on top of a standard curve group (as indicated by the  icon):



Method 2: Use Shortcut Menu

You may also add sample groups to standard curves right-clicking a group in the Plates tree, and using the shortcut menu:

1. In the Plates tree, right-click the standard group you wish to add to open the shortcut menu.
2. Hover over the Add as Standard Curve option, and then select the name of the experiment:



Change the Curve Fitting Method

Belysa® immunoassay curve fitting software provides several regression methods for fitting standard curve data, including:

- 4PL (standard and robust models)
- 5PL (standard and robust models)
- Linear
- Cubic Spline
- Competitive

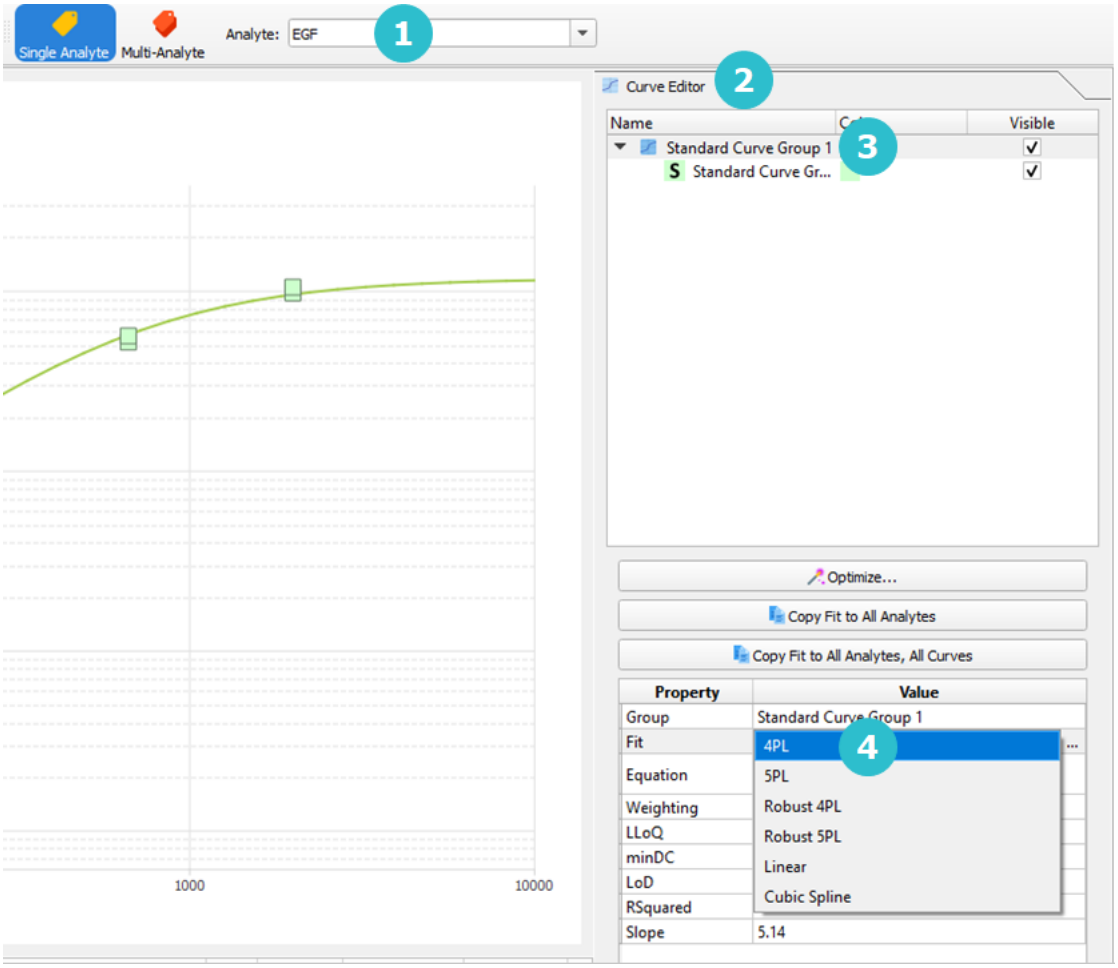
For a more detailed description of the differences between the different regression options, see **Mathematical Reference**.

How to change the curve fit for a single analyte

In single-analyte view

1. Select an analyte from the **Analyte** list on the toolbar, or press **Ctrl-K** on the keyboard and then type the name of the analyte.
2. Go to the **Curve Editor** tab in the right panel
3. In the standard curve tree, click the name of the curve you wish to modify

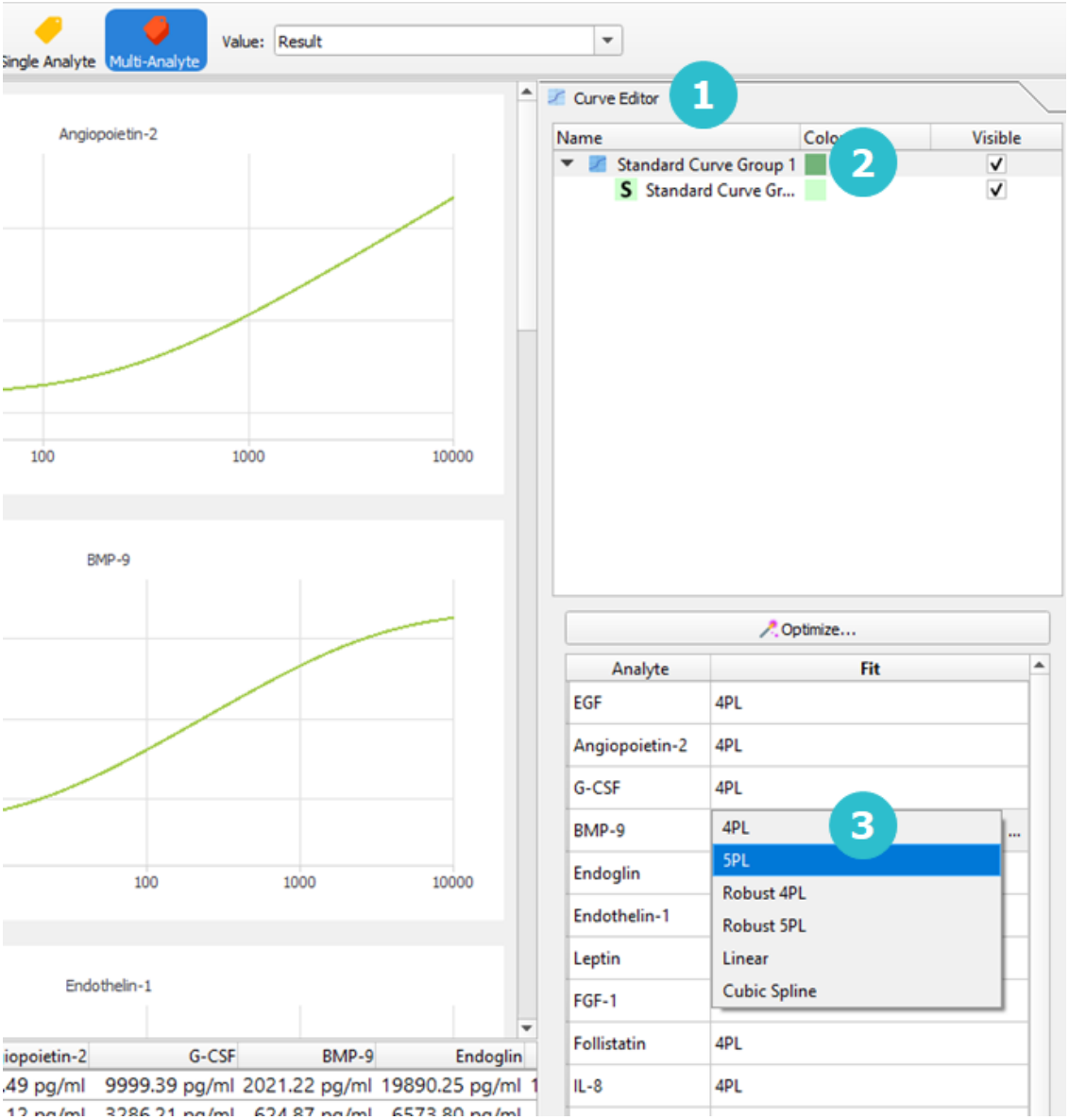
4. In the curve details table, find the **Fit** row and double-click the current value to activate the **Curve Fit** list. Select a new curve fit and the results will update automatically:



In multi-analyte view

1. Go to the **Curve Editor** in the right panel
2. In the standard curve tree, click the name of the curve you wish to modify
3. Double-click in the **Fit** column next to the name of the analyte whose curve fit you wish to modify.

This activates the **Curve Fit** list. Select a new curve fit and the results will update automatically:

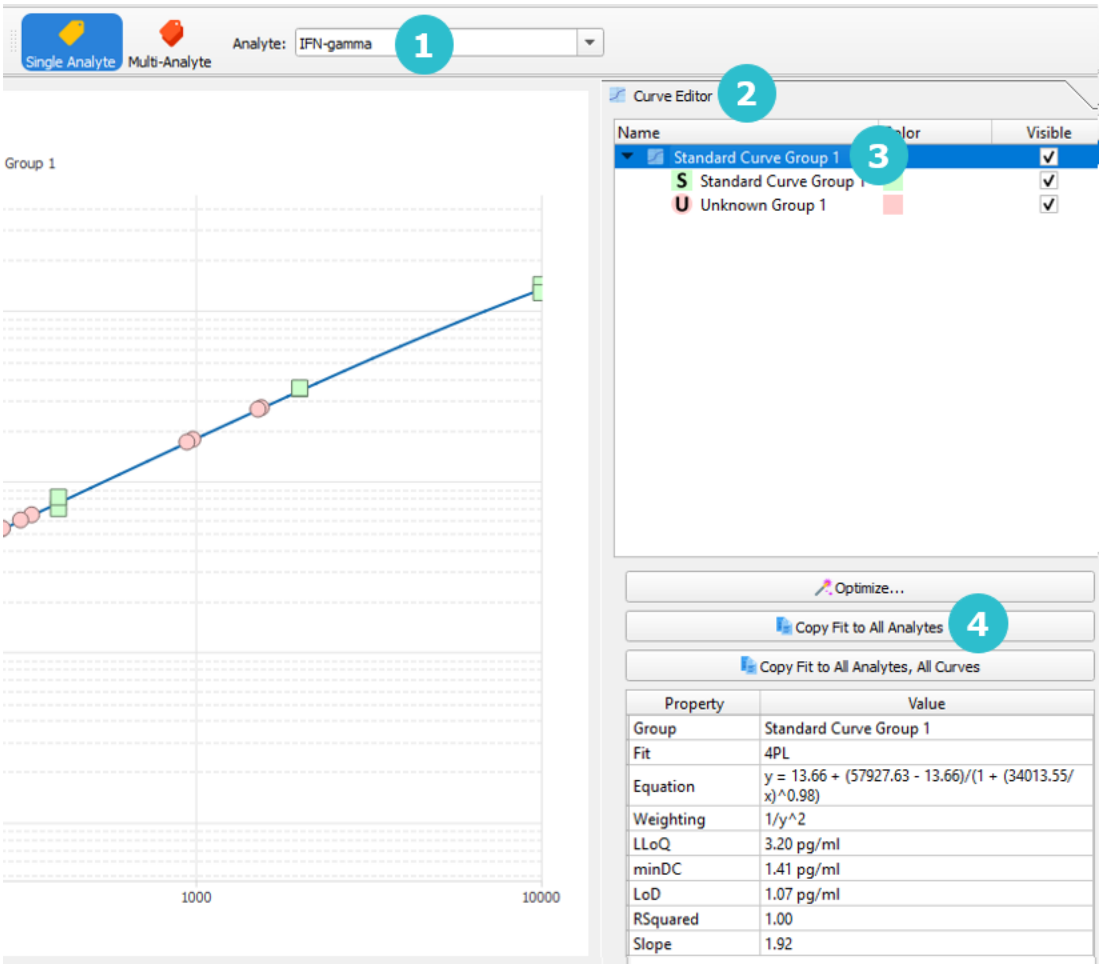


How to copy a curve fit between analytes

In single-analyte view

1. Select an analyte from the **Analyte** list on the toolbar, or press **Ctrl-K** on the keyboard and then type the name of the analyte
2. Go to the **Curve Editor** in the right panel
3. In the standard curve tree, click the name of the curve you wish to modify

4. Select the **Copy Fit to All Analytes** button to copy the current analyte's fit to all other analytes for all curves in this experiment

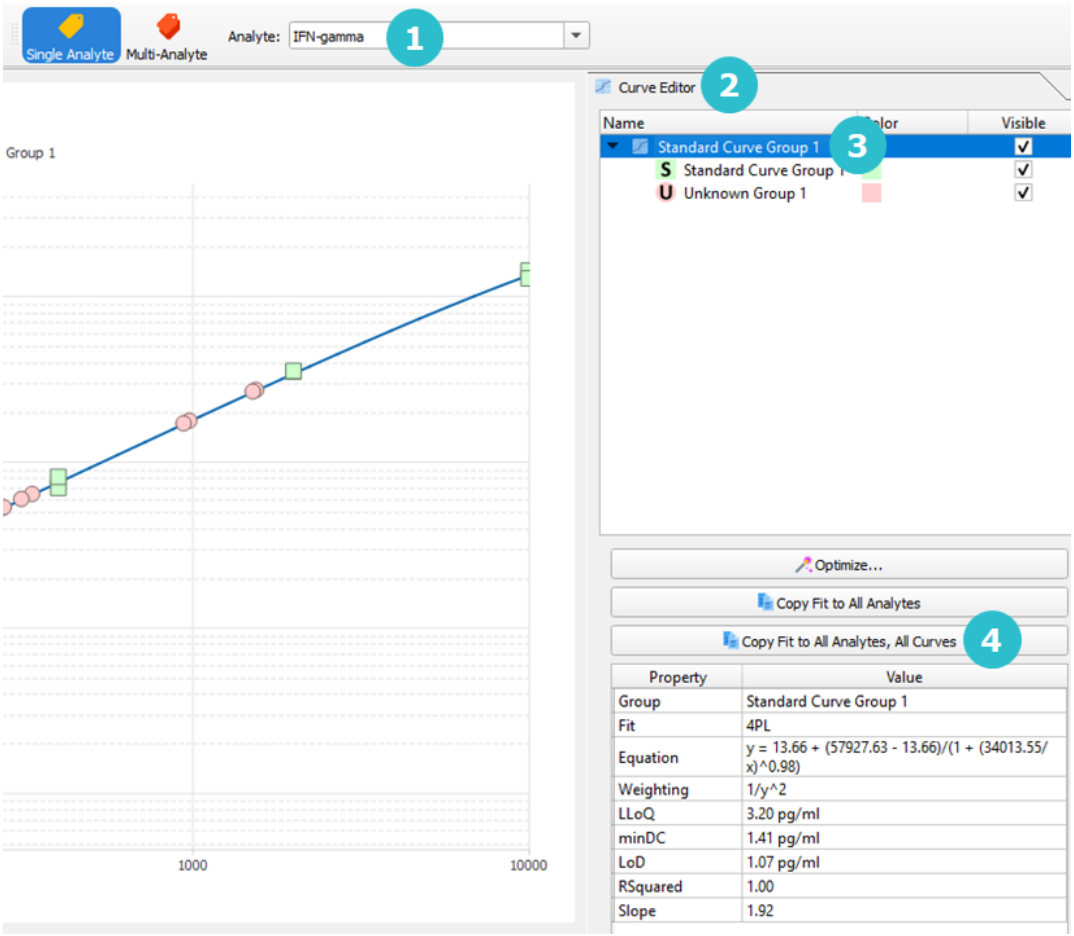


How to copy a curve fit to all curves

In single-analyte view

1. Select an analyte from the **Analyte** list on the toolbar, or press **Ctrl-K** on the keyboard and then type the name of the analyte
2. Go to the **Curve Editor** in the right panel
3. In the standard curve tree, click the name of the curve you wish to modify
4. Select the **Copy Fit to All Analytes, All Curves** button to copy the current analyte's fit to all

other analytes for all curves in this experiment

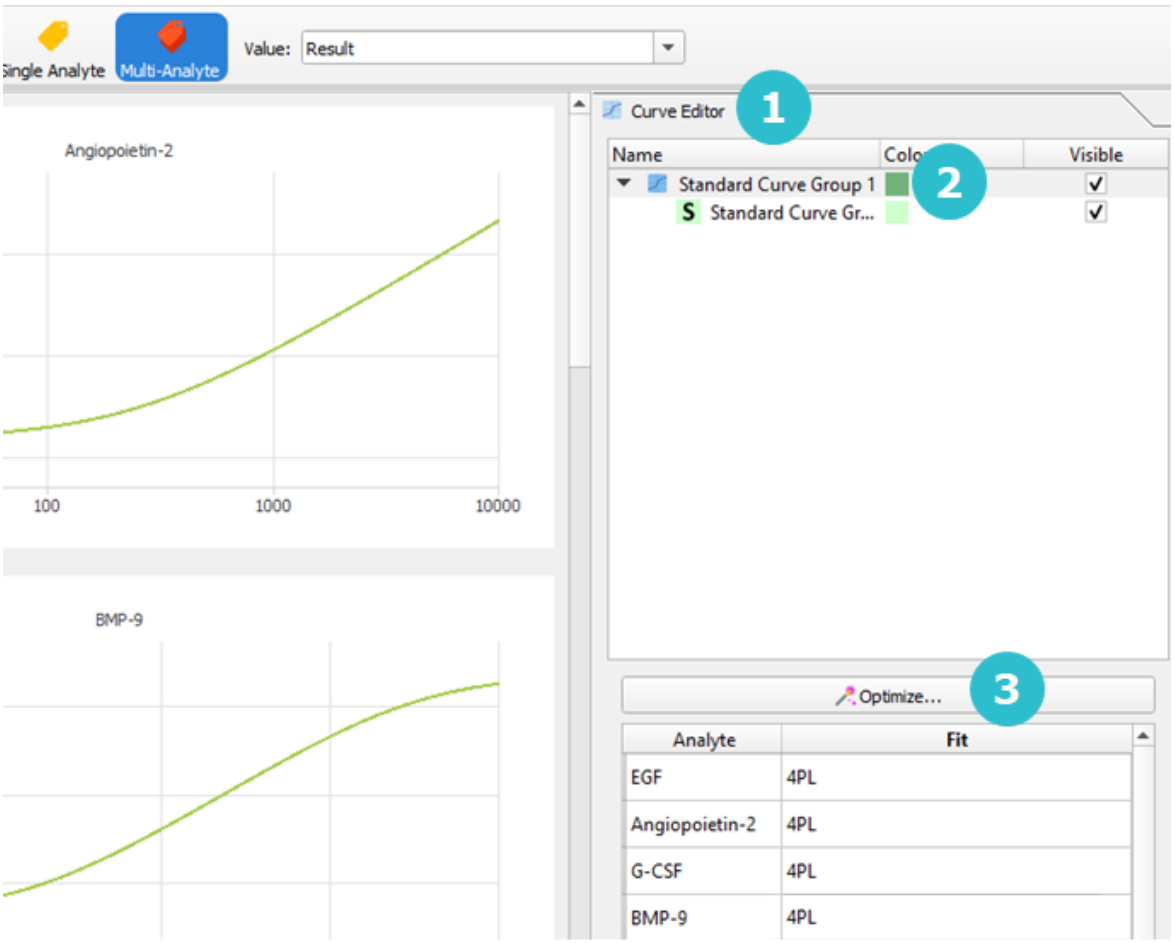


Optimize Curve Fit

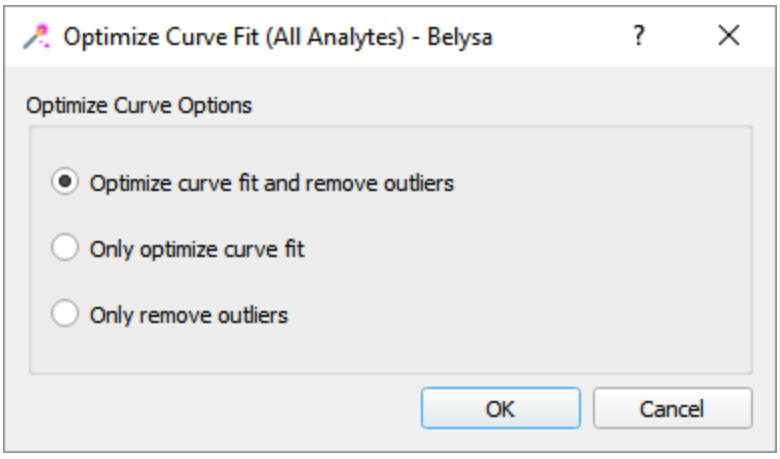
Belysa® immunoassay curve fitting software can automatically select the best fit and identify outliers for your standard curve data. For detailed information on how curve fit and outlier optimization are performed, see **Curve Optimization**. Note that curve optimization always operates on all analytes, regardless of whether you are in single-analyte or multi-analyte view (see **Single and Multi-Analyte Views**).

How to optimize the curve fit and/or outliers

1. In either single-analyte view or multi-analyte view, go to the **Curve Editor** in the right panel
2. In the standard curve tree, click the name of the curve you wish to modify
3. Select **Optimize..**



4. The **Optimize Curve Fit (All Analytes)** dialog will appear:



The options for curve fit optimization are as follows:

Option	Description
Optimize curve fit and remove outliers	Performs both curve fit optimization and outlier removal on the selected curve for all analytes
Only optimize curve fit	Performs only the curve fit optimization method on the selected curve for all analytes
Only remove outliers	Performs only the outlier removal method on the selected curve for all analytes

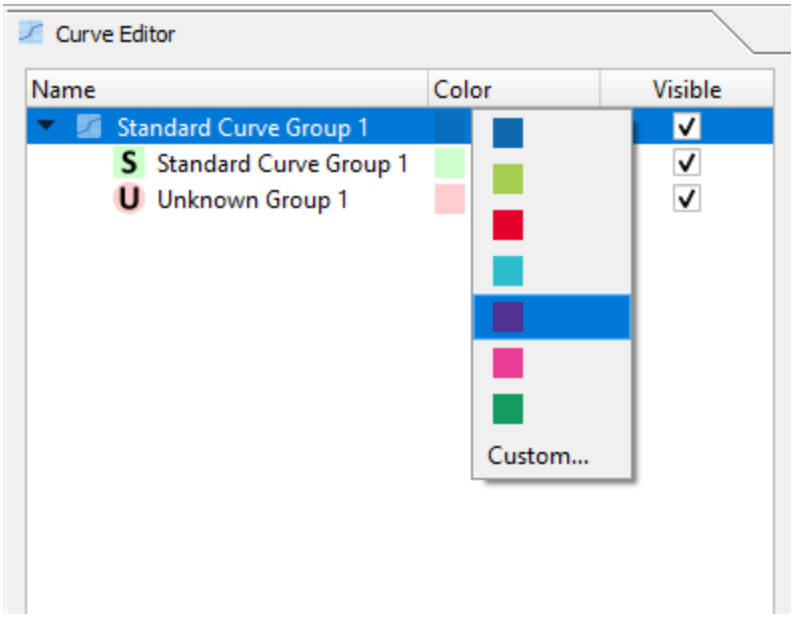
Outliers removed by the optimization method will have their Exclude Reason updated to read "Set by optimize outliers command".

Change Curve Plot Appearance

When viewing the results of an experiment, you can customize the appearance of the standard curve plot.

How to Customize Standard Curve Plots

1. Go to the **Curve Editor** tab in the right panel of the experiment results screen
2. At the top of the **Curve Editor** tab, locate the standard curve tree. Click the arrows next to the curve names to view sample groups:



3. Change the appearance of the plot in one or more of the following ways:

Change Color

a. Double-click the colored rectangle in the **Color** column next to the element whose color you wish to change. In the **Color** list, select the desired color. Alternatively, select **Custom...** to choose a custom color. Note: color settings for groups will be saved to the Results file.

Toggle Visibility

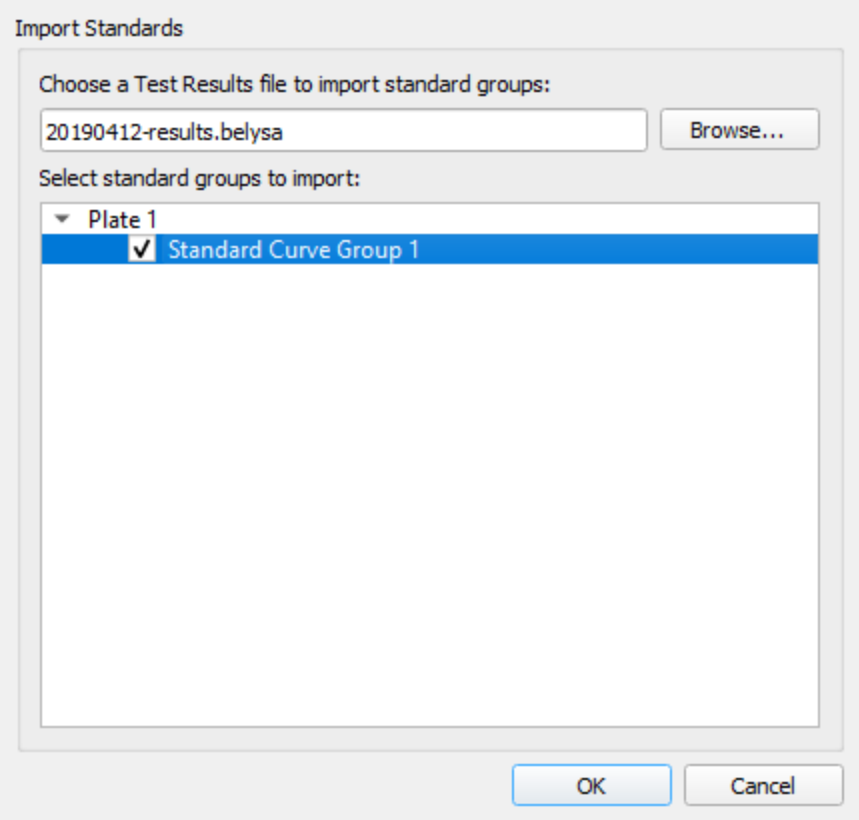
a. Select or clear the **Visibility** check box next to the group's name to show or hide the group on the plot. Selecting the check box next to a standard curve will show or hide all sample groups associated with that curve. Note: visibility settings for groups are not saved to the Results file.

Import an External Standard

Curve fits from external files may be used to quantify sample groups contained in the currently opened Results file, and/or be included in relative potency calculations.

How to import an external curve

1. When viewing experiment results, go to **File** > **Import** > **Import External Curve...**
2. In the Import External Curve dialog, select the **Browse...** button and choose a Belysa® software Results file containing the external curve fit
3. If the external Results file contains at least one analyte in common (as determined by ID) with the currently opened file, you may select one or more standard groups to import:



4. When you are finished selecting groups to import, select **OK**. The external standard groups will be added to the current experiment named with an "Ext." prefix.

   Note: Imported external standards will not be saved to the Results file. You should export any reports needed for this experiment before closing the file.

Edit Experiment Name and Details

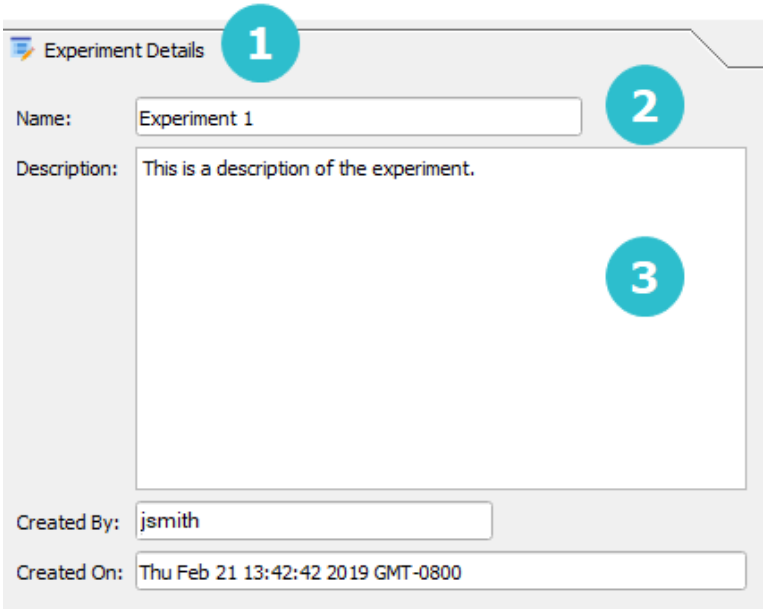
The following details are available for each experiment:

- Name
- Description
- Created By (automatically populated with user name)
- Created On (automatically populated with time experiment was created)

How to change experiment details:

1. Go to the **Experiment Details** tab
2. Enter a new experiment name in the **Name** box

3. Enter an experiment description in the **Description** box



Notes

- Experiment names have a maximum length of 100 characters
- When you are finished editing the experiment name or description, select an area outside of the text box to commit your changes

Plates

A *plate* in Belysa® immunoassay curve fitting software represents a 96-well or 384-well microplate, depending on the format of the source data. Each well on the plate is referenced by its *location*, represented as its row letter (A, B, C, etc.) plus its column number (1, 2, 3, etc.). For example, location A3 refers to the well in row A column 3.

To view and edit well information, including groupings and replicates, use the **Plate Map Editor**. Other common tasks involving plates include:

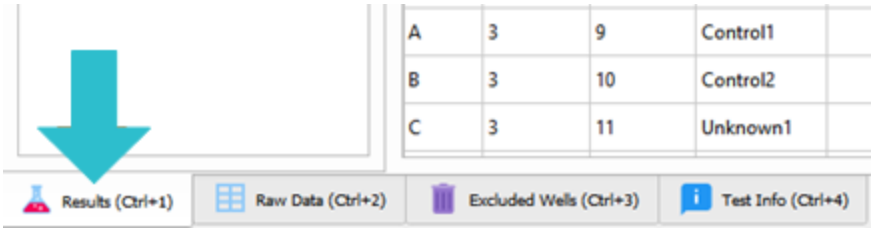
- **Create Groups**
- **Cut, Copy, & Paste Wells**
- **Clear Well Information**

Create Groups

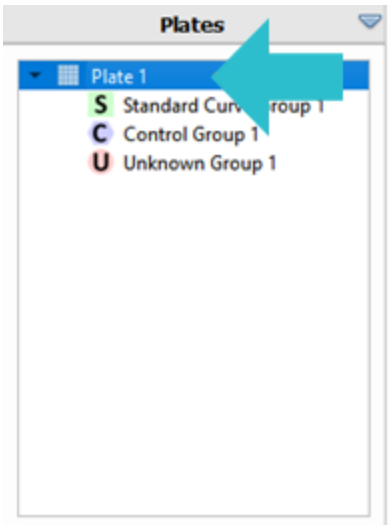
Plates may contain one or more groups of wells. Groups can be added to experiments in order to obtain results.

How to Create a Group

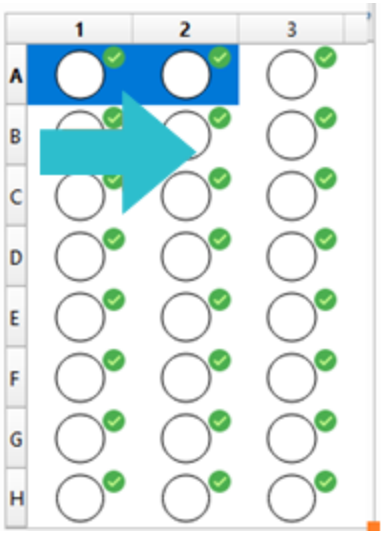
1. At the bottom of the screen, go to the **Results** tab or press **Ctrl+1** on your keyboard:



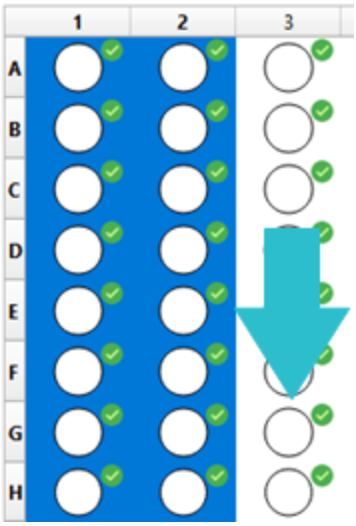
2. Select the name of the plate you wish to edit. This will display the plate map:



3. Select wells to group. Belysa® immunoassay curve fitting software can automatically populate replicate count and direction based on how you select the wells to group. For example, to create a group with 8 replicate groups, each with a replicate count of 2, arranged horizontally, you could first select two columns like this by dragging with the mouse:



and then drag down to complete the selection:

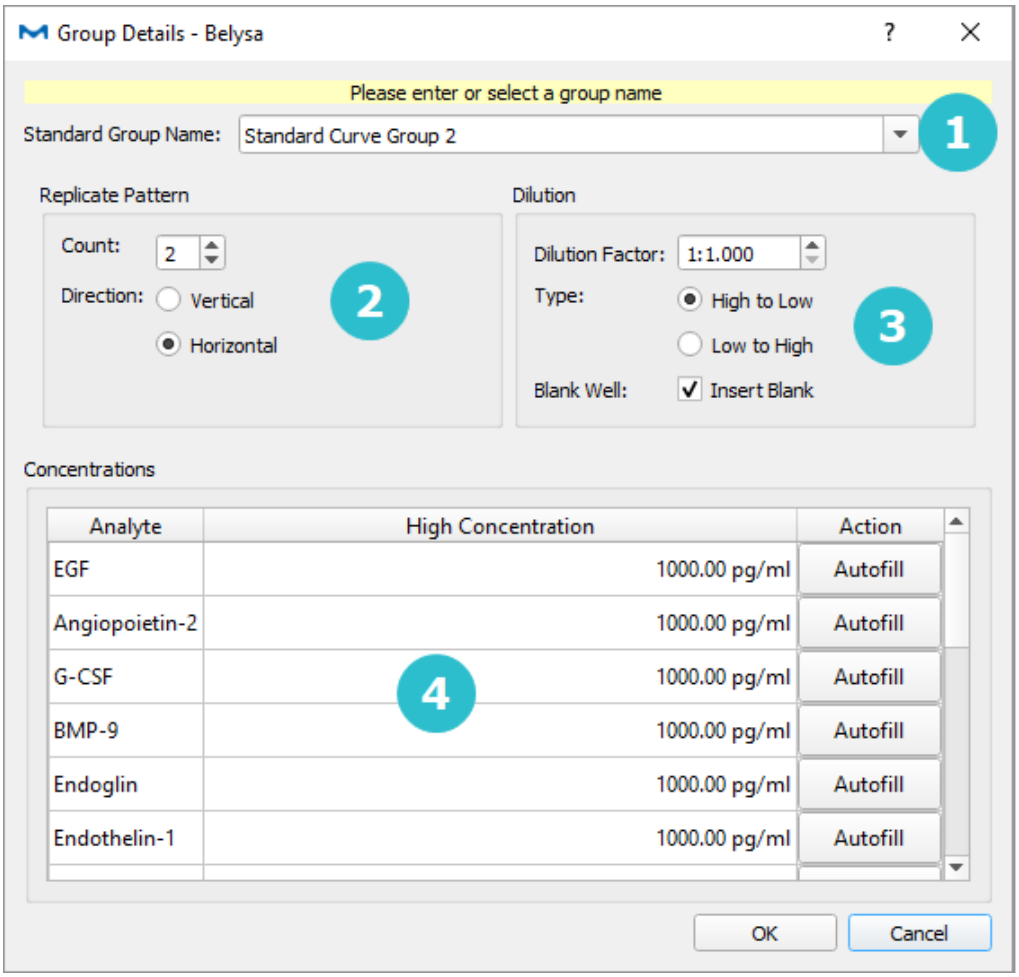


It is also possible to change the replicate count and direction in the next step, so while following these selection guidelines may be convenient they are not required.

4. Select group type and enter information. Once you have selected wells for the group, select one of the three group types by clicking the appropriate icon on the toolbar, right-clicking and selecting the group type from the shortcut menu, or by using a keyboard shortcut:

Icon	Shortcut	Type	Description
S	Ctrl+D	Standard	In a Standard well, you have a known concentrations of analyte in a standard diluent, values that are used to form standard curves.
C	Ctrl+F	Control	Similar to the Standard, these wells have known concentrations of analyte in a sample matrix; used to measure run-to-run variability.
U	Ctrl+G	Unknown	The Unknown well is where you are measuring for an unknown concentration.

In the resulting dialog window, enter additional information about the group:



1. Enter a unique name for the new group. To append wells to an existing group instead of creating a new one, use the dropdown to select the name of the existing group.
2. Enter the desired replicate pattern. These settings are pre-populated based on how you selected the wells, but you may change them if desired:
    - Count - number of replicates in the replicate group
    - Direction - whether to group replicates going down the plate(vertical) or across the plate(horizontal)
3. Enter dilution information:
    - Dilution Factor - the ratio of the dilution, such as 1:2, 1:3, etc. For Standard and Control groups, this will be used to calculate Expected Concentration values. For Unknown groups, this will be used as a multiplier to adjust the interpolated result.

- Type (Standard and Control only) - specifies whether the highest concentration is at the top/left of the plate (High to Low), or at the bottom/right of the plate (Low to High)
- Blank Well (Standard and Control only) - if checked, the lowest Expected Concentration in a group of diluted samples is automatically set to 0.00 (blank)

4. Enter high concentration for each analyte (Standard and Control only). Selecting the **Autofill** button will copy an analyte's high concentration to all analytes below it.

Clear Well Information

The Clear Wells action will remove the following information about a well:

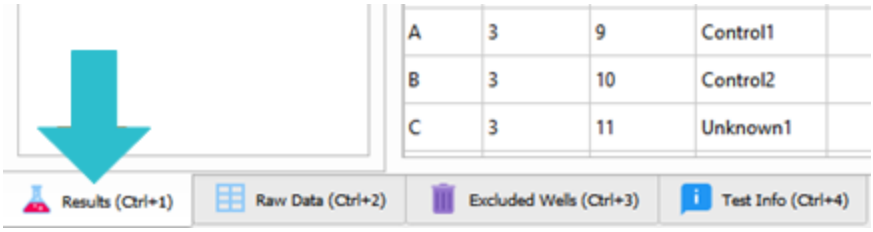
- Group
- Replicate number
- Sample ID
- Expected Concentration
- Dilution Factor

The following information will not be cleared:

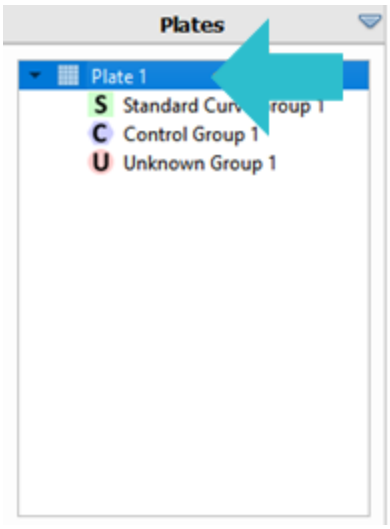
- Response (MFI), other raw data
- Run Status
- Excluded status and reason

How to Clear Well Information

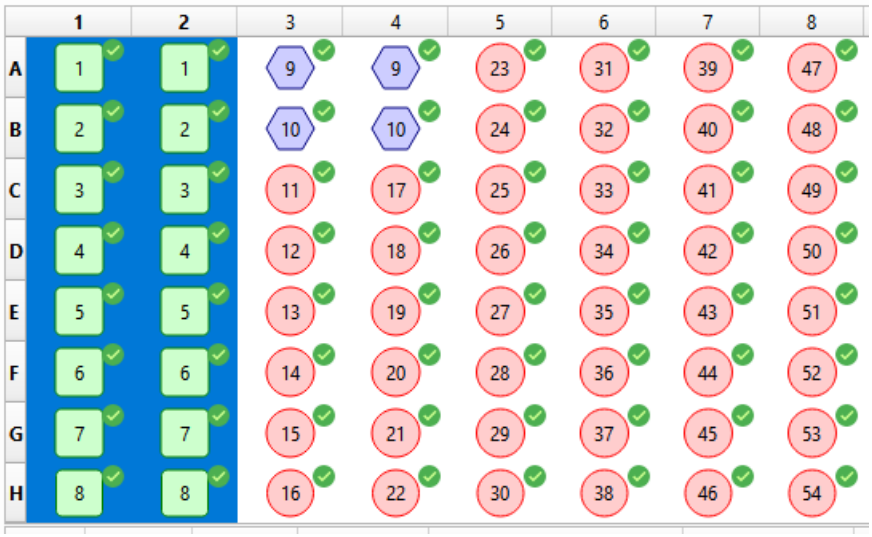
1. At the bottom of the screen, go to the **Results** tab or press **Ctrl+1** on your keyboard.



2. Click the name of the plate you wish to edit. This will display the plate map for the plate.



3. Select wells to clear. When clearing wells, the order in which you select wells doesn't matter.



4. Select Clear Wells on the toolbar, or right-click and select the Clear Wells from the shortcut menu to clear well information

Cut, Copy, & Paste Wells

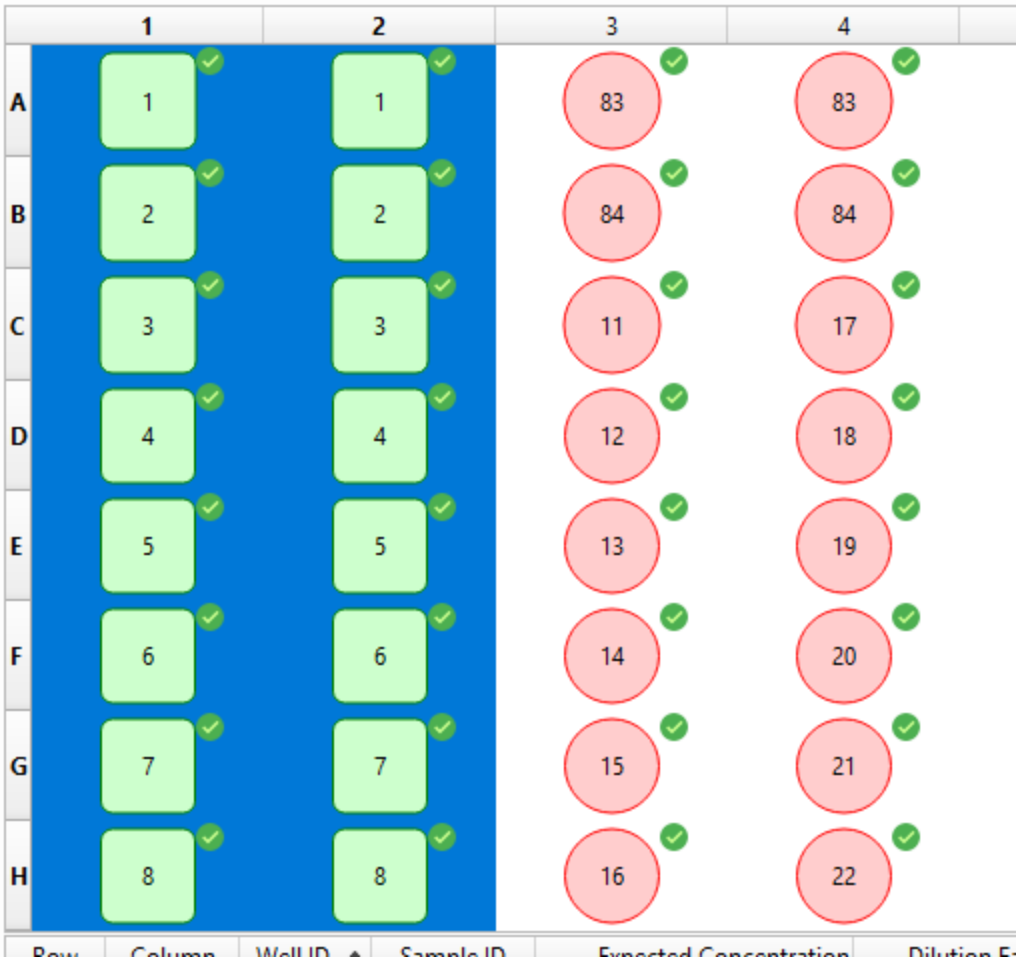
You can cut, copy, and paste well formatting from one part of a plate to another in order to quickly create multiple groups. This is useful when your source data does not contain formatted plate information (i.e. all unknown wells). When you paste wells, the following information is transferred:

- Group type
- Replicate pattern

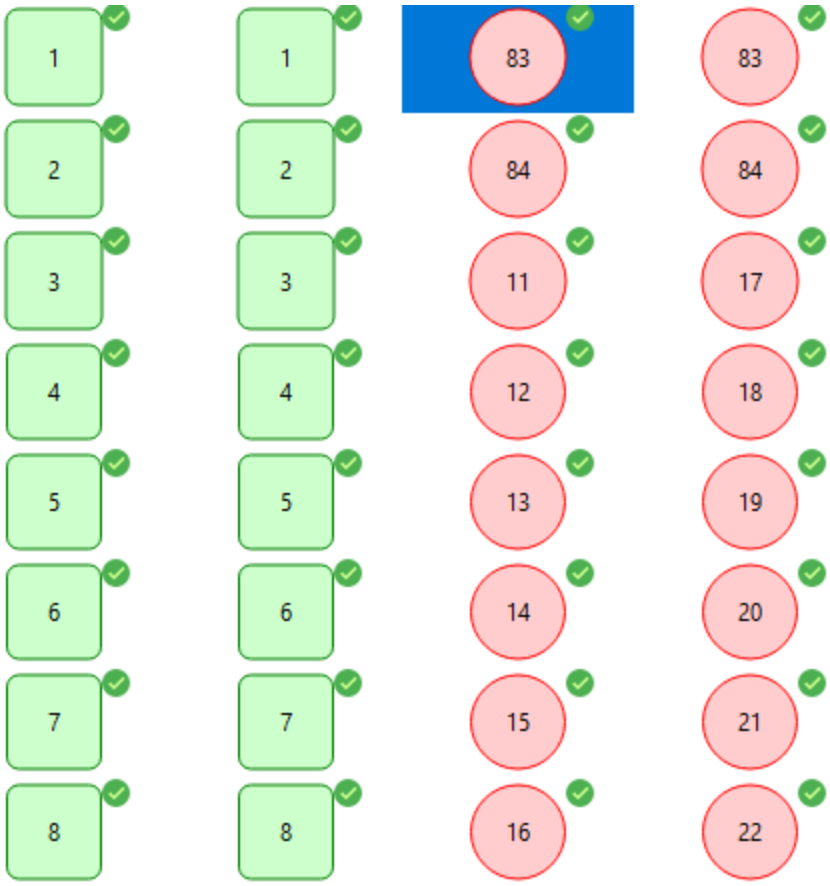
- Sample ID
- Expected Concentration (for Standards and Controls)
- Dilution Factor (for Unknowns)

How to Cut, Copy, & Paste Wells

1. Select some wells on the plate map editor



2. Select **Cut** or **Copy** on the toolbar to cut or copy wells. Alternatively, use the keyboard shortcuts **Ctrl+X** for cut and **Ctrl+C** for copy. If you choose to cut wells, the highlighted wells will be cleared (see **Clear Well Information**).
3. Select the well at the top-left corner of where you would like to paste the cut or copied well information



4. Select **Paste** to paste well information. A new group will be created with the same replicate pattern, sample IDs, and concentrations as the wells you cut or copied:

Renumber Replicates

When viewing the plate map, wells are numbered such that wells with the same number will be have their results averaged together when viewing experiment results. Wells with the same number are known as *replicates*. As you add and remove groups on the plate, the replicate number for new groups continues to increment (1, 2, 3, etc.). Renumbering replicates will make the replicate numbers start at 1 without changing the replicate pattern.

Note: This action does not affect results in any way, it only changes the numbers assigned to each replicate group

How to renumber replicates

1. In the **Plates** tree, select the name of the plate you wish to edit. This will display the plate map:

2. Select Renumber Replicates (1) from the toolbar

3. Observe that the replicate numbers have now restarted from 1:

Edit Plate Name and Details

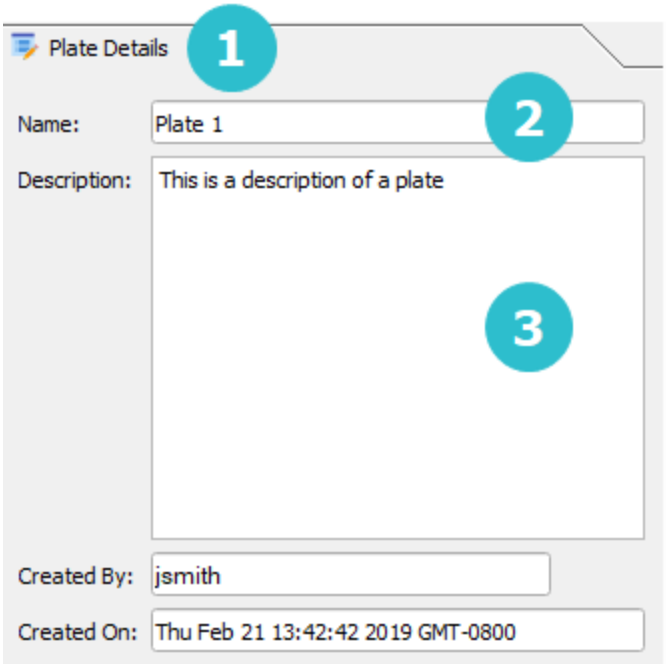
The following details are available for each plate:

- Name
- Description
- Created By (automatically populated with user name)
- Created On (automatically populated with time experiment was created)

How to change plate details:

1. Go to the **Plate Details** tab
2. Enter a new plate name in the **Name** box

3. Enter an plate description in the **Description** box



Notes

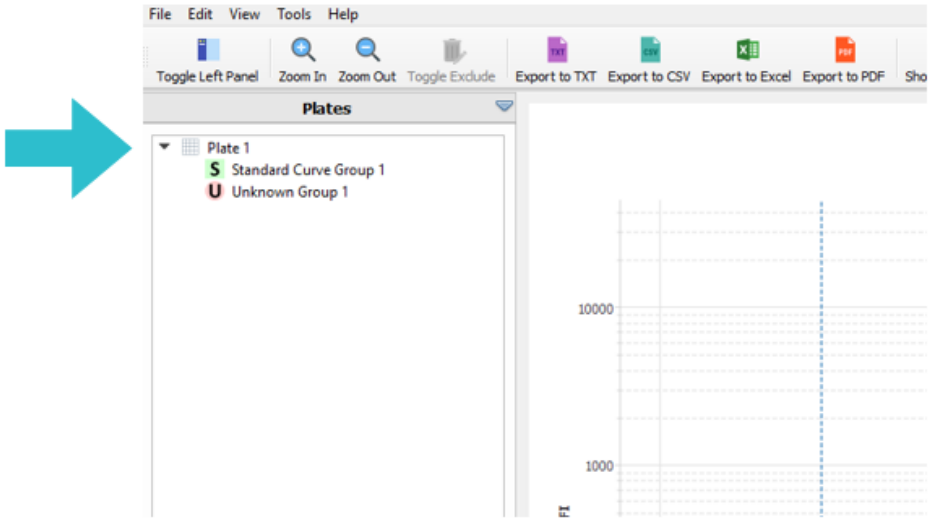
- Plate names have a maximum length of 100 characters
- When you are finished editing the plate name or description, select an area outside of the text box to commit your changes

Rename Plates and Groups

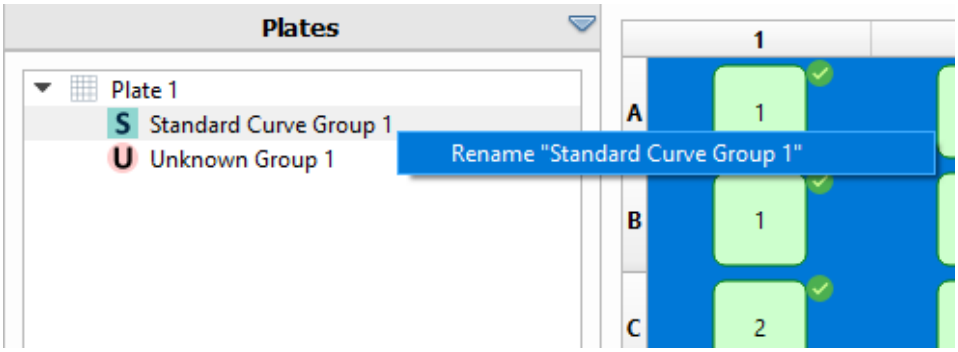
You can give plates, quadrants, and groups custom names to help organize your results.

How to rename a plate, quadrant, or group

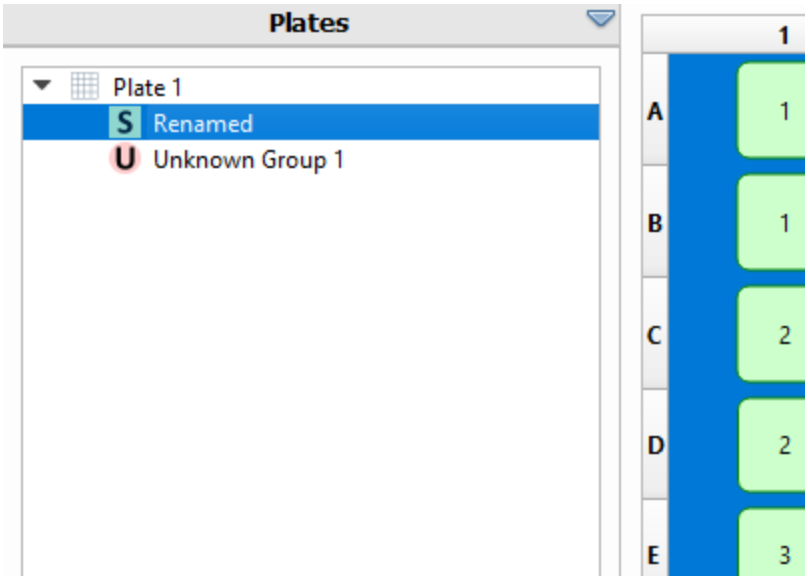
1. Go to the **Results** tab and find the **Plates** tree:



2. If necessary, expand the plate or quadrant to show all sub-items
3. Right-click a plate, group, or quadrant and select "Rename <current name>" from the shortcut menu



4. Type in a new name for the item and press **Enter**:

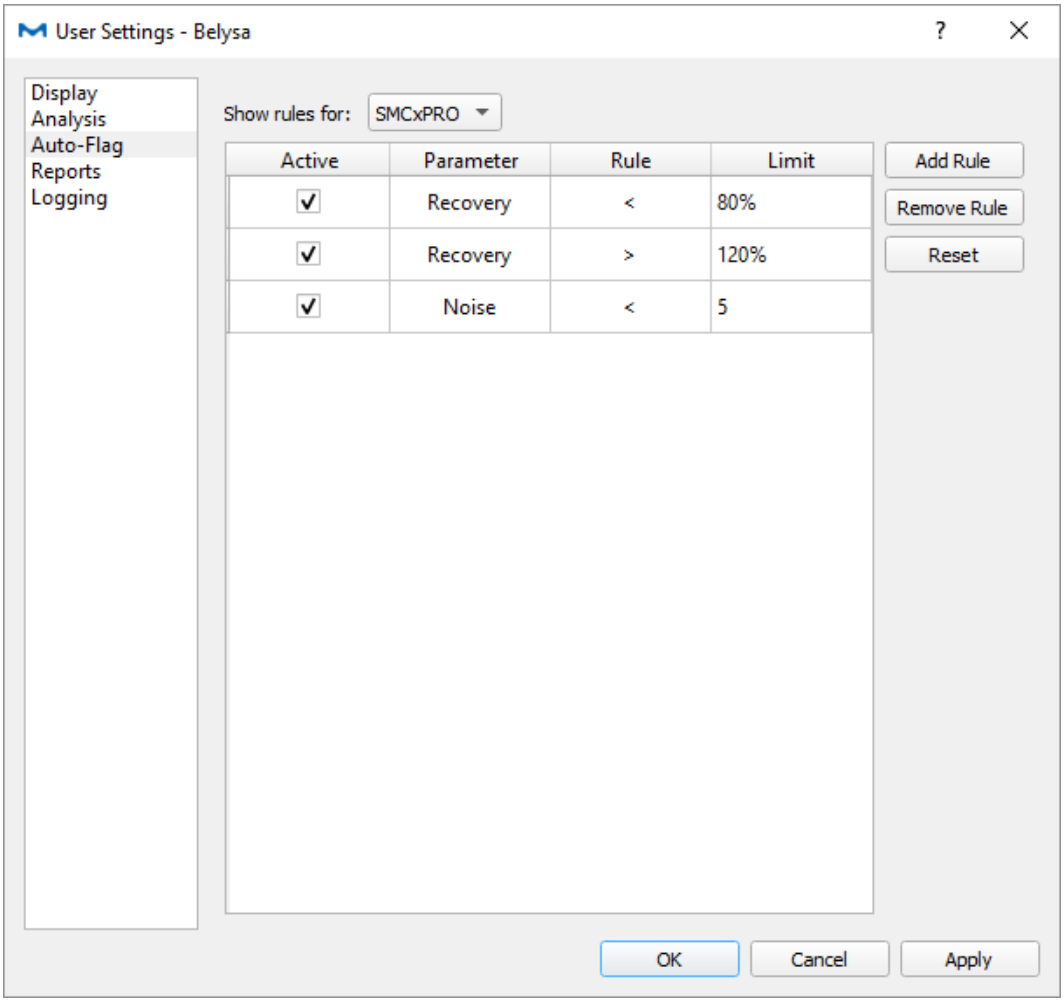


Auto-Flagging

Belysa® immunoassay curve fitting software can automatically flag data that falls outside certain thresholds. When a value is flagged, the icon will appear next to the value in the table.To learn how to manage your auto-flag rules, see **Create, Update, and Delete Auto-Flag Rules**. To exclude all wells with certain flagged values, see the Exclude All Flagged action in the **Raw Data Toolbar**.

Create, Update, and Delete Auto-Flag Rules

To view your currently defined auto-flag rules, open the User Settings dialog by going to **Tools > Settings** and selecting **Auto-Flag** from the list (see **Settings and Preferences**):



Each auto-flag rule has the following editable components:

Column	Description
Active	Selecting this box applies the rule to your results. Clear the box to temporarily suspends

	running this rule.
Parameter	The statistic to which this rule applies
Rule	The type of comparison that will be applied to the parameter value when compared to the rule's limit value. The choices are: • < Less than • <= Less than or equal to • > Greater than • >= Greater than or equal to • = Equal to • ≠ Not equal to
Limit	The threshold value to which the parameter will be compared

For example, the rule shown below will show a flag icon next to any Recovery value that is less than 80%:

Active	Parameter	Rule	Limit
☑	Recovery	<	80%

You can set up different auto-flag rules for different instrument data types. Before changing your auto-flag rules, ensure that you have selected the correct data type from the **Show rules for** box.

Once you have selected the data type, you can alter your auto-flag rules. To add a new auto-flag rule, select the **Add Rule** button. A new row will be added to the rule list. To change an auto-flag rule, click in cell you wish to change and enter or select a new value. To remove an auto-flag rule, select any cell in the row you wish to remove and select the **Remove Rule** button. To reset rules for this data type back to their factory default values, select the **Reset** button.

Changes to auto-flag rules will be applied as soon as you select the **Apply** or **OK** buttons.

Exclude and Include Wells

Belysa® immunoassay curve fitting software provides the ability to exclude certain well data from results calculations. This can be used to suppress outlier data or data that fails to meet certain quality thresholds (see **Auto-Flagging**). You can exclude some or all analyte data for a particular well. When you exclude data, the data is excluded from all **Experiments** in the file.

There are multiple ways to exclude and include data in the application:

- To exclude data while viewing experiment results, see **Quantitative Experiment Results**
- To exclude data while viewing raw data, see **Raw Data Table**
- To exclude data while viewing the standard curve, see Standard Curve Plot
- To review excluded data, see **Excluded Wells Tab**

Show and Hide Table Columns

Many data tables in Belysa® immunoassay curve fitting software provide the ability to customize which columns are visible and what order they appear in. In some cases, these preferences can be saved for all subsequent files you open.

How to show and hide table columns

To show and hide table columns, go to the **Table Columns** tab when viewing **Experiment Results** or the **Raw Data Tab**

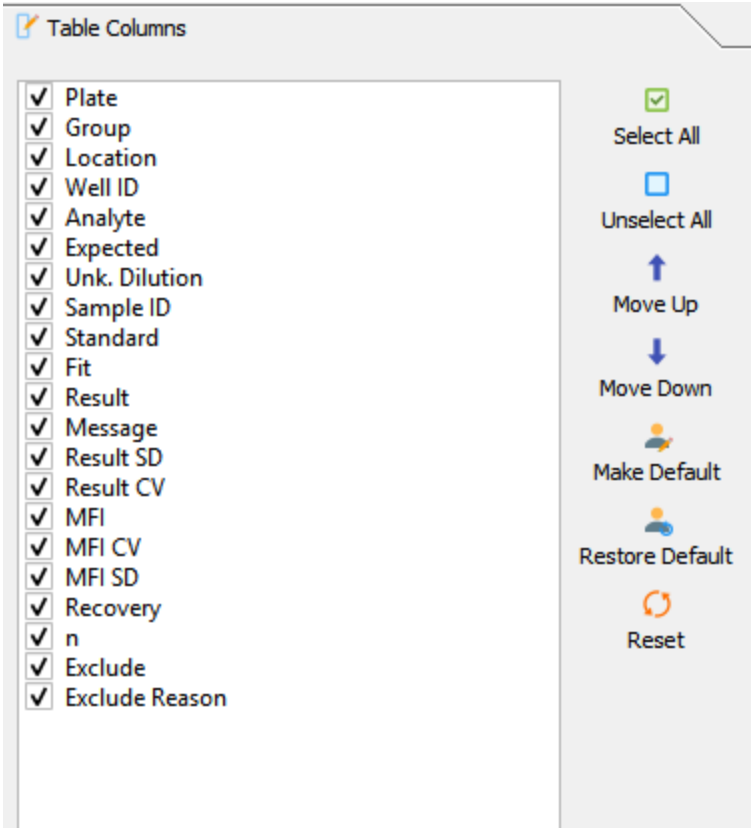


Table columns can be customized in the following ways:

- To hide a table column, clear the check box next to the column's name. Select the check box to show it again. To hide all columns, select the **Unselect All** button. To show all columns, select the **Select All** button.
- To rearrange columns, select the column name so that is highlighted and select the **Move Up** and **Move Down** buttons. Alternatively, you can drag and drop the columns into different orders in this tab or in the table itself

- To save the current selections as your default for subsequent files, select the **Make Default** button (note that this option is not available for multi-analyte tables). To restore your default settings, select the **Restore Default** button
- To revert back to the application default settings, select the **Reset** button
- To sort the table by different columns, click on the column's header in the data table

Reports

Belysa® immunoassay curve fitting software can save your data and analysis settings to a Results file (.belysa), as well as produce reports in tab-separated text (.txt), comma-separated text (.csv), Excel, and PDF formats. To learn more about producing reports, see the following sections:

- **Save Results File**
- **Export to Text or CSV**
- **Export to Excel**
- **Export to PDF**

Save Results File

Belysa® immunoassay curve fitting software results are saved as a Results file (.belysa). This file preserves the following information:

- All plate and experiment data, with the exception of external standard curve data
- Curve fit selections
- Curve color
- Analyte names, units, and precision

To save a Results file, select **File > Save** or **File > Save As...** from the menu bar.

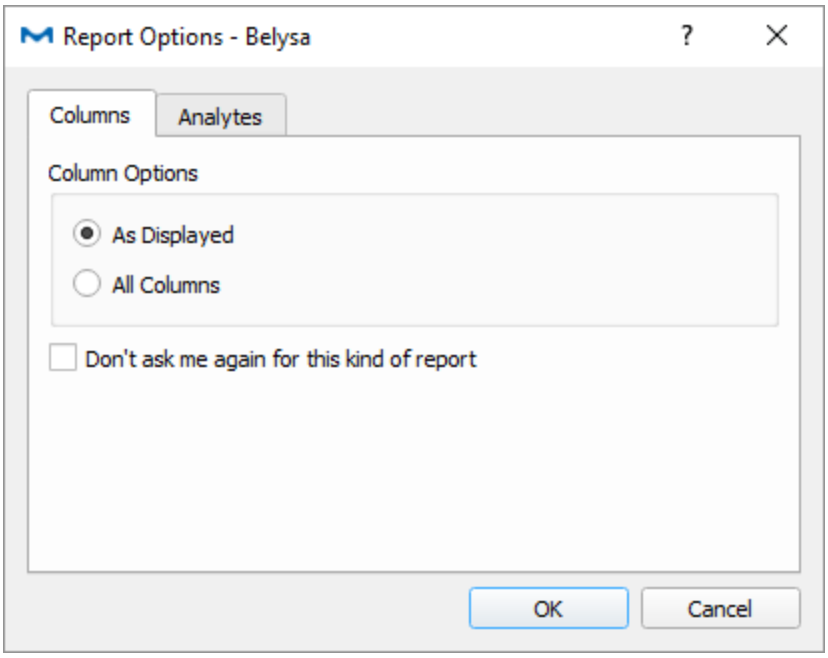
Export to Text or CSV

Exporting data to text files (tab-separated .txt or comma-separated .csv) is supported by the **Experiment Results** view and the **Raw Data Tab**. You can export data in single-analyte format or multi-analyte format (see **Single and Multi-Analyte Views**). In single-analyte format, each row displays all of a single analyte's data for a single well. In multi-analyte format, each row displays a single statistic (i.e. Recovery) for a single well across all analytes.

To export data to a text file, select **Export to TXT** 

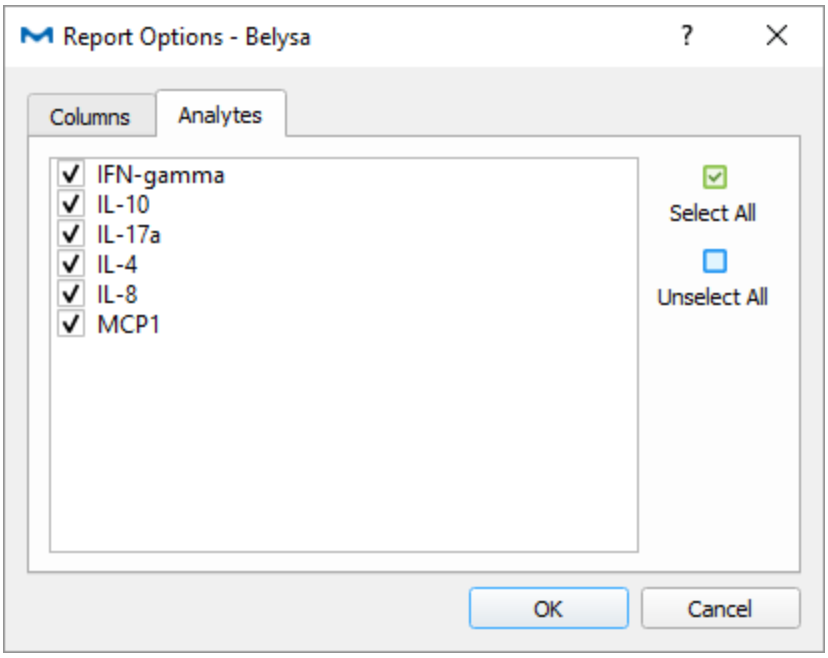
or **Export to CSV** 

on the toolbar. Depending on your preferences, the **Report Options** dialog may appear:



Use the **Columns** tab to select what data will be included in the report. Select **As Displayed** if you want the arrangement and sorting of the data columns in your report to match the way you have configured the columns on the screen. Select **All Columns** to use the application default columns and sorting. Select **Don't ask me again for this kind of report** to remember your choice for all subsequent text reports (both tab-separated and comma-separated formats). You can change the remembered setting later in **Settings and Preferences**.

In single-analyte view, the **Analytes** tab appears where you can choose to include all or some analytes in the report:

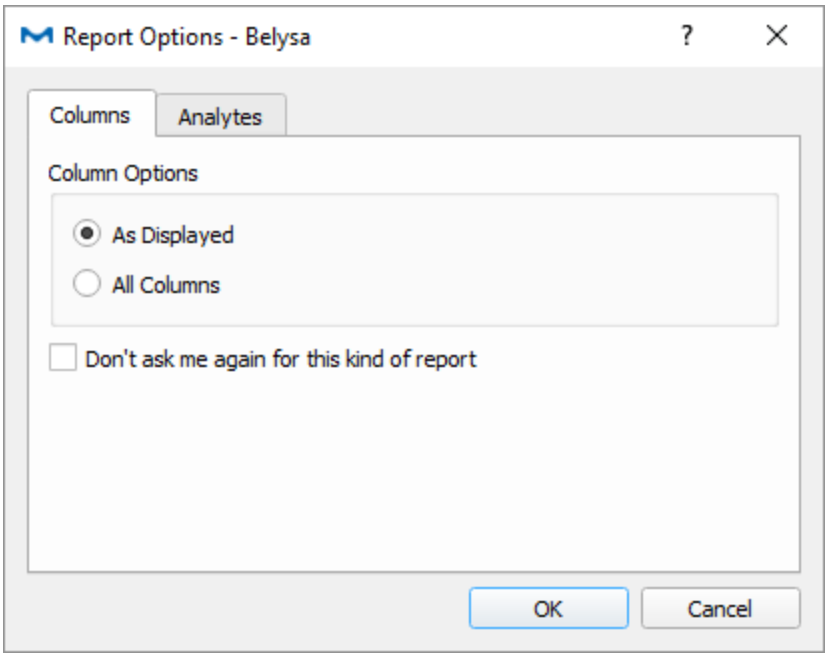


To filter certain analytes from multi-analyte reports, hide columns for those analytes and select the **As Displayed** column option (see **Show and Hide Table Columns**).

Export to Excel

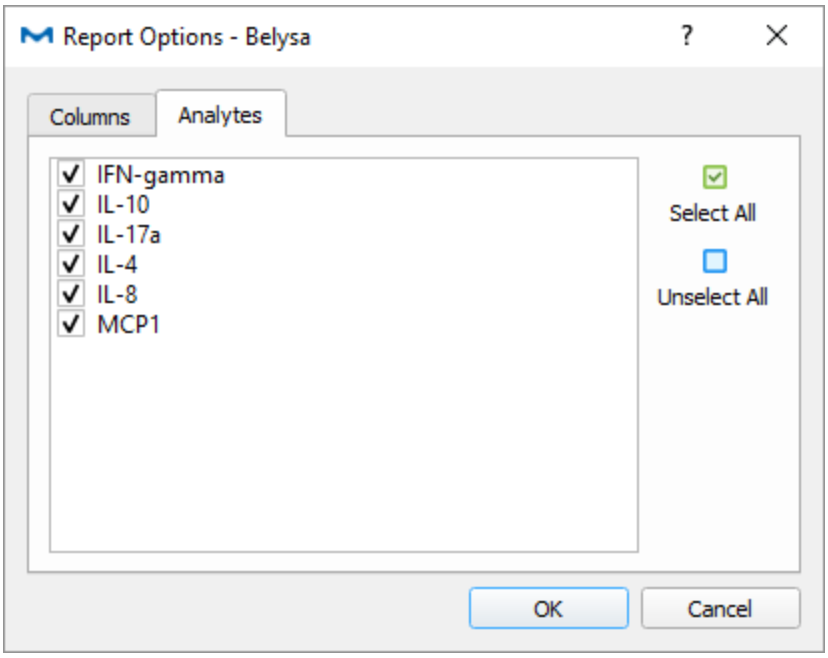
Exporting to a Microsoft® Excel workbook (.xlsx) is supported by the **Experiment Results** view and the **Raw Data Tab**. You can export data in single-analyte format or multi-analyte format (see **Single and Multi-Analyte Views**. In single-analyte format, each sheet contains all statistics for an individual analyte in the file. In multi-analyte format, each sheet contains a particular statistic (i.e. Recovery) for all analytes.

To export data to Excel, select **Export to Excel** on the **Experiment Toolbar**. Depending on your preferences, the **Report Options** dialog may appear:



Use the **Columns** tab to select what data will be included in the report. Select **As Displayed** if you want the arrangement and sorting of the data columns in your report to match the way you have configured the columns on the screen. Select **All Columns** to use the application default columns and sorting. Select **Don't ask me again for this kind of report** to remember your choice for all subsequent Excel reports. You can change the remembered setting later in **Settings and Preferences**.

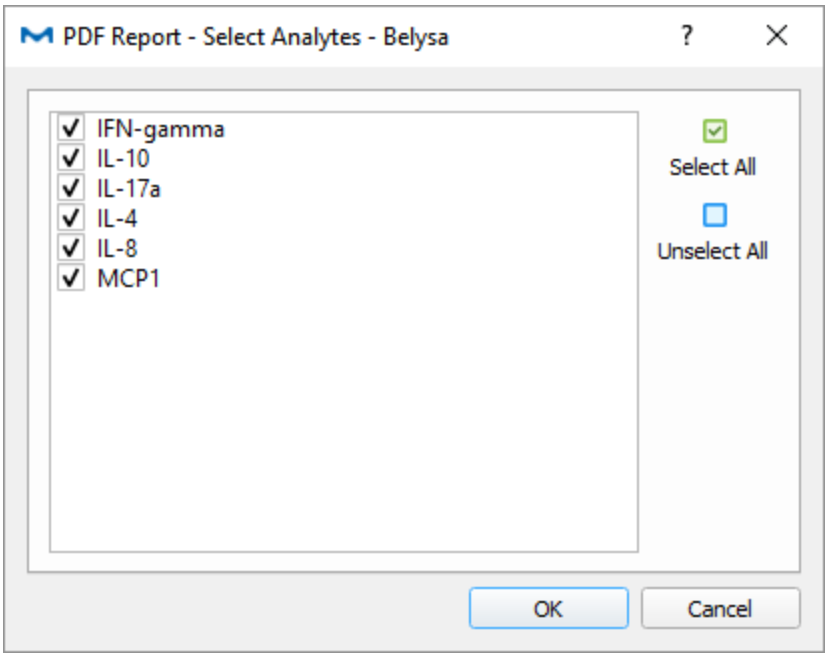
In single-analyte view, the **Analytes** tab appears where you can choose to include all or some analytes in the report:



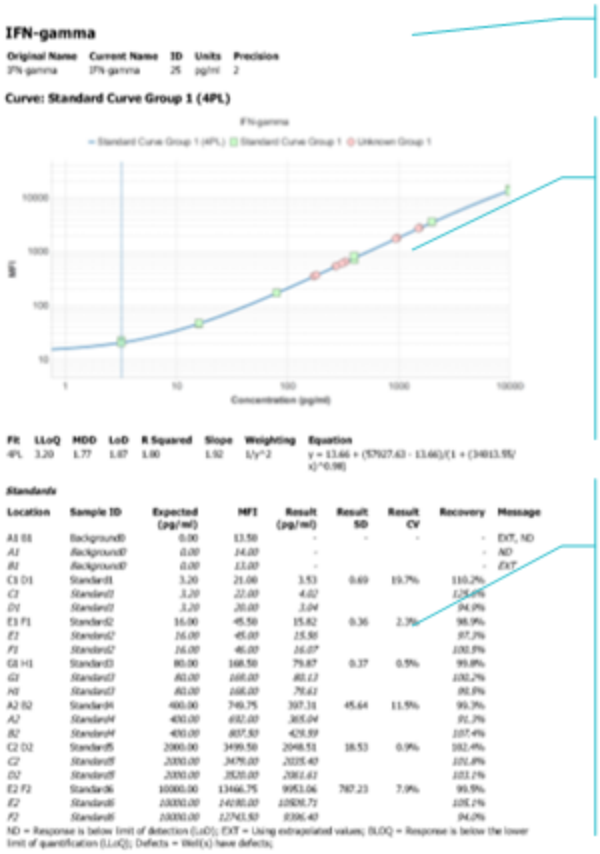
To filter certain analytes from multi-analyte reports, hide columns for those analytes and select the **As Displayed** column option (see **Show and Hide Table Columns**).

Export to PDF

The Export to PDF function creates a PDF document version of your **Quantitative Experiment Results**. To export your quantitative experiment data to a PDF file, select **Export to PDF** on the **Experiment Toolbar**. The **Select Analytes** dialog appears where you can choose to include all or some analytes in the report:



The PDF report contains sections for header information, experiment results, and excluded wells. The experiment results section is organized by analyte, and then by the results for each standard curve in the experiment (if the experiment contains more than one standard curve):



IFN-gamma

Original Name	Current Name	ID	Units	Precision
IFN-gamma	IFN-gamma	25	pg/ml	2

Curve: Standard Curve Group 1 (4PL)

IFN-gamma

Standard Curve Group 1 (4PL) Standard Curve Group 1 Unknown Group 1

MFI

Concentration (pg/ml)

Fit	LLoQ	MDD	LoD	R Squared	Slope	Weighting	Equation
4PL	3.20	1.77	1.07	1.00	1.02	1/y^2	y = 13.66 + (57927.63 - 13.66)/(1 + (34913.55/x)^0.98)

Standards

Location	Sample ID	Expected (pg/ml)	MFI	Result (pg/ml)	Result SD	Result CV	Recovery	Message
A1 B1	Background0	0.00	13.50	-	-	-	-	EXT, ND
A1	*Background0*	*0.00*	*14.00*	-			-	*ND*
B1	*Background0*	*0.00*	*13.00*	-			-	*EXT*
C1 D1	Standard1	3.20	21.00	3.53	0.69	19.7%	110.2%	
C1	*Standard1*	*3.20*	*22.00*	*4.02*			*125.6%*	
D1	*Standard1*	*3.20*	*20.00*	*3.04*			*94.9%*	
E1 F1	Standard2	16.00	45.50	15.82	0.36	2.3%	98.9%	
E1	*Standard2*	*16.00*	*45.00*	*15.50*			*97.3%*	
F1	*Standard2*	*16.00*	*46.00*	*16.07*			*100.5%*	
G1 H1	Standard3	80.00	168.50	79.87	0.37	0.5%	99.8%	
G1	*Standard3*	*80.00*	*169.00*	*80.13*			*100.2%*	
H1	*Standard3*	*80.00*	*168.00*	*79.61*			*99.5%*	
A2 B2	Standard4	400.00	746.75	397.31	45.64	11.5%	99.3%	
A2	*Standard4*	*400.00*	*692.00*	*365.04*			*91.3%*	
B2	*Standard4*	*400.00*	*807.50*	*429.59*			*107.4%*	
C2 D2	Standard5	2000.00	3499.50	2048.51	18.53	0.9%	102.4%	
C2	*Standard5*	*2000.00*	*3479.00*	*2035.40*			*101.8%*	
D2	*Standard5*	*2000.00*	*3520.00*	*2061.61*			*103.1%*	
E2 F2	Standard6	10000.00	13466.75	9953.06	767.23	7.9%	99.5%	
E2	*Standard6*	*10000.00*	*14180.00*	*10509.75*			*105.1%*	
F2	*Standard6*	*10000.00*	*12743.50*	*9396.40*			*94.0%*	

ND = Response is below limit of detection (LoD); EXT = Using extrapolated values; BLOQ = Response is below the lower limit of quantification (LLoQ); Defects = Well(s) have defects;

Analyte information

Standard curve plot and statistics

Results for standards (back-calculated), controls, and unknowns obtained using above standard curve

Interface

The Belysa® immunoassay curve fitting software user interface is primarily divided into several tabs:

- **Results Tab**
    - The **Results Tab** is where you can view interpolated concentrations, change curve fitting algorithms, and edit the plate map.
- **Raw Data Tab**
    - The **Raw Data Tab** is where you can view additional data about each well, as well as auto-exclude flagged wells.
- **Excluded Wells Tab**
    - The **Excluded Wells Tab** is where you can view wells that have been excluded from the results, and re-include them if desired.
- **Test Info Tab**
    - The **Test Info Tab** contains header information about the file, such as when the test was conducted. It also is where you can change analyte names if desired.

Additionally, you may wish to modify **Settings and Preferences** or switch between single-analyte and multi-analyte views using the **Analyte View Toolbar**.

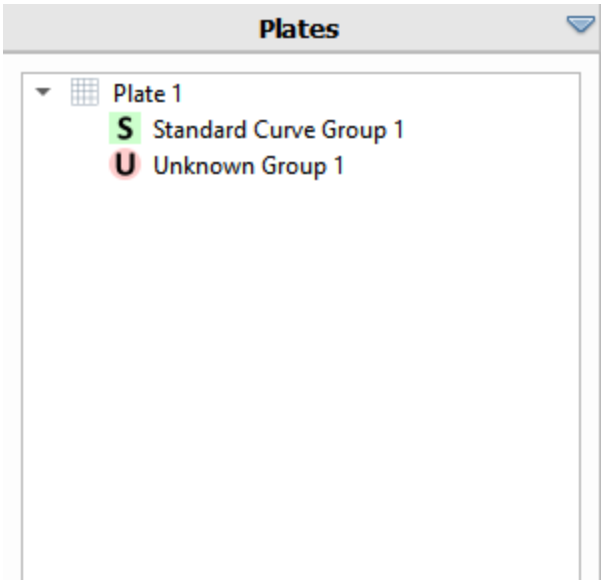
Results Tab

The Results tab is where you can edit plate map information and examine experiment results. The main parts of the results tab are:

- **Plate Tree**
- **Experiments Tree**
- **Plate Map Editor**
- **Experiment Results**

Plate Tree

The Plate Tree displays each plate present in your Results file, along with any groups that reside on the plate.



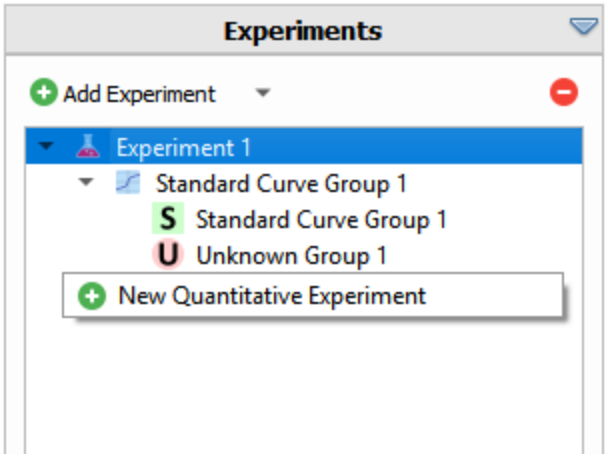
To rename a plate or group using the plate tree, see **Rename Plates and Groups**.

Experiments Tree

The Experiments tree displays all **Experiments** and associated well groups that are contained within your Results file.

How to create an experiment using the Experiments Tree

To create an experiment using the Experiments Tree, select the **Add Experiment** button and select the type of experiment you wish to create (see **Create an Experiment**). Alternatively, you may right-click on the tree to activate the shortcut menu and select **New Quantitative Experiment** (relative potency experiments can only be created using the **Add Experiment** button).



How to remove an experiment or group using the Experiments Tree

To remove an experiment from your Results file, remove a standard curve from an experiment, or remove a sample group from a standard curve, select the item you wish to remove and then select the Remove button. You can also select the Remove option from the shortcut menu activated by right-clicking in the Experiments Tree:

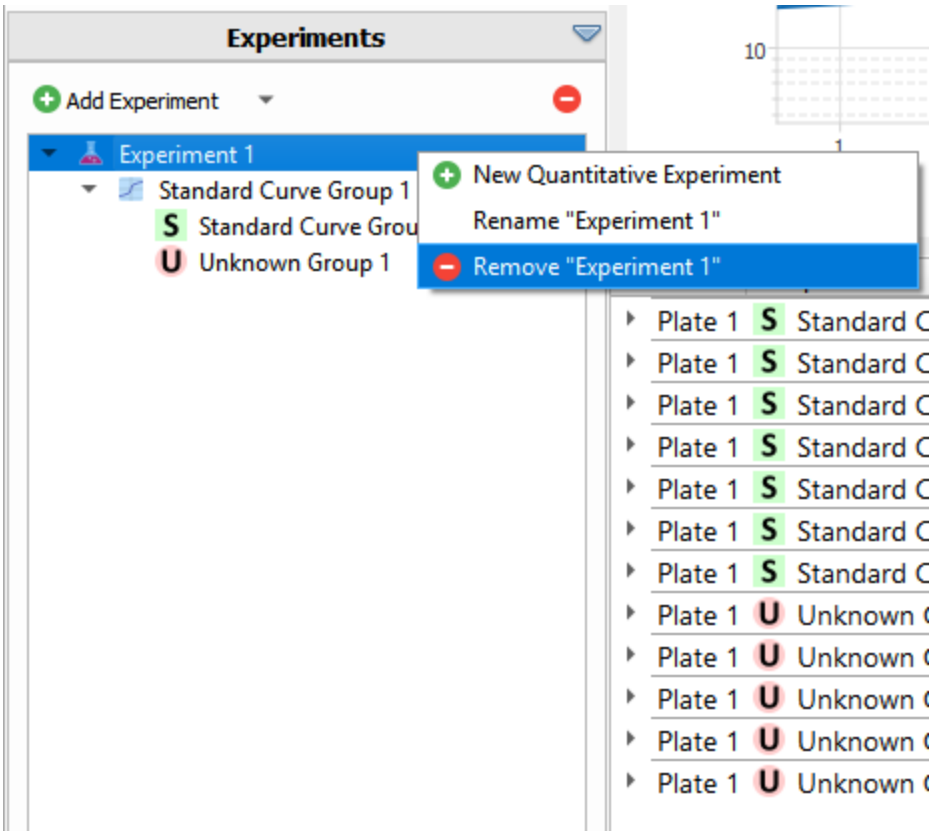


Plate Map Editor

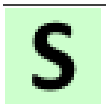
The Plate Map Editor allows you to create groups and edit well information, such as sample ID, expected concentration, and dilution factor for a particular plate (see **Plates**).

The main parts of the Plate Map Editor are:

- **Plate Toolbar**
- **Plate Map**
- **Well Data Table**
- **Editor Options Tab**
- Plate Details Editor (see **Edit Plate Name and Details**)

Plate Toolbar

The plate toolbar can be found on the **Plate Map Editor** and provides access to the following actions:

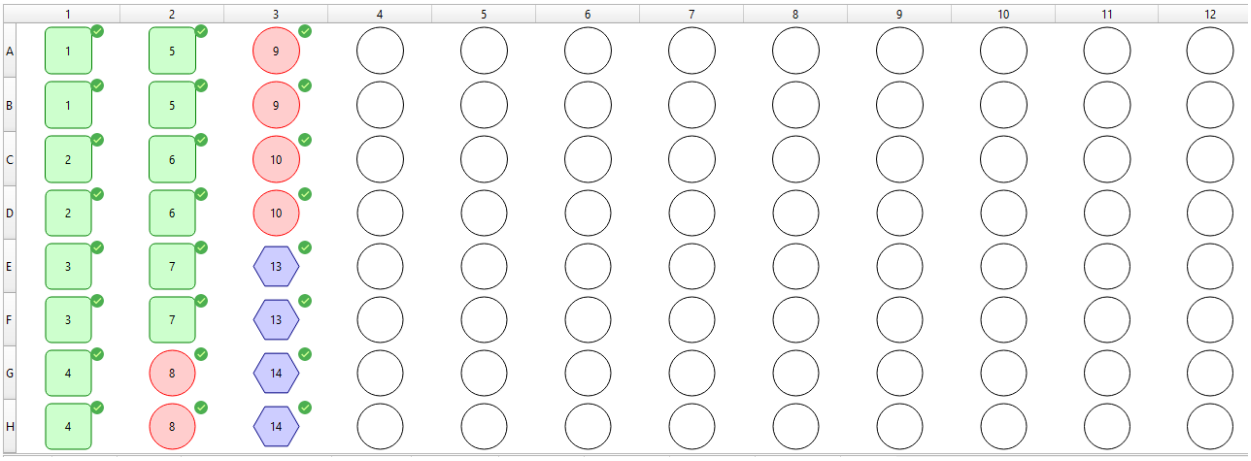
Icon	Title	Shortcut	Description
	Toggle Left Panel	Alt+1	Show or hide the left panel
	Group As Standard	Ctrl+D	Groups the selected wells as standards. See **Create Groups**.
	Group As Control	Ctrl+F	Groups the selected wells as controls. See **Create Groups**.
	Group As Unknown	Ctrl+G	Groups the selected wells as unknowns. See **Create Groups**.
	Clear Wells		Clears the selected wells. See **Clear Well Information**
	Cut Wells	Ctrl+X	Cuts the selected wells. See **Cut, Copy, & Paste Wells**.
	Copy Wells	Ctrl+C	Copies the selected wells. See **Cut, Copy, & Paste Wells**.
	Paste Wells	Ctrl+V	Pastes the selected wells. See **Cut, Copy, & Paste Wells**.

Icon	Title	Shortcut	Description
	Renumber Replicates		Renumbers replicates on the plate. See **Renumber Replicates**.
	Show Replicates		Show all replicates in the well data table
	Hide Replicates		Hide all replicates in the well data table
	Toggle Right Panel	Alt+2	Show or hide the right panel

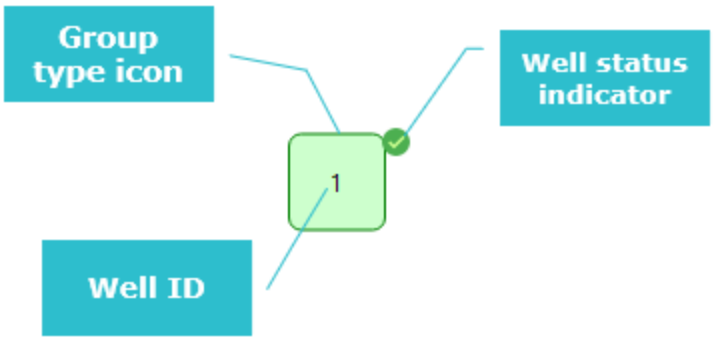
Plate Map

Group and Well Information

The plate map is found on the **Plate Map Editor** and displays information about each well on the plate:



Each well is represented as an icon with a particular color, shape, and indicator:



Element	Meaning
Group type icon	A green square represents a standard well. A red circle represents an unknown well. A blue hexagon represents a control well.
Well status indicator	A green check mark means that the well was included in the source data and has valid data. A red cross means that the well was included in the source data, but the result is invalid (for example due to an instrument error). No indicator means that the well was not included in the source data.
Well ID	A number representing which replicate group this well is a part of. Wells with the same Well IDs are replicates of one another.

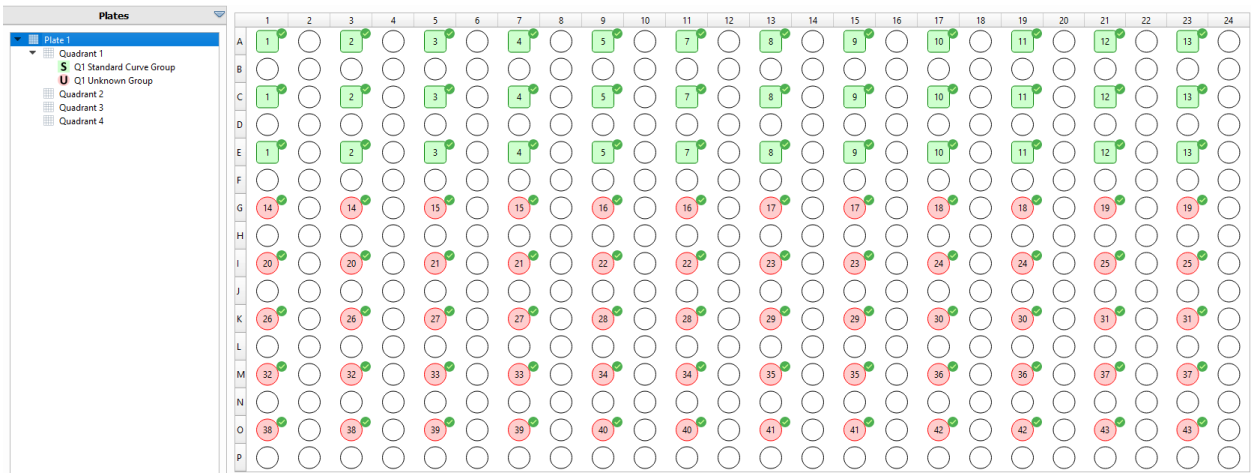
You can select wells on the plate map by clicking or dragging with the mouse. You may hold down the **Ctrl** or **Shift** keys while selecting wells to make disjoint selections. Once you have selected wells, you can do things like **Create Groups**, **Clear Well Information**, or **Cut, Copy, & Paste Wells**.

384-Well Plates and Quadrants

When source data is provided in 384-well plate format, Belysa® immunoassay curve fitting software offers two views of the plate map.

384-Well View

The first view shows all 384 wells in a single table. You can access this view by selecting the plate name in the **Plate Tree**:



Quadrant View

384-well plates are further subdivided into four 96-well *quadrants*, where each quadrant contains the following locations:

- Quadrant 1: A1, A3, ..., C1, C3,...
- Quadrant 2: A2, A4, ..., C2, C4,...
- Quadrant 3: B1, B3, ..., D1, D3,...
- Quadrant 4: B2, B4, ..., D2, D4,...

To view the plate map for a particular quadrant, select the quadrant name under the plate in the **Plate Tree**

Well Data Table

The well data table can be found on the **Plate Map Editor** and allows you to view and edit well information such as sample IDs, expected concentrations, and dilution factors.

Single-Analyte View

Here is an example of the well data table in single-analyte view:

Row	Column	Well ID ▲	Sample ID	Expected Concentration	Dilution Factor	Group Name
A	1	1	Background0	0.00		S Standard Curve Group 1
C	1	2	Standard1	3.20		S Standard Curve Group 1
E	1	3	Standard2	16.00		S Standard Curve Group 1
G	1	4	Standard3	80.00		S Standard Curve Group 1
A	2	5	Standard4	400.00		S Standard Curve Group 1
C	2	6	Standard5	2000.00		S Standard Curve Group 1
E	2	7	Standard6	10000.00		S Standard Curve Group 1
G	2	8	Unknown1		1.00	U Unknown Group 1
A	3	9	Unknown2		1.00	U Unknown Group 1
C	3	10	Unknown3		1.00	U Unknown Group 1

The table below describes the columns available for display in the table. If the column is marked as editable, you may edit the value by double-clicking it in the table, or by copying and pasting a new value over the old one (e.g. from Excel).

Column Name	Description	Editable?
Row	The row for this well	No
Column	The column for this well	No
Well ID	A number representing which replicate group this well is a part of	No
Sample ID	A user-editable identifier for the well	**Yes**
Expected Concentration	The expected concentration of this well (if applicable)	**Yes**
Dilution Factor	The dilution factor of this well (unknowns only). Results for unknown wells are multiplied by this scaling factor.	**Yes**
Group	The name of the group to which this well belongs	No

Multi-Analyte View

Here is an example of a quantitative results table in multi-analyte view:

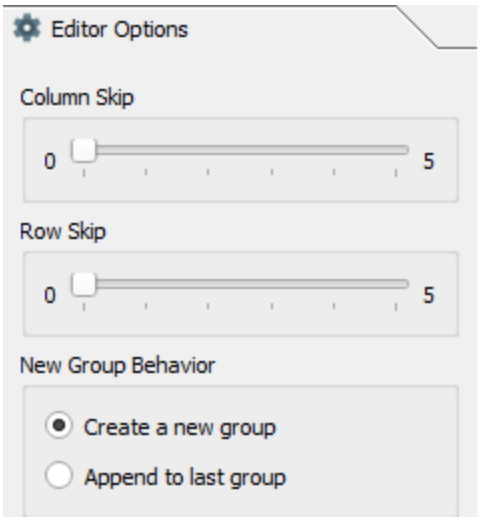
Row	Column	Well ID	Group Name	Sample ID	IFN-gamma	IL-10	IL-17a	IL-4	IL-8	MCP1
A	1	1	S Standard Curve Group 1	Background0	0.00 pg/ml	0.00 pg/ml	0.00 pg/ml	0.00 pg/ml	0.00 pg/ml	0.00 pg...
C	1	2	S Standard Curve Group 1	Standard1	3.20 pg/ml	3.20 pg/ml	3.20 pg/ml	3.20 pg/ml	3.20 pg/ml	3.20 pg...
E	1	3	S Standard Curve Group 1	Standard2	16.00 pg/ml	16.00 pg/ml	16.00 pg/ml	16.00 pg/ml	16.00 pg/ml	16.00 p...
G	1	4	S Standard Curve Group 1	Standard3	80.00 pg/ml	80.00 pg/ml	80.00 pg/ml	80.00 pg/ml	80.00 pg/ml	80.00 p...
A	2	5	S Standard Curve Group 1	Standard4	400.00 pg/ml	400.00 pg/ml	400.00 pg/ml	400.00 pg/ml	400.00 pg/ml	400.00 ...
C	2	6	S Standard Curve Group 1	Standard5	2000.00 pg/ml	2000.00 pg/ml	2000.00 pg/ml	2000.00 pg/ml	2000.00 pg/ml	2000.00...
E	2	7	S Standard Curve Group 1	Standard6	10000.00 pg/ml	10000.00 pg/ml	10000.00 pg/ml	10000.00 pg/ml	10000.00 pg/ml	10000.0...
G	2	8	U Unknown Group 1	Unknown1						
A	3	9	U Unknown Group 1	Unknown2						
C	3	10	U Unknown Group 1	Unknown3						
E	3	11	U Unknown Group 1	Unknown4						
G	3	12	U Unknown Group 1	Unknown5						

In this mode, a single statistic is shown for all analytes. The statistic can be changed by selecting a new value from the statistic list (see **Analyte View Toolbar**). The available statistics for the well data table are (see Single-Analyte section for descriptions):

- Expected Concentration
- Dilution Factor

Editor Options Tab

The editor options tab is located on the **Plate Map Editor** and gives you more flexibility when laying out groups.



Control	Function
Column Skip	Number of columns to skip when selecting wells on the **Plate Map**

Row Skip	Number of rows to skip when selecting wells on the **Plate Map**
New Group Behavior	When set to **Create a new group**, the Create Group Dialog will be configured to create a new group every time. When set to **Append to last group**, the Create Group Dialog will be configured to append wells to the most recently created group of the same type (if at least one group of that type has been created in the current session). See **Create Groups**.

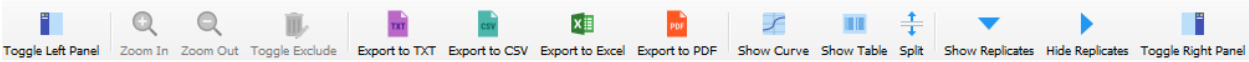
Experiment Results

The Experiment Results view contains plots and tables showing data about a quantitative or relative potency experiment (see **Experiments**). This is also where you can find and exclude outliers from your data, change the curve fit, and produce reports.

The main parts of the Experiment Results view are:

- **Experiment Toolbar**
- **Standard Curve Plot**
- **Quantitative Experiment Results** or **Relative Potency Results** tables
- **Curve Editor**
- Experiment Details tab (see **Edit Experiment Name and Details**)
- Table Columns tab (see **Show and Hide Table Columns**)

Experiment Toolbar



The experiment toolbar provides access to the following actions:

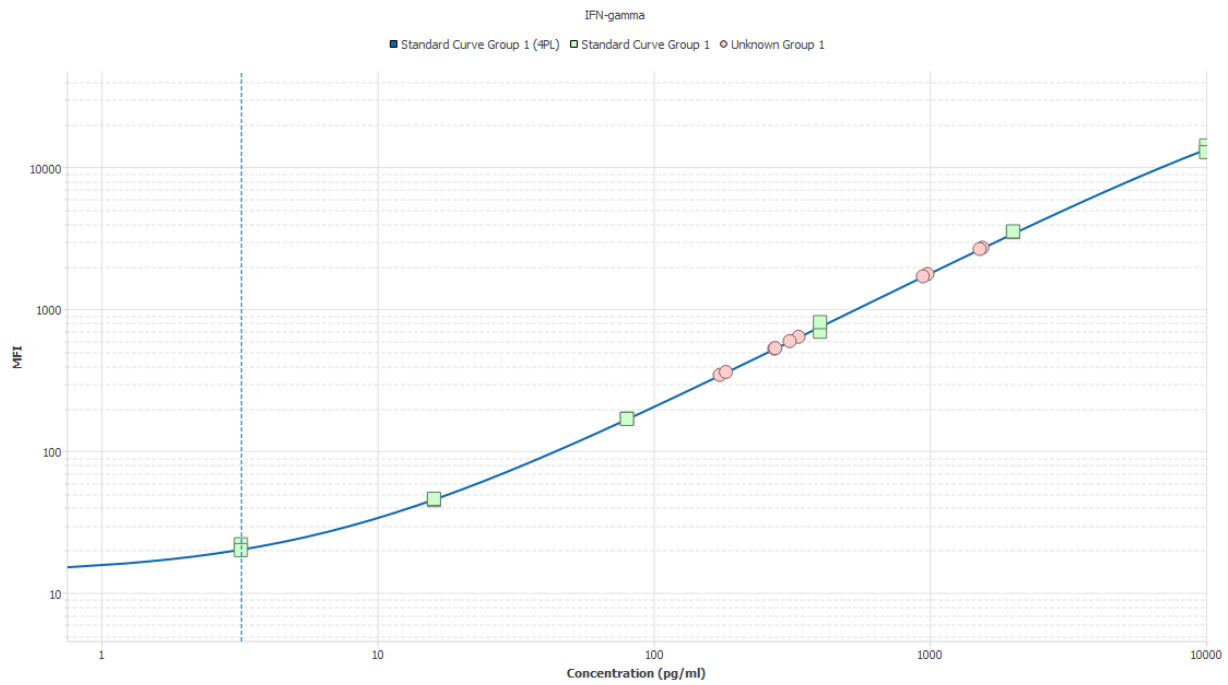
Icon	Title	Shortcut	Description
	Toggle Left Panel	Alt+1	Show or hide the left panel
	Zoom In	+	Zoom in on the standard curve plot
	Zoom Out	-	Zoom out on the standard curve plot
	Toggle Exclude	O	Exclude or include the selected point on the standard curve plot

Icon	Title	Shortcut	Description
	Export to TXT		Save a report for this experiment in tab-separated plain text format (*.txt)
	Export to CSV		Save a report for this experiment in comma-separated variable format (*.csv)
	Export to Excel		Save a report for this experiment in Excel workbook format (*.xlsx)
	Export to PDF		Save a report for this experiment in PDF format (*.pdf). Available for quantitative experiments only.
	Show Curve	Alt+3	Hide the results table and show only the standard curve plot
	Show Table	Alt+4	Hide the standard curve plot and show only the results table
	Split		Split the view between the standard curve plot and the results table
	Show Replicates		Show all replicates in the results table
	Hide Replicates		Hide all replicates in the results table
	Toggle Right Panel	Alt+2	Show or hide the right panel

Standard Curve Plot

The standard curve plot displays all standard curves and sample groups contained in the experiment (see **Experiments**).

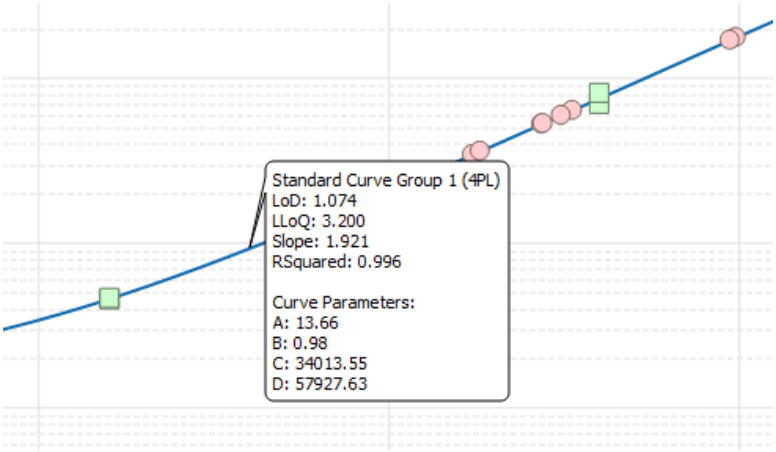
The axes will automatically adjust to best fit the range of your data. Logistic curve fit methods will automatically use a log scale for the X and Y axis, while the Linear curve fit will use linear scale axes.



Here are some things you can do with the standard cure plot:

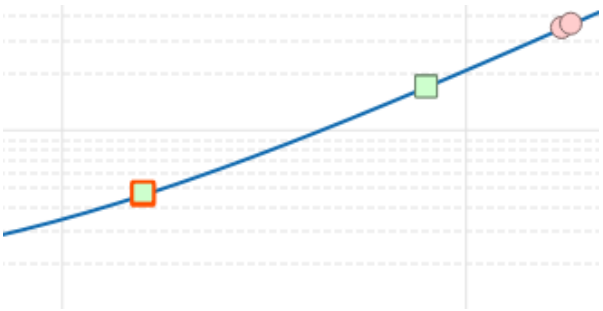
Display Curve and Point Information

Hovering over a curve or sample point with the mouse will display information about the curve or point:



Exclude and Include Wells

To exclude a point from the standard curve plot, select the point by clicking on it. Or, select multiple points by clicking and dragging a box:



The selected points are highlighted on the standard curve plot. To exclude selected wells, press the **O** key on the keyboard, or select **Toggle Exclude** from the toolbar (see **Experiment Toolbar**).

Excluded wells are displayed with a dotted-outline and a faded color:

To re-include the wells, select them and press the **O** key on the keyboard, or select **Toggle Exclude** from the toolbar.

Export as Image File

To export the standard curve plot as an image file, right-click on the standard curve plot to open the shortcut menu, then select an export option:

- **Copy Curve Plot** - copies the current plot to your clipboard
- **Save Curve Plot as PNG** - saves the curve plot as a high-resolution PNG image file
- **Save Curve Plot as SVG** - saves the curve plot as a scalable vector graphics file, suitable for printing at any resolution

Note that the saved image will reflect your current zoom and pan settings. Before saving, ensure that the chart has the desired content visible.

Change Plot Appearance

To toggle the visibility of curves or to change colors, see **Change Curve Plot Appearance**

Quantitative Experiment Results

The quantitative results table shows you interpolated concentrations for sample groups in your experiment. Wells that are part of the same replicate group are rolled-up into single rows in the table, showing average values where appropriate. To see individual replicate results, expand the row by selecting the arrow in the Plate column.

Single-Analyte View

Here is an example of a quantitative results table in single-analyte view:

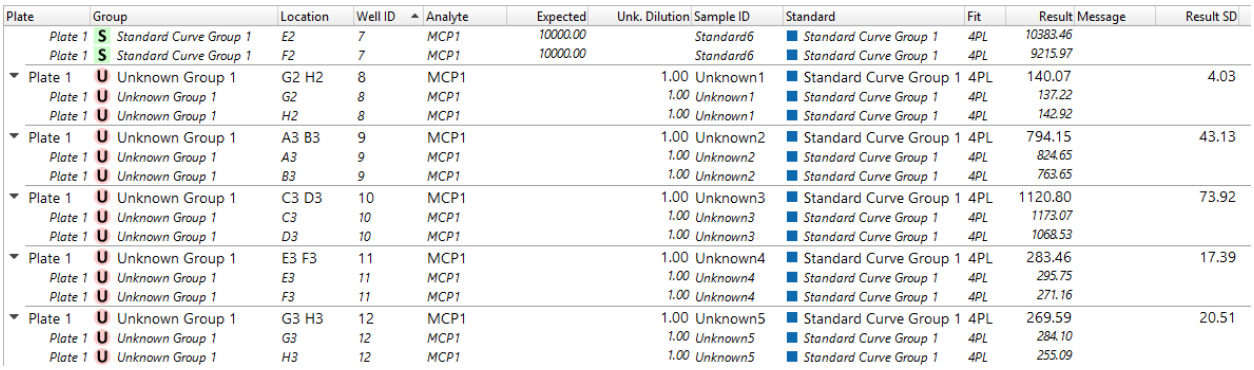


Plate	Group	Location	Well ID	Analyte	Expected	Unk. Dilution	Sample ID	Standard	Fit	Result	Message	Result SD
Plate 1	S *Standard Curve Group 1*	*E2*	*7*	*MCP1*	*10000.00*		*Standard6*	*Standard Curve Group 1*	*4PL*	*10383.46*		
Plate 1	S *Standard Curve Group 1*	*F2*	*7*	*MCP1*	*10000.00*		*Standard6*	*Standard Curve Group 1*	*4PL*	*9215.97*		
Plate 1	U Unknown Group 1	G2 H2	8	MCP1		1.00	Unknown1	Standard Curve Group 1	4PL	140.07		4.03
Plate 1	U *Unknown Group 1*	*G2*	*8*	*MCP1*		*1.00*	*Unknown1*	*Standard Curve Group 1*	*4PL*	*137.22*		
Plate 1	U *Unknown Group 1*	*H2*	*8*	*MCP1*		*1.00*	*Unknown1*	*Standard Curve Group 1*	*4PL*	*142.92*		
Plate 1	U Unknown Group 1	A3 B3	9	MCP1		1.00	Unknown2	Standard Curve Group 1	4PL	794.15		43.13
Plate 1	U *Unknown Group 1*	*A3*	*9*	*MCP1*		*1.00*	*Unknown2*	*Standard Curve Group 1*	*4PL*	*824.65*		
Plate 1	U *Unknown Group 1*	*B3*	*9*	*MCP1*		*1.00*	*Unknown2*	*Standard Curve Group 1*	*4PL*	*763.65*		
Plate 1	U Unknown Group 1	C3 D3	10	MCP1		1.00	Unknown3	Standard Curve Group 1	4PL	1120.80		73.92
Plate 1	U *Unknown Group 1*	*C3*	*10*	*MCP1*		*1.00*	*Unknown3*	*Standard Curve Group 1*	*4PL*	*1173.07*		
Plate 1	U *Unknown Group 1*	*D3*	*10*	*MCP1*		*1.00*	*Unknown3*	*Standard Curve Group 1*	*4PL*	*1068.53*		
Plate 1	U Unknown Group 1	E3 F3	11	MCP1		1.00	Unknown4	Standard Curve Group 1	4PL	283.46		17.39
Plate 1	U *Unknown Group 1*	*E3*	*11*	*MCP1*		*1.00*	*Unknown4*	*Standard Curve Group 1*	*4PL*	*295.75*		
Plate 1	U *Unknown Group 1*	*F3*	*11*	*MCP1*		*1.00*	*Unknown4*	*Standard Curve Group 1*	*4PL*	*271.16*		
Plate 1	U Unknown Group 1	G3 H3	12	MCP1		1.00	Unknown5	Standard Curve Group 1	4PL	269.59		20.51
Plate 1	U *Unknown Group 1*	*G3*	*12*	*MCP1*		*1.00*	*Unknown5*	*Standard Curve Group 1*	*4PL*	*284.10*		
Plate 1	U *Unknown Group 1*	*H3*	*12*	*MCP1*		*1.00*	*Unknown5*	*Standard Curve Group 1*	*4PL*	*255.09*		

The table below describes the columns available for display in the table. If the column is marked as editable, you may edit the value by double-clicking it in the table, or by copying and pasting a new value over the old one (e.g. from Excel).

Column Name	Description	Editable?
Plate	The name of the plate on which this well resides	No
Group	The name of the group to which this well belongs	No
Location	The well's row and column location on the plate	No
Analyte	The name of the analyte whose results are being displayed	No
Well ID	A number representing which replicate group this well is a part of	No
Expected	The expected concentration of this well (if applicable)	**Yes**
Unk. Dilution	The dilution factor of this well (unknowns only). Results for unknown wells are multiplied by this scaling factor.	**Yes**
Sample ID	A user-editable identifier for the well	**Yes**
Standard	The name of the standard group used to obtain results for this well	No
Fit	The curve fit method used to obtain results for this well	No
Result	The interpolated concentration for this well as determined by the standard curve	No
Message	If populated, contains one or more codes containing feedback from the curve fitting algorithm	No

Column Name	Description	Editable?
Result SD	Result standard deviation	No
Result CV	Result coefficient of variation	No
MFI or Response	Raw instrument response for this well. Exact definition varies depending on source of data.	No
MFI or Response CV	MFI/Response coefficient of variation	No
MFI or Response SD	MFI/Response standard deviation	No
Recovery	The ratio of interpolated result to expected concentration, expressed as a percentage	No
n	The number of non-excluded replicates included in this roll-up	No
Exclude	An indication of whether or not this well (or one replicate of a duplicate or triplicate) has been excluded. A checked box means that the well or entire replicate group has been excluded. A partially filled box means that one or more, but not all, replicates have been excluded	**Yes**
Exclude Reason	A user-provided reason why the well was excluded	**Yes**

Multi-Analyte View

Here is an example of a quantitative results table in multi-analyte view:

Plate	Group	Location	Well ID	Sample ID	Standard	IFN-gamma	IL-10	IL-17a	IL-4	IL-8
Pl...	S Standard Curve Group 1	F2	7	Standard6	Standard Curve Group 1	9396.40	9590.38	8977.35	9872.35	9098.36
Plate 1	U Unknown Group 1	G2 H2	8	Unknown1	Standard Curve Group 1	177.91	139.49	146.48	126.50	143.89
Pl...	U Unknown Group 1	G2	8	Unknown1	Standard Curve Group 1	173.31	136.95	148.47	128.63	141.58
Pl...	U Unknown Group 1	H2	8	Unknown1	Standard Curve Group 1	182.51	142.03	144.49	124.37	146.19
Plate 1	U Unknown Group 1	A3 B3	9	Unknown2	Standard Curve Group 1	961.43	721.43	805.09	651.88	789.80
Pl...	U Unknown Group 1	A3	9	Unknown2	Standard Curve Group 1	980.30	709.04	781.92	647.34	790.18
Pl...	U Unknown Group 1	B3	9	Unknown2	Standard Curve Group 1	942.55	733.83	828.26	656.43	789.42
Plate 1	U Unknown Group 1	C3 D3	10	Unknown3	Standard Curve Group 1	1530.70	860.94	1241.24	1189.85	759.69
Pl...	U Unknown Group 1	C3	10	Unknown3	Standard Curve Group 1	1548.11	852.35	1280.83	1197.60	757.99
Pl...	U Unknown Group 1	D3	10	Unknown3	Standard Curve Group 1	1513.29	869.53	1201.65	1182.10	761.38
Plate 1	U Unknown Group 1	E3 F3	11	Unknown4	Standard Curve Group 1	303.83	164.47	254.38	222.40	152.57
Pl...	U Unknown Group 1	E3	11	Unknown4	Standard Curve Group 1	334.48	186.42	267.26	232.82	164.97
Pl...	U Unknown Group 1	F3	11	Unknown4	Standard Curve Group 1	273.17	142.52	241.49	211.99	140.18
Plate 1	U Unknown Group 1	G3 H3	12	Unknown5	Standard Curve Group 1	293.02	169.67	222.85	219.26	146.28
Pl...	U Unknown Group 1	G3	12	Unknown5	Standard Curve Group 1	275.37	167.33	233.81	208.13	147.39
Pl...	U Unknown Group 1	H3	12	Unknown5	Standard Curve Group 1	310.67	172.00	211.89	230.39	145.18

In this mode, a single statistic is shown for all analytes. The statistic can be changed by selecting a new value from the statistic list (see **Analyte View Toolbar**). The available statistics for quantitative experiment results are (see Single-Analyte section for descriptions):

- Expected
- MFI/Response
- MFI/Response CV
- MFI/Response SD
- Result

- Recovery
- Result CV
- Result SD

Messages

The Message column may display additional information about how a result has been interpreted by the xPRO software. The possible values for the Message column are:

Icon	Name	Description
	EXT (above curve)	The response for this well is near or above the top of the standard curve, and the interpolated concentration is obtained via linear extrapolation
	EXT (below curve)	The response for this well is below the standard curve, and the interpolated concentration is obtained via linear extrapolation
	ND	Non-Detect – the interpolated concentration is below the **LoD**
	BLOQ, or generic warning	When accompanied by the "BLOQ" label, this indicator

Icon	Name	Description
		means that the interpolated concentration is below the **LLoQ**. When displayed on a roll-up or average row, it means that one or more of the unexcluded replicates in this replicate group have message values.
	Defects	SMCxPRO® data files only - the software has detected likely defects in this well that may be affecting results

Non-Detect Flag

If an individual replicate is flagged as Non-Detect (ND), then by default its interpolated concentration and recovery are not calculated and are left blank. If a replicate group includes any replicates that are flagged as Non-Detect, then the mean interpolated concentration, SD, CV, and mean recovery for the group are not calculated until all Non-Detect replicates are excluded. You can override this behavior in **Analysis**.

The ND Flag has no effect on the Standard Curve calculation and all points are used unless marked as Outlier.

Relative Potency Results

The relative potency results table shows you how one or more curves compares to a designated reference curve. In a relative potency experiment (see **Create an Experiment**), the first curve added is always the reference curve.

Single-Analyte View

Here is an example of relative potency results in single-analyte view:

Curve	Parallelism	Relative Potency	CI Low	CI High
STD2	0.998	0.743	0.569	0.969
STD3	1.002	0.743	0.583	0.947
STD4	0.991	0.666	0.482	0.918
STD5	0.985	0.594	0.456	0.774
STD6	0.999	0.874	0.565	1.353

The table below describes the columns available for display in the table. More detailed explanations of each calculation can be found in **Mathematical Reference**.

Column Name	Description
Curve	The name of the curve being compared to the reference curve
Parallelism	A measure of the statistical similarity between the two curves
Relative Potency	A unitless measure obtained from a comparison of the dose-response relationships of a test curve to a reference curve
CI Low	The lower bound of the 95% confidence interval for the slope difference between the linear regions of the test and reference curves
CI High	The upper bound of the 95% confidence interval for the slope difference between the linear regions of the test and reference curves

Multi-Analyte View

Here is an example of a relative potency results table in multi-analyte view:

Curve	IL-22	IL-9	Analyte 3	Analyte 27	Analyte 28	Analyte 33	Analyte 35	Analyte 37	Analyte 39	Analyte 43	Analyte 45	A
STD2	0.998	1.055	1.003	0.994	0.995	1.001	0.954	0.999	0.988	0.995	1.003	
STD3	1.002	1.050	0.997	0.986	1.001	1.015	1.050	0.995	0.987	1.000	0.994	
STD4	0.991	0.940	1.013	0.984	1.006	0.995	1.050	1.002	0.985	1.007	1.002	
STD5	0.985	1.053	0.988	0.986	1.007	1.003	1.033	0.995	0.981	1.008	0.991	
STD6	0.999	0.992	0.996	1.012	1.012	1.003	0.993	1.002	1.005	1.001	0.999	

In this mode, a single statistic is shown for all analytes. The statistic can be changed by selecting a new value from the statistic list (see **Analyte View Toolbar**). The available statistics for relative potency experiment results are (see Single-Analyte section for descriptions):

- Parallelism
- Relative Potency
- CI Low
- CI High

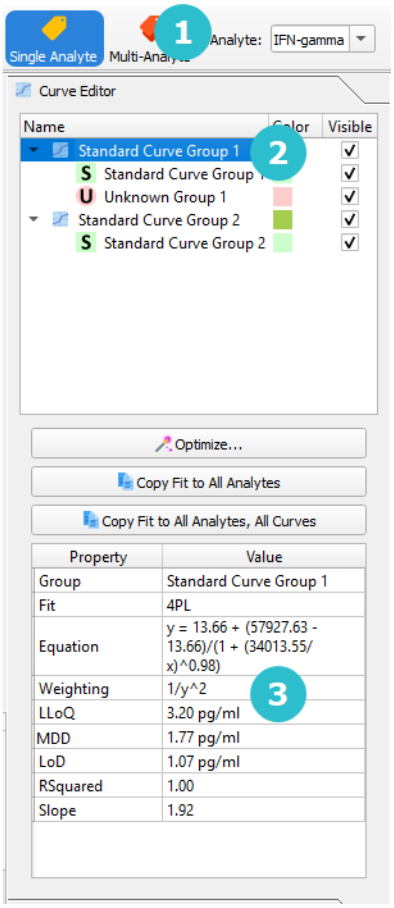
Curve Editor

The Curve Editor tab allows you to alter the appearance and properties of standard curves in your **Experiments**, including color, visibility, and curve fit. The Curve Editor displays different information depending on whether or not you are in single-analyte or multi-analyte view (see **Single and Multi-Analyte Views**).

Single-Analyte View

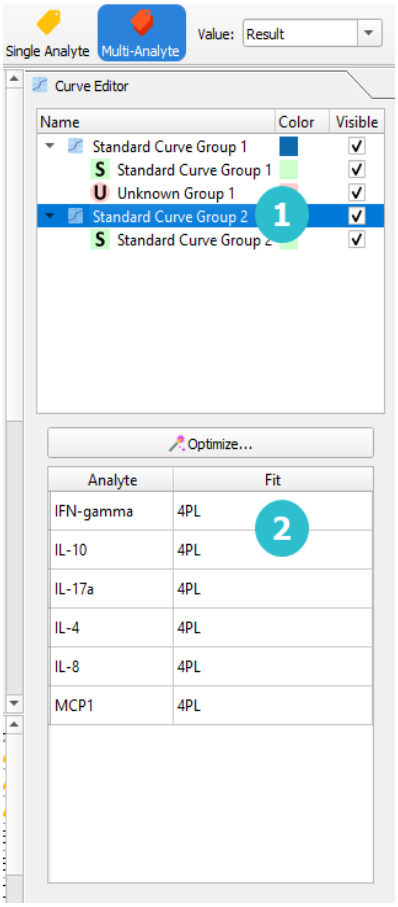
In single-analyte view, the Curve Editor displays information for the currently selected analyte for the currently selected curve. For explanations of the values presented in this table, see **Curve Quality Statistics**. In general, to view information about or edit a curve, follow this procedure:

1. Select the analyte whose curve you wish to view or edit
2. Select the name of the curve
3. The displayed information will reflect the analyte and curve selected in #1 and #2, respectively:



Multi-Analyte View

In multi-analyte view, select a curve (#1) and the Curve Editor displays the curve fit for all analytes for that curve (#2). Each analyte's curve fit can be adjusted independently. The statistic filter does not affect what is shown in the Curve Editor.



Usage

Common tasks performed using the Curve Editor tab include:

- **Change Curve Plot Appearance**
- **Change the Curve Fitting Method**

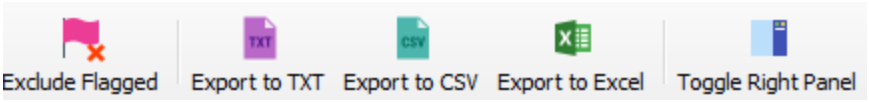
Raw Data Tab

The Raw Data tab displays information about each well in the data file. In addition to the instrument response value used for quantitative interpolation, this tab may display other meta-data about each well, for example defect count or temperature. The exact set of columns available in the raw data table will vary with the source of the data. The Raw Data table is designed to facilitate identifying and excluding outliers in your data (see **Auto-Flagging**).

The main parts of the Raw Data tab are:

- **Raw Data Toolbar**
- **Raw Data Table**
- Table Columns tab (see **Show and Hide Table Columns**)

Raw Data Toolbar



The Raw Data toolbar provides access to the following actions:

Icon	Title	Shortcut	Description
	Exclude Flagged		Exclude all currently visible wells that have one or more flags (see **Auto-Flagging**). Note: in single-analyte view, this will only exclude data from the currently selected analyte. To exclude all wells with flags for all analytes, select this action while in multi-analyte view.
	Export to TXT		Save a raw data information in tab-separated plain text format (*.txt)
	Export to CSV		Save a raw data information in comma-separated variable format (*.csv)
	Export to Excel		Save a raw data information in Excel workbook format (*.xlsx)
	Toggle Right Panel	Alt+2	Show or hide the right panel

Raw Data Table

The raw data table displays detailed information about each well and provides you an easy way to find and exclude outliers.

Single-Analyte View

Here is an example of the Raw Data table in single-analyte view:

Flag	Plate	Location	Well ID	Count	MFI	Exclude	Exclude Reason
	Plate 1	E12	11	80	43.00	☐	
	Plate 1	D3	12	179	26.00	☐	
	Plate 1	F12	12	168	36.00	☐	
⚠	Plate 1	E3	13	5 🚩	40.00	☐	
✓	~~Plate 1~~	~~G12~~	~~13~~	~~10~~ 🚩	~~42.00~~	☑	Low bead count
	Plate 1	F3	14	55	54.00	☐	
	Plate 1	H12	14	47	52.00	☐	
⚠	Plate 1	G3	15	33 🚩	39.00	☐	
	Plate 1	H3	16	154	45.00	☐	
	Plate 1	C4	17	58	64.50	☐	
	Plate 1	D4	18	81	52.00	☐	
	Plate 1	E4	19	46	44.00	☐	
⚠	Plate 1	F4	20	28 🚩	34.00	☐	
	Plate 1	G4	21	189	25.00	☐	
	Plate 1	H4	22	184	31.00	☐	
	Plate 1	A5	23	81	66.00	☐	
	Plate 1	B5	24	60	85.50	☐	

The columns are described in the table below:

Column Name	Description	Editable?
Flag	Indicates whether or not this row has any value that has been flagged, and if so, whether that row has been excluded. The ⚠ icon indicates that the row has one or more flagged columns. The ✓ icon indicates that the row has one or more flagged columns, but that the data has been excluded from results. No icon means that there are no flagged columns in this row. Note: in single-analyte view, the Flag column only indicates whether or not the currently visible analyte has flagged data. To see flags for all analytes, use multi-analyte view (see **Single and Multi-Analyte Views**).	No
Plate	The name of the plate on which this well resides	No
Location	The well's row and column location on the plate	No

Column Name	Description	Editable?
Well ID	A number representing which replicate group this well is a part of	No
Exclude	An indication of whether or not this well (or one replicate of a duplicate or triplicate) has been excluded. A checked box means that the well or entire replicate group has been excluded. A partially filled box means that one or more, but not all, replicates have been excluded	**Yes**
Exclude Reason	A user-provided reason why the well was excluded	**Yes**

Multi-Analyte View

Here is an example of the raw data table in multi-analyte view:

Flag	Plate	Location	Well ID	EGF	Angiopoietin-2	G-CSF	BMP-9	Endoglin	Endothelin-1	Leptin
	Plate 1	G1	7	217	257	239	302	310	366	303
	Plate 1	H1	8	181	241	239	291	328	362	319
	Plate 1	H2	8	222	264	230	282	312	353	300
	Plate 1	A1	1	221	228	202	293	235	330	269
	Plate 1	F1	6	243	234	242	314	320	315	311
	Plate 1	A3	9	197	215	204	253	303	314	262
	Plate 1	B1	2	180	181	216	248	259	313	285
	Plate 1	C1	3	202	236	220	308	282	302	270
	Plate 1	E2	5	157	212	223	256	267	299	269
	Plate 1	H5	30	208	232	230	295	304	293	294
	Plate 1	A4	9	222	211	214	244	257	291	263
	Plate 1	E7	43	162	227	78	240	249	291	258
	Plate 1	D1	4	176	249	195	239	265	288	271
	Plate 1	H7	46	172	207	154	277	252	285	276
⚠	Plate 1	F5	28	164	248	22 ⚑	274	281	283	261
	Plate 1	B2	2	181	179	197	213	241	281	277

In this mode, a single statistic is shown for all analytes. The statistic can be changed by selecting a new value from the statistic list (see **Analyte View Toolbar**). The available statistics for the raw data table vary depending on the data source.

Excluded Wells Tab

The Excluded Wells tab displays all wells and analytes that have been excluded from results calculations for any reason (see **Exclude and Include Wells**). It also provides a convenient way to restore all excluded data.

The main parts of the Excluded Wells Tab are:

- **Excluded Wells Toolbar**
- **Excluded Wells Table**

Excluded Wells Toolbar

The Excluded Wells toolbar contains a single action called **Restore All**  . In single-analyte view this action will restore all excluded wells for the currently selected analyte, or in multi-analyte view this action will restore all excluded wells for all analytes (see **Single and Multi-Analyte Views**).

Excluded Wells Table

The Excluded Wells table displays analyte and well data that have been removed from calculations across all **Experiments**. You can use this table to re-include data that has been excluded, or see a quick overview of all your excluded wells and analytes. Note that only excluded wells and analytes are included in this table. To exclude data for a well or analyte that does not appear in this table, use the **Raw Data Table** or the **Quantitative Experiment Results**.

Single-Analyte View

Here is an example of the Excluded Wells table in single-analyte view:

Plate	Location	Well ID	Group	Sample ID	Exclude	Reason
Plate 1	C1	2	Standard Curve Group 1	Standard1	☑	Count is < 55
Plate 1	B2	5	Standard Curve Group 1	Standard4	☑	Count is < 55
Plate 1	C5	18	Unknown Group 1	Unknown11	☑	Count is < 55

The table below describes the columns in the table:

Column Name	Description	Editable?
Plate	The name of the plate on which this well resides	No
Location	The well's row and column location on the plate	No
Well ID	A number representing which replicate group this well is a part of	No
Group	The name of the group to which this well belongs	No
Sample ID	A user-editable identifier for the well	No

Exclude	If checked, this analyte's data for this well has been excluded from calculations.	**Yes**
Reason	A user-provided reason why the analyte/well was excluded	**Yes**

Multi-Analyte View

Here is an example of a quantitative results table in multi-analyte view:

Plate	Location	Well ID	Group	Sample ID	12 EGF	13 FGF-2	14 Eotaxin	15 TGF-a	18 G-CSF	19 FLT-3L	20 GM-CSF
Plate 1	A1	1	Standard Curve Group 1	Background0	☐	☐	☐	☐	☑	☐	☐
Plate 1	A2	5	Standard Curve Group 1	Standard4	☐	☐	☐	☐	☐	☐	☐
Plate 1	A3	9	Control Group 1	QC2	☐	☐	☐	☐	☐	☐	☑
Plate 1	A4	13	Unknown Group 1	Unknown6	☐	☐	☐	☐	☐	☐	☐
Plate 1	A5	17	Unknown Group 1	Unknown10	☐	☐	☐	☐	☐	☐	☐
Plate 1	A6	21	Unknown Group 1	Unknown14	☐	☐	☐	☐	☐	☐	☐
Plate 1	A7	25	Unknown Group 1	Unknown18	☐	☐	☐	☐	☐	☐	☐
Plate 1	A8	29	Unknown Group 1	Unknown22	☐	☐	☐	☐	☐	☐	☐
Plate 1	B1	1	Standard Curve Group 1	Background0	☐	☐	☐	☐	☐	☐	☐
Plate 1	B2	5	Standard Curve Group 1	Standard4	☑	☐	☐	☐	☑	☐	☐
Plate 1	B3	9	Control Group 1	QC2	☐	☐	☐	☐	☐	☐	☑

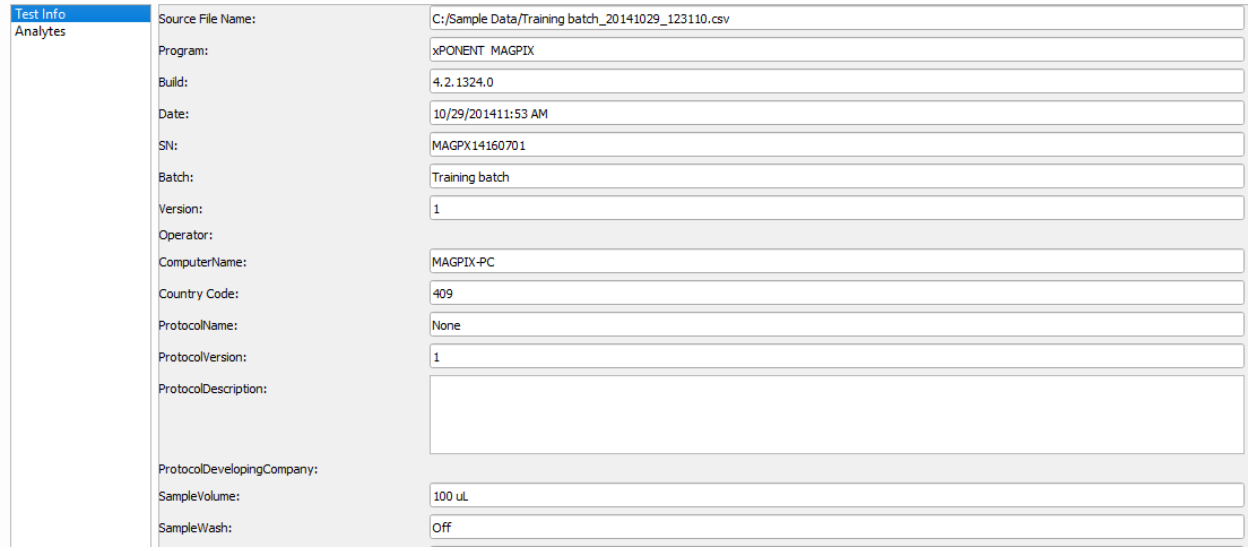
In multi-analyte view, each row represents a specific well on a plate and the columns are labeled for each analyte. A well (row) must have at least one excluded analyte to appear in the table. To re-include a particular analyte, clear the box in the cell at the intersection of the well's row and the analyte's column. To change the reason for exclusion: double-click in the table cell for the desired well and analyte, enter a new reason, and press **Enter**.

Test Info Tab

The Test Info tab displays extra information about the data file, such as when the test was run. For some data formats this information may be referred to as "header data" or "metadata." The Test Info tab also allows you to view and edit information about the analytes in your file. There are two sections on the Test Info tab:

Test Info

Select **Test Info** to view header information about the file. The exact fields will vary based upon the source of the data.



Analytes

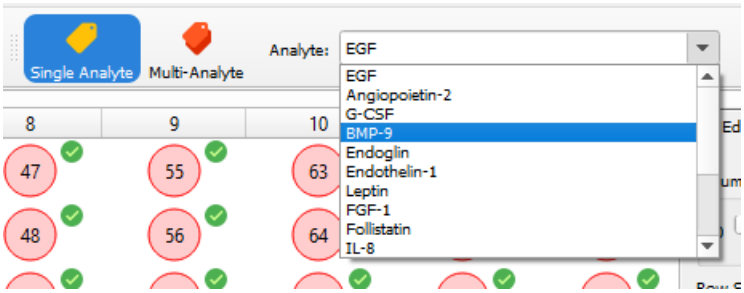
Select **Analytes** to view information about the analytes in this file:

Column Name	Description	Editable?
Original Name	The name of the analyte as reported in the original source data	No
Current Name	The name of the analyte as displayed in the software. By default this is the same as the Original Name, but can be changed to a new name if desired.	**Yes**
ID	A number uniquely identifying the analyte in addition to its name. For Luminex® data files this is equivalent to the bead region.	No
Units	The units of measure used to express concentration values for this analyte	**Yes**
Precision	The number of decimal places used to express concentration values for this analyte. Intermediate calculations are carried out using full double precision, and then the results are rounded to this number of decimal places. To avoid confusion due to small values appearing as zero, ensure that precision is set to an appropriate number of decimal places for the quantities and units you are working with.	**Yes**

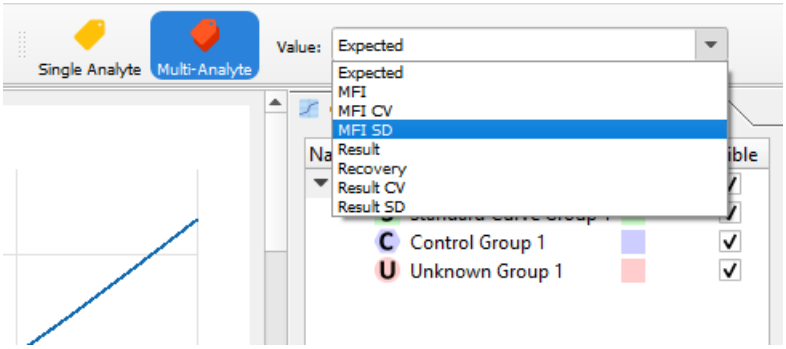
Analyte View Toolbar

The Analyte View toolbar allows you to switch between single and multi-analyte views of your data (see **Single and Multi-Analyte Views**). You can switch between analytes (in single-analyte view) or statistics (in multi-analyte view) by selecting different values in the analyte drop-down box, or typing in the name of the analyte and pressing **Enter**.

Here is an example of selecting different analytes in single-analyte view:



And here is an example of selecting different statistics in multi-analyte view:



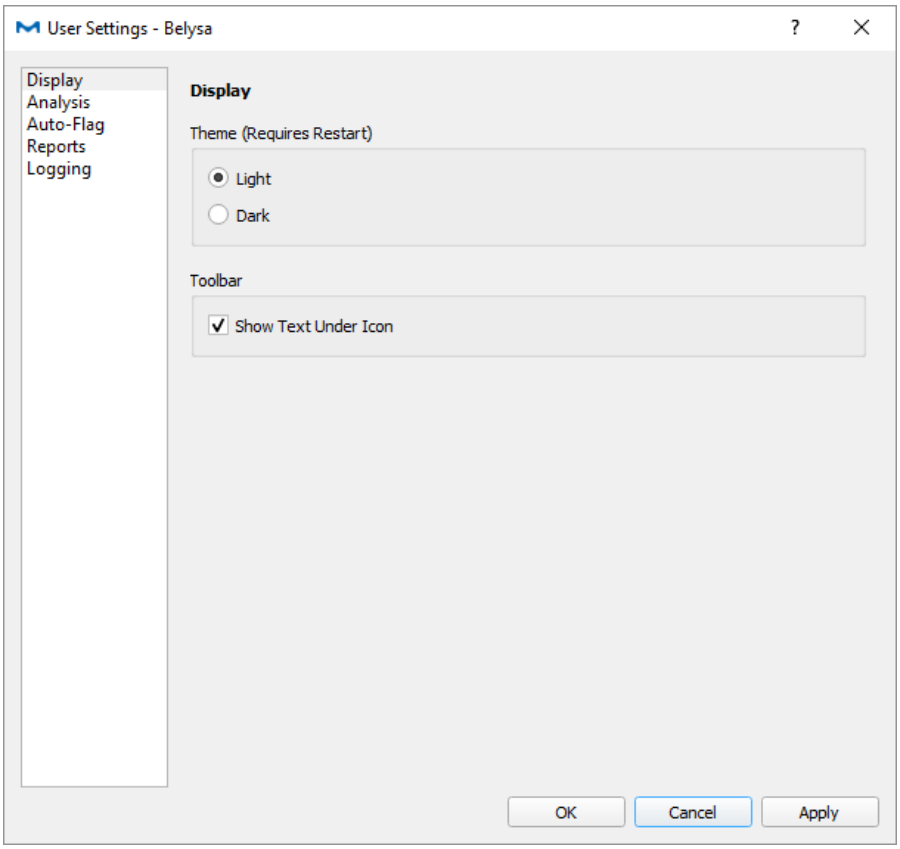
You can use the keyboard shortcuts **Ctrl+E** and **Ctrl+R** to switch between single and multi-analyte views, respectively. To quickly change between analytes or statistics, press **Ctrl+K** to move the cursor to the drop-down box and highlight the current value.

Settings and Preferences

Belysa® immunoassay curve fitting software provides several configuration options to optimize your workflow and customize your experience. These options can be accessed by selecting **Tools > Settings** from the menu bar.

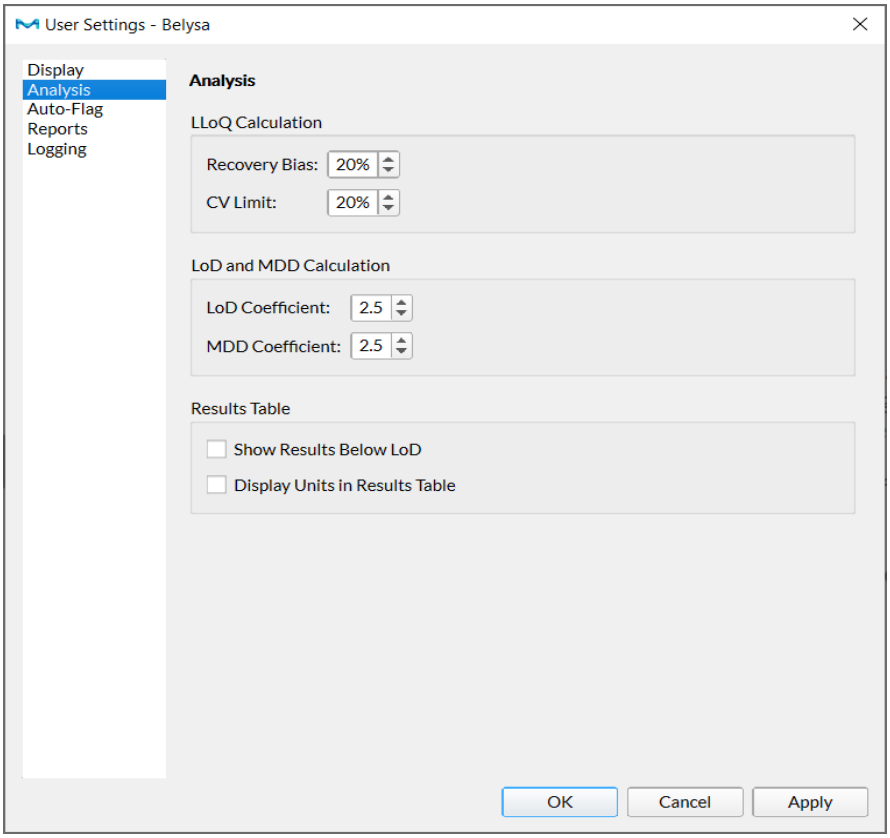
The User Settings dialog is divided into sections:

Display



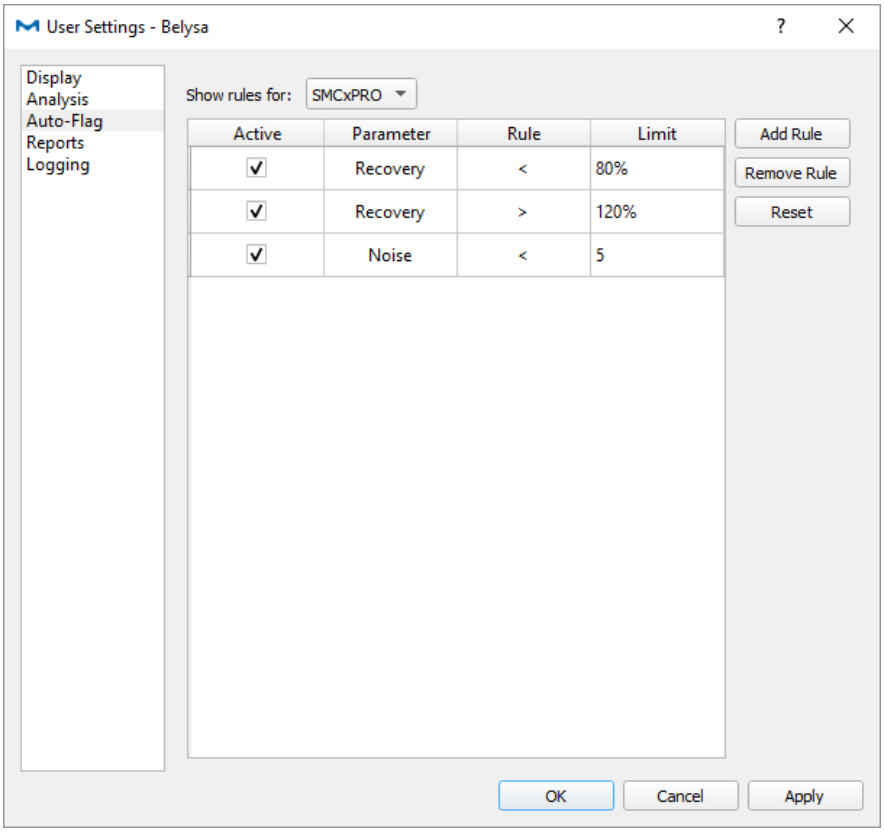
Setting	Description	Default Value
Theme	Choose a light or dark appearance. Requires a restart of Belysa® software to take effect.	Light
Toolbar	When **Show Text Under Icon** is selected, toolbar icons include a text description of the action under the icon. When cleared, only the icon is shown. Try clearing this box to conserve space on smaller screens.	Checked

Analysis



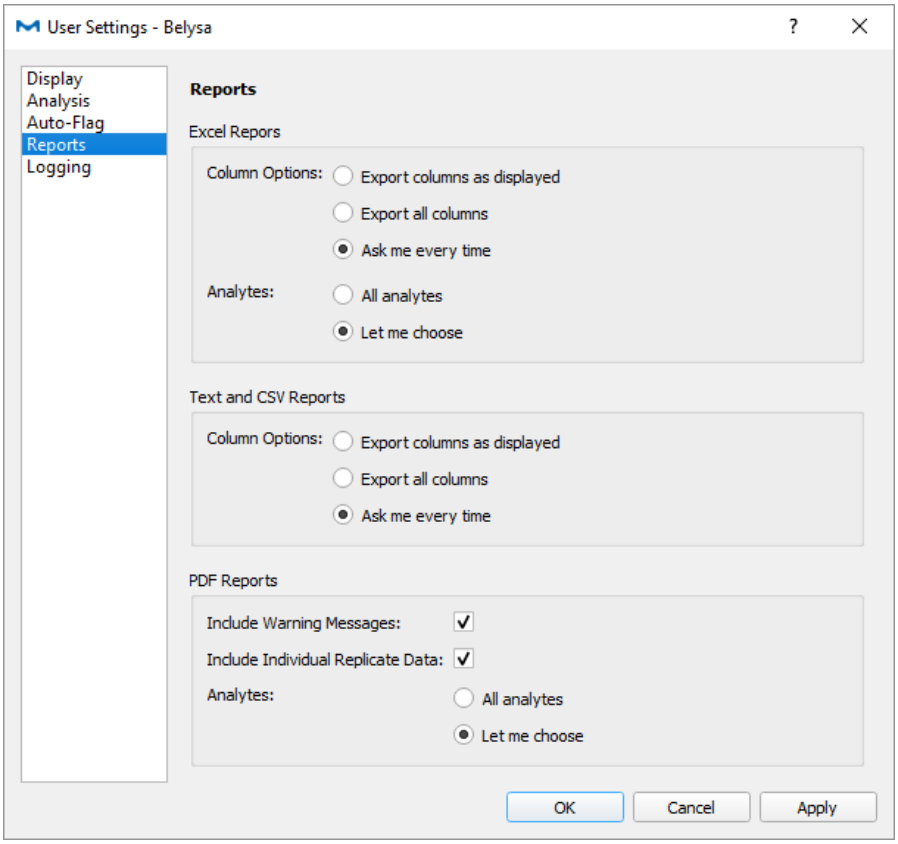
Setting	Description	Default Value (s)
LLoQ Calculation	Sets the recovery bias and CV% limit values used when calculating **LLoQ**. For example, a recovery bias of 20% will use a recovery range of 80%-120% when calculating LLoQ. A CV limit of 20% will accept points with CV less than or equal to 20%.	Recovery Bias = 20% CV Limit = 20%
LoD and MDD Calculation	Sets the coefficients used when calculating **MDD** and **LoD**. Valid range 1.0-5.0.	LoD Coefficient = 2.5 MDD Coefficient = 2.5
Show Results Below LoD	When selected, displays values for results below the **LoD**. When cleared, values below the LoD will be reported as "-" in results tables.	Cleared
Display Units in Results Table	When selected, units of measure will be included in results tables.	Cleared

Auto-Flag



The Auto-Flagging tab lets you set highlighting rules for different data types. See **Auto-Flagging**.

Reports



Excel Reports

Setting	Description	Default Value
Column Options	Controls which columns will appear in the report. When **Export columns as displayed** is selected, the report's columns and sorting will match the way you have configured your experiment results. When **Export all columns** is selected, the report will include all columns with a default sort order. When **Ask me every time** is selected, the software will prompt you to choose an option each time you generate a report.	Ask me every time
Analytes	Allows you to select which analytes are included in the report. Select **All Analytes** to include every analyte in the file in the report. Select **Let me choose** to only include some analytes in the report.	Let me choose

Text and CSV Reports

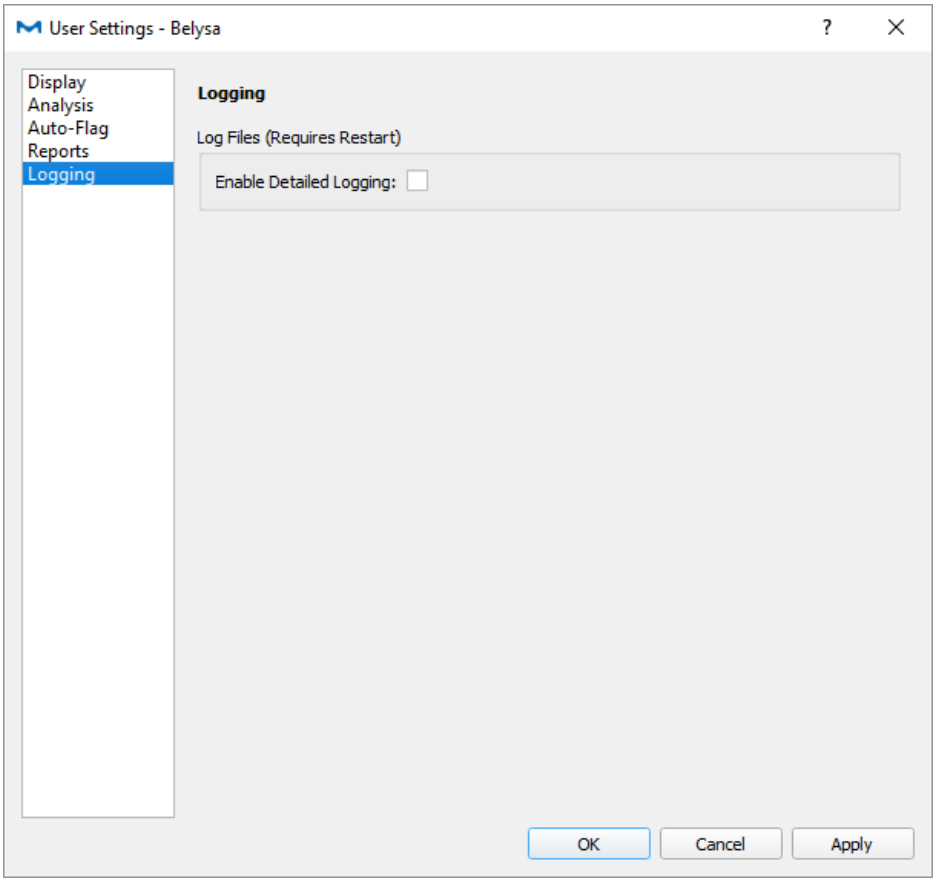
Setting	Description	Default

		Value
Column Options	Controls which columns will appear in the report. When **Export columns as displayed** is selected, the report's columns and sorting will match the way you have configured your experiment results. When **Export all columns** is selected, the report will include all columns with a default sort order. When **Ask me every time** is selected, the software will prompt you to choose an option each time you generate a report.	Ask me every time

PDF Reports

Setting	Description	Default Value
Include Warning Messages	Select this box to include warning messages from the curve fitting algorithm in the report	Selected
Include Individual Replicate Data	Select this box to include individual replicate data in the report. When cleared, only averages for each replicate group will be reported.	Selected
Analytes	Allows you to select which analytes are included in the report. Select **All Analytes** to include every analyte in the file in the report. Select **Let me choose** to only include some analytes in the report.	Let me choose

Logging



Setting	Description	Default Value
Enable Detailed Logging	Select this box to write log messages to a file, for example to send to technical support.	Cleared

Additional Information

This section contains additional useful information about Belysa® immunoassay curve fitting software:

- The **Mathematical Reference** section contains detailed explanations of the various curve fitting and statistical methods used in the application
- The **Keyboard Shortcuts** table provides a quick reference for keyboard shortcuts used in the application

Mathematical Reference

See the following sections for detailed information on the mathematical and statistical methods used in Belysa® immunoassay curve fitting software:

- For information on 4PL and 5PL curve fits, see **Logistic Curve Fitting**
- For other curve fit options (such as linear and cubic spline), see **Other Curve Fitting Methods**
- For information on how curve fit statistics such as LoD and LLoQ are calculated, see **Curve Quality Statistics**
- For relative potency experiments, see **Relative Potency**

Logistic Curve Fitting

Belysa® immunoassay curve fitting software provides several logistic curve fitting methods:

Method	Description
4PL	The default curve fit selection. Four-parameter logistical interpolation of the standard curve, of sigmoid shape, to fit the signals of fluorescent responses with respect to the expected analyte concentration.
5PL	A five-parameter logistical regression of the standard curve, sigmoid shape. This is essentially a variation of the 4PL, except it may be asymmetrical about the center of the curve.
Robust 5PL / 4PL	The corresponding curve equation is the same as 4PL/5PL, but the numerical procedure to fit the 4 or 5 parameters has similar robustness to potential outliers in the standard data.

The equation for the five-parameter logistic curve (5PL) is given by one of the following formulas:

$$y = f(x;p) = a + \frac{d-a}{(1+(\frac{c}{x})^b)^g} \quad \text{(Equation 1a)}$$

$$y = f(x;p) = d + \frac{a-d}{(1+(\frac{x}{c})^b)^g} \quad \text{(Equation 1b)}$$

where y is the response, x is the concentration, and p≡[a, b, c, d, g] is the vector of parameters defined as:

- a – The bottom or the lower asymptote of the curve
- d – The top or the upper asymptote of the curve
- c – The mid-range concentration or the concentration at the inflection point
- b – The slope of the curve at its midpoint or the rapidity of the curve transition between asymptotes
- g – The asymmetry factor or the asymmetry around the inflection point

By setting g = 1, the standard curve becomes four-parameter logistic curve (4PL). For 4PL model, c is also called EC50, the effective concentration corresponding to the middle point response between a and d.

Weighted Least Squares Fitting Model

Using the standard curve calibration data, the parameters [a, b, c, d, g] in Equations 1a and 1b are estimated by the weighted least squares (WLS) fitting model:

$$Y_{i,j} = f(x_i; p) + \sigma w_i^{-\frac{1}{2}} \epsilon_{i,j} \qquad i = 1, \ldots N, j = 1, \ldots m_i \quad \text{(Equation 2)}$$

where N is the number of standard concentration points, m_i is the number of replicates at each concentration point x_i, $Y_{i,j}$ is the measured response for the jth replicate of the ith standard concentration, f(x_i; p) is the regression mean function given by (1), w_i is the weight proportional to the experimental error with the assumption of Var($Y_{i,j}$) = σ^2 w_i^{-1} with each response, and ϵ_{ij} is the independent random errors with a standard normal distribution.

Standard and Robust Variations

The WLS model (2) is generally fitted by minimizing the sum of squares of the errors (SSE) given by:

$$R = \sum_{i=1}^{N} \sum_{j=1}^{m_i} w_i [RE_{i,j} - f(c_i; p)]^2 \quad \text{(Equation 3)}$$

Belysa® software uses the Nelder-Mead simplex algorithm to search the parameter space of p for the local minimum solution. Besides standard plate input data, the optimal solution of parameter *p* for the fitted logistic curve model also depends on other heuristic factors including the initial values for *p*, the weighting method, the iteration stopping criteria, etc.

Belysa® software extracts the response $RE_{i,j}$ for each well scan. There are two different choices to formulate the fitting procedure that depend on fitting logistic functions, different weighting schemes w_i, or using different error functions.

Standard 5PL (4PL) Procedure

5PL (4PL) procedure uses the extracted signals or responses, $RE_{i,j}$, for corresponding concentrations, c_i, as the standard data to fit a 5PL (4PL) logistic formulation as in (1) by an iterative procedure with the fitting error and weighting functions in (3) defined as follows.

$$w_i^{-1} = f(x) = \begin{cases} [RE_{i,j}]^2, & RE_{i,j} > \text{Threshold} \\ [mean(RE_{i,j})]^2, & \text{Otherwise} \end{cases} \qquad Error_{i,j} = [RE_{i,j} - f(c_i; p)]^2 \quad \text{(Equation 4)}$$

Note: the weighting factor w_i is set to be the inverse of the square of the individual response $RE_{i,j}$ for well response above the pre-defined threshold, otherwise it is set to be the inverse of the square of the mean response of the ith replicate group.

Robust 5PL (Robust 4PL) Procedure

The Robust 5PL (Robust 4PL) procedure is similar to 5PL (4PL) except for using the absolute difference (L1 distance) between the measured response and the fitted value for the residue error along with the inverse of the median response in a replicate group as the weight in (3).

$$R = \sum_{i=1}^{N} \sum_{j=1}^{m_i} w_i |RE_{i,j} - f(c_i; p)| \quad \text{(Equation 5)}$$
$$w_i^{-1} = median(RE_{i,j})$$

These two different formulation changes are specifically designed to reduce the influence of the outlier data on the residue error.

Back calculation of the concentration from the response

$$[C] = x = f^{-1}(y;p) = c\left(\left(\frac{d-a}{y-a}\right)^{\frac{1}{g}} - 1\right)^{-\frac{1}{b}} \quad \textbf{(Equation 6a)}$$

$$[C] = x = f^{-1}(y;p) = c\left(\left(\frac{a-d}{y-d}\right)^{\frac{1}{g}} - 1\right)^{\frac{1}{b}} \quad \textbf{(Equation 6b)}$$

Equations 6a and 6b above define how concentration [C] can be computed from a given response y or $RE_{i,j}$. Equations 6a and 6b correspond to the logistic curve fit Equations 1a and 1b, respectively. There are several special cases where concentration may be obtained via alternate means:

1. When the response y is equal or larger than the upper asymptote, d, the back calculation of inverse fitted logistic curve by (6) gives positive infinity (+∞). Two nearby standard group points are used to perform linear extrapolation to compute [C].
2. When the response y is equal or smaller than the lower asymptote, a, the back calculation of inverse fitted logistic curve by (6) is not well defined. Two nearby standard group points are used to perform linear extrapolation to compute [C].
3. For the top of curve standard group, or when the response y is very close to the upper asymptote from below, the back-calculated concentration is the value that results in better recovery between the equation (6) and the linear extrapolation by the nearby standard group points.

Other Curve Fitting Methods

Linear regression

The simplest model to analyze empirical data is to fit a linear regression line in the form of Y = a + bX, where X is the explanatory variable (Standards' expected concentration) and Y is the dependent variable (Standards' measured response). The method of weighted least-squares is used to find model parameters a (line y-intercept) and b (line slope) by minimizing the sum of the weighted squares of the vertical difference between each data point and the line.

Cubic Spline

A cubic spline curve is a piecewise cubic curve with continuous second derivative. The word "spline" actually refers to a thin strip of wood or metal that went through the desired data points when curves were designed for ships and planes at one time. A four knots (3 segments) cubic spline curve with weighting is implemented based on Netlib library function, dc2fit. Unlike the other curve types, the fitted cubic spline is represented by three cubic curves formulated by three 4-elements vectors: X knots (concentrations), Y knots (responses), and YP knots (first derivative of responses). For most assay data, cubic spline curve is usually not the model that produces the best recovery and C.V., instead it can be used to check against complicated (e.g. multiphase) or abnormal (wavy) relationship between the response and the concentration in the data.

In Belysa® software, the equation for a cubic spline curve is displayed as follows:

$$k_x = (x_1, x_2, x_3, x_4)$$
$$k_y = (y_1, y_2, y_3, y_4)$$
$$k_{yp} = (yp_1, yp_2, yp_3, yp_4)$$

where kx is the the X (concentration) knot vector, ky is the Y (response) knot vector, and kyp is the Y's first derivative knot vector.

Curve Quality Statistics

Belysa® immunoassay curve fitting software provides several statistics that can be useful to evaluate your standard curve and assay performance. These can be found on the user interface in the **Curve Editor**.

Equation

Each standard curve is defined by its equation. Belysa® software displays the equation for each curve in plain-text format. The equations for logistic curve fits are described in the **Logistic Curve Fitting** section, and equations for other curve fits are described in the **Other Curve Fitting Methods** section.

Weighting

The weighting function affects how the curve fit parameters are calculated and describes how the variance changes in relation to the concentration. For most curve fit options, the weighting function used is $1/y^2$. For Robust curve fit options, the weighting function used is 1 divided by the median value of the replicate group (or the average if 2 or fewer replicates).

LLoQ

The LLoQ, Lower Limit of Quantification (Quantitation), is defined as the standard with the lowest concentration satisfying three conditions:

a. The back-calculated analyte concentration CV is less than or equal to 20% (or custom value specified in **Analysis**)
b. The recovery rate of back-calculated analyte concentration is in [80,120]% (or custom value specified in **Analysis**)
c. All the standards above the LLoQ that also satisfy conditions a and b.

MDD

The MDD (Minimum Detectable Dose) concentration is defined as follows:

$$\text{MDD} = S(\mu(\text{blank response}) \pm C \times \sigma(\text{blank response}))$$

where *S* is the inverse of the standard curve function and *C* is a user-configurable coefficient (see **Settings and Preferences**). The standard deviation of the blank response is subtracted from the mean in the case of competitive assays, otherwise it is added. If no blank wells are present in the standard curve or interpolation fails, a value of "N/A" is reported.

LoD

The LoD (Limit of Detection) is the lowest concentration of an analyte that can be reliably detected from background noise. LoD is defined as:

$$\text{LoD} = L(\mu(\text{blank response}) \pm C \times \sigma(\text{blank response}))$$

where *L* is the inverse of the function defined by the least squares regression line that best fits the first three standard groups (starting from the blank) and *C* is a user-configurable coefficient (see **Settings and Preferences**). The term containing the standard deviation of the blank response is subtracted from the mean in the case of competitive assays, otherwise it is added. If no blank wells are present in the standard curve or interpolation fails, a value of "N/A" is reported.

R-squared

R-squared, also called the coefficient of determination, is a value to measure how much variation of response variable is explained by the fitted model, defined as:

$$\text{R-squared} = 1 - \frac{\sum_i (y_i - f_i)^2}{\sum_i (y_i - \bar{y})^2}$$

$$\text{where } y_i \text{ is response, } f_i \text{ is the fitted value, } \bar{y} = \frac{1}{n}\sum_{i=1}^{n} y_i$$

Usually the closer R-squared value to 1, the better fit the model is to the data, but one needs to combine R-squared value with the residual variance to guard against any bias in the fitting.

Slope

Slope is defined as the slope of the least square regression line using all replicates of non-blank standards except the highest concentration standard. Slope provides a measure of how steeply the response increases with respect to concentration for standards in the low-to-high concentration segment.

Curve Optimization

Automatic best curve fitting and outlier removal (optimization) is a procedure to decide the best curve that fits the standard data with the identified outliers being removed. The following criteria in descending importance order are followed to determine the best curve (see **Curve Quality Statistics** for definitions):

1. Lowest LLoQ point with the smallest concentration
2. Lowest LLoQ total error as the sum of CV and Recovery
3. Highest R-Squared
4. Model with fewest number of parameters

The optimization procedure is a multipass iterative process to find the best fit that meets the above criteria. These following rules are used to end the searching process:

1. Current LLoQ point is already the point before the blank point
2. Number of removed outliers reaches the pre-defined limit

3. No more replicates are eligible to be removed because:
    a. Each replicate group must have at least two replicates
    b. It is not allowed to remove the whole replicate group
    c. At current LLoQ point, it only searches its immediate neighbors which does not give a better outcome

The detection of outliers is based on the assumption that the curve fit residuals are normally distributed, therefore, any replicates with outlying residual errors are potential outliers to be considered for removal. More specifically, the algorithm is as follows:

1. Global outliers are first identified as those replicates that have curve fit residual outlying beyond the Tukey's outlier test, and then removed from the standard data.
2. Potential local outliers are then iteratively inspected point by point starting from current LLoQ point. The decision to remove the identified local outlier is based on if it improves the LLoQ with the local outlying criteria as:
    a. The local outliers value has the largest deviation from the mean that is beyond at least one standard deviation, or
    b. The local outlier is the replicate which produces the largest residual from the least squares linear regression fitting with local groups

Relative Potency

Relative potency assays help provide assurance of the quality and consistency of biopharmaceutical products. Assuming that the Standard and Test materials contain samples with biologically similar activity, the Test sample can be expected to behave like a concentration or dilution of the Standard and parallelism between the Test and Standard concentration-response segments should be present. Log relative potency is determined to be the horizontal displacement between the curves at the same Test and Standard assay response. The key assumption about the Test and Standard curves is also termed as statistical similarity, which assesses the parallelism of standard and test samples in parallel-line or parallel-curve models. Our implementation adopts the parallel-line model for relative potency determination[1].

Given two curves corresponding to two samples, one Standard sample and the other Test sample, here are the steps to calculate the relative potency and its confidence interval.

1. Select the linear range (LR) of dose-response curves as the concentration points that fall between pre-specified START and END groups.
2. Calculate the 95% confidence interval (CI) for the slope difference between the two least squares fitted lines. To measure the statistical similarity between the two curves, use equivalent test procedure to compare the CI against the pre-determined equivalent interval (EI).

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[1]Bortolotto, E & Rousseau, R & Teodorescu, B & Wielant, A & Debauve, G. (2015). Assessing similarity with parallel-line and parallel-curve models: Implementing the USP development/validation approach to a relative potency assay. BioProcess International. 13.

3. Establish statistical similarity by computing the slope ratio between the Standard and the Test sample as the parallelism.
4. Finally, the relative potency is calculated using the Standard slope as the common slope for both least squares fitted lines:

   RP = exp(Test_intercept – Standard_intercept)/Standard_slope)
5. The 95% confidence interval (CI) of RP is estimated by using Fieller's Theorem[1]
6. RP value of 1 is regarded as the completely equivalency between the Standard and the Test samples; RP value of less than one indicates the Standard sample is more potent than the Test sample, or vice versa.

[1]Buonaccorsi, J. P. (2014). Fieller's Theorem. In Wiley StatsRef: Statistics Reference Online (eds N. Balakrishnan, T. Colton, B. Everitt, W. Piegorsch, F. Ruggeri and J. L. Teugels). doi:10.1002/9781118445112.stat05858

Keyboard Shortcuts

General

Shortcut	Description
Ctrl+O	Open a file
Ctrl+S	Save current file
Ctrl+W	Close current file
Ctrl+Q	Quit application
Ctrl+1	Switch to **Results Tab**
Ctrl+2	Switch to **Raw Data Tab**
Ctrl+3	Switch to **Excluded Wells Tab**
Ctrl+4	Switch to **Test Info Tab**
F1	Show help
Alt+1	Toggle left panel
Alt+2	Toggle right panel
Ctrl+E	Switch to single-analyte view
Ctrl+R	Switch to multi-analyte view
Ctrl+K	Change filter value
Ctrl+Shift+P	Jump to first plate
Ctrl+Shift+E	Jump to first experiment
Ctrl+Z	Undo
Ctrl+Y	Redo

Plate Map

Shortcut	Description
Ctrl+D	Create standard group from selected wells
Ctrl+F	Create control group from selected wells
Ctrl+G	Create unknown group from selected wells
Ctrl+X	Cut selected wells
Ctrl+C	Copy selected wells
Ctrl+V	Paste selected wells

Experiment Results

Shortcut	Description
Alt+3	Show curve full screen
Alt+4	Split view between curve and table
Alt+5	Show table full screen
+	Zoom in on chart
-	Reset chart zoom
O	Exclude/include selected point on chart
Alt+[Drag]	Zoom in on box in chart