

Part III: MARS Data Analysis

Version 5.10

BMG LABTECH

The Microplate Reader Company

This manual was designed to guide users through the software features.
Although these instructions were carefully written and checked, we cannot accept responsibility for problems encountered when using this manual. Suggestions for improving this manual will be gratefully accepted.
BMG LABTECH reserves the right to change or update this manual at any time. The Revision-Number is stated at the bottom of every page.

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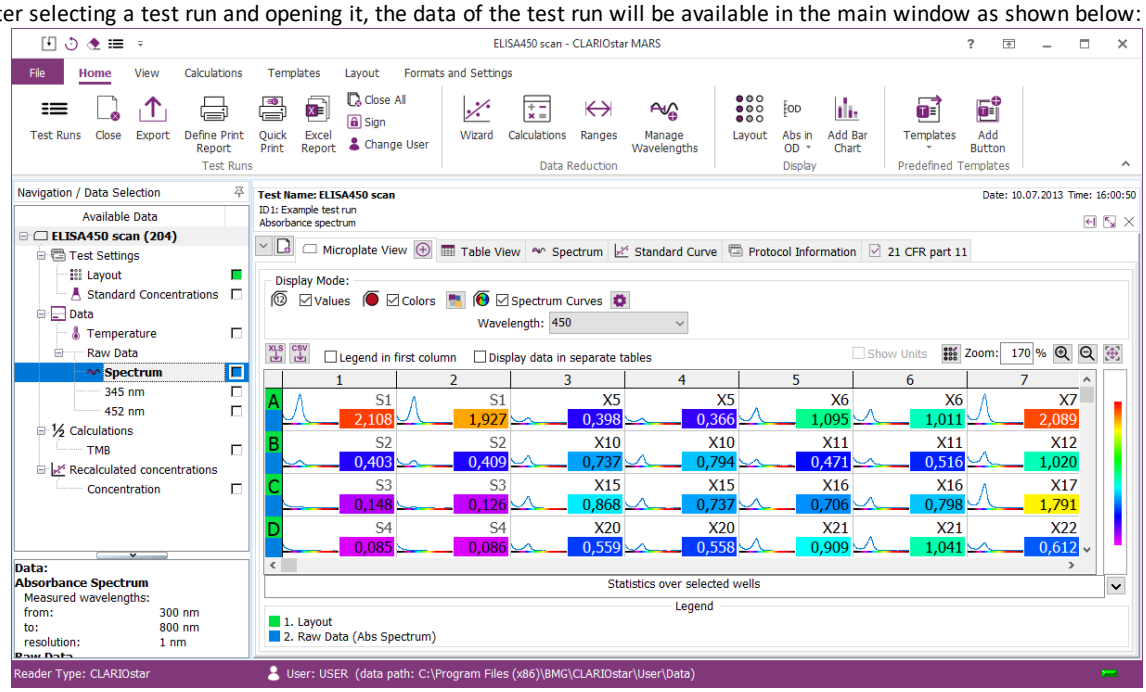
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1 Overview

1.1 Main Screen of MARS

After starting MARS, either from the control software or directly, you will see a window with all available test runs (see chapter 2: *Manage Test Runs*) of the logged in user (chapter 1.2 *Login*).
After selecting a test run and opening it, the data of the test run will be available in the main window as shown below:



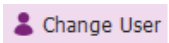
The window is divided in two areas: The navigation tree on the left side and the working area on the right side. The working area displays your data in different ways, providing several pages which you can access by clicking on the relevant tabs on the top of the working area, e.g. Microplate View (the default page), Table View, Spectrum, Standard Curve, etc. How data is displayed in each page is explained in detail in the chapter *Explore Data*.
The ribbon with its task-oriented tabs and groups gains you access to all available task.
The status bar at the bottom of the screen shows the reader series used to generate the data and the details of the user logged in with the data path showing where the data is stored. The status icon on the right side of the status bar shows if the application is busy (red) or ready to accept user activities (green).
To check the version numbers of the software and the modules used, click the File tab and then click Info.
These version numbers are needed when completing a technical support request.

1.2 Login

1.2.1 Login at Start Up

When starting MARS from the voyager software you do not need to select a login user again. The software automatically starts with the same user as used in the voyager software.
If a user with the limited rights is used, some of the functions are not available in MARS.
More details about th elogin functions of the window can be found in the help of the voyager software.

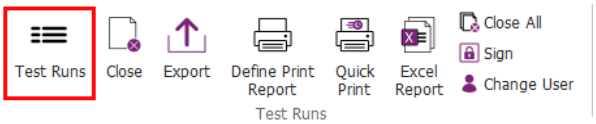
1.2.2 Changing the User

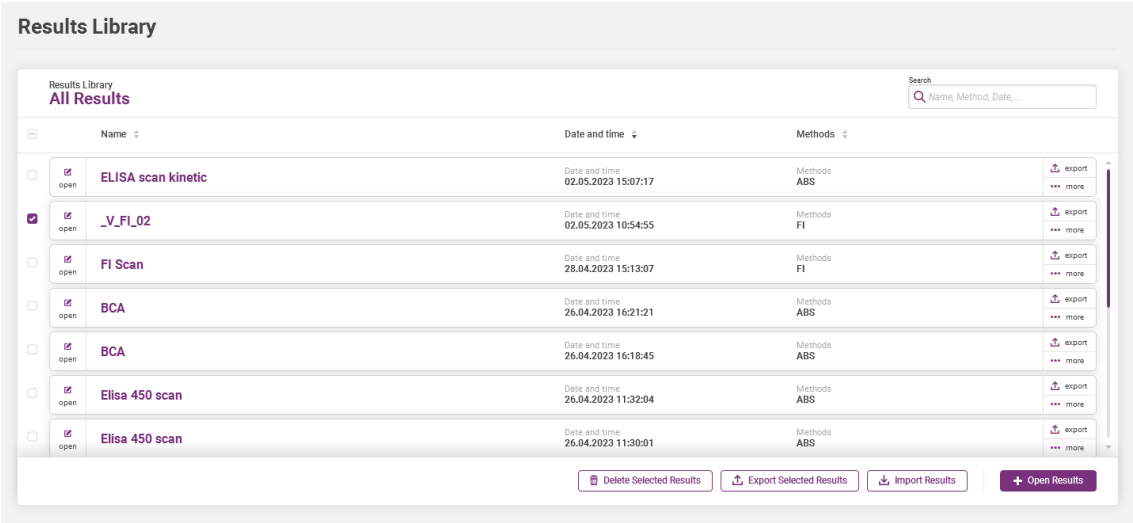
To change to a new user account in MARS you can either click on the status bar showing the current user or by clicking *Change User* 

in the *Test Runs* group on the *Home* tab of the ribbon..
The login window of the voyager software appears, and you can log into the desired user.

2 Manage Test runs

The *Manage Test Runs* window shows you all available test runs of the current user.
You can reach the *Manage Test Runs* window by clicking *Test Runs*in the *Test Runs* group on the *Home* tab of the ribbon or click *Test Runs* in the *File* menu.





Open a single result in the list by clicking on the open icon at the left side of the test run or click the Open Results buttons on the end of the list to open all selected results at once.
Find more details about the results library in the voyager manual.

2.1 Import / Export Test Runs

2.1.1 Import Test Runs

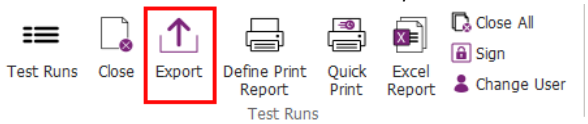
Using the import function from voyager, you can import results. Open the voyager result library and click on the *Import Results*button at the end of the list. A standard file window opens where you can select a file with the test runs you want to import into the current users data.

2.1.2 Export Test Runs

You can export one or more test runs as one file to exchange it between users.

> **Note:** If making a support request regarding the test run(s) it is useful to include exported data that demonstrates the problems experienced as this can help in finding and solving the problem.

If you want to export only the currently opened and active test run (the one which is selected in the navigation tree), click *Export* in the *Test Runs* group on the *Home* tab of the ribbon or click the *Export* item in the *File* menu:



A standard file window will appear where a file destination can be selected to save the exported test runs. Define a name for the file (or accept the proposed one) and press the *Save* button. The extension of the file name will be added automatically and is always . Please do not change the extension manually as this will mean that the created file will not be recognized by the software when trying to re-import the file.

2.2 Test Run Settings

Each test run comes with its own list of settings. These settings store most of the display parameters and the performed calculations for that test run as well as settings for print and Excel reports. The first time a test run is opened, default settings are assigned to the test run. Checks are performed and the default settings are determined using the first condition that matches the defined criteria as below:

- If the test run has already its own setting file, it will be used (e.g. from earlier opening the test run).
- If the test protocol which created the test run has a template (see chapter 5: *Using Templates*), the setting will be generated according to that template.
- If there is a default template for the type of measurement method (see chapter 5: *Using Templates*) the setting will be generated according to that template.
- If none of the above conditions are met, a new standard setting file will be generated.

Important settings for a test run could be to show:

- All performed calculations (like blank correction, replicate statistics, standard calculations...). If the test run contains a measured spectrum all manually defined wavelengths are stored in the setting file.
- Defined Variables (see chapter 4.2.1: *Define and Use Variables*)
- The selected nodes in the navigation tree and the content filter tree (see chapter 3.1: *Navigation Tree* and 3.2: *Content Filter Tree* to read more details about selecting nodes to display data).
- The displayed View (Microplate View / Table View / Signal Curve / Spectrum Curve ...) and the special settings of the view.
- The defined print report layout and pages
- A changed microplate layout
- The defined Excel report settings and pages

If you change settings of a test run and you close the test run, you will be asked if you want to save these settings. If you confirm these settings, you will be able to continue your work after reopening the test run at the same point as you closed it.
If you changed the layout of a test run (see chapter 6.1: *Changing Layout*) the changed layout will also be saved then.

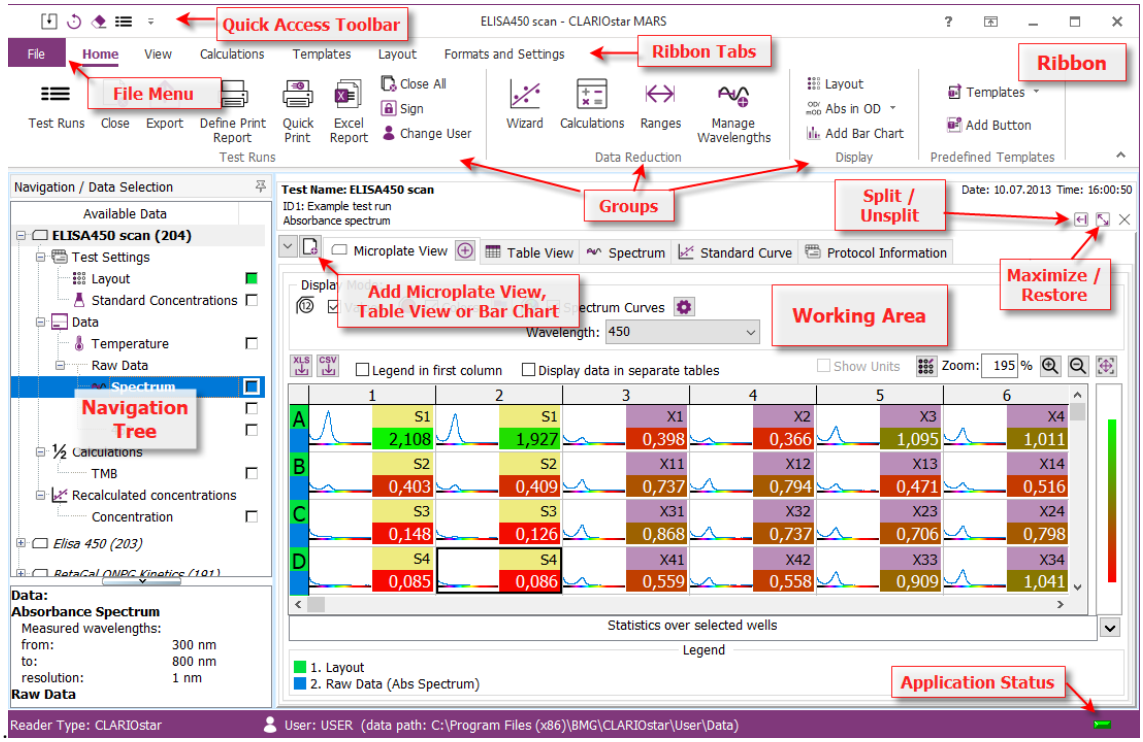
Note: Read Only users cannot change settings or the layout of a test run.

To remove all performed settings (even an assigned template) of an opened test run, select in the quick access toolbar. The test run settings will be deleted, and the format will be reconfigured to default settings using rule 3 of the list above.

To reset settings without applying default settings use the clear button ( ) in the quick access toolbar.
Resetting settings of a test run does not reset a changed layout. To reset both, settings and the layout, select the menu entry *Reset Layout* in the *Test Run Layout* group on the *Layout* tab of the ribbon.
In addition you can reset settings of closed results, if you select the more menu entry *Reset Test Run Settings and Changed Layout* on the pop-up menu of the Manage Test Runs window after you've selected the test runs in the list.
If you export or import a test run, the settings (including a changed layout) will be exported / imported as well.

3 Explore Data

After opening one or more test runs you can explore the data using the commands on the different ribbon tabs, the *Quick Access Toolbar* and the *File* menu:



The *ribbon* with its tabs, the *Quick Access Toolbar* and the commands in the *File* Menu gains you access to the complete functionality of the software. All the available data (raw data and created data) are listed in the navigation tree on the left side of the screen. After selecting one or more data nodes in the navigation tree, you will see the data in the working area. Use the *Maximize* and *Restore* button on the top right area of the working area to maximize the displayed working area or to restore a maximized working area to the normal size. If the working area is maximized, the navigation tree will be collapsed and the test run information on top of the page disappears. The navigation tree can be manually reopened again.

There can be many different pages in the working area where data can be inspected using different formats. Data can be obtained using the following tabs (the list is not complete and depends on the available data and calculations):



If a bar chart was added to the test run, the tab Bar Chart will appear after the *Table View* tab.

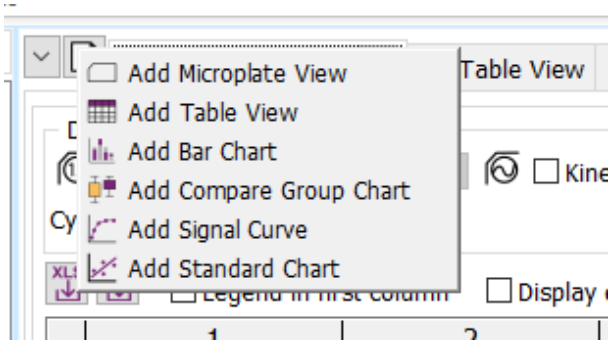
If the data has well scanning data and areas inside the well where defined, the tab Area Statistics will appear after the *Table View* tab or *Bar Char* tab (if available).

If the data has a measured spectrum the tab Spectrum will also appear. A notifications tab (Measurement notifications) appears, if one or more warnings occurred during the measurement of the test run.

If the data comes from a BMG LVis Micro Drop plate, *Microplate View* tab is replaced by the *LVis Plate View* tab LVis Plate View .

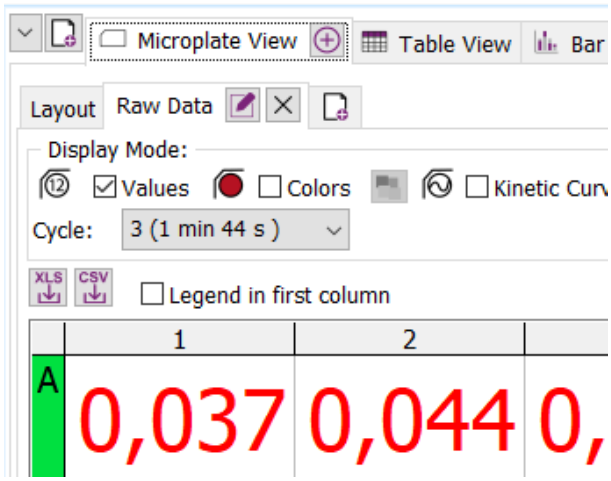
For most of the views, there can be more than one tab/page. You can add a further view for one of these views with the add view button:

After clicking this button, a small menu opens, where you can select the type of the view you want to add (the list with the available pages in the screen shot below is not complete).



In addition, you can add a further view by clicking the add button on the tab ().

After adding a further view, a new row with tabs for each view appears. You can select the desired view by clicking on the according tab.



The name of the tab can be changed if you click on the button beside the selected tab: 
An added view can be deleted with the X button. For some of the views, the last view cannot be deleted.

Clicking the () button on the right side of the tabs will add a further view.
You also can split the working area into two parts to display two different pages side by side. In addition, the right side of the split-view can be undocked from the main window and moved to any position on the screen. To split the working area, press the split button: 
To go back to the unsplitted view, press the unsplit button: 
Read more details about the possibility to display data in a split view here: Split the view to display data side by side.

To change the visible page, click on the tab of the page you want to open. Only pages with available data will have a tab, e.g. the tab for the Standard Curve will only appear when a standard calculation has been performed.
Each page is explained in detail on the according help page:

Microplate View / LVis Plate View

Data is displayed in a grid according to the microplate format. For BMG LVis Micro Drop measurements, the grid has two columns and eight rows.

Table View

Data is displayed in an Excel-like table format.

Bar Chart

Displays a chart with bars (2D or 3D) of the selected wells or of the complete microplate.

Area Statistics

Displays statistic data of defined areas inside wells of a well scanning test run.

Spectrum Curve

Displays a chart with the spectral curve(s) of the selected well(s)

Signal Curve

Displays a chart with the kinetic data of the selected well(s)

Standard Curve

The standards data are shown plotted in graphs with fitted curve(s).

Enzyme Kinetic Fit(s)

The results (e.g. saturation curve) of enzyme kinetic calculations are shown plotted in graphs with fitted curve(s) (Michaelis-Menten, Lineweaver-Burk, ...)

Binding Kinetics

The results of a kinetic rate equation are shown plotted in a graph.

Compare Groups

The results of a compare data group comparison like Anova or T-Test are shown plotted in a bar graph, scatter- or jitter plot, beeswarm or violine plot.

Quantal Response Fit Curve

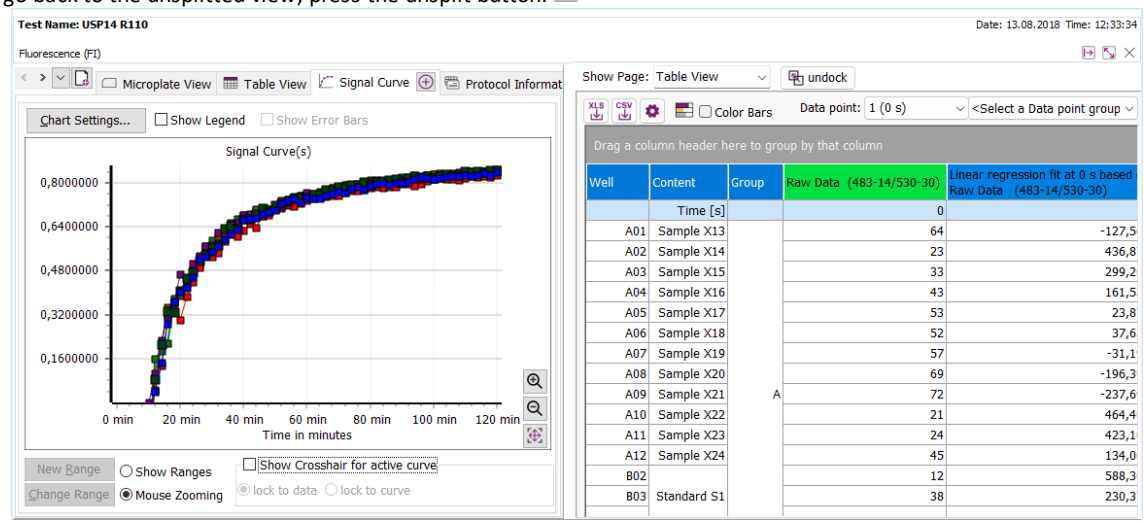
The results of a quantal response calculation (Logit, Probit, Spearman-Kärber...) are shown plotted as chart series with data points.

Protocol Information

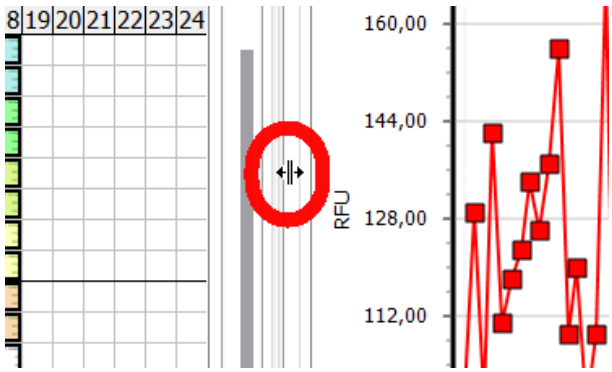
Displays the settings used in the test run protocol.

3.1 Split view to display data side by side

To go back to the unsplitted view, press the unsplit button: 



In the right split window, you can change between the displayed page with the *Show Page* drop down control. Not all pages are available on the right side, but you can always combine all available data pages to be displayed side by side.
If you select a page to be displayed on the right side, which is already displayed on the left side, the page on the left side will be changed to the microplate page.
If you select a page to be displayed on the left side, which is already displayed on the right side, the split window will close.
You can change the size of the right page with the slider control between the two pages. Move the mouse over the control until the cursor changes to the size cursor, click the left mouse button and move the slider in the desired direction:

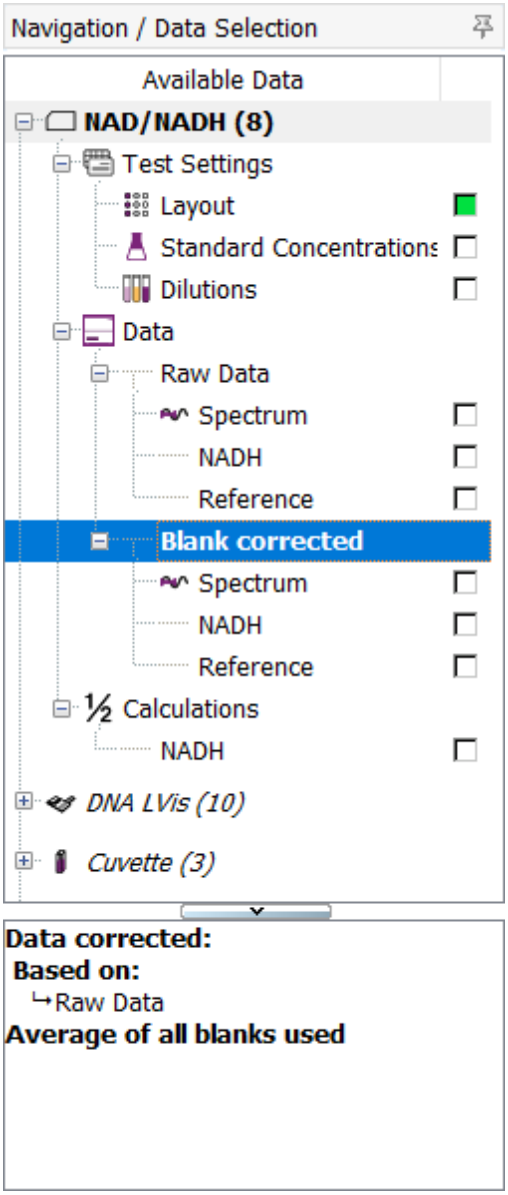


If you press the undock button, the right side of the split view will be moved into a separate window, that can be moved and resized to any position and size on the screen. Closing the window will also close the split view. Press the dock button on the window, to move it back into the split area of the main window.

> **Note:** The navigation tree on the left side off the main window is connected to the page displayed on the left side of the split view. You cannot change the selected data on the page of the right side of the split view, if the page is displayed on the right side. To change the displayed data, move it back to the left side, change the data and then move it back to the right side.

3.2 Navigation Tree

The navigation tree is the main tool used to select the data displayed in the working area (see chapter 3: *Explore Your Data*).



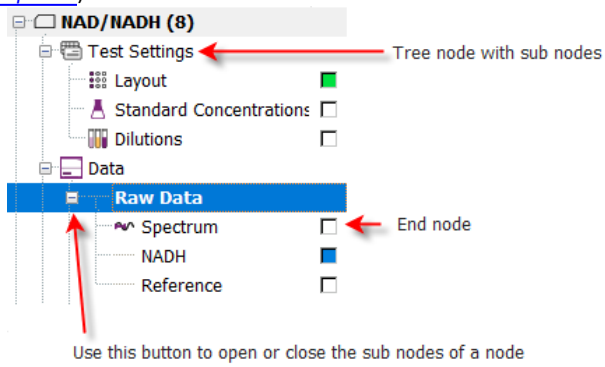
It contains two sections.
The upper section displays the tree with all opened test runs and sub nodes. Each test run has further sub nodes showing all available data (measured or calculated). If more than one test run is opened, the last opened test run is always shown at the top of the tree. Read more about this tree in the section 3.1.1: *Using the Tree*.

The border can be changed to alter the partitioning of the sections. (drag using the mouse to its new position)

The lower section contains two parts, the first containing a detail window, which displays detailed information about the selected node in the tree above (the selected node is indicated by a bold caption and a surrounding dotted border).
The second part shows a further selection tree, called a content filter tree. The data shown here is dependent upon the selected page in the working area.

3.2.1 Using the Tree

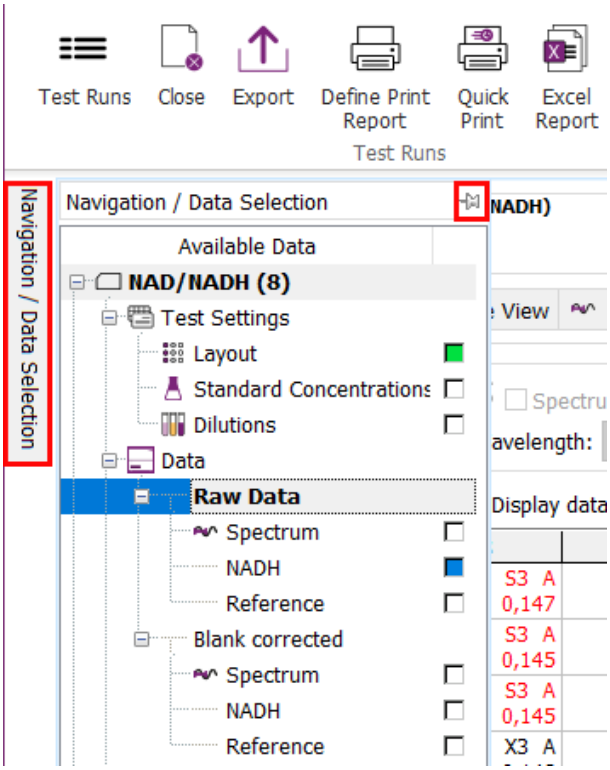
A tree is a hierarchical structure with a set of linked nodes. Each node in the tree has zero or more sub nodes. If there are no sub-nodes it represents data that can be displayed in the working area. The top node in the tree represents an opened test run. Each opened test run is represented by its own tree. The number of nodes and the kind of data the end nodes represent depends on the kind of test run (Layout, measurement mode...) and the calculations defined by the user or a template (see chapter 5: *Using Templates*).



The tree of the current test run is expanded (all sub nodes are visible) and the caption of the topmost node is shown in bold.

Common Functions

You can expand or close each node in a tree that contains sub nodes by clicking on the button (⊞ for expand, ⊟ for close) before the node. If you select the tree of a different test run, then this test run becomes the current test run and will be expanded automatically. The tree of the previous current test run will be closed (but not removed).
The header of the navigation area has a little pin on the right border. You can use this pin to change between auto hide or fix to the area by clicking on the pin:



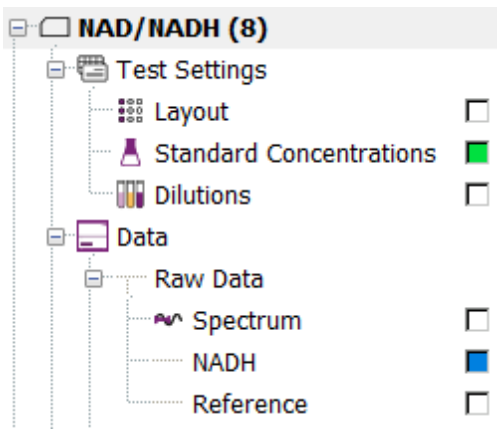
If the pin is fixed (📌) then the area is fixed and can only change in width by dragging the separating border using the mouse between the navigation tree and the working area.

> **Note:** There is a maximum size of the navigation area when fixed. The size depends on the size of the working area, the size of the main window and the resolution of your screen.

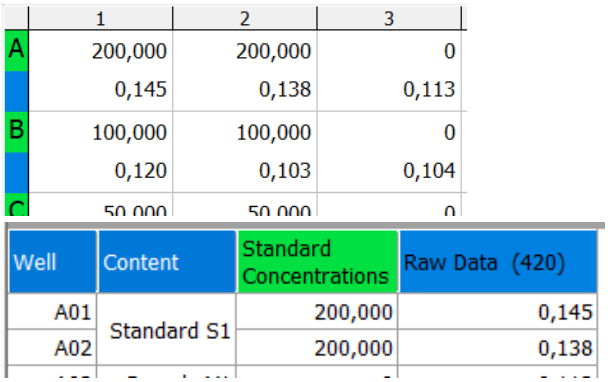
If the pin is unfixed (like in the image on the left) the area will automatically be closed if you move the mouse out of this area. Moving the mouse to the little navigation tab on the left side of the application window (this is only displayed when the pin is not fixed), the navigation tree will appear again.

In auto hide mode, there is more room left for the working area as the tree will be displayed above the working area if it appears.

The whole navigation tree can also be dragged by clicking on its header whilst holding the left mouse button down to move it away from its position. The navigation tree will then become a separate window that can be moved and sized in any direction. It is possible to close the window from here. To reopen it again press Ctrl+N or click *Show Navigation Tree* in the *Navigation* group on the *View* tab of the ribbon.
Selecting Nodes
To display your data in the working area you have to select the corresponding node in the navigation tree and - if you display a chart (like a bar chart view, the signal curve view, the standard curve view, the enzyme kinetic fit(s) view the binding kinetics fit view or the spectrum curve view) also the content in the content filter tree. Depending on the active page in the working area, the navigation tree can have two different modes to select nodes.
Selecting data for microplate or table view
If the microplate view or the table view is active, the navigation tree has a column, called *Row*. Behind each selectable end node, the column *Row* contains a little box. If a node is selected, its box is colored.



Each color of the selected nodes represents a data row in the microplate view or a data column in the table view:



	1	2	3
A	200,000	200,000	0
	0,145	0,138	0,113
B	100,000	100,000	0
	0,120	0,103	0,104
C	50,000	50,000	0

Well	Content	Standard Concentrations	Raw Data (420)
A01	Standard S1	200,000	0,145
A02		200,000	0,138

To select a node in this mode

- click with the mouse into the box behind the node you want to select or
- use the menu item *Select/Deselected Node* either in the *Navigation* group on the *View* tab or in the pop-up menu of the tree.

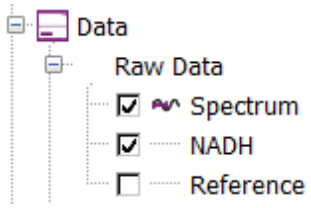
A selected node will now be deselected (the box is white then) and a deselected box will be selected by using the next free available color. That means, if you have already used green and blue for other nodes, the red color will be used for this node. If no colors are left, the node will not be selected. You can select one row and deselect all selected nodes at once. Therefor you have to click on the row you want to select (it must be an unselected row) and select the *Select row (Deselect all others)* menu item in the pop-up menu (opened with a click on the right mouse button). If you want to deselect all nodes (without selecting a new row), click on a selected node, open the pop-up menu and select the menu item *Deselect all rows*. You can change the number of selectable rows up to 10. How to do this, you can read in the section MARS Settings / Navigation Tree Settings

Changing the position of displayed rows

In the microplate view or the table view you can change the row or column positions of the displayed data with the mouse. You can do this either in the displayed legend below the tables by clicking on the according data entry in the legend and move the entry to the new position (in the legend). Alternatively, you can select the row you want to move in the microplate view by clicking on one of the according color in the first column of the microplate table and moving it to the new position. In the table view you can move the column by clicking on its header and moving it to the new position (see chapter 2.2.3: *Change the Position of a Column*).

Selecting data for curve charts

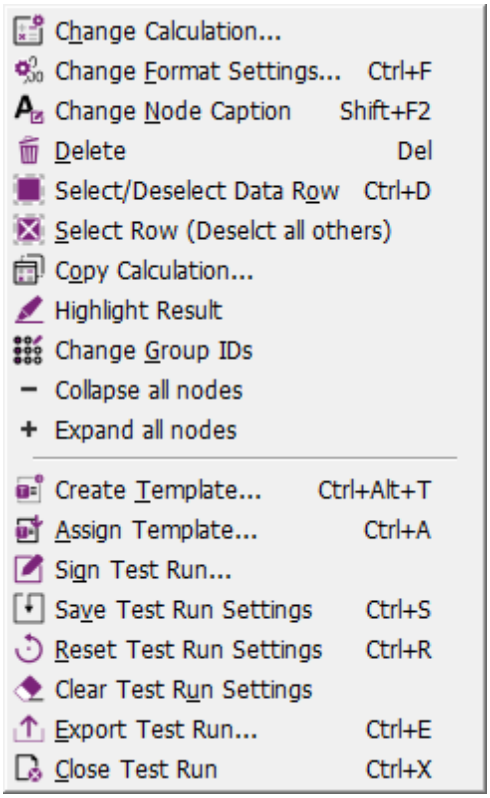
If a chart is active such as the signal curve view, spectrum curve view, standard curve view , enzyme kinetic fit curve or binding kinetics fit curve, the column *Row* in the tree will vanish and a small check box will appear before each selectable node. The data you wish to be displayed can be selected or deselected by clicking on the check boxes of the available nodes.



Note: Only nodes that can be selected for the active chart have a check box, e.g. if a test run has kinetic data and the signal curve view is active, only nodes with kinetic data will have a check box.

Tree pop-up Menu

If you move the mouse in the navigation tree area and press the right mouse button, a pop-up menu opens which gives access to important functions linked to test runs and tree nodes:



Opens a window to change calculation parameters (only available for calculation nodes).

Opens a window to change the number format setting of the selected data.

Change the displayed name/title of the node (only available for nodes referring to performed calculations)

Deletes the node (if not the basis of a calculation)

Selects or deselects a node (if selectable). Alternatively, you can double click on the node.

Deselects all selected nodes at once (if opened on a selected node) / Select node and deselect all other rows (if opened on a not selected node)

Copy calculation (create a second node with the a new calculation but with the same settings - only available for calculation nodes).

Highlights the Result Node in the display area (Microplate View, Table View)

Opens a window to change the group IDs (only available if the layout contains groups)

Collapse (fold) all open nodes at once.

Expand (unfold) all nodes at once.

Creates a template with the current settings of the current test run.

Assigns a template to the current test run and overwrites its settings.

Signs the current test run (Note: not yet available for MARS together

with voyager)

Saves the test run settings.

Resets the settings of the test run to the default values

Removes all settings of the test run.

Exports the current test run (see chapter 2.3.2: *Export Test Runs*)

Closes the current test run

All the functions are also available in the ribbon.

Note: The pop-up menu shows only these functions which are independent from the selected node or applicable on the selected node.

Change the caption of a generated node

The captions of the nodes in the tree are generated by the program. If the node represents data or the result of a calculation, you can change the caption of the node.

Therefore, you need to select the node in the tree and open the tree pop-up menu by pressing the right mouse button. Select *Change Node Caption* in the menu and the representation of the node changes into edit mode and the desired caption can be entered.

If an empty caption was entered, the text *<empty>* will be used automatically as an empty value is not allowed.

You can change at any time back to the original node caption. Therefore, you have to select the node again, open the pop-up menu and select *Restore Original Node Caption*.

Note: If you change the caption of a node in a dual channel or multiple chromatic measurement, all nodes representing data of the same channel/chromatic in all following nodes will be changed as well.

Summary of all possible nodes

Each test run consists of static nodes which are generated when a test run is opened. These are the nodes for the test settings and the nodes for the raw data. They cannot be removed. The test setting nodes represents data that was defined in the control software.

Default templates perform some calculations like blank corrections and replicate statistics. The other nodes are created either by the user manually or by using a template. See chapter 5: *Using Templates*.

, *test run name*	Topmost nodes. Represents the test run. The displayed icon depends on the type of test run (microplate, LVis plate) The name is the name of the test run followed by its internal test run id. If Detailed Captions is selected (*View* group on the ribbon, section *Navigation*), the plate IDs of the test run are also part of the name.
Test Settings	Parent node for all test settings sub nodes
Layout	Select this node to display the layout in the microplate view
Group IDs	Represents defined group IDs. See chapter 6.1: Changing Layout.
Standard Concentrcuvetteations	Represents the concentration values of the standards or controls (appears only if standards where defined in the layout or controls with a standard concentration was defined. See chapter 6.1.3: *Changing Concentrations, Dilutions and Sample IDs*).
Dilutions	Represents the dilution factor for the wells (appears only if dilution factor > 1 was defined)
Sample IDs	Represents the sample IDs of the samples (appears only if sample IDs where defined for the samples)
Injections	Parent node for all injection volume nodes (appears only if there have been injections in the test run)
Volume *n*	Sub nodes of Injections, for each defined injection volume (*n* is the number of the volume)
Data	Parent node for the raw data and the corrected raw data

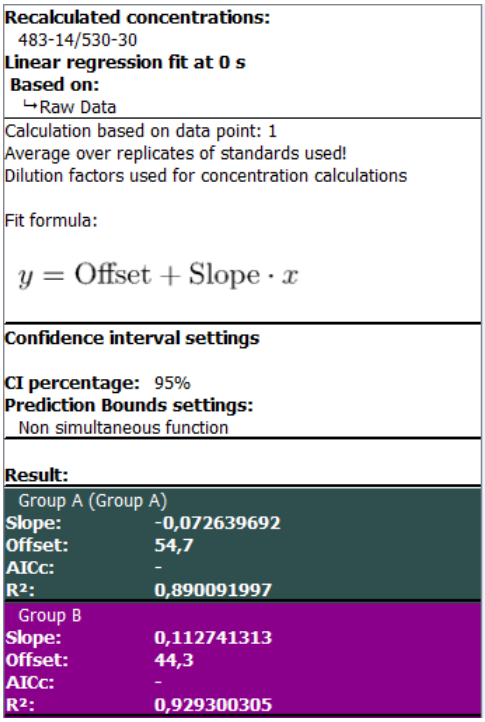
Temperature	Represents the temperature during the test run measurement. (Appears only if temperature monitoring or incubation was set in the control software)
Oxygen %	Represents the O^2concentration during the test run measurement. (Appears only if an ACU with O^2 monitoring was used during the measurement)
Carbon dioxide %	Represents the CO^2concentration during the test run measurement. (Appears only if an ACU with CO^2 monitoring was used during the measurement)
Raw Data	Represents the raw data. If the test run has only one measured wavelength, the used Filter(s)/Wavelength is displayed in brackets behind the node name. If the test run is a well scan test run the hint Well scan is added in brackets behind the node name.
Wavelength: *lambda/filter*	This is always an end node and a possible sub node of each data node of each data node (raw data or calculated data). Appears if the test run contains more than one measured wavelength, or if it is a spectrum test run and you added a wavelength to the test run (see chapter 3.12.1: *Adding, Changing or Removing Wavelengths*). The used filter(s) or the added lambda value of the wavelength is also part of the nodes name. If the test run was measured with the PHERAstar series, the number of the used optic module is displayed in brackets after the wavelength information.
parallel	Only for fluorescence polarization measurements. Represents the parallel measurement channel
perpendicular	Only for fluorescence polarization measurements. Represents the perpendicular measurement channel
Spectrum	Appears only for test runs with a measured spectrum. Represents the spectrum curves.
Spectrum calculations Sum Maximum Minimum Local maxima Local minima Inflection points Average Slope Maximum of slope	Represents the calculated data taken from a spectrum range. The range used for the calculation is displayed behind the calculation method (e.g. Sum of Range 1). See chapter 4.18: *Spectrum Calculations*. Performed calculations of the types *Local maxima*, *Local minima* and *Inflection points* can have more than one sub nodes, for each calculated minima, maxima or inflection point.
Blank corrected	Represents the blank corrected raw data (if the layout contains blanks)
Blank corrected (all groups) Neg. control correction Neg. control correction (all groups)	Represents the data of performed corrections (see chapter 4.4: *Corrections*).
FP calculations Polarization Anisotropy Intensity	Represents the calculations available for fluorescence polarization measurements (see chapter 4.6.1: *FP Calculations*).
TR Fret Calculations Ratio DeltaF	Represents the ratio and the DeltaF calculation for time resolved fluorescence (dual emission) test runs (see chapter 4.6.2: *TR-FRET Calculations*).
Statistics	Represents the calculated statistical data for replicates (see

σ Well statistics Average Standard deviation Standard deviation n % CV % CV n Minimum Maximum Median Sum No. of Values	chapter 4.5: Statistics), layout groups or for selected wells (see chapter 4.19: *Statistic over Wells*).
Well Scan statistics Well Scan Areas statistics	Represents the calculated statistical data for scan-points in a well of a well scan test run (see chapter 4.20: *Well Scan statistics*). The Areas statistics represents the statistical data for defined areas in a well of a well scan test run.
Curve Smoothing Moving average - Width: *b* (Range *n)*	Represents the smoothed signal curve for a defined range, using the moving average method. The moving width of the method is *b*. The number of the used range is *n*. See chapter 4.7: *Curve Smoothing*
Kinetic fit calculations Linear regression fit Logarithmic fit Exponential fit Double logarithmic fit 4/5-Parameter fit Segmental regression fit 2nd Polynomial fit 3rd Polynomial fit Hyperbola fit of Range *n*	Represents a curve fit calculation based on the range *n* of the signal curve. See chapter 4.9: *Kinetic Fit Calculations*.
Kinetic fit result parameters *parameter* of *fit method* (Range *n*)	Represents the parameter calculation of a curve fit calculation based on the range *n* of the signal curve. The calculated *parameter* and the used *fit method* are part of the node name. See chapter 4.9: *Kinetic Fit Calculations*.
Kinetic calculations Slope Time to threshold Time to max Sum Average Maximum Minimum Standard deviation Standard deviation n % CV % CV n Maximum of slope Time to max slope Median	Represents the calculated data taken from a kinetic range. The range used for the calculation is displayed behind the calculation method (e.g. Slope of Range 1). See chapter 4.8: *Kinetic Calculations*.
Standards calculations Linear regression fit 4/5-Parameter fit Cubic spline fit Point to point fit Segmental regression fit	Represents the recalculated concentrations taken from the standard curve fit results (see chapter 4.10: *Standard Calculation / Curve Fitting*

2nd polynomial fit 3nd polynomial fit Hyperbola fit	
Concentration calculations Difference Ratio known/calc Ratio calc/known Percentage deviation	Represents the result of performed calculations based on known and recalculated concentrations (only available if a standard fit was performed). See chapter 4.11: *Concentration Calculations*.
Calculations data 1 / data 2 data 1 - data 2 data 1 * data 2 data 1 + data 2	Represents the results of performed calculations as displayed (*, /, +, or -). *Data 1* and *data 2* will be replaced by the input data selected for the calculation (see chapter 4.12: *Data Calculations*).
Validations good / bad good / bad / unknown	Represents the result of a performed validation (see chapter 4.13: *Validations*).
Assay Quality Z' based on *Cnt1* and *Cnt2* Signal to blank (*Cnt)* Signal to noise (*Cnt)* Percentage calculation	Represents the result of a performed assay quality calculation (Z', signal to blank, signal to noise and percentage calculation). *Cnt*, *Cnt1* and *Cnt2* will be replaced by the selected content on which the calculation is based on (for Signal to blank *Cnt* is the used blank, for Signal to noise, *Cnt* is the used noise see chapter 4.14: *Assay Quality*)
User Defined Formula <entered formula name>	Represents the result of a performed calculation based on a entered well based formula (see chapter 4.15: *User Defined Formula*)
Enzyme Kinetic Michaelis-Menten fit Lineweaver-Burk fit Eadie-Hofstee fit Scatchard fit Hanes-Woolf fit	Represents the result of a performed enzyme kinetic calculation (see chapter 4.16: *Enzyme Kinetic Calculation*).
Curve Scaling Scaled curve (Range *n*)	Represents the result of a performed curve scaling calculation (see chapter 4.17: *Curve Scaling*).
Binding Kinetics Kinetic rate equation	Represents the result of a performed kinetic rate equation (see chapter 4.25: *Binding Kinetics Calculation*).
Curve Analysis Area under Curve Differentiation Integration	Represents the result of a performed curve analysis equation (see chapter 4.24: *Curve Analysis*).
Parallel Line Analysis Slope-ratio fit Parallel line fit 4/5 parameter	Represents the result of a performed parallel line analysis equation (see Parallel Line Analysis calculationchapter 4.17: *Parallel Line Analysis*)
Compare data groups unpaired t-Test One-way ANOVA paired t-Test repeated measures one-way ANOVA	Represents the result of a performed data comparison (see Compare Data Groups calculationchapter 4.??: *Compare Data Groups*)

3.2.2 Detailed Information on the Selected Node

The detail window is shown under the navigation tree, if neither the signal curve view nor the spectrum curve view or the bar chart view is active. In this case, the area contains the content filter tree.
The detail window displays detailed information to a selected node in the navigation tree (if available).

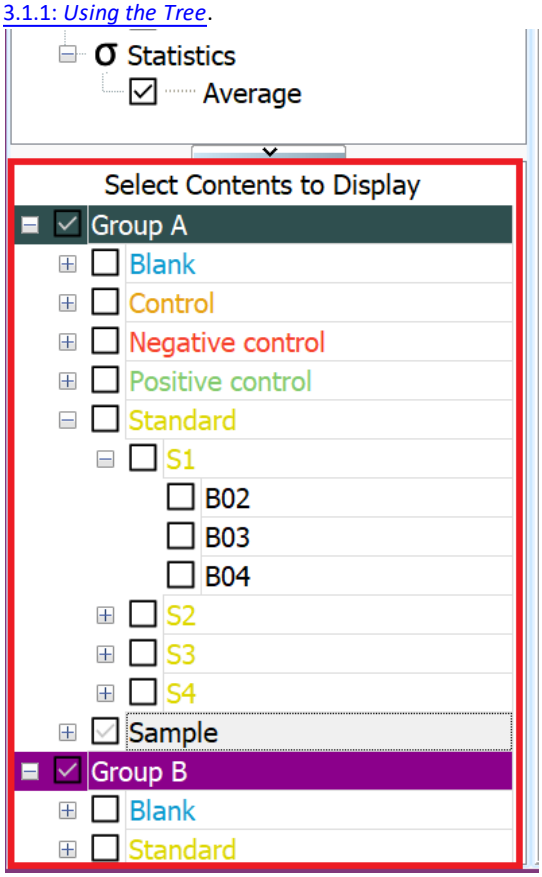


The type of data displayed depends on the represented data of the selected node.
For each performed calculation, it contains the workflow for that calculation from the last input data down to the first input data which leads to the result..
In case of the linear fit in the screen shot shown below, tthe linear fit was performed on a kinetic calculation (Average) of a full range.
The kinetic calculation was performed based on the raw data

Following the workflow are the parameters of the calculation.
In addition to the standard curve fit data, it also displays the performed fit formula and the result fit parameter.

3.3 Content Filter Tree

The content filter tree is part of the navigation area containing the navigation tree. Read more about trees in the section 3.1.1: *Using the Tree*.



The content filter tree replaces the area where the detailed window is shown if you change to either the signal curve page (for kinetic test runs) or the spectrum curve page (for absorbance spectrum test runs only) or if you open a bar chart page in the working area.
The navigation tree is used to select the data you want to view (i.e. blank corrected raw data). The content filter tree lets you select the wells you want to display in the graph of the working area.
If you've already selected wells in the microplate view, the LVis view these wells are also selected in the content filter tree when it appears.
In addition, the content filter tree allows you to select groups of wells, for example replicates or a series of wells that have received the same treatment. The tree is organized hierarchically with the end nodes representing the wells. The parent nodes of the end nodes represent the replicates of wells (only applicable where replicates were defined in the layout). The next level groups all elements of the same content type (i.e. all samples or all standards). The highest level (topmost nodes) represent the groups (Only if groups are defined), otherwise the root node is visible, representing all wells.

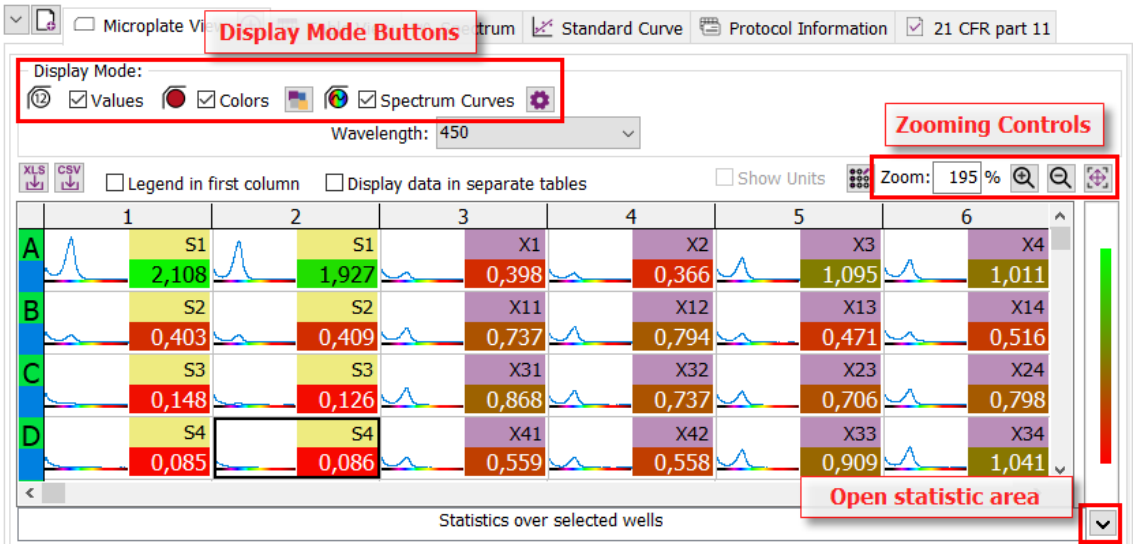
If the layout contains layout groups, you can click with the mouse on the group node to add or change group ids.

Clicking on the check box shown before the node representing a well or group of wells will select them for use. The highlighted well (all wells representing samples of group A in the screen shot above) in the content tree corresponds to the selected curve in either the signal curve or the spectrum curve charts. Changing the selected curve in the chart will also highlight the corresponding well in the tree and will expand the parent nodes.
If the tree is too large to fit in the area, a scroll bar is displayed on the right side of the tree to change the visible part of the tree. The size of the visible area for the content filter tree can also be increased by moving the splitter above the tree ( ) upwards.

3.4 Microplate View

The initial page on the working area for measured microplates is the *Microplate View* page.

Note: If the open test run is a BMG LVis Micro Drop measurement, the title changes to LVis Plate View.

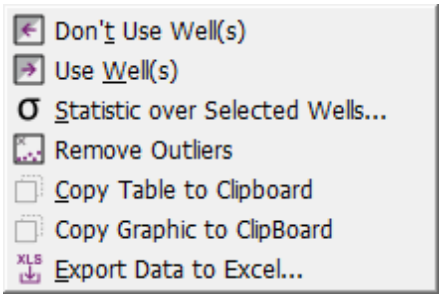


In this view, data is displayed according to the defined microplate layout. The navigation tree can be used to select the data you want to see.
The upper section of the page displays detailed information of the test run: the name of the test run, the measurement date and time, the defined test run ID's (ID1-ID3), the measurement mode and if the test run is signed or manipulated.
At the bottom of the page you see the legend for the displayed data.

With the Excel button ( ), you can export the displayed data to Excel (see chapter 3.22: *Export Data*) (you need to have installed a Microsoft Excel version 97 or higher on your PC).

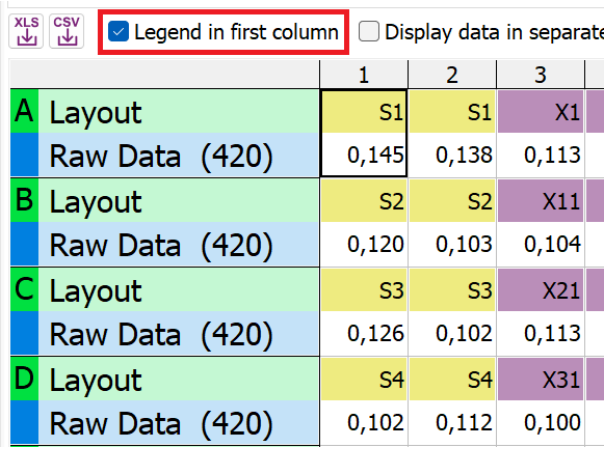
With the ASCII Export button ( ), you can export the displayed data into a text file. The data are stored in the comma separated value (CSV) format (see chapter 3.22: *Export Data*).

With the change layout button ( ) above the microplate table, you can directly open the window to change the test runs layout (see chapter 6: *Change Test Run Layout*).
Popup Window
The *Microplate View* page has a popup menu that can be reached by pressing the right mouse button in the main window:



Don't use the selected well(s) (see Exclude Wells)
Reuse excluded wells (see Exclude Wells)
Perform a statistic over the selected wells (see Statistic over Wells)
Open the Outlier Detection dialog (see Outlier Detection)
Copy the *Microplate View* as text to the clipboard.
Copy the *Microplate View* as a graphic to the clipboard.
Export the data to Excel (does the same as the Excel button)

Display legend in first column
Check this button to display the legend in the first column of the grid, for each row:

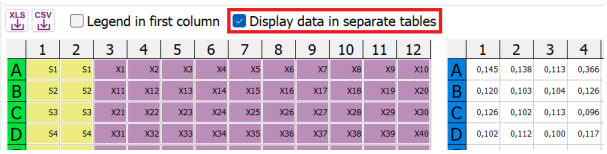


		1	2	3
A	Layout	S1	S1	X1
	Raw Data (420)	0,145	0,138	0,113
B	Layout	S2	S2	X11
	Raw Data (420)	0,120	0,103	0,104
C	Layout	S3	S3	X21
	Raw Data (420)	0,126	0,102	0,113
D	Layout	S4	S4	X31
	Raw Data (420)	0,102	0,112	0,100

This is useful for non-colored printing reports, to see the description of the data according to the row in the *Microplate View*.

Display data in separate tables

If more than one row is displayed in the microplate view, the data can also be displayed in a separate table for each selected row:



	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10
B	S2	S2	X11	X12	X13	X14	X15	X16	X17	X18	X19	X20
C	S3	S3	X21	X22	X23	X24	X25	X26	X27	X28	X29	X30
D	S4	S4	X31	X32	X33	X34	X35	X36	X37	X38	X39	X40

	1	2	3	4
A	0,145	0,138	0,113	0,366
B	0,120	0,103	0,104	0,126
C	0,126	0,102	0,113	0,096
D	0,102	0,112	0,100	0,117

the screen will be divided, and a smaller microplate will appear for each selected data. The microplates are arranged automatically to make the most of the space available.

3.4.1 View Modes

The *Microplate View* page can display the data in up to five different modes. You can change the mode with the view mode buttons found above the microplate grid.

Depending on the test runs measurement method there can be up to four modes available for a test run.

If groups are defined in the layout, the background of a microplate grid well will be drawn in a unique color for each group.

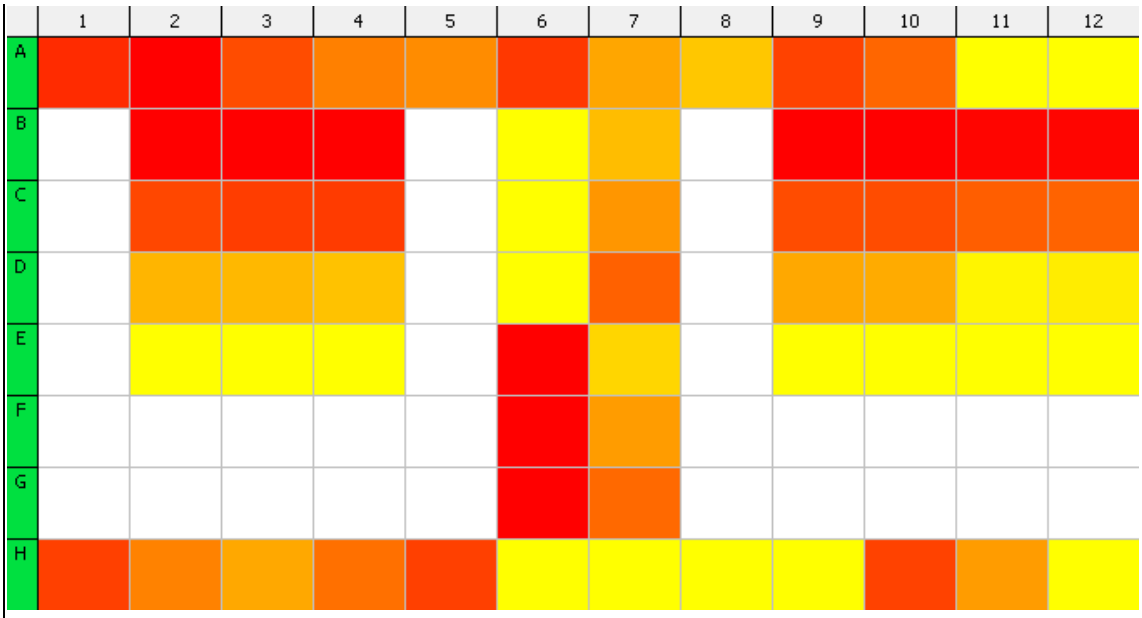
Value mode

Displays the values of the selected data nodes that can be expressed in one number. If the test-run is a kinetic, the data for a selected loop time point can be displayed in each well (see section Kinetic Test Runs). The screenshot at the top of this page shows the *Microplate View* in value mode.

If concentration values are displayed in the grid and a unit is defined for concentrations, you can display the unit behind the value if you check the *Show Units* control above the grid.

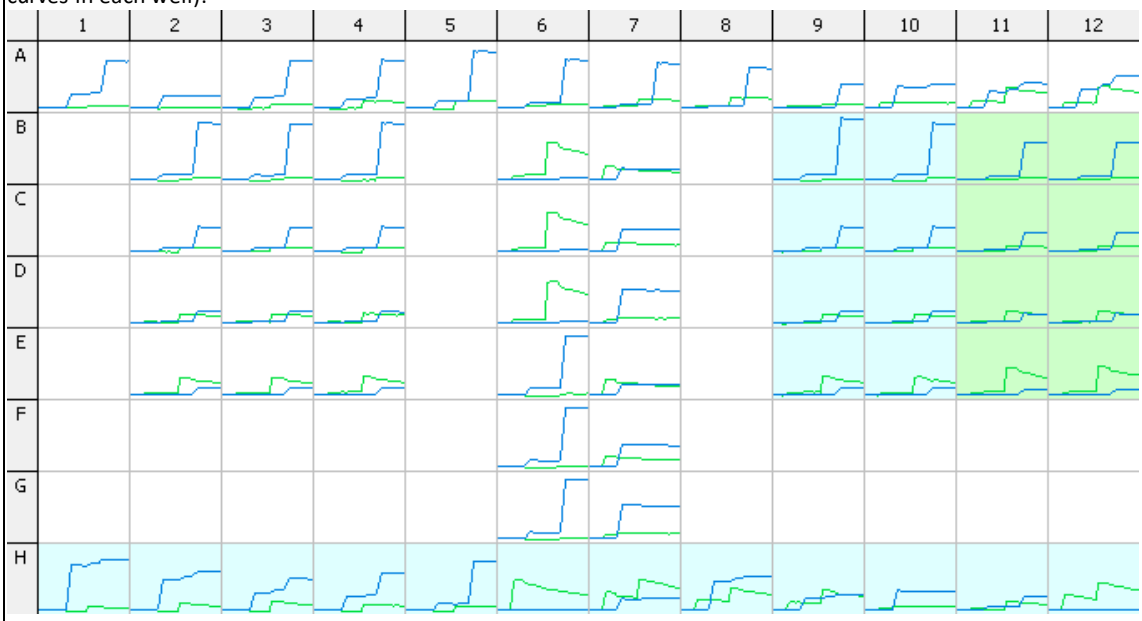
Color mode

Displays the values in different color modes: Good/Bad decision (one color for all values above a threshold, one color for the other values), Three colors (two limits defining the borders for the three colors) or Color gradient, which displays the values in different shades of colors between a defined range. You can enter and change the settings for the color view mode in the color settings window. To open the window, press the color button ( ) behind the color mode check box. To adjust the color settings, you can use the color range selector on the right side of the microplate view. This screen shot is an example for data displayed in Color gradient mode:



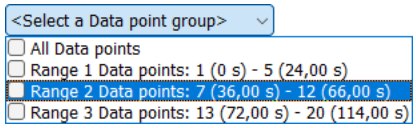
Kinetic mode

This mode is only available for kinetic test runs. It displays kinetic curves in the wells used for each selected data node that can be applied to the kinetic data (i.e. Blank corrected values or multiple wavelengths will show as multiple curves in each well):



The scaling for the horizontal and vertical axis depends on the scale settings. Default vertical scaling is the minimum and maximum values of all displayed curves. The default horizontal (time) scaling starts from the first iteration to the last one. The scaling can be changed in the *Curve Scaling Settings* dialog which can be opened with the settings button ( ) on the right side of the *Kinetic Curves* check box.

If kinetic ranges are defined (see chapter 4.1 Ranges) and only the kinetic mode is selected, you can select whether you want to see all loop iteratrions or if you want to see only a cut-out of the kinetic defined by one or more ranges with the drop down list on the right side above the table:

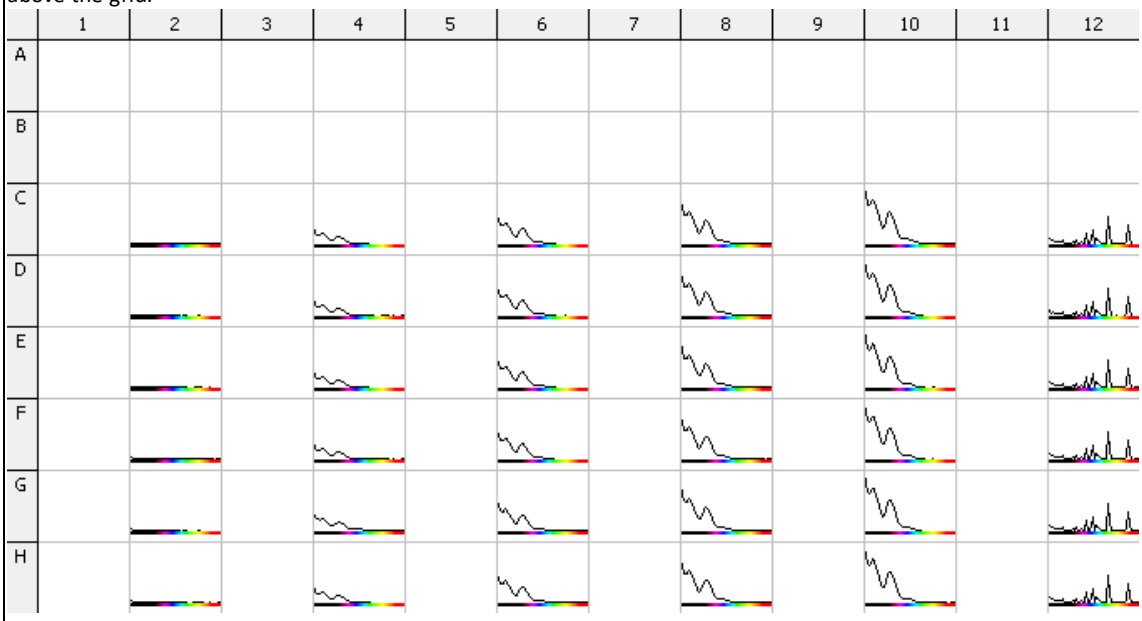


The curves color is defined by the selected row in the navigation tree. For better contrast you can define to print all curves in black. This can be defined in the MARS options dialog.

Spectrum mode

This mode is only available for measured spectra. It displays the spectrum curve of the selected spectrum node for each well. On the bottom of each well, a small spectrum bar is displayed that gives you an overview of the measured

. If the measurement has a kinetic, you can select the loop time point with the drop down list for loops on the top left side of the grid, or you can display the spectra of each loop iteration at once (overlapping) if you check the *All* control above the grid.

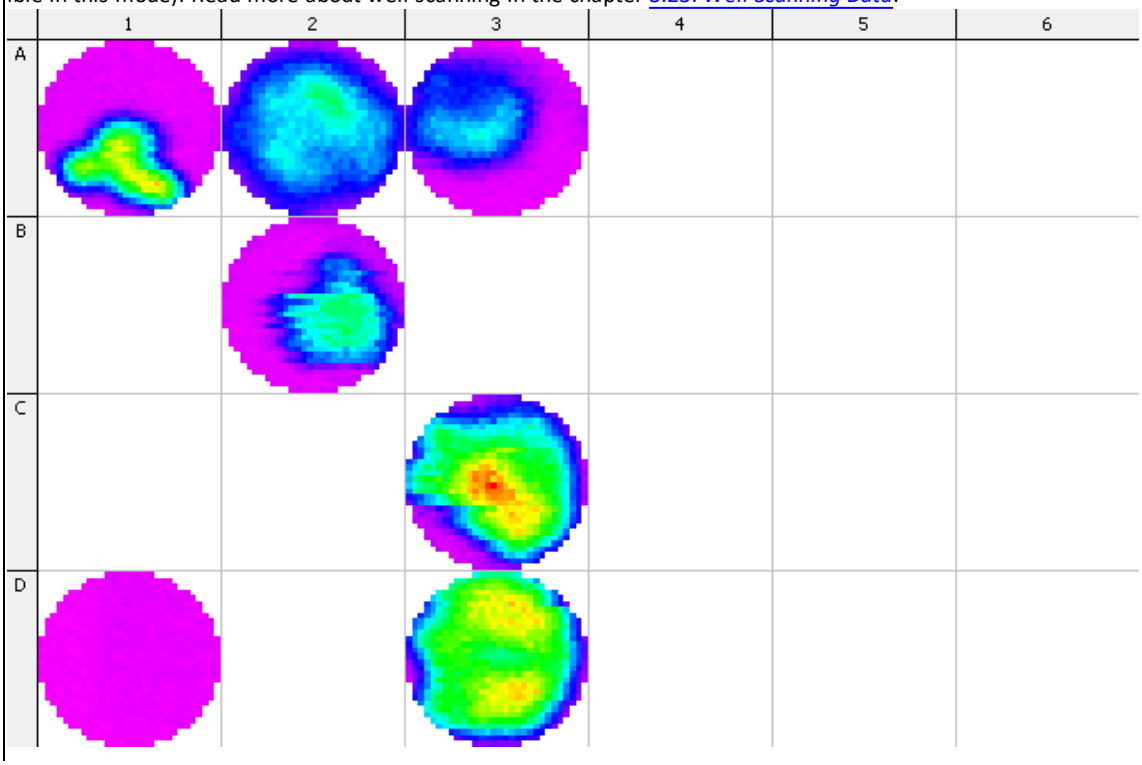


The scaling for the horizontal and vertical axis depends on the scale settings. Default vertical scaling is the minimum and maximum values of all displayed curves. The default horizontal (wavelength) scaling starts from the first wavelength to the last one. The scaling can be changed in the *Curve Scaling Settings* dialog which can be opened with the settings button ( ) on the right side of the *Spectrum Curves* check box.

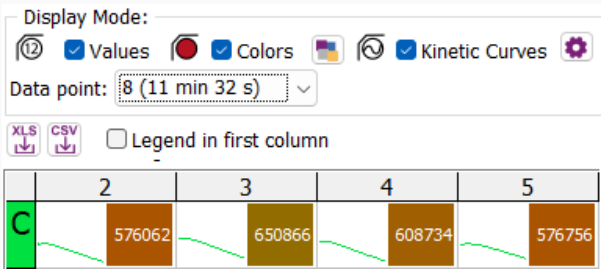
Well scan mode

This mode is only available if your test run contains well scanning data. It displays each scanned point in the well in a color, defined with the color settings window. The data can be displayed in the same three modes like in the color mode. Only raw data can be displayed using this mode. Meaning that the selected nodes in the navigation tree have no influence on the displayed data in this mode.

If your measurement contains more than one measured wavelength (dual channel or multiple wavelength test run), you can select the wavelength you want to display, with the drop-down list on the top of the microplate grid (only visible in this mode). Read more about well scanning in the chapter 3.23: *Well Scanning Data*.



The available view modes can be combined if you select more than one of the checkbox controls. If you combine the view modes, you will see all selected view modes side by side in each grid cell. If you combine the color mode with the value mode, the value will be shown above the color (the color is used as a background color for the value mode).

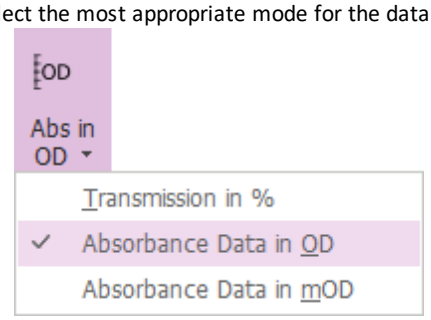


Kinetic Test Runs

You can display an overview of the kinetic curves for each well with the *Kinetic View Mode* (see chapter 3.3.1: *View Modes*). In the Value View Mode, and in the Color View Mode, you can select the loop iteration you want to inspect using the kinetic drop down list at the top left of the microplate grid: Cycle: 23 (37 min 27 s) . Select the '*All*' check box and in *Color Mode* a color bar representing the data of each loop iteration will be shown in each well.

Absorbance Data

Absorbance measurement data and curves can be shown as OD values, as milliOD values (mOD) or as transmission values (in % transmission). An additional button appears in the *Display* group on the *Home* tab of the ribbon when an absorbance test run is open allowing the user to select the most appropriate mode for the data to be expressed.

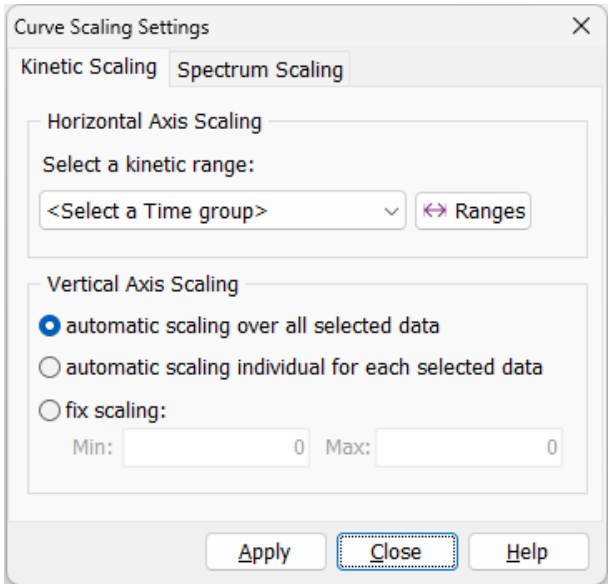


You can also find a command button for each of the three modes in the *Working Area* group on the *View* tab.

> **Note:** All absorbance calculations in MARS are internally based on OD values. Switching between OD/mOD/Trans only relates to the representation of the values. When calculating with two absorbance values in OD, e.g. when two absorbance values are multiplied, the calculation result can no longer be converted according to the display setting. For example, if an absorbance value of 0.8 OD is multiplied by an absorbance value of 0.6 OD, the corresponding result is 0.48 (OD^2). However, if the display in MARS is set to 'Abs in mOD' the two absorbance values are displayed as 800 and 600 mOD but the calculation result, remains 0.48. There is no conversion to mOD^2 (i.e. 480,000 mOD^2).

Scale Settings for Kinetic and Spectrum Curves

The settings for the horizontal and vertical axis scaling of the displayed curves in the microplate view can be adjusted. Select the settings button ( ) for either the kinetic curves or the spectrum curves to open the Curve Scaling Settings dialog:



Horizontal Axis Scaling: select a range (kinetic or wavelength) to define the scale (if there is no range with the desired scale, you can create a new range using the *Ranges* button).
Vertical Axis Scaling: You can choose between three settings:

- automatic scaling over all selected data (minimum and maximum is calculated from all selected data over all wells)
- automatic scaling individual for each selected data (minimum and maximum is calculated separately for each selected data but for all wells).
- fix scaling (enter the desired minimum and maximum value for the axis scaling).

3.4.2 Selecting Wells

You can select one or more wells in the *Microplate View* using the mouse. To select one well, just click on it with the left mouse button. To select an area of adjacent wells, press the left mouse button over the first well you want to select, and keep it pressed dragging the mouse cursor over to the last well of the area before releasing the mouse button.
To select a collection of wells allotted over the microplate grid, press the Ctrl-Key on your keyboard and click with the left mouse button on each well you want to select.
To select a whole row or a column on the grid, click on the appropriate row letter or column number.
The selected wells are indicated by a black border around the well.
A double click on a well performs an action that depends on the preset viewing mode:

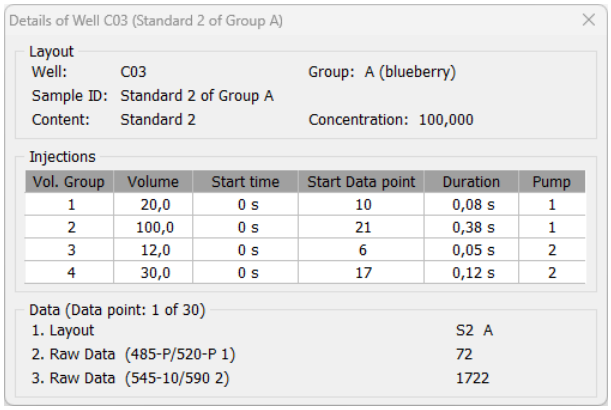
View Mode	Action
Value mode Color mode	Opens a window with detail information of the well (see chapter 3.3.3: *Details of a Well* below)
Kinetic curve mode	Changes to the signal curve view page and displays the selected well(s) in the chart.
Spectrum curve mode	Changes to the spectrum curve view page and displays the selected well(s) in the chart.
Well scan mode	Opens a window with a detailed view on the well scanning values of the well (see chapter 3.23: *Well Scanning Data*)

The selection of one or more wells also leads to a selection of the associated nodes in the content filter tree.

3.4.3 Details of a Well

The window, *Details of Well <WellName>* appears after double clicking on a well in the *Microplate View*, if the *Value Mode* or *Color Mode* is in use. Alternatively, you can click *Well Details* in the *Working Area* group on the *View* tab of the ribbon, which will also work if the other view modes are in use.

Note: If more than one well is selected, the details of the first selected well will be displayed.



The detail window shows layout information of the well and content such as, associated group, sample ID and concentrations (if available). If the test run includes injections, then the volumes used, and the injection time values are also displayed in a table for each well.
The bottom part of the window displays the values of the selected nodes in the navigation tree for that well.

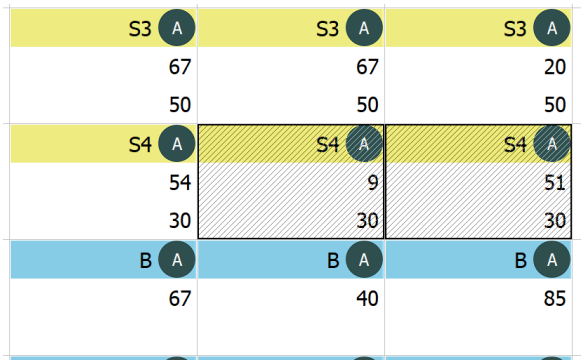
Note: If the test run was created with a NEPHELOstar reader, the injection information Start time (plate mode tests only) and Duration are not available.

3.4.4 Zooming

If there are many data values shown in one well or if using a microplate format with 384, 1536 or 3456 wells, the values in one well can appear very small and become difficult to read. To overcome this, it is possible to zoom the visible section of the microplate grid from displaying the whole plate up to displaying only one well.
Use the zooming controls shown at the bottom right of the grid to change the zoom factor (in Percent). You can either press the Zoom In or the Zoom Out buttons to zoom into the grid or out of the grid in predefined steps (25 %) or by entering a zoom factor in the entry field. The entered value will be adjusted to display only whole wells.
To reset the view to the whole plate (100%) setting, press the button.

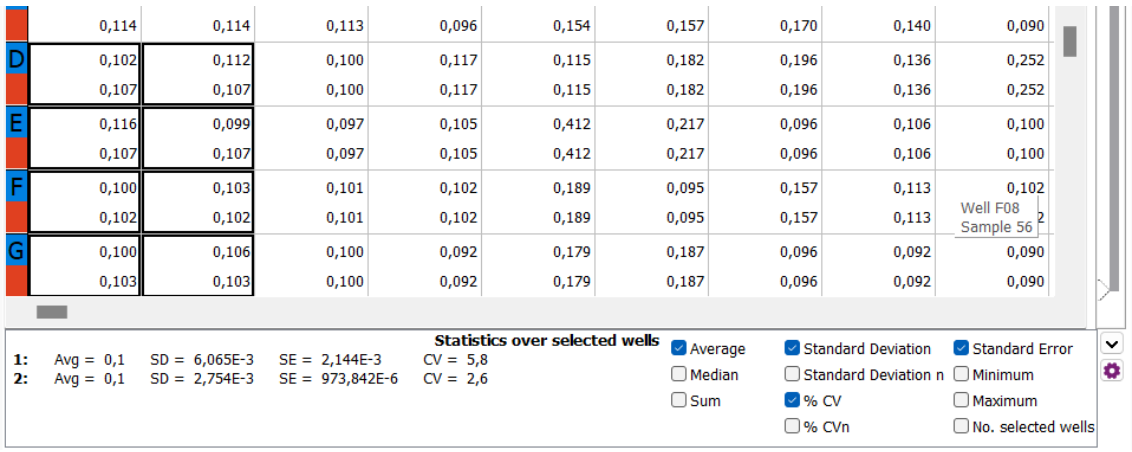
3.4.5 Exclude Wells

If there are outliers within your test run data, you can exclude these wells from the evaluation by applying a toggle to the usage state of the wells you do not wish to use. Wells can be set to be excluded, or these unused wells can be set to be used again by pressing the Ctrl-T keys on your keyboard or by clicking on the right mouse key to use the pop-up menu, after selecting the wells in the *Microplate View*. You can also find a *Toggle Well* command in the *Working Area* group on the *View* tab of the ribbon.
Unused wells are displayed with diagonal gray stripes. Only the raw data values and the layout values are displayed (also in gray):



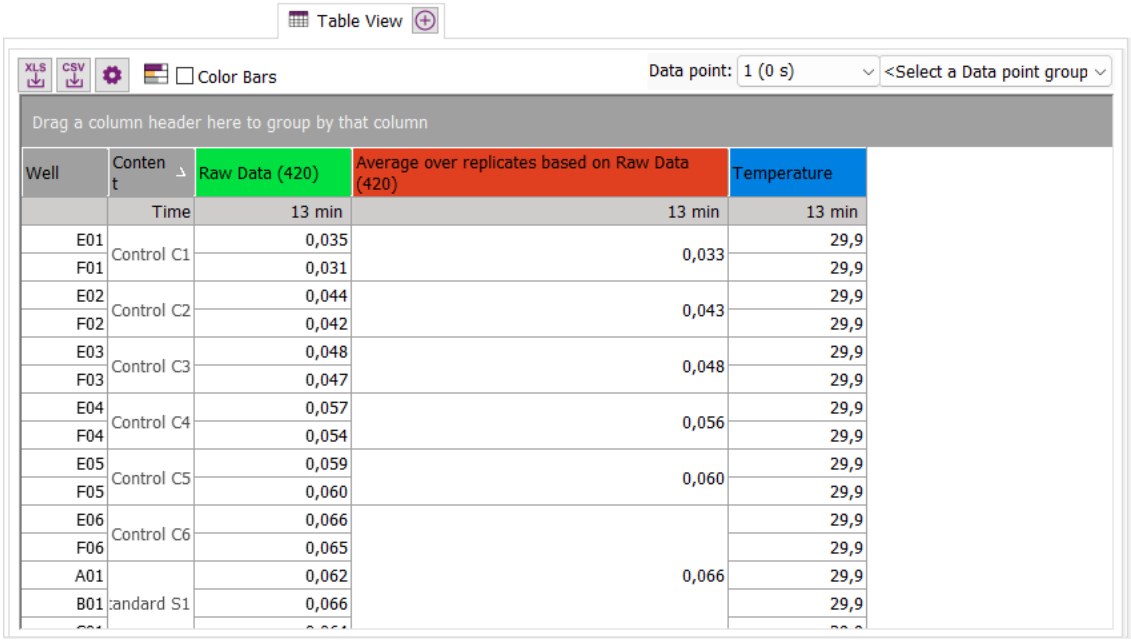
3.4.6 Statistic over selected wells

Below the microplate view a section can be displayed to show statistical data of the selected wells. Click on the button on the right side below the microplate view to open the section. Click on the button to define the statistic values you want to see. For each selected data row in the microplate view, the statistic values for the selected wells will be displayed. The section can be closed with the button .
.



3.5 Table View

The table view page displays the data in a table form with a row for each well and a column for each selected data node in the navigation tree.



The first columns are predefined with the well name (alternative two columns with row and column name of the well) and the content of the well. If there are groups defined in the layout, a further column with the group name is also shown. To the right of the predefined columns, the columns corresponding to the data nodes as selected in the navigation tree are displayed.

The legend under the table shows a description of the selected data nodes. The color of the selected row in the navigation tree is displayed in the legend before the corresponding line and is also used as background color for the header of the appropriate column.

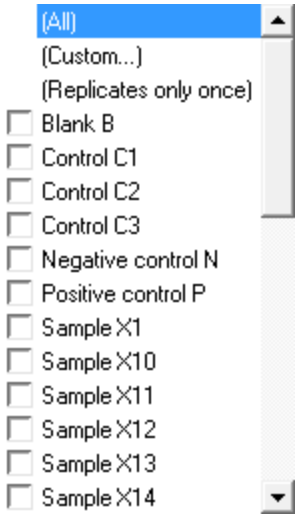
If groups are defined in the layout, the background of the row will be drawn in the color unique to the associated group.

If a well is not used (see chapter 3.3.5: *Exclude Wells*) the row of the well is shown with a light gray background and a dark gray text color.

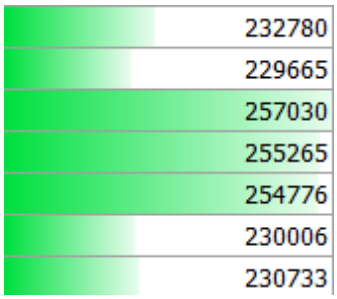
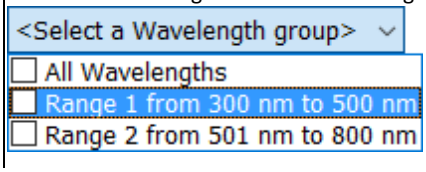
You can order, filter or move each data column in the table. Grouping is only possible with the Column *Content* and *Group*. You cannot move columns into or inside a column sequence (columns with the same header color are a sequence of loop iterations or wavelength and cannot be divided or resorted). You can hide the fix columns and the column *Well* can be split into the two columns *Well Row*and *Well Col* (see Table View Settings).

More information is given about common table functionality in the chapter 2.2: *Group and Filter Test Runs* .

The column *Content* has a special filter function. Move the mouse over the column header and press the appearing arrow button. The filter list for the column opens:



The filter (*Replicates only once*) displays only the first well of each replicate series. This filter is useful if the data to display are based on replicate statistics.
There are further controls shown above the table:

	Export the table to Excel (you must have installed an Excel version on your computer) (See chapter 3.22: *Export Data*)
	Export the table into a text file. The data of the table are stored in the comma separated value (CSV) format (See chapter 3.22: *Export Data*).
	Open the Table View Settings page to change further table view options.
	Click this control to add color bars inside the column cells with data (like the Excel data bars): 
Wavelength	This option appears only for spectrum scan test runs and is only enabled if in the *Select a Wavelength group* box is nothing selected. Select which wavelength you want to display. **Note:** If you have a lot of spectrum data (many wells and a large measured spectrum especially in combination with kinetic data), the creation of the table with spectrum or blank corrected spectrum data may take some time (a progress bar with an abort button will appear)!
Select a Wavelength group	This control appears only for spectrum scan test runs. With the drop-down list, you can select the wavelength range you want to see in the table. If at least one entry is checked, a column for each wavelength of the selected ranges is created (only if the selected data contains spectral data). The wavelength is shown in the *Wavelength* row. You can combine the selection of the ranges or you can select *All Wavelengths* to display all wavelengths. To see only one wavelength in the table, deselect all selected entries and select the wavelength with the Wavelength control on the left. 

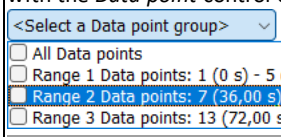
Content	Raw Data (Abs Spectrum)	Raw Data (Abs Spectrum)	Raw Data (Abs Spectrum)	Raw Data (Abs Spectrum)
Time [s]	0	300	600	900
Wavelength	600	600	600	600
Blank B	0.365	0.354	0.322	0.306
Negative con	0.084	0.084	0.082	0.083

Data point

This option appears only for kinetic test runs and is only enabled if the *All Data points* box is not checked. Select which loop data point you want to display.

Select a Data point group

This control appears only for kinetic test runs. With the drop-down list, you can select the interation range you want to see in the table. If at least one entry is checked, a column for each data point of the selected ranges is created (only if the selected data contains loops). The header of the column is expanded with the data point number, if detailed header is not checked. If the loop has less than 65 Iterations, you also can select single data points to display. The time value is shown in the *Time* row. You can combine the selection of the ranges or you can select *All Data points* to display all data points. To see only one data point in the table, deselect all selected entries and select the data point with the *Data point* control on the left.



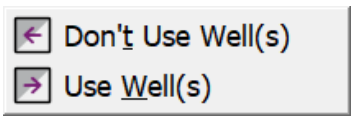
Group	1 - 1	1 - 2	1 - 3	1 - 4
e	0 min	0 min 30 s	1 min	1 min 30 s
0 A	-2842	-3909	-699	-4755

With the table view option 'grouped by kinetics' you can decide, if the kinetic data should be displayed successively for the selected data (like displayed above) or if the data should be ordered by selection followed by loop data point:

Content	Raw Data (600)	Temperature	Raw Data (600)	Temperature	Raw Data (600)	Temperature
Time [s]	0	0	300	300	600	60
Blank B	0.365	37	0.354	37.1	0.322	3
Negative con	0.084	37	0.084	37.1	0.082	3

pop-up Window

The *Table View* page has a pop-up menu that can be reached by pressing the right mouse button when the mouse is over the table:



Don't use the selected well(s) (see Exclude Wells)
Reuse excluded wells (see Exclude Wells)

3.6 Bar Chart

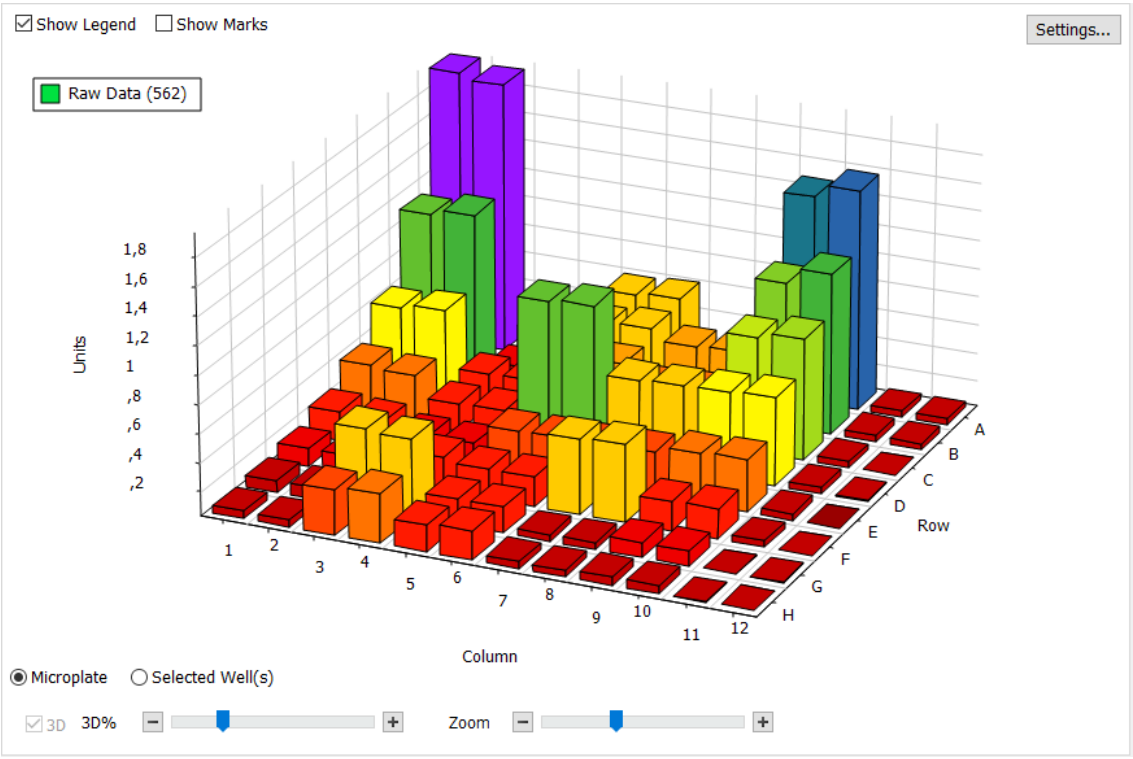
The bar chart page displays data as bars in a chart. You can add a bar chart page by clicking  in the *Display* group on the *Home* tab of the ribbon or by clicking the add view button  and select the *Add bar chart* entry.

To change settings like axis settings, color settings, titles and marks, click the *Settings...* button. In addition to the chart legend, Marks for each series can be displayed. The default mark text can be changed in the *Settings* dialog on the *Marks* tab (see Change Marks Settings).

You can create two kinds of bar charts from the data selected in the navigation tree.

3.6.1 3D bar chart from the whole microplate

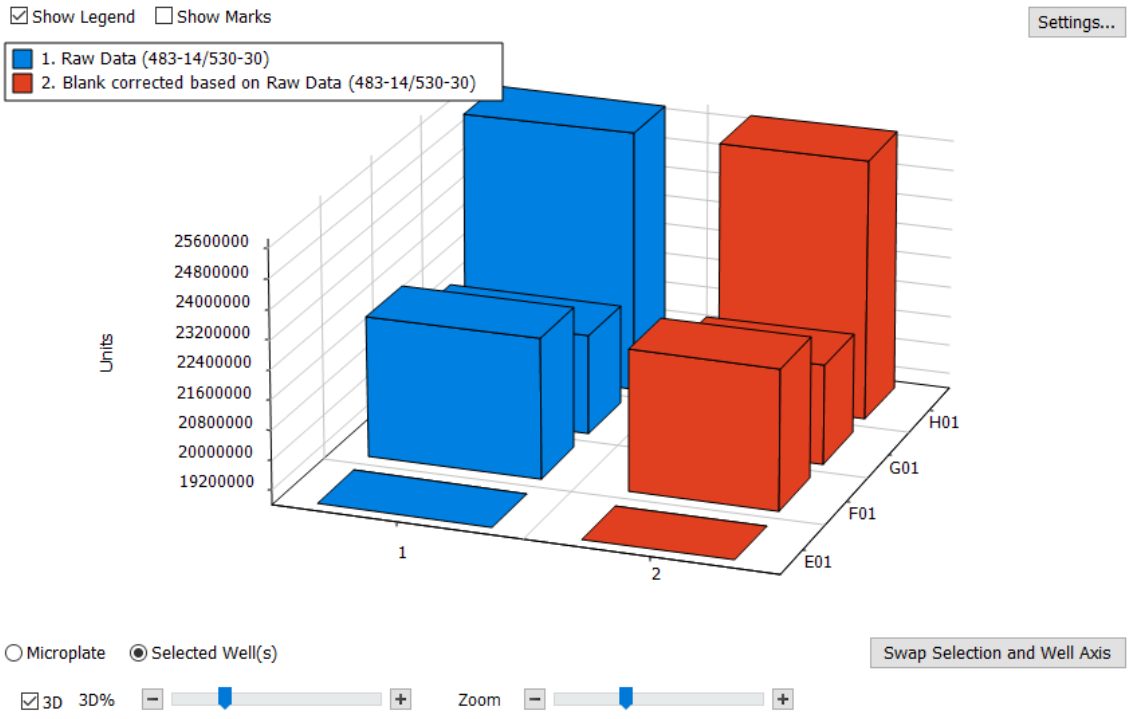
Select the *Microplate* control at the bottom of the chart:



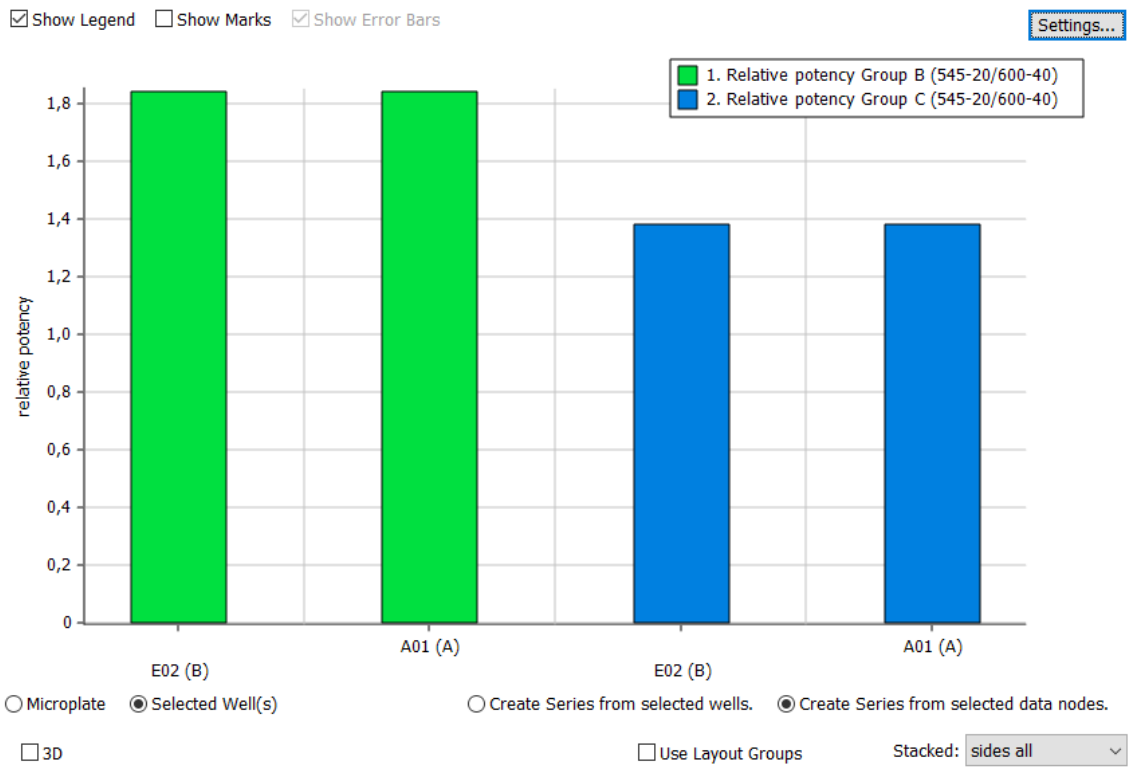
The chart displays a bar for each measured well in the microplate view according to the value for this well based on the selected data. You can adjust the 3D settings of the chart, using the 3D% and the Zoom slider. To change the rotation of the chart, you can use the mouse by clicking into the chart and move the mouse.

3.6.2 Bar chart based on selected wells

Select the *Selected wells* control on the bottom of the chart.
The chart displays a bar only for the selected wells, but also one for each selected data row. You can choose between 3D or 2D bars.
3D bar charts of selected wells

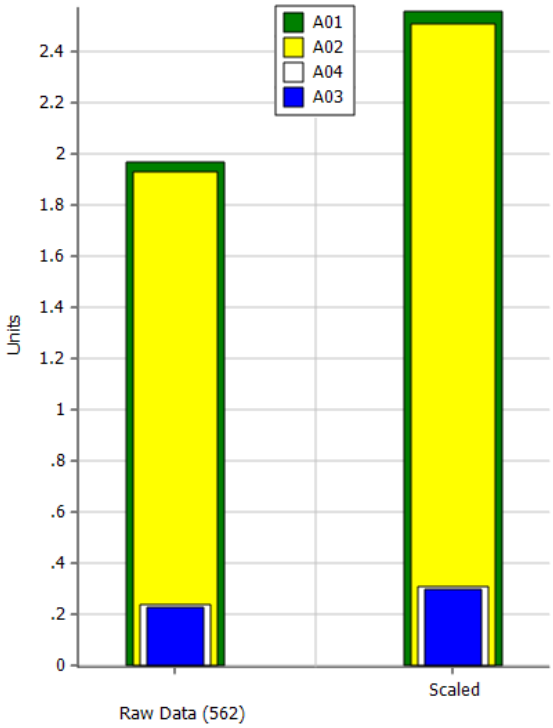


You can swap the x and z axis in the chart with the *Swap Selection and Well Axis* button. You can adjust the 3D settings of the bar chart using the same controls as for the microplate bar chart, described above.
2D bar charts of selected wells



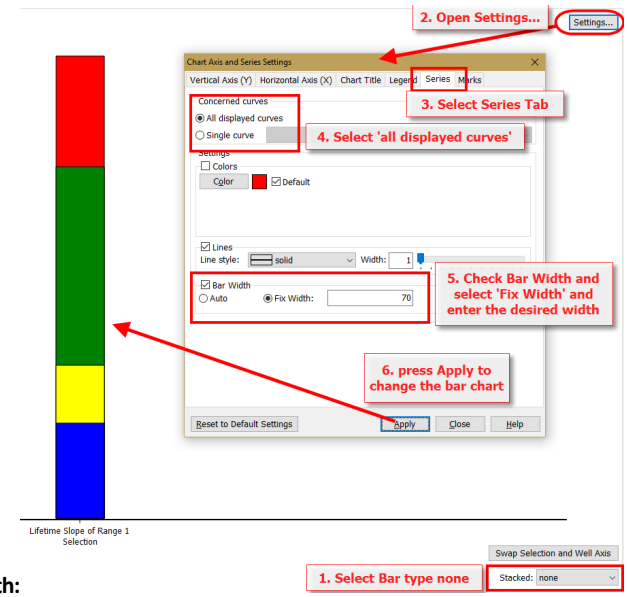
In this mode, bar charts are created based on the selection made in the navigation tree and on the selected wells/contents in the content tree. Each selected data base represents a new bar series set with a bar for each selected well, if *Create Series from selected data* is selected. If *Create Series from selected wells* is selected, a bar series for each selected well will be added to the chart, with bars for each selected data. If the layout contains groups, you can check *Use Layout Groups*, to create a new series for each group.
If the selected wells are replicates and the selected data is an average calculation over replicates, error bars can be added to these bars.
The bar series and the single bars of each series can be arranged in different ways. Use the drop-down control *Stacked* to choose between these different options:

Option	**Meaning**
none	Each bar series is placed one behind the other, starting with the first data bar of each bar series followed by the second and so on. To see all the bars, the size of the bars increases the more the bar is in the background. The bar series are placed side by side. The width of the bars can also be set to one size. See both types of bar charts here:



1. Auto-width:

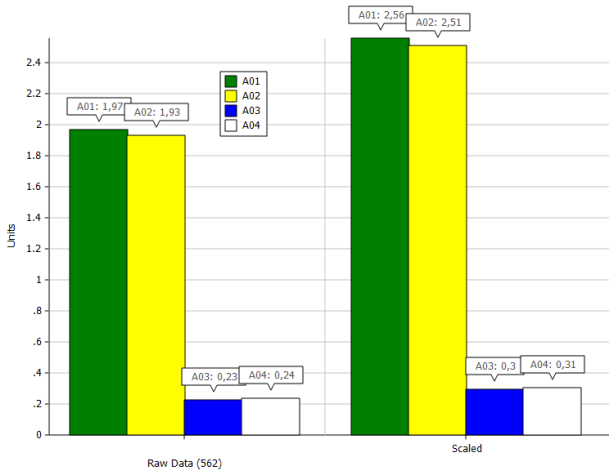
2. Fix (same) width:



> **Note:** If only one bar series is selected, *none* displays the same as *sides* and *sides all*. If the number of series points (bars) are different between single bar series, this can lead to an unexpected mixing of the single bars into bars ordered one behind the other. It is recommended to use *none* only for bar series with the same amount of data points.

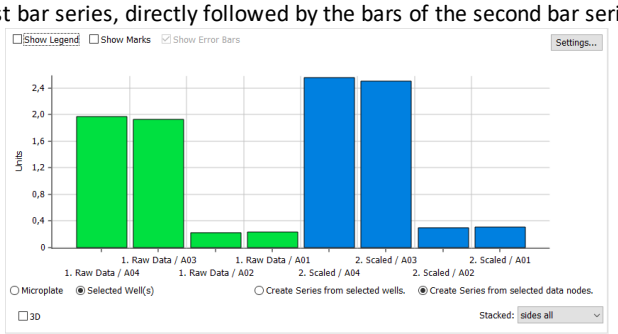
sides

Each bar of one bar series is placed directly side by side. Between two or more bar series, there is a space, if the chart is big enough, otherwise the series overlap:



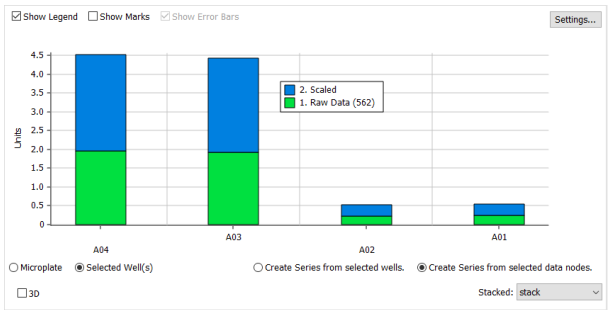
The bars are placed side by side (with a little space) for all bar series, starting with the bars of the first bar series, directly followed by the bars of the second bar series...

sides all



These three options will stack the bars, that means, each bar is placed above the other. If stack or stack 100% is selected, the bar series are stacked. If all bar series have for example 3 data points (each data point represents a bar), the bars for the first data point of each series are stacked, the bars for the second data point of each series are stacked, and so on. If self stacked is selected, the data points itself are stacked and a stacked bar is drawn for each bar series. Stack 100% draws each bar as a percentage of the whole stack (= 100%).

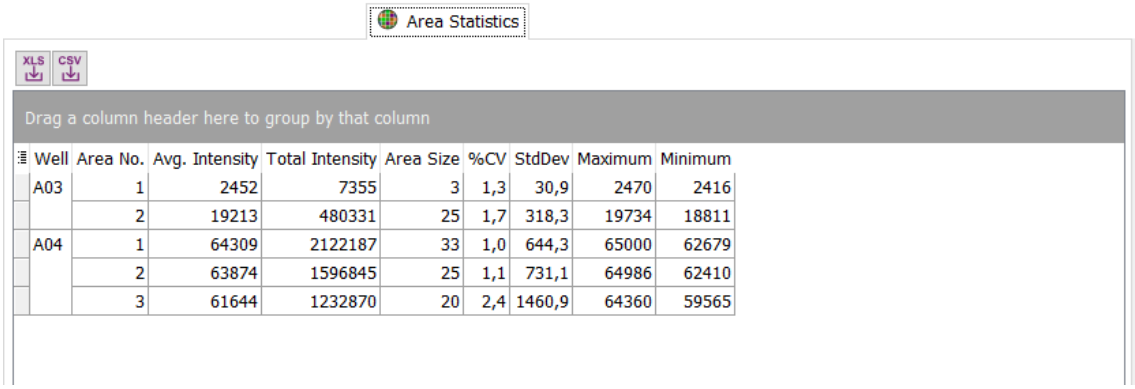
stack / stack 100% / self stacked



> **Note:** If any of the stack modes is selected and the number of series points (bars) are different between single bar series, this can lead to an unexpected mixing of the single bars into stacked bars. It is recommended to use stacked bars only for single bar series or for bar series with the same amount of data points.

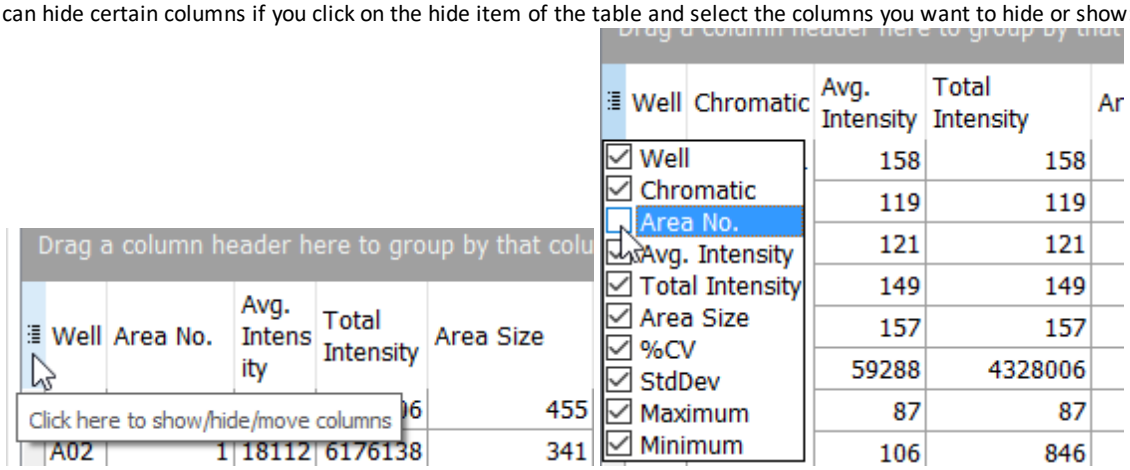
3.7 Well Scan Area Statistic Table

This page is only visible for well scan test runs with defined areas.
The area statistics view page displays the statistical data of all defined areas in a table with a row for each well. Each statistic value is represented by a table column.



Well	Area No.	Avg. Intensity	Total Intensity	Area Size	%CV	StdDev	Maximum	Minimum
A03	1	2452	7355	3	1,3	30,9	2470	2416
	2	19213	480331	25	1,7	318,3	19734	18811
A04	1	64309	2122187	33	1,0	644,3	65000	62679
	2	63874	1596845	25	1,1	731,1	64986	62410
	3	61644	1232870	20	2,4	1460,9	64360	59565

You can order, filter or move each column in the table. Grouping is only possible with the Column *Content* and *Group*.
You can hide certain columns if you click on the hide item of the table and select the columns you want to hide or show:



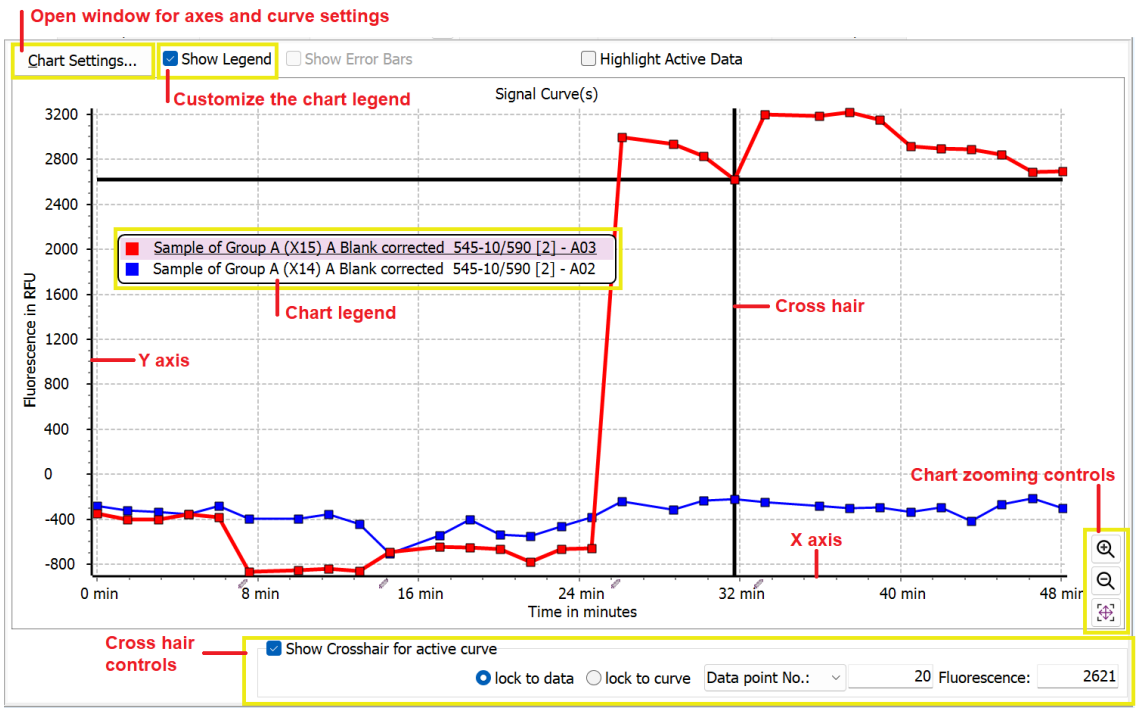
More information is given about common table functionality in the chapter 2.2: *Group and Filter Test Runs* .
There are further controls shown above the table:

XLS	Export the table to Excel (you must have installed an Excel version on your computer) (See chapter 3.22: *Export Data*)
CSV	Export the table into a text file. The data of the table are stored in the comma separated value (CSV) format (See chapter 3.22: *Export Data*).

3.8 Common Chart Functions

The subjects described in this chapter apply to all five kinds of chart used in MARS:
The signal curves chart (available for kinetic test runs), the spectrum curves chart (only for spectrum measurements), the standard fit curves chart (available after a performed standard calculation or parallel line analysis), the enzyme kinetic fit curves chart (available after a performed enzyme kinetic calculation) and the binding kinetic fit curves (available after a performed binding kinetic fit calculation).
All these charts contain the following elements and functions:

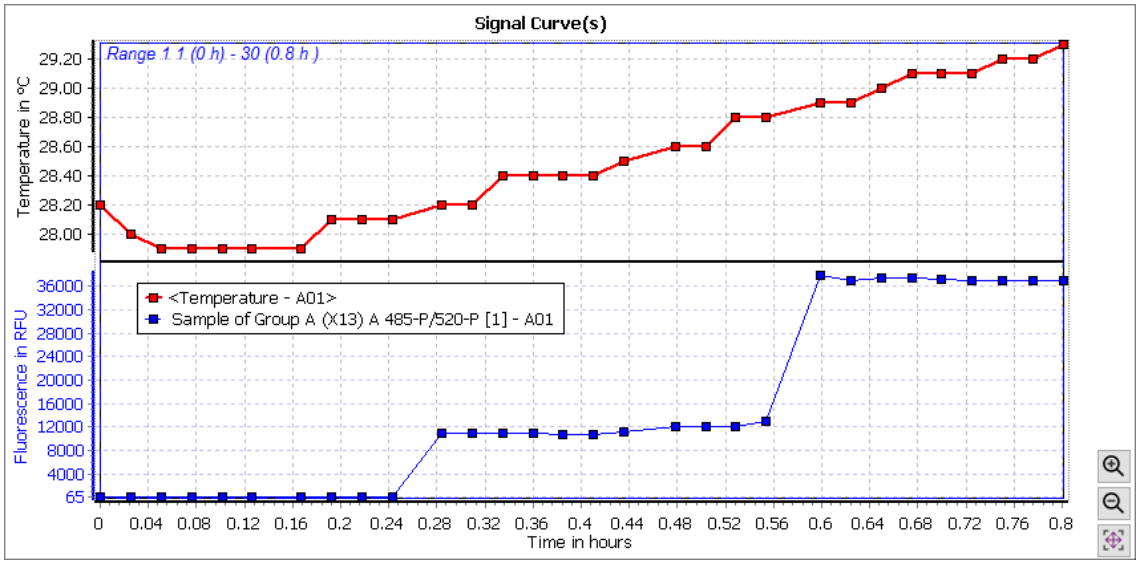
- A chart with at least one X-axis and one Y-axis and selected curves in it
- A legend explaining the displayed curves
- A control box to hide or show the legend
- Crosshair functionality
- Zooming possibilities
- A dialog to change the settings of axes and curves.



The chart title and the title font can be changed on the *Chart Title* tab of the chart settings dialog box. Click *Chart Settings...*, select the *Chart Title* tab and enter the desired settings (see chapter 3.9: Chart Title Settings).

3.8.1 Chart Axes and Curves

A chart consists of one X axis and one or more Y axis (e.g. the signal curves chart has Y axes for each signal curve with a different unit value). If the chart has more than one Y axis, they are shown as different charts one on top of the other. Each axis can be customized:



Each curve in the chart has its own color and the data points of the curve (if it is a data curve and not a calculated fit curve) have an initial shape.

Click on the curve or click on the associated legend entry to select a curve in the chart. The selected curve has a bold line and two enclosing brackets (<...>) in the legend. The corresponding node in the navigation tree and - if visible - the corresponding well node in the content filter tree will also be selected.

You can change the color and the style of the curve with the chart settings dialog box (pressing the *Chart Settings...* button, see chapter 3.8: *Change Chart Curve Settings*).

You can define each axis according to the start and stop value, the increment value, the name, the scaling (logarithmic or linear) and the number format settings for the displayed axis labels, by opening the chart settings dialog box to change these attributes (see chapter 3.7: *Change Settings of a Chart Axis*).

The title of the chart can also be changed in the chart settings dialog box.

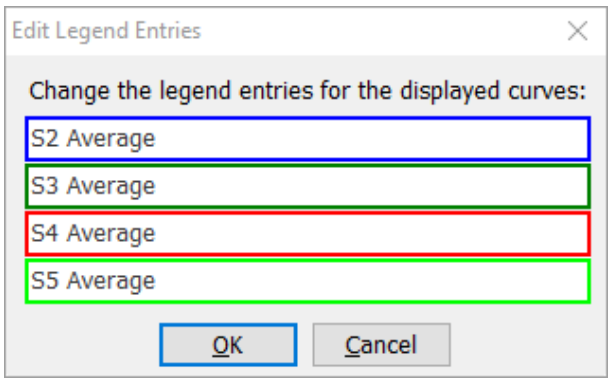
3.8.2 Chart Legend

The chart legend contains an entry for each displayed curve or data point series in the chart describing the curve.

To hide the legend, uncheck the *Show Legend* check box.

Changing chart legend entries

You can change the text of legend entries manually. Click on the *Edit Legend* item in the chart popup menu or open the chart settings dialog box select the Legend tab and click the *Edit legend contents* button to open the *Edit Legend Entries* dialog box:



Click in one of the entry fields and enter the desired legend entry. The legend entry is part of the test run setting. Save the test run settings, to make your changes persistent.

Note: There is a maximum of 80 signs you can enter for each legend entry.

You can also change the font of legend entries, the legend title and the legend position in the chart settings dialog box. Select the Legend tab and define the desired settings (see chapter 3.10: Chart Legend Settings).

3.8.3 Crosshair

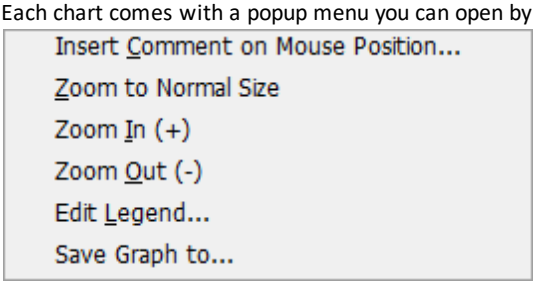
The crosshair consists of a horizontal and a vertical line on the chart. The intersection of the two lines is always a point on the selected curve. Check the *Show crosshair for active curve* to display the crosshair. The x and y values of the intersection point are displayed in the two fields of the crosshair control group. To set a position for the crosshair to a given x value, enter that value into the entry field for the x intersection point.
You can move the crosshair along the curve in x direction by dragging its vertical line with the mouse and dropping it at the desired position or by using the cursor keys ← and → on the keyboard. There are two modes moving the crosshair along the curve:

Lock to data	The crosshair jumps from one measured data point to the next data point when you move it.
Lock to curve	The crosshair moves also between data points along the curve (linear connection between adjacent data points).

Change the mode by clicking on the corresponding radio button.

3.8.4 Chart Popup Menu

Each chart comes with a popup menu you can open by clicking the right mouse button.



You can insert up to five comments at the current mouse position (see chapter 3.6.6: *Chart Comments*)
The menu items for zooming apply to the zoom functionality of the chart (see chapter 3.6.5: *Zooming*)
Click Edit Legend, to change the text of the chart legend entries.
You can create an image out of the chart and save it into a file on your file system. Define the desired file format in the subsequent dialog.

3.8.5 Zooming

It is easy to zoom in on a chart using the mouse: using the left mouse key drag the cursor from the top left of the chosen area out to the bottom right corner of the zoom area. When the mouse button is released the chart will then zoom into the highlighted area.

Note: Zooming using the mouse in the signal curve window is not possible if ranges are displayed (see chapter 3.11: *Signal Curve*).

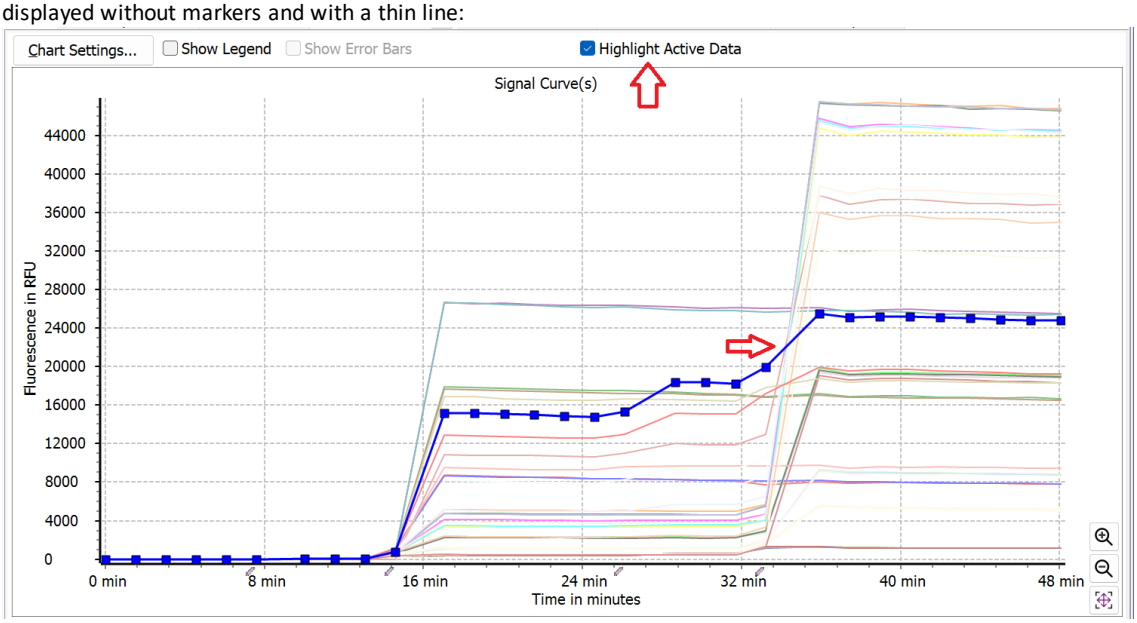
There are zooming buttons on the right side of the chart that can also be used. Clicking on these buttons will zoom into the center of the chart in predefined steps. If you use the Zoom In (+) item on the popup menu, the position of the mouse is used as new center of the chart before zooming.
To move the zoomed area, press and hold the shift key on the keyboard and hold the left mouse button over the chart. To change the positioning of the zoomed area, press the left mouse button whilst holding down the shift key and move the mouse.

To reset the chart to its normal size, double click on the chart or press the  button.

> **Note:** Resetting the zoom re-establishes the state before zooming but will not change start and end values of the axis to the initial values if you've changed them with the axis settings window!

3.8.6 Highlight Active Data

For some charts (if several data are displayed) a single series can be highlighted if the *Highlight Active Data* check box is checked. The highlighted series is displayed in a slightly thicker line and with markers for each data point. The other series are displayed without markers and with a thin line:



The highlighted data can be changed by clicking on the data in the chart or by selecting the data in the chart legend.

3.8.7 Chart Comments

A chart can have up to five individual comments. To add a new comment, move the mouse to the position on the chart where the upper left corner of the comment should be placed and press the right mouse button. Use the *Insert Comment on Mouse Position* item on the appearing popup menu to add the comment. Enter the text and click outside the comment window after entering the desired comment.

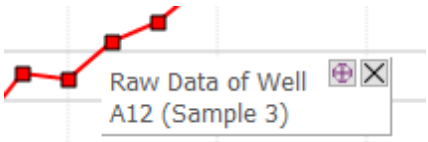
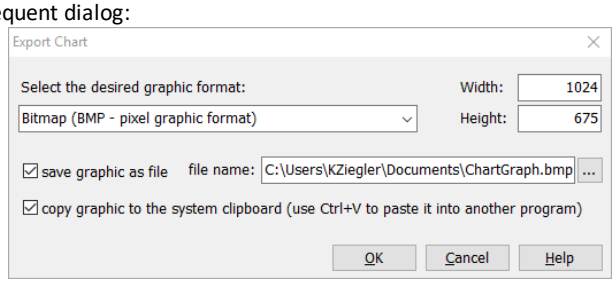


Chart comments can be removed by moving the mouse over the comment and press the appearing  button, and moved by the  button..

3.8.8 Export Chart into an image file

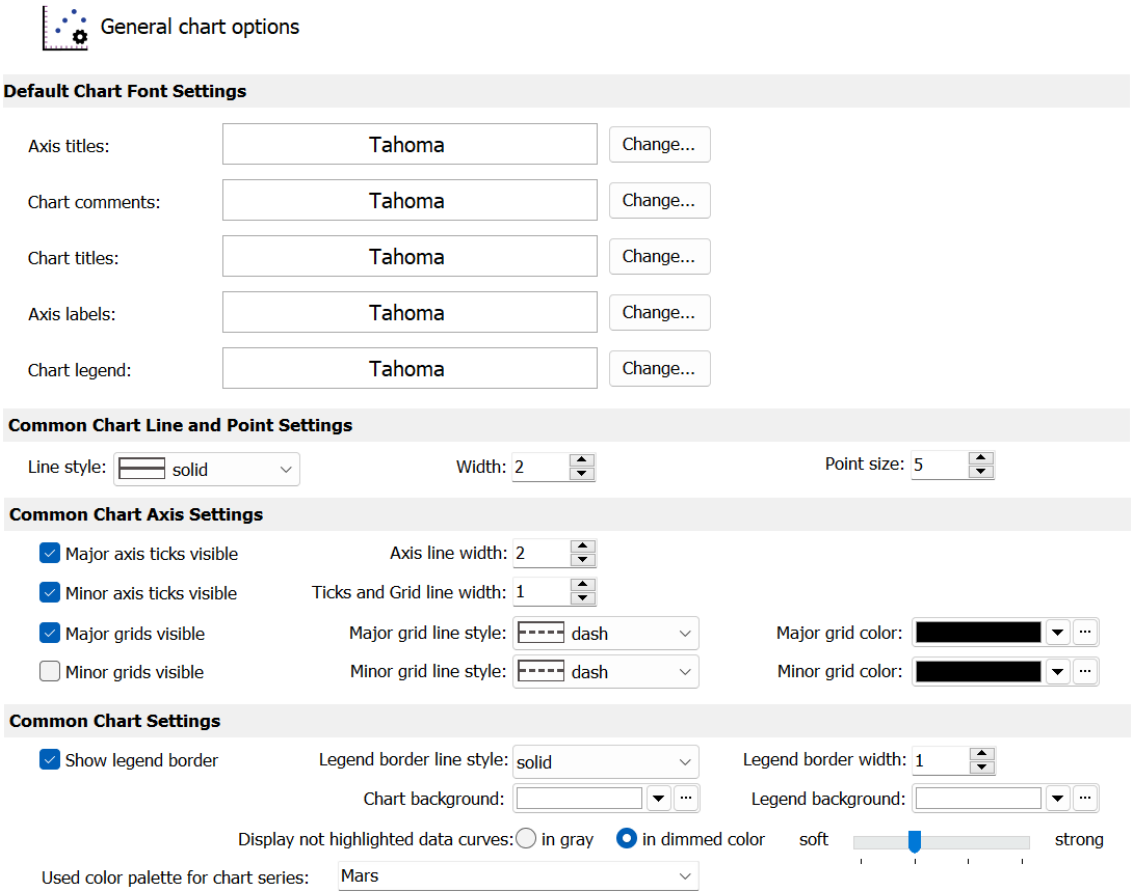
To export the chart into an image file, use the menu item 'Save Graph to...' in the chart's popup menu. Define the settings for the image file in the subsequent dialog:



Select the desired graphic format	Choose between one of the offered graphic formats: • Bitmap (pixel based graphic format - default file extension: bmp) • Windows Meta File (vector graphic format - default file extension: wmf) • Enhanced Meta File (vector graphic format - default file extension: emf) • Scalable vector graphics (vector graphic format - default file extension: svg)
Width / Height	Define the desired width and height (in pixels) of the graphic.
save graphic as file	If checked, the graphic will be saved in to the file, entered in the file name field (including the desired directory). Use the ... button to select a directory.
copy graphic to the system clipboard	If checked, the graphic will be copied into the windows clipboard and can be pasted (use the key combination Ctrl+V) in any other application that allows to import graphics

3.9 General Chart Options

The *General Chart Options* window lets you change the default settings for charting in the MARS. The window can be opened by clicking the *Charts*command in the *Settings* group of the *Formats and Settings* tab, open the MARS options menu and select the *Charts* section, or press the *Edit Default Settings* button on the settings dialog of the different charts.



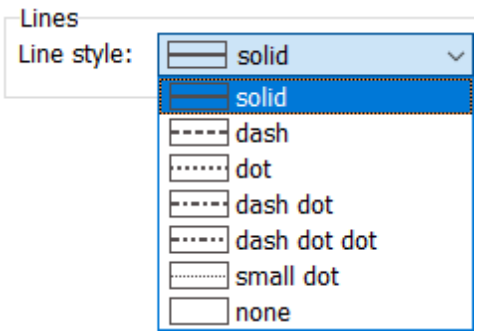
The default settings will be used for new created charts or if the *Reset Settings* button is pressed on the charts settings dialog.

3.9.1 Default chart Font Settings

For different sections in the chart default font settings can be defined here. Click on the *Change...* button for the according chart section the font settings should be changed. A dialog will open up where the font and its attributes can be selected. After applying the dialog, a preview of the selected settings is shown.

3.9.2 Common Chart Line and Point Settings

Line style	Select the default style of the line between the data points:



Width	Enter the desired default line width for series lines between data points
Point size	Enter the desired size of the data point shape in pixel.

3.9.3 Common Chart Axis Settings

Define the default settings for the visibility, color, style and width of axis lines, ticks and grids .

3.9.4 Common Chart Settings

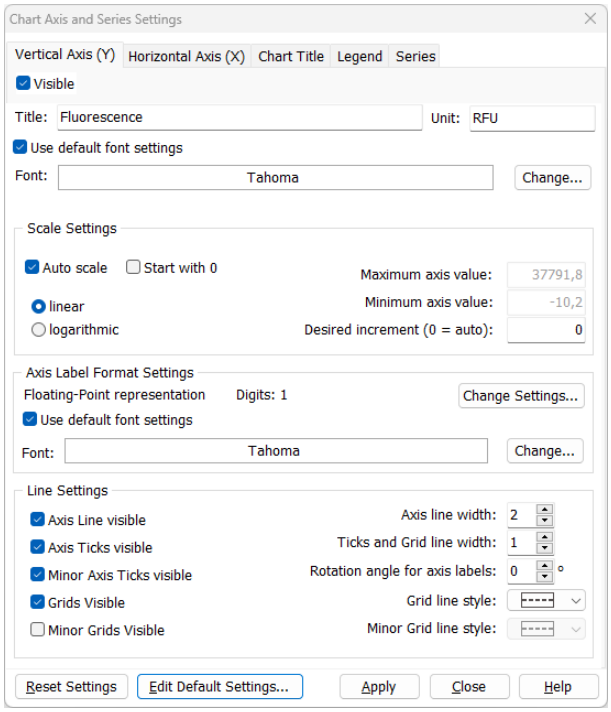
Show legend border	If checked, a border around the legend box is drawn.
Legend border line style / Legend border width	If *Show legend border* is checked, define the style and the width of the border
Legend background	Define the background color of the legend
Chart background	Define the background color of the chart.
Display not highlighted data curves	Define how data curves are displayed if they are not highlighted (see the *Highlight Active Data* setting in Common Chart Functions). ° in gray: all curves except the highlighted one are displayed in gray ° in dimmed color: the curve will be drawn with dimmed color if the curve is not highlighted. With the slider you can define if the color should be dimmed soft or strong.
Used color palette for chart series	Select between the offered color palettes to define the used colors for chart series.

3.10 Change Settings of a Chart Axis

If you want to change settings of any chart (like the scaling of an axis) just click on the appropriate axis in the chart. Alternatively, you can click the *Chart Settings* button above the chart and select the tab of the axis you want to change.
The *Chart Axis and Series Settings* dialog box opens and lets you customize all settings of the axis. Select the group of axes you want to customize with one of these tabs:

- Vertical Axis(Y): all Y axes (one or more)
- Horizontal Axis(X): the X axis (only one)
- Depth Axis (Z): the Z axis (only available in spectrum 3D charts).

:



Note: Changes will be overtaken only, if you press the *Apply* button or if you change the axis group page. To reset all applied changes to default settings, press the *Reset to Default Settings* button.

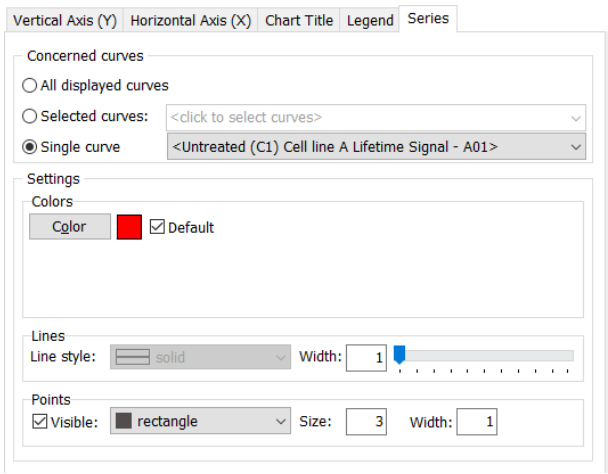
You can change the following attributes:

Visible	Uncheck this control if you want to hide the axis.
Select axis	This entry field is only shown if the Vertical Axis(Y) group was selected. Select the Y-Axis you want to customize.
Title/Unit	Displays the title of the axis. To change the title, enter the new title in this field. Enter a unit into the unit field. If a unit is entered the title will be supplemented with 'in *Unit*' (where *Unit* is replaced by the entered unit).
Font	Shows the used font for the axis title. Click Change to change the font. If *Use default font settings* is checked the default axis title font is used (see General Chart Options).
Scale Settings	Controls in this group box define the scaling settings of the axis.
Auto scale	If this control is checked, the axis calculates the minimum and maximum value automatically. The calculation is based on all visible curves in the chart. The entry fields for the maximum and minimum values are then disabled.
Start with 0	Is only enabled when *Auto scale* is checked. If it is checked, the axis scaling starts always with 0. Only the maximum value is calculated automatically. **Note:** If the values of all visible curves are less than zero, the maximum value is zero and the minimum value is calculated automatically. **Note:** If the scaling of the axis is logarithmic, Start with 0 is disabled (because log10 (x) is only valid for x>0).
Maximum axis value **Minimum axis value**	If *Auto scale* is not checked, you can enter the desired maximum and minimum value of the axis here.
Desired increment	Enter the increment value for the axis scaling here. The chart uses that increment value as minimum step between axis labels. The chart will use this value as the starting axis labels step. If there is not enough space for all labels, a bigger one will be calculated. The value must be a positive number. If you enter 0, the chart tries to find the best increment step. **Note:** If the axis scaling is logarithmic, the increment value will be calculated automatically and cannot be entered.
linear / logarithmic	Change between linear or logarithmic axis scaling. The logarithmic axis scaling is based

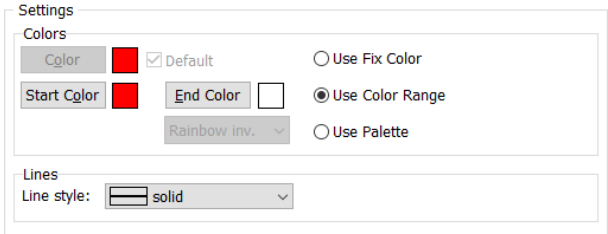
	on 10. If the axis scaling has values less than 0, a logarithmic scaling is not possible.
Axis Label Format Settings	Displays the current number format settings for the axis labels. To change the settings, press the *Change Settings* button. How to change the number settings is described in chapter 3.25.6: *Number Format Settings for Data Nodes and Chart Axes*. The format settings for time values (X-Axis of the signal curve) are defined in the global time format options.
Font	Shows the used font for the axis labels. Click Change to change the font. If *Use default font settings* is checked the default axis labels font is used (see General Chart Options).
Line Settings	Define the visibility, style and width of axis lines, ticks and grids .

3.11 Change Chart Curve Settings

The series settings page lets you customize the settings of any curve in the chart. Click on the *Chart Settings...* button above the chart and select the *Series* tab:

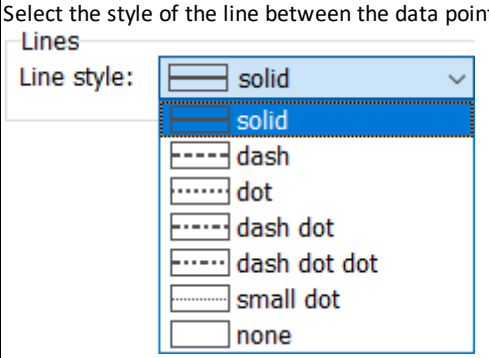
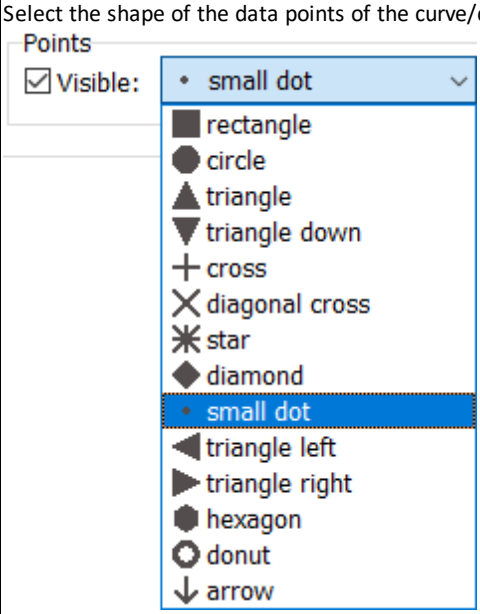


For three-dimension charts (3D charts) the settings part of the window looks different:



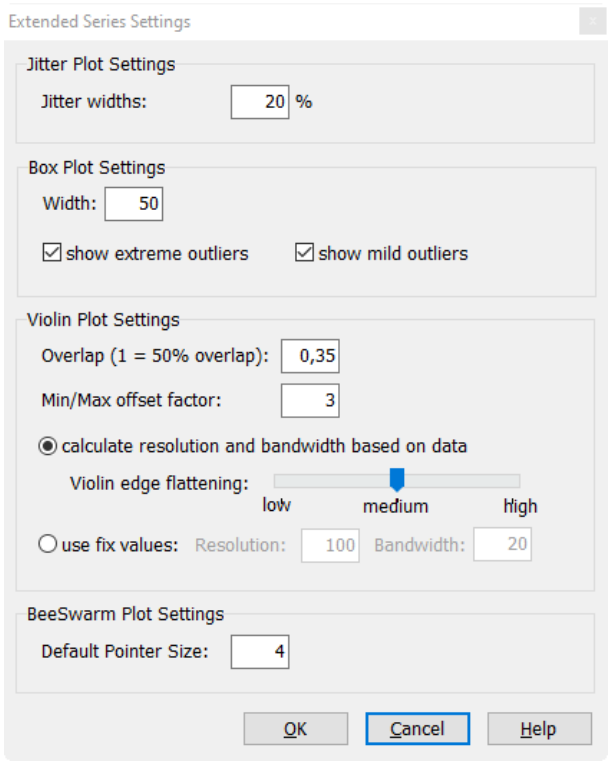
Select the curve or the group of curves you want to change:
If you want to make changes for more than one curve, select the *All displayed curves* radio button to change the settings for all curves currently displayed in the chart or select *Selected curves* to apply the settings only to a subset of the curve in the chart. If *All displayed curves* or *Selected curves* is selected, a check box appears at the top of each settings group. Click the check box of the kind of settings (color, line or point style) you want to change for the selected curves.
If *Selected curves* is selected, you can select the curves with the drop-down list named: <click to select curves>. A list with all available curves is displayed, with a check box behind each curve. Use the mouse to select the desired curves. Click with the left mouse button on a curve entry to select or deselect the curve.
If you want to change the settings of a single curve, click the *Single curve* radio button and select the curve you want to change using the drop-down list control beside the radio button.
The changes will be overtaken only if you press the *Apply* button of the dialog box.
Changeable settings:

Color	Define the color of the line and data points. If *Default* is checked, the color is selected automatically (only available for 2D charts).
For 3D charts only:	
Use Fix Color	Select this control to define a single color for the shape. If *Default* is checked, the color is selected automatically.
Use Color Range	Select this control to define a color range for the shape from a start color to an end color.
Start Color	Define the start color of the three-dimension shape color range. The color of the

	shape fades from the start color for the highest values to the end color for the lowest value (only available for 3D charts).
End Color	Define the end color of the three-dimension shape color range. The color of the shape fades from the start color for the highest values to the end color for the lowest value (only available for 3D charts).
Use Palette	Select this control to define the color for the shape based on a color palette.Use the drop-down control to select the desired color palette.
Lines	Defines the look of the line between data points.
Line style	Select the style of the line between the data points: 
Width	Enter the desired line width or adjust the desired line width with the track control (not available for 3D shapes).
Points	Defines the look of the data points of the curve/data series (not enabled for calculated curves like the standard curve and not available for 3D charts or bar charts).
Visible	Data points are visible if this check box is checked.
Point shape	Select the shape of the data points of the curve/data series: 
Size	Enter the desired size of the data point shape in pixel (not for small dot).
Width	Enter the desired line width of the data point shapes border line or the data point shapes draw line (depending on the selected shape, but not for small dot).

3.11.1 Extended Series Settings (compare group plots only)

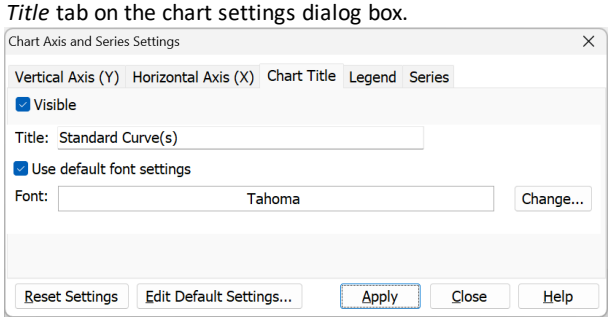
If the dialog was opened for compare group plots, you can change special plot settings if you press the *Extended Series Settings* button:



Jitter Plot Settings:
Jitter widths (%): the points will be distributed inside the entered percentage area of the available area for the group (the available area depends on the number of displayed groups and the size of the chart).
BoxPlot Settings:
Widths (%): defines the default widths of the box (can be changed for each box with the series points width field above.
show extreme outliers: if checked, extreme outliers will be shown in the box plot
show mild outliers: if checked, mild outliers will be shown in the box plot
Violin Plot Settings:
Overlap: defines the allowed overlapping factor between different violine plots. A lower value separates the different plots more than a higher value.
Min/Max offset factor: a low factor can lead to a cut of at the bottom and the top of the plot. A higher factor stretches the violins upper and lower part.
calculate resolution and bandwidth based on data or use fix values: the bandwidth and the resolution defines the shape of the violin. In general the best is to let MARS calculate this values automatically. Try to change the parameters and see the influence of the shape.
BeeSwarm Plot Settings:
Default pointer size: defines the default size of the bee swarm dots (can be changed for each plot with the series points width field above.

3.12 Chart Title Settings

The Title and the used font of the chart title can be changed. Click on the *Chart Settings...* button above the chart you want to change and select the *Chart Title* tab on the chart settings dialog box.



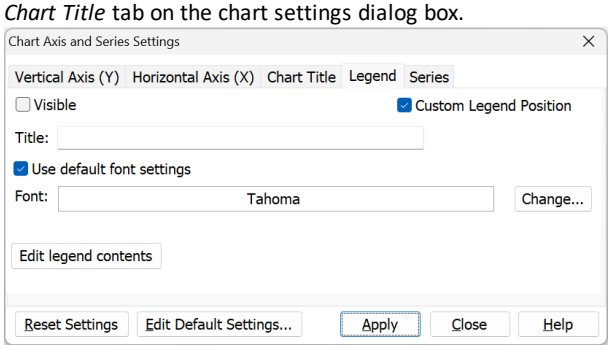
Note: Changes will be overtaken only, if you press the *Apply* button. To reset all applied changes to default settings, press the *Reset to Default Settings* button.

Changeable settings:

Visible	If checked, the chart title is visible.
Title	Change the chart title here.
Font	The used font is displayed here. To change the font, click the *Change...* button. If *Use default font settings* is checked the default legend font is used (see General Chart Options).

3.13 Chart Legend Settings

The title and the used font of the chart legend can be changed. Click on the *Chart Settings...* button above the chart you want to change and select the *Chart Title* tab on the chart settings dialog box.



Note: Changes will be overtaken only, if you press the *Apply* button. To reset all applied changes to default settings, press the *Reset to Default Settings* button.

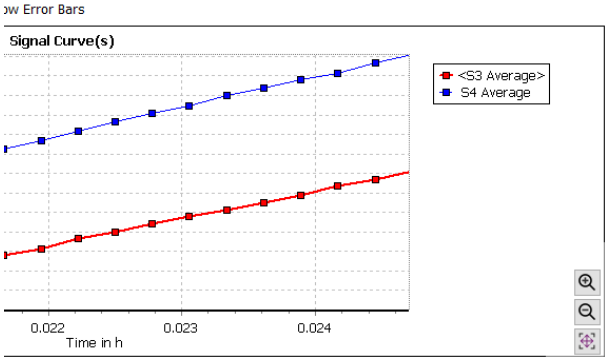
Changeable settings:

Visible	If checked, the chart legend is visible.
Custom Legend Position	Define the position of the legend (see section below)
Title	Change the legend title here or leave it empty if no title is wanted.
Font	The used font is displayed here. To change the font, click the *Change...* button. If *Use default font settings* is checked the default legend font is used (see General Chart Options).
Edit legend contents	Click this button to change the entries of the chart legend. Details are described in Common Chart Settings, chapter Changing chart legend entries .

If the *Custom Legend Positioning* is checked, the legend is drawn upon the chart and can be moved to any position over the chart using the mouse (move the mouse cursor over the chart to drag it and release the mouse button to drop it at the desired position).
If the check box is unchecked, the legend will be displayed beside the chart (defaulting to the right side of the chart). The chart size will be reduced so that the legend and the chart will both fit on the screen. You can change the position of the legend to the four places:

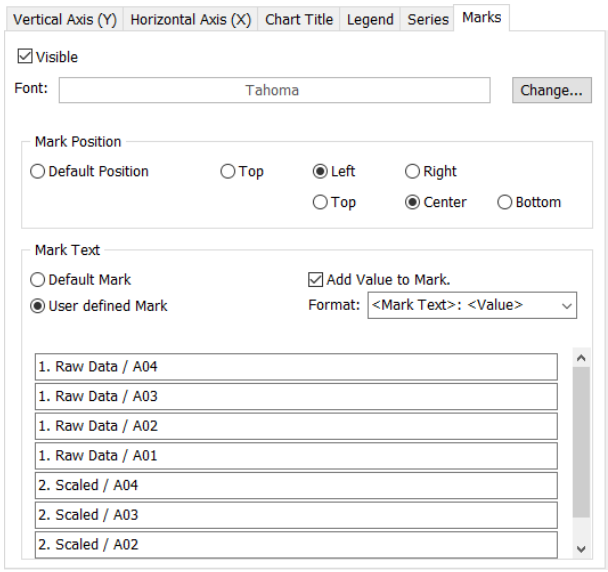
- on top of the chart
- left side of the chart
- right side of the chart
- under the chart

Change the position using the mouse by dragging it to the new position and then drop it.



3.14 Bar Chart Marks Settings

The marks settings page lets you customize the settings of the displayed marks for each bar in a bar series of a bar chart. Click on the *Settings...* button above the chart and select the *Marks* tab:



Note: Changes will be overtaken only, if you press the *Apply* button. To reset all applied changes to default settings, press the *Reset to Default Settings* button.

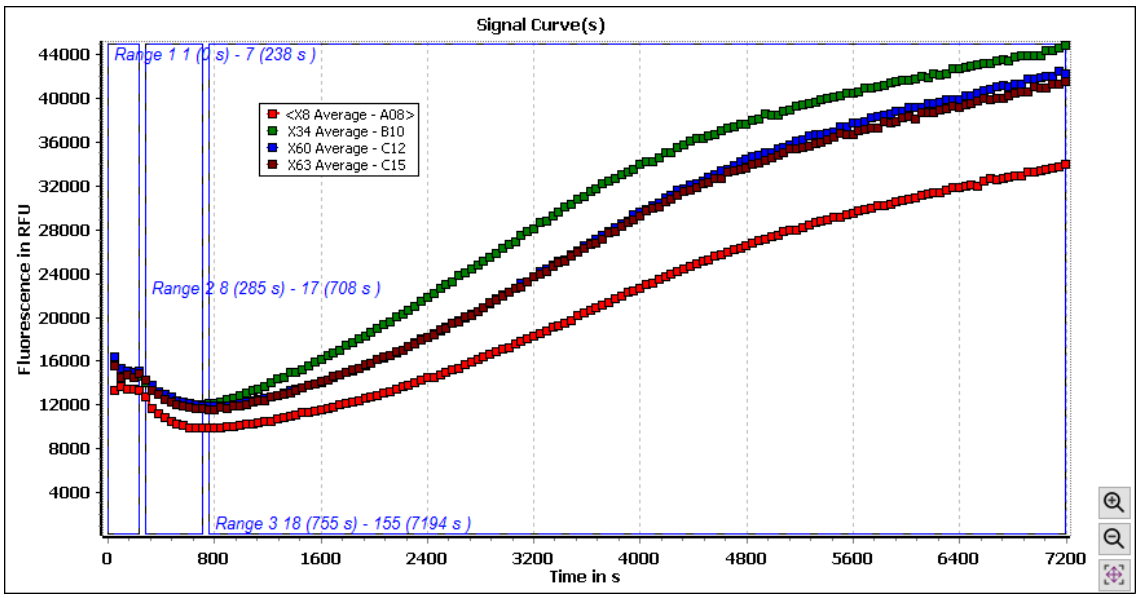
Changeable settings:

Visible	If checked, the marks are visible.
Font	The used font is displayed here. To change the font, click the *Change...* button
Mark Position	Change the position, where the mark is drawn. You can choose between these positions: • *Default Position*: the software decides, depending on the chart, where the mark is drawn • *Top*: on the top of the bar • *Left*: on the left side of the bar • *Right*: on the right side of the bar. If *Left* or *Right* is selected, you can define if the mark is drawn on the top end, in the middle or on the bottom end of the bar.
Mark Text	Define the text, displayed as marks: • *Default Mark*: the software decides, depending on the chart, the content of the mark. If *Add Value to Mark* is checked, the numeric value of the bar is added to the mark text. Define with the format list, how the value and the mark text should be combined. • *User defined Mark*: Enter an individual text for each mark of each bar.

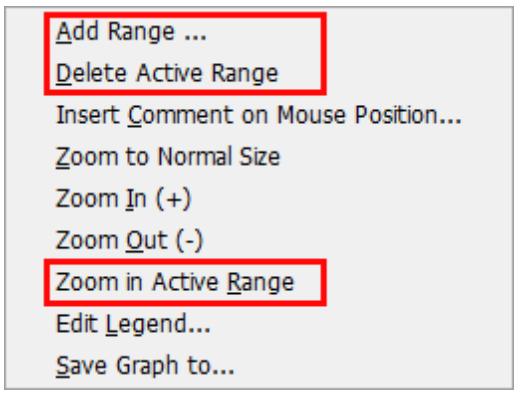
3.15 Signal Curve

In case your test run contains kinetic data, it can be viewed by clicking on the *Signal Curve* tab in the working area. This view can also be obtained by double clicking on a well in the *Microplate View*, when the curve view mode is active.
The signal curve chart will plot the kinetic data for all data nodes selected in the navigation tree against time. Only the data of wells either selected in the *Microplate View* or in the content filter tree, will be displayed. The data points of one well in the chart are connected by a thin linear line. The line can be removed in the curve settings window.

Note: If there is no node or well selected the working area will appear empty!



In addition to the common chart functions (zooming, crosshair function, axis scaling...), the chart for the signal curves will also display user defined ranges. Each range is shown as a dashed blue rectangle with the name and the start and stop loop iteration as a caption. If the range represents a baseline section of the kinetic (see chapter 4.4.4: *Baseline Corrections*), the border of the range will be red. To select a range, click onto the range using the mouse, the selected range will then change to a non-dashed bold rectangle. It is only possible to select one range at a time.
If performing the kinetic calculation *Time to threshold*, a red horizontal line will appear in the chart to mark where the threshold position is on the Y axis.
If performing the kinetic calculation *Maximum of slope*, a line with that slope in the same color as the series will appear. The line intersects the series at the time position of the maximum slope.
The pop-up menu for the signal curve chart is expanded to include special range functions (see also the section 3.11.1: *Range Functions in the Chart*):



To zoom in the chart using the mouse as described in the chapter 3.6.5: *Zooming*, the *Mouse Zooming* mode must be activated using the respective buttons available under the chart. Whilst using the zooming feature the ranges selected will be hidden, to see the ranges again, they can be activated by clicking on the *Show Ranges* button.

Note: The 'Mouse Zooming mode' can also be used to temporarily hide the ranges, if a better look of the data needed.

3.15.1 Range Functions in the Chart

The chart can be zoomed to the size of the active range using the *Zoom in Active Range* function found in the charts pop-up menu. This will show only the data lying within the defined range (including the range borders).
To change a range in the chart, you first have to select it. In the next three sections it is explained how to change the ranges in the chart directly. In addition, you can view, add and change ranges with the range window.
The Change Range button under the chart opens the range window as well as the *Ranges* button in the *Data Reduction* group on the *Home* tab of the ribbon. (See chapter 4.1: *Ranges*).
Changing Range Position
Move the mouse cursor into the active range (the mouse cursor will then change to a small hand point). Click into the range using the right mouse button and hold the button. Move the mouse and the range will follow the mouse. Leave the mouse button when the range has reached the desired position.
Changing Range Size

To change the size of the active range (changing the first or final loop iteration used in the range) you must first move to the border line that you would like to change. When the mouse is over the border, the mouse cursor will change to an icon with two arrows (<->). Click the left mouse button and hold it, moving the mouse to the position of the range start / stop iteration number and release the mouse button.

> **Note:** If a range is changed that has already been used to perform any calculations (like a kinetic calculation), the calculations will be updated in line with the new range details. This will in turn also influence any calculation based on the updated calculations. Recalculation may mean that there is a small delay after adjusting the range borders (this will be indicated with a message box).

Adding and Deleting Ranges

When opening a kinetic test run for the first time, at least one range will be created by default (read more about predefined ranges in the chapter 4.1.1: *Predefined Ranges*). If you want to add a further range press the New Range button or select the *Add Range* item from the pop-up menu by pressing the right mouse key in the active chart.

The default range shown in the chart will be already selected. Its borders are set from the first iteration to the last iteration, the borders and their position can then be changed as described in the Changing Range Size and Changing Range Position sections.

To delete a range, select the *Delete Active Range* item from the pop-up menu. A range that has been used to perform a calculation will not be deleted unless the calculation is deleted first.

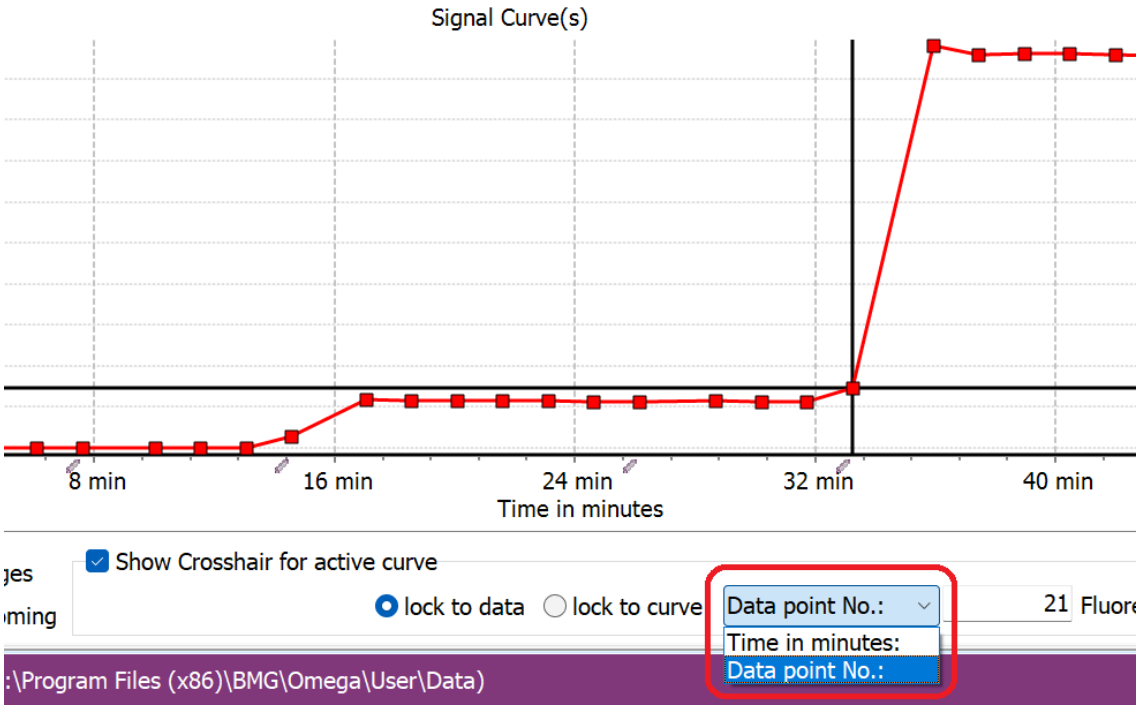
3.15.2 Show Error Bars

Above the chart there is a further control available to show error bars in the chart. The check box can be checked, if replicates are defined in the layout and the displayed signal curve is based on an average calculation over replicates.

If the error bar check box is enabled and checked, you can see a hint on the right side of the check box. It shows the current setting for the error bar calculation. Clicking on the hint opens the Calculation Settings dialog where you can change the settings for the error bar calculation.

3.15.3 Show data point number on crosshair position

The crosshair (if activated, see: common chart functions (crosshair function)) for signal charts can display either the time value or a data point number of the crosshair position. To change between time value and data point number, select the desired setting with the drop down control behind the time entry field in the crosshair section:



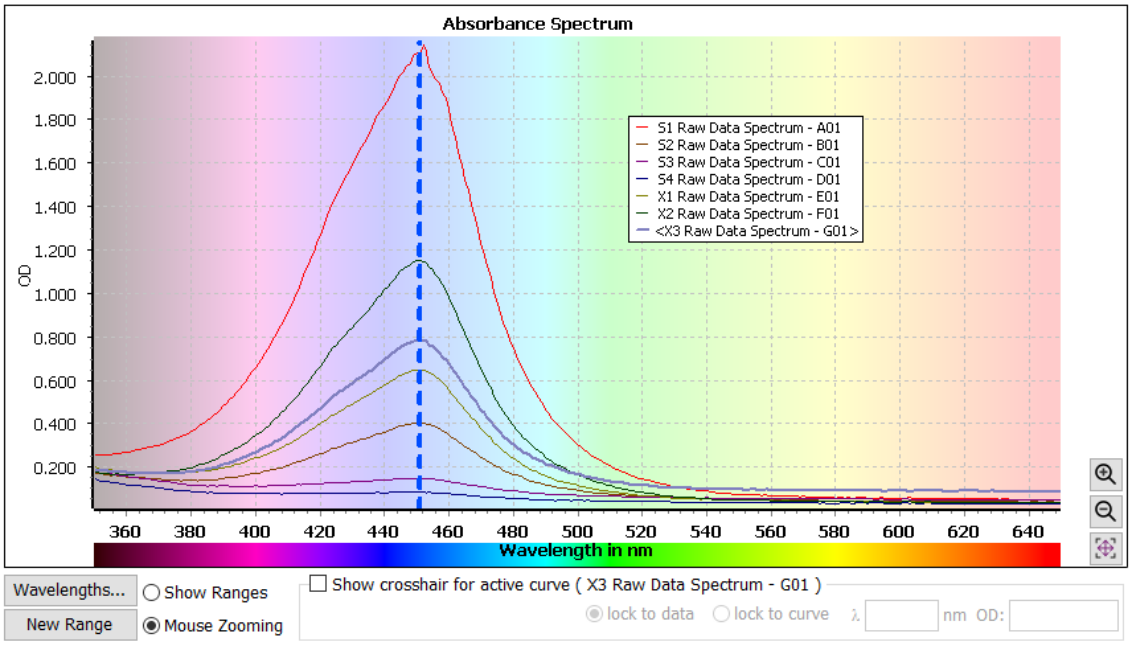
If you changed the settings to display data points, you can enter a data point number into the entry field to set the crosshair position to the entered data point.

3.16 Spectrum Curve

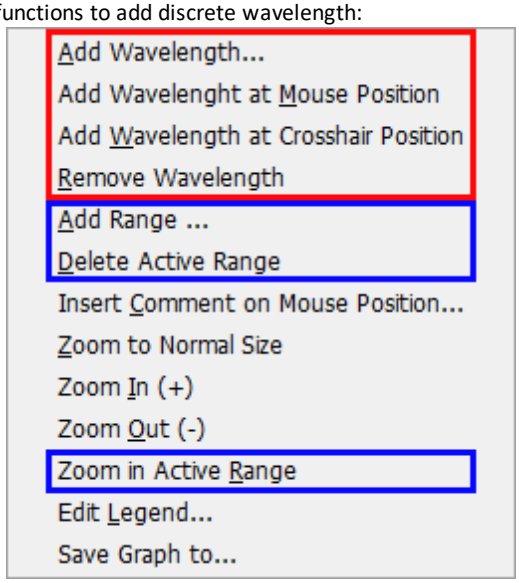
The spectrum curve chart will be available if your test run contains spectra data. The chart can be accessed by clicking on the *Spectrum* tab on the working area or by double clicking on a well in the Microplate View, if the spectrum view mode is active.

The spectrum curve chart plots the spectrum data of all selected spectrum nodes in the navigation tree against lambda (wavelength). Data will be shown for all wells which have been selected in the Microplate View or in the content filter tree. The data points of one well are connected by a thin line. It is possible to remove the line using the Curve Settings window.
For each added discrete wavelength, a dashed vertical line at the position of the lambda value on the X axis will be displayed. The color of the dashed line relates to the natural color of the lambda value. If you don't want to see the lines shown in the lambda-color you can display them in gray by changing the corresponding setting in the Spectrum Display Settings window.

Note: If no well or node is selected, the working area appears empty!



The background of the chart is lightly colored according to the lambda values of the X axis. To change the intensity of the background or to select a white background, use the Spectrum Display Settings window.
In addition to the common chart functions (zooming, crosshair function, axis scaling...) it is also possible within this chart to add discrete wavelength data to the test run (see next section: Adding a Discrete Wavelength) and the chart will also display user defined ranges.
Each range is shown as a dashed blue rectangle with the name and the start and stop wavelength as a caption. To select a range, click onto the range using the mouse, the selected range will then change to a non-dashed bold rectangle. It is only possible to select one range at a time.
If performing the spectrum calculations *Local minima*, *Local maxima* or *Inflection points*, a black vertical line for each calculated minima, maxima of inflection point at its wavelength position will be displayed in the chart.
The pop-up menu for the spectrum curve chart is expanded to include special range functions (see also the section 3.9.1: *Range Functions in the Chart*) and functions to add discrete wavelength:



To zoom in the chart using the mouse as described in the chapter 3.6.5: *Zooming*, the *Mouse Zooming* mode must be activated using the respective buttons available under the chart. Whilst using the zooming feature the ranges selected will be hidden, to see the ranges again, they can be activated by clicking on the *Show Ranges* button.

Note: The 'Mouse Zooming mode' can also be used to temporarily hide the ranges, if a better look of the data needed.

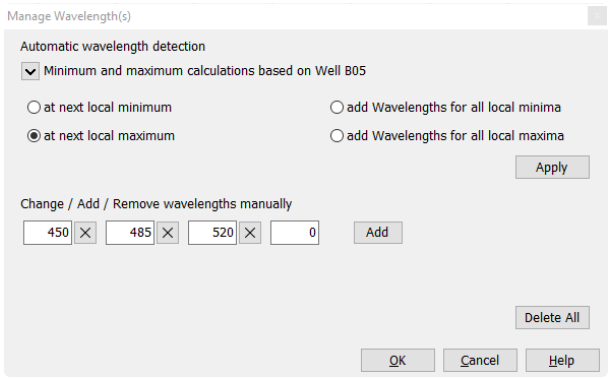
Read more about range functions in the chart in the appropriate section in the signal curve chapter as the described function is also valid for the spectrum curve (section 3.11.1: *Range Functions in the Chart*).

3.16.1 Adding, Changing or Removing Wavelengths

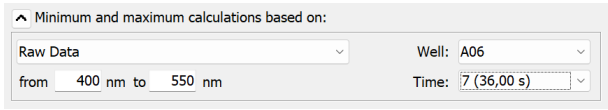
Note: At least one discrete wavelength from a spectrum will be needed to perform calculations other than spectrum calculations or spectrum kinetic calculations, as these calculations are not possible using the full spectrum data.

Note: If there is a combined excitation and emission scan, each discrete wavelength is assigned to either the excitation OR the emission scan!

To add one or more new discrete wavelength to the test run, press the *Wavelengths...* button shown under the chart or open the pop-up menu and select one of the offered items *Add Wavelength*, *Add Wavelength at Mouse Position* or *Add Wavelength at Crosshair Position*. You can add as many wavelengths as needed (The maximum number allowed = the total of all measured data points).
If *Add Wavelength at Mouse Position* or *Add Wavelength at Crosshair Position* was selected, a wavelength will be added at the current x-axis position of either the mouse or the vertical crosshair line.
The following window will be shown if the *Wavelengths...* button or *Manage Wavelengths* in the *Data Reduction* group of the *Home* ribbon menu was pressed:



The window offers two ways to manage wavelengths:
1. Automatic wavelength detection:
If you want to add one or more wavelength at maxima or minima of the selected spectrum curve, select the according radio control and press the *Apply* button. The selected spectrum curve can be changed if you press the  button and open the spectrum curve selection control:



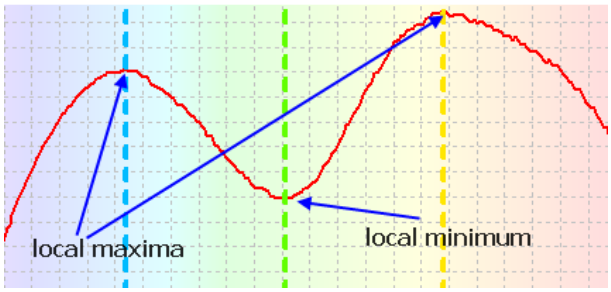
The control is closed if you open the window from the spectrum curve page with a selected spectrum curve. If you open the window from any other page, using the menu entry *Manage Wavelengths* in the *Data Reduction* group of the *Home* ribbon menu, the control is already opened.
The *from* and *to* Lambda entry fields are used to define the range on which the minima and maxima calculations are based on. If the spectrum chart is visible, the current X-Axis settings are used as preset values for this range.
The following calculations are based on the selected spectrum curve:

at next local minimum:	Searches for the minimum value of the active spectrum curve in the defined lambda range. If there is a discrete wavelength selected already at that point, the next higher minimum will be found and selected.
at next local maximum:	Searches for the maximum value of the active spectrum curve in the defined lambda range. If there is a discrete wavelength selected already at that point, the next lower maximum will be found and selected.
add Wavelengths for all local minima	Tries to find out all minima of the active spectrum curve.

Adds a wavelength for each found local minima. A local minimum is defined as a significant lower value between higher values.

add Wavelengths for all local maxima

Tries to find out all maxima of the active spectrum curve. Adds a wavelength for each found local maxima. A local maximum is defined as a significant lower value between higher values.



The added wavelengths will appear in the *Change / Add / Remove wavelengths manually* section and can be changed there:
2. Change, Add or Remove Wavelength(s):
For each discrete wavelength already added, an entry field and a delete button are shown. The value can either be changed or the discrete wavelength can be removed from the test run with the delete button.

An empty entry field is show (with no delete button behind) to enter new discrete wavelength. After pressing the add button, the wavelength will be added, and the empty entry field can be used to enter further wavelengths. If the crosshair is visible in the spectrum chart, the X value of the crosshair can be used as default value and the empty entry field is preset with this X value. To delete all wavelengths at once, press the *Delete All* button.

Note: Press the OK button to apply the changes to the test run.

3.16.2 Change the Lambda Value of a Discrete Wavelength

It is possible to change the lambda value of an added wavelength directly in the chart:

- Move the mouse over the wavelength line in the chart, the mouse cursor will then change to two arrows (<->).
- Click and hold the left mouse button.
- Move the line to the new position and release the mouse button.

Alternatively you can use the *Manage Wavelength* window (see chapter 3.12.1: *Adding, Changing or Removing Wavelengths* above) to change the lambda value of one or more added wavelength(s) at once.

Note: If a wavelength is changed that has already been used to perform any calculations, the calculations will be updated in line with the new wavelength details. This will in turn also influence any calculation based on the updated calculations. Recalculation may mean that there is a small delay after adjusting the wavelength (this will be indicated with a message box).

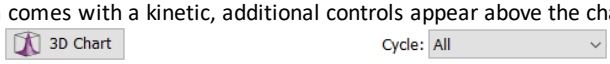
3.16.3 Deleting a Discrete Wavelength

To delete a discrete wavelength, even after performing calculations, open the pop-up menu of the spectrum chart and select the menu item *Remove Wavelength*, or select the raw data node for that wavelength in the navigation tree and press the *Del* key on your keyboard (this is the same as selecting the *Delete* menu item in the navigation tree pop-up or the *Delete Node* button in the *Navigation* group on the *View* tab of the ribbon.

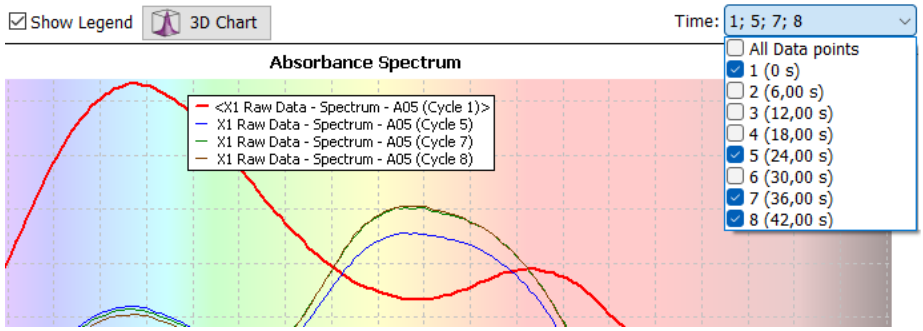
To delete more than one wavelength at once, you can also use the *Manage Wavelength* window (see chapter 3.12.1: *Adding, Changing or Removing Wavelengths* above).

3.16.4 Spectra and Kinetics

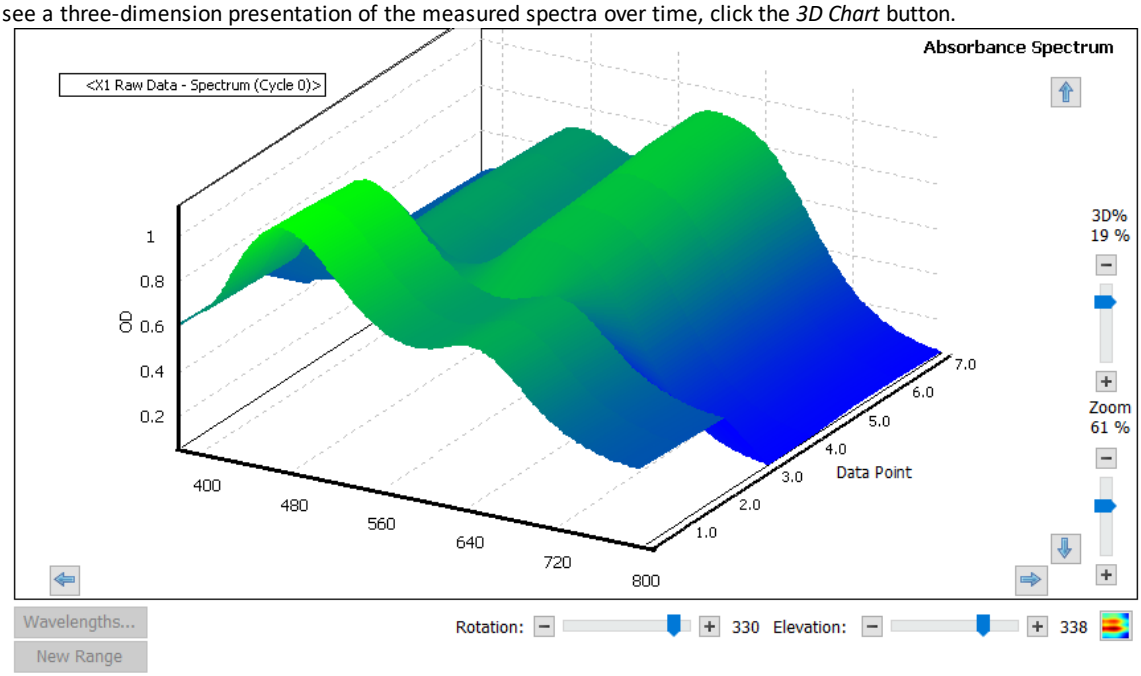
If the measurement of spectra comes with a kinetic, additional controls appear above the chart:



Select the displayed spectra for certain time values with the drop-down menu on the top right side. You can select one data point, all data points or only certain data points to compare them in one chart.

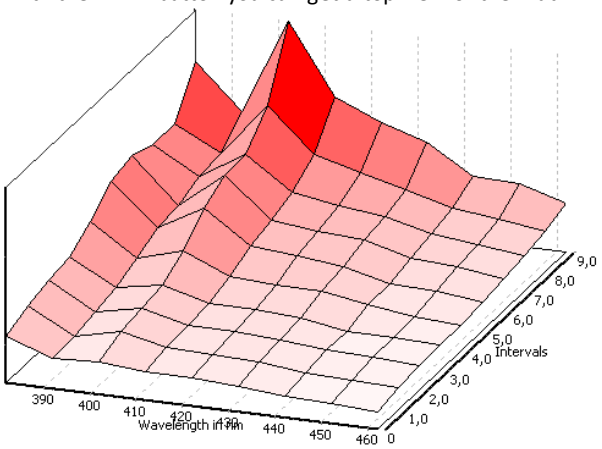


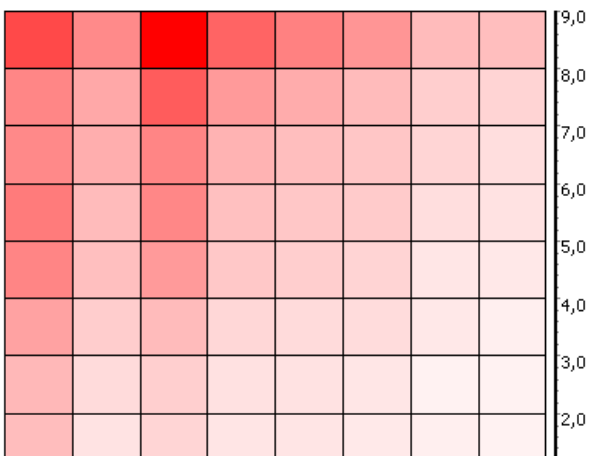
To see a three-dimension presentation of the measured spectra over time, click the *3D Chart* button.



Use the controls *Zoom*, *Rotation* and *Elevation* to adjust the chart to the desired position. With the *3D%* control, the depths of the chart can be adjusted.
Use the arrow buttons to move the chart.

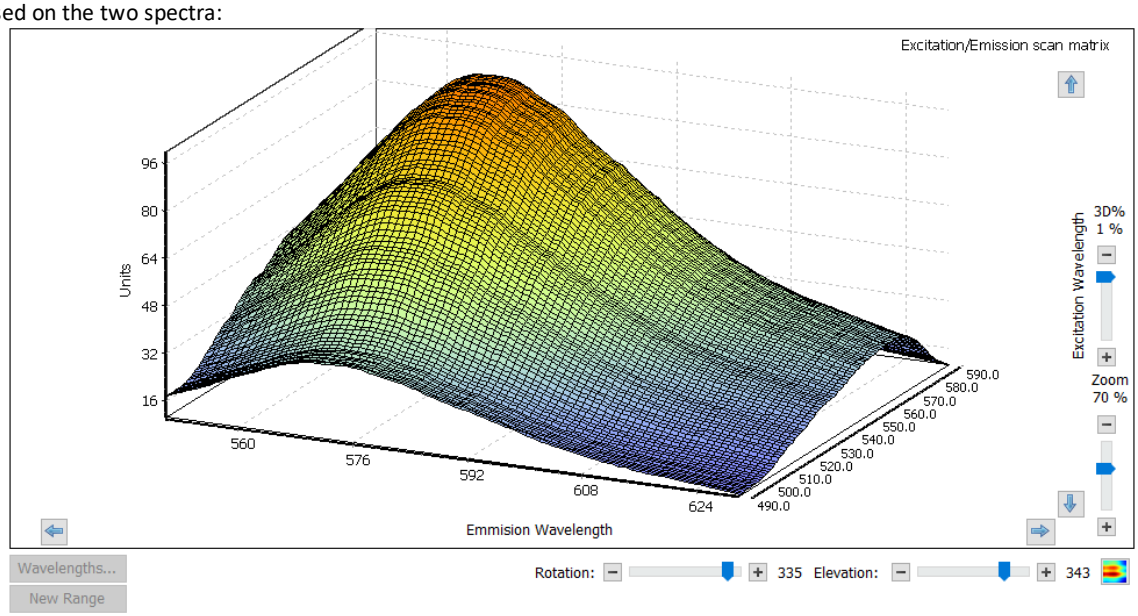
With the  button you can get a top view of the matrix:





3.16.5 Combined excitation and emission spectra

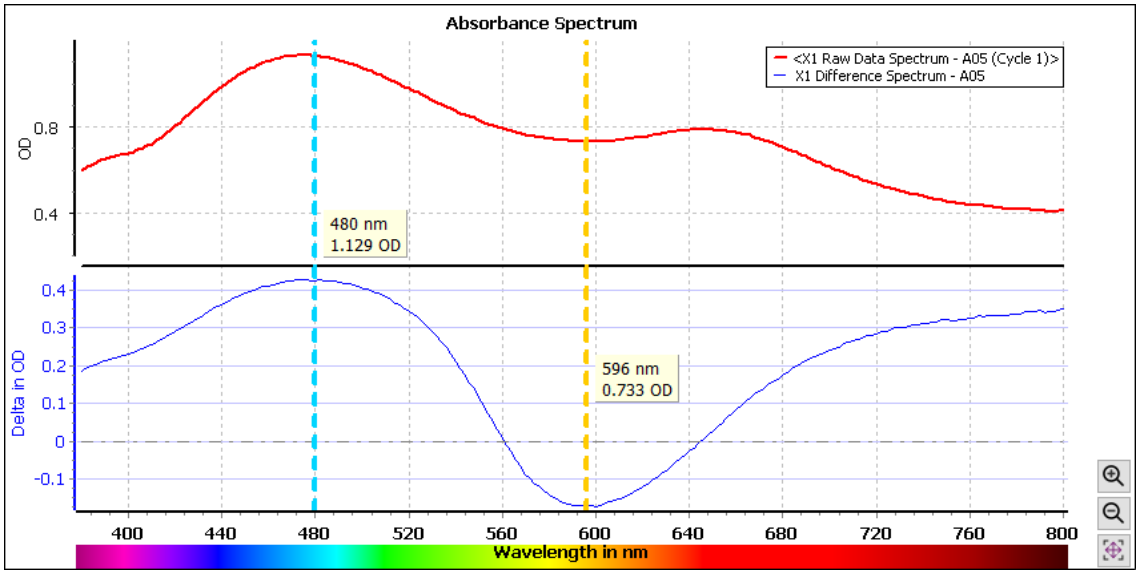
If the measurement is an excitation and emission scan, you can also change to a three-dimension presentation where one axis is the excitation wavelength and the depth axis is the emission wavelength. Thee three-dimension matrix is calculated based on the two spectra:



Note: In the three-dimension mode, the add wavelength function is disabled and the lines for already added wavelength are hidden.

Calculated Spectra

For each performed spectrum calculation type (difference or ratio) between two kinetic data points, an additional chart with its own Y axis is drawn:



Export Fluorescence and Luminescence Spectra

Note: This section is for readers with a build in monochromator only!

Fluorescence and Luminescence Spectra can be exported to a fluorophore database associated to the current user. The fluorophores in this database can be used to set up the monochromator of the CLARIOstar or the VANTAstar.

To export an excitation or emission scan, select the desired scan curve in the spectrum chart and click the Export Fluorophore button above the chart. Read more details about exporting scan curves in the chapter 3.13: Export a Fluorophore.

3.16.6 Export a Fluorophore

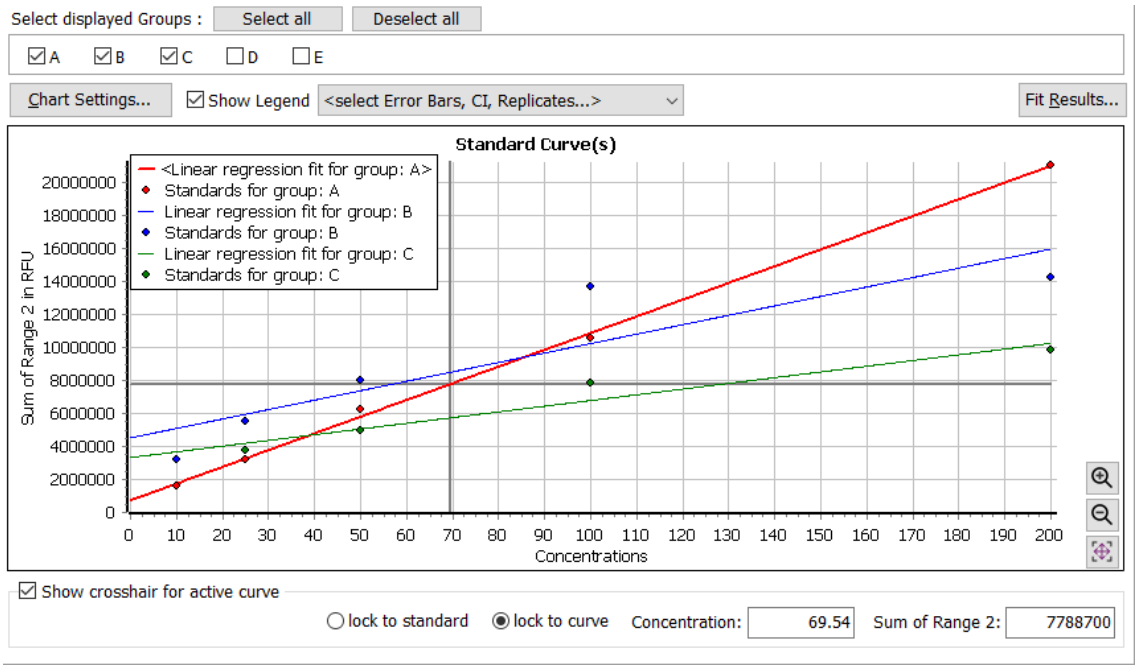
Fluorescence and Luminescence Spectra can be exported to a fluorophore database associated to the current user. The fluorophores in this database can be used to set up the monochromator of the CLARIOstar or VANTAstar.
To export an excitation or emission scan, select the desired scan curve in the spectrum chart and click the Export Fluorophore button above the chart. A dialog opens and you need to enter a name for your fluorophore. If the data come from a combined excitation and emission scan or it is an emission curve from a luminescence test run, the data will be added automatically to the fluorophore database. If you have only a single excitation scan curve or a single emission scan curve (fluorescence test runs only) you will be asked to enter a file name and a location where the data will be saved. The type of a file name is *.BCF. This is the internal format to import data into the fluorophore database. After you've saved the excitation curve in the BCF file, you can open the associated emission scan and export this scan curve into a second *.bcf file. After exporting the data, you get a confirmation that the export succeeded. If you click on the *'Open Toolbox'* button in this confirmation dialog, you will be asked to select both created files and they will be imported into the fluorophore data base as one fluorophore.
The exported data will be scaled automatically to 0-100%.

To see all the individual fluorophores of the current user, click on the button to open the fluorophore manager.
Read more about managing fluorophores in the chapter 4.3.6: *Fluorophore Manager* of the SMARTControl Software Manual Part II.

3.17 Standard Curve

The standard curve chart option will be available if you have performed a standard calculation or a parallel line analysis calculation. To view the chart, click on the *Standard Curve* tab in the working area.
The standard curve chart will plot the fit result curves and the standards for all selected standard fit and parallel line analysis nodes in the navigation tree against concentration. The color and style of the curves and standards can be changed using the Curve Settings window.

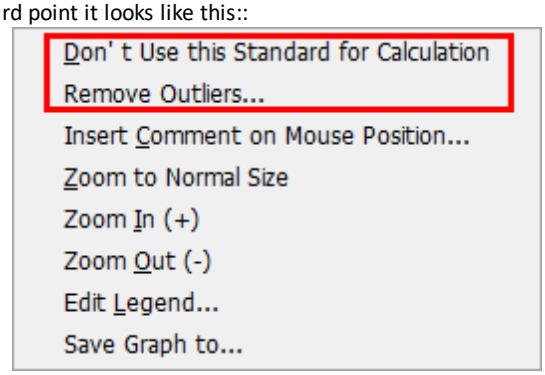
Note: If nothing is selected, the working area will appear empty!



When the standard curve chart is visible, the detailed window under the navigation tree will be visible instead of the content filter tree. The detailed window shows the fit parameters and fit results of the selected data node in the navigation tree.

To see the fit results of all displayed standard curves, open the fit result window with the Fit Results... button on the top right corner of the chart.
In addition to the common chart functions (zooming, crosshair function, axis scaling...), the standard curve chart has a check box bar to select or deselect the fit results for single groups. This bar will appear only if groups were defined in the layout. To select all groups or to deselect all groups with one click, use the two buttons *Select All* and *Deselect All* above the chart.
Check or uncheck the box of the group you want to see or hide.
If more than six groups are defined, the check boxes are replaced by a drop-down menu to select the desired groups.

The pop-up window of the standards curve comes with additional entries to modify the fit. If the pop-up is opened when the mouse cursor is over a standard point it looks like this::



Don't Use this Standard for Calculation

If the mouse position is over a data point of a standard, this item is active and can be used to remove this standard from the fit calculation. All replicates of this standard will be toggled out (this can be seen on the *Microplate View*).

Remove Outliers...

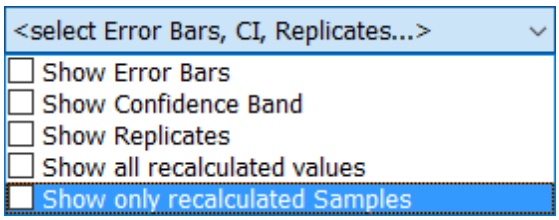
If you have more than 5 replicates for the standards of the selected standard curve, you can find and remove outliers. Read more about the used outlier detection method in chapter 3.26: *Outlier Detection*.

If the pop-up is opened, when the mouse cursor is over a fit curve, it allows you to change the fit model directly.
If the chart contains a 4 or 5-Parameter standard fit and the crosshair is shown, you can see the EC or IC value of the crosshair position beside the Y value of the crosshair below the chart. Enter the EC or IC value you want to see, and the crosshair will move to that position (if it is in the charts range).

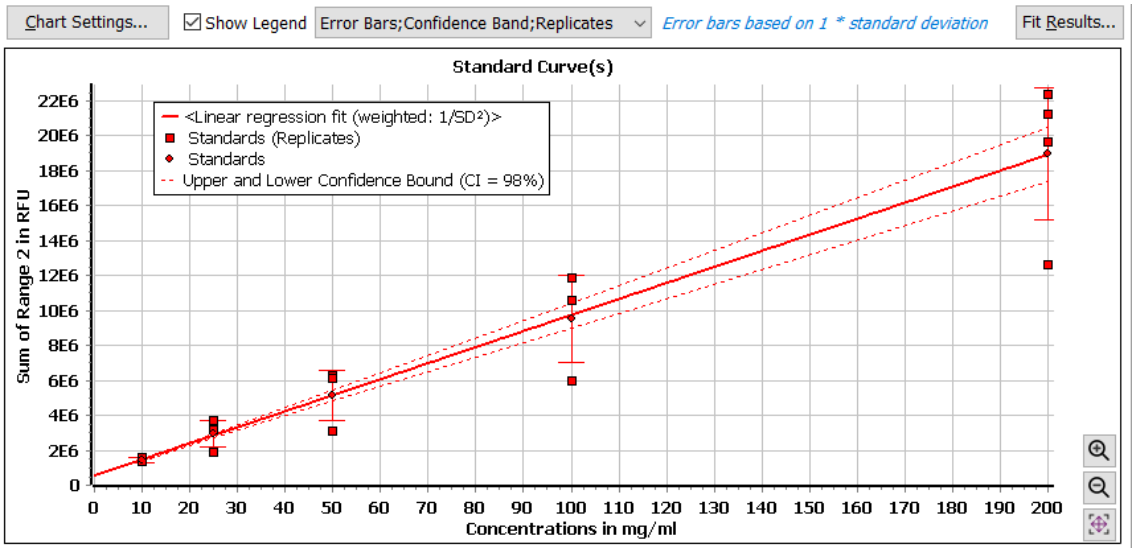
Note: Use the 'lock to curve' setting to get the exact position.

3.17.1 Error Bars, Replicates of Standards, Confidence Band and Recalculated Values

Above the chart there is a further control available to show error bars, confidence band, replicates of standards and recalculated values in the chart.
Open the drop-down list and check on of the available controls:



Show Error Bars: error bars will be displayed for the standards. If error bars are selected, you can see a hint on the right side of the control. It shows the current setting for the error bar calculation. Clicking on the hint opens the Calculation Settings dialog where you can change the settings for the error bar calculation.
Show Confidence Band: the calculated confidence band will be displayed for each standard curve.
Show Replicates: if replicates are defined for the standards, the replicates can be displayed for each standard.
Show all recalculated values: display the recalculated values of all wells on the fitted curve (as gray points on the curve).
Show only recalculated Samples: display the recalculated values of the samples only on the fitted curve (as gray points on the curve).
In this chart error bars, replicates and confidence band are displayed:

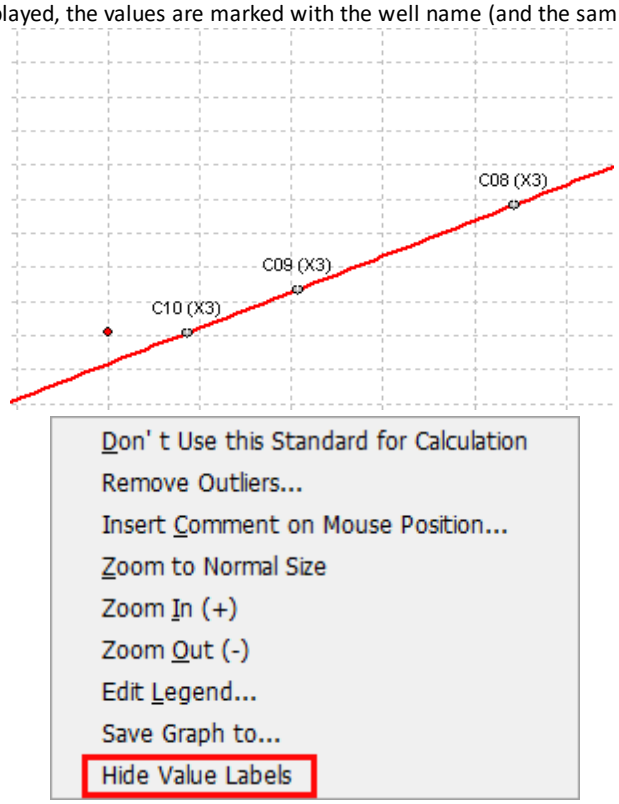


The parameters for the calculation of the confidence intervals (e.g. CI = 95%) are defined in the standard calculation dialog (see chapter 4.10: *Standard Calculation*) or in the parallel line analysis calculation dialog. The confidence band is also defined by the confidence interval settings.

To view the error bars the following conditions must be fulfilled:

- You must have defined replicates for your standards in the layout of the test run.
- The Y-Axis scaling is not logarithmic.
- The standard deviation of the replicates may not be zero.
- The standard fit may not have a user generated replicate statistic in its base processes (see chapter 4: *Perform Calculations*).

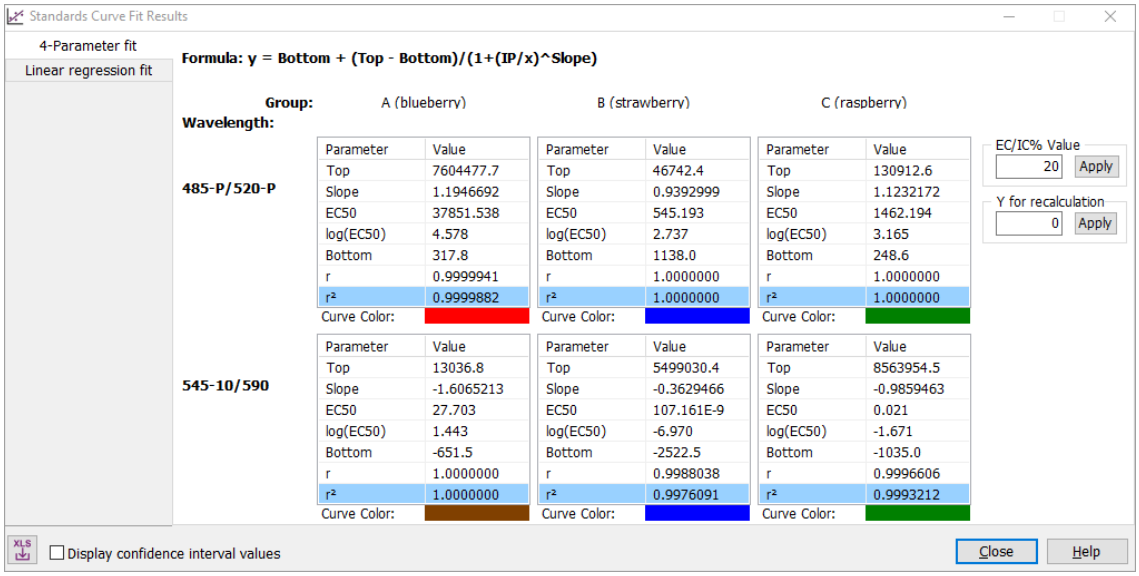
If recalculated values are displayed, the values are marked with the well name (and the sample ID for the well, if defined):



To hide the marks, open the standard curve pop-up menu (press the right mouse button) and select *Hide Value Labels*.

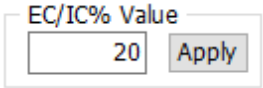
3.17.2 Fit Result Window

To view the fit result parameters of all displayed standard curves, press the *Show fit Results* button and a window showing the results will open:

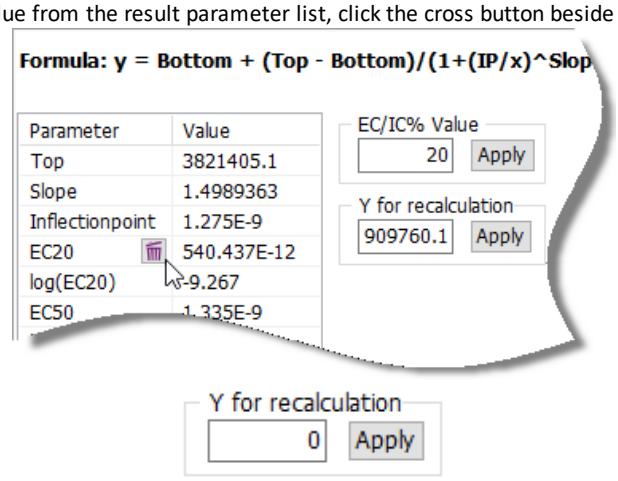


The window contains a page for each performed standard fit and for each parallel line analysis. To open a page of a standard fit, click on the appropriate tab on the top of the window.
The first line of the window displays the applied fit formula.
The fit results are organized in a kind of table, where the columns represent the groups from the layout and the rows represent the different wavelength data.
Each result on its own has a small table whose rows represent the parameters of the fit. The parameters shown are dependent upon the fit performed. The parameter qualifying the fit result is r^2 and AICc. The r^2 row is highlighted with a blue background. You can find more details on certain fit parameter in the section Fit model comparison using Akaike's information criterion (AICc).
Check *Display confidence interval values* to display also the values for the confidence interval.

To export all results to Excel, press the  Button.



If the fit model of a standard fit is a 4 or 5-Parameter fit, you can add additional EC/IC values to the fit result parameters. Enter the desired value in the control on the right top of the window and press the apply button.
To remove an added EC/IC value from the result parameter list, click the cross button beside the parameter:

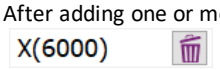


For standard fits, you can enter additional Y values to get the calculated x values and add this value to the fit parameter list. If the value cannot be calculated, you can get one of the following results:

Text	Meaning
n.a.	Not available - recalculation not possible
<< std range	The calculated concentration value for this Y value is under the defined limit for this calculation method. (see Limitations for Recalculated Concentrations table)
>> std range	The calculated concentration value for this Y value is above the defined limit for this calculation method. (see Limitations for Recalculated Concentrations table)

<< Y range	The Y value is either under the domain of the fit or under the defined limit for this calculation method. (see Limitations for Recalculated Concentrations table)
>> Y range	The Y value is either above the domain of the fit or above the defined limit for this calculation method. (see Limitations for Recalculated Concentrations table)
ambiguous	The Y value is ambiguous, that means that it fits to more than one concentration value.

After adding one or more Y-Values to the fit result list, you can remove them with the delete button beside the parameter:

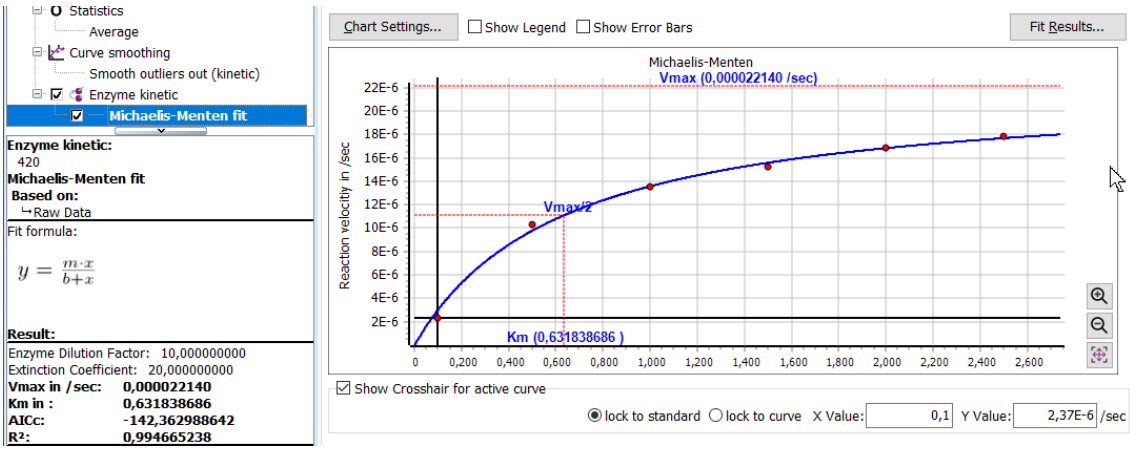


The fit result table for parallel line analysis calculations looks a little bit different. It is explained in the chapter Parallel Line Analysis.

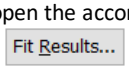
3.18 Enzyme Kinetic Fit Curves

The enzyme kinetic curve chart option will be available if you have performed an enzyme kinetic calculation. To view the chart, click on the *Enzyme Kinetic Fit(s)* tab in the working area.
Read more about how to use the MARS Data Analysis software for the determination of Km and Vmax in chapter 4.16: *Enzyme Kinetic Calculations*.
The enzyme kinetic fit chart will plot the result curves of enzyme kinetic calculations such as Michaelis-Menten plot, Lineweaver-Burk plot etc. for all selected enzyme kinetic fit nodes in the navigation tree. The color and style of the curves and standards can be changed using the curve settings dialog box.

Note: If nothing is selected, the working area will appear empty!



When the enzyme kinetic fit chart is visible, the detailed window under the navigation tree will be visible instead of the content filter tree. The detailed window shows the fit parameters and fit results such as Vmax and Km of the selected data node in the navigation tree.
There can be different types of enzyme kinetic fits. Each type is displayed in its own chart. To change between the chart views, use the arrow buttons up  and down  on the right side of the chart to select the previous/next chart. You also can click directly on the data node of an enzyme kinetic equation in the navigation tree to open the according chart.

To see the fit results of all calculated enzyme kinetic fits, open the fit result window with the 

button on the top right corner of the chart.
In addition to the common chart functions (zooming, crosshair function, axis scaling...), the enzyme kinetic fit chart has a check box bar to select or deselect the fit results for single groups. This bar will appear only if groups were defined in the layout.
Check or uncheck the box of the group you want to see or hide.
If more than six groups are defined, the check boxes are replaced by a drop-down menu to select the desired groups.

If a Michaelis-Menten chart is displayed, a line for the calculated Vmax and Km value can be displayed in the chart. Select the *View* tab in the ribbon and activate the *Show Km and Vmax in plot* control on the *Chart* group to add the lines to the chart.

3.18.1 Error Bars

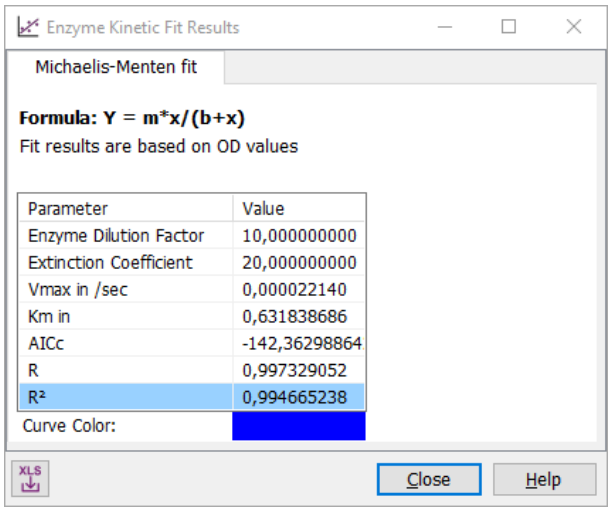
Above the chart there is a further check box available: *Show Error Bars.*
If the *Show Error Bars* is checked the error bars will be displayed. As a default, the error bars are based on the standard deviation of the y values. The current setting is displayed on the right side of the check box (if checked). You can click on this hint to change the error bar settings.

To view the error bars the following conditions must be fulfilled:

- You must have defined replicates for your standards in the layout of the test run.
- The standard deviation of the replicates may not be zero.
- The enzyme kinetic fit may not have a user generated replicate statistic in its base processes (see chapter 4: *Perform Calculations*).

3.18.2 Fit Result Window

To view the fit result parameters of all calculated enzyme kinetic fits, press the *Show fit Results* button and a window showing the results will open:



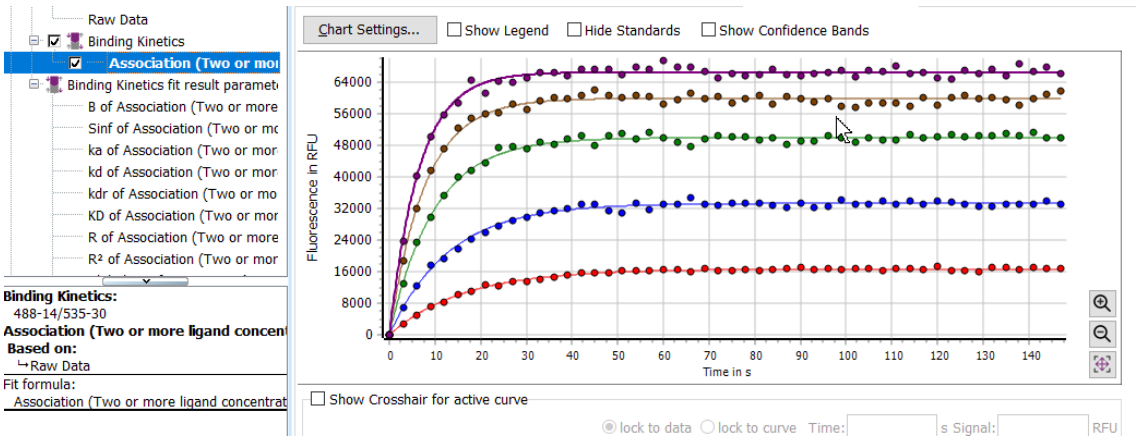
The window contains a page for each performed fit and displayed fit. To open a page of a fit, click on the appropriate tab on top of the window.
The first line of the window displays the applied fit formula.
The fit results are organized in a kind of table, where the columns represent the groups from the layout and the rows represent the different wavelength data.
Each result on its own has a small table whose rows represent the parameters of the fit. The parameters shown are dependent upon the fit performed. The parameter qualifying the fit result is r^2. Its associated row is highlighted with a blue background.

To export all results to Excel, press the Button.

3.19 Binding Kinetics Fit Curves

The binding kinetics curve chart option will be available if you have performed a binding kinetic equation. To view the chart, click on the *Binding Kinetics Curve* tab in the working area.
The color and style of the curves and standards can be changed using the curve settings dialog box.

Note: If nothing is selected, the working area will appear empty!



When the binding kinetics fit chart is visible, the detailed window under the navigation tree will be visible instead of the content filter tree. The detailed window shows the selected fit model of the selected data node in the navigation tree.
In addition to the common chart functions (zooming, crosshair function, axis scaling...), the binding kinetics fit chart has a check box bar to select or deselect the fit results for single groups. This bar will appear only if groups were defined in the layout.

Check or uncheck the box of the group you want to see or hide.
If more than six groups are defined, the check boxes are replaced by a drop-down menu to select the desired groups.

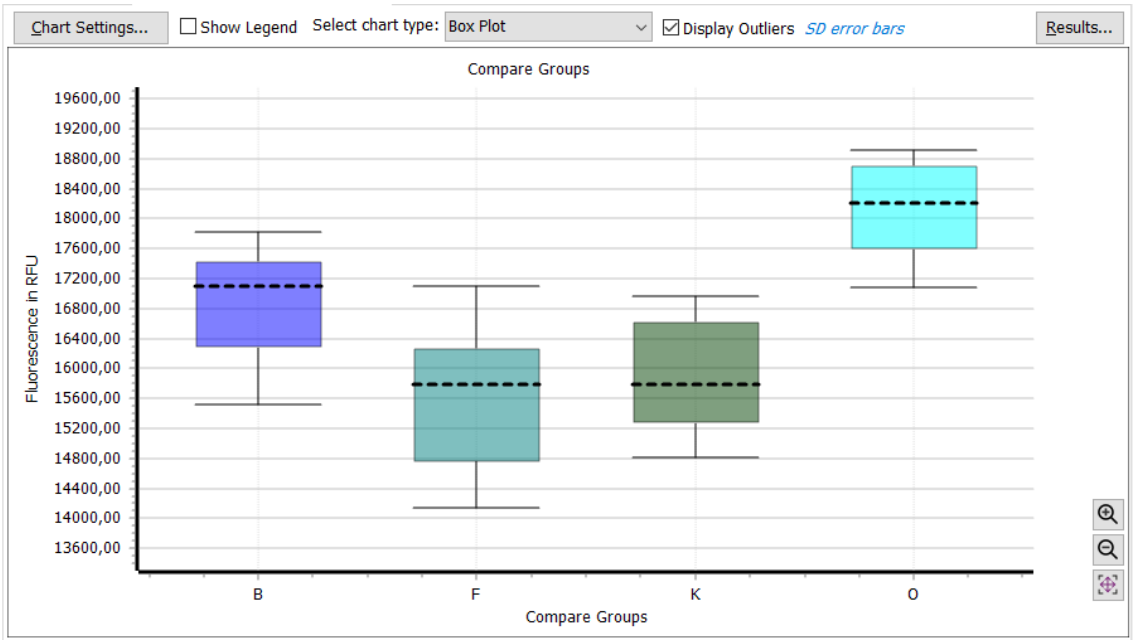
Select the check box *Hide Standards* to see only the fit curves without the data points of the standards.

Select the *Show Confidence Bands* check box to display also the upper and lower confidence bands in the chart.

3.20 Compare Data Plots

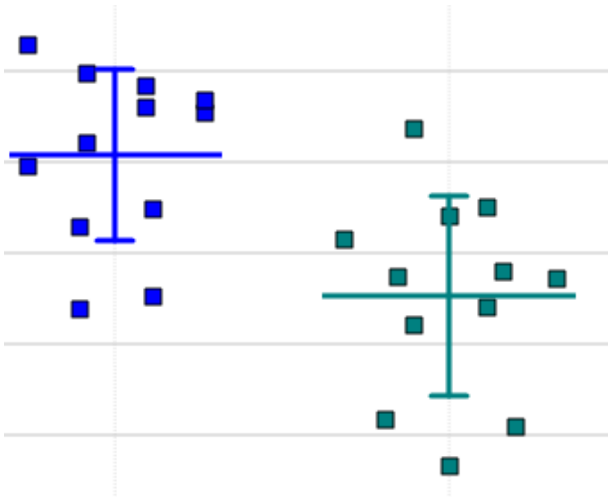
The compare groups plot option will be available if you have performed a compare data group comparison. To view the plot, click on the *Compare Groups* tab in the working area.
The color and style of the curves and standards can be changed using the curve settings dialog box.

Note: If nothing is selected, the working area will appear empty!



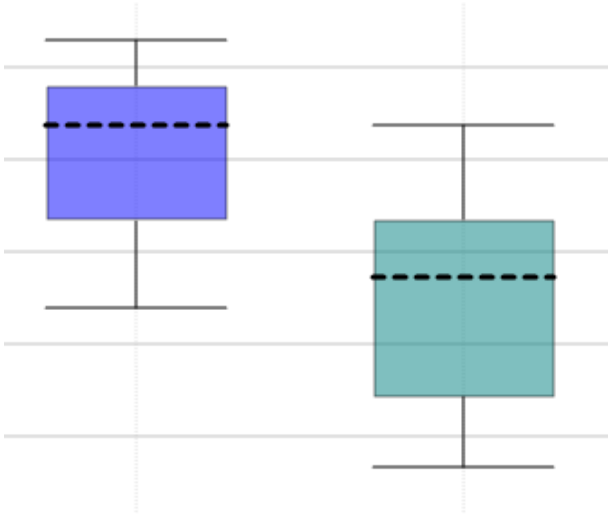
When the compare data group chart is visible, the detailed window under the navigation tree will be visible instead of the content filter tree. The detailed window shows the selected calculation model of the selected data node in the navigation tree.
To see the comparison results, open the result window with the *Results...* button on the top right corner of the chart.
In addition to the common chart functions (zooming, crosshair function, axis scaling...), the compare data group chart allows you to change the displayed type of plot with the pull down control *Select chart type* above the chart:

3.20.1 Jitter Plot



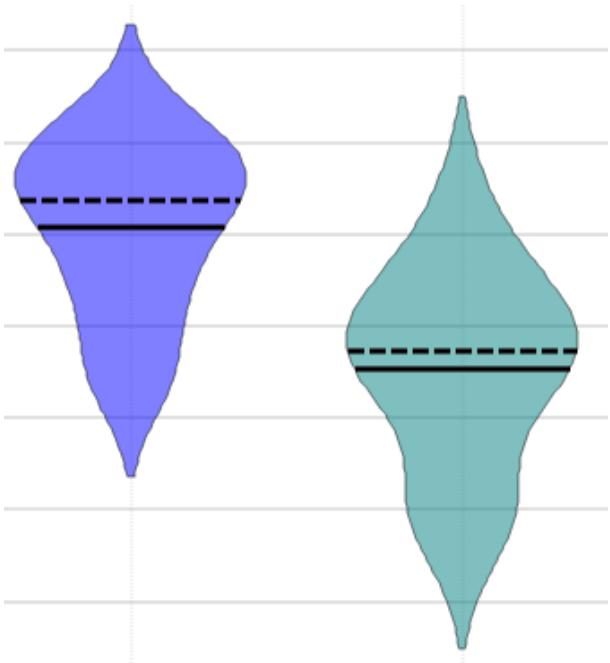
The jitter plot shows the data groups with its real y value but distributes the values on the x axis based on an algorithm to avoid overlapping of data points with same or similar y-values. The mean is marked in the plot with a small solid line.

3.20.2 Box Plot



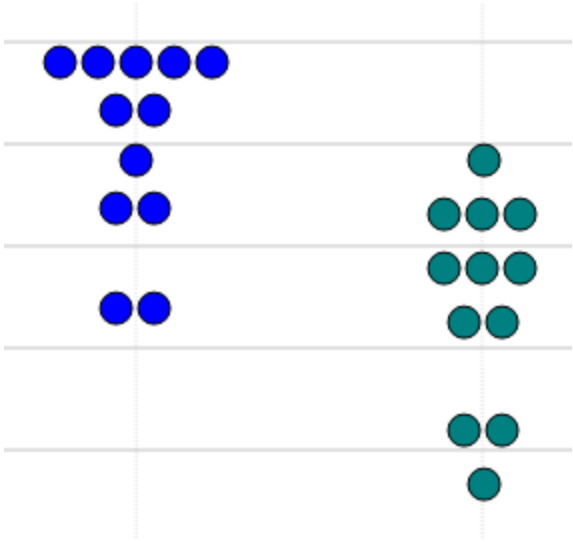
A box plot shows the data groups through their quartiles.The extending lines (whiskers) indicate the variability outside the upper and lower quartiles. If the check box Display outliers above the chart is checked, outliers are plotted as individual points (define the outliers you want to see in the extended series settings dialog). The median is marked in the plot with a small, dashed line.

3.20.3 Violin Plot



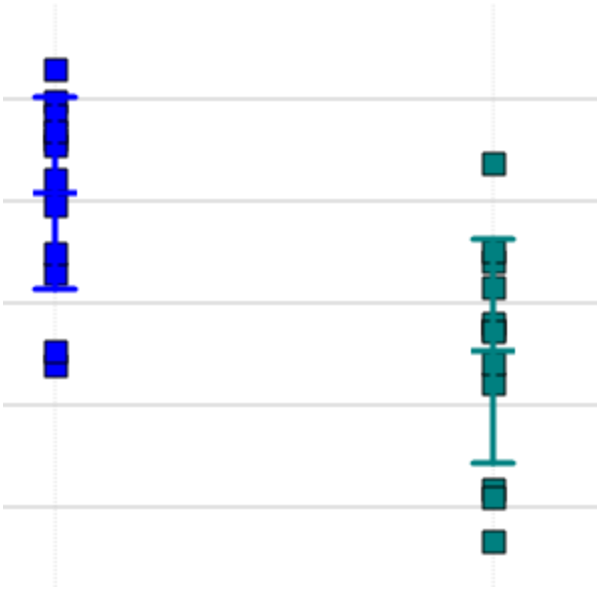
A violin plot is similar to a box plot, with the addition of a rotated kernel density plot on each side. It has to markers. The dashed line is the median of the data and the solid line is the mean.

3.20.4 Bee Swarm



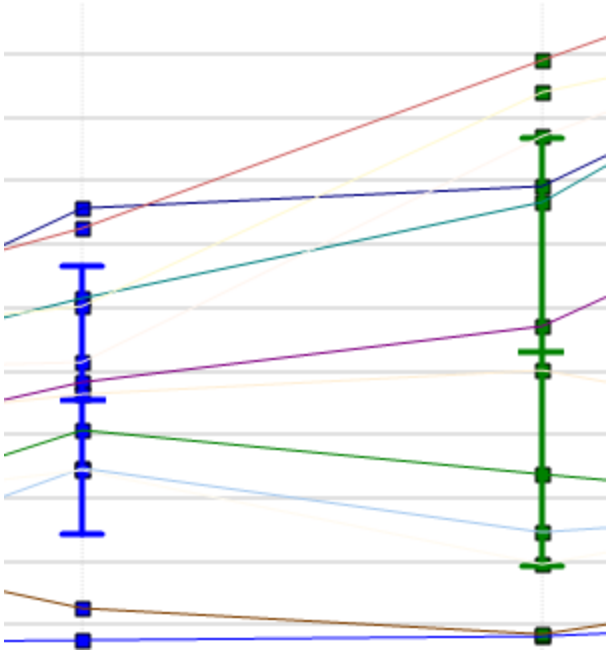
The bee swarm plot is similar to the jitter plot but distributes the data by grouping them in y-lines depending on the original y-value.

3.20.5 Scatter Plot



The scatter plot plots each data point of one group on the same x-position and on the y-position that relates to its y-value. The mean is marked in the plot with a small solid line.

3.20.6 Scatter Plot (connected)



The connected scatter plot is only available if a paired t-test or a repeated-measures one-way ANOVA was performed. In addition to the normal scatter plot, corresponding data points are connected (e.g. to indicate that they come from the same well).

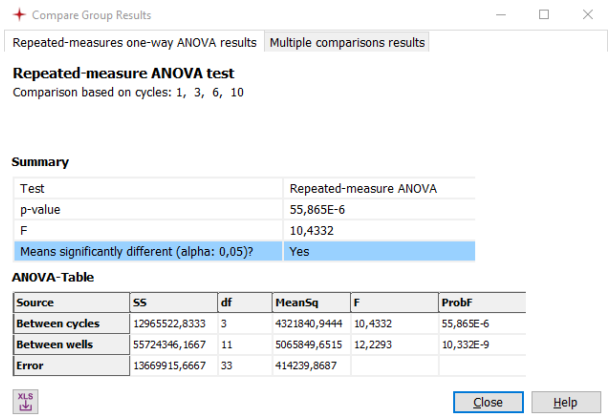
3.20.7 Error Bars

Above the chart there is a further check box available if Scatter or Jitter-plot is selected: *Show Error Bars.*
If the *Show Error Bars* is checked the error bars will be displayed. As a default, the error bars are based on the standard deviation of the y values. The current setting is displayed on the right side of the check box (if checked). You can click on this hint to change the error bar settings.
To view the error bars the following conditions must be fulfilled:

- You must have defined replicates for your standards in the layout of the test run.
- The standard deviation of the replicates may not be zero.
- The enzyme kinetic fit may not have a user generated replicate statistic in its base processes (see chapter *: Perform Calculations*).

3.20.8 Compare Result Window

To view the result parameters the comparison, press the *Results...* button and a window showing the results will open:



The window contains a page for each performed and displayed comparison. To open a page of a comparison, click on the appropriate tab on the right side of the window.
The first lines of the window display the performed test and the groups that where compared.
The results are organized in one table for the t-Test and in two tables for the ANOVA test. The first table shows the overall results of the comparison (like the p-value), the second table shows additional ANOVA results.

If *Apply post test* was selected, a second page with the multiple comparison result table will be available, where the result of each compared group pair is shown.

Repeated-measures one-way ANOVA results | Multiple comparisons results

Tukey-Kramer after Repeated-measure ANOVA

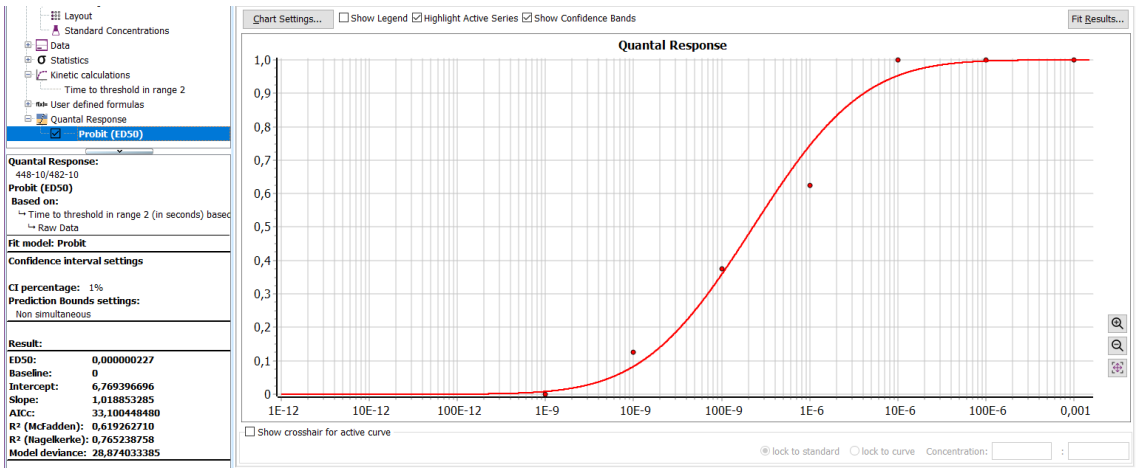
1st group	2nd group	95% CI min	Difference of mean	95% CI max	p-value	Difference significant?
Cycle: 1	Cycle: 3	-955,3567	-493,0833	-30,8099	0,0358	yes
Cycle: 1	Cycle: 6	-1514,8113	-799,3333	-83,8554	0,0277	yes
Cycle: 1	Cycle: 10	-2582,6610	-1434,2500	-285,8390	0,0143	yes
Cycle: 3	Cycle: 6	-893,6087	-306,2500	281,1087	0,4332	no
Cycle: 3	Cycle: 10	-1948,8675	-941,1667	66,5341	0,0695	no
Cycle: 6	Cycle: 10	-1224,0032	-634,9167	-45,8301	0,0338	yes

To export all results to Excel, press the XLS Button.

3.21 Quantal Response Fit Curves

The quantal response curve chart option will be available if you have performed a quantal response calculation. To view the chart, click on the *Quantal Response* tab in the working area.
Read more about how to use the MARS Data Analysis software for the quantal response analysis in chapter X.XX: *Quantal Response Analysis*.
The quantal response chart will plot the result curves of quantal response analysis such as Spearman-Kärber, Logit or Probit plot etc. for all selected quantal response nodes in the navigation tree. The color and style of the curves and standards can be changed using the curve settings dialog box.

Note: If nothing is selected, the working area will appear empty!

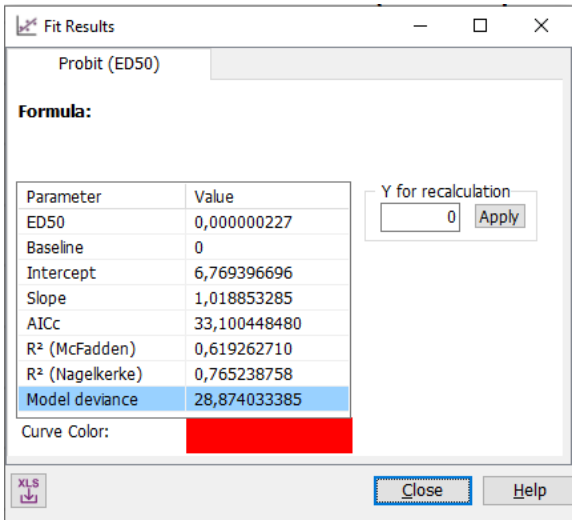


When the quantal response chart is visible, the detailed window under the navigation tree will be visible instead of the content filter tree. The detailed window shows the fit parameters and fit results such as Baseline and Intercept of the selected data node in the navigation tree.

To see the fit results of all performed quantal response analysis, open the fit result window with the Fit Results... button on the top right corner of the chart.
In addition to the common chart functions (zooming, crosshair function, axis scaling...), the quantal response chart has a check box to show confidence bands.

3.21.1 Fit Result Window

To view the fit result parameters of all quantal response calculations, press the *Show fit Results* button and a window showing the results will open:

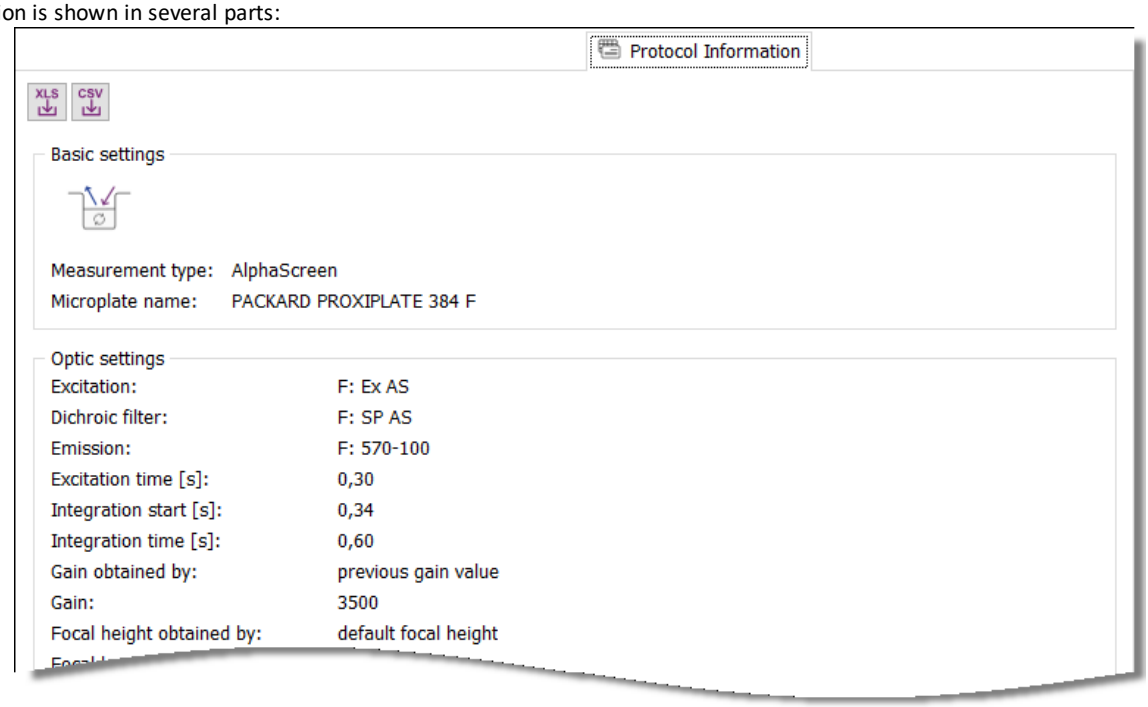


The window contains a page for each performed fit and displayed fit. To open a page of a fit, click on the appropriate tab on top of the window.
The first line of the window displays the applied fit formula (not all fit models do have a fit formula. If no formula is available, the sections is empty).
The fit results are organized in a kind of table, where the columns represent the groups from the layout and the rows represent the different wavelength data.
Each result on its own has a small table whose rows represent the parameters of the fit. The parameters shown are dependent upon the fit performed. The parameter qualifying the fit result is r^2.

To export all results to Excel, press the XLS Button.

3.22 Protocol Information

This page shows all information regarding the measurement protocol used to create the current test run data. The information is shown in several parts:



Basic settings
This gives some information about the measurement type and the plate used to create the test run
Kinetic (Plate mode / Well mode) or Endpoint settings
This gives the kinetic/end point information of your test run, like number of flashes, number of loop iterations, loop duration etc.
Optic settings
The optical properties like filter names of excitation / emission or the wavelength range of the test run are shown here.
Injection settings
This part shows the settings of the pump parameters used and is only displayed if you had defined injections in the test run protocol.

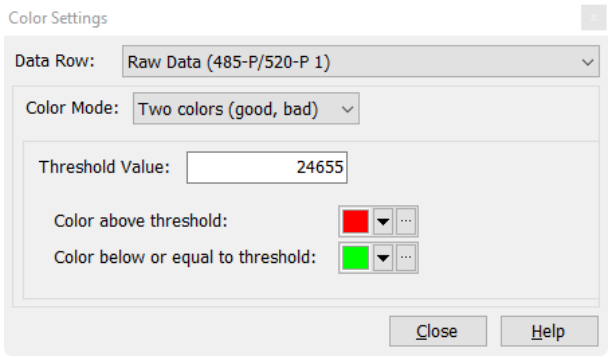
Shaking settings
If there were defined shaking methods in the test run protocol, the information is displayed here.
General settings
These include test run properties like positioning delay, reading direction or temperature control where used.
Comment
Any comment recorded in the test protocol will be displayed here, a comment can also be changed, or a comment can be newly created in this display area.

The settings can be exported to Excel ( ) and to an ASCII text file ( ).

3.23 Color Settings

If the *Color View Mode* in the Microplate View is active or if the Well Scanning window is open for well scan test runs, the *Color Settings* window will then be available.

Press the button behind the *Colors* check box or the *Color Mode Settings* button in the *Working Area* group on the *View* tab to open the *Color Settings* dialog box. On the Well Scanning dialog box, press the *Colors* ( ) button to open the dialog box:

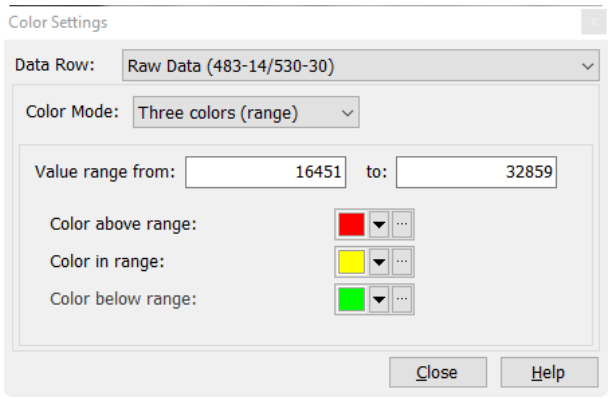


Each displayed data row in the microplate view can have its own color settings. Select the data row at the top of the dialog for which the color mode settings should be changed.
If the *Color View Mode* is active the settings selected will affect the way the data in the *Microplate View* will be shown.
To change the display mode, select one of the three color modes available:

3.23.1 Two Colors (Good, Bad)

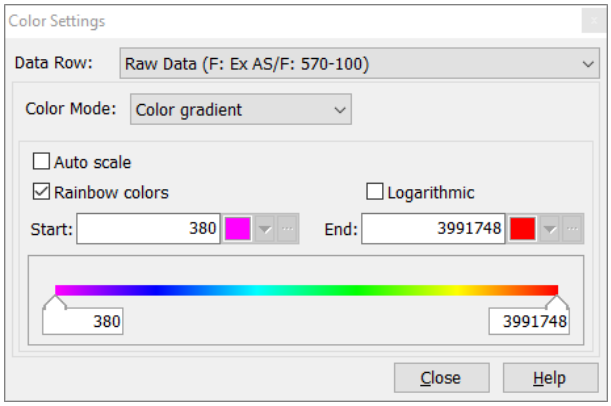
To show a good / bad (pass / fail) decision, you should choose this option to display a color for all values under a certain threshold and to display a different color for all values above the selected threshold. It is possible to select the two colors in use and to change the threshold value.

3.23.2 Three Colors (Range)



This uses the same concept as 'Two colors', but here you can also define a range 'in-between' to be displayed in a third color.

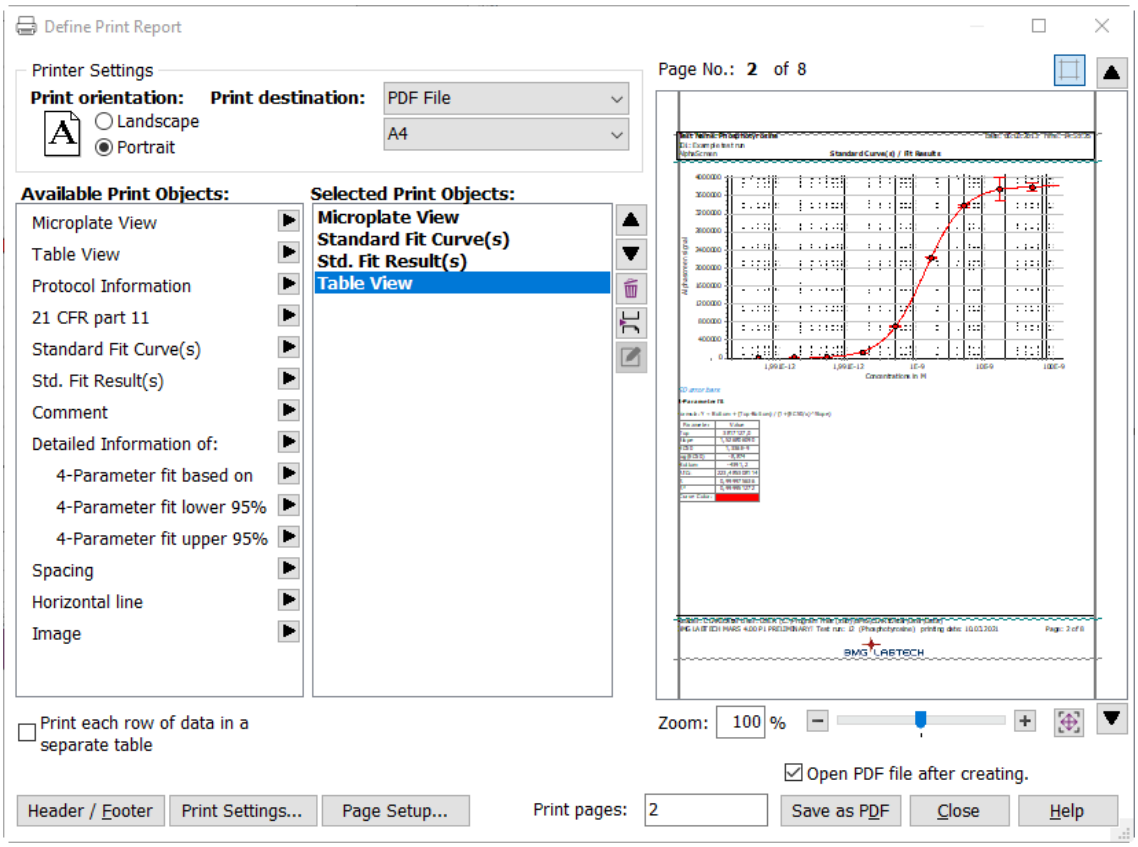
3.23.3 Color Gradient



The measurement values will be displayed using different shades of colors or gray levels. The start and end color of the scale can be defined by the user, it is also possible to use colors from the rainbow spectrum. The range of values displayed can be defined allowing the user to select the start and the end values to enlarge the range of the color gradient used.
The auto scaling function when applied will set the start and the end values of the range automatically to the minimum and maximum measurement values at the selected wavelength for the whole plate (based on the selected data row).

3.24 Printing Your Data

To create a printed report of the measured and calculated data open the *Define Print Report* window. Click  in the *Test Run* group on the *Home* tab of the ribbon or open the *Print* menu in the *File* menu and click *Define Print* to open the window.



The window is separated in four sections described below: *Printer Settings, Available Print Object List, Selected Print Object List* and *Report Preview*.
Press the *Print* button to generate and print the report.
The *Header / Footer* button opens a window to define the header and footer to be printed on each page as described later in this chapter.
Press the *Print Settings...* button to change common print settings (chapter 3.21.5: *Print Settings*).
Click *Page Setup* to define page margins (see Define Page Margins below).
To print only certain pages, use the *Print pages* entry field. Type the page numbers and/or page ranges separated by comma. For example, type 1, 3-5, 7 to print the pages 1, 3, 4, 5 and 7. Leave the field empty to print all pages!

Note: There is a limit of maximum 200 print pages.

Printer Settings

In the *Printer Settings* section of the window select the printer and the print orientation (landscape or portrait). To change further settings of the printer, press the *Printer Setup*... button to open the systems *Print Setup* window. The print destination can also be a PDF file or an HTML file. The name of the *Print* button changes to *Saves as PDF* and *Save as HTML* respectively if PDF or HTML is selected as print destination. In this case you will be asked to enter a file name after pressing the button. If available, MARS opens the Acrobat Reader application with the generated PDF file if *Save as PDF* was pressed: If *Save as HTML* was pressed, MARS opens the default HTML-Viewer (e.g. Microsoft Internet Explorer) with the generated HTML pages.

Available Print Objects

The list *Available Print Objects* holds all present printable objects (such as tables, charts, settings...). By double clicking on an object or by pressing the small arrow button (), you can add the object to the *Selected Print Object* list.
If an object is shown in blue, it is at least added once to *the Selected Print Objects*. If an object cannot be added it is shown in gray. This can be if its maximum number of selections is reached or if the object was not initialized yet (to initialize an object, open the appropriate page in MARS).
If the microplate view displays more than one row, a check box below the list is shown. Check this control to print each data row in its own microplate table:

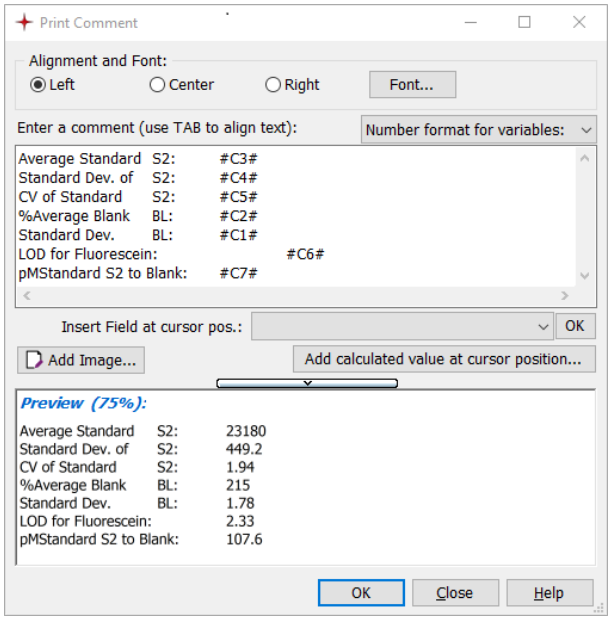
Print each data row in a separate table	print result
checked	<table><tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th></tr><tr><td>A</td><td>3.5</td><td>1.229</td><td>0.324</td><td>1.719</td><td>0.325</td><td>0.306</td></tr><tr><td>B</td><td>1.575</td><td>0.443</td><td>0.296</td><td>0.392</td><td>2.205</td><td>1.841</td></tr><tr><td>C</td><td>1.604</td><td>0.303</td><td>0.301</td><td>0.459</td><td>1.786</td><td>1.599</td></tr><tr><td>D</td><td>3.5</td><td>1.055</td><td>0.341</td><td>2.132</td><td>0.313</td><td>0.361</td></tr></table><table><tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th></tr><tr><td>A</td><td>3.154</td><td>0.553</td><td>0.109</td><td>0.338</td><td>0.129</td><td>0.102</td></tr><tr><td>B</td><td>0.21</td><td>0.218</td><td>0.092</td><td>0.177</td><td>0.937</td><td>0.42</td></tr><tr><td>C</td><td>0.209</td><td>0.115</td><td>0.088</td><td>0.228</td><td>0.908</td><td>0.184</td></tr><tr><td>D</td><td>2.629</td><td>0.486</td><td>0.139</td><td>0.778</td><td>0.098</td><td>0.139</td></tr></table>
not checked	<table><tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th></tr><tr><td>A</td><td>3.5 3.154</td><td>1.229 0.553</td><td>0.324 0.109</td><td>1.719 0.338</td><td>0.325 0.129</td><td>0.306 0.102</td></tr><tr><td>B</td><td>1.575 0.21</td><td>0.443 0.218</td><td>0.296 0.092</td><td>0.392 0.177</td><td>2.205 0.937</td><td>1.841 0.42</td></tr><tr><td>C</td><td>1.604 0.209</td><td>0.303 0.115</td><td>0.301 0.088</td><td>0.459 0.228</td><td>1.786 0.908</td><td>1.599 0.184</td></tr><tr><td>D</td><td>3.5 2.629</td><td>1.055 0.486</td><td>0.341 0.139</td><td>2.132 0.778</td><td>0.313 0.098</td><td>0.361 0.139</td></tr></table>

If a chart is added to the print report and the chart is selected in the selected print objects, a check box below the list is shown (*Create one chart for each series*). Check this control to print each curve of the chart in its own chart instead of all curves in one chart. Be careful with many curves, because this increases the number of print pages substantially.

Selected Print Objects

The *Selected Print Objects list* shows the objects to be printed. The order of the objects in the list defines the sequence in the report. The position of an object in the list can be changed with the  and  buttons at the right side of the list. You can also select the object in the list and move it with the mouse to its new position.

To remove an object, press the  button.
Page breaks are added automatically, if needed. To enforce a page break between two objects, select the object before the desired page break and press the  button.
Print Comments
If you add a Comment object from the available print objects to the report, a dialog opens where the comment can be entered and formatted:



With the *Alignment and Font* controls, you can define the format of the comment. In the preview area at the bottom of the dialog, you can see the resulting comment.

To insert predefined values like date, time, test run information and many more at the cursor position, use the drop-down menu *Insert field at cursor pos.*. The available fields are a subset of the fields, available for header and footer (see *Available fields and their meanings* table later in this chapter). After selecting an entry from the list, press the *OK* button to insert the field into the comment.

With the *Add calculated value at cursor position...* button any value available in MARS can be added to the comment at the cursor position. See chapter 4.2.1: *Define and Use Variables* to read more about calculated values and system variables.

If the comment contains such a value, you can see detailed information of this variable value, if you move with the mouse pointer over the place holder of this variable.

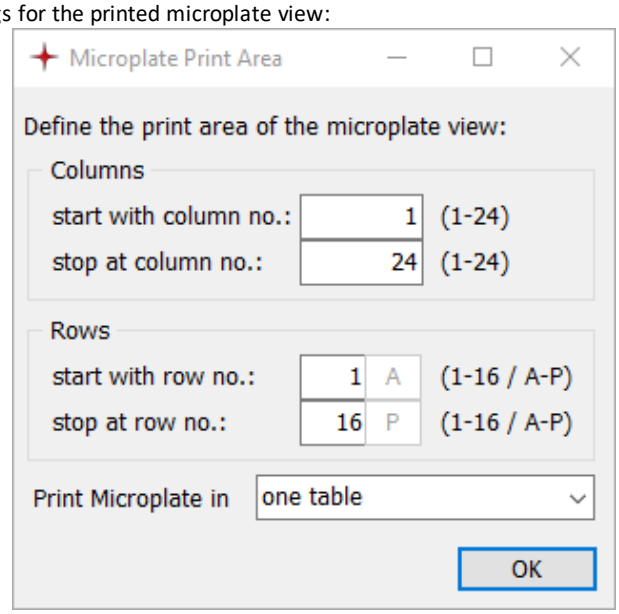
You can change the way the number of such a value is printed (number of digits, scientific format...). Select the variable in the *Number format for variables* drop-down list and define the settings in the appearing dialog.

You can add a (small) image to the comment. Click *Add Image...* to select an image from the file system. The added image can be placed/moved and re-sized with the mouse. Move the mouse over the image in the preview area of the comment dialog and click the left mouse button, to move the image. Move the mouse over a border or a corner of the image and click the left mouse button to change the size of the image. It can be removed from the comment, if you move the mouse over the image and press the small X button that appears, when the mouse is placed over the image.

To change a comment in the report, click on the comment object in the selected print object list.

Print microplate view

After you've added a microplate view to the print report, you can click on the microplate view object in the 'Selected Print Objects' list to change settings for the printed microplate view:

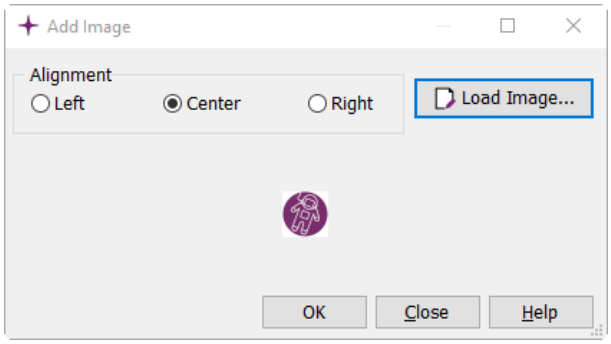


Enter the start column number and the stop column number to reduce the printed part of the microplate to the desired columns. Do the same with the rows.

If you want to divide the microplate into more than one table, select the desired number of tables in the drop-down control 'Print Microplate in'. For plate sizes between 24 and 384 you can also select 4 tables, for higher plate sizes you can also select 16 tables.

Add an image to the print report

You can add one or more images to the print report by selecting the image entry in the list of the available print objects. After selection it, a dialog is opened, where you can define the alignment of the image and where you can load the image from the file system:



You can change the size of the added image in the print output.

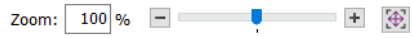
After moving the mouse over the image in the preview area, a blue dashed line is shown around the image. Click on the border of the line with the left mouse button, hold the mouse button and move the mouse to change the image size.

3.24.1 Preview

At the right side of the window, a preview window is shown. The preview will be updated each time the report will be changed. You can resize the window to enlarge the preview section.

Use the  and  buttons or the slider control on the right side of the preview to change the displayed print page.

Click  above the preview to see the page margins in the preview window as dashed lines. Click it again to hide the lines.

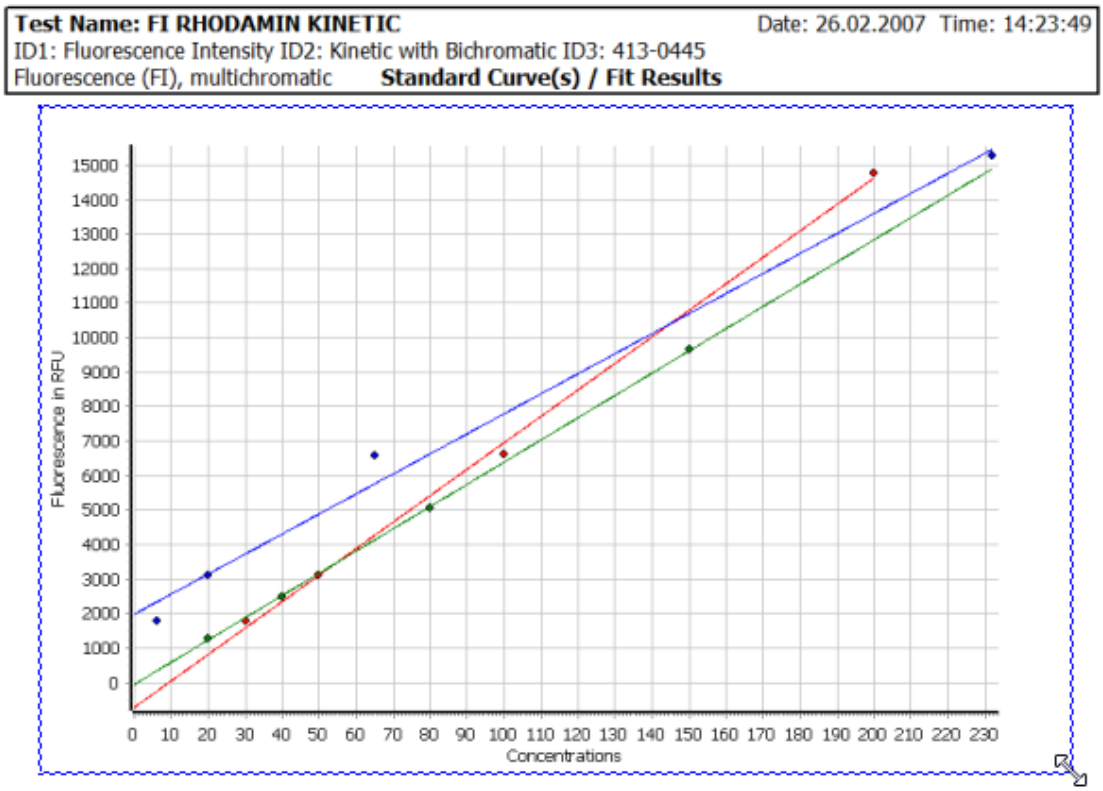


Use the Zoom controls to set the desired zoom factor in percent for the print preview between 10 and 1000% (100% = full size).

Change the size of charts in the print output:

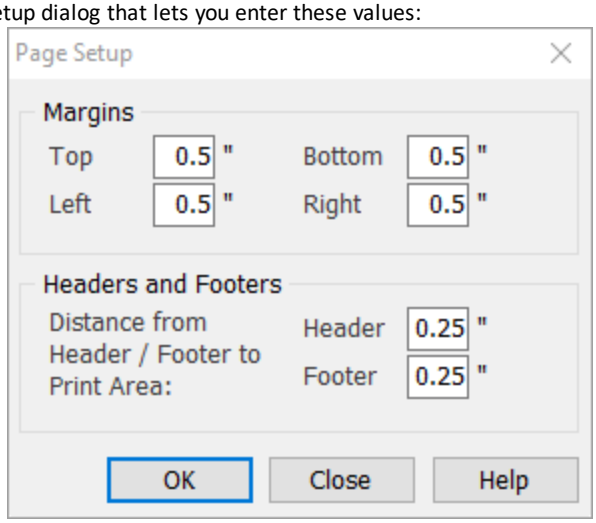
You can change the size of charts like the signal curve, the standard curve, the spectrum curve or the enzyme kinetic fit curve in the print output.

After moving the mouse over the chart in the preview area, a blue dashed line is shown around the chart. Click on the border of the line with the left mouse button, hold the mouse button and move the mouse to change the chart size.



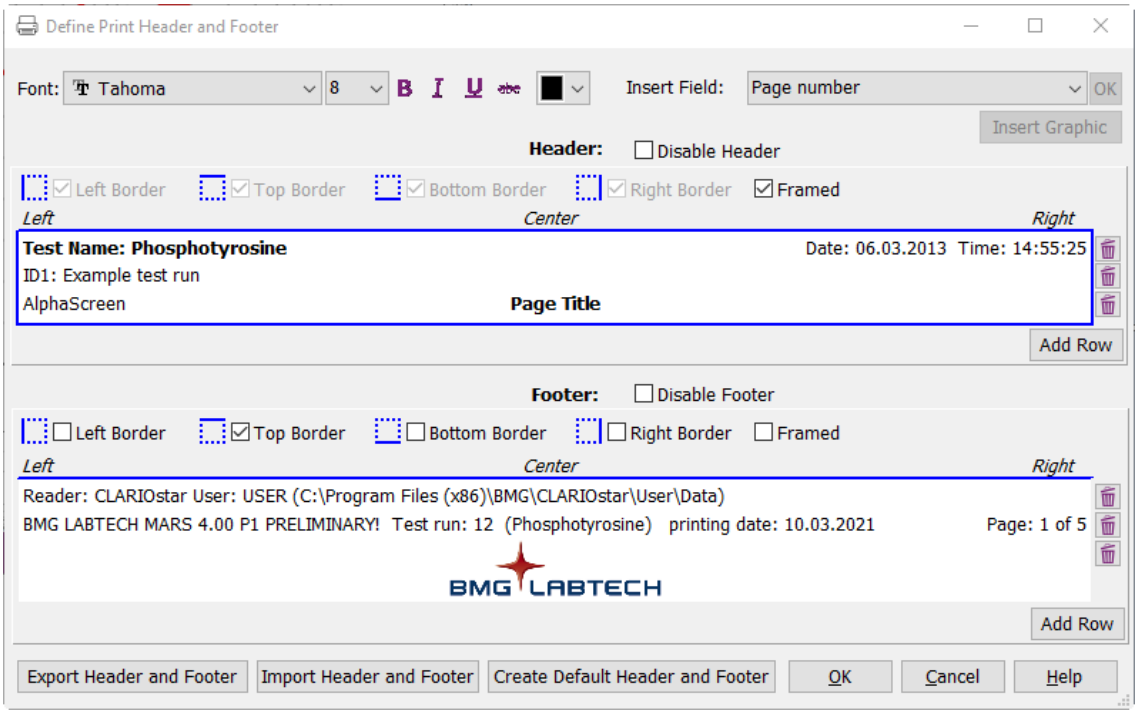
3.24.2 Define Page Margins

The MARS report generator lets you define six margins:
The left, right, top and bottom page margins and two margins that define the space between the header or footer text and the print area.
If the page margins are shown in the small preview window (see section above), you can change the margins with the mouse. Move the mouse cursor over the margin you want to change. The mouse cursor changes to a line with two arrows (⟺ or ⇳) if the mouse is over the line. Click the left mouse button and move the mouse to the desired position of the margin.
Alternatively, you can enter the margins in inch or cm (depending on the regional settings of the operating system). Click *Page Setup* to open the page setup dialog that lets you enter these values:



3.24.3 Define Print Header and Footer

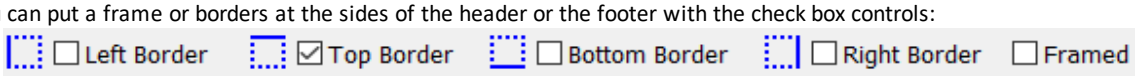
The printed header and footer of the generated report can be changed and defined individually. A set of predefined headers can be managed to change between different headers and footers quickly. Press the *Header/Footer* button on the Define Print Pages window to open the *Define Header and Footer* window:



The window comes with a section to define the header and a section to define the footer. Both, defining the header and the footer work the same way.
A header or footer exists of one or more lines/rows. Each row is separated in three parts (section cells): A left part, a middle part and a right part. The text alignment depends on the part of the row in which the text was entered:

Section Cell position	Text Alignment
Left	Left
Middle	Center
Right	Right

You can add new rows with the *Add Row* button, and you can remove a row with the delete button () at the right side of the concerned row.
You can put a frame or borders at the sides of the header or the footer with the check box controls:



The border lines will be printed in black.
You can disable the complete header or footer by checking the *Disable Header* / *Disable Footer* check box control.

Enter header or footer text:

To add a row to the header or footer, press the Add Row button. Move the mouse over the part of the row, where the position of entered text should be until you see a framed section. Click on this section and a blinking edit curser is show. Now you can enter the desired text.

> **Note:** If the section contains a graphic, you will see no frame. If you click on the graphic a small delete button appears on the top right corner of the graphic. You can delete the graphic with this button and enter the text afterwards. In one section cell text and graphic content cannot be combined.

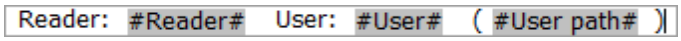
Change the font style of the entered text in one section cell:

Select the concerned section cell like described in *Enter header or footer text* above and change the font settings with the font settings controls at the top of the window.
The font settings can be defined for each section cell separately.

Use fields in header or footer rows:

Each section cell of a header or footer row can contain text combined with fields. Use the fields to enter page numbers, date, time or test run information and many more to the header or footer.

To insert a field into a cell, select the desired field in the drop-down menu *Insert field* and select the cell like described in *Enter header footer text* above. Set the curser in the cell do the desired position of the field and press the OK button on the right side of the *Insert field* drop-down menu.
Unlike normal text, fields are displayed in gray and enclosed in # if a section cell is selected. To remove a field, click on the field when its section cell is selected. A small delete button appears on the top right corner of the field. Press the button to remove the field.
If a section cell with fields is not selected, MARS replaces the field name with the field value if possible (e.g. instead of #NoPages# a 5 is shown if the report has 5 pages).
A selected section cell with fields:



The same section cell but not selected:

Reader: OMEGA User: USER (c:\program files\bmg\omega\user\Data\)

Available fields and their meanings:

Field	Meaning
Page number (#PageNo#)	Current page number
Number of total pages (#NoPages#)	Total number of pages the report created.
Title of the page (#PageTitle#)	The page title is created automatically, depending on the objects on the page (e.g. if the page contains the microplate and the table view, the page title is *Microplate View / Table View*)
Current date (#Date#)	The date when the report was created.
Current time (#Time#)	The time when the report was created.
Used reader series (#Reader#)	The reader series/family which created the measurement data (e.g. Omega, PHERAstar)
Used reader type (#ReaderType#)	The type of the reader which created the measurement data (e.g. POLARstar Omega, PHERAstar *FS*).
Serial number of used reader (#SerialNo#)	The serial number of the reader which created the measurement data.
BMG User (#User#)	The BMG User.
BMG User directory (#User path#)	The path where the measurement data are stored.
Test Run number (#TrNo#)	The number of the test run in the test run data base.
Test Run name (#TrName#)	The name of the test run.
Test Run ID1 (#ID1#)	The first test run id.
Test Run ID2 (#ID2#)	The second test run id.
Test Run ID3 (#ID3#)	The third test run id.
Test Run Measurement Date (#TRDate#)	The date when the test run was measured.
Test Run Measurement Time (#TRTime#)	The time when the test run was measured.
Test Run Measurement Method (#TRMethod#)	Used test method of the test run.
Print 'signed' if the Test Run is signed (#IsSigned#)	Prints the text *signed* if the test runs is signed. If not, nothing is printed.
Print 'manipulated' if the Test Run is manipulated	Prints the text *manipulated* if the test run is manipulated. If not, nothing is printed.
Name of the application (MARS) (#ApplicationName#)	Prints *MARS*.
Program Version (#Version#)	Version number of the application.

Field	Meaning
Reader Company Name (#Company#)	Name of the reader company (BMG LABTECH)
Windows User	The name of the windows user.

Use graphics in header or footer rows:

A section cell can also contain a graphic instead of text. To insert a graphic into a section cell, select the cell (see *Enter Header or Footer text* above) and press the *Insert Graphic* button at the top of the window. A file dialog opens. Select a valid graphic file and press the *Open* button. The graphic will be inserted in the section cell. If the cell contains text or fields, they will be deleted.
To remove an inserted graphic, select the section cell; click on the graphic and press the appearing delete button on the top right corner of the graphic. Inserting a field in a section cell with a graphic will also replace the graphic by the field.

Create default Header and Footer

Press the *Create Default Header and Footer* button to overwrite the current header and footer with default ones. The default header and footer can be changed afterwards - this will not change the stored defaults (i.e. you can press the default button again to cancel the changes).

3.24.4 Export and Import Header and Footer

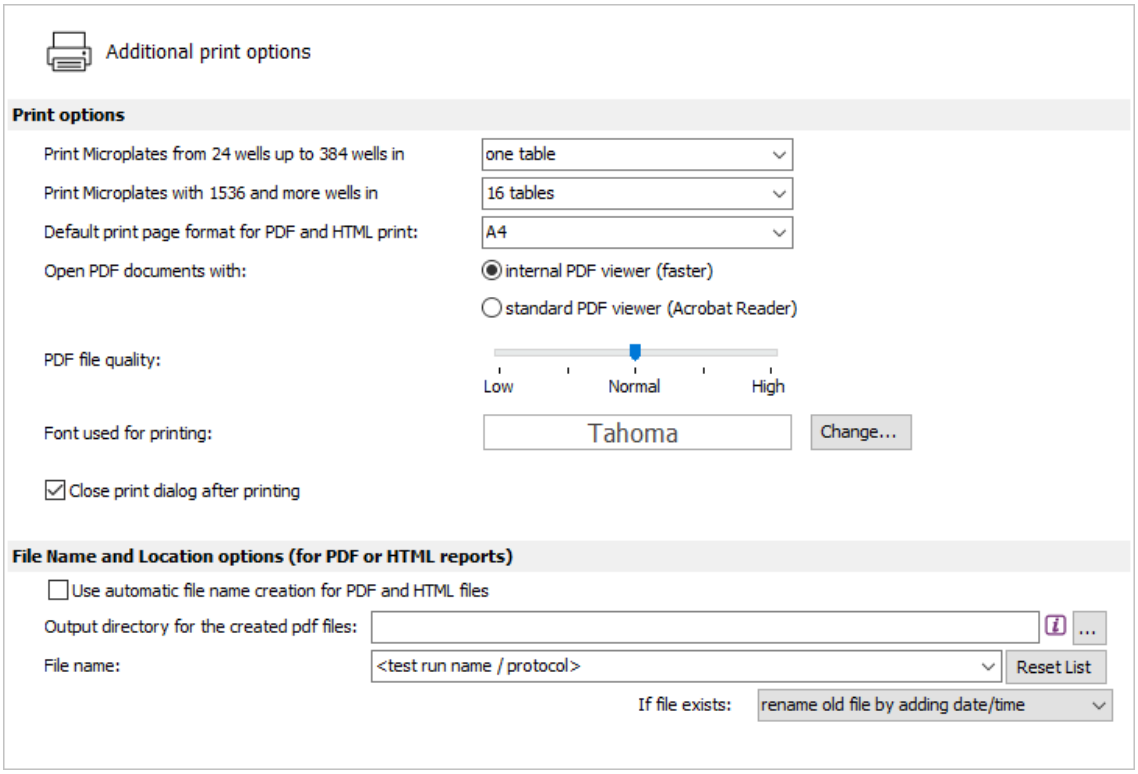
The current header and footer can be exported to store it in a file. Thus, you can create different header/footer settings. These settings can be imported again and will overwrite the current header/footer settings.
To export the current settings, press the *Export Header and Footer* button. An *Export Dialog* opens. Select the desired drive and folder and enter a file name (the extension is created automatically and must be MHF so that it can be imported again). Press the *Save* button to create and save the file with the header and footer settings.
To import a saved setting file, press the *Import Header and Footer* button. An *Import Dialog* opens. Select the *.MHF file containing the desired header and footer settings and press the *Import* button.

3.24.5 Quick Print Function

If you want to print data directly without open the *Define Print Report* window, you can use the *Quick Print* function in MARS. Press  to start the printing of the data. Quick print uses the current defined print settings of the opened test run. If you have never defined or changed the print settings for this test run, default settings are used. With the default print settings always the currently displayed data page will be printed out (i.e. if the table view page is displayed, the table view page will be printed out).

3.24.6 Print Settings

The *Print Settings* window lets you change basic settings for printing. Click *Printing* in the *Settings* group on the *Formats and Settings* tab of the ribbon to open the window or press the *Print Settings...* button on the *Define Print Pages* window or open the MARS options menu and select the *Print* section.



Print options

Print Microplates from 24 wells up to 384 wells in

The microplate view can be divided in more than one table, if the size of the plate is bigger than 12 wells. For plate sizes between 24 wells up to 384 wells, use this drop-down menu to decide if the microplate should be printed in one table or in four tables (this is a default setting for the print report. This setting can be overwritten for each printed microplate in the print report.).

Print Microplates with 1536 and more wells in

Defines the default setting, whether microplates with 1536 or 3456 wells should be printed in one, four or 16 tables. This setting can be overwritten for each printed microplate in the print report.

Default print page format for PDF and HTML print

Select the desired default page format (A4, Letter...) when creating a PDF or HTML print report.

Open PDF documents with

Select *internal PDF viewer* if no Acrobat Reader is installed. To open the created PDF files with the Acrobat Reader, select the second option. If this option is selected and no Acrobat Reader is installed, the PDF file will be created but not opened.

PDF file quality

For the internal PDF viewer, you can adjust the PDF quality. Higher quality means larger PDF files.

Font used for printing

Define the used default font for all print objects in the print report. Note that the header and footer settings are not influenced by this setting.

Close print dialog after printing

Check this control if the MARS print dialog should close automatically after the report was printed (or saved as PDF/HTML) successfully. If the print was aborted or an error occurred, the dialog will stay open even if this control is activated.

File Name and Location options (for PDF or HTML reports)

Use automatic file name creation for PDF and HTML files

Check this control to save the PDF and HTML files automatically after creating. Path and file name settings below will be used.

Output directory for the created pdf files	Enter the directory path, where the generated files should be stored. Use the button to open an explorer window to select the destination directory. The directory can also contain automatic text to sort the exported files. The same syntax like for the file name is used (see below - except the automatic file name creation).
File name	Enter the name of the file without file extension or use the drop-down list, to select one of the proposed file names. You can use either constant typed in text or automatically generated text (enclosed in < and >). You can combine constant and automatic text. See the description of the syntax for automatic file name creation section in the *File Export Settings* chapter. Each new name entered by the user will be added to the drop-down list for further usage. To reset the list to its default entries, click *Reset List*.
If file exists	Define the behavior what MARS should do, if there is already a file with the same name: • *rename old file by adding date/time*: the existing file will be renamed by adding the current date and time to the file name. • *overwrite old files*: the existing file will be overwritten (without prompting)

3.25 Export Data

In the next two sections it is explains how to create a single and quick data export into a text file or to Excel either from the microplate or from the table view page. If you want to define a complete Excel report for a single test run, read the last section (3.22.3) in this chapter.

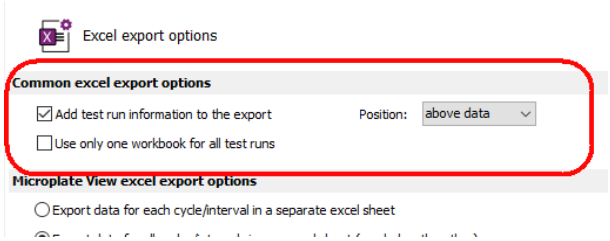
3.25.1 Export Displayed Data

Export to Excel

Data can be exported into Excel from the microplate view, table view and the protocol information by clicking on the Excel button  shown on the upper left side of the page.

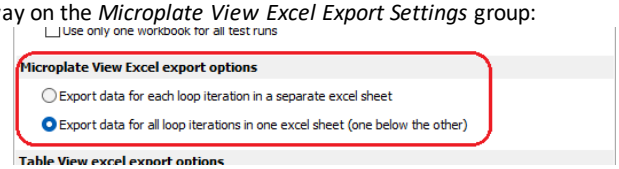
> **Note:** If Microsoft Excel is not installed on the PC, the Excel export is not available, and the appropriate controls and buttons are disabled.

Each export sheet created shows at the top of the page the detailed information of the test run as in the upper section of the evaluation software. If you don't want to export the information or if you want to have this information at the end of the data, open the Excel Settings dialog box.
1. Click *Excel Export* in the *Settings* group on the *Formats and Settings* tab or open the MARS options menu and select the *Excel Export* section.
2. Select the desired behavior in the *Common Excel Export Settings* section:



If more than one end node or calculation in the navigation tree is selected in the microplate view, each item will be displayed in a separate table within the Excel sheet.
If well scanning data are displayed in the microplate view, a scan matrix with the measured values of each well will be exported to Excel.
If the exported test run has more than one loop iteration, you can select the data points, you want to export (displayed data point, all iterations, a range or manually selected loop iterations). If you decided to export more than one loop iteration, there are two ways the data for each loop iteration will appear in Excel:
1. For each loop iteration an Excel sheet will be created. The sheet name is a combination of the iteration number and time value of the appropriate iteration.

2. All loop iterations are shown in the same Excel sheet. The data group for each iteration is exported one below the other. A data group consists of one or more tables in the microplate format (a table for each selected data node). Each data group has a header containing the iteration number and the appropriate time value.
To define, which export way should be used, open the Excel Settings dialog box:
1. Click *Excel Export* in the *Settings* group on the *Formats and Settings* tab or open the MARS options menu and select the *Excel Export* section.
2. Select the desired export way on the *Microplate View Excel Export Settings* group:



If the test run generates more than 250 columns in the table view page, the data will be distributed on different sheets if the used Excel version limits the number of columns. Alternatively, you can transpose the table before exporting it (rows and columns will be swapped), if you select the option *Transpose Table.*

Export to a Text File

Data displayed in the table view, in the microplate view or in the protocol information can be exported to a text file by clicking the 

button on the top of the according page. The generated file is stored in the comma separated value format. That means, each row of the table represents a row in the text file and the column values of each row are separated by a separator character. The destination directory, the file name, the preferred file extension (CSV, TXT or user defined) and the separator can be defined with the File Export Settings window.
The file can be created including the test run information header and available fit result tables or without this information.
The file name can be generated automatically (*TRno+[Unique Test Run Number]+.+[Selected Extension])* or defined by the user.
The generated files can be opened with any text editor or with Excel. If the file extension is CSV and the separator is ',' for English systems or ';' for e.g. German system, Excel recognizes the file as data file and separates the columns into Excel columns.
See an example of a CSV file taken from the table view including the header below:

```
User: USER;Path: C:\Programme\BMG\OMEGA\User\Data\;Test run no.: 407
Test name: FAST;Date: 29.07.2008;Time: 17:16:34
ID1: Export Test;
Fluorescence (FI)

Well Row;Well Col;Content;good / bad based on Raw Data;Z' based on S2 and S1 based on Raw Data;Raw Data;
A;1;Standard S1;-;n.a.;260000;
A;2;Standard S2;-;n.a.;260000;
A;3;Standard S3;-;;260000;
A;4;Standard S4;-;;260000;
A;5;Standard S5;-;;260000;
```

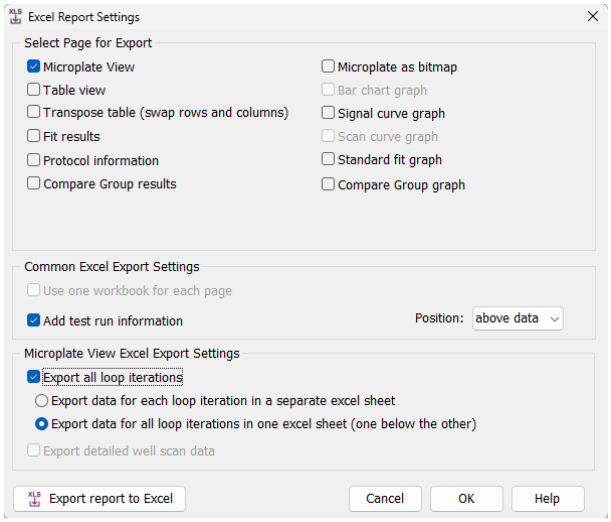
3.25.2 Exporting Fit Results

If the data exported from the microplate or table view contain the result of a standard fit calculation or an enzyme kinetic calculation, an additional sheet in Excel will be created with the result parameter of the standard fit calculation.
To export the result parameters of all performed standard fit calculations or enzyme kinetic calculations, open the fit result window on the corresponding chart and press the 

button.

3.25.3 Define an Excel Report

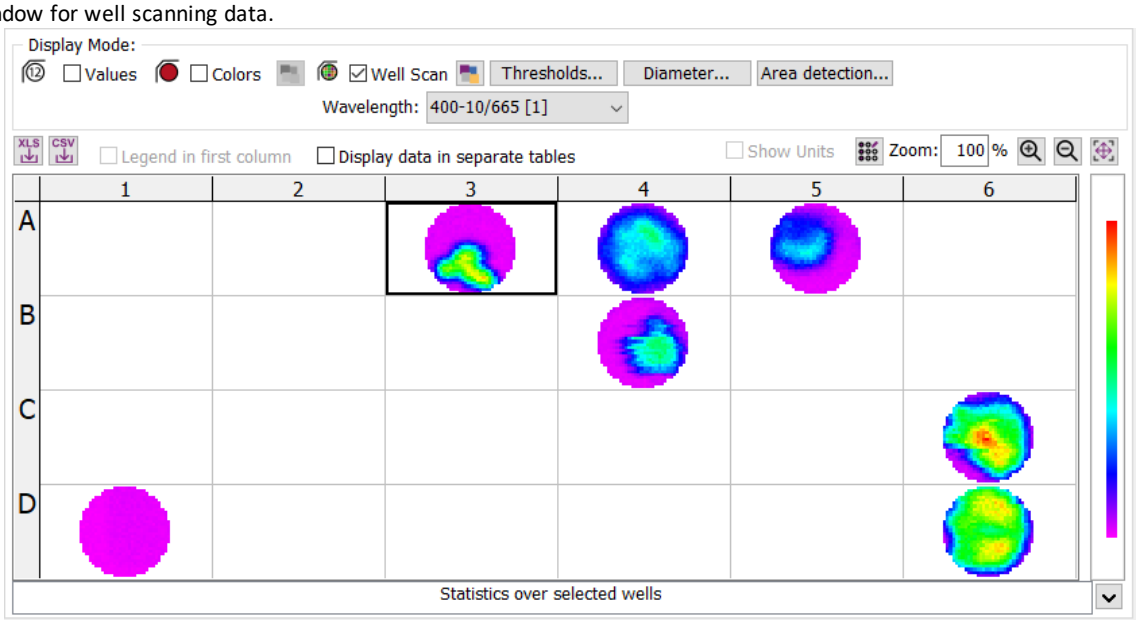
If you want to export not only a single data sheet, you can combine up to all available data pages in one Excel report. Press the *Excel Report* button in the *Test Runs* group on the *Home* tab of the ribbon to define the report:



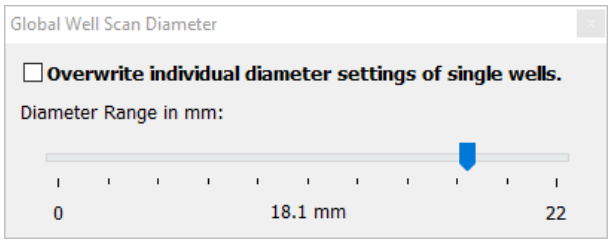
Define the data to export and the special settings for each exported page. The entered report configuration is valid for the opened test run and will be saved together with the test run settings.
The available settings are the same as for the single page export described in the two sections above. In addition, the option *Use one workbook for each page* allows defining, if all exported data should be copied in one Excel workbook, or if a new Excel workbook should be created for each exported data page.

3.26 Well Scanning Data

If the test run contains well scanning data, an additional view button in the *Microplate View* will become visible. Pressing this button will show an overview of the scanned wells. The values are mapped to colors defined with the color settings window for well scanning data.



If more than one wavelength was measured (dual emission or multi-chromatic test runs), you must choose the wavelength to view using the drop-down menu found above the microplate grid.
You can double click on a well to see a zoomed view of the measurement values along with additional information. In this detailed view it is possible to change the scan diameter used for the well calculations of this well, individual thresholds for this well, exclude single scan-points and define areas inside the well.
With the button *Thresholds...* you can define thresholds to exclude scan-points valid for all wells (read section Using thresholds to exclude scan-points in this chapter.
With the *Diameter...* button you can set a new used well diameter for all wells:



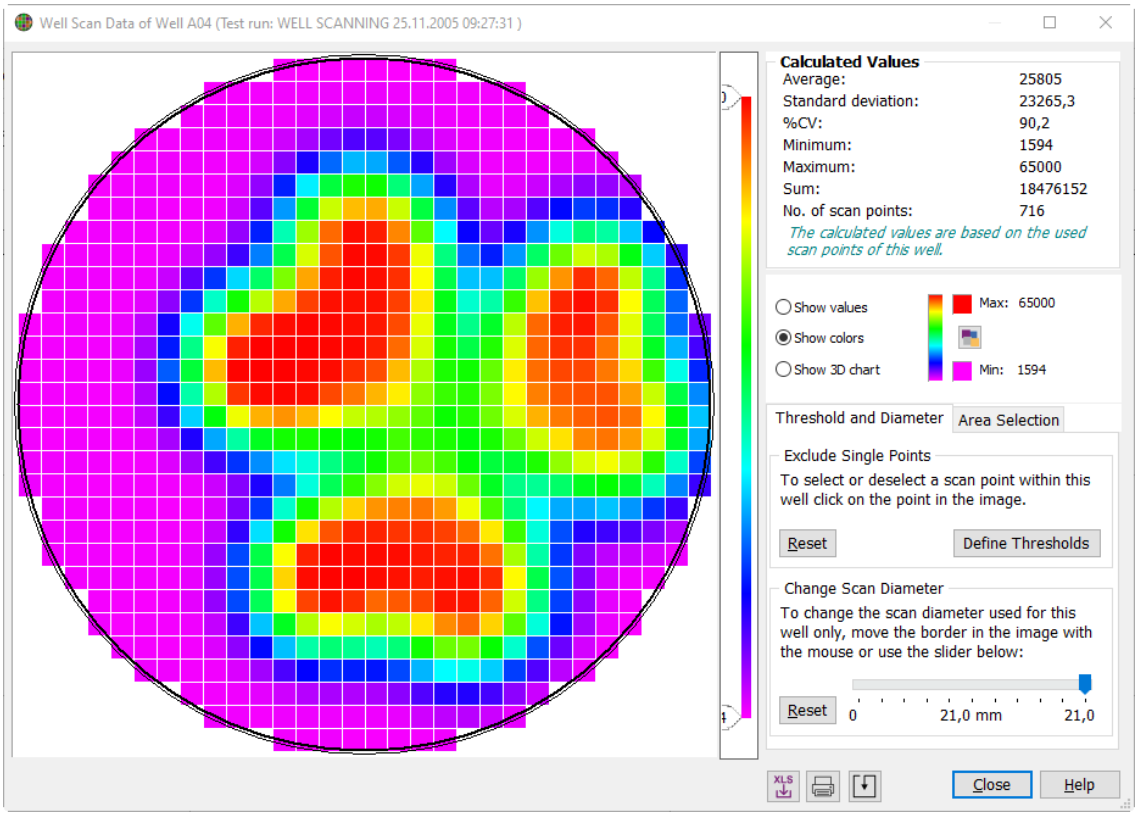
Use the *Overwrite individual diameter settings of single wells* check box to overwrite the individual well diameter settings. If this control is not checked, individual well diameter settings (if defined) will be preserved.
The used scan diameter describes the diameter of a circle (for a round well shape) or of a square (for a square well) that defines the area within the measured data points are used for further calculation.
Changing the diameter size allows users to reduce this area to exclude potential inaccurate readings from the edge of the well.

Use the *Area detection...* button to detect areas inside the well. Read more about area detection in the section Well Scan Area detection at the end of this chapter.

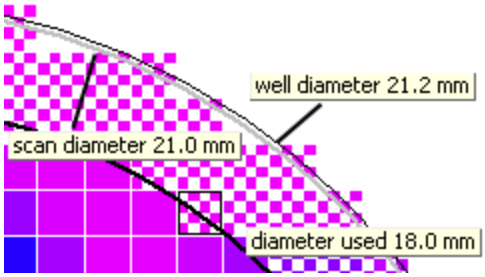
Clicking on the Excel button will export a scan matrix with the measured values of each well to Excel.

3.26.1 Detailed View of Well Scanning Data for a Selected Well

Double clicking on the well in the *Microplate View* when the *Well Scanning View Mode* is active, will open the detail window for this well.



In this window a zoomed view of the selected well will be shown along with some other additional information. When moving the mouse cursor over a scan-point, a hint will appear showing the measurement value of the point. The picture contains three circles (for round wells) or squares (for square wells) with the following descriptions:



The fat black line: Shows the scan diameter used for calculations on the well. All scan-points that fall outside of this line will not be used and are marked to indicate them as not in use.
The fat grey line: Shows the physical scan diameter. This is the diameter used by the reader as a limit when the well is scanned. Only scan-points of the defined matrix whose center lies inside this area are measured. The scan diameter is selected in the protocol settings of a test run in the reader control software.
The thin black line: Shows the border of the well as defined in the microplate database.
When the mouse cursor is moved over one of these border lines, a hint will be displayed showing the identifier and size of the border.

Information and controls on the right side of the Window

Calculated Values:
It contains statistic values for this well like average, standard deviation, %CV, minimum, maximum, sum and number of used scan-points.

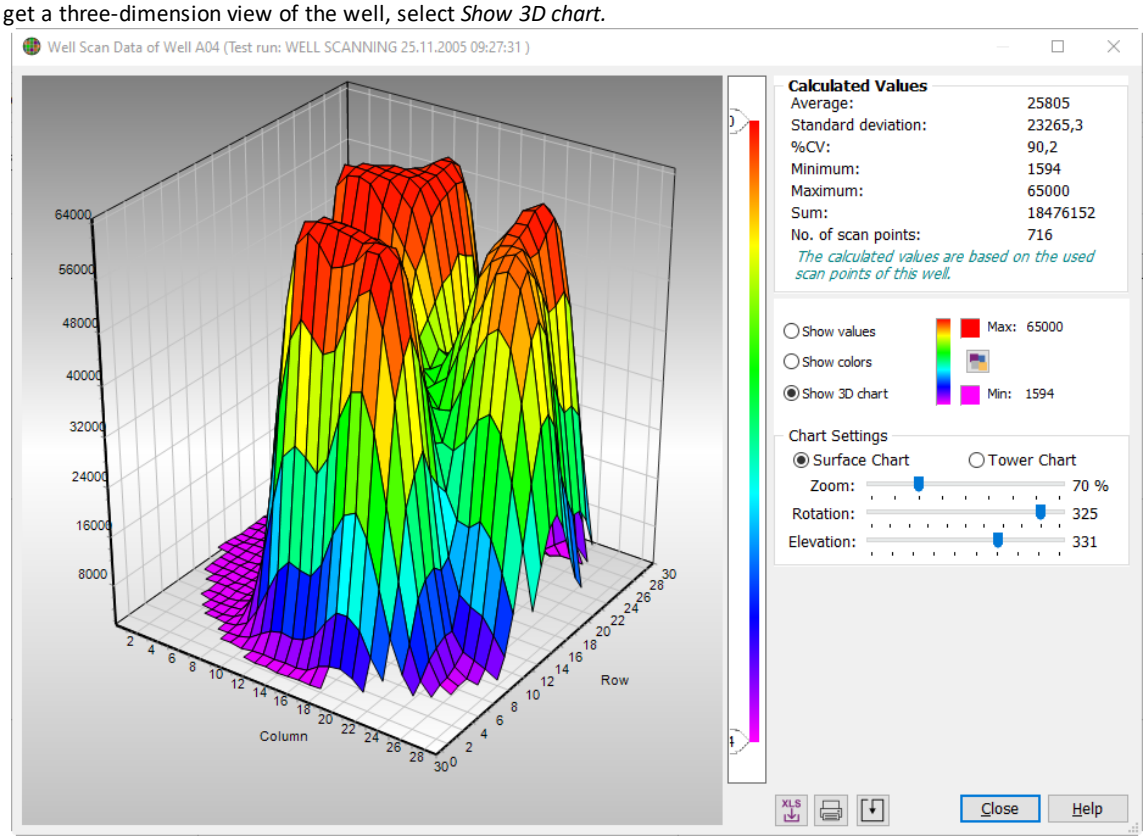
View Settings

To display the values of each measured scan-point, select *Show values*. The image will change and the values for each scan-point will be shown instead of a colored square. It is recommended to maximize the window when using this function so that the font can be displayed in a readable size.

To change back to viewing the data in color mode select *Show colors*.

Press the *Colors* item to change the selected color mode and its settings (see chapter 3.20: *Color Settings*). The color legend shows the color gradient between the minimum value (*Min:*) and the maximum value (*Max:*) of a selected well. To adjust the color settings, you can use the color range selector on the right side of the chart.

To get a three-dimension view of the well, select *Show 3D chart.*

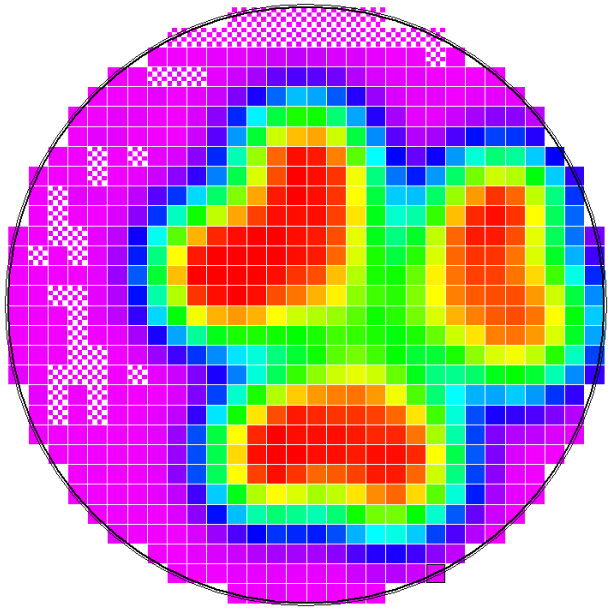


The section *Threshold and Diameter - Area/Cell Selection* on the window is then replaced by the section *Chart Settings* to change the zoom value, the rotation and the elevation of the chart.

Threshold and Diameter - Area/Cell Selection

The *Threshold and Diameter* tab offers controls to exclude single scan-points form this well for further calculations.

Exclude Single Points

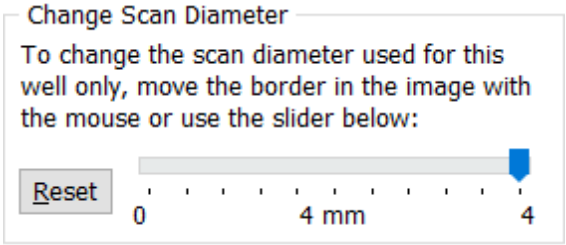


Single scan-points can be excluded from the selected well by clicking on them. Clicking on an excluded scan-point will reactivate the point within the calculations for the well. Excluded (unused) scan-points have a checked pattern on them.

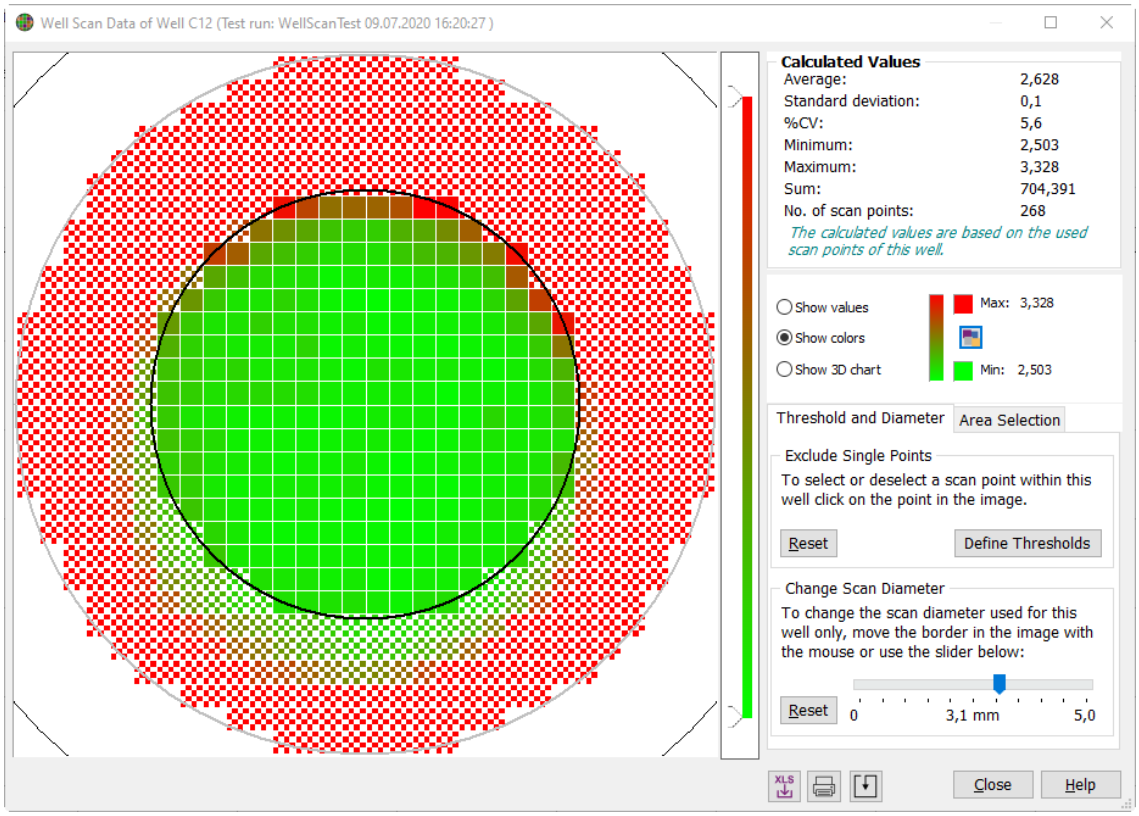
Note: Scan-points that have been excluded using the Scan Diameter Used function will not be reactivated by clicking on them. To reintroduce these points the scan diameter must first be increased.

Pressing the *Reset* button will change back the state of each scan-point to 'used', if its center lies inside the area defined by the *Scan Diameter* in use.
To define thresholds for excluding scan-points, press the *Define Thresholds* button. For more details on how to use thresholds to exclude scan-points see *Using thresholds to exclude scan-points* below.

Change Scan Diameter



The slider control is used to change the diameter. Use the mouse to move the slider and change the diameter. Data points falling outside of the selected area will be displayed in a gray pattern indicating, that these points will not be used for calculating the result value of the well scanned.
Alternatively, the border in the image can be moved by using the mouse to change the diameter. Move the mouse over the fat black line in the image until a hint showing *diameter used xxx mm* appears and the mouse cursor changes to two arrows. Press the mouse button and the color of the border will change to blue, it can then be moved to the desired size before releasing the mouse button.
The *Reset* button sets the diameter back to the scan diameter value.



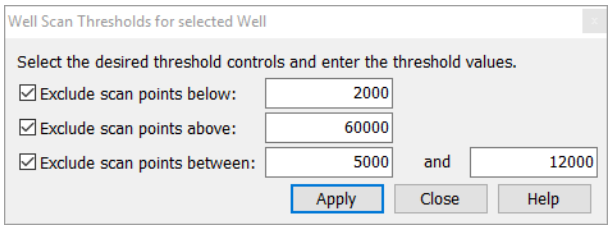
To export the measured values to Excel, press the  button. A matrix of the scanning matrix dimension will be generated in Excel and filled with the values of all the scan-points measured.

To print the window, press the *Print* button  . A window to define the print destination (printer, pdf or html file,...) print orientation, page margins and header or footer contents will be opened (see chapter 3.21: *Printing Your Data*)

Click the save button  to save the well scan detail as a bitmap file or to the clipboard.

Using thresholds to exclude scan-points

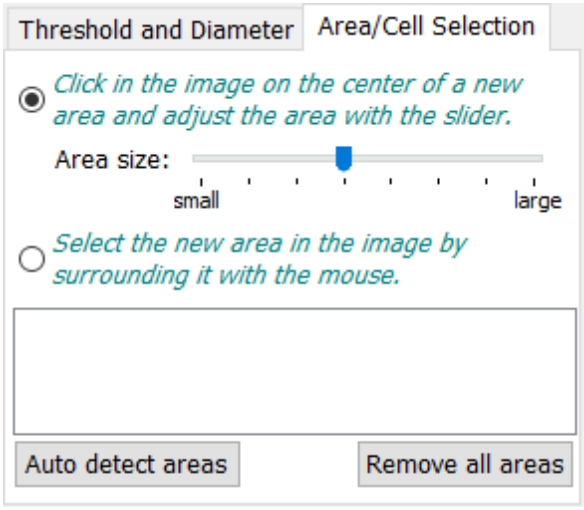
Beside reducing the used scan diameter and clicking on single scan-points, there is a third way to exclude scan-points from further calculation.
You can define thresholds to exclude single scan-points with the window that is show after pressing the *Define Thresholds* button:



There can be four threshold values which can be combined: One to exclude values below a certain number, one to exclude scan-points above a certain number and two to exclude scan-points between these numbers.
To use a certain threshold, activate the according control by checking its check box.
If the *Global Well Scan Thresholds* dialog is used, an additional check box control at the top of the dialog is available: *Overwrite individual threshold settings of single wells*. If this check box is checked, the global threshold settings will overwrite the individual settings. If this control is not checked, individual threshold settings (if defined) will be preserved.

Well Scan Area detection

The area detection is used to find and highlight areas inside the well. There can be one or more areas inside a well. This can be a group of cells located on different positions inside the well.



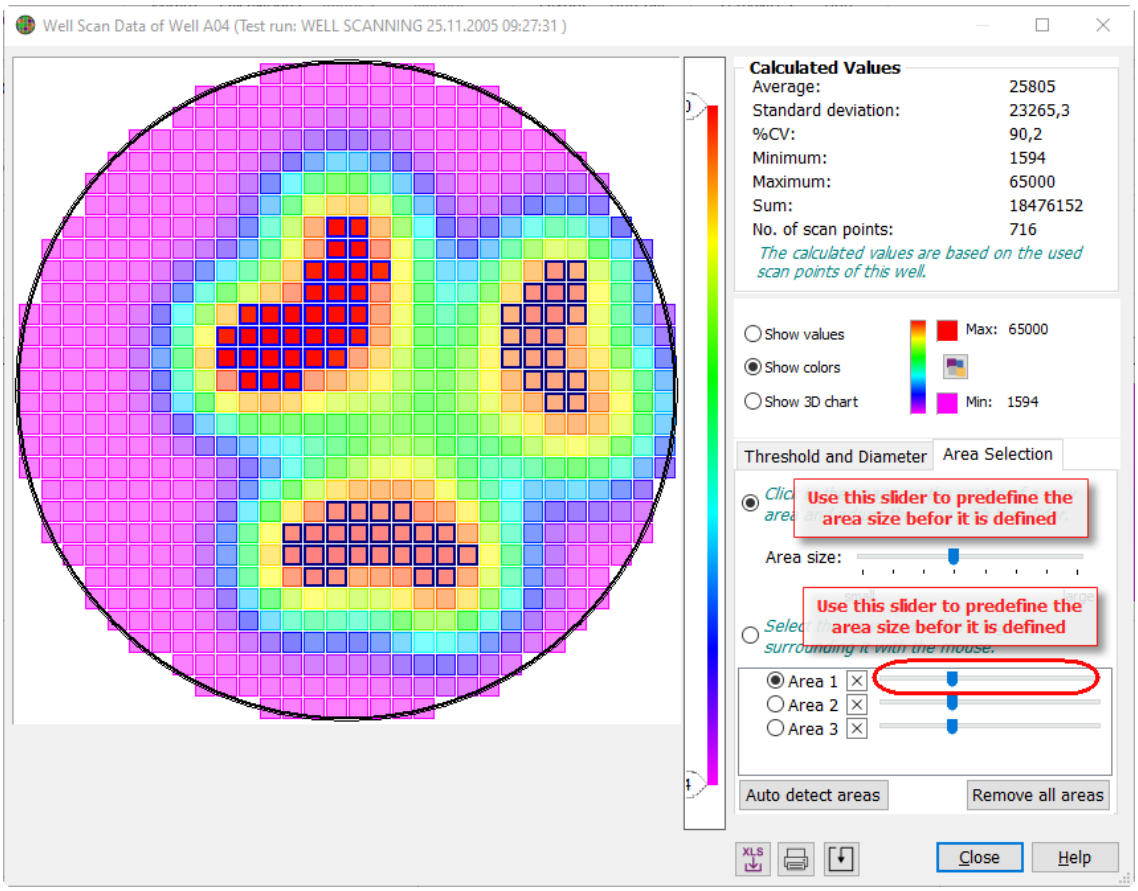
There are three ways available to find and define areas:

1. Select a scan-point inside the area

Select the first control *(Click in the image on the center of a new area and adjust the area with the slider)*

Click on a significant scan-point inside the area you want to define. The area will then be selected automatically, based on the settings of the Area size slider.

The defined area will appear below the controls. You can use the appearing slider on the right side of the area control to adjust the size of the area:



2. Manually define the border of the area

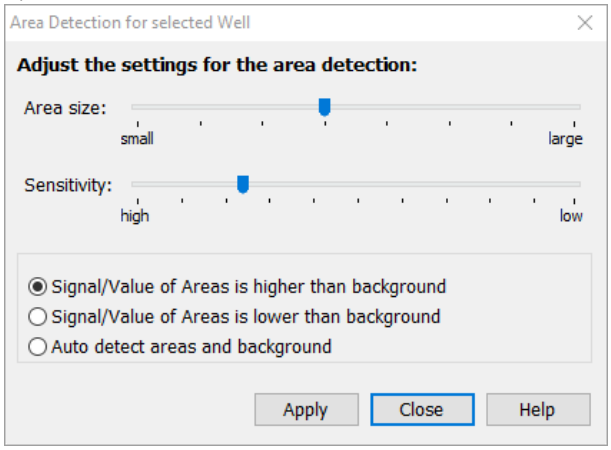
Select the second control *(Select the new area in the image by surrounding it with the mouse)*

Use the mouse to surround the desired area with the mouse inside the scan image by pressing the left mouse button. Keep the mouse button pressed if you define the area border. After releasing the mouse button, the area will be defined according to the border you drew with the mouse.

Manually defined areas do not have a size slider. You can add or remove single scan-points at the border of these areas with a right click on the scan-point you want to add/remove.

3. Detect areas automatically

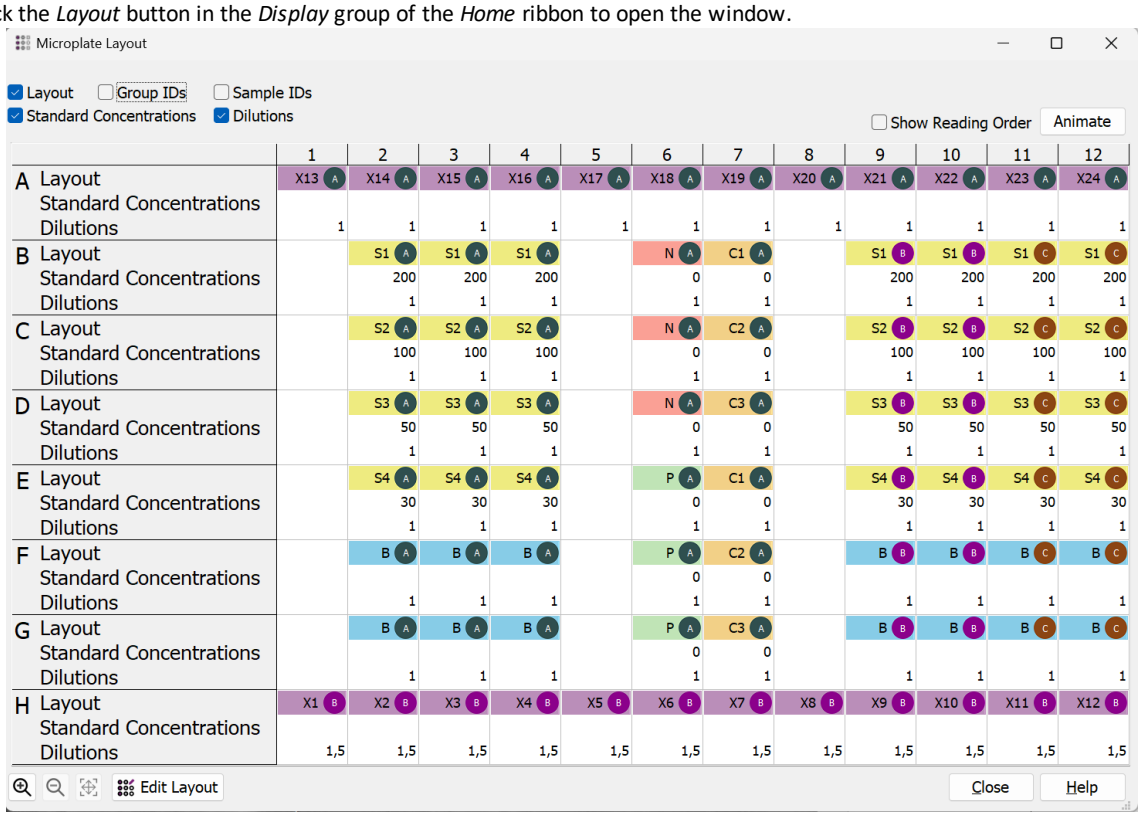
You can let MARS try to detect the areas inside the well automatically. The result of the automatic detection is depending of the significance of the signal and how good the areas are divided from each other. Please check the detected areas after the auto-detection procedure to verify the result. You can adjust the detected areas in their size, and you can remove single areas by clicking on the red cross on the right side of the area name (area1, area2...). You can also add further areas to the detected ones by using one of the first two methods.
To start the automatic detection, click on the *Auto detect areas* button. The *Auto Detect Areas Settings* box opens:



Define the default area size and the sensitivity for the detection. You can repeat the detection with different settings, to get the settings for the best result. The best settings can be individual for each test run. A repeat of the auto-detection will delete all already detected areas.
If the expected areas have a higher signal than the background, choose the first mode (*Signal/Value of Areas is higher than background*), if it is lower, choose the second one. If it is not clear or if you want MARS to find out, choose the last mode (*Auto detect areas and background*). Press *Apply* to start the detection and validate the result.
If the area detection was started for all wells, the box has an additional button *Remove all areas* to remove already detected areas for all wells.

3.27 View Microplate Layout

The microplate layout window shows the layout data of the plate. This is a useful tool to get a quick view on the layout even if the microplate view is not the active page.
Click the *Layout* button in the *Display* group of the *Home* ribbon to open the window.



		1	2	3	4	5	6	7	8	9	10	11	12
A	Layout	X13 A	X14 A	X15 A	X16 A	X17 A	X18 A	X19 A	X20 A	X21 A	X22 A	X23 A	X24 A
	Standard Concentrations												
	Dilutions	1	1	1	1	1	1	1	1	1	1	1	1
B	Layout		S1 A	S1 A	S1 A		N A	C1 A		S1 B	S1 B	S1 C	S1 C
	Standard Concentrations		200	200	200		0	0		200	200	200	200
	Dilutions		1	1	1		1	1		1	1	1	1
C	Layout		S2 A	S2 A	S2 A		N A	C2 A		S2 B	S2 B	S2 C	S2 C
	Standard Concentrations		100	100	100		0	0		100	100	100	100
	Dilutions		1	1	1		1	1		1	1	1	1
D	Layout		S3 A	S3 A	S3 A		N A	C3 A		S3 B	S3 B	S3 C	S3 C
	Standard Concentrations		50	50	50		0	0		50	50	50	50
	Dilutions		1	1	1		1	1		1	1	1	1
E	Layout		S4 A	S4 A	S4 A		P A	C1 A		S4 B	S4 B	S4 C	S4 C
	Standard Concentrations		30	30	30		0	0		30	30	30	30
	Dilutions		1	1	1		1	1		1	1	1	1
F	Layout		B A	B A	B A		P A	C2 A		B B	B B	B C	B C
	Standard Concentrations						0	0					
	Dilutions		1	1	1		1	1		1	1	1	1
G	Layout		B A	B A	B A		P A	C3 A		B B	B B	B C	B C
	Standard Concentrations						0	0					
	Dilutions		1	1	1		1	1		1	1	1	1
H	Layout	X1 B	X2 B	X3 B	X4 B	X5 B	X6 B	X7 B	X8 B	X9 B	X10 B	X11 B	X12 B
	Standard Concentrations												
	Dilutions	1,5	1,5	1,5	1,5	1,5	1,5	1,5	1,5	1,5	1,5	1,5	1,5

Data can be selected to be viewed using the controls above the grid. Some controls only appear when the according data exists in the layout, i.e. if the test run has no injections, the volume group controls will not be visible.

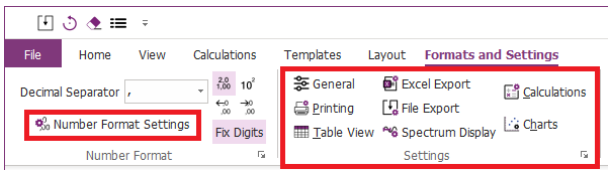
The grid can be zoomed in and zoomed out using the buttons shown. To reset to a view of the whole plate use the whole plate button .
If the reading order of the wells was recorded by the reader, you can display the reading order with the control *Show Reading Order*. Click the *Animate* button to see a visual animation of the reading order.
The size and the position of the window can also be changed and is stored during the whole program session.
Click the Edit Layout button to change the layout of the test run (see Changing Layout).

3.28 Settings

General Settings for MARS, Excel- or file export, number formats or spectrum display can be found in the options dialog:

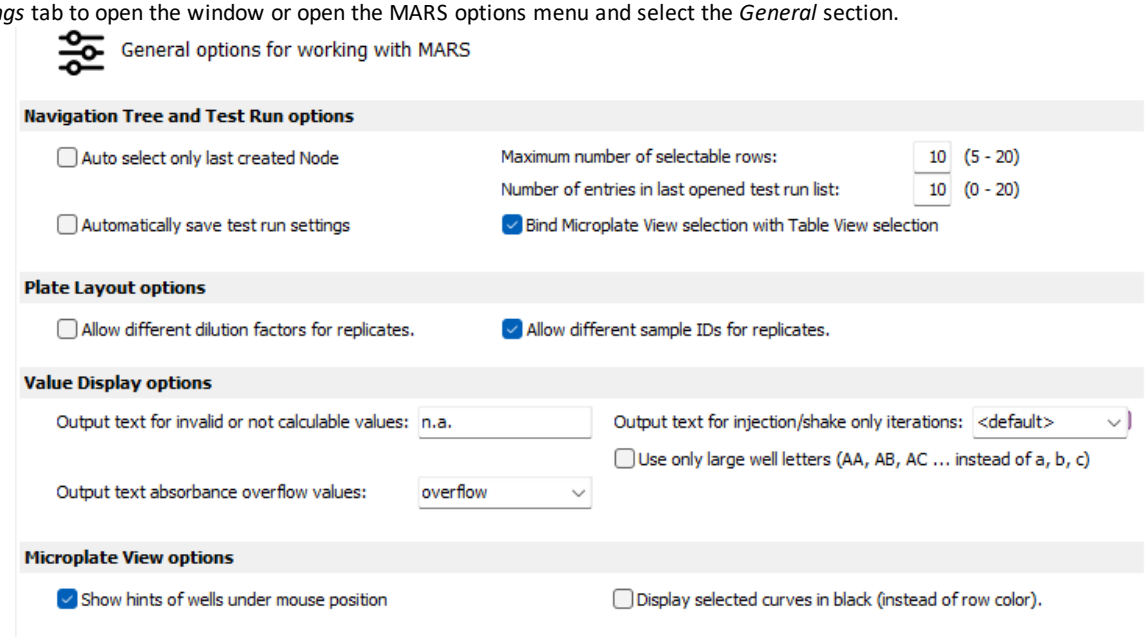
- Open register *File*
- Select Item *Options*

A second way is to open the register *Formats and Settings* and select the option you want to see in the settings group:



3.28.1 MARS Settings

The MARS settings dialog box lets you change general settings. Click *General* in the *Settings* group on the *Formats and Settings* tab to open the window or open the MARS options menu and select the *General* section.



Click *OK* to apply the changes and close the window.

Navigation Tree and Test Run options

Auto select only last created Nodes

If this control is checked (default), the behavior of the navigation tree is like this: If a new node or group of nodes is added, previous selections are deselected and only the new node(s) is selected in the display.
If the box is not checked, the previous selections will remain active in the display if there are free selectable rows left (see *Maximum number of selectable rows* below). New nodes will be selected automatically. If no more rows/colors are left for allocation, the previous selections will be deactivated to make room as new nodes are added.

Maximum number of selectable rows

Enter the number of rows that can be selected in the navigation tree if the microplate view or the table view is visible. The minimum and default is 5 and the maximum is 20.

Number of entries in last opened test run list	Enter the number of entries, shown in the *Recent* list in the *File* menu. The minimum is 0, default and maximum are 10.
Automatically save test run settings	Saves the test run settings automatically when closing the test run.
Bind Microplate View selection with Table View selection	If this control is checked (default), the selection of the displayed data for the microplate view defines also the selection of the displayed data for the table view and vice versa. This is only valid for the first microplate view and the first table view. If more than one microplate view or table view is added, the selection for these views is always individual.

Plate Layout options

Allow different dilution factors for replicates	In standard mode, each replicate gets automatically the same dilution factor. Set this control to enter different dilution factors for each replicate. You can change the dilution factor of wells within the change layout (chapter 6.1.3) procedure of MARS.
Allow different sample IDs for replicates	In standard mode, each replicate gets automatically the same sample ID. Set this control to enter different sample IDs for each replicate. You can change the sample ID of wells within the change layout (chapter 6.1.3) procedure of MARS.

Value Display options

Output text for invalid or not calculable values	Enter a text to be displayed if the value of a well is invalid, not available or not calculable. The default text is *n.a.* (not available).
Output text for injection/shake only iterations	For loop iterations with no measurement data (injection only or shake only iterations), define what to be displayed instead of the measurement value: - <default>: displays *Inj*. for injection only iterations, *Shake* for shake only iterations. - 0: display always the value 0. - <empty>: display nothing - <injection volume>: for injection only iterations: display the injected volume.
Output text for not available chromatic values	This setting is only available if the used reader has a build in monochromator. Define the displayed text for a selected chromatic where no value is available (only possible if an excitation and emission scan was performed).
Use only large well letters	For microplate formats 1536 and 3456: If this control is checked, the well letters a, b, c, ... are replaced with AA, BB, CC wherever they appear (microplate view, table view, print report, file export, Excel export...)
Output text for absorbance overflow values	Define the text that will be displayed, when an overflow value in an absorbance measurement appears. Select a text in the drop down list or enter your own text to be displayed. Select <empty> in the list if nothing should be displayed, select <overflow value>, if the overflow value in OD should be displayed.

Microplate View options

Show hints of wells under mouse position	If selected, a hint will be displayed for the well under the

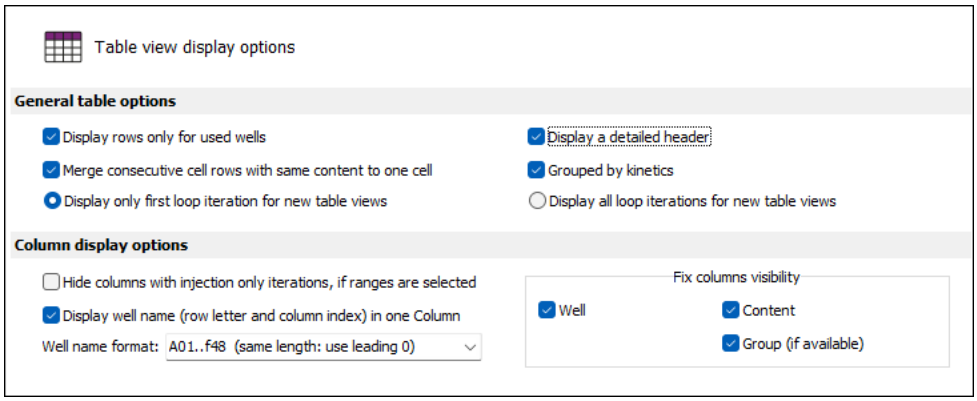
mouse position.

Display selected curves in black (instead of row color)

Shows all selected kinetic or spectrum curves always in black (for higher contrast) instead of the color of the row selection.

3.28.2 Table View Settings

The *Table view display options* window lets you change the display settings of the table view .The window can be opened by clicking the *Table View* command in the *Settings* group of the *Formats and Settings* tab or open the MARS options menu and select the *Table View* section.



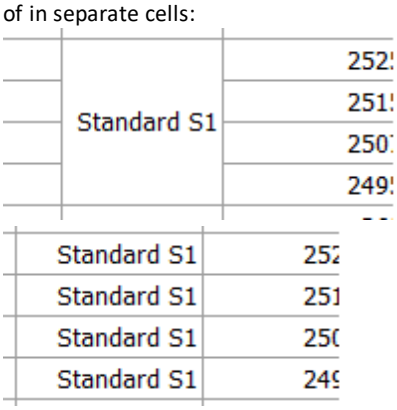
General table options

Display rows only for used wells

If this checkbox is unchecked, a row for each well in the micro-plate is displayed, even if it is not used in the layout of the test run.

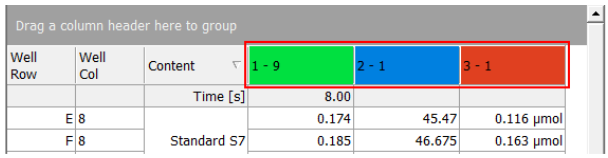
Merge consecutive cell rows with same content to one cell

Display cell rows, with the same content as one cell instead of in separate cells:



Detailed header

If this checkbox is checked, the header of the data rows shows the text of the legend for this column. If it is unchecked, only the number of the row in the legend is displayed in the header:



Grouped by kinetics

If the test run contains kinetic data, the table view displays the data grouped by kinetics (displays each loop data point for the selected data first) if this control is selected (default). If not selected, the table view displays the data grouped by the selected data (displays the selected data together, loop data point by loop data point).

Display only first loop iteration for new tables

If the selected data contains loops, check this control to see

initially only the first loop data point of the selected data (you can select further/different data points to be displayed above the table view)

or

Display all loop iterations for new table views

To display all loop data point s at once in the table, select this control (this can make the creation of the table view slow, if many loop iterations are displayed).

> **Note:** The selected option will only be applied to new table views. All changes you make on existing table views will remain.

Column display options

Hide columns with injection only iterations, if ranges are selected

If selected, no columns will be created for injection or shake only iterations, if a kinetic range is selected to be displayed in the table view.

Display well name (row letter and column index) in one Column

Displays the well row and well column information in one cell *Well* (default) instead of two separate columns (*Well Row* and *Well Col*).

Well name format

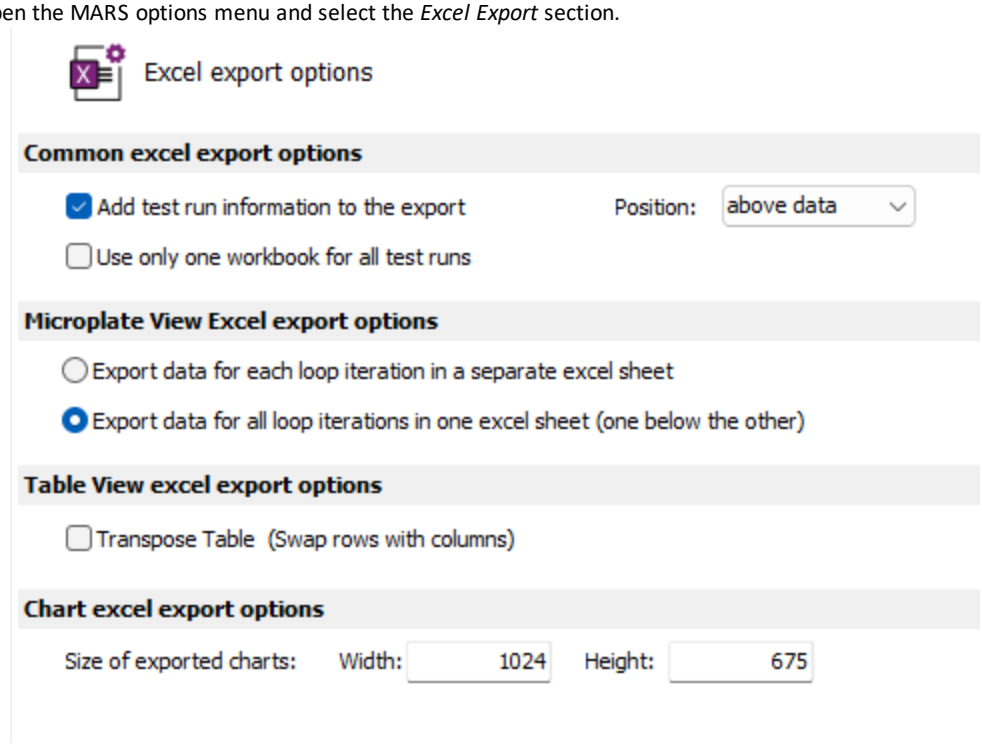
Defines the displayed format for the well column content. Select the desired display format in the drop-down list.

Fix columns visibility

Show or hide the fix table view columns Well, Content and Group.

3.28.3 Excel Export Settings

The Excel export settings window lets you change the settings for data export to Excel.
To open the window, select the *Formats and Settings* Tab on the ribbon and press the *Excel Export* button on the *Settings* group or open the MARS options menu and select the *Excel Export* section.



Common Excel export options

Add test run information to the export

Check this control to add further information about the exported data. These are:

- Username and data directory
- test run no., test run name, measurement date and time
- plate ID1- ID3
- Measurement method.

Position	If *Add test run information to the export* is checked, define if the information should be exported *above* or *below* the exported data.
Use only one workbook for all test runs	Check this control, if data from different test runs should be exported into one Excel workbook.

Microplate View Excel export options

Export data for each loop iteration in a separate Excel sheet	Each loop iteration will be exported to its own Excel sheet but in one Excel document. The name of the sheet is generated using the iteration number and the corresponding time value.
Export data for all loop iterations in one Excel sheet (one below the other)	Data for all loop iterations are exported to one Excel sheet as tables or group of tables (if the microplate contains more than one data row) one below the other.

Table View Excel export options

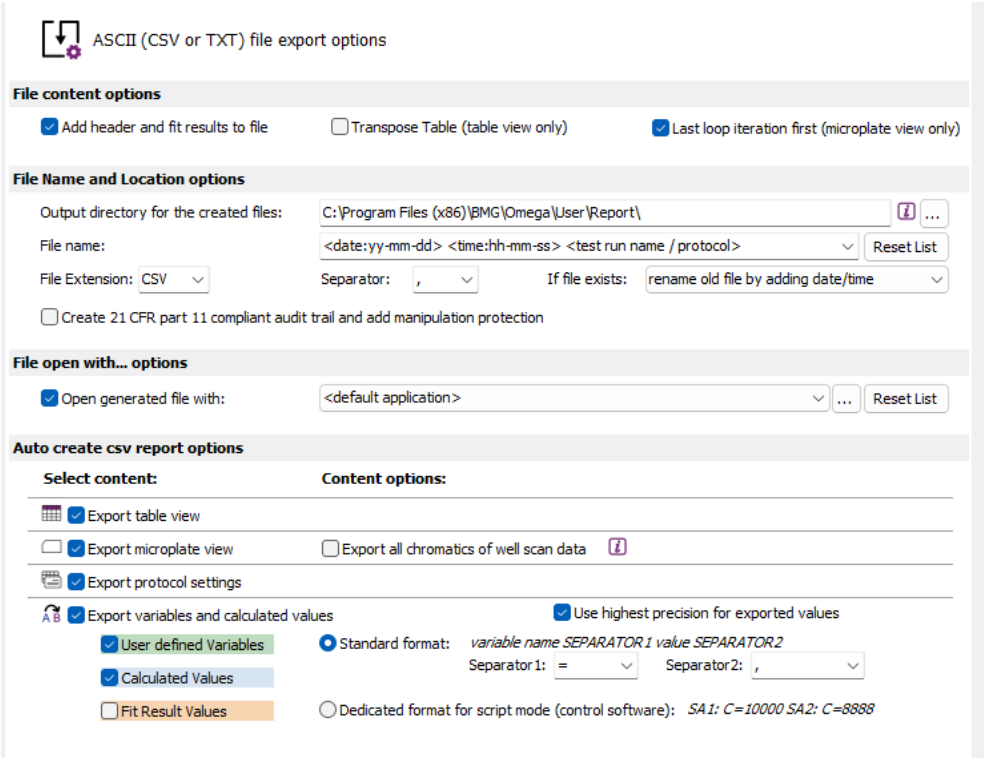
Transpose Table	For tables with many columns and only a view rows it could be useful to swap the columns with the rows before exporting the data to Excel. Tables with more than 250 columns (e.g. Spectrum tables or tables with more than 250 iterations) cannot be exported into one Excel sheet for Excel version older than Excel 2007 if the *Transpose Table* option is not selected.

Chart Excel export options

Size of exported charts	Define the default width and height for charts exported to excel.

3.28.4 File Export Settings

The *File Export Settings* window lets you change the settings for text file export of the MARS microplate view, table view or protocol settings. The window can be opened by clicking the *File Export* command in the *Settings* group of the *Formats and Settings* tab or open the MARS options menu and select the *File Export* section.



The text file will be generated as *Comma Separated Value* (CSV) file, which means, each row of the table is a row in the file and the columns of each row are separated by a separator in the file. The file can be opened with any text editor (e.g. notepad) and, if the text file is stored with the extension CSV, it can also be opened with Excel.

File content options

Add header and fit results to file

Check this control if test run information and - if available - standard fit results should be added to the file. The information will be stored above the table data in the file.

Transpose Table

This function is only valid for the MARS table view. For tables with many columns and only a view rows it could be useful to swap the columns with the rows before exporting the data. Check this control to transpose the rows with the columns of the table.

Last loop iteration first

This function is only valid for the MARS microplate view if the test run contains kinetic data. If selected, the export starts with the data of the last iteration in the generated file instead of the first iteration.

File Name and Location options

Output directory for the created text files

Enter the directory path, where the generated files should be stored. Use the button to open an explorer window to select the destination directory. The directory can also contain automatic text to sort the exported files. The same syntax like for the file name is used (except the automatic file name creation).

File name

Enter the name of the file without file extension or use the drop-down list, to select one of the proposed file names. You can use either constant typed in text or automatically generated text (enclosed in < and >). You can combine constant and automatic text. See the description of the syntax for automatic file name creation below. Each new name entered by the user will be added to the drop-down list for further usage. To reset the list to its default entries, click *Reset List*.

File Extension

Select the extension of the file. You can choose either CSV (which is the usual ending for those kinds of files) or TXT (which indicates, that the file is an ASCII text file) or you can enter an extension you like.

Separator

Select or enter a separator. The separator separates the column values of each row. The standard separators are: , and ;, depending on the operating system language (English, German, French...).

If file exists

Define the behavior what MARS should do, if there is already a file with the same name:

- *rename old file by adding date/time*: the existing file will be renamed by adding the current date and time to the file name.
- *overwrite old files*: the existing file will be overwritten (without prompting)
- *append data (with separation line)*: the new export data will be appended to the file separated by a dashed line.
- *append data (without separation line)*: the new export data will be appended to the file separated only by an empty line.

Create 21 CFR part 11 compliant audit trail and add manipulation protection

If you use this option, there will be an additional file created for each ASCII file (same file name, but with additional file extension '.at'). This file will contain an anti-manipulation hash value and the audit trail for the data stored in the connected ASCII file. The anti-manipulation hash value will protect the whole ASCII file and the audit trail entries (see FDA 21 CFR part 11 compliance). The hash value will be created when the ASCII data file is created. It can be checked using the 'Check Data Integrity' tool, which will be installed with the BMG software.

> **Note:** This functionality is not yet available for MARS used with Voyager software.

Automatic file name creation:

The file name can be generated automatically, using the following options:

<automatic filename creation>	MARS creates the filename automatically, combining the fix text *TRno* with the test run id. **Example**: *TRno3.CSV* (assuming the selected file extension is CSV).
<test run name / protocol> or <test run name>	Name of the test run (and the protocol the test run was created with)
<method>	Name of the measurement method, e.g. *Fluorescence Intensity* or *Luminescence*
<ID1> ... <ID3>	Plate ID1 ... Plate ID3
<date:yyyy-mm-dd>	insert test run creation date in long format (using four sings for the year, two for the month and two for the day)
<date:yy-mm-dd>	insert test run creation date in short format (using two sings for the year, two for the month and two for the day)
<time: hh-mm-ss>	insert test run creation time in long format (using two sings for the hours, two sings for the minutes and two signs for the seconds)
<time: hh-mm>	insert test run creation time in short format (using two sings for the hours and two sings for the minutes)
<test run id>	insert the test run id (automatically created when the test run was created)

The default setting for the file name creation is a combination of date, time and test run name: *<date:yyyy-mm-dd> <time: hh-mm-ss> <test run name / protocol>.*

File open with ... options

Note: This setting is not used, if MARS is running in automatic mode or if the text files are generated from the manage test runs list. In these cases, the generated files are only saved at the defined location.

Open generated file with	Check this control to open the generated file automatically. You can either select or enter a program to open the file or you can find the operating system the default program for the generated file. To define the program, enter the name or browse the directories after pressing the  button to find the desired program. Select *<default application>* and the operating system opens the file with the program, linked to the defined file extension. If *<open folder only>* is selected, the windows explorer starts with the folder opened, where the generated file is stored. Each new application selected or entered by the user will be added to the drop-down list for further usage. To reset the list to its default entries, click *Reset List*.

Auto create csv report options

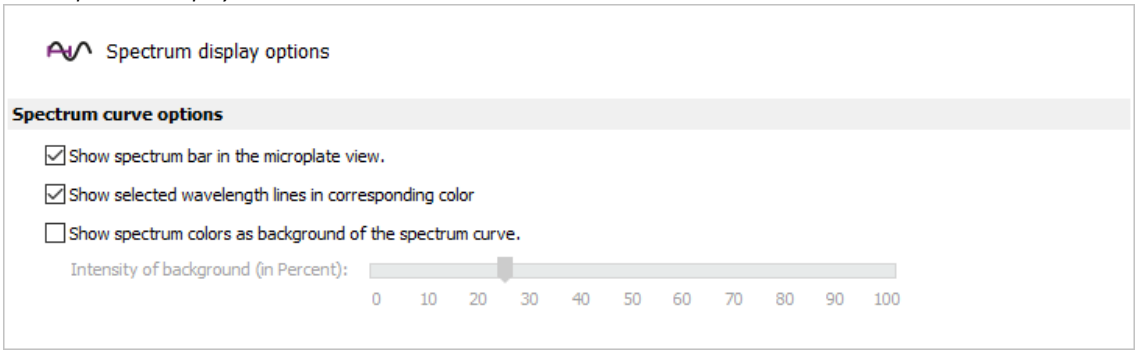
Note: This setting is only used, if MARS is running in automatic mode or if the text files are generated from the manage test runs list.

Export table view	If selected, the generated file contains the content of the MARS table view.
Export microplate view	If selected, the file contains the content of the MARS microplate view. **Export all chromatics of well scan data**: If the test run contains well scan data with more than one chromatic, only the first chromatic will be exported if this control is not checked. Check this control to export the well scan data for each chromatic.
Export protocol settings	If selected, the file contains the content of the protocol information page
Export variables and calculated values	If selected, variables and calculated values will be integrated into the export. Select the type of variables you want to export (user defined variables, calculated values and fit result values).

You can choose between the standard format where the name and the value will be exported using the selected separators, or the dedicated script mode format, which can be used in the control software script language.

3.28.5 Spectrum Display Settings

The *Spectrum Display Settings* window lets you change the settings for spectrum chart presentation. Click *Spectrum Display* in the *Settings* group on the *Format and Settings* tab of the ribbon to open the window or open the MARS options menu and select the *Spectrum Display* section.



Spectrum curve options

Show spectrum bar in the microplate view — If checked, a small spectrum bar is displayed under the spectrum curve in each well used in the microplate view, if the spectrum view mode is active.

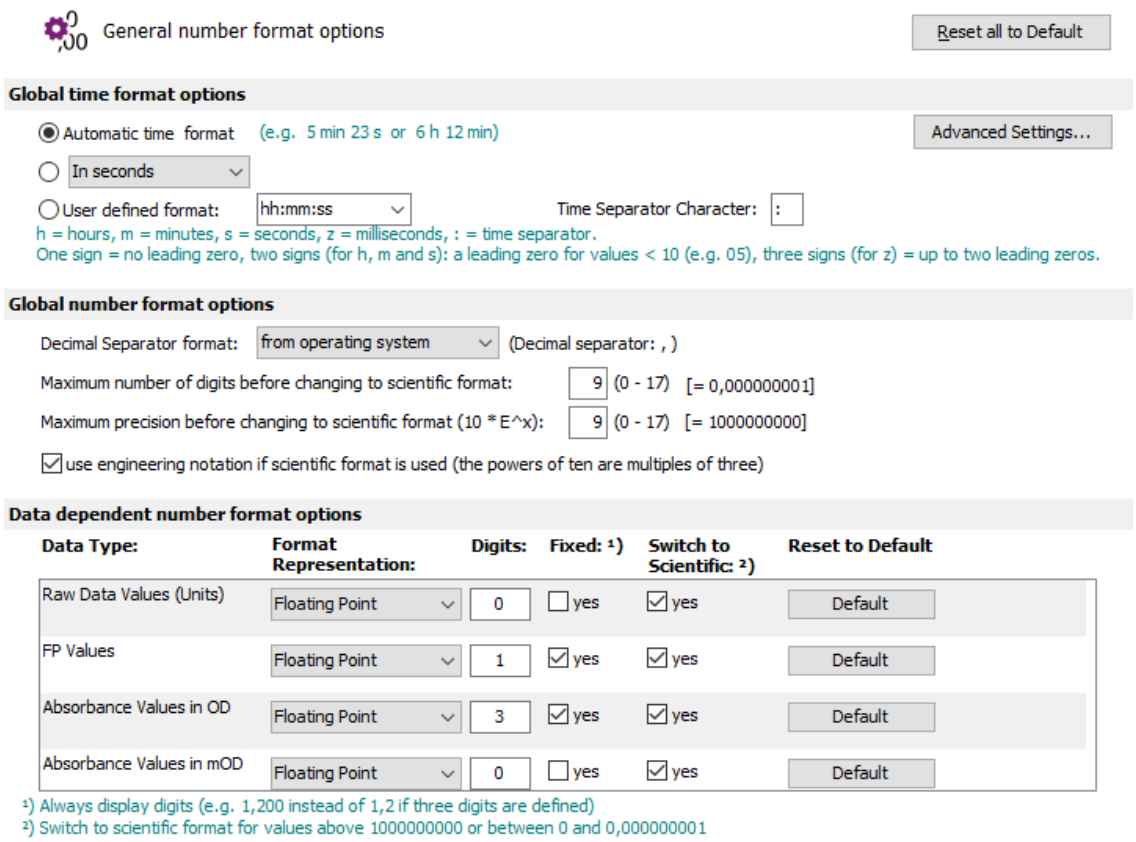
Show selected wavelength lines in corresponding color — If checked, the wavelength lines viewed in the spectrum curve chart will be displayed in the color of their lambda value.

Show spectrum colors as background of the spectrum curve — If checked, a light background, colored according to the lambda value of the X axis is displayed in the spectrum curve chart.

Intensity of background — This sliding bar is enabled, when the spectrum color background above is checked. The intensity of the background can be changed using the slider.

3.28.6 Number Format Settings

The presentation of numerical data is based on general number format settings. These settings are defined with the *Number Format Settings* window. Click *Number Format Settings* in the *Number Format* group on the *Formats and Settings* tab of the ribbon to open the window or open the MARS options menu and select the *Number Formats* section.
To change the presentation of certain number formats, use the function described in chapter 3.25.6.



Press the *Reset all to Default* button to reset all changes to default settings.

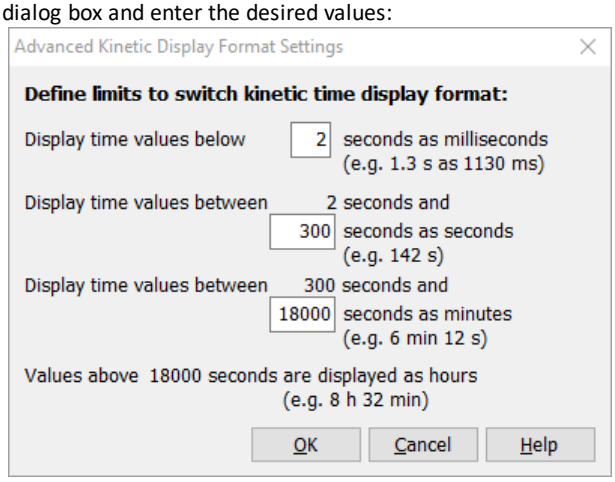
Global time format options

Use automatic display format

Select this mode to use the automatic time format function. Time values are displayed in one of the four ways shown in the table below:

Maximum displayed time value	value displayed in
< 2 seconds	ms (milliseconds)
>= 2 seconds and < = 5 minutes	s (seconds)
> 5 minutes and < 5 hours	min (minutes) (e.g. 24 min 13 s)
>=5 hours	h (hours)

You can change the default limits to switch between the different display formats: Click *Advanced Settings...* to open the *Advanced Kinetic Display Format Settings* dialog box and enter the desired values:



In seconds, In minutes, in hours

Select this mode to display time values always in seconds or in minutes or in

hours.

User defined format

Select this mode if you want to define your own time display format. Use the drop-down list to select one of the proposed formats or enter your own format. See the table below, how to define the format:

h	hours. Only as many signs as needed are displayed.
hh	hours. Minimum two signs are displayed (02 instead of 2).
m	minutes. Only as many signs as needed are displayed.
mm	minutes. Minimum two signs are displayed (02 instead of 2).
s	seconds. Only as many signs as needed are displayed.
ss	seconds. Minimum two signs are displayed (02 instead of 2).
z	milliseconds. Only as many signs as needed are displayed.
zz	milliseconds. Minimum two signs are displayed (02 instead of 2).
zzz	milliseconds. Minimum three signs are displayed (002 instead of 2).

Note: Using the formats s,z or ss,zzz is only useful for time values below one minute, because the seconds are only displayed up to 59. The same is for minutes above 59 (for e.g. m:ss,zzz).

Time Separator Character

If the user define format is selected, you define the separator between time values (separator between hours, minutes, seconds and milliseconds) in this field.

Note: The time format settings are ignored for ALPHASCREEN, TRF and TRF advanced test runs!

Global number format options

Decimal Separator Format

Select from the drop-down menu the style of numbers used, either in the native format (defined by the operating system) or the English number format. The used style defines the decimal separator. The used separator is displayed behind the drop-down menu.

Maximum number of digits before changing to scientific format

Defines the maximum number of displayed digits. If the displayed value has more than the maximum number of displayed digits and *Switch to Scientific* (see below) is enabled for this value, the value is displayed in scientific format (e.g. maximum digits = 5, *0.00000123* is displayed as *1.23E-6*).

Maximum precision before changing to scientific format

Defines the maximum displayed value in floating point format. If the absolute displayed value is greater than the $10 * 1^n$ (n= maximum precision value) and *Switch to Scientific* (see below) is enabled for this value, the value is displayed in scientific format (e.g. n = 5, *2300000* is displayed as *2.3E6*)

use engineering notation of scientific format is used

Check this box (default) if in scientific format the powers of then should be multiples of three (e.g. *230000* is *0.23E6* instead of *2,3E5*).

Data dependent number format options

MARS can display numerous types of data. For the different number types, the standard display settings can be defined separately. For each data type, the following settings can be defined:

Format Representation

Use the drop-down menu to define whether the data should be displayed in floating point style (like *1.243*) or in scientific style (like *1.2E-3*).

Digits

Only enabled if floating point style is selected. Enter the maximum number of digits to be displayed for the number (the number will be rounded if necessary).

Fixed

Only enabled if floating point style is selected. If checked, the number will always be displayed with the entered number of digits. Missing digits are displayed as 0 (e.g. 1.2 is displayed as 1.200 if three digits are entered)

Switch to scientific	**Only enabled if floating point style is selected**. If checked, the presentation of the number will be switched to a scientific representation if the number exceeds the defined minimum or maximum limits for floating point representation. The limits are defined in the *General Number Format Settings* section above.

Press the *Default* button to reset the settings for this data type to default settings.
The last entry in the list ***Not specified Values*** is used for all numbers where none of the other entries in the list is valid.

3.28.7 Number Format Settings for Data Nodes and Chart Axes

The presentation of numerical data is based on general number format settings. These settings are defined with the *Number Format Settings window*. Click the *Default Number Format Settings* button in the *Number Format* group on the *Formats and Settings* tab to open the *Number Format Settings* dialog box.
In addition, the presentation of certain numerical data can be changed with the *Change Format Settings* dialog box.
These data can be:
1. The displayed data of each node in the navigation tree that refers to numerical data. For data nodes certain settings can also be changed directly with the controls in the *Number Format* group on the *Formats and Settings* tab:

2.0
1.00 Display data in floating point presentation

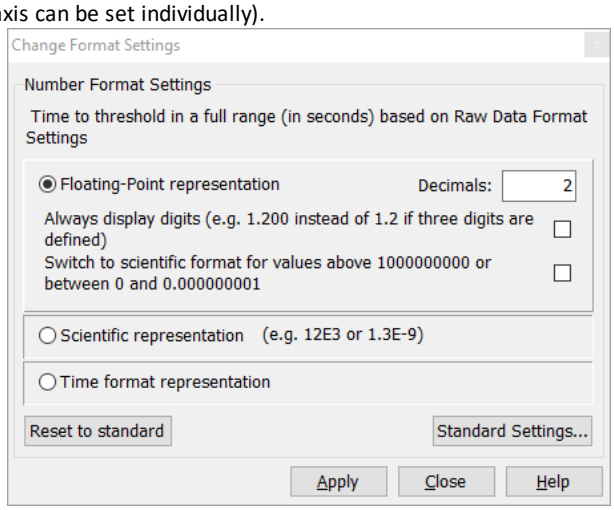
10^2 Display data in scientific presentation

→0
.00 Increase the number of maximum displayed decimal places

←0
.00 Reduce the number of maximum displayed decimal places.

Fix Digits If pressed (highlighted), the number of displayed digits is fix (see Always display digits below)

2. Axis labels of charts (each axis can be set individually).



To open the *Change Format Settings* dialog box for data nodes in the navigation tree, you have to select the concerned node in the tree and open the *Change Format Settings...* menu item in the popup menu of the navigation tree or click the dialog box launcher in the *Number Format* group on the *Formats and Settings* tab.
To change the number format settings of displayed axis labels, press the *Change Settings* button on the *Axis settings window* to open the *Change Format Settings* window for this axis.
With the *Reset to standard* button the settings can be changed to the standard format settings defined in the *Number Format Settings window*.
The *Standard Settings...* button will open the *Number Format Settings window*.
To use the entered settings, press the *Apply* button.

Changeable Settings

The data can be displayed in a floating-point representation or in scientific representation:
Floating-point representation
The floating-point representation displays data in the normal format like *1.25* with a certain number of digits separated by the preset decimal separator.

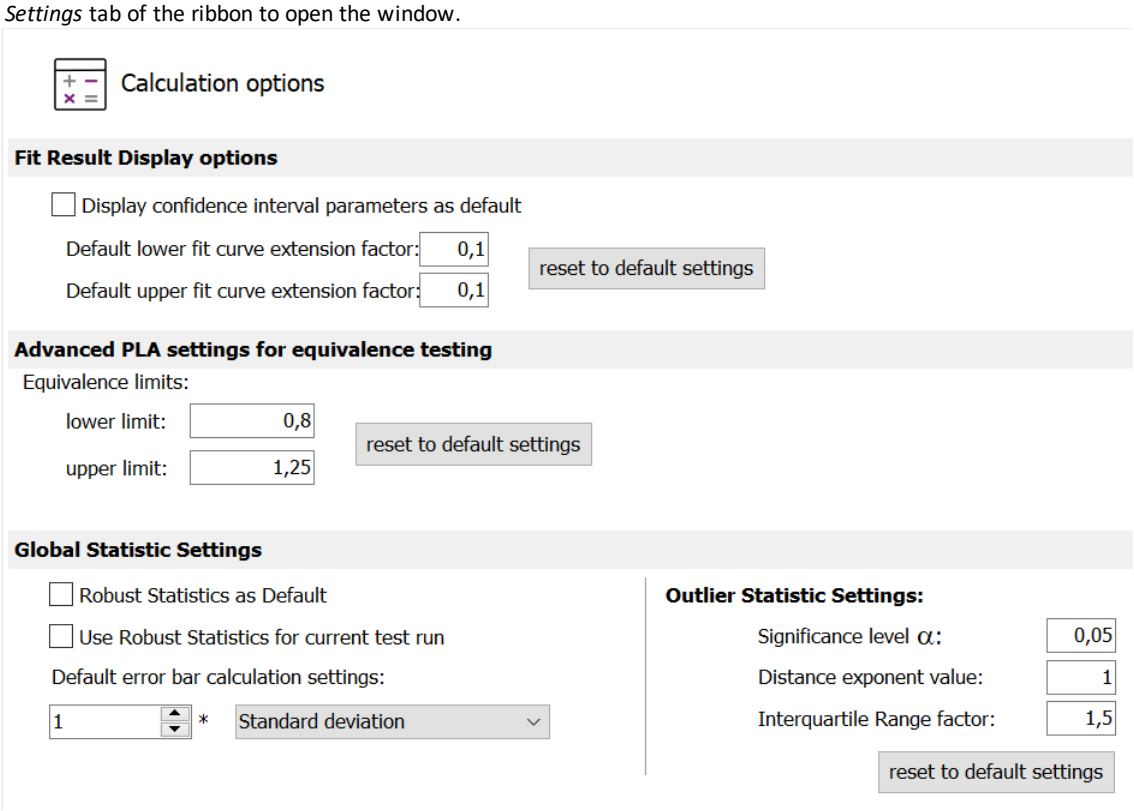
Digits	Enter the maximum number of digits to be displayed for the number (the number will be rounded if necessary).
Always display digits	If checked, the number will always be displayed with the entered number of digits. Missing

	digits are displayed as 0 (e.g. 1.2 is displayed as 1.200 if three digits are entered)
Switch to scientific format...	If checked, the presentation of the number will be switched to a scientific representation if the number exceeds the defined minimum or maximum limits for floating point representation. The limits can be defined in the *Number Format Settings window*.

Scientific representation
The scientific representation displays data always in a format like *8.5E12* or *1.25E-9* where *8.5E12* means $8.5*10^{12}$ and *1.25E-9* means $1.25*10^{-9}$.
Time format representation
The time format representation displays data as time values. The format settings for time values can be set with the global time format options.

3.28.8 Calculation Settings

The *Calculation options* window lets you change basic settings for fit calculations, parallel line fit calculations, global statistic calculations and default settings for the error bar calculation. Click *Calculations* in the *Settings* group on the *Formats and Settings* tab of the ribbon to open the window.



Click *OK* to apply the changes and close the window.

Fit Result Display options

Display confidence interval parameters as default	The fit result window for standard fits will display the parameter values for the confidence interval beside the other fit result parameters when first opened.
Default lower fit curve extension factor	This factor determines how far the fit curve is drawn below the smallest standard. The value is used as standard value but can be overwritten by an individual factor for each standard fit.
Default upper fit curve extension factor	This factor determines how far the fit curve is drawn above the highest standard. The value is used as standard value but can be overwritten by an individual factor for each standard fit.

Press the *reset to default settings* button to return to the default factors (0.1 for the upper and lower factor)

Advanced PLA settings for equivalence testing

Equivalence limits:
For an equivalence test of a PLA calculation, the used lower and upper limits can be changed. For more details, read the chapter Parallel Line Analysis.

lower limit	Enter the value for the lower equivalence limit.
upper limit	Enter the value for the upper equivalence limit.

Press the *reset to default settings* button to return to the default limits (0.8 for the lower limit and 1.25 for the upper limit)

Global Statistic Settings

The first two check box controls define if robust statistic should be used for statistical methods like average, standard deviation and percent coefficient of variation (%CV). Read more about robust statistic in the chapter Robust Statistics.

Robust Statistics as Default	Robust statistics is used for all new test runs
Use Robust Statistics for current test run	Robust statistics is used for the currently opened and selected test run only.

Default error bar calculation settings

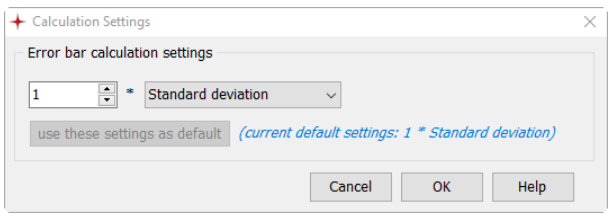
As a default, the error bar calculation is based on the standard error. You can change the default calculation for all error bar here.
Use the drop-down control to select the calculation method for the error bar. This can be standard deviation, standard deviation n, standard error, standard error n, CI mean or CI median.
For the standard deviation and the standard error methods, you can define that the error bar value should be multiplied with a factor.
For the CI based methods, you can define the CI% factor (0-100).
You can enter the desired factor in the entry field left to the drop-down control.
If you want different error bar settings for certain charts, you can change the settings for that chart in the according chart dialog.

Outlier Statistic Settings

Significance level α	Probability of identifying a value as outlier although it is actually none (only applicable for Grubbs-Test, GESD-Test and Dixon-Test).
Distance exponent value	Determines the kind of distance function used for outlying curve identification (e.g. 1: City-block distance, 2: Euclidean distance).
Interquartile Range factor	This factor for the Tukey test is multiplied by the interquartile range and added to the 75 % quartile and subtracted from the 25 % quartile and determines the upper and lower limit from which a data point is identified as outlier.

Press the *reset to default settings* button to return to the default values.

3.28.9 Error Bar Calculation Settings



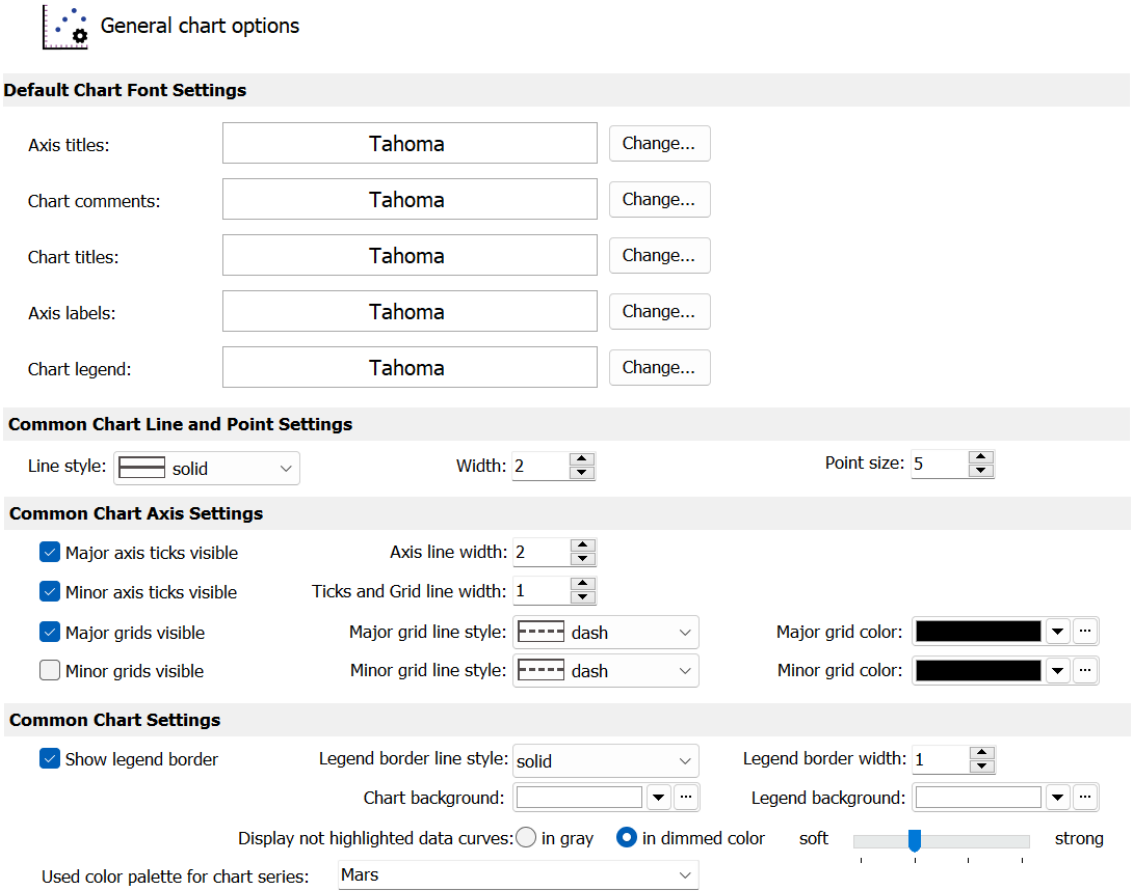
Error Bar Calculation Settings

As a default, the error bar calculation is based on the standard error. Sometimes a different error bar is desired. You can change the calculation for the error bar here.
Use the drop-down control to select the calculation method for the error bar. This can be standard deviation, standard deviation n, standard error or standard error n, CI mean or CI median.
For the standard deviation and the standard error methods, you can define that the error bar value should be multiplied with a factor.

For the CI based methods, you can define the CI% factor (0-100).
You can enter the desired factor in the entry field left to the drop-down control.
If you click on the button *use these settings as a default*, you can define that these settings should always be used. Otherwise the settings will only be used for the current chart.

3.28.10 General Chart Options

The *General Chart Options* window lets you change the default settings for charting in the MARS. The window can be opened by clicking the *Charts*command in the *Settings* group of the *Formats and Settings* tab, open the MARS options menu and select the *Charts* section, or press the *Edit Default Settings* button on the settings dialog of the different charts.



The default settings will be used for new created charts or if the *Reset Settings* button is pressed on the charts settings dialog.

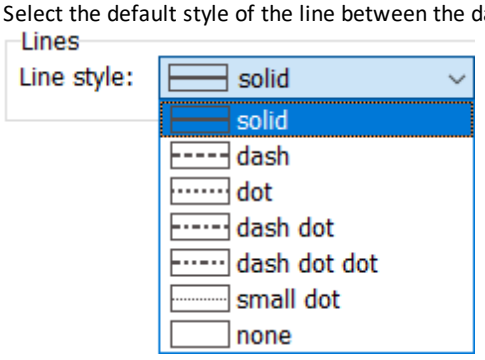
Default chart Font Settings

For different sections in the chart default font settings can be defined here. Click on the *Change...* button for the according chart section the font settings should be changed. A dialog will open up where the font and its attributes can be selected. After applying the dialog, a preview of the selected settings is shown.

Common Chart Line and Point Settings

Line style

Select the default style of the line between the data points:



Width	Enter the desired default line width for series lines between data points
Point size	Enter the desired size of the data point shape in pixel.

Common Chart Axis Settings

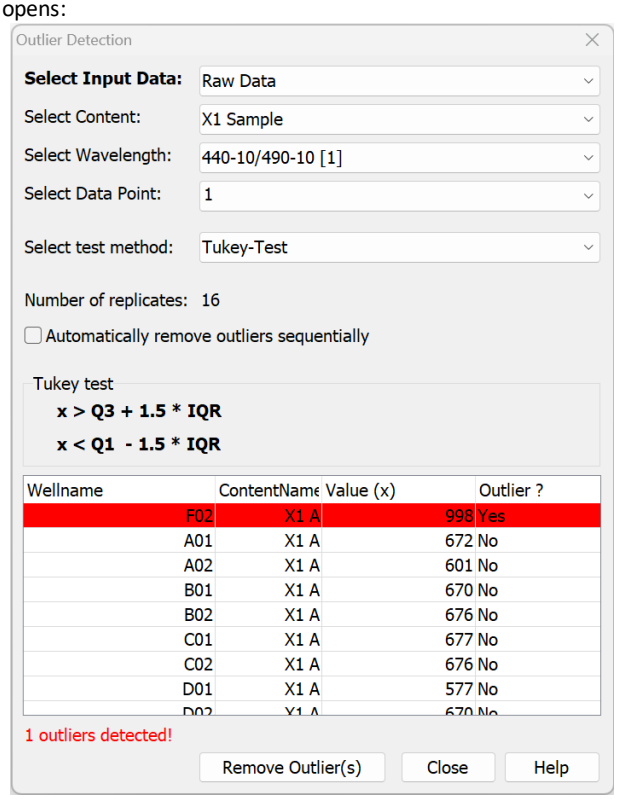
Define the default settings for the visibility, color, style and width of axis lines, ticks and grids .

Common Chart Settings

Show legend border	If checked, a border around the legend box is drawn.
Legend border line style / Legend border width	If *Show legend border* is checked, define the style and the width of the border
Legend background	Define the background color of the legend
Chart background	Define the background color of the chart.
Display not highlighted data curves	Define how data curves are displayed if they are not highlighted (see the *Highlight Active Data* setting in Common Chart Functions). ° in gray: all curves except the highlighted one are displayed in gray ° in dimmed color: the curve will be drawn with dimmed color if the curve is not highlighted. With the slider you can define if the color should be dimmed soft or strong.
Used color palette for chart series	Select between the offered color palettes to define the used colors for chart series.

3.29 Outlier Detection

To identify outliers in a group of replicates in a defined and efficient manner, you can select between different outlier detection methods. To perform a statistical relevant test, a replicate group with at a minimum number of wells is needed. The needed number depends on the selected method. To start the outlier detection, select at least one replicate in the microplate view and open the pop-up menu with a right mouse click. Select the menu entry *Remove Outliers...*. You also can click on the  button on the *Single Calculations* group in the *Calculations* ribbon menu.
The outlier detection window opens:



Select input data: Select the input data for the outlier test. This can be the result of any calculation performed using replicates and output data in numbers.

Select Content: Select a dedicated content (= replicate group) or select <all contents>: When MARS performs an outlier test on dedicated contents, it considers all data points falling among the selected content category as replicates while when choosing <all contents>, MARS steps through each content category and performs an outlier test for each content category separately. Nevertheless, the results of all these separate outlier tests are summarized in a whole big table in the Outlier detection window.
Select Wavelength: If the test run contains more than one wavelength data set, select the wavelength you want to check for outliers.
Select Data Point: If the test run contains kinetic data, select the loop iteration you want to check for outliers. MARS can identify and remove outlying kinetic curves if the <all> option is checked.
Select test method: Select the desired outlier detection method.

After the selection of input data, content, wavelength, loop iteration and method is completed, the outlier detection starts immediately.

Read more details about the usage and the different detection methods in the chapter Outlier identification methods.

> **Note:** You can access the outlier dialog also by clicking the right mouse button over standard markers on the Standard Curve page.

4 Perform Calculations

When opening a test run measurement in MARS for the first time, a default view will be displayed showing the measured raw data, the blank corrected data (if blanks were defined in the protocol settings) and the calculated averages of replicates (if replicates were defined in the protocol settings). See what happens, when you open a test run the first time in the chapter 2.5: *Test Run Settings*.
To further evaluate the data, MARS provides numerous calculation methods to choose from. Most calculations can be combined together, and all intermediary results can be viewed.
Each calculation performed creates a new data node in the navigation tree. For calculations defined for data using more than one wavelength, the calculation will be performed for each wavelength (except for calculations where arithmetic operations between two wavelengths are used).
The calculation results can be viewed directly in either the microplate view or the table view, as a new data node is added to the navigation tree automatically after performing a calculation.
The performed calculation steps for a result data node are displayed in the detailed information window under the navigation tree. The steps are displayed as a hierarchical series with the last performed calculation shown at the top:

Standards calculations:
Wavelength: 545-10, 590 (No. 2A)
Linear regression fit
Based on:
↳ Sum of Range 1
↳ Average
↳ Blank corrected
↳ Raw Data

The top lines describe the last performed calculation for this node.
The **Based on** list shows all calculations performed successively starting with the latest. The hierarchy shows which calculation was performed on the output data of the previous calculation. The last line is always the raw data node because all calculations are based on the measured data.

To perform a new calculation, click *Calculations* in the *Data Reduction* group on the *Home* tab of the ribbon or select the required calculation method directly by clicking the corresponding control in the *Single Calculations* group on the *Calculations* tab. The calculation window will then open. If selecting the desired calculation in the *Single Calculations* group, the page of the selected calculation method will open automatically in the calculation window.
If the test run measured has standards in the layout and you want to perform a standard calculation (curve fitting), in most cases it is possible to use the standard calculation wizard to get a quick and easy result. Read how to use the wizard in the chapter 4.21: *Standard Calculation Wizard*.
After selecting several wells in the microplate view, calculations can be performed using the statistic over selected wells option. This feature becomes available, after two or more used wells are selected in the microplate view. To perform the calculation, select the menu item *Statistic over selected wells...*from the microplate view pop-up menu or by clicking *Statistic over Selected Wells* in the *Common* group of the *Calculations* tab.
For test runs containing kinetic data, it is important to define ranges before creating calculations using the kinetic data. Read how to handle and use ranges in the chapter 4.1: *Ranges*.
To change the parameters of a previously performed calculation, this can be done by opening the node pop-up window, by clicking on the calculation using the right mouse key. You can then select the *Change calculation* option from the menu to change any parameter of the calculation except the input data.
If the parameters of a calculation are changed and there are further calculations whose input data are the output data of the changed calculation, the corresponding calculations will also be recalculated.

4.1 Ranges

Ranges are used for test runs containing kinetic data or a measured spectrum.
A range defines an extract of your kinetic or spectrum data. A range can include the complete measurement data down to a single loop iterationl/wavelength of a measurement.
A full range over the whole kinetic/spectra data is always available. In addition, you can define ranges with any possible start and stop value.
It is possible to define more than one range, and ranges can overlap. See how to define and manage ranges in the section Define a Range.
When having defined a new range, a calculation can then be selected and the input data for that calculation method defined. See the chapters 4.8: *Kinetic Calculations* and 4.18: *Spectrum Calculations*, how to perform calculations based on a range.
It is possible to define more than one calculation on the same range (e.g. both the slope and the average may be needed from the same range).
Kinetic ranges are displayed in the signal curve chart and in the range window. Spectrum ranges are displayed in the spectrum curve chart and in the range window.
Each range gets its own number starting with one. If unused ranges are deleted (ranges used to perform existing calculations cannot be deleted), the ranges will then be renumbered to compensate for this.

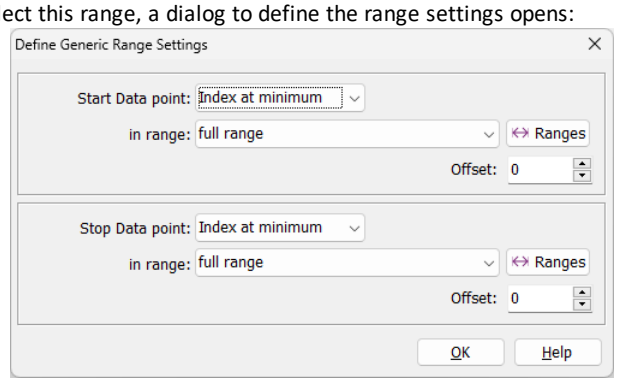
For kinetic ranges, it is possible to define a baseline range. It defines the loop iterations for a baseline correction. The baseline range can be differentiated from the other ranges by its name (baseline ranges are called baseline *#no*., normal ranges are called range *#no*. *#no* is the number of the range) and by its color in the signal curve chart as it will appear red instead of blue.

4.1.1 Predefined Ranges

For each kinetic or spectrum test run a full range for the whole kinetic/spectrum measurement is always available. This range cannot be changed or deleted.
If the test run contains injections, additional kinetic ranges will be defined: Creating one range before the injection and one range after the injection (including the injection iteration). These are ranges that can be changed and deleted.

4.1.2 Individual ranges

For calculations based on ranges (like the *Kinetic Calculations* and the *Spectrum Calculations)* you can also define individual ranges. Individual ranges can have a start and/or stop value that is not predefined. This allows you to define ranges that can be different for each well. The second last entry in the range selection box of the calculation definition page is always the individual range. If you select this range, a dialog to define the range settings opens:

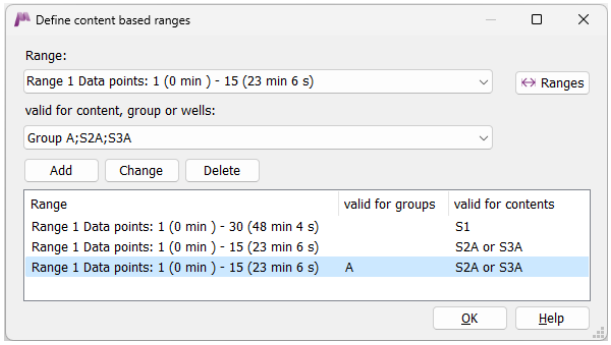


Define the settings for the start and the stop index for the range:

Index at minimum	Finds the index position of the minimum value in the selected range. Use the *Offset* control to enter an offset index to the resulting index.
Index at maximum	Finds the index position of the maximum value in the selected range. Use the *Offset* control to enter an offset index to the resulting index.
Index at threshold	Returns the index position when the value in the selected range reaches the entered threshold the first time. If the threshold is not reached, the last index will be returned. Use the *Offset* control to enter an offset index to the resulting index.
Index from variable	The index is defined by the selected variable. Read more about variables in chapter 4.2.1: *Define and Use Variables*.Use the *Offset* control to enter an offset index to the resulting index.
First index	Returns the first index of the measurement (= 1). In combination with the offset value, you can define any fix start/stop index for the range. Use the *Offset* control to enter an offset index to the resulting index.
Last index	Returns the last index of the measurement. In combination with the offset value, you can define any fix start/stop index for the range. Use the *Offset* control to enter an offset index to the resulting index.
Index at injection	Only for kinetic test runs with at least one injection defined: Returns the index of the selected injection iteration. Use the *Offset* control to enter an offset index to the resulting index.

4.1.3 Content based ranges

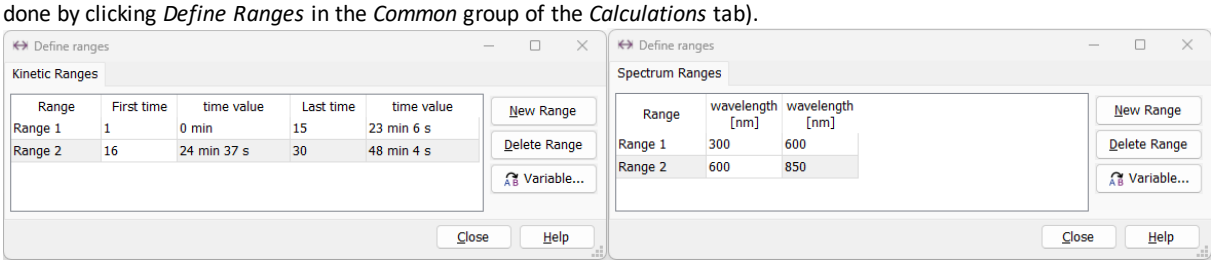
For calculations based on ranges (like the *Kinetic Calculations* and the *Spectrum Calculations)* you can also define content-based ranges. Content-based range means, that you can define different ranges for different contents used in the same calculation. The last entry in the range selection box of the calculation definition page is always the content-based range. If you select this range, a dialog to define the range settings opens:



Select the desired real range first and then select in the drop don control *valid for content, group or wells* for which content the selected range should be used. Click *Add* to add this definition to the list. Add a selection of ranges and contents for each combination of content-based ranges you need.

4.1.4 Define a Range

To define a new range use the range functionality options shown on the signal curve chart and on the spectrum curve chart to add, move or re-size a range or use the range menu.
You can open the window by clicking *Ranges* in the *Data Reduction* group on the *Home* tab of the ribbon (the same can be done by clicking *Define Ranges* in the *Common* group of the *Calculations* tab).



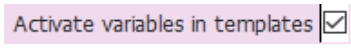
The window shows up to two tabs, one for kinetic ranges (if the test run contains kinetic data) and one for spectrum ranges (if the test run is an absorbance spectrum measurement). On each page you see a list with all ranges and their start and stop time points, respectively their start and stop wavelength.
To change the borders of a kinetic range, click in the time field you want to change and enter the new time point value, or for a spectrum range, click in the wavelength field you want to change. These values can also be changed up or down, using the small spin control that appears on the right side of the field when you click on it.
It is possible to add a new range by clicking on the *New Range* button. The borders of the new range will cover from the first iteration to the last, respectively from the first measured wavelength to the last. The border values can then be changed as described above.
To delete a range, select the range or ranges you wish to delete and press the *Delete Range* button.
With the *Variable...* button, you can use a variable or a value from another calculation to set the range start or stop iteration/wavelength. Read more about variables in chapter 4.2.1: *Define and Use Variables*.
Any changes made to a range that has had a calculation performed on it will result in calculation being recalculated according to the amended range.

4.2 Variables

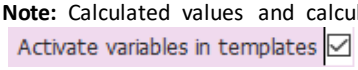
4.2.1 Define and Use Variables

With MARS you can define variables to use them in calculations. During the calculation, the variable will be replaced by a certain value.
MARS knows three types of different variables:

- **User defined variables**
- **Calculated values**
- **Calculated fit parameters**

To use variables in calculations, the check box 

on the *Calculations* ribbon menu in the *Variables and Calculated Values* group must be checked. You can find the same group with the button in the *Templates* ribbon menu.

Note: Calculated values and calculated fit parameters can be used in the user definable formula, even if the 

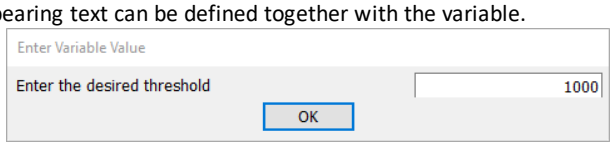
is not checked. For all other calculation, the check box needs to be checked.

Parameters of a calculation that can be set by a variable are marked with an additional button ( ) on the left side of the parameter entry control. To assign a variable to the parameter, click on the button and select the desired variable. See section Select a Variable in this chapter for more details.

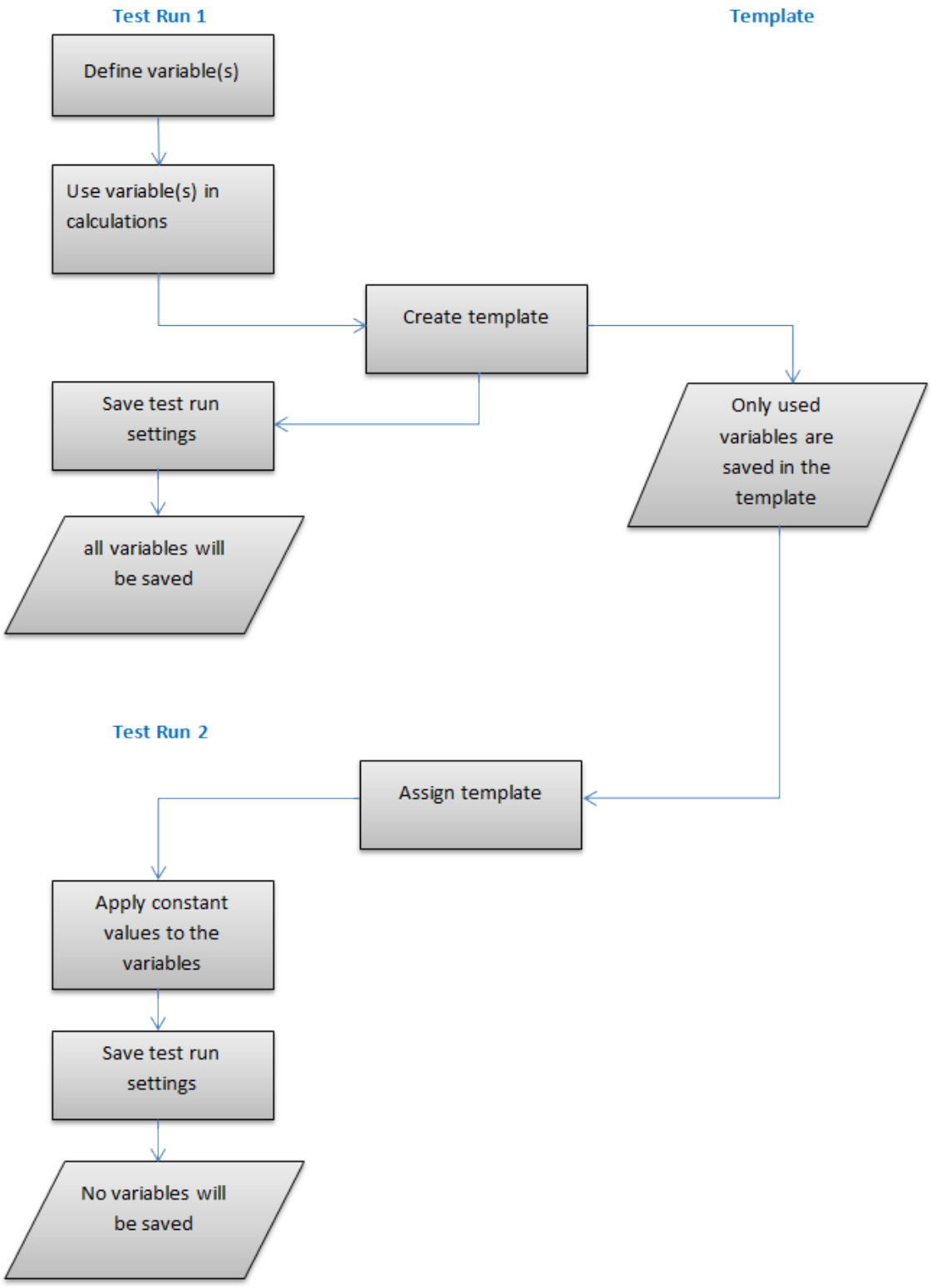
Note: The buttons are only visible, if the Activate variables in templates control is checked.

User defined variables

A user defined variable describes a placeholder for a value, entered by the user. This kind of variable is useful only together with templates. If a template was defined to perform always the same calculations, but only few parameters (e.g. an extinction coefficient or a threshold value) changes from test run to test run or from time to time, you can use a variable for this parameter. You can define a default value for the variable, and you can decide if the variable has to be entered for each new test run or not.
If a variable need to be entered and you assign the template to a test run, a dialog comes up and asks the user to enter the value for the variable. The appearing text can be defined together with the variable.



If the value of a variable changes only sometimes and not for each test run, you define a variable that does not need to be entered (see *Create a new user defined variable* below) and define a default value for this variable. To change the default value in the template, you can select the template in the Manage Templates window and click on the *Edit Parameters* button. Read the Manage Variables section below to find out how to change the default value of a variable.
See the flowchart below how variables are used together with templates:



Calculated Values

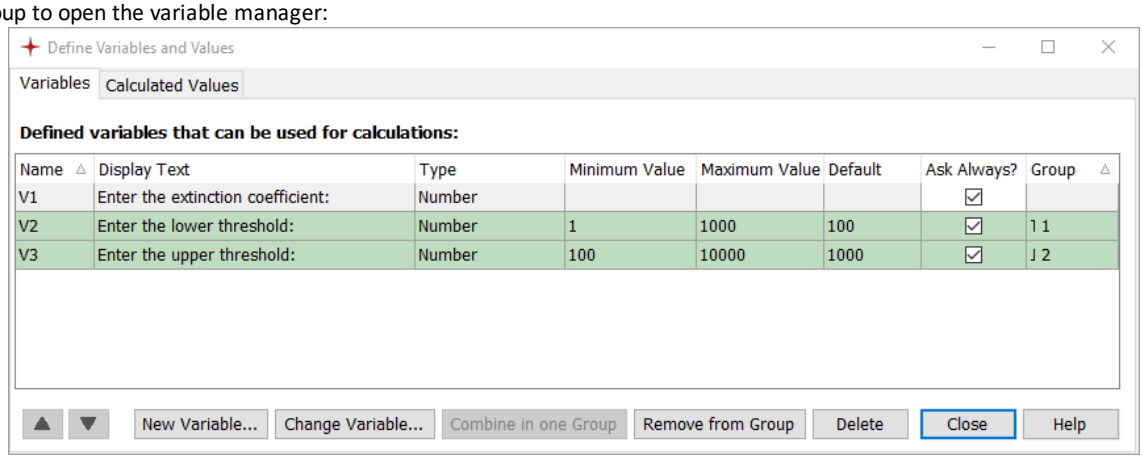
Calculated values can hold any value available for the test run. This can be either a measured value for a certain well or content, or a calculation result for a well or content. These kinds of variable allow you to define calculations between different processes. Read the section *Add a new calculated value variable* below, to see how to define such a variable.

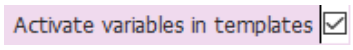
Calculated fit parameters

Calculated fit parameters can hold parameter values of calculated standard fits for the current test run. These kinds of variable are available, as soon as standard calculations are done for this test run. They can be directly selected for further calculations (see section *Select a Variable* below).

4.2.2 Manage Variables

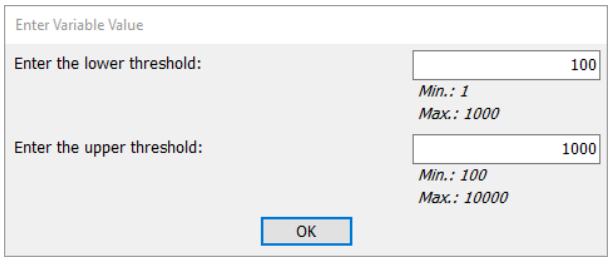
To see and change all defined variables or to define new variables, you need to open the variable manager. Click on the  button on the *Calculations* ribbon menu (or on the *Templates* ribbon menu) in the *Variables and Calculated Values* group to open the variable manager:



If the  control is checked, you see two tabs (pages) on the window: One for the user definable variables (*Variables* tab) and one for the calculated values (*Calculated Values* tab). Otherwise you see only the *Calculated Values* tab.

Variables tab

The table shows all defined variables (if you open the table the first time for the test run, the list is empty). You can change the parameters (*Display Text, Type, Minimum Value, Maximum Value, Default and Ask Always* (= needs to be entered)) directly in the table or you can select a variable and click *Change Variable* to open a dialog with the parameter details. The dialog and the meaning of each parameter are explained in the Create a new user defined variable section below.
You can group user defined variables together so that the values for these variables (if they must be entered) can be entered in one dialog:

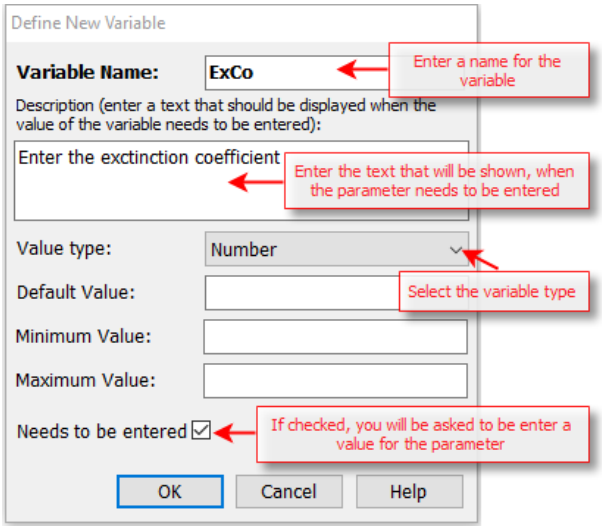


Grouping variables

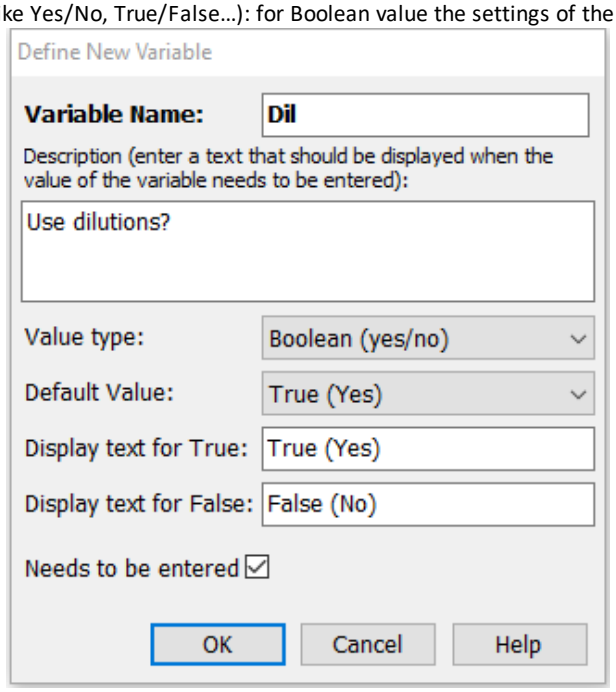
If you use more than one variable that must be entered in a template, there will come up a window for each variable. Sometimes the variables are used in the same calculation and are associated. In this case you maybe want to see them in one dialog. You can do this if you create a variable group with these variables.
You can have more than one variable group if necessary.
To group variables together in one group, select the variables in the table and click *Combine in one Group*. The number displayed in the group column shows the order of the variables in the dialog. You can change the order of the variable in a group with the  and  buttons. To remove a variable from a group, select the variable and click *Remove from Group*.
Click *New Variable...* to create a new user defined variable:

Create a new user defined variable

After you have clicked the *New Variable...* button on the *Manage Variables* window or on the *Select Variable or Value* window, the window to define a new variable comes up:



The default variable name (V1, V2, V3...) can be changed to a user defined variable name.
Enter the text that will be shown for this variable in the first text field of the window. If you want to user numbers in the text, where the decimal and/or thousand separators should be taken from the windows system settings, use #@ instead of the decimal separator and #* instead of the thousand separator: 1#*000#@25 instead of 1000.25.
The Value type of the variable can be:
1. Number (any floating point of integer value)
2. Boolean (any logical value like Yes/No, True/False...): for Boolean value the settings of the variable changes:



You can enter a display text for the true state and the false state. Boolean entry fields which can be used as parameters in templates are:
True/False Overview table for logical entry fields in MARS

Calculation	Field Caption	True	False
Content Based Correction	average or median	median	average
Baseline Correction	Correction Mode	division	subtraction
Kinetic Calculations	Slope Direction	Rising	Falling
	Start Time	Begin of selected range	Measurement begin
Spectrum Calculations	Slope Direction	Rising	Falling
Statistics	Absolute value only	absolute value	real value

3. Text: Can be used where text parameters are used.
4. Enumeration: You can select between these enumeration types:

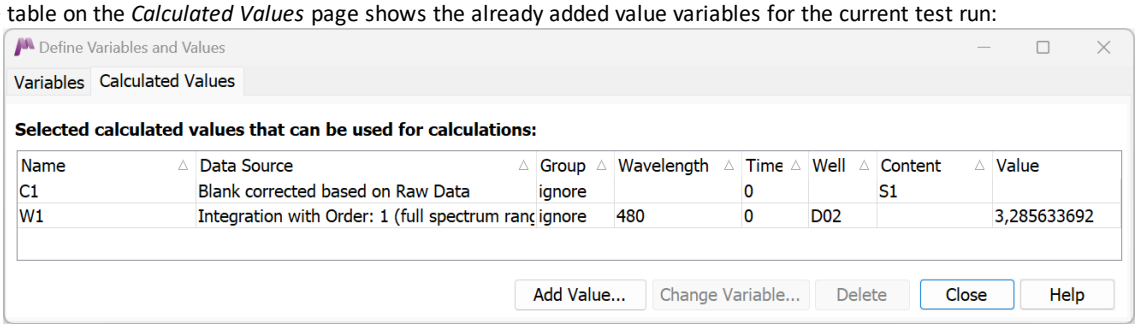
- Slope Type (/hour, /min, /sex, /ms, /µs)
- Statistic calculation (Average, Standard deviation, …)
- Enzyme kinetic calculation (Michaelis-Menten, Lineweaver-Burk, …)
- Series type for plausibility checks
- Data calculations (minus, divided by, plus, multiplied by)
- Concentration calculations (Difference, Ratios, Percentage)

Press OK, to save the variable.

Note: The variable is part of the test run setting and will be saved together with the other settings. If you close the test run and open another one, you will no longer see the variable you just defined for the test run before.

Calculated Values tab

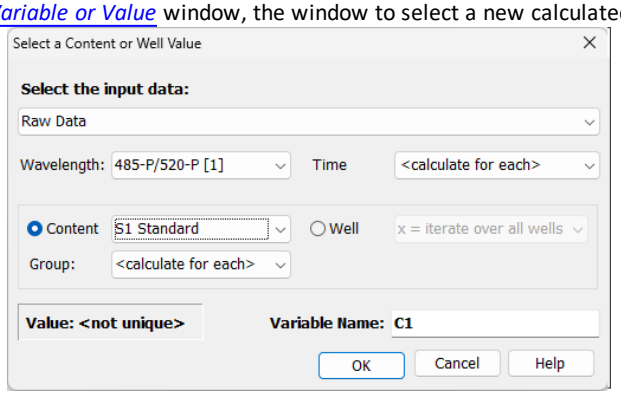
The table on the *Calculated Values* page shows the already added value variables for the current test run:



Calculated values are content (starting with a **C** in the name if no user defined variable name was used) or well values (starting with a **W** in the name if no user defined variable name was used) from any performed calculation of the current test run. The *Data Source* column shows the calculation which is the base for the data value.
If groups are defined in the test run layout, the *Group* column shows (important only for content values) the group of the variable. If the group column is empty, the average value over all groups is used.
If the test runs contain more than one wavelength, the *Wavelength* column shows the wavelength data base for the variable. If the wavelength column is empty, the value of the variables is calculated separately for each wavelength.
For kinetic test runs, the *Time* column shows the selected time point for this variable (0 means, the value iterates over the time points).
For content values, the *Content* column shows the selected content, for well values the *Well* column shows the selected well.
If the value variable does not iterate over groups, wavelength, time points or wells, the *Value* column shows the current value for this variable. If the *Well* column contains an x, the value of the variable iterates over each well.
To add a new calculated value variable to the list, press the *Add Value...* button, to change the parameters of an existing variable, press the *Change Variable...* button.

Add a new or change an existing calculated value variable

After you have clicked the *Add Value...* or the *Change Variable...* button on the *Manage Variables* window or the *Add Value...* button on the *Select Variable or Value* window, the window to select a new calculated value comes up:



Use the controls to select any value of all available input data for the current test run:

Select the input data:	Select the input data process for the value variable.
Wavelength	If the selected input data process contains data of more than one wavelength, select the desired wavelength data. If you select the entry <calculate for each>, the value variable will be calculated for each wavelength.

Time	If the selected input data process contains kinetic data, you can select a certain time data point to define the value, or you can select <calculate for each> to iterate the value over the loop iterations.
Content	If you select a content, the value will be calculated as the average of all wells of the selected content.
Group	If the layout contains groups and **Content** is selected, you can select the group of the desired content or you can select <ignore groups> and the average of all wells of the selected content without considering the group information will be calculated or you can select <calculate for each> and the average of all wells of the selected content with the same group as the currently calculated well will be used.
Well	Select a certain well for the value variable. If you want to iterate over all wells, select the *x = iterate over all wells* entry.
Value	If the selected value variable defines a certain value (not iterating over the wavelength, loop iterations or well), the resulting value of the variable is displayed here.
Variable Name	The default variable name (C1, C2, C3...) can be changed to a user defined variable name.

Press *OK*, to add the calculated value variable to the selection list.

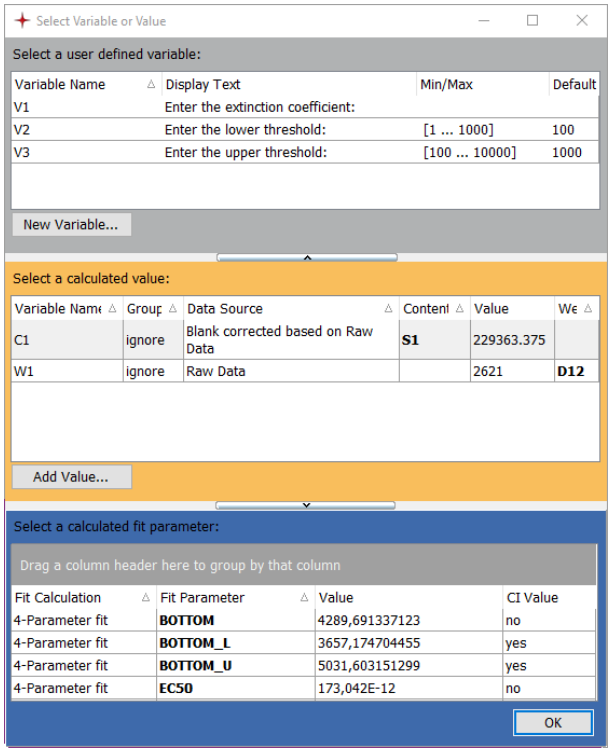
Note: The calculated value variable is part of the test run setting and will be saved together with the other settings. If you close the test run and open another one, you will no longer see the variable you just defined for the test run before.

4.2.3 Select a Variable

After you have defined one or more user defined variables or calculated values, you can use them in calculations.
Create the calculations you want and use the variable for the calculation parameters you want to have as a parameter in the template: Each parameter of a calculation that can be used as a parameter in the template has a small icon on the right side of the parameter ({v}):



If you click on this icon, a window with all available variables that matches for this parameter is shown:



The window shows two or three tables (the table with the user defined variables is only visible if the control is Activate variables in templates ☑ checked).

The green table shows all user defined variable. If no variable is defined you can create a new one with the *New Variable...* button (see *Create a new user defined variable* above).

The blue table shows all defined calculated values. To add more calculated values, press the *Add Value...* button (see *Add a new calculated value variable* above).

The orange table shows the available calculated fit parameters. With the CI Value column, you can filter the result to hide or show result parameters based on CI values.

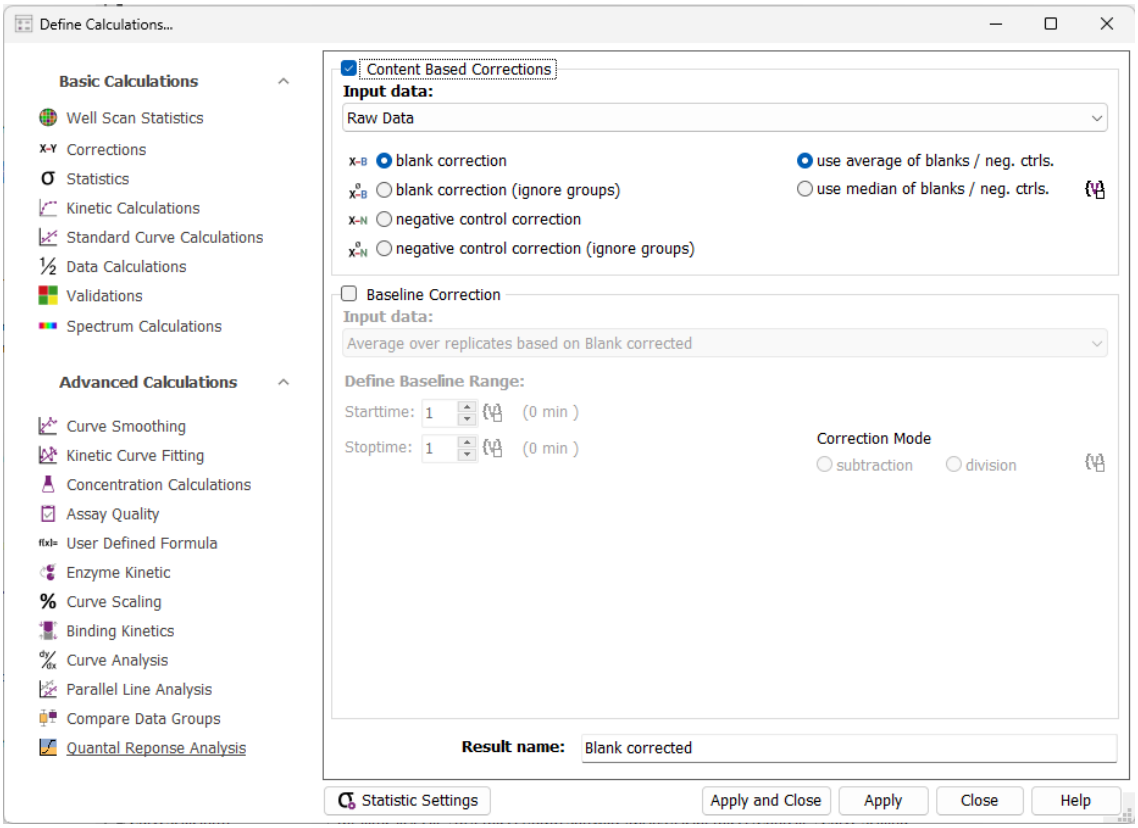
Select the variable you want to use and click *OK*.

The icon changes and the control to enter the parameter is disabled. To remove the assigned variable from the parameter, click the icon again:



4.3 Calculations

To perform a single calculation, select the calculation from the calculation window, define the properties of the calculation and perform the calculation by pressing the *Apply* or the *Apply and Close* button in the window.



Enter a name for the resulting calculation data in the entry field ***Result name:*** at the bottom of the window. The name will be used in the Navigation Tree and in the legend of the Microplate and Table View pages to identify the data.
Depending on the test run the following calculations can be performed:
Basic calculations:

- Blank corrections
- Negative control corrections
- Baseline corrections
- Statistics
- FP calculations
- TR-FRET calculations
- Kinetic calculations (Calculations based on ranges over loop iterations)
- Standard curve calculation (Curve fitting)
- Data calculations (Arithmetic operations between wavelength or output data of other calculations)
- Validations (Classifies the data in good / bad / unknowns...)
- Spectrum Calculations (Calculations based on wavelength ranges over a spectrum)
- Well Scan Statistics (If the test run contains well scan data)

Advanced calculations:

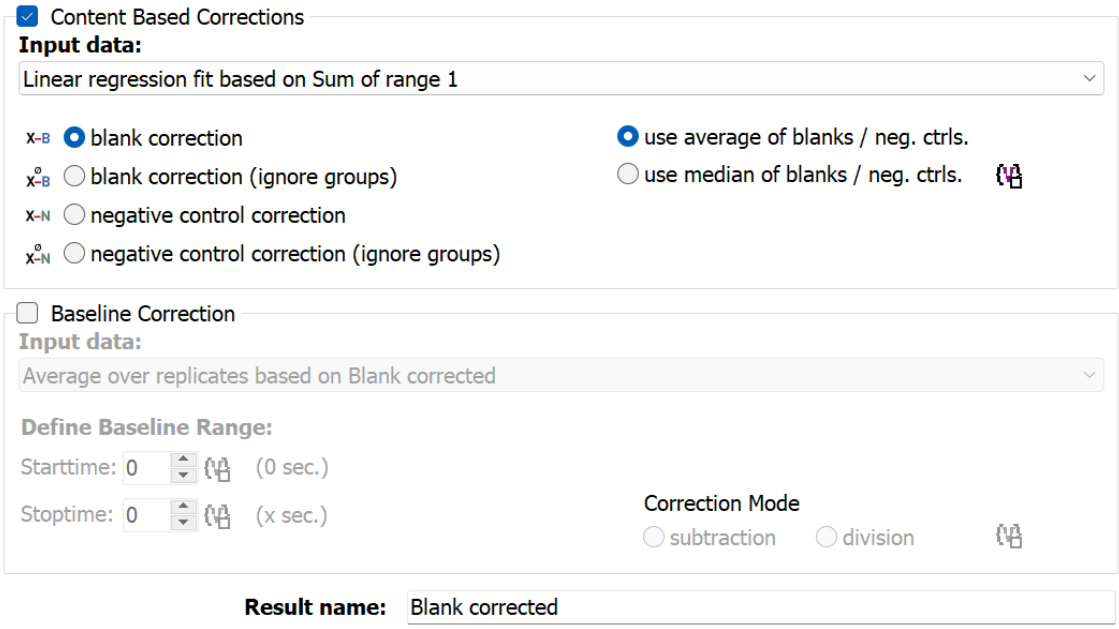
- Curve Smoothing (Curve smoothing for the signal curves and spectrum curves)
- Kinetic Curve Fitting (Curve fitting based on ranges over signal curves)
- Concentration calculations (Calculations based on known and calculated concentrations)
- Assay Quality (Z', signal to blank, signal to noise...)
- User Defined Formula (Formula generator based on well values)
- Enzyme Kinetic (Performs an enzyme kinetic calculation to calculate Km and Vmax)
- Curve Scaling (Converts a signal curve to a percentage presentation)
- Binding Kinetics (Binding kinetic fitting like kinetic rate equation)
- Curve Analysis (Area under Curve, Differentiation and Integration of curves)
- Parallel Line Analysis (PLA)
- Compare Data Groups (T-Test and ANOVA)
- Quantal Response Analysis (Probit, Logit, Spearman-Kärber, Reed-Muench, Dragstedt-Behrens)

The subdivision into **basic** and **advanced** calculations helps to simplify the window and allows you to find better the most often used calculations. You can change the division if you want to have some of the advanced calculations in the basic calculation section and vice versa. Just click the desired item in the menu and move it with the mouse to the desired section and position. The changed division will be saved - individually for each user - and restored after restart of the program.
To open or collapse a section click on the double arrows on the right side of the section caption.
Special calculations:
1. Well statistics:

Statistic over selected wells (Not found within the *Define Calculations* window, but if wells are selected in the microplate view, this calculation will be performed when the menu item *Statistic over Selected Wells...* in the microplate views pop-up menu or in the corresponding menu item under the *calculations* menu is selected).
Details of each single calculation are explained in the appropriate chapters of the calculation method.
To perform more than one calculation, just apply the defined calculation and change to the page of the next calculation you want to perform. The data of the newly created calculation will be immediately available to be used as input data for the next calculation.
To perform only one calculation at once, press the *Apply and Close* button to close the calculation window after the calculation was performed.
2. Remove Outliers:
If enough replicates are used, outliers can be removed automatically. How to find and remove outliers can be found in the chapter 3.26: Outlier Detection.
Using Variables in Calculations:
If a parameter entry control of a calculation shows this element , it can be set by a variable. Press the button to open a new window with all available variables. An explanation of all different kind of variables and how to use them in calculations and templates can be found in chapter 4.2.1: *Define and Use Variables*.
The dialog with the available variables shows only variables that can be used for the appropriate parameter.
The variable button ( ) is only visible, if variables for templates are activated. To activate variables for templates, check the Activate variables in templates button on the *Calculations* ribbon menu in the *Variables and Calculated Values* group. You can find the same group with the button in the *Templates* ribbon menu.
Statistic Settings:
To change some predefined settings for statistic calculations like the calculation of error bars and the settings for robust statistics, click the *Statistic Settings* button to open the Calculation Settings page.

4.4 Corrections

The corrections page contains two groups. One page shows corrections made using the blank and negative controls, and the other for baseline corrections.



4.4.1 Content-Based Corrections

There are two groups of content-based corrections: The blank corrections and the negative control corrections.
If the layout contains more than one blank or negative control you can define if the average or the median of all blanks/negative controls should be used for the correction with the radio control on the right side of the window.
Input data: Select the input data for the content-based correction. This can be the raw data or the result of any calculation.

4.4.2 Blank Corrections

Blank Correction
If there are blanks defined in the layout of the test run, it is then possible to perform a blank correction when you check the *blank correction* check box. If no blanks are available, the checkbox will be disabled.
The blank correction calculates the average or the median of all available blanks and subtracts the value from the raw data.

Blank Correction (Ignore Groups)
If you have groups in addition with blanks, the normal blank correction would calculate the averages or the median of the blanks from each group and subtract the appropriate values from the corresponding groups.
If you want to calculate the average or the median of the blanks of all groups and subtract this value from all raw data, this can be done using the *blank correction (ignore groups)* option. This option is only available if you have groups with blanks.

4.4.3 Negative Control Corrections

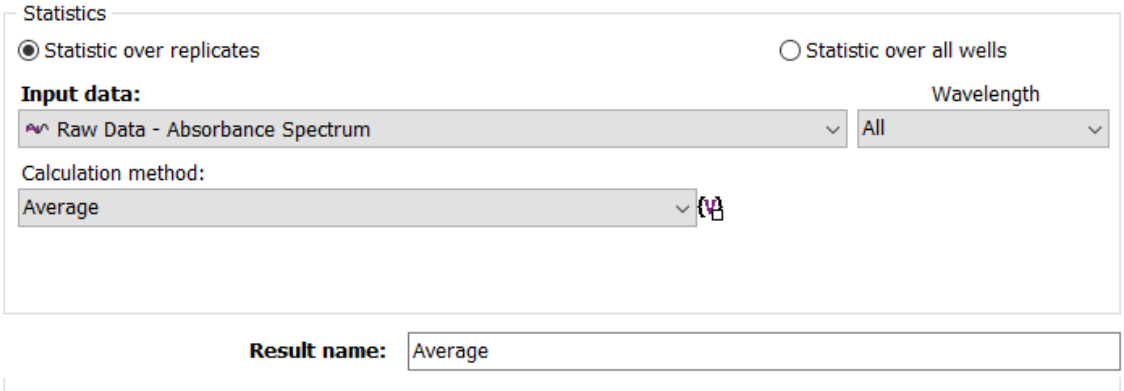
Negative Control Correction
If there are negative controls defined in the layout of the test run, it is possible to perform a correction using the negative controls. Check the *negative control correction* checkbox. If no negative controls are available, the checkbox will be disabled.
The negative control correction calculates the average or the median of all available negative controls and subtracts the value from the raw data.
Negative Control Correction (Ignore Groups)
If you have groups in addition to negative controls, the normal negative control correction will calculate the averages or the median of the negative controls from each group and subtracts the appropriate values from the corresponding groups.
If you want to calculate the average or the median of the negative controls of all groups and subtract this value from all raw data, this can be done using the *negative control correction (ignore groups)* option. This option is only available if you have groups with negative controls.

4.4.4 Baseline Corrections

If a test run has kinetic data, this option is enabled to perform a baseline correction.
The baseline correction will calculate the average of the values in the baseline range and subtract or divide this value from all the kinetic data of the selected input data.
Use the **Correction Mode** control to define if the values should be subtracted or divided.
Input data: Select the input data for the baseline correction. This can be the output result of any calculation performed using kinetic data or the raw data.
Define Baseline Range: Define the start and stop iteration of the baseline range. The borders of the baseline range can even be changed after applying the calculation. Open the signal curve chart, and see that the base line range is shown as a red border range. Change the borders of the range as described in the chapter 3.11.1: *Range Functions in the Chart*.
The calculation will be automatically updated.

4.5 Statistics

The statistics page σ is used to calculate statistics over replicates or over layout groups.



If the selected input data has replicates and layout groups, use the radio box controls *Statistic over replicates / Statistic over layout groups* to define if the statistic is calculated based on replicates or based on layout groups.
If the statistic is based on all wells or based on layout groups, the well contents (like samples, standards, blanks or controls) are ignored and all wells of one group (statistic over layout groups) or all wells of the plate (statistics over all wells) are used for the statistic calculation.
Input data: Select the input data for the statistic. This can be the result of any calculation performed with output data in numbers.
Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry *All* in the list, the calculation will be performed for each wavelength.
Calculation method: Select the calculation method for the statistic.
Available methods:
Average: Calculates the average (mean) of all replicates of the same content (statistic over replicates) or of all wells of the same layout group (statistic over layout groups)

Standard deviation: Calculates the standard deviation based on samples (in this case replicates of the same content or wells of the same group). The standard deviation is a measure of how widely values are dispersed from the average value. The SD value is calculated using the following formula:

$$\sqrt{\frac{\sum (x-\bar{x})^2}{(n-1)}}$$

Note: The standard deviation is the recommended standard deviation method, as the measured data applies to samples.

Standard deviation n: Calculates the standard deviation based on an entire population (in this case replicates of the same content or wells of the same group). The SD n value is calculated using the following formula:

$$\sqrt{\frac{\sum (x-\bar{x})^2}{n}}$$

Standard error: Calculates the standard error of the mean based on samples (in this case replicates of the same content or wells of the same group). The standard error is calculated using the following formula (standard deviation divided by the square root of the number of replicates):

$$\frac{SD}{\sqrt{n}}$$

Standard error n: Calculates the standard error n of the mean based on samples (in this case replicates of the same content or wells of the same group). The standard error n is calculated using the following formula (standard deviation n divided by the square root of the number of replicates):

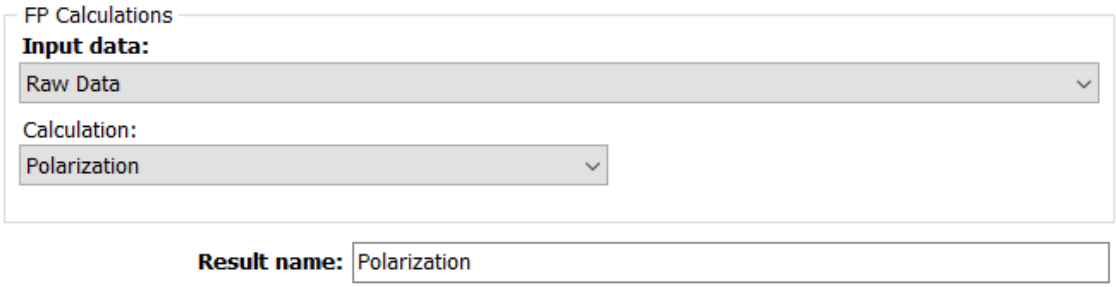
$$\frac{SDn}{\sqrt{n}}$$

%CV: Calculates the standard deviation of the replicates of the same content (respectively wells of same group) divided by their average and multiplies this number by 100 to express the result as a percentage.
%CV n: Calculates the standard deviation n of the replicates of the same content (respectively wells of same group) divided by their average and multiplies this number by 100 to express the result as a percentage.
If the method %CV or %CVn is selected a further control appears: ☐ Absolute values only (positive values only) Check this control if you want to see only absolute (positive) %CV values.
Minimum: Finds the minimum value of the replicates of the same content (respectively wells of same group).
Maximum: Finds the maximum value of the replicates of the same content (respectively wells of same group).
Median: Finds the median value. The median is described as the number separating the higher half of the replicates from the lower half. The median is found by arranging all the values to compare from lowest value to highest value and picking the middle one. If there is an even number of values, the median is not unique, so the mean of the two middle values is taken.
Sum: Calculates the sum of the values of all replicates of the same content (respectively wells of same group).
No. of Values: Returns the number of replicates for one content-type (respectively wells of same group).
CI mean: Calculates the confidence interval for the mean of the replicates/data. The confidence interval is a range of values that is likely to contain a population mean with a certain level of confidence.
CI percentage: Enter the level of confidence in percentage for the calculation (0-100). Usually, 95 is used.
CI median: Calculates the confidence interval for the median of the replicates/data. The calculation differs between the upper and the lower value (which can be different when the calculation is based on the median and not on the mean).
CI percentage: Enter the level of confidence in percentage for the calculation (0-100). Usually, 95 is used.
calculate lower CI value / calculate upper CI value: select the desired output: Lower or Upper CI value. If you want to see both values, just perform a second calculation.

4.6 FP and TR-FRET Calculations

These calculation methods (★) are only available if the test run is either a fluorescence polarization measurement (FP calculations) or a TR FRET measurement (TR-FRET calculations) using two measurement channels simultaneously (not available on all readers!). Using these calculations, the two measurement channels will be compared against each other. The available calculation operations differ, depending on the measurement methods:

4.6.1 FP Calculations



For fluorescence polarization measurements the polarization values are calculated automatically when the test run is opened. Using this calculation method, further calculations can be performed on the parallel and perpendicular raw data.
Input data: Select the input data for the calculation. This can be the result of any calculation which obtains the parallel and perpendicular channel data.
Calculation: Select the calculation you want to perform:
Available methods:
Polarization: Calculates the polarization values in mP from the two measured channels (parallel and perpendicular).

1000 * (parallel - perpendicular) / (parallel + perpendicular)

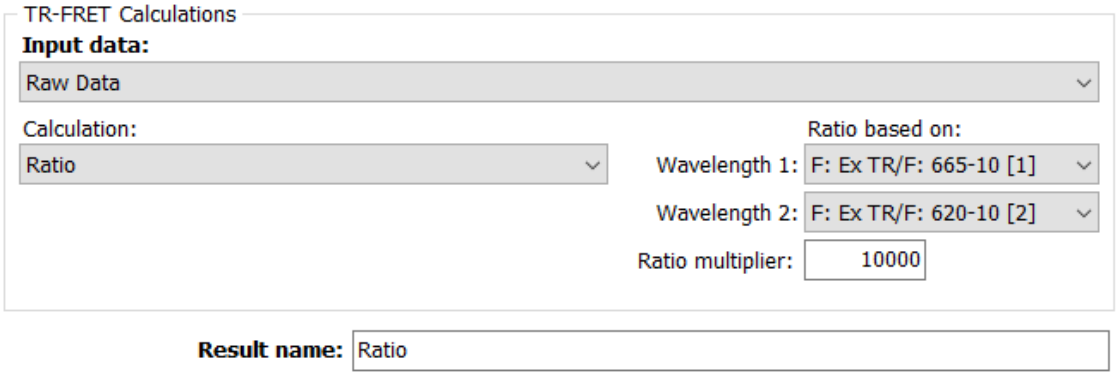
Anisotropy: Calculates the anisotropy values in mA from the two measured channels (parallel and perpendicular).

1000 * (parallel - perpendicular) / (parallel + 2 * perpendicular)

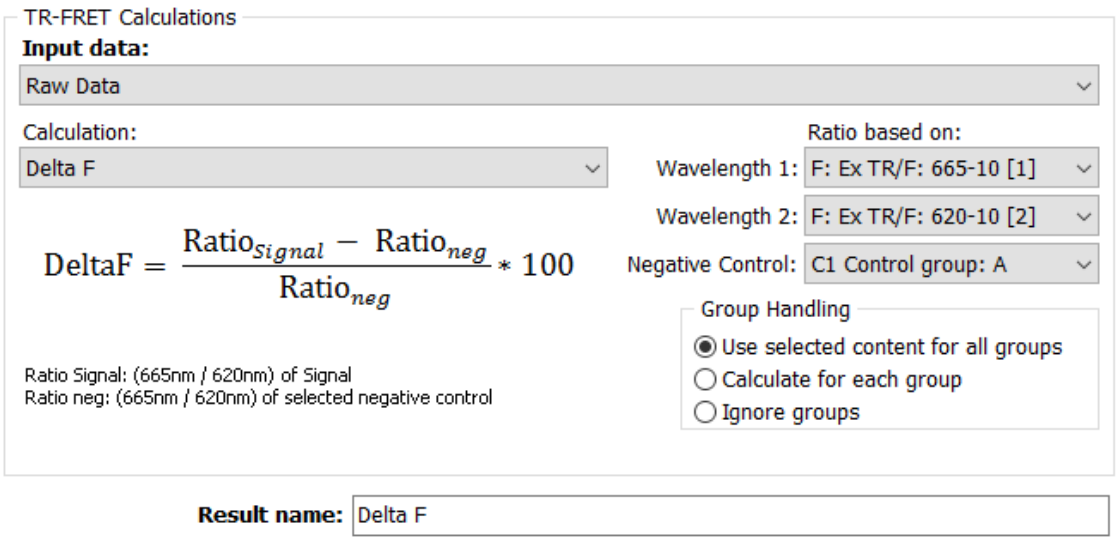
Intensity: Calculates the intensity values from the two measured channels (parallel and perpendicular).

parallel + 2 * perpendicular

4.6.2 TR-FRET Calculations



Input data: Select the input data for the calculation. This can be the result of any calculation which obtains data from at least two measured wavelengths.
Ratio based on: Select the first (numerator) and the second (denominator) wavelength for the ratio calculation.
Calculations: Select the calculation you want to perform:
Available methods:
Ratio: Calculates the ratio between the two selected wavelengths/channels. Enter a multiplier for the ratio calculation in the field *Ratio multiplier*.
Delta F:



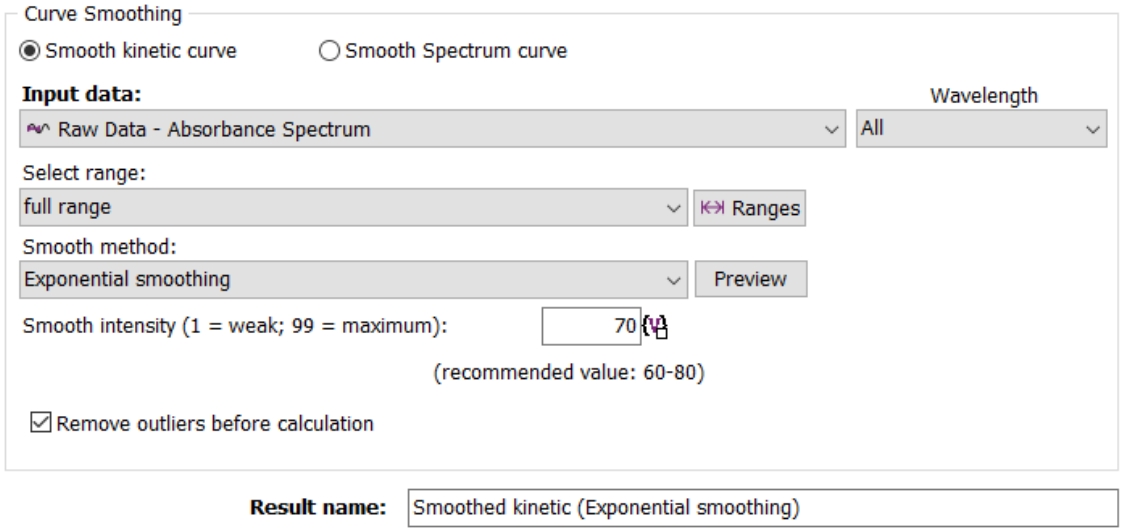
Calculates the DeltaF value. If the layout contains a negative control, this content will be selected as negative control. If the layout has no negative control or if you want to perform the calculation based on a different content, you can change the content if you select another entry in the drop-down list *negative control*.
If the layout contains groups, you must decide, how to handle groups with the Group Handling control:

Use selected content for all groups	Use the selected content of a group for the calculation even if the calculated well is in a different group.
Calculate for each group	Use the selected content of the group of the calculated well. If the group of the well does not have the selected content, no calculation is done for that well.
Ignore groups	Ignore the groups and calculate the result as if no groups where defined.

The formula for DeltaF is shown in the screen shot above.
Ratio Signal is the ratio of the signal for which the DeltaF value is calculated and Ratio neg is the Ratio of the signal of selected negative control (Ratio means: [Value for wavelength 665nm] divided by [Value for wavelength 620nm]).

4.7 Curve Smoothing

With the curve smoothing page one of two smooth calculations can be performed for the signal curves of kinetic test runs or the spectrum curves of a spectrum scan test run.



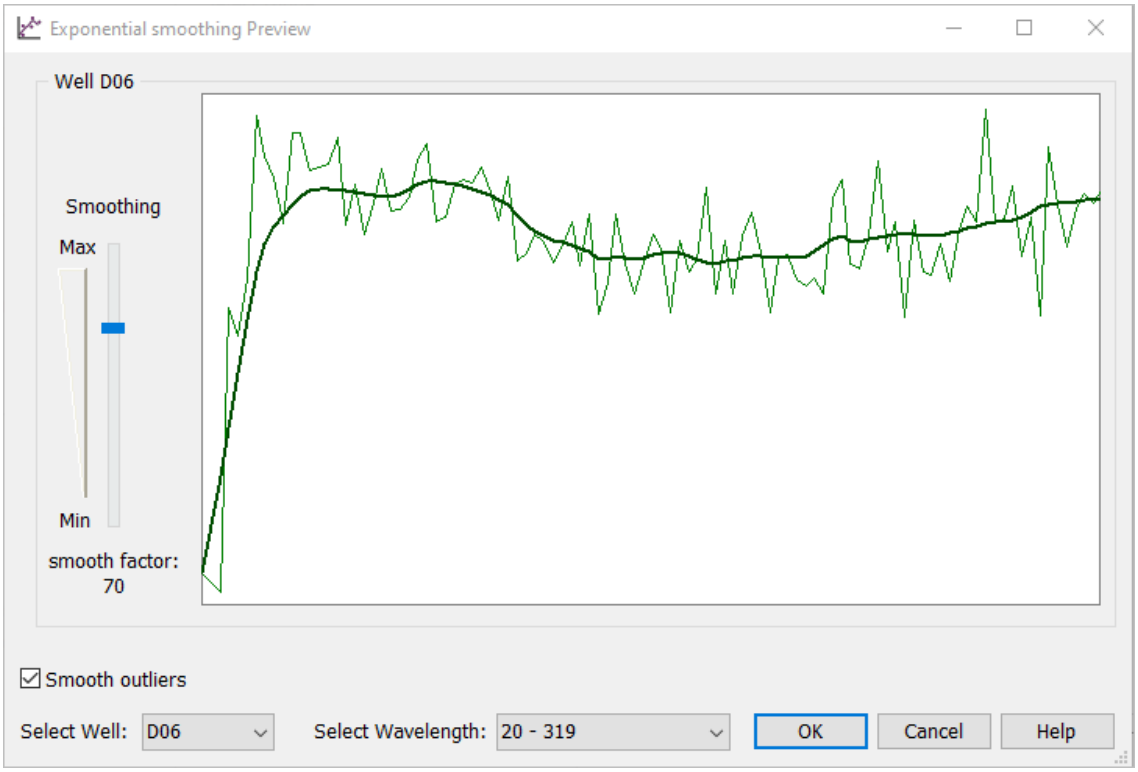
Input data: Select the input data for the calculation. This can be the raw data or the result of any calculation that obtains kinetic data or spectra data.
If the input data has both, kinetic and spectra data, you can select if the smoothing should be calculated based on the kinetic or based on the spectra curve with the radio control above the input data drop-down list: *Smooth kinetic curve / Smooth Spectrum curve*

Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry *All* in the list, the calculation will be performed for each wavelength.
Select range: The input data for a curve smoothing calculation are always defined by the first time point and the last time point of a kinetic range or by the first wavelength and the last wavelength of a spectrum range. It is possible to have one or more ranges defined. The full range is always available and covers the total measurement. See the chapter 4.1: *Ranges*, how to define a range. All defined ranges are listed in the drop-down list with their start and stop time point or wavelength (depending on the selected input data). Select a range for the calculation from this list. Only ranges with at least three values are shown, because the minimum number of values for the curve analysis is three. To view, create or change a range, press the Ranges button to open the range window. You can also define an individual range or a content-based range, where the start and/or stop time point is not fix. Select the *Individual Range* or *Content based ranges* in the drop-down list to use and define this type of range. Read more about individual and content based ranges in the chapter 4.1: *Ranges*.
Smooth method: Select the smooth method you want to perform:
Available methods:
Moving average: Calculates the smoothed curve using a window of a defined width that slides over the curve and calculates the average of the values inside the windows. To remove outliers before smoothing, check the control *Remove outliers before calculation.*
Number of moving Intervals: Enter the number of intervals used for the moving window over the curve (box car width). The number must be odd and minimum three. The maximum number is defined by the number of values in the selected range minus one.
Exponential smoothing: Calculates the smoothed curve using an adapted form of the double exponential smoothing method. To remove outliers before smoothing, check the control *Remove outliers before calculation.*
Smooth intensity: Enter a number between 1 for weak smoothing and 99 for extreme smoothing. A good smooth intensity is between 60 and 80.
Smooth outliers out: Removes outliers in the curve, using a window of a defined width that slides over the curve and calculates the average of the values inside the windows. If outliers are detected in the defined window, the value will be changed to fit to the rest of the data points in the defined window. The used detection method follows the Tukey-test. Read more details about this test in the Outlier identification methods chapter.
Number of moving Intervals: Enter the number of intervals used for the moving window over the curve (box car width). The number must be odd and minimum three. The maximum number is defined by the number of values in the selected range minus one. Entering the value is only possible, if the next control (calculate optimal box car width) is not selected
calculate optimal box car width: If this control is checked, the optimal box car width is calculated automatically.

You can open a preview window to see how entered smoothing parameters affect the curve. Press the Preview button to open the preview window.

4.7.1 Preview of the smoothed curve

After pressing the preview button, you see a window containing a graph with the smoothed curve:



The preview shows the curve and the smoothed curve of the first used well. The thin curve is the original curve. The bold curve is the smoothed curve.
Move the slider on the left to change the width of the moving window (box car) - if the selected method is moving average - and see how this affects the smoothing of the curve(s). If you've found the best width, press *OK* to take this value as parameter on the calculation window.
You can change the displayed well if you change the selected entry of the drop-down list *Select Well.*

Note: Pressing *OK* on the preview window will not perform the smoothing calculation. You must press the *Apply* button on the calculation dialog in addition.

4.8 Kinetic Calculations

The kinetic calculations page is only enabled if the test run contains kinetic data.



Input data: Select the input data for the kinetic calculation. This can be the raw data or the result of any calculation that obtains kinetic data.
Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry *All* in the list, the calculation will be performed for each wavelength.

Kinetic range: The input data for a kinetic calculation are always defined by the first time point and the last time point of a range. It is possible to have one or more ranges defined over your kinetic. The full range is always available and covers the total measurement. See in the chapter 4.1: *Ranges*, how to use and define a range. All defined ranges are listed in the drop-down list with their start and stop time point. Select a range for the calculation from this list. To view, create or change a range, press the Ranges button to open the range window. You can also define an individual range or a content-based range, where the start and/or stop time point is not fix. Select the *Individual Range* or *Content based ranges* in the drop-down list to use and define this type of range. Read more about individual and content-based ranges in the chapter 4.1: *Ranges.*

Calculation method: Select the calculation method for your kinetic calculation.

Available methods:

Slope: Calculates the linear regression curve for the kinetic points in the selected range and gives the corresponding slope value for each well. If slope is selected in the method list, a further drop-down list appears beside the list to select the units for the result: The list contains five entries: **slope/hour, slope/min, slope/sec, slope/ms, slope/µs**.

Time to threshold: Calculates the time taken from the first time point in the selected range for the curve to reach a given threshold for each well. Enter the threshold value in the entry field *Threshold*, this option appears beside the method drop-down list when this method is selected. If a kinetic curve does not reach the entered threshold, n.a. will be printed as result. You can define, what you want to see instead of n.a. in the result table with the *Result, if threshold is not reached* drop-down menu.

To create binary output data (1 if threshold reached, 0 if not), check the control *create binary output data*. You need binary data as input data for the quantal response calculation.

Time to max: Calculates the time taken for the maximum value to be reached in the selected range for each well.

Sum: Calculates the sum of all kinetic points within the selected range for each well.

Average: Calculates the average of all kinetic points within the selected range for each well.

Maximum: Finds the maximum value of all kinetic points within the selected range for each well.

Minimum: Finds the minimum value of all kinetic points within the selected range for each well.

Standard deviation: Calculates the standard deviation based on samples (in this case all kinetic points in the selected range for each well). The SD value is calculated by the following formula:

$$\sqrt{\frac{\sum (x-\bar{x})^2}{(n-1)}}$$

Note: The standard deviation is the recommended standard deviation method, because the measured data applies to samples.

Standard deviation n: Calculates the standard deviation based on an entire population (in this case all kinetic points in the selected range for each well). The standard deviation is a measure of how widely values are dispersed from the average value. The SD n value is calculated by the following formula:

$$\sqrt{\frac{\sum (x-\bar{x})^2}{n}}$$

%CV: Calculates the standard deviation of all kinetic points in the selected range for each well divided by the average of all kinetic points in the selected range for each well and multiplies this number by 100 to express the result as a percentage.

%CV n: Calculates the standard deviation n of all kinetic points in the selected range for each well divided by the average of all kinetic points in the selected range for each well and multiplies this number by 100 to express the result as a percentage.

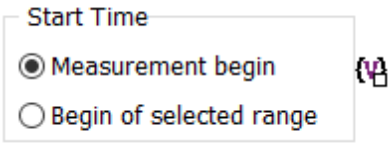
Maximum of slope: Finds the maximum slope value of all kinetic points within the selected range for each well. The slope is calculated using the entered width: A linear regression fit with the number of iterations entered in the width entry is performed starting with the first time point of the range up to the last. The maximum value of all calculated slopes is the result. You can decide with the *Slope Direction* control whether the rising or the falling maximum value should be calculated.



Time to max slope: Calculates the time taken for the maximum slope value to be reached in the selected range for each well. Use the same controls as for Maximum of slope to define the calculation width and the slope direction.

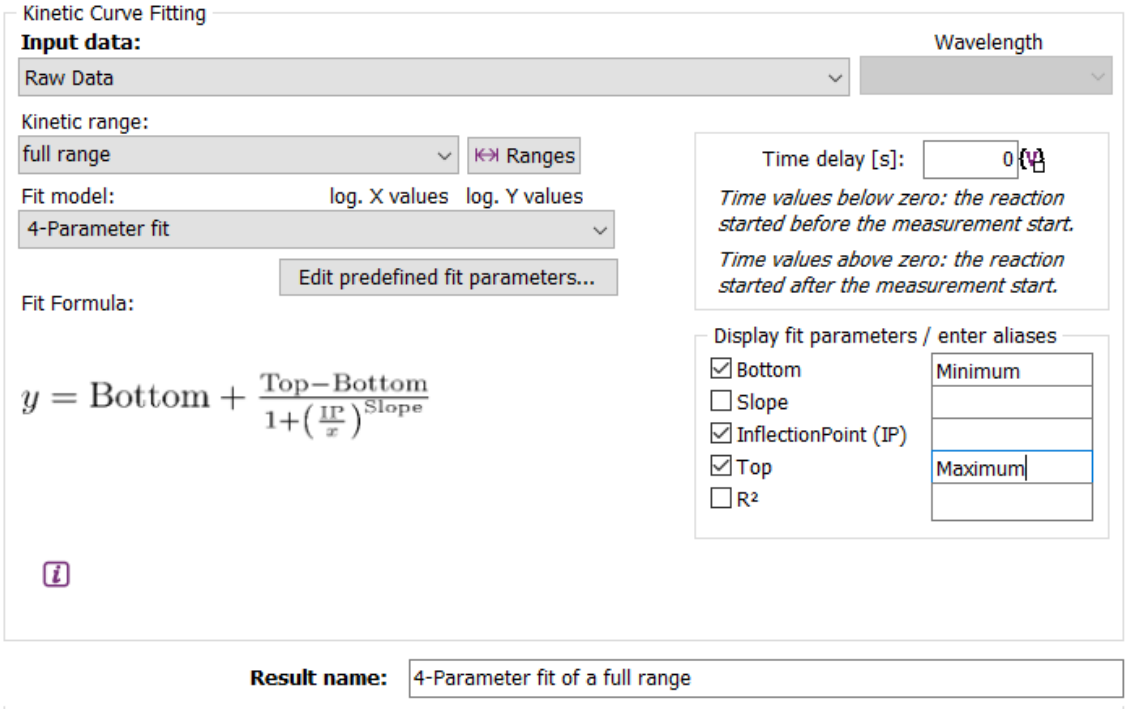
Median: Finds the median value of all kinetic points in the selected range for each well. The median is described as the number separating the higher half of the values from the lower half. The median is found by arranging all kinetic values in the defined range of one well from lowest value to highest value and picking the middle one. If there is an even number of values, the median is not unique, so the mean of the two middle values is taken.
Data point index at...: Like the time to max, time to threshold or time to max slope method, you can have the same calculations with the data point index instead of the time value as result. This can be useful in combination with individual ranges, if you want to use this index as a start or stop value for a range for further calculations. If a kinetic curve does not reach the entered threshold, n.a. will be used as result. You can define, what you want to use instead of n.a. in the result table with the *Result, if threshold is not reached*

If one of the methods ***Time to...*** was selected, you can define whether the start time for the time to calculation should be from begin of the measurement or the start time of the range for this calculation:



4.9 Kinetic Fit Calculations

The kinetic fit calculation page allows you to perform a formula fit based on a range of a signal curve. The fit will be calculated for each measured well in the plate. Each well has its own result fit curve and (optional) result fit parameter(s).



The result of each calculation is a fitted curve in the defined range which can be used like any other signal curve for further calculations. Outside the defined range, the original signal curve remains.

> **Note:** You can combine different fit models in one signal curve if you define different non overlapping ranges and calculate the different fit models in the different ranges, using one fit calculation result as input data for the next one.

Input data: Select the input data for the calculation. This can be the raw data or the result of any calculation that obtains kinetic data.
Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry All in the list, the calculation will be performed for each wavelength.

Kinetic range: The input data for a kinetic fit calculation are always defined by the first time point and the last time point of a range. It is possible to have one or more ranges defined over your kinetic. The full range is always available and covers the total measurement. See in the chapter 4.1: *Ranges*, how to use and define a range. All defined ranges are listed in the drop-down list with their start and stop time point. Select a range for the calculation from this list. To view, create or change a range, press the 

button to open the range window. You can also define an individual range where the start and/or stop time point is not fix. Select the *Individual Range* in the drop-down list to use and define this type of range. Read more about individual ranges in the chapter 4.1: *Ranges.*

Fit model: Select the curve fitting model for the calculation. Each curve fitting model has a set of parameters that describes the fit result. You can define which parameter should be used as result. See Select fit parameters / enter aliases below. Some of the fit models have different fit formulas, if the x or y values should be logarithmic. For these fit models you will find an entry in the drop-down list for each combination of logarithmic and non-logarithmic x or y values, where a different fit formula is used. Below the drop-down list you see the selected fit formula.

Available models:

Linear regression fit: Calculates a straight line through the kinetic range with minimum r² value. The result describes the line with the parameters *m* (slope) and *b* (offset):

$$y = mx + b$$

Logarithmic fit: Calculates a logarithmic curve through the kinetic range with minimum r² value. The result describes the curve with the parameters *m* (scale) and *b* (offset):

$$y = m \log x + b$$

Exponential fit: Calculates an exponential curve through the kinetic range with minimum r² value. The result describes the curve with the parameters *k*, *b* and *Offset*:

$$y = \text{Offset} + b \cdot e^{k \cdot x}$$

Double logarithmic fit: Calculates a logarithmic curve through the kinetic range with minimum r² value, where the y values are also logarithmic. The result describes the curve with the parameters *m* and *b*:

$$\log y = m \log x + b$$
$$\equiv$$
$$y = e^{m \log x + b} * \ln 10$$

4-Parameter fit: Calculates the dose response curve in the kinetic range with minimum r² value. Result parameters are *Bottom*, *Top*, *Slope*, *IP* (point of inflection) for the formulas (the first one for logarithmic y values, the second one for linear y values):

$$y = Bottom * \left(\frac{Top}{Bottom}\right)^{\frac{1}{1+\left(\frac{IP}{x}\right)^{Slope}}}$$

$$y = Bottom + \frac{Top - Bottom}{1 + \left(\frac{IP}{x}\right)^{Slope}}$$

5-Parameter fit: Calculates the dose response curve with a symmetry factor in the kinetic range with minimum r² value. Result parameters are *Bottom*, *Top*, *Slope*, *IP* (point of inflection) and *Symmetry* for the formulas (the first one for logarithmic y values, the second one for linear y values):

$$y = \text{Bottom} * \left(\frac{\text{Top}}{\text{Bottom}}\right)^{\frac{1}{\left[1+\left(\frac{IP}{x}\right)^{slope}\right]^{Sym}}} \qquad y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{\left[1 + \left(\frac{IP}{x}\right)^{Slope}\right]^{Sym}}$$

Segmental regression fit: The result of the segmental regression is an intersection point (time value) that divides the kinetic range into two sections. For each section, a linear regression fit will be performed. If no intersection point will be found, the result is a simple linear regression fit, otherwise the result are two different linear regression fits. One for time values below the intersection point and the other one for time values above this intersection point. Each combination of logarithmic and linear x and y values has its own fit formula:

$$y = mx + b$$
$$y = m \log x + b$$
$$\log y = mx + b$$
$$\log y = m \log x + b$$

If two fits are calculated, each fit has its own offset (b) and slope (m) as output parameters.
***2nd polynomial fit**:* Calculates a quadratic polynomial curve according to the kinetic range. The result parameters are *b* (offset), *c1* (multiplier 1) and *c2* (multiplier 2) for the fit formulas:

$$y = b + c1x + c2x^2$$
$$y = b + c1\log x + c2(\log x)^2$$
$$\log y = b + c1\log x + c2(\log x)^2$$
$$\log y = b + c1x + c2x^2$$

***3rd polynomial fit**:* Calculates a third order polynomial curve according to the kinetic range. The result parameters are *b* (offset), *c1* (multiplier 1), *c2* (multiplier 2) and *c3* (multiplier 3) for the fit formulas:

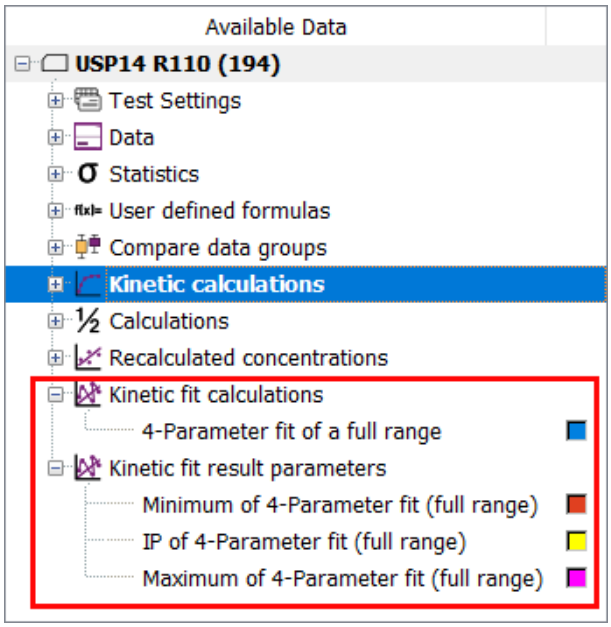
$$y = b + c1x + c2x^2 + c3x^3$$
$$y = b + c1\log x + c2(\log x)^2 + c3(\log x)^3$$
$$\log y = b + c1\log x + c2(\log x)^2 + c3(\log x)^3$$
$$\log y = b + c1x + c2x^2 + c3x^3$$

Hyperbola fit: Calculates a hyperbola in the kinetic range with minimum r² value. Each combination of logarithmic and linear x and y values has its own fit formula:

$$y = \frac{mx}{b+x} \quad y = \frac{m\log x}{b+\log x} \quad \log y = \frac{m\log x}{b+\log x} \quad \log y = \frac{mx}{b+x}$$

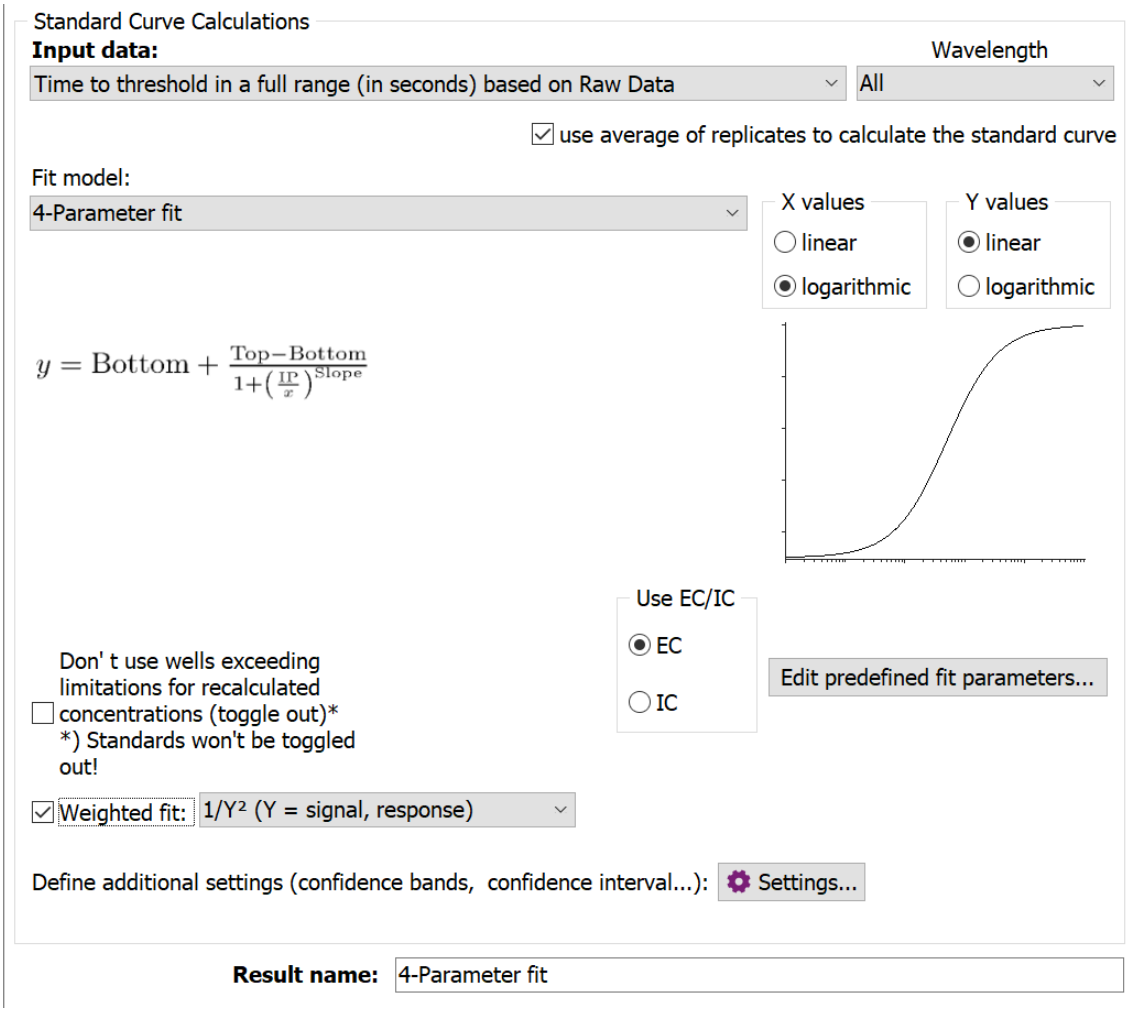
User defined fit: Define your own fit formula, if none of the predefined formulas fulfill your requirement. If this entry is selected, a button *Edit Formula...* appears. Click on this button to define the formula and the fit parameters. Details are explained in the chapter 4.26 User defined fit formulas.

Edit predefined Fit Parameter: Press this button to open a dialog to enter the settings for the predefined fit parameters. Details are explained in the section Initial guess values and constraints.
Display fit parameters / enter aliases:
In addition to the fit result curve you can create a result node for each available fit parameter. Check the check box control for the parameters you want to use as result. If you want to use a different name for the result parameter, enter the alias name in the entry field beside the parameter.
After performing the calculation new nodes appear in the navigation tree. One for the fitted curve and one for each result parameter you have selected:



4.10 Standard Calculations

The standard calculation page is only enabled if the layout of the test run contains standards. If the test run is a measured spectrum, at least one discrete wavelength must be added to the test run, to enable the standard calculation page.



Input data: Select the input data for the standard calculation. This can be the result of any calculation generating end point data. If the selected input data has kinetic data, you need to select the time point to define the data base for the fit. If the layout contains replicates of the standards and the selected data is not based on a replicate calculation, you can define that the calculation is based on the average of the replicates if you check the control *use average of replicates to calculate the standard curve*.

Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry *All* in the list, the calculation will be performed for each wavelength.

Fit model: Select the curve fitting model for the standard calculation. Each curve fitting model has a set of parameters that describes the fit result and is used for the concentration recalculation of the samples. See the section Fit Results below for more information.

Available models:

Linear regression fit: Calculates a straight line through the standards with minimum r^2 value. The result describes the line with the parameters *m* (slope) and *b* (offset):

$$y = mx + b$$

If ***force line through zero*** is selected the linear regression fit calculates a straight line with Y=0 for X=0:

$$y = mx$$

4-Parameter fit: Calculates the dose response curve for the standards. Result parameters are *Bottom*, *Top*, *Slope*, *EC50/IC50* and *log(EC50)/log(IC50)* for the formula:

$$y = Bottom * \left(\frac{Top}{Bottom}\right)^{\frac{1}{1+\left(\frac{IP}{x}\right)^{Slope}}}$$

$$y = Bottom + \frac{Top - Bottom}{1 + \left(\frac{IP}{x}\right)^{Slope}}$$

The first formula is used if logarithmic Y values are used, the second is for linear Y values (see *Linear or logarithmic X / Y Values* below)

5-Parameter fit: Calculates the dose response curve with a symmetry parameter for the standards. Result parameters are *Bottom*, *Top*, *Slope*, *Inflection point*, *EC50/IC50*, *log(EC50)/log(IC50)* and *Symmetry* for the formula:

$$y = \text{Bottom} * \left(\frac{\text{Top}}{\text{Bottom}}\right)^{\frac{1}{\left[1+\left(\frac{IP}{x}\right)^{\text{Slope}}\right]^{\text{Sym}}}}$$

$$y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{\left[1 + \left(\frac{IP}{x}\right)^{\text{Slope}}\right]^{\text{Sym}}}$$

The first formula is used if logarithmic Y values are used, the second is for linear Y values (see *Linear or logarithmic X / Y Values* below)

advanced 5-Parameter fit: Select the *5-Parameter fit* model and click the ***Use advanced 5 parameter fit*** to get this fit model. It calculates the dose response curve with a symmetry parameter for the standards, but without moving the point of inflection. Result parameters are *Bottom*, *Top*, *Slope*, *EC50/IC50*, *log(EC50)/log(IC50)* and *Symmetry* for the formula:

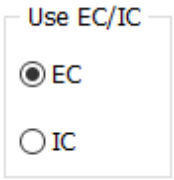
$$y = Bottom * \left(\frac{Top}{Bottom}\right)^{\frac{1}{\left[1+\left(2^{\frac{1}{Sym}}-1\right)*\left(\frac{IP}{x}\right)^{Slope}\right]^{Sym}}}$$

$$y = Bottom + \frac{Top - Bottom}{\left[1 + \left(2^{\frac{1}{Sym}} - 1\right) * \left(\frac{IP}{x}\right)^{Slope}\right]^{Sym}}$$

The first formula is used if logarithmic Y values are used, the second is for linear Y values (see *Linear or logarithmic X / Y Values* below)

The variable *IP* (point of inflection) in the 4- and 5-Parameter fit formula is the *EC50/IC50* value. The *EC50/IC50* is the concentration value where the signal is in the middle between Top and Bottom.

If the 4-Parameter fit or the 5-Parameter fit is selected, additional controls appear on the window:



Use EC/IC: Defines if EC or IC value should be calculated.

Cubic spline fit: A spline is a special function defined piecewise by polynomials. The cubic spline calculates polynomial fit curves between two adjacent standards. The result is a continuous and differentiable curve with each standard lying on the curve. Therefore, the result is not just one formula but a set of polynomial formulas and r^2 is always 1.

Point to point fit: The point-to-point fit calculates linear regression fits between two adjacent standards. The result is in fact a continuous but not a differentiable curve. Each standard is lying on the curve therefore the result r^2 is 1.

Segmental regression fit: The result of the segmental regression is an intersection point (concentration value) that divides the standards into two sections. For each section, a linear regression fit will be performed. If no intersection point will be found, the result is a simple linear regression fit, otherwise the result are two different linear regression fits. One for concentration values below the intersection point and the other one for concentration values above this intersection point. Each linear fit has its own offset and slope as output parameters.

2nd polynomial fit*:* Calculates a quadratic polynomial curve according to the standards. The result parameters *are b* (offset), *c1* (multiplier 1) and *c2* (multiplier 2) for these fit formulas (one formula for each combination of linear and logarithmic X and Y values. See *Linear or logarithmic X / Y Values* below):

$$y = b + c1x + c2x^2$$

$$y = b + c1\log x + c2(\log x)^2$$

$$\log y = b + c1\log x + c2(\log x)^2$$

$$\log y = b + c1x + c2x^2$$

3rd polynomial fit*:* Calculates a third order polynomial curve according to the standards. The result parameters are *b* (offset), *c1* (multiplier 1), *c2* (multiplier 2) and *c3* (multiplier 3) for these fit formulas (one formula for each combination of linear and logarithmic X and Y values. See *Linear or logarithmic X / Y Values* below):

$$y = b + c1x + c2x^2 + c3x^3$$
$$y = b + c1\log x + c2(\log x)^2 + c3(\log x)^3$$
$$\log y = b + c1\log x + c2(\log x)^2 + c3(\log x)^3$$
$$\log y = b + c1x + c2x^2 + c3x^3$$

Hyperbola fit: Calculates a hyperbola for the standards with minimum r^2 value. The result parameters are *m* and *b* for these fit formulas (one formula for each combination of linear and logarithmic X and Y values. See *Linear or logarithmic X / Y Values* below):

$$y = \frac{mx}{b+x} \quad y = \frac{m\log x}{b+\log x} \quad \log y = \frac{m\log x}{b+\log x} \quad \log y = \frac{mx}{b+x}$$

User defined fit: Define you own fit formula, if none of the predefined formulas fulfill your requirement. If this entry is selected, a button *Edit Formula...* appears. Click on this button to define the formula and the fit parameters. Details are explained in the chapter 4.26 User defined fit formulas.

Linear or logarithmic X / Y Values: Use the check boxes available to define whether the fit result is shown using either linear or a logarithmic scaling for the according axes.

Depending on the X and Y Values, a logarithmic calculation may not be possible (i.e. for values <= 0). In this case the hint *Calculation of fit parameters not possible* appears where the fit result parameters are normally shown.

> **Note:** Using logarithmic X values will influence the fit result of a point to point fit, a linear regression fit and a segmental regression fit. Using logarithmic Y values will influence the fit result of all fit models.

Use dilution factor for standards calculation: This option appears if there is at least one dilution factor > 1 defined in the layout. If this checkbox is checked, the dilution factor is used for the recalculation of the concentration values meaning that the result will be multiplied by the dilution factor defined

Use result of group ... for calculation: This option appears if you have more than one group with standards. It is possible to select that the standard fit result of one group be used for the recalculation for all other groups. If the '–' entry is selected, then every group will use its own fit result for the recalculation of the concentration values.

Don't use wells exceeding limitations for recalculated concentrations (toggle out): If this option is set, a well will be automatically toggled out, if a concentration value cannot be calculated with the resulting fit formula and the value of the well or if the recalculated concentrations exceeds the limitations (see Limitations for Recalculated Concentrations). Standards used to calculate the fit will not be toggled out, because this would change the fit.

Edit predefined Fit Parameter: Press this button to open a dialog to enter the settings for the predefined fit parameters. Details are explained in the section Initial guess values and constraints.

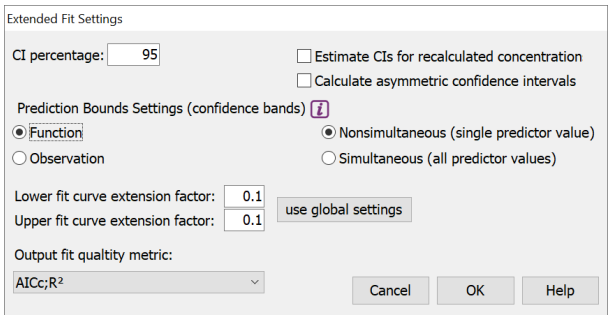
Weighted fit

Check this control if the weight of each standard should not be the same. You can give less weight to less precise standards and more weight to more precise standard. Select one of the offered weighting methods to define, how the weighting for each standard should be calculated.

Confidence Interval Settings and additional fit Settings

For all fit models except the point to point and the cubic spline fits, confidence intervals and confidence band are calculated automatically. Confidence intervals are calculated for each standard and for each fit result parameter. The confidence band can be displayed in the standard curve chart together with the error bars. The confidence values for the fit result parameters are displayed as two additional columns in the fit result table.

To define or change the confidence bands and interval settings, click on the settings button to open the setting dialog for confidence bands and intervals:



CI percentage: Define the percentage value for the confidence interval calculation. A lower value results in a wider interval band.

Estimate CIs for recalculated concentrations: Check this control to add the lower and upper CI values to the recalculated concentrations. The values will appear as additional data nodes in the navigation tree.
Calculate asymmetric confidence intervals: Asymmetric confidence intervals are based on the evaluation of profile likelihoods. Simply said it is the sum-of-squared error (SSE) profile evaluated for each individual fit parameter. Starting from the optimal solution (i.e. the value of the fit parameters where the SSE is minimal) a given fit parameter is sequentially varied in positive and negative direction (seen from the optimal value) while all the other fit parameters are again optimized. Once the SSE-profile reaches a certain threshold, depending on the confidence level set by the user, the corresponding parameter value at the threshold denotes the lower and upper confidence limit of that parameter, respectively.
Please note that asymmetric confidence intervals are regarded as more accurate precision metrics of fit parameters than asymptotic confidence intervals (default in MARS). However, the former ones take longer to compute and probably either one or none of the confidence limits exist. Then the corresponding entry in the Fit Results table will be empty.
Please note that only fit parameter confidence intervals are asymmetric (if checked) and not the fit curve's confidence bands.
Prediction Bounds Settings: Select between the different settings:
Observation or Function Bounds: Provides a confidence interval for a new observation or for a new function (curve).In general, observation intervals are wider than function intervals, because of the additional uncertainty of predicting a new response value (the function plus random errors).
Non-simultaneous or Simultaneous Bounds: Provides a confidence interval using a single predictor value (non-simultaneous) or all predictor values (simultaneous). In general, simultaneous intervals are wider than non-simultaneous intervals, because of the additional uncertainty of bounding values for all predictors.
Use the ***Lower fit curve extension factor*** and the ***Upper fit curve extension factor*** to define how far the fit curve is drawn below the smallest standard and above the highest standard. Press ***use global settings*** to reset the values to the global extension factors (defined in the Calculation Settings Dialog).

Output fit quality metric:

In addition to the fit quality metrics R^2 and AICc (Akaike's information criterion corrected) that are always output, you can define further quality metrics to be added to the output in the fit results (see next chapter).
See the whole list of possible metrics:

Shortcut	Quality Metric
AIC	Akaike's information criterion
AICc	Akaike's information criterion, corrected for small sample sizes
R^2	R-squared
R^2 adj	Adjusted R-squared (R-squared, adjusted with the number of predictors)
BIC	Bayesian information criterion
MSE	Mean-square error
RMSE	Root mean-squared error

4.10.1 Fit Result

After performing a standard curve fit, the fit result can then be inspected on the standard curve chart. If this is the first standard calculation of the test run, the tab for the standard curve page will appear following a successful fit. You can find more details on certain fit parameter in the section Fit model comparison using Akaike's information criterion (AICc).
If a fit fails for a group of standards of the test run, the hint *Calculation of fit parameters not possible* appears on the detailed window for the fit result and on the fit result window. The reason for a failed fit could either be a wrong axis scaling (logarithmic instead of linear), or to few standards to calculate the fit (see Limitations for Recalculated Concentrations)
If the calculation of a concentration value fails for a well, you will see in the microplate or table view a text message that indicates the reason for the failure:
In the microplate and the table view, the result of the concentration calculation for the contents, based on the fit result will be displayed.

Text	Meaning
n.a.	Not available - recalculation not possible (normally the fit itself was not possible)

<< std range	The calculated concentration value is under the defined limit for this fit model. (see Limitations for Recalculated Concentrations table below)
>> std range	The calculated concentration value is above the defined limit for this fit model. (see Limitations for Recalculated Concentrations table below)
<< Y range	The input value is either under the domain of the fit or under the defined limit for this fit model. (see Limitations for Recalculated Concentrations table below)
>> Y range	The input value is either above the domain of the fit or above the defined limit for this fir model. (see Limitations for Recalculated Concentrations table below)
ambiguous	The input value is ambiguous, that means that it fits to more than one concentration value.

Limitations for Recalculated Concentrations:
In general, fit curves are drawn in a defined range below the lowest and above the highest defined standard for the fit. The lower and upper fit curve extension factor for that range can be defined in the Calculation Settings.
For some of the fit models you cannot use these limits. See the used limits in the table below.

Fit model	input (y) minimum	input (y) maximum	concentration minimum	concentration maximum	min. no. of standards
Linear regression	no limit (but > 0 if log Y)	no limit	lower fit curve extension factor (but >= 0)***	upper fit curve extension factor	1
4-Parameter	f(x) where x is lowest std concentration - lower fit curve extension factor	f(x) where x is highest std concentration + upper fit curve extension factor	lower fit curve extension factor (but > 0)	upper fit curve extension factor	4
5-Parameter	f(x) where x is lowest std concentration - lower fit curve extension factor	f(x) where x is highest std concentration + upper fit curve extension factor	lower fit curve extension factor (but > 0)	upper fit curve extension factor	5
Cubic spline	min Y of calculated splines	max Y of calculated splines	minX*	maxX*	4
Point to point	minY*	maxY*	minX*	maxX*	2
Segmentalregression	no limit (but > 0 if log Y)	no limit	lower fit curve extension factor (but >= 0)***	upper fit curve extension factor	4
2nd polynomial	no limit (but > 0 if log Y)	no limit	lower fit curve extension factor (but >= 0)***	upper fit curve extension factor	3**
3nd polynomial	no limit (but > 0 if log Y)	no limit	lower fit curve extension factor (but >= 0)***	upper fit curve extension factor	4**
Hyperbola	no limit (but > 0 if log Y)	m	lower fit curve extension factor (but >= 0)***	upper fit curve extension factor	3
User Defined Fit	depending on the fit	depending on the fit	if possible, lower fit	if possible, upper fit	number

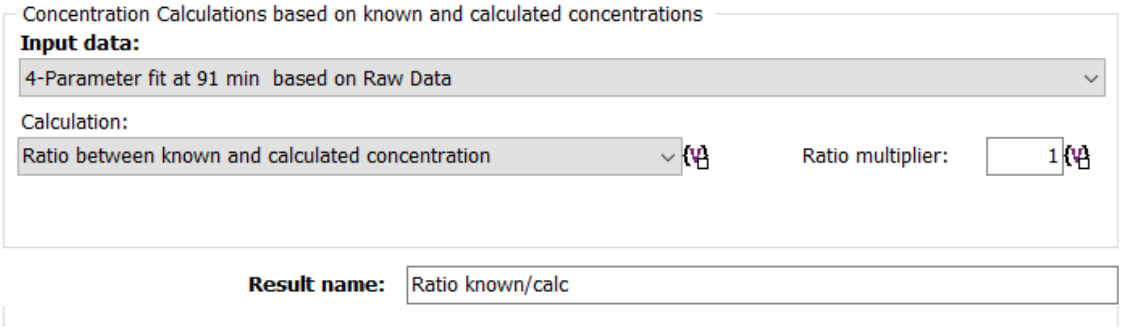
Fit model	input (y) minimum	input (y) maximum	concentration minimum	concentration maximum	min. no. of stand-ards
	formula	formula	curve extension factor r (but >= 0) ***	curve extension factor	of fit para-meters

*) **minY** = minimum value of standards; **maxY** = maximum value of standards; **minX** = minimum standard concentration; **maxX** = maximum standard concentration.
**) if less standards are defined, the degree of the polynomial fit will be reduced (e.g. from 3nd to 2nd if only 3 standards are available or from 2nd to linear regression if only 1 or 2 standards are available)
***) >0 for logarithmic x values.

Note: To calculate and display confidence limits, you need one more standards as a minimum as defined in the ***min. no. of standards*** column

4.11 Concentration Calculations

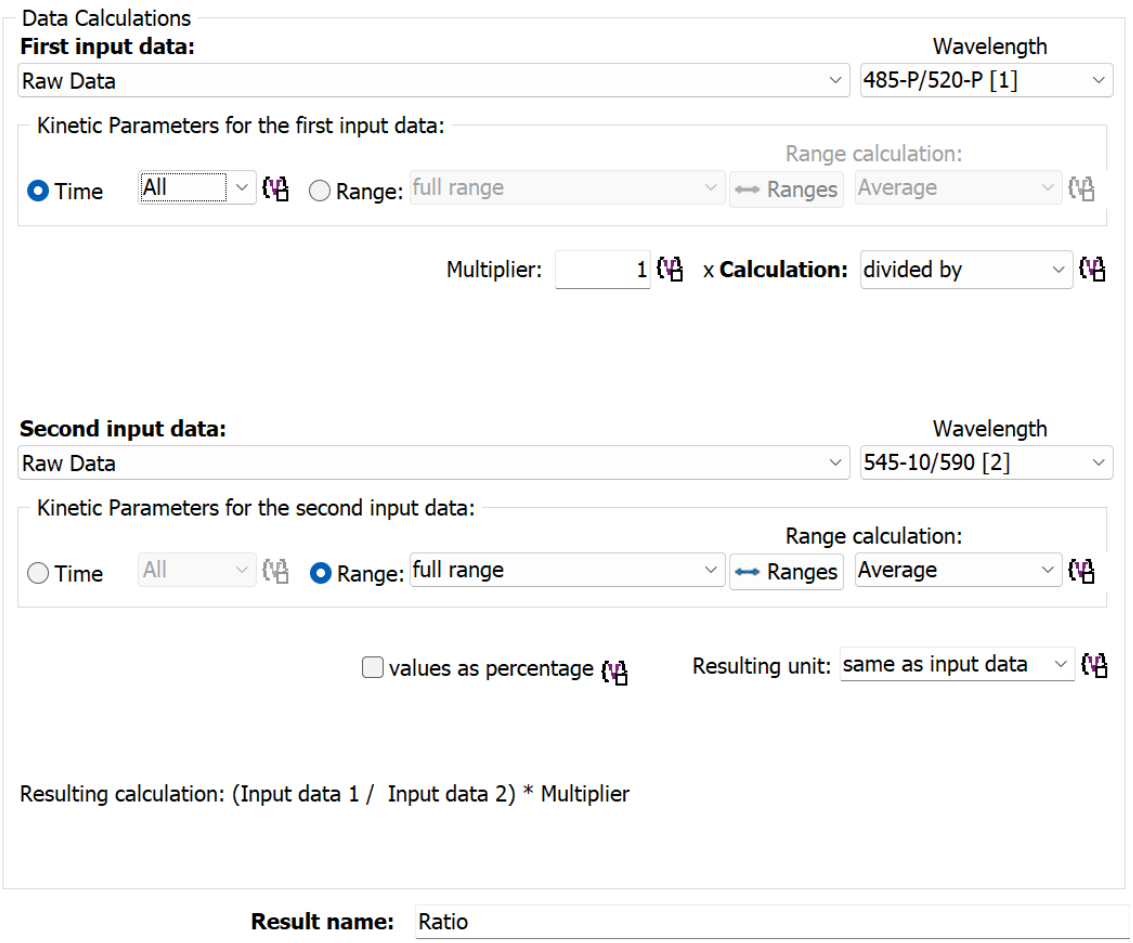
The concentration calculation page allows you to perform arithmetic operations based on recalculated concentration values (by a standard curve fit calculation) and known concentrations (standard concentrations).
With these calculations only values of wells which contain a standard with a defined standard concentration value are considered.



Input data: Select the input data for the calculation. This can be the result of any standards calculation with concentration values as result data.
Calculation: Select the calculation method for your calculation.
Available methods:
Difference of calculated and known concentration: Subtracts the calculated concentration value from the standard concentration value.
Ratio between known and calculated concentration: Calculates the ratio between the known and the calculated concentration. The result will be multiplied with the entered ratio multiplier.
Ratio between calculated and known concentration: Calculates the ratio between the calculated and the known concentration. The result will be multiplied with the entered ratio multiplier.
Percentage deviation of calculated from known concentration: Calculates the deviation in percent of the calculated concentration from the known standard concentration. If the calculated value is less than the standard concentration, the percentage value is expressed as negative value.
Ratio Multiplier: Enter a ratio multiplier for the calculation.

4.12 Data Calculations

The data calculation page allows you to perform arithmetic operations between either two different data inputs or between two sets of wavelength data for the same data input.



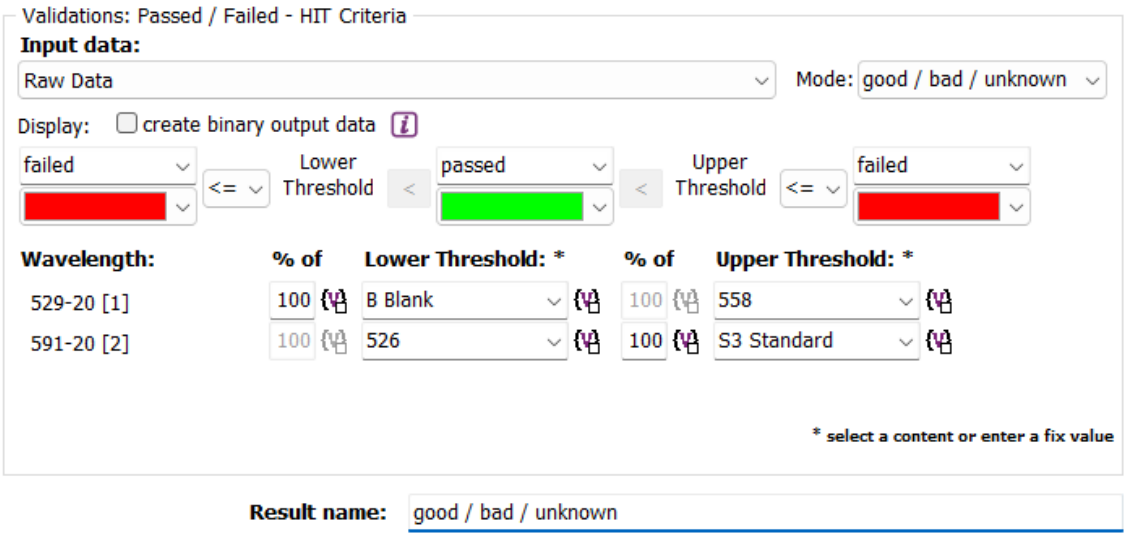
First input data: Select the first input data for the data calculation. This can be the result of any calculation which outputs the data as numbers.
Second input data: Select the second data input for the data calculation. This can be the result of any calculation which outputs the data as numbers.
Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry *All* in the list, the calculation will be performed for each wavelength (wavelength 1 of the first input data with wavelength 1 of the second input data, wavelength 2 of the first input data with wavelength 2 of the second input data and so on). If the selected input data contains spectra data and you select spectrum, the calculation will be done based on the spectrum curve and the result is again a spectrum curve.

Kinetic Parameters: If the selected input data contains kinetic data, the Kinetic Parameters controls are visible and can be used to define how to handle the kinetic data:
Time: select a certain time data point to use only this value for the calculation. Select all, to do the calculation for all iterations (the result is than a kinetic curve).
Range: Select a range and a calculation to calculate a certain value for the data points in the range and use this value for the data calculation.

Multiplier: Enter a multiplier for the calculation.
Calculation: Select the calculation method for your data calculation.
Available methods: ***minus, divided by, plus, multiplied by***.
values as percentage: This control appears if the selected calculation method is *'divided by'*. Select it to calculate the ration as a percentage value. The *Multiplier* will then be set to 100 and the resulting unit is %.
Resulting unit: Select the unit type of the calculation result.

4.13 Validations

The validations page lets you classify your data. Similar to the color modes for the *Microplate View*, the data can be grouped into good or bad (pass or fail) categories:



Input data: Select the input data for the validation. This can be the result of any calculation which outputs the data as numbers.
Mode: select the kind of validation: **good / bad, good / bad / unknown, gradient** or **delta band**.
For each measured wavelength you can enter a **threshold** (for the good / bad mode) or a **lower** and an **upper threshold** (for the good / bad / unknown mode) or a **start** and a **stop** value (for the gradient mode) or **threshold** and a **delta band** value (for the delta band mode). Enter a threshold value that divides the good from the bad data or defines the lower and upper boundary of your classification. You can also select contents out of the pull-down list that defines the threshold value. Use the ***% of*** entry field to define a percentage of a content value (e.g. 20 % of the blank value). In the delta band mode, you define a threshold and a delta value which defines a range around the threshold. You can also define if the delta range is an absolute value or a percentage of the threshold value.
Create binary output data: To create binary output data, click this control. The result data will only be 0 or 1. Define for the each result (below threshold, above threshold, between thresholds), if the result should be a 0 or a 1. Binary output data are used (and needed) as input data for the quantal response calculation.
Display: Text and background color is defined here that will be shown with the result of the classification. For the ***good / bad*** and the ***good / bad / unknown*** modes, you can define if wells, exceeding the defined thresholds should not be used further on. Therefore, the drop-down list for the displayed text has the entry *<Don't Use Well>*.
If *<Don't Use Well>* is selected, the subjacent color control changes to a list control that allows you to define if only wells with special contents should be set to unused.
Another special meaning has the entry *<Value>*. If this item is selected, the value of the well for the selected input data is displayed together with the selected color as background.
If a number is entered, this will be used as result value for the well that matches the condition.
If ***gradient*** mode is selected, the drop-down list contains the additional entry *<Percentage>*. If you select this entry, not the value but a percentage representation of the value based on the entered start and stop values will be displayed.

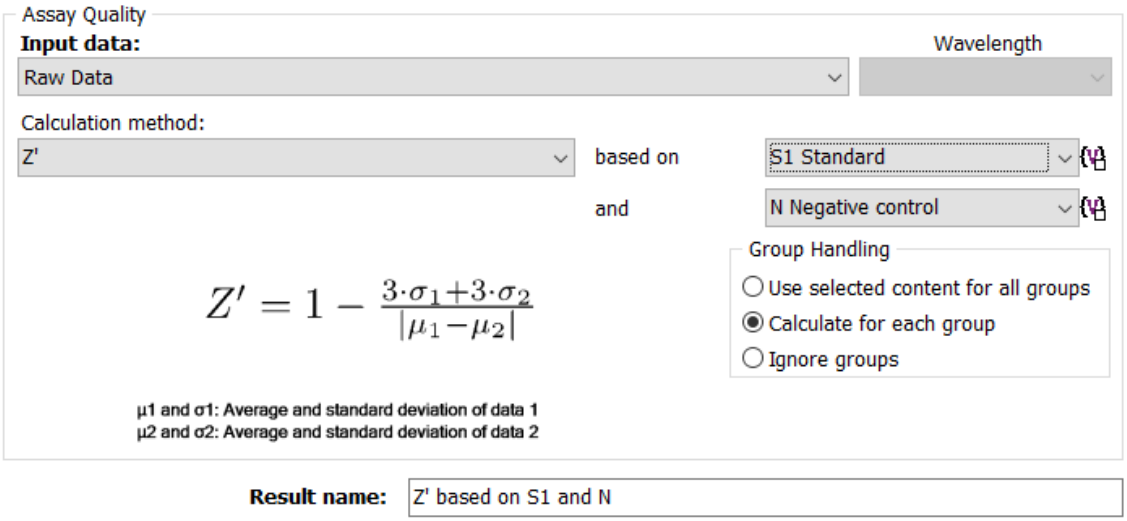
<= or < (Lower) Threshold (not for the *gradient* mode or *delta band* mode): Select the text displayed for the values under/equal to the (lower) threshold out of the drop-down list, or enter any text into the entry field and select a according background color.


: Use this control to decide if the value should be lower or equal or real lower.
<= or < Upper Threshold (only for the*good / bad / unknown* mode): Select the displayed text for the values above the (upper) threshold out of the drop-down list, or enter any text into the entry field and select an according background color.


: Use this control to decide if the value should be higher or equal or real higher.
between Lower Threshold and Upper Threshold (only for the *good / bad / unknown* mode): Select the displayed text for the values between the lower and the upper threshold out of the drop-down list, or enter any text into the entry field and select an according background color.
Start Color / End Color (only for the *gradient* mode): Select the background color for the start and for the stop value. All values between these two values will be displayed with a background color between the two selected colors according to the percentage of the position of the value between the start and the stop value. Select **Rainbow colors** if the background color should follow the color of the rainbow spectrum.
Outside Band / Inside Band (only for the *delta band* mode): Select the displayed text and background color for values outside the defined delta value around the threshold and for values inside the defined band.

4.14 Assay Quality

The assay quality calculations page provides several methods to analyze your measurement.



Input data: Select the input data for calculating the assay quality. This can be the raw data or the result of any calculation.
Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry *All* in the list, the calculation will be performed for each wavelength.
Calculation method: Select the calculation method for your assay quality.
Available methods:
Z' (Z prime): Calculates the Z prime value based on the reference contents you define. Select a content out of each drop-down list for each of the data on which the Z prime calculation is based on. The formula for the calculation is:

$$Z' = 1 - \frac{(3 \cdot \sigma 1 + 3 \cdot \sigma 2)}{|\mu 1 - \mu 2|}$$

µ 1 is the average of the values over the replicates of the first reference content
σ 1 is the standard deviation over the replicates of the first reference content.
µ 2 is the average of the values over the replicates of the second reference content
σ 2 is the standard deviation over the replicates of the second reference content.

Signal to blank: Calculates the Signal to blank ratio for each well. You can select the content which represents the blank value with the *select blank* drop-down list. If the layout contains blanks, it will be preselected. The formula for the calculation is:

$$S/B = \frac{\mu_{signal}}{\mu_{background}}$$

µ signal is the average of the values over the replicates of the content for which the S/B value is calculated.
µ background is the average of the values over the replicates of the blank.

Signal to noise: Calculates the Signal to noise ratio for each well. You can select the content which represents the noise value with the *select noise* drop-down list. If the layout contains blank, it will be pre-selected. The formula for the calculation is:

$$S/N = \frac{\left|\mu_{signal} - \mu_{background}\right|}{\sqrt{\sigma_{signal}^{\,2} + \sigma_{background}^{\,2}}}$$

µ signal is the average of the values over the replicates of the content for which the S/N value is calculated.
σ signal is the standard deviation over the replicates of the content for which the S/N value is calculated.
µ background is the average of the values over the replicates of the noise (blank).
σ background is the standard deviation over the replicates of the noise (blank).

Note: It is important to have enough replicates for a reasonable Z', Signal To Blank or Signal To Noise calculation. Make sure that the input data for these calculation methods are NOT the result of a replicate statistic or based on a replicate statistic!

Percentage calculation: Set the value of each well in a percentage relation between a 0% and a 100% reference. You can select the content which represents the 0% reference value and the content which represents the 100% value. If the 0% value or the 100% value should be a fixed number (like 0% = 0), you can enter the value in the related entry field.
In addition, you can enter a percentage value to use e.g. 90% of the 100% reference or 110% of the 0% reference. The formula for the calculation is:

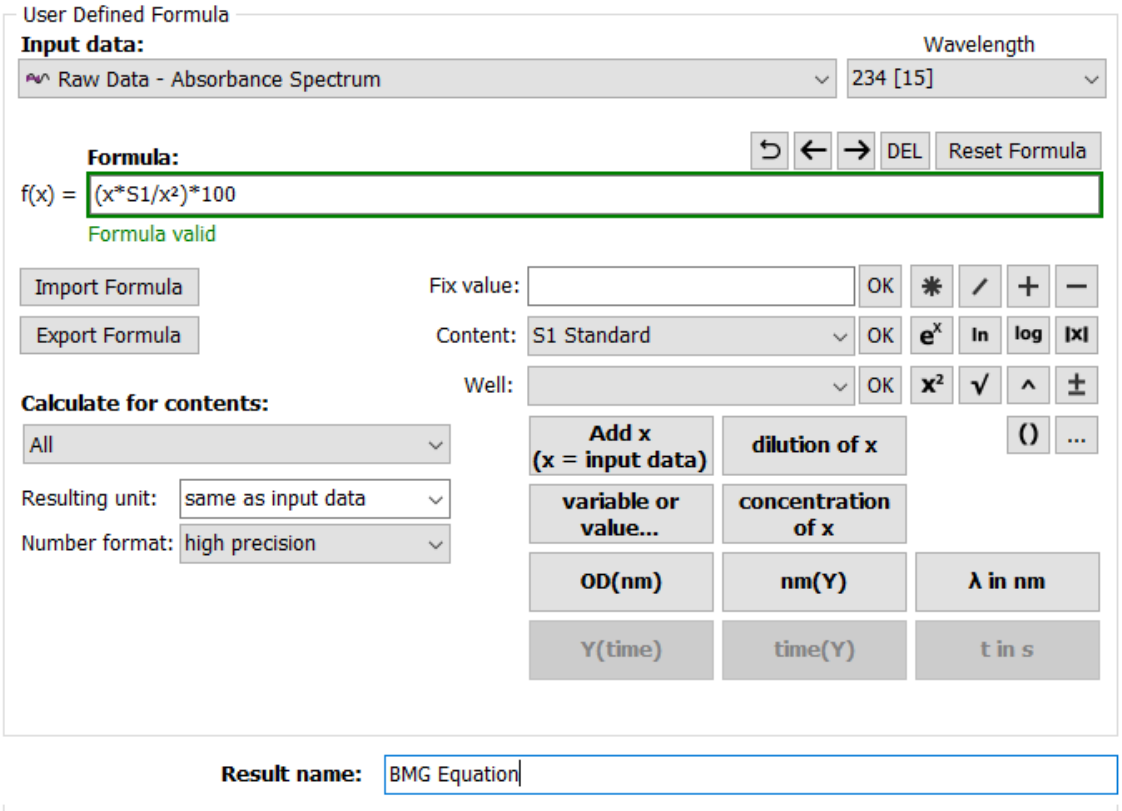
$$V\% = \frac{Signal - \mu_{0\%}}{\mu_{100\%} - \mu_{0\%}} \times 100$$

μ 0% is the average of the values over the replicates of the 0% reference.
μ 100% is the average of the values over the replicates of the 100% reference.
Group Handling: If the layout of the test run contains groups, you must decide how groups are handled:

Use selected content for all groups	Use the selected content of a group for the calculation even if the calculated well is in a different group.
Calculate for each group	Use the selected content of the group of the calculated well. If the group of the well does not have the selected content, no calculation is done for that well.
Ignore groups	Ignore the groups and calculate the result as if no groups where defined.

4.15 User Defined Formula

The user defined formula calculation page f(x)= allows you to define and perform arithmetic operations based on an entered equation. The result of the equation for each well defines the result data of the created process.



Input data: Select the input data for the data calculation. This can be the result of any calculation which outputs the data as numbers. The input data defines the used X value in the formula for each well and each wavelength (if the input data comes with more than one wavelength).
Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry *All* in the list, the calculation will be performed for each wavelength.

Calculate for contents: Select the contents for which the formula is valid. The formula is applied and calculated for all wells containing the selected contents. You can select one or more contents in the drop-down menu.
Result name: Enter a name that identifies the formula process. The name defines the name of the process and node name of that process in the navigation tree.

Resulting unit: Enter the resulting unit of the entered formula. This can be any entered text, or you can select one of the entries of the drop-down menu: Use the entry *Same as input data*, if the unit of the formula result is the same as the unit of the input data. Select *none* if the formula has no unit or you don't know the unit. If nothing is selected, *same as input data* is used.
Number format: Enter the desired number format. You can select between 'defined by resulting unit', 'high precision', 'define format...'.
- *defined by resulting unit*: the number format settings are defined by the selected resulting unit.
- *high precision*: returns numbers with a high number of decimal digits.
- *define format...*: a dialog opens, where the desired number format can be defined (see described in chapter 3.25.6.)

If nothing is selected, "high precision" is used, which returns numbers with a high number of decimal digits.

> **Note:** The selected unit affects the used format to display the numbers only if Number format is set to 'defined by resulting unit'. See chapter 3.25.5: Number Format Settings.

4.15.1 Enter a formula

Formula: The field formula shows the entered formula. The color frame and the text below the field show if the formula is valid. A syntax check is performed after each entry made into the formula.

Color	Text	Meaning
green	Formula valid	The formula is valid. The apply button is enabled and can be pressed if the formula is completely entered.
orange	Formula incomplete / Open bracket	The formula is incomplete. An operator or a parameter for an operator is missing or a bracket is not closed. Please complete the formula.
red	Syntax error	The combination of the entered operators, variables and constants is not valid. Please correct the entry to make the formula valid.

Use the operator buttons, the entry fields and the drop-down menus (see description below) to enter the formula. Numbers and simple operators can also be typed in directly in the formula field. In addition, you can type in the first letter of a desired function and will get e selection of all available functions starting with this letter. Select the desired function from this list to add it to the formula. You can set the cursor in the field with the mouse and you can delete entries with the backspace and the DEL key on the keyboard.

Description of the buttons above the formula entry field:

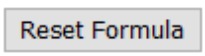

Undo the last step(s).


Sets the cursor one position to the left.


Sets the cursor one position to the right.



Deletes the content (an operator, bracket, variable or constant value) on the left side of the cursor (if you move the mouse over the button, the content that will be deleted is marked with a blue background in the formula field).



Clears the formula entry field.

Adding operators and brackets to the formula (operator buttons):

To add operators to the formula, press the according operator button. The operator will be inserted at the current cursor position. If an operator has two parameters (like division) the operator must be between these two operators, e.g. x / S1 (first enter the x, using the *x (well value)* button, then press the division operator button, and then enter the S1, using the content drop-down menu (how to enter variables and constant values see below). If the operator has one parameter, the parameter for most of these operators is behind the operator only for the x^2 operator, the parameter comes before the operator and for brackets the parameter is enclosed between the brackets. A parameter can be a variable, a constant value or a formula on its own (enclosed in brackets if needed).

Operator Button	No. Parameters	Operator description
*	2	Multiplication between the two parameters
/	2	Division: First parameter is the numerator; second parameter is the denominator.
+	2	Addition between the two parameters
−	2	Subtraction between the two parameters
e^x	1	Calculates the power of the parameter based on the Euler's number.
ln	1	Calculates the natural logarithm of the parameter
log	1	Calculates the logarithm based on 10 ($\log_{10}(x)$) of the parameter
|x|	1	Calculates the absolute value of the parameter (e.g. *-5* becomes *5*, but *5* remains *5*)
x^2	1	Calculates the square of the parameter.
√	1	Calculates the square root of the parameter.
^	2	Calculates the power of the two parameters. The first parameters are the mantissa, the second is the exponent of the power calculation.
±	1	Negates the parameter: *-5* becomes *5* but *5* becomes *-5*
( )	1	Encloses the parameter in brackets.
...	-	Opens a dialog with additional functions. See section *additional functions* below.

Adding variables and constant values to the formula:

You cannot add constant values or variable names directly into the formula field with the keyboard. Use the controls described in this section instead to enter your formula. The value or variable will be inserted at the current cursor position in the formula entry field.

Fix value

Use the entry field to enter fix number values. Press the OK button beside the field to add the value to the formula.

Content

Open the drop-down menu and select a content. Press the OK button to add the content as a variable to the formula. The value of the content, valid for the selected input data will be used in the formula. If replicates are available, the average value over the replicates is used. For each group of contents (Standards, Samples...), there is a separate entry with the extension *iterate over*, indicated with a # after the content type letter (S#, X#, ...) in the formula. Select this content, if the index of the content should follow the index of the content of the well, for that the value is calculated. Example: If the formula is x-S# and the wells content is X2, the value of S2 will be used at the position of S# in the formula (X2-S2 will be calculated for that well).

If the layout contains groups, a further drop-down menu appears on the right side of the content drop-down menu. You can select between certain group, <ignore groups> or < calculate for each>:

<ignore group>: The average of all the wells with the selected content over all groups will be calculated and used in the formula. The variable in the formula is marked with an i: S1i, or X2i...

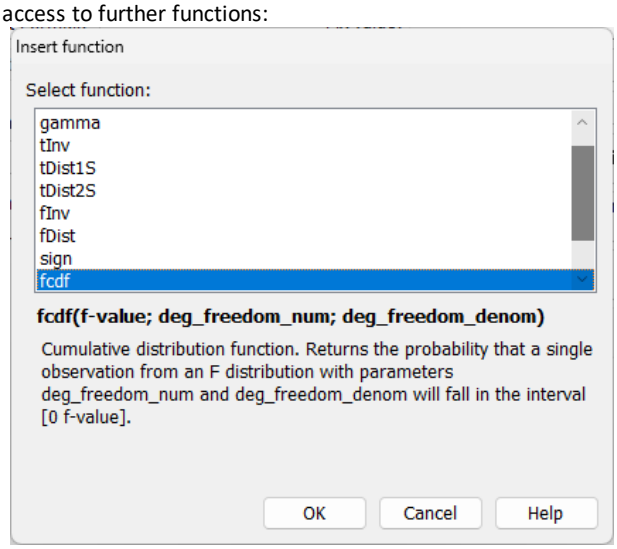
<calculate for each>: The average of the wells with the selected content of the group for the current well will be calculate and used in the formula (if the result for a well of group A is calculate, the average of the content for group A is used, if the result for a well of group B is calculated, the average of the content for group B is used, and so on). The variable in the formula has no further mark: S1, X2...

GROUPNAME: The average of the wells with the selected content of the group GROUPNAME will be used in the formula, regardless of the group of the currently calculated well. The variable in the

	formula is marked with the name of the group: S1A, X2B...
Well	Open the drop-down menu and select a well. Press the OK button to add the well as a variable to the formula. The value of the well, valid for the selected input data will be used in the formula.
Add x (x = input data)	The variable x will be added to the formula, when you press this button. X is replaced with the value, valid for the selected input data of the currently calculated well, when the formula is applied.
dilution of x	The term dil(x) will be added to the formula, when you press this button. Dil(x) is replaced with the dilution value of the currently calculated well. If no dilution values where defined, a dilution of 1 is used.
concentration of x	The term conc(x) will be added to the formula, when you press this button. Conc(x) is replaced with the concentration value of the currently calculated well, if available/defined. If the well is not a standard with concentration value, no calculation for this well is done.
value... or **variable or value...**	Press this button to add a value that comes from any other calculation in this test run or a variable to the formula. Read the chapter 4.2.1: *Define and Use Variables* to see, what variables and values are and how to use them. In the formula, variables are displayed as V1, V2, V3..., values are displayed as C1, C2..., W1, W2... if they represent content or well values. If the value represents a fit result parameter, the name of the parameter is displayed in the formula. If you move the mouse over a variable or a value in the formula, you get a detailed hint with information about the variable or value.
OD(nm)	Returns the OD value for a given Lambda value in nm if the value is in the valid range of the measured spectrum (only for Spectrum measurements).
nm(Y)	Returns the nm value of the given Y value of the spectrum curve. The nm value is the value of the index which is the nearest to the given Y value (only for Spectrum measurements).
λ in nm	If the selected input data is a spectral scan, the current wavelength value of the iteration over the wavelength will be used in the formula.
Y(time)	Returns the signal value of a given time value if the time value is in the valid range of the measured kinetic (only for kinetic measurements).
time(Y)	Returns the time value of the given Y value of the signal curve. The time value is the value of the index which is the nearest to the given Y value (only for kinetic measurements).
t in s	If the selected input data has kinetic data, the current time value of the iteration over the time will be used in the formula.

Additional functions:

Press the ... button to get access to further functions:



Select the desired function from the list to add it to the formula at the current cursor position.

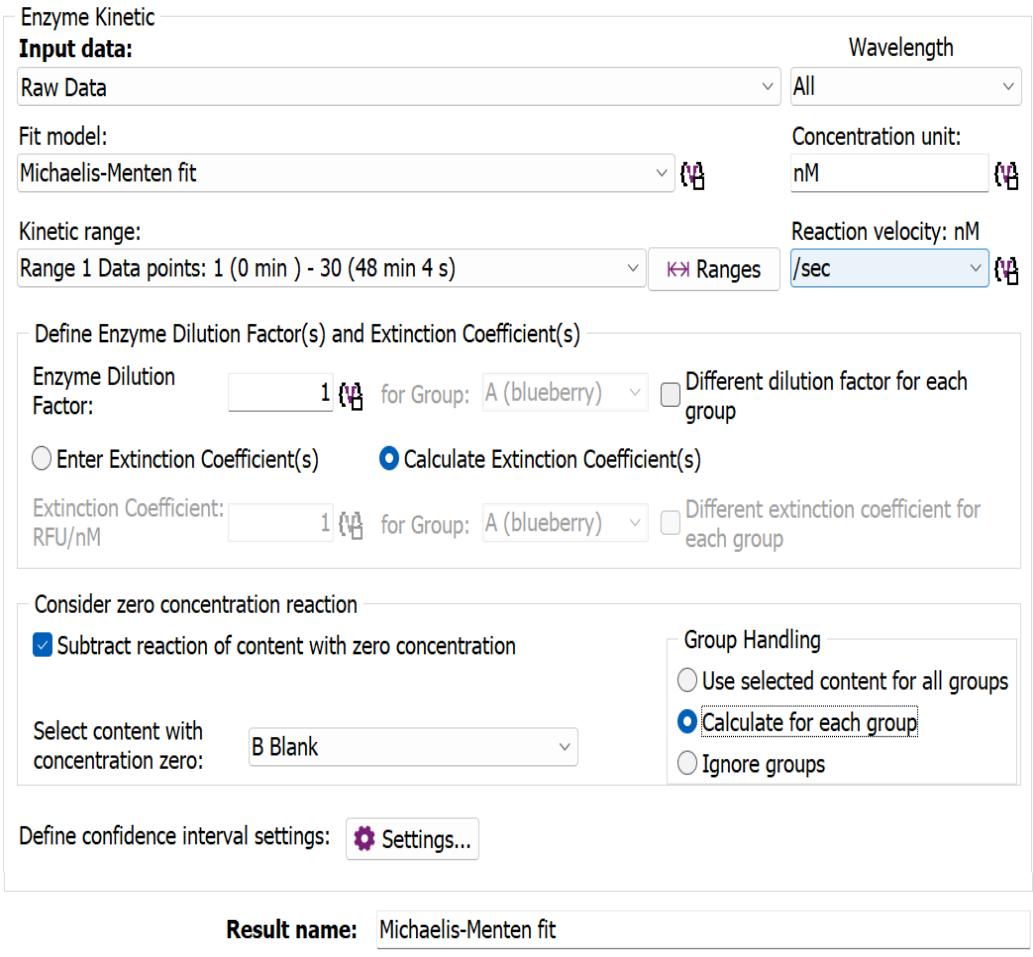
4.15.2 Export and Import a Formula

If a formula is valid it can be exported to a file to save it and use it for further test runs. To use a previously saved formula you can import the formula:

Import Formula	Press this button to open a file dialog and to import a saved formula. Files containing a formula have the extension MWF. Only files with this extension can be selected and opened.
Export Formula	If the entered formula is complete and valid, you can press this button to export the formula to a BMG Labtech formula file (with the file extension MWF). A file dialog opens. Enter the name for the file (the extension will be added automatically) and press Save to save the formula to the file.

4.16 Enzyme Kinetic Calculations

The enzyme kinetic calculation page is only enabled if it is a kinetic measurement and the layout of the test run contains standards. Read more about enzyme kinetic and how to set an experiment and use MARS to evaluate the measurement in Chapter 4.16.4: *How to Perform an Enzyme Kinetic Experiment*.
The enzyme kinetic calculation algorithm calculates the reaction velocity over the concentration first. Based on this it performs a hyperbola or a linear fit - depending on the selected fit model -, to evaluate Vmax and Km.



Input data: Select the input data for the enzyme kinetic calculation. This can be the result of any calculation that keeps the kinetic data.
Wavelength: If the selected input data comes with more than one wavelength (multi chromatic, dual channel or spectrum), select the wavelength for the calculation.
Fit model: Select the desired enzyme kinetic equation to perform the fit of the reaction rate over the concentration.
Available models:
Michaelis-Menten fit : The saturation process describes a hyperbola. The Michaelis-Menten fit calculates directly the desired variables Km and Vmax based on this hyperbola:

$$y = \frac{Vmax * x}{Km + x}$$

Where Y is the reaction velocity and X is the concentration.

Lineweaver-Burk: Transforms the plot of the reaction velocity (V) over concentration ([S]) to 1/V (Y-Axis) over 1/[S] (X-Axis) and performs a linear regression fit:

$$y = mx + b$$

where *VMax = 1/b* and *Km = m/b*

Eadie-Hofstee: Transforms the plot of the reaction velocity (V) over concentration ([S]) to V (Y-Axis) over V/[S] (X-Axis) and performs a linear regression fit:

$$y = mx + b$$

where *VMax = b* and *Km = -m*

Scatchard: Transforms the plot of the reaction velocity (V) over concentration ([S]) to V/[S] (Y-Axis) over V (X-Axis) and performs a linear regression fit:

$$y = mx + b$$

where *VMax = -b/m* and *Km = -1/m*

Hanes-Woolf: Transforms the plot of the reaction velocity (V) over concentration ([S]) to [S]/V (Y-Axis) over [S] (X-Axis) and performs a linear regression fit:

$$y = mx + b$$

where *VMax = 1/m* and *Km = b/m*

Concentration unit: Enter the concentration unit. If the concentration unit is already defined, you can see and change it here.

Kinetic range: Select the range for the calculation of the reaction velocity (maximum slope in the range). The full range is always available and covers the total measurement. See in the chapter 4.1 *Ranges*, how to define a range. All defined ranges are listed in the drop-down list with their start and stop iterations. Select a range for the calculation from this list. To view, create or change a range, press the Ranges button to open the range window. You can also define an individual range or a content-based range, where the start and/or stop iteration is not fix. Select the *Individual Range* or *Content-based ranges* in the drop-down list to use and define this type of range. Read more about individual and content-based ranges in the chapter 4.1: *Ranges.*

Reaction velocity: Define if the reaction velocity is volume per microsecond, millisecond, second, minute or hour.

4.16.1 Define Enzyme Dilution Factor(s) and Extinction Coefficient(s)

Enzyme Dilution Factor: If the enzyme was diluted, enter the dilution factor in this field.

> **for Group**: (only visible if the layout contains more than one group) If groups are defined, a dilution factor for each group can be entered (the control **Different dilution factor for each group** must be checked) or the same dilution factor for all groups can be used. To enter the dilution factor for a certain group, select the group with the drop-down control and enter the desired factor.

Enter Extinction Coefficient(s): If this control is selected, the extinction coefficient to transfer the OD values to concentration volumes must be entered below. Read more about how to find out the extinction coefficient with MARS in chapter 4.16.4: *How to Perform an Enzyme Kinetic Experiment* .

Calculate Extinction Coefficient(s): If this control is selected, the extinction coefficient to transfer the OD values to concentration volumes are calculated automatically. Therefore, a linear regression fit is performed on the last iteration of the selected range, where the slope of the fit defines the coefficient. This is only possible in long-time enzymatic reactions in which the substrate is completely converted into product indicated by no more change in signal. Read more about how to find out the extinction coefficient with MARS in chapter 4.16.4: *How to Perform an Enzyme Kinetic Experiment* .

Extinction Coefficient: If **Enter Extinction Coefficient(s)** is selected above, this field is enabled, and the extinction coefficient can be entered in this field.

> **for Group**: (only visible if the layout contains more than one group) If groups are defined, an extinction coefficient for each group can be entered (the control **Different extinction coefficient for each group** must be checked) or the same extinction coefficient for all groups can be used. To enter the extinction coefficient for a certain group, select the group with the drop-down control and enter the desired value.

4.16.2 Consider zero concentration reaction

Subtract reaction of content with zero concentration: If you have a background reaction at concentration zero, that needs to be subtracted from the reaction with concentration, check this control and select the content which contains the substance with concentration zero with the next control:

Select the content with concentration zero: Select the content from the drop-down list that contains the substance that shows the reaction with no concentration.

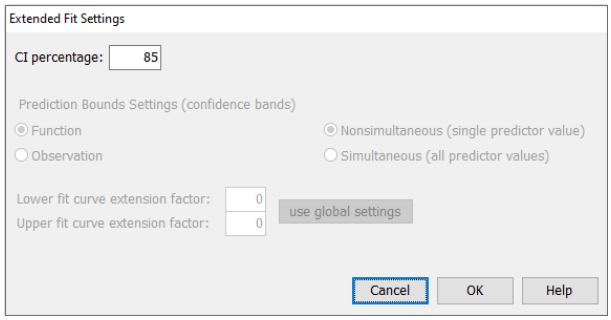
Group Handling: If the layout of the test run contains groups, you have to decide how groups are handled:

Use selected content for all groups	Use the selected content of a group for the calculation even if the calculated

	well is in a different group.
Calculate for each group	Use the selected content of the group of the calculated well. If the group of the well does not have the selected content, no calculation is done for that well.
Ignore groups	Ignore the groups and calculate the result as if no groups where defined.

Confidence Interval Settings

For all fit models, confidence intervals are calculated automatically for each fit result parameter which is not fix.
To define or change the confidence interval settings, click on the settings button to open the setting dialog for confidence intervals:



CI percentage: Define the percentage value for the confidence interval calculation. A lower value results in a wider interval band.

> **Note:** The Prediction Bounds Settings and the fit curve extension factors are not available for the enzyme kinetics calculation.

4.16.3 Calculation Result

After performing an enzyme kinetic calculation, the result can then be inspected on the enzyme kinetic fit chart. If this is the first enzyme kinetic calculation of the test run, the tab for the enzyme kinetic fit curve page will appear following a successful calculation.
The calculated Vmax and Km values are displayed in the detailed window and can be displayed and exported on the enzyme kinetic fit result window.
In the microplate and the table view, the calculated reaction velocity for each well is shown for that calculation node.

4.16.4 How to Perform an Enzyme Kinetic Experiment

This chapter describes how to set an experiment and how to use the MARS Data Analysis software for the determination of Km and Vmax.

Outline

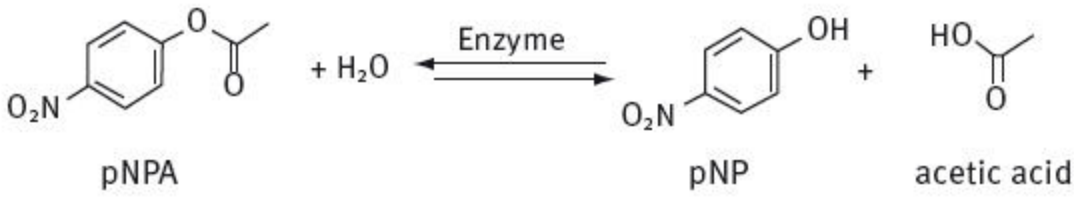
1. Definition of Km and Vmax
2. Preparation of the standard curve
3. Enzymatic measurements using different substrate concentrations
4. Evaluation using the MARS Data Analysis software

Definition of Km and Vmax

Km (Michaelis-Menten constant) is equivalent to the substrate concentration at which the reaction velocity reaches half of the maximal velocity. Km describes the affinity of enzymes to a specific substrate.
Vmax stands for the maximal velocity of an enzymatic reaction.

Preparation of the standard curve

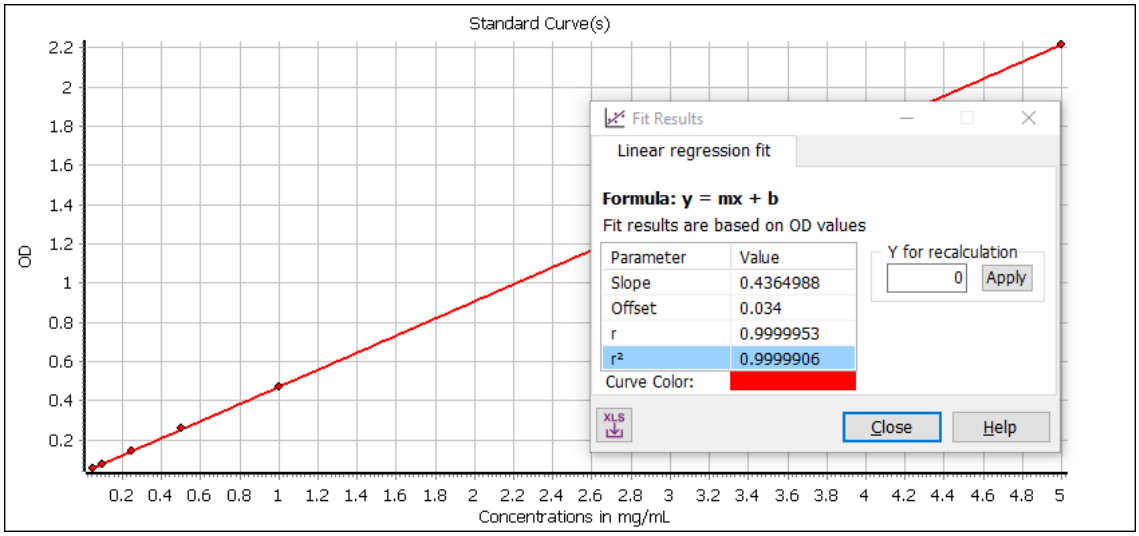
A usual enzymatic reaction contains a substrate that is converted into a product with the help of an enzyme. To determine the conversion from substrate to product it is necessary that the substrate changes its spectral properties during the reaction. Either the substrate absorbs at a special wavelength and does not give a signal when it is converted to the product or the substrate gives no signal, but the product does. The following figure shows a reaction example in which the product gives a measurable absorbance signal:



The product pNP (p-nitro phenol) is a yellow substance and shows an absorbance maximum at about 410 nm whereas the substrate pNPA (p-nitro phenyl acetate) does not show any absorbance at 410 nm.

Self-made standard curve

Before the enzymatic measurement will start a standard curve of the substrate/product should be taken. The slope of the standard curve will later be necessary for the enzymatic kinetic calculations. An example for the pNP standard curve is given here:



Software made standard curve

In case that the enzymatic reaction is run until the substrate is completely converted into the product, it is possible that the software creates the standard curve using the endpoint values of the wells. Please note that this option is only useful when the substrate is completely converted into the product indicated measurement values that are no longer increasing or decreasing (plateau). This kind of kinetic measurements usually take several hours.

Enzymatic measurements using different substrate concentrations

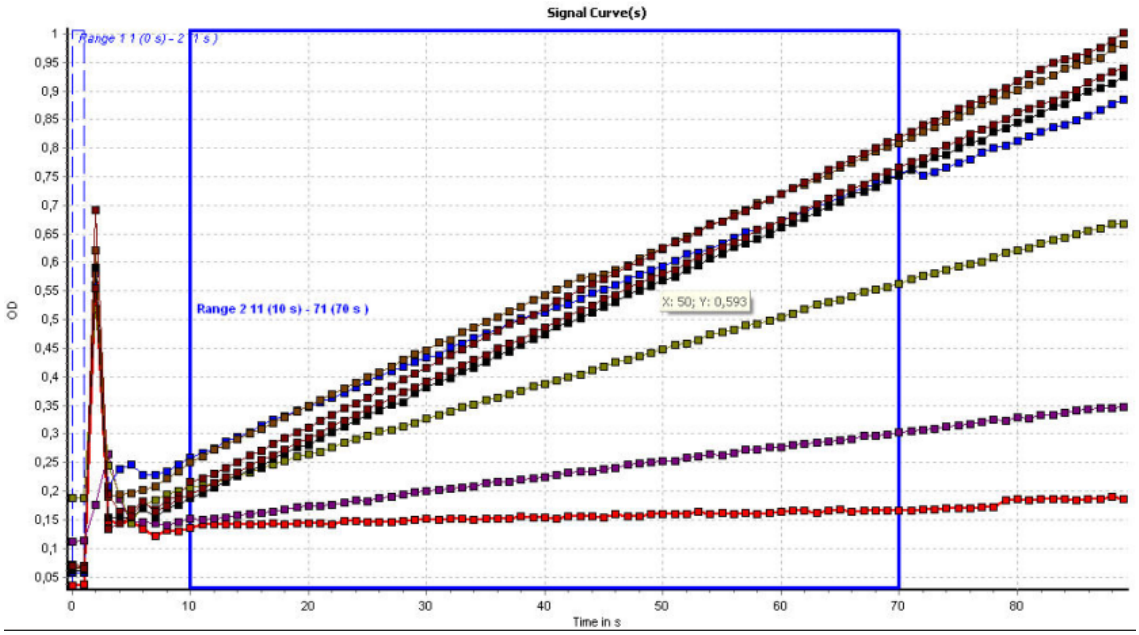
Usually equal amounts of enzyme solution and buffer are transferred into the wells. Different concentrations of substrate are added using the onboard injectors. A possible layout is given here:

	1	2	3	4	5	6	7	8	9	10	11	12
A Layout												
Standard Concentrations												
E Layout		S1	S2	S3	S4	S5	S6	S7		N	B	
Standard Concentrations		0.025	0.05	0.1	0.15	0.175	0.188	0.2				
F Layout		S1	S2	S3	S4	S5	S6	S7		N	B	
Standard Concentrations		0.025	0.05	0.1	0.15	0.175	0.188	0.2				
G Layout		S1	S2	S3	S4	S5	S6	S7		N	B	
Standard Concentrations		0.025	0.05	0.1	0.15	0.175	0.188	0.2				
H Layout												
Standard Concentrations												

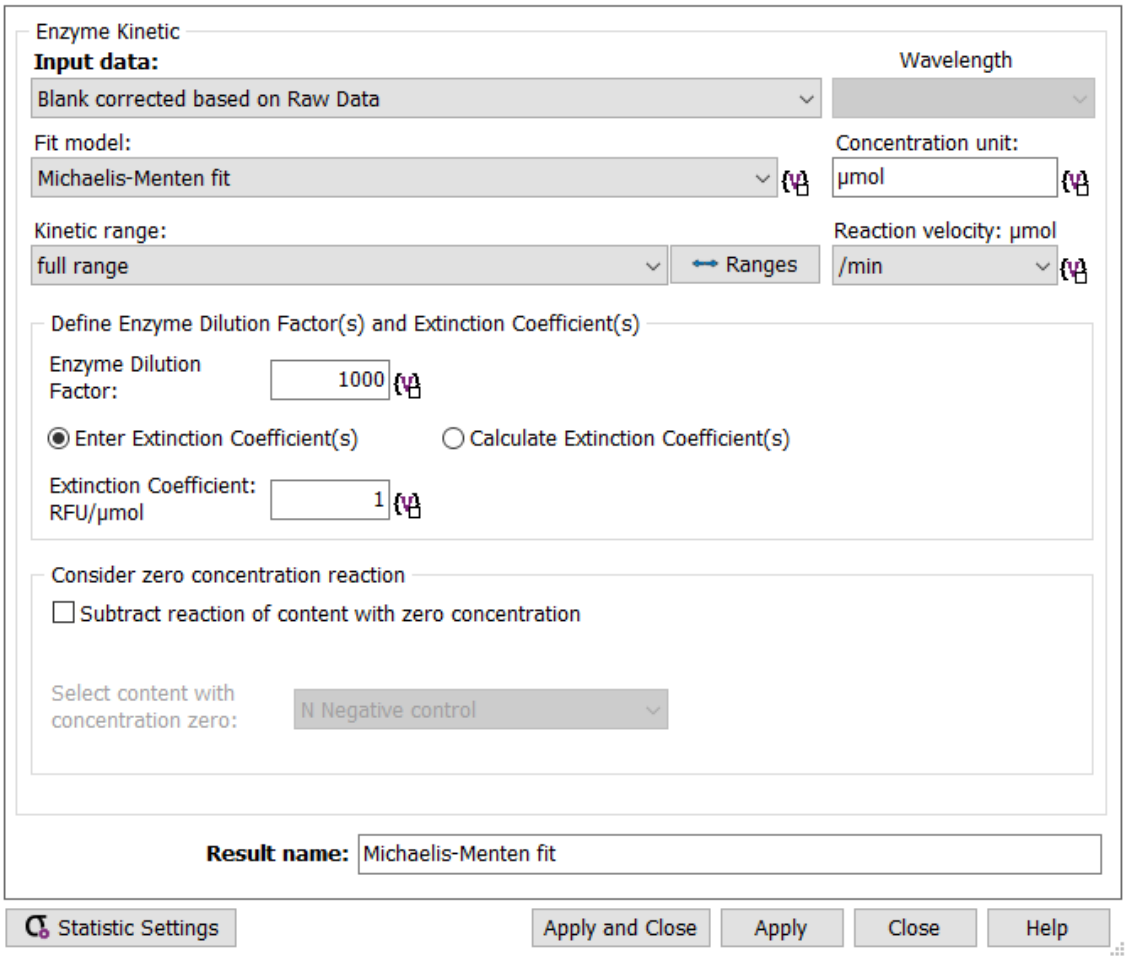
The blank should contain everything except the enzyme. The negative control should contain everything except the substrate.

Evaluation using the MARS Data Analysis software

After measurement, the results can be found in the MARS Data Analysis software. First it is necessary to look at the signal curve. An example is given here:



Choose a range that is useful for the calculation of the maximum slope. The range should be right shifted from the injection peaks.
After that, open the calculation window and click the tab Enzyme Kinetic. The following window will appear:



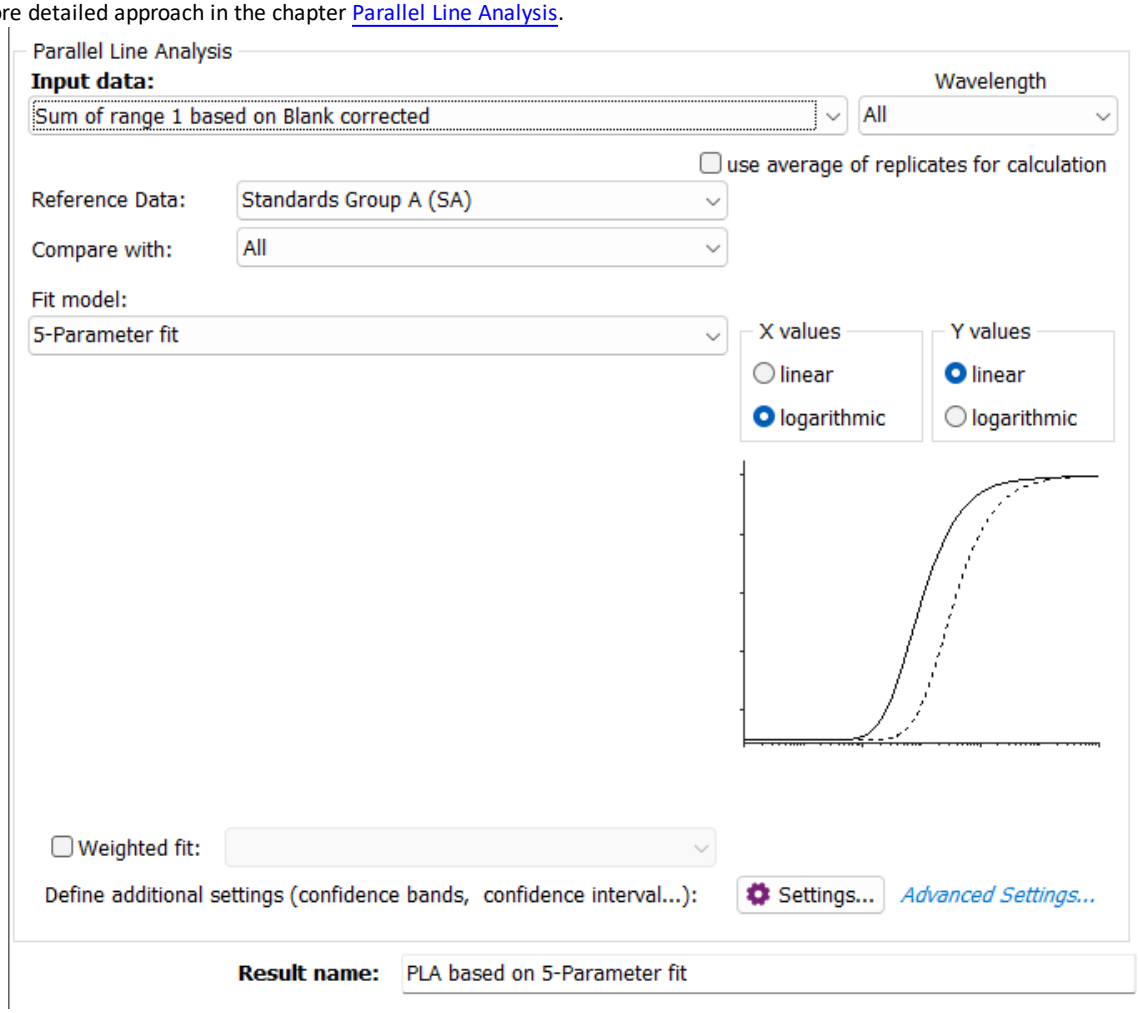
Now you must choose the input data – that is usually blank corrected raw data or average based on blank corrected data. For the fit model you can choose between the Michaelis-Menten-Fit, Lineweaver-Burk, Eadie-Hofstee, Scatchard and Hanes-Woolf.
It is very important to type in the unit of the substrate concentrations used. In this case it was µmol per minute. Select the correct range that was defined before in the Signal Curve window. In case that the enzyme was diluted the dilution factor should be announced.
The extinction coefficient can either be typed in (the slope of the standard curve) or can be calculated automatically.

Note: Please note that the automatic calculation of the extinction coefficient is only possible in long-time enzymatic reactions in which the substrate is completely converted into product indicated by no more change in signal.

Consider zero concentration reaction:
Subtract reaction of content with zero concentration: If you run a background reaction at a concentration of zero (but that is not identical to the blank), and you want to subtract this value from all other values, check this control.
Select the content with concentration zero: Select the content that contains the substance with zero concentration from the drop-down list (e.g Positive Control).
Group Handling: If the layout of the test run contains groups, you must decide how groups are handled:
Use selected content for all groups: Use the selected content of a group for the calculation even if the calculated well is in a different group.
Calculate for each group: Use the selected content of the group of the calculated well. If the group of the well does not have the selected content, no calculation is done for that well.
Ignore groups: Ignore the groups and calculate the result as if no groups where defined.

4.17 Parallel Line Analysis calculation

The parallel line calculation page is only enabled if the layout of the test run contains standards. If the test run is a kinetic measurement, a kinetic calculation must be performed first to enable the standard calculation page. If the test run is a measured spectrum, at least one discrete wavelength must be added to the test run, to enable the standard calculation page. For PLA calculation the layout must contain at least two layout groups with standards in both groups. You can find a more detailed approach in the chapter Parallel Line Analysis.



Input data: Select the input data for the parallel line calculation. This can be the result of any calculation generating end point data. If the layout contains replicates of the standards and the selected data is not based on a replicate calculation, you can define that the calculation is based on the average of the replicates if you check the control *use average of replicates for calculation*.
Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry *All* in the list, the calculation will be performed for each wavelength.
Reference Data: Select the group with the reference standards.
Compare with: Select the test group(s) to be compared.

Fit model: Select the fitting model for the parallel line analysis. Each fitting model has a set of parameters that describes the fit result and is also used for the concentration recalculation of the samples of the group. See the section Fit Results below for more information.
Available models:
Slope-ratio fit: Calculates a straight line through the standards with minimum r^2 value. The result describes the line with the parameters *m* (slope) and *b* (offset):

$$y = mx + b$$

Parallel line fit Calculates a straight line through the standards with minimum r^2 value with logarithmic x and logarithmic y values. The result describes the line with the parameters *m* (slope) and *b* (offset):

$$\log y = m \log x + b$$

4-Parameter fit: Calculates the dose response curve for the standards. Result parameters are *Bottom*, *Top*, *Slope*, *EC50/IC50* and *log(EC50)/log(IC50)* for the formula:

$$y = Bottom * \left(\frac{Top}{Bottom}\right)^{\frac{1}{1+\left(\frac{IP}{x}\right)^{Slope}}}$$

$$y = Bottom + \frac{Top - Bottom}{1 + \left(\frac{IP}{x}\right)^{Slope}}$$

The first formula is used if logarithmic Y values are used, the second is for linear Y values (default).
5-Parameter fit: Calculates the dose response curve with a symmetry parameter for the standards. Result parameters are *Bottom*, *Top*, *Slope*, *Inflection point*, *EC50/IC50*, *log(EC50)/log(IC50)* and *Symmetry* for the formula:

$$y = \mathrm{Bottom} * \left(\frac{\mathrm{Top}}{\mathrm{Bottom}}\right)^{\frac{1}{\left[1+\left(\frac{IP}{x}\right)^{Slope}\right]^{Sym}}}$$

$$y = \mathrm{Bottom} + \frac{\mathrm{Top} - \mathrm{Bottom}}{\left[1 + \left(\frac{IP}{x}\right)^{Slope}\right]^{Sym}}$$

The first formula is used if logarithmic Y values are used, the second is for linear Y values (default).
The variable *IP* (point of inflection) in the 4- and 5-Parameter fit formula is the *EC50/IC50* value. The *EC50/IC50* is the concentration value where the signal is in the middle between Top and Bottom.
Probit fitand ***Logit fit***: for more details on this two calculations read the section Logit and probit regression in the chapter Quantal Response.

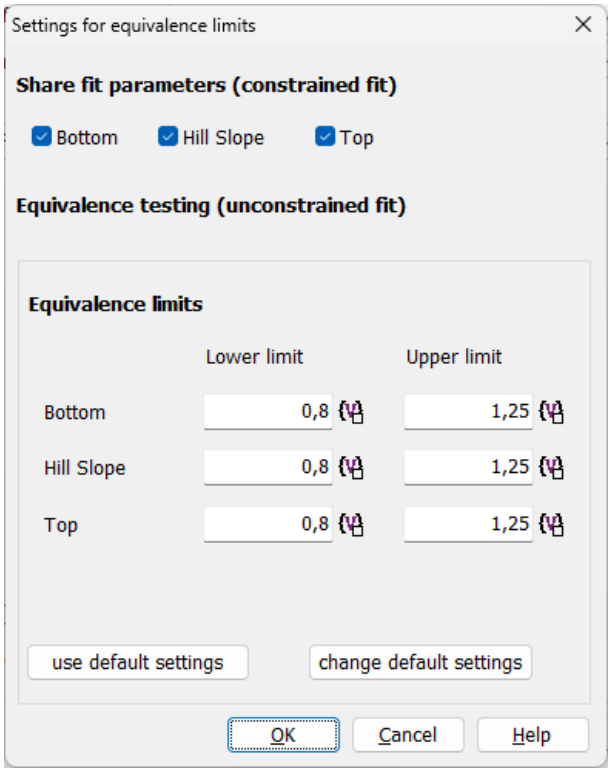
Linear or logarithmic X / Y Values: Use the check boxes available to define whether the fit result is shown using either linear or a logarithmic scaling for the according axes.
Depending on the X and Y Values, a logarithmic calculation may not be possible (i.e. for values <= 0). In this case the hint *Calculation of fit parameters not possible* appears where the fit result parameters are normally shown.

> **Note:** Using logarithmic X values will influence the fit model of the two linear fits. Using logarithmic Y values will influence the fit result of all fit methods.

Advanced Settings

If the 4-Parameter fit or the 5-Parameter fit is selected, you can define advanced fit parameters by clicking the *Advanced Settings...* hyper link.

You can define fit parameters to be shared for constrainded fit or you can define the equivalence limits for equivalence testing (unconstrained fit):



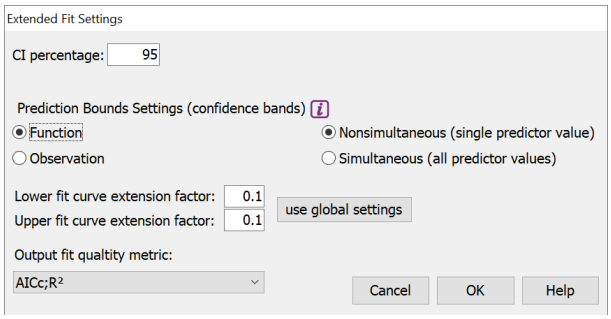
Read more about the fit settings in the chapter Parallel Line Analysis.

Weighted fit

Check this control if the weight of each standard should not be the same. You can give less weight to less precise standards and more weight to more precise standard. Select one of the offered weighting methods to define, how the weighting for each standard should be calculated.

Confidence Interval Settings and additional fit Settings

For all fit models, confidence intervals and confidence band are calculated automatically. Confidence intervals are calculated for each standard and for each fit result parameter. The confidence band can be displayed in the standard curve chart together with the error bars. The confidence values for the fit result parameters are displayed as two additional columns in the fit result table.
To define or change the confidence bands and interval settings, click on the settings button to open the setting dialog for confidence bands and intervals:



CI percentage: Define the percentage value for the confidence interval calculation. A lower value results in a wider interval band.
Prediction Bounds Settings: Select between the different settings:
Observation or Function Bounds: Provides a confidence interval for a new observation or for a new function (curve). In general, observation intervals are wider than function intervals, because of the additional uncertainty of predicting a new response value (the function plus random errors).
Non-simultaneous or Simultaneous Bounds: Provides a confidence interval using a single predictor value (non-simultaneous) or all predictor values (simultaneous). In general, simultaneous intervals are wider than non-simultaneous intervals, because of the additional uncertainty of bounding values for all predictors.
Use the ***Lower fit curve extension factor*** and the ***Upper fit curve extension factor*** to define how far the fit curve is drawn below the smallest standard and above the highest standard. Press ***use global settings*** to reset the values to the global extension factors (defined in the Calculation Settings Dialog).

Output fit quality metric:

In addition to the fit quality metrics R^2 and AICc (Akaike's information criterion corrected) that are always output, you can define further quality metrics to be added to the output in the fit results (see next chapter).
See the whole list of possible metrics:

Shortcut	Quality Metric
AIC	Akaike's information criterion
AICc	Akaike's information criterion, corrected for small sample sizes
R^2	R-squared
R^2 adj	Adjusted R-squared (R-squared, adjusted with the number of predictors)
BIC	Bayesian information criterion
MSE	Mean-square error
RMSE	Root mean-squared error

4.17.1 Fit Result

After performing a parallel line analysis, the fit result can then be inspected on the standard curve chart. If this is the first parallel line or standard calculation of the test run, the tab for the standard curve page will appear following a successful fit.
If a fit fails for a group of standards of the test run, the hint *Calculation of fit parameters not possible* appears on the detailed window for the fit result and on the fit result window. The reason for a failed fit could either be a wrong axis scaling (logarithmic instead of linear), or a smaller number of standards to calculate the fit (see Limitations for Recalculated Concentrations)
If the calculation of a concentration value fails for a well, you will see in the microplate or table view a text message that indicates the reason for the failure:
In the microplate and the table view, the result of the concentration calculation for the contents, based on the fit result will be displayed.
In drug screening the efficacy of a substance A is often compared against that of a reference substance B by using the ratio of equivalent doses:

$$\text{rel. potency} = \frac{\text{dose}_B}{\text{dose}_A}$$

This ratio of doses at which the biological system gives the same biological activity or measured response is referred to as relative potency. The different substances are often referred to as preparations and MARS also adopted this terminology. Dose-response curves are typically sigmoid, i.e. at some point, increasing the dose does not increase the response of the biological system anymore. That means dose-response curves fade out at higher doses reaching an upper asymptote. They also fade out at lower doses as the biological system does not react at all due to homeopathic (low) doses. In bioassays various doses are typically applied as a dilution series with logarithmic spacing. In between the lower and upper asymptote, there is an almost linear increase of the responses with the log¬(doses). This part is often used for fitting in order to estimate the relative potency of a test substance relative to a reference one. Visually the log(relative potency) is the horizontal shift of the dose-response curves

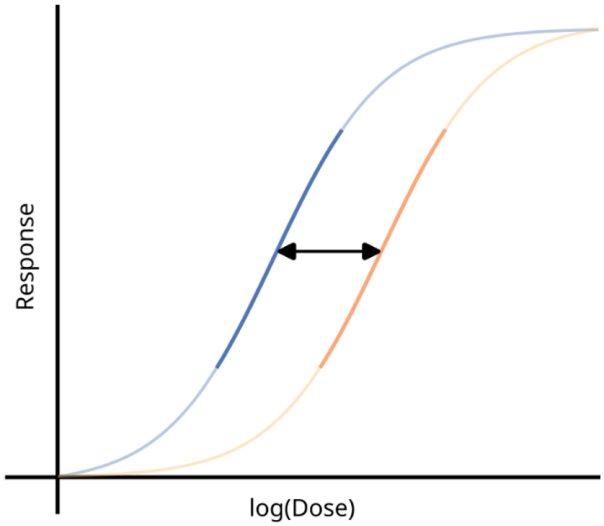


Figure 4.1: Two dose-response curves where one is the shifted version of the other. The horizontal shift (double arrow) corresponds to the log(relative potency).

The above equation then changes to:

$$\log(\text{rel. potency}) = \log(\text{dose}_B) - \log(\text{dose}_B)$$

taking into account that doses are typically log-spaced.
However, it is only reasonable to calculate the relative potency if the dose-response curve is indeed a shifted copy of the reference dose-response curve or if at least both curves are statistically similar enough to be regarded as equivalent. There are mainly two approaches that test for the similarity/equivalence of these curves, i.e. a so-called difference testing approach and an equivalence testing approach. We're going to touch on both approaches starting with the former one being preferably reported in the European Pharmacopeia.

Difference testing

Herein the two (or more) dose-response curves are fit with a constrained fit model, e.g. linking the slope of the parallel-line model between the preparations. Different fit models typically share different fit parameters. For example in the slope-ratio model (for details, see below), the preparations share a common intercept instead of a common slope. In any case, a (weighted) ANOVA is used to assess the validity of the assay, i.e. proofing that the regression is significant and that the non-linearity and non-parallelism are not significant. Internally, the (weighted) ANOVA also takes into account the unconstrained model in which none of the fit parameters is shared. E.g. it checks if not sharing the slope lead to a significant better fit than with sharing the slope between the preparations. Therefore it performs an F- or Chi²-squared test based on the corresponding sum-of-squared errors (SSE) of the models. The corresponding p-values are also reported in the ANOVA table in the PLA fit results. These two approaches give the exact same p-value for non-parallelism in case of the parallel-line model but not in case of the 4P and 5P model. The European Pharmacopeia describes a linearization by logit-transformation of the sigmoidal data sets after the fit parameters had been estimated by conventional non-linear regression. The ANOVA is performed on the transformed data, being the reason why the ANOVA table for the 4P/5P model has a non-linearity term, too. Data from quantal bioassays are treated in a similar way, i.e. by performing a linearizing transformation on the data.

Looking at the ANOVA (Analysis of Variance) table

In any case it is useful to pay attention to the conclusions that can be drawn from the ANOVA table.
The ANOVA table is typically read from bottom to top.

Source	SS	DF	MS	ChiSquared	p-Value
Preparation	0.0007569	1	0.0007569	0.5296564	0.4667514
Regression	9.4305450	1	9.4305450	6599.5171069	0
Non-Parallelism	0.0000656	1	0.0000656	0.0458740	0.8304050
Non-Linearity	0.0127084	16	0.0007943	8.8933703	0.9177396
Treatments	9.4440758	19	0.4970566	6608.9860077	0
Residual	0.0285795	20	0.0014290	20.0000000	0.4579297
Total	9.4726553	39	0.2428886	6628.9860077	0

Figure 4.2: Exemplary weighted ANOVA table.

The Residual error denotes the variance that cannot be explained by the full model (i.e. the model including all regressors) and can be helpful in identifying outlying results as its value tends to be unreasonably big in these cases. The residual error either corresponds to the pure error (within-group variation) in case of multiple replicates per dose or it corresponds to the lack-of-fit error in case of no replicates per dose. Correspondingly, the F-/Chi²-values relate to the pure error and lack-of-fit error, respectively. In the "no replicate" case, the residual error and the non-linearity rows in the ANOVA table are the same.

The Treatments effect corresponds to the overall dose-response effect, i.e. if there is a change in response at all when changing the dose levels of the preparations. This is often already seen when looking at the corresponding dose-response chart. In classical textbooks on statistics the ANOVA term corresponding to Treatments is typically called among-groups effect. Together with the Residual error term it forms the Total error sum-of-squares that is also listed in the ANOVA table at the very bottom. The Treatments effect can is further decomposed into the following effects:

The Non-Linearity term in the ANOVA table tells whether the linear regression model (at least of the transformed data) is appropriate and that the data would not have been better described e.g. by a quadratic model. For the linear model to be valid, the non-linearity p-value should be non-significant (e.g. above 0.05). In general, non-linearity can only be determined if each preparation has at least three doses[1]

The Non-Parallelism term in the ANOVA table can be used to "proof" that the assumption of parallel regression lines is valid. In this case the non-parallelism p-value is non-significant. A high p-value (e.g. above 0.05) indicates that the unconstrained model (i.e. in which no parameters are shared) does not significantly fit the data better than the constrained model.

The Regression term in the ANOVA table simply contains information about if the common slope of the regression lines is significant (i.e. different from zero). If the p-value is significant (e.g. below 0.05) the slope is likely to be different from zero.

The Preparations term in the ANOVA table contains information on the difference in intercepts of the regression lines. If the corresponding p-value is reasonably big (e.g. > 0.05), there is likely only a minor difference in potencies of the preparations.

It is worth mentioning that if there are no replicates in the microplate layout or if the user chose to average the replicates ahead of the PLA, then the (weighted) ANOVA will report the actual residual error of the unconstrained model instead of the pure error derived from the replicates.

Also note that the ANOVA assumes a completely randomized design (i.e. block-related effects, like microplate edge effects, are assumed to be negligible).

> **Note:** With MARS 5.00 the PLA feature is supposed to be fully compliant with the calculations described in the European Pharmacopeia. In contrast to former MARS versions, which calculated the non-parallelism validity criterion according to Gottschalk and Dunn, with MARS 5.00 the non-parallelism criterium calculation has changed and additional validity criteria can be considered such as non-linearity and regression. These are all summarized in a (weighted) ANOVA table (see also description above). However, if it would still be required to calculate the non-parallelism criterium the "old" way, there are ways to do that also in MARS 5.00 (ask our support@bmglabtech.com for details).

Equivalence testing

The other approach to test for equivalence of the bioassays curves is an equivalence test. An equivalence test is the preferred approach by the US Pharmacopeia.

It can be chosen to assess parallelism in which all curves are fitted independently with the unconstrained model. Subsequently, the fit parameters (e.g. the slope) between the preparations are compared based on confidence intervals (CIs) for the ratios of the corresponding fit parameters in relation to the reference curve. What ratios are formed depends on the fit model chosen. For the Parallel-line, the Logit and the Probit model the ratio of the slopes of the test curve(s) to the reference curve are calculated. For the 4-Parameter model the ratios for the Hill Slope, the Bottom parameters and the Top values are calculated. For the 5-Parameter model additionally the asymmetry parameter is considered[1]. For the Slope-ratio model the ratios of intercepts are calculated. If the confidence intervals of the ratios are fully contained within user-defined equivalence limits, the fit parameters can be considered equivalent, and the corresponding curves are (sufficiently) parallel to the reference curve. In this case it is valid to "accept" the relative potencies derived from the fit parameters of the corresponding constrained model.

[1]With only two different doses the maximum possible model is a linear one. To fit a quadratic model would already require at least three different doses. Thus, with only two doses it is impossible to answer the questions, if a non-linear model would have fitted the data better.

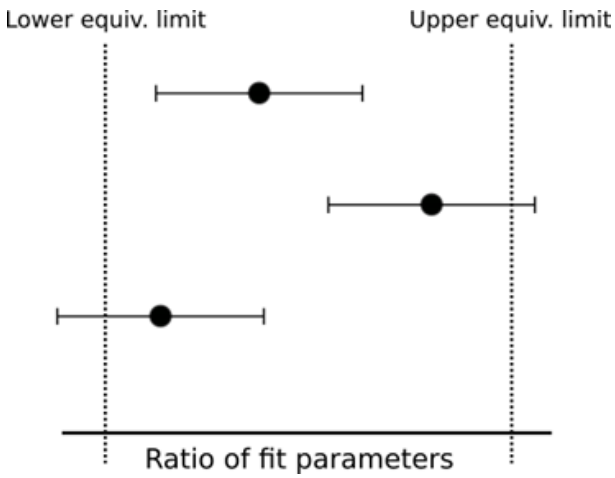


Figure 4.3: Principle of an equivalence test. The ratios of the fit parameters and the corresponding confidence intervals are compared against pre-defined equivalence limits. Only if all the confidence intervals are contained within the equivalence intervals (dashed line) the reference and the test curve can be considered parallel.

Assess parallelism using PLA in MARS

To perform PLA in MARS, open the Calculations Window in the Home tab and click on Parallel Line Analysis near the bottom on the left-hand side (see below).

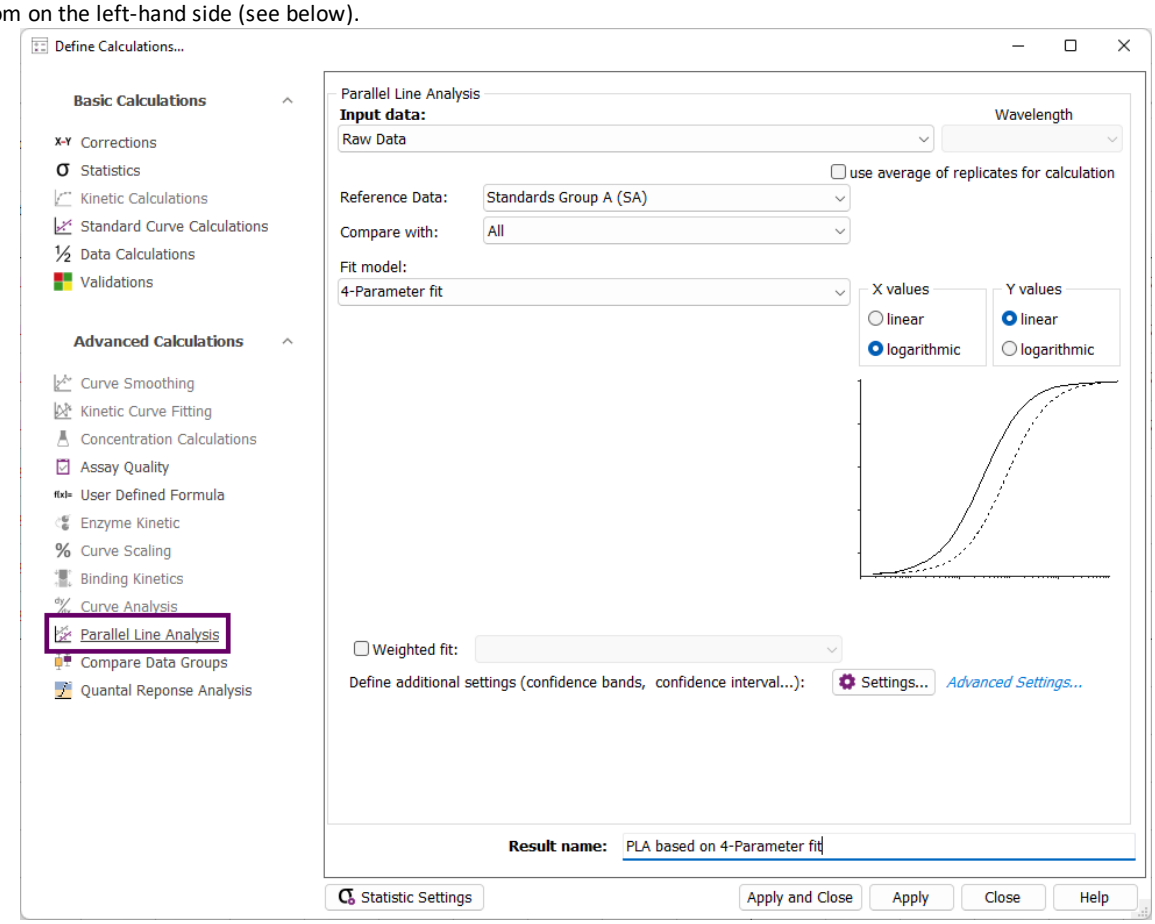
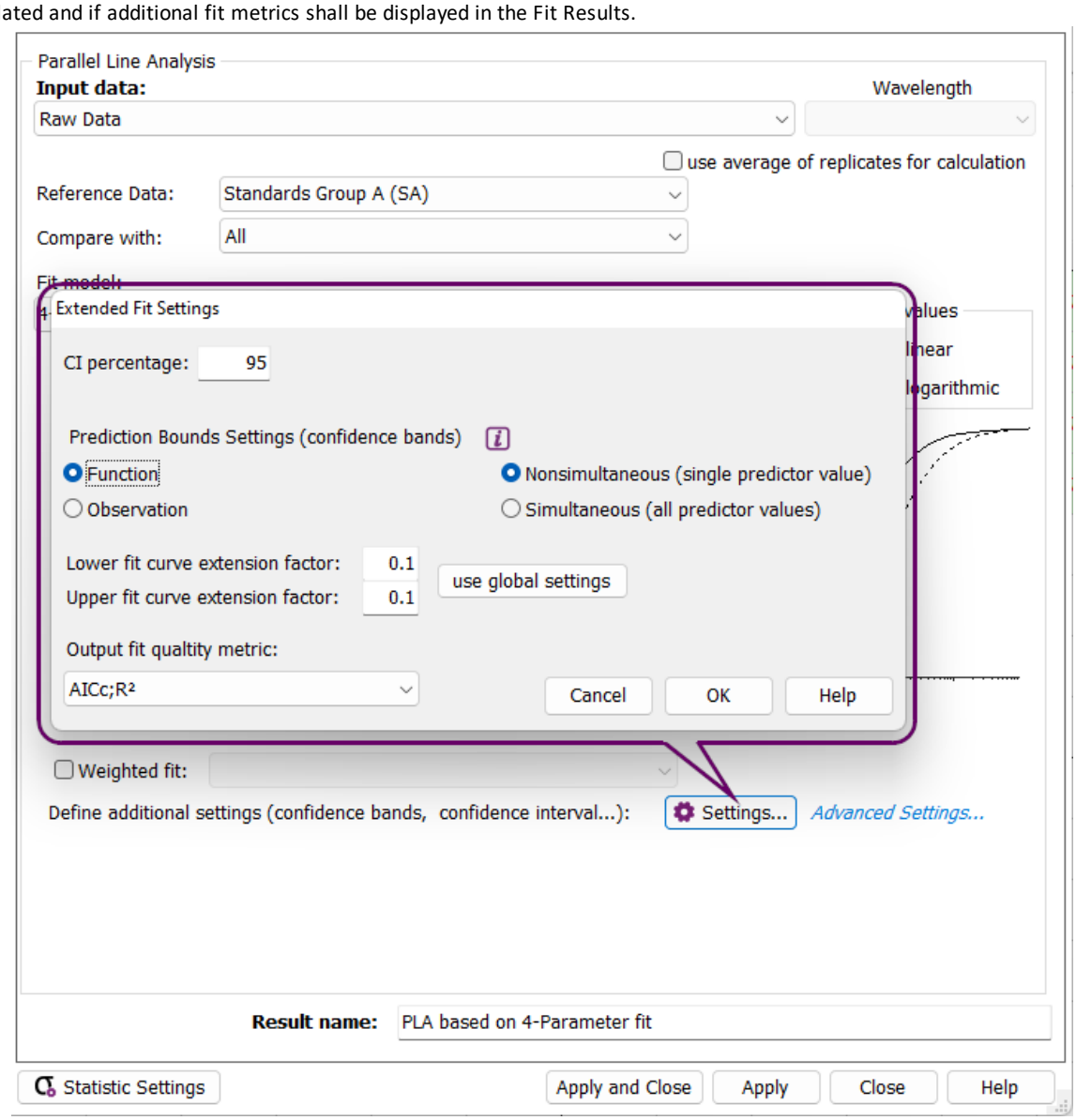


Figure 4.4: Parallel line analysis in MARS is available via the Calculations button.

Choose the Input data for the analysis and set the corresponding Reference Data and the test group(s) with which the Reference Data shall be compared.
Note: The PLA feature in MARS is only accessible if standards with at least two groups are present in the microplate layout.

> **Note:** The PLA feature in MARS is only accessible if standards with at least two groups are present in the microplate layout.

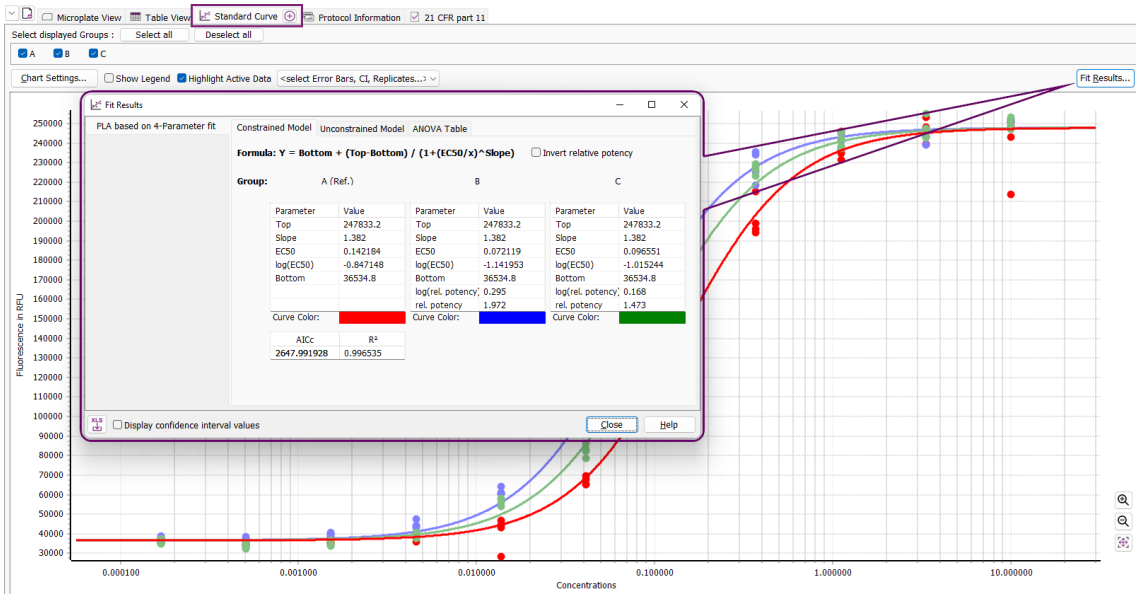
PLA-assays/Bioassays typically have multiple independent replicates. In this case averaging them first using the use average of replicates for calculation is inappropriate and is thus not checked by default. One might consider this option when technical replicates are used in the microplate layout instead. In the Fit model dropdown select an appropriate fit model, either for quantitative responses (i.e. Parallel-line, Slope-ratio, 4-Parameter or 5-Parameter fit) or one of the two quantal models (i.e. Probit or Logit fit model). The latter two models are only appropriate for binary data, i.e. data that is a results of a validation or time-to-threshold calculation with binary output option selected. Select the Weighted fit option where appropriate. Common weighting options for dose-response curves include $1/Y^2$ and $1/Yhat^2$. Please note that when weighting is applied, the ANOVA table reports Chi^2-values instead of F-values in its 5th column. The confidence level is set to 95 % by default but can be changed under Settings… Herein one can also change the way confidence bands/intervals are calculated and if additional fit metrics shall be displayed in the Fit Results.



The CI percentage not only denotes the confidence level of the two-sided confidence intervals (1-α/2) of the fit parameters (e.g. with α=0.05) but also denotes the CI percentage of the confidence level of the (1-2α) two-sided confidence intervals for the ratios of the fit parameters. Thus, setting CI percentage to 95 % corresponds to a 5 % equivalence test applying 90 % confidence intervals for the ratios.
For the 4-Parameter and 5-Parameter there is an Advanced Settings option to choose the parameters that shall be shared for the constrained model or that shall be compared for the equivalence test on the unconstrained model.

Note: With MARS 5.00 the (log) relative potency is always estimated, no matter if the validity criteria are met or not. This is because in the end it is up to the user to accept or reject the assay. The statistics can only guide the user.

The Fit Results which are accessible from the standard curve page contain three different tables: one contains the results of the constrained model. Another contains the fit results of the unconstrained model including the ratios of selected fit parameters and a third one contains the (weighted) ANOVA results.



All Fit Results can be exported to Excel by clicking the corresponding XLS-button at the bottom of the Fit Results window. Please note that (log) relative potencies are always reported in the table for the constrained model as they are always calculated from the constrained model's fit parameters.

4.17.2 Literature

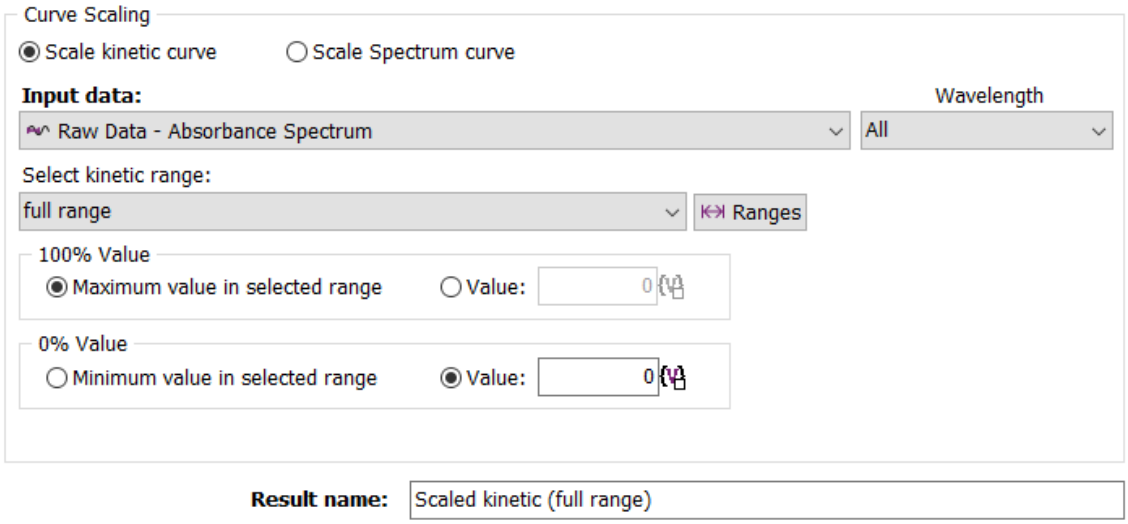
1. Council of Europe, "Statistical analysis of results of biological assays and tests," European Pharmacopoeia, pp. 551-579, 2011.
2. P. G. Gottschalk and J. R. Dunn, "Measuring Parallelism, Linearity, and Relative Potency in Bioassay and Immunoassay Data," Journal of Biopharmaceutical Statistics, pp. 437-463, 2005.
3. The United States Pharmacopeial Convention, "<1034> Analysis of biological assays," First Supplement to USP 35-NF 30, pp. 5186-5200, 2012c.
4. The United States Pharmacopeial Convention, "<1032> Analysis of Biological Assays," First Supplement to USP 35–NF 30, pp. 5160-5174, 2012a.
5. "Using Software to Estimate Relative Potency," United States Department of Agriculture Center for Veterinary Biologics Standard Operating Policy/Procedure, Ames, IA, 2015.

Interact with the PLA-feature by following the instructions in the following demonstration:

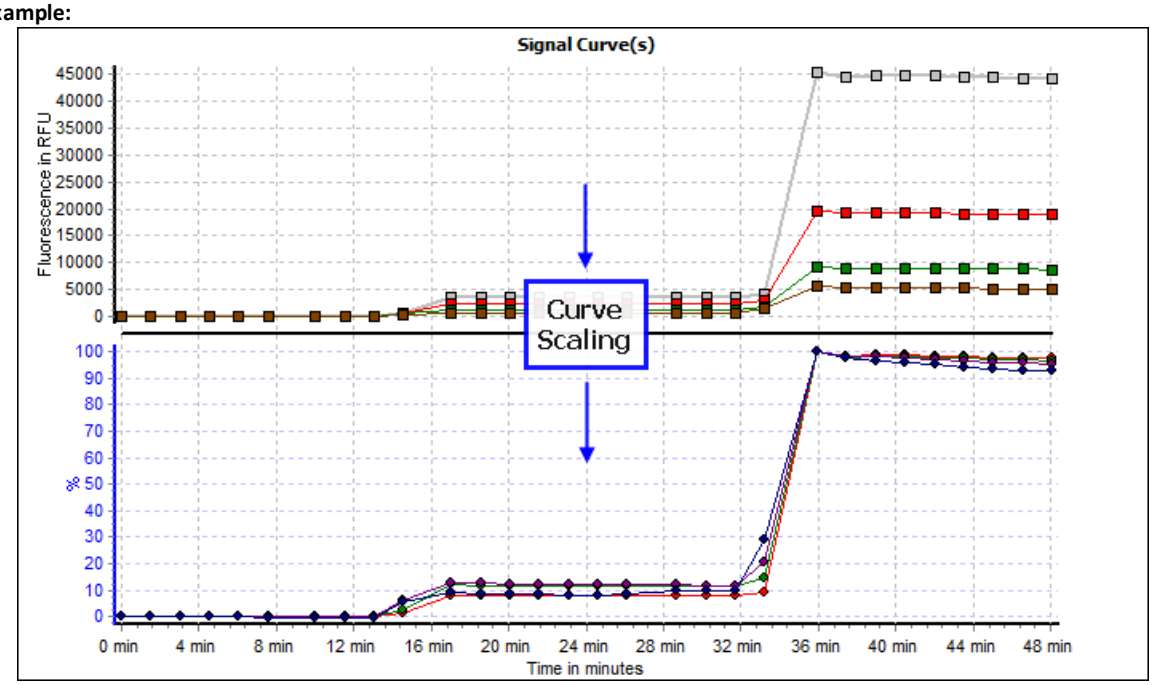
[1]For the 4-/5-Parameter model the ratios of bottom values might not be considered for the assay validity evaluation as their uncertainty (variance) is often rather big and this violates the estimation of the ratios' confidence intervals (calculated according to Fieller's theorem). See also a publication on this from the USDA.

4.18 Curve Scaling

The Curve Scaling % calculation converts a signal curve or a spectrum curve to a percentage presentation between a defined maximum (100%) value of the curve in the selected range and a defined minimum (0%) value. This calculation can be used to normalize the curves:

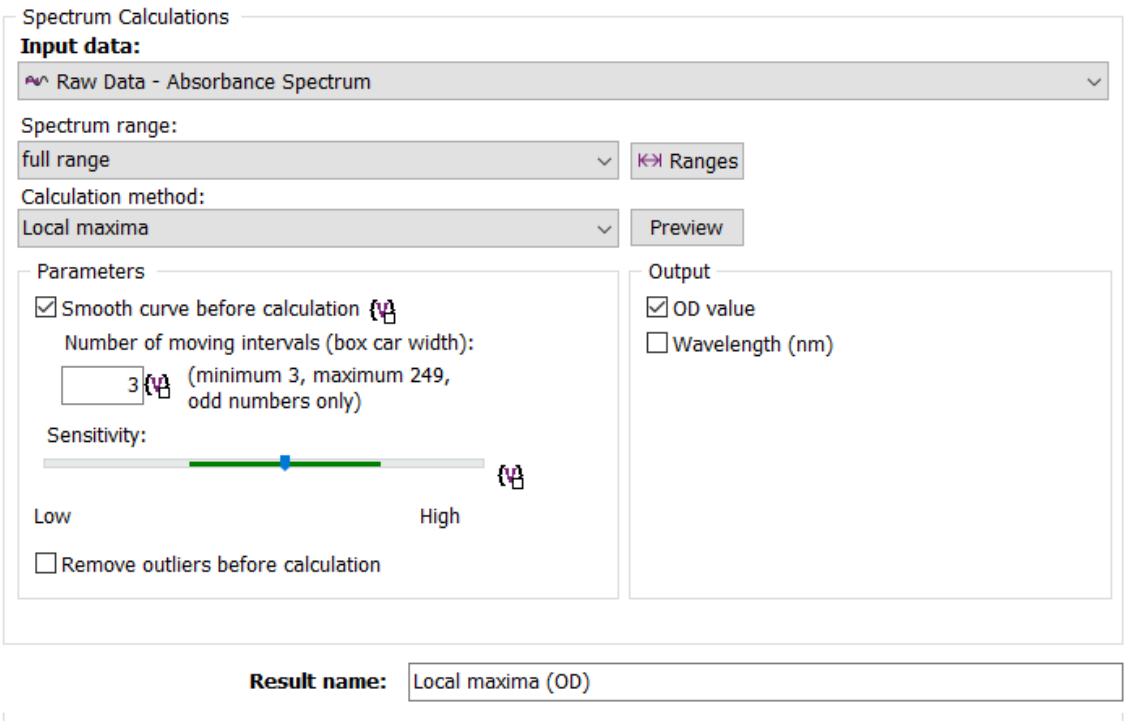


Input data: Select the input data for the calculation. This can be the raw data or the result of any calculation that obtains kinetic data or spectra data.
If the input data has both, kinetic and spectra data, you can select if the scaling should be calculated based on the kinetic or based on the spectra curve with the radio control above the input data drop-down list: *Scale kinetic curve / Scale Spectrum curve*
Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry *All* in the list, the calculation will be performed for each wavelength.
Select range: The input data for a curve scaling calculation are always defined by the first time point and the last time point of a kinetic range or by the first wavelength and the last wavelength of a spectrum range. It is possible to have one or more ranges defined. The full range is always available and covers the total measurement. See the chapter 4.1: *Ranges*, how to define a range. All defined ranges are listed in the drop-down list with their start and stop time pointor wavelength (depending on the selected input data). Select a range for the calculation from this list. Only ranges with at least three values are shown, because the minimum number of values for the curve analysis is three. To view, create or change a range, press the Ranges button to open the range window. You can also define an individual range or a content-based range, where the start and/or stop time point is not fix. Select the *Individual Range* or *Content-based ranges* in the drop-down list to use and define this type of range. Read more about individual and content based ranges in the chapter 4.1: *Ranges.*
100% Value: Select if the 100% value comes from the maximum of the curve in the selected range or if it is a fix value. Fix values can be entered in the *Value* entry field. The fix value can also come from a defined variable (see Variables).
0% Value: Select if the 0% value comes from the minimum of the curve in the selected range or if it is a fix value. Fix values can be entered in the *Value* entry field. The fix value can also come from a defined variable (see Variables).
Example:

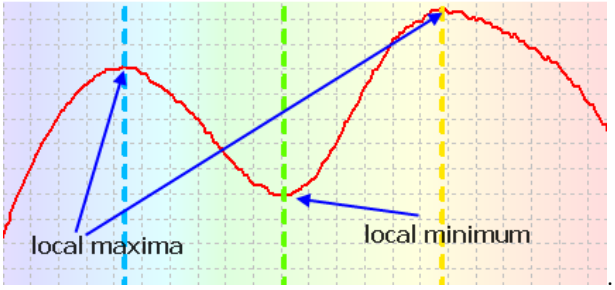


4.19 Spectrum Calculations

The spectrum calculation page allows you to define a calculation based on a range over a measured spectrum. You can only select it, if the test run is a spectrum scan test.

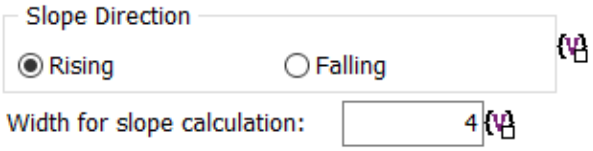


Input data: Select the input data for the calculation. This can be any process with spectrum data.
Spectrum range: The input data for a spectrum calculation are always defined by the first wavelength and the last wavelength of a range. It is possible to have one or more ranges defined over your spectrum. The full range is always available and covers the total measurement. See in the chapter 4.1: *Ranges*, how to define a range. All defined ranges are listed in the drop-down list with their start and stop wavelength. Select a range for the calculation from this list. To view, create or change a range, press the Ranges button to open the range window. You can also define an individual range or a content-based range, where the start and/or stop wavelength is not fix. Select the *Individual Range* or *Content-based ranges* in the drop-down list to use and define this type of range. Read more about individual and content based ranges in the chapter 4.1: *Ranges*.
Calculation method: Select the calculation method for your spectrum calculation.
Available methods:
Sum: Calculates the sum of all measurement values for all measured wavelengths within the selected range for each well.
Maximum: Finds the measured maximum value within the selected range for each well.
Minimum: Finds the measured minimum value within the selected range for each well.
Local maxima: Calculates all local maxima within the selected range for each well. See an example of local maxima or minima in the picture below.
For each maximum found, a node in the workflow tree will be created to see the associated value(s) of the maximum for each well (and iteration if it is a kinetic test run). See the section Extended parameters below for more details. In the same section you can find an explanation of the additional parameters you can use for this calculation method.
Local minima: Calculates all local minima within the selected range for each well. See an example of local maxima or minima in the picture below.
For each minimum found, a node in the workflow tree will be created to see the associated value(s) of the minimum for each well (and iteration if it is a kinetic test run). See the section Extended parameters below for more details. In the same section you can find an explanation of the additional parameters you can use for this calculation method.



Inflection points: Calculates all inflection points within the selected range for each well.
For each inflection point found, a node in the workflow tree will be created to see the associated value(s) of the inflection point for each well (and iteration if it is a kinetic test run). See the section Extended Parameters below for more details. In the same section you can find an explanation of the additional parameters you can use for this calculation method.
Average: Calculates the average of all measured values within the selected range for each well.

Slope: Calculates the linear regression curve for the measured points in the selected range and gives the corresponding slope value for each well.
Maximum of slope: Finds the maximum slope value of all measured points within the selected range for each well. The slope is calculated using the entered width: A linear regression fit with the number of measured values entered in the width entry in the *Parameters* group is performed, starting with the first wavelength of the range up to the last. The maximum value of all calculated slopes is the result. You can decide with the *Slope Direction* control in the *Parameters* group whether the rising or the falling maximum value should be calculated.



Wavelength at threshold: Calculates the wavelength taken from the first wavelength in the selected range for the curve to reach a given threshold for each well. Enter the threshold value in the entry field *Threshold*, this option appears under the smooth controls when this method is selected. If a spectral curve does not reach the entered threshold, n.a. will be printed as result. You can define, what you want to see instead of n.a. in the result table with the *Result, if threshold is not reached* drop-down menu.

Parameters: Every calculation method has at least to settings available in the parameters group. Some of the calculations can have further settings. These settings are explained together with the calculation above.
Smooth curve before calculation: You can smooth the curve before the calculation to reduce the effect of the noise in the measurement. Check this control and define the ***number of moving intervals*** to smooth the curve.
Remove outliers before calculation: Check this control to remove outliers in the curve before the calculation will be performed.

Note: More details on curve smoothing and outliers can be found in the chapter Curve Smoothing.

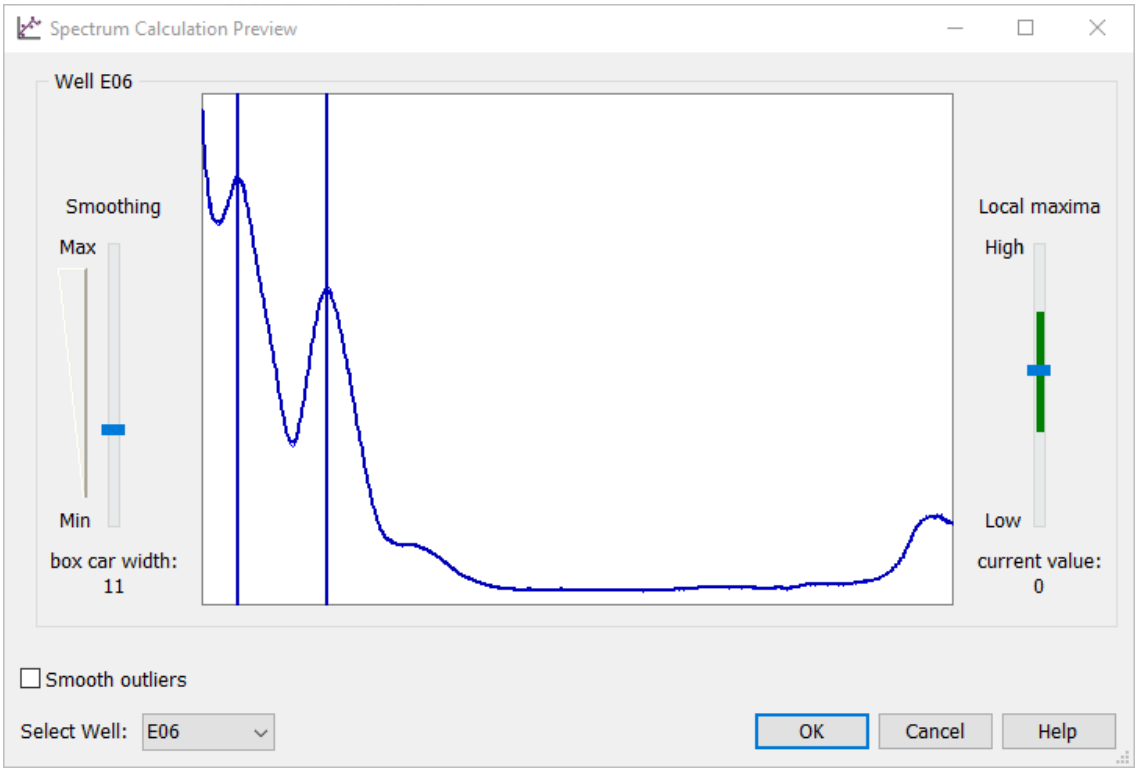
Output: Select the desired output values. The available output depends on the selected calculation method. Each selected output type will be displayed in a separate node or group of nodes in the navigation tree.
Available output types:
OD value: This type is available for all calculation methods except *Wavelength at threshold*. It shows the associated OD Value for the given calculation result.
Wavelength (nm): Not available for the calculation methods *Sum*, *Average* and *Slope*. It shows the associated Wavelength for the given calculation result.
Slope direction: Only available for the calculation method *Inflection points*. The result can have the values -1 for a falling direction, 0 for no slope and 1 for a rising direction.
Slope: Only available for the calculation method *Maximum of slope*. It shows the calculated maximum slope value.

4.19.1 Extended parameters

If one of the calculation methods ***Local maxima***, ***Local minima*** or ***Inflection points*** is selected, in the *Parameters* group on the left side of the window appears a sensitivity slider control:
Use the ***Sensitivity*** slider to define the sensitivity of the calculation. Depending on the spectrum used as input data, a high sensitivity can lead to many (wrong) hits because of the noise of the curve. On the other hand, a very low sensitivity could lead to a bad result as well because of missing real minima, maxima or inflection points.
Consider that the calculation is done for each valid well of the selected input data. This means, that each well has its own minima, maxima and inflection points and the number of calculated results can differ between the wells. The number of created result nodes in the navigation tree will be the highest number of calculated results for all wells in all loop iterations (if loops with more than one iterations was measured).
Therefore, if you have measured empty or blank wells, they should be excluded from these calculations. Use blank corrected data as input data and toggle out wells with high noise and less information before executing this calculation.

4.19.2 Preview the smoothed curves

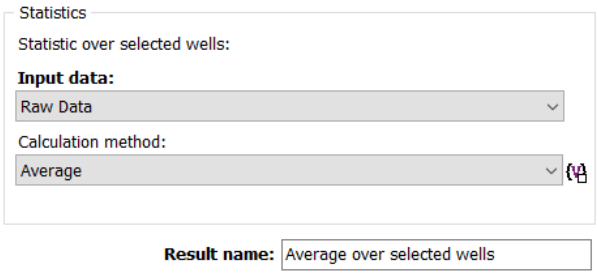
If you are not sure, how the position of the slider and the entered interval number are affecting the result, you can press the ***Preview*** button to open a preview of the result of the calculation:



Use the sliders on the left and right side to see how the result changes. The left slider is only available, if the ***Smooth curve before calculation*** control was checked on the calculation window.
Change the displayed well with the ***Select Well*** drop-down list to see the result for different wells and - if a kinetic with more than two loop iterations was measured - select the displayed time point with the ***Select Time*** drop-down list. If the positions of the sliders are optimized and the result is good, press ***OK*** to assign the settings to the calculation window.

4.20 Statistic over Wells

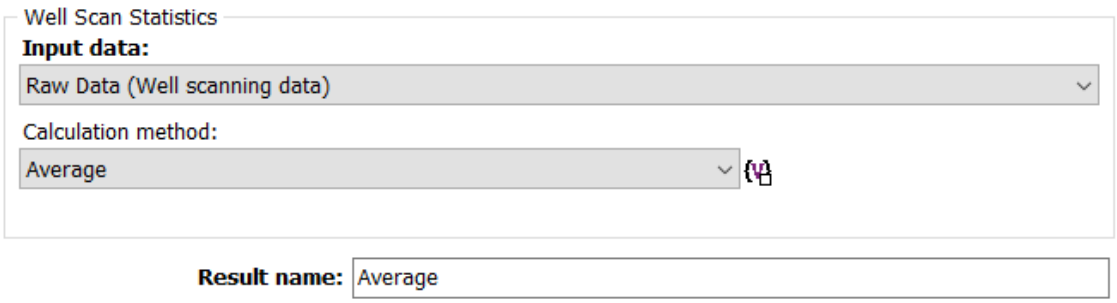
You can perform a statistic over wells, after selecting two or more wells in the *Microplate View*. Select the menu item *Statistic over selected wells...* in the *Microplate Views* pop-up menu or in the corresponding menu item under the *calculations* menu to open this window:



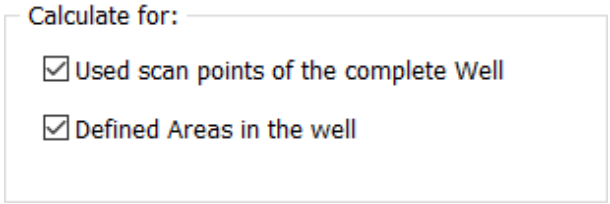
Input data: Select the input data for the well statistic. This can be the result of any calculation which outputs the data as numbers.
Calculation method: Select the calculation method for your well statistic.
Available methods: ***Average, Standard deviation, Standard deviation n, Standard error, Standard error n, %CV, %CV n, Minimum, Maximum, Median, Sum, No. of Values***.
The available methods are the same as for the replicate and group statistics. For details on the methods, see the chapter 4.5: *Statistics*.
Press the *OK* - button on the window to perform the calculation.

4.21 Well Scan Statistics

The well scan statistics page is used to calculate statistics over scan-points inside a well of a well scan test run.



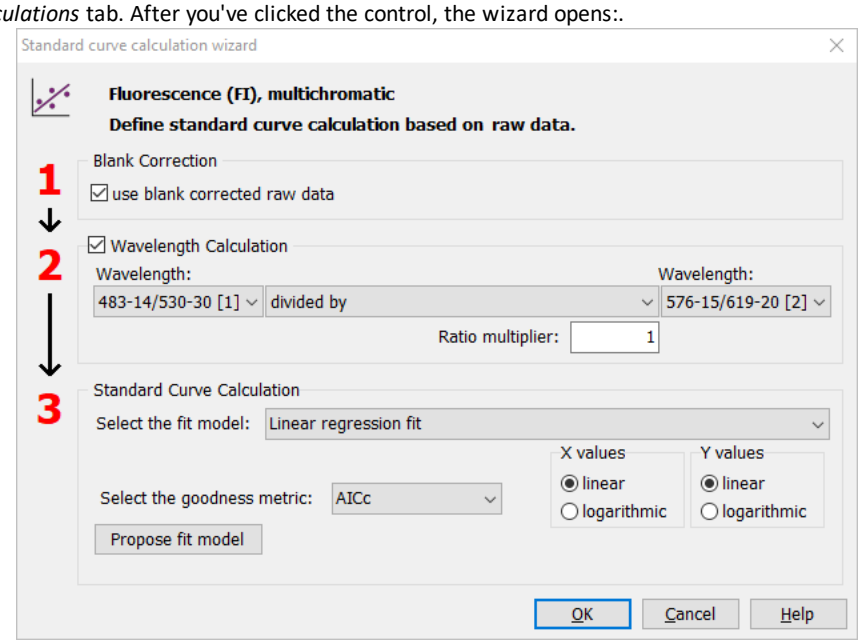
Input data: Select the input data for the well scan statistic. This can be the result of any calculation that obtains well scan data (usually only raw data).
Calculation method: Select the calculation method for your well statistic.
Available methods: ***Average, Standard deviation, Standard deviation n, Standard error, Standard error n, %CV, %CV n, Minimum, Maximum, Median, Sum, No. of scan points***.
The available methods are the same as for the replicate and group statistics. For details on the methods, see the chapter *4.5: Statistics*.
Press the *OK* - button on the window to perform the calculation.
If areas where defined inside at least one of the wells (see chapter *3.23: Well Scanning Data*, section: *Well Scan Area detection*) an additional calculation method is available: ***No. of areas***. This returns the number of areas defined in the well.
Furthermore, you can decide if the calculation should be performed for the scan points of the entire well or only for the scan points of the defined areas inside a well:



Note: The well scan statistics average is automatically calculated and displayed, if a well scan test run will be opened the first time.

4.22 Standard Calculation Wizard

In most cases it is not necessary to perform each calculation step by step.
Use the standard calculation wizard instead.
The wizard recommends the suitable calculation steps to apply to your standard calculation process.
Click *Wizard* in the *Data Reduction* group on the *Home* tab of the ribbon. You can find the same control also in the *Common* group on the *Calculations* tab. After you've clicked the control, the wizard opens:.



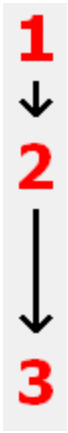
4.22.1 When Can You Use the Wizard?

To use the wizard, the test run must fulfill the following conditions:

- The layout must contain one or more standards
- End point test runs may have a maximum of two measured wavelength
- Kinetic test runs may have a maximum of one measured wavelength (including single wavelength FP- measurements) and not more than one injection.

4.22.2 How the Wizard Works

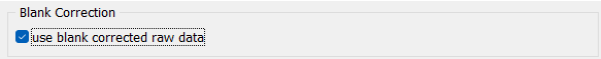
The wizard recommends calculations depending on the layout and measurement of the test run.
Each available calculation with its parameters is displayed in a separate box on the window.
The user can decide whether he wants to perform the recommended calculations or not by checking or un-checking the check box for each calculation.



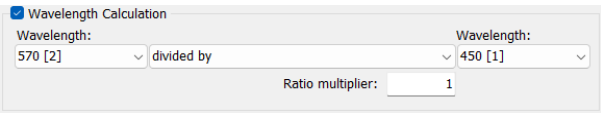
The sequence of the calculation is shown from top to bottom and is indicated by the numbers on the left side of the wizard window. This means that the result of a calculation can be used as the input data for the next performed calculation in the sequence.

The parameters of the single calculations are similar to performing the calculation separately by using the calculation window.

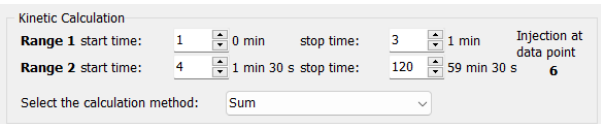
Possible Calculations in the Wizard
Blank Correction



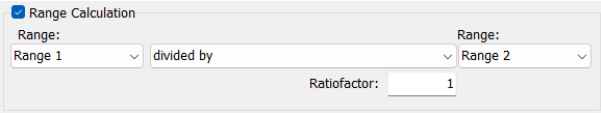
This option will appear if blanks are defined in the layout. When the blank correction is recommended by the wizard, it is checked and always the first calculation step. Uncheck the check box if you do not want to perform a blank correction before creating the standard calculation.
Wavelength Calculation



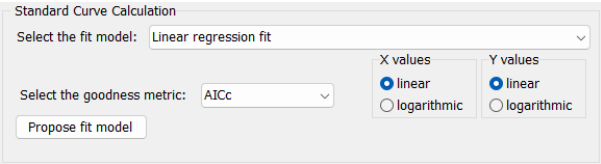
This option will appear only for end point test runs with two measured wavelengths. If you want to plot a standard curve based on a calculation performed on the wavelengths such as the ratio between them, leave the *Wavelength Calculation* check box checked and select the arithmetic operation you wish to perform from the drop-down list between the two wavelengths.
The available calculations are: *minus, divide by*, *plus* or *multiplied by*.
If there is a need to swap the wavelengths, select the wavelength in one of the wavelength drop-down lists. The entry for the other wavelength is updated automatically.
To multiply the result of a division by a constant value, enter the value into the entry field *Ratio multiplier*.
Kinetic Calculation



This group appears only for kinetic test runs. If the test run has an injection, you see two ranges in the group and the injection data point number. Otherwise you see only one range.
If only one range is visible, the borders of the range are from the first to the last iteration number of the measurement.
If there are two ranges, the first range includes the iterations before the injection, the second range the iterations after the injection (including the injection).
To change the borders of the ranges use the spin buttons beside the entry fields for the start and stop time point or enter numbers into the entry field.
Select the calculation method for the kinetic calculation(s). Read more about kinetic calculation methods in the chapter 4.8: *Kinetic Calculations*.
If there are two ranges, two kinetic calculations will be created, one for each range using the same calculation method.
A kinetic calculation cannot be unchecked, as it is necessary to perform a kinetic calculation before you can perform a standard calculation when you have kinetic test runs.
Range Calculation



This option will only appear if the kinetic test run has more than one range selected or has one injection. In this case you have two ranges with two kinetic calculations for the two ranges.
It is possible to perform an arithmetic calculation on the two kinetic calculations (called range calculation in the wizard as the ranges are defining the kinetic calculations).
Select the calculation method using the drop-down list between the two ranges.
The available calculations are: *minus, divide by*, *plus* or *multiplied by*.
It is possible to swap the ranges if needed, select a range in one of the range drop-down lists. The entry for the other range will be updated automatically.
If you want to multiply the result of a division by a constant value, enter the value into the entry field *Ratio multiplier*.
If you do not want to perform a range calculation, uncheck the check box *Range Calculation*. In this case the standard calculation will be performed for both kinetic calculations.
Standard Calculation



The last calculation step is always the standard calculation.
Select the curve fitting model you want to perform using the model drop-down list.
If the layout contains dilution factors for at least one sample, the user can decide whether they want to use the dilution factor for the recalculation of the concentration values or not.
Using the two buttons shown for the *X values* and *Y values* the user can decide whether to use a linear or logarithmic scale for each axis.
Read more about standard curve calculation in the chapter 4.10: *Standard Calculations*.

Let MARS automatically find a proper fit model
MARS' wizard offers the ability to propose a fit model based on the data you selected. Click Propose fit model and MARS will fit various models and return the fit model which best suits your data. Therefore, one can choose different fit quality metrics such as the Akaike information criterion (AIC), the Bayesian information criterion (BIC), the R^2, the adjusted R^2, the mean-squared error or the root mean squared error. It is worth mentioning that (due to technical reasons) the fit models are not simultaneously explored for linear and logarithmic y-axis scaling combinations. One must actively choose the y-axis scaling (linear or log). Once this is done, the optimal x-axis scaling (linear and logarithmic) will be automatically determined together with the most suitable model. If the test run layout contains multiple groups, e.g. A,B,C, in practice it could occur that the groups A and B share the same fit model while group C would actually fit better to another model (e.g. a model that give a lower AICc compared to the model of group A and B). In this case MARS advocates the former model, i.e. that best fits group A and B. However, if all groups would lead to a different best fit model, MARS selects the one whose AICc is minimal among the different groups.
Please note that the AICc might be a good metric to start with to find a proper fit model, as it balances between goodness of fit and complexity of the fit model (i.e. number of fit model parameters). Using the R^2, MARS will always return the fit model whose resulting fit curve is closest to the data points. This tends to be the model with most fit model parameters.
Please note that a proposed model might not be in accordance with what was expected due to various reasons:
Probably there were too less data points n for more complex models (i.e. models with more fit parameters).
Probably the expected model is not correct.
Probably the data was poor (e.g. noisy).
etc.
Concerning the first point it is important to know that the AICc can only be calculated for models whose number of fit parameters is less than n-2 (n: number of data points). For all other metrics, there need to be more data points than fit model parameters.
If there are good reasons to stick to a certain fit model, although the procedure proposes a different one, it is worth thinking about where the discrepancy comes from and probably stick to the expected model.
For more details on fit model comparison and the AIC, see chapter Fit model comparison and fit quality metrics.
The resulting fit model will automatically be selected together with the linear or logarithmic scale settings for X- and Y-Values.

After defining all the parameters of the calculations, press the *OK* button to perform the calculations.
When the calculation of the standard curve has been completed, the page with the standard curve will be displayed in the working area.

If more flexibility is needed to define your calculation than the wizard provides, you can perform the required calculations step by step using the calculations window.

4.23 ORAC Evaluation

The ORAC assay is used to determine the antioxidant capacity of samples. Often Trolox® (a water-soluble analogue of vitamin E) is used as a standard by which all other antioxidant compounds are compared. There are three templates available that can be used to automatically calculate the Trolox® Equivalents (TE) of the samples.

The ORAC templates can be used if the following criteria are fulfilled:

1. All samples that are not a blank or a control have to be defined as standards using different groups for different substances: The Layout can look like this:

		1	2	3	4	5	6	7	8	9	10	11	12
A	Layout												
	Standard Concentrations												
B	Layout		X10 A	B A	B A	X1 A	X2 A	X3 A					
	Standard Concentrations												
C	Layout		S1 A	S1 A	S1 A	S1 B	S1 B	S1 B	S4 E	S5 E	S6 E		
	Standard Concentrations		200	200	200	200	200	200	25	12,5	200		
D	Layout		S2 A	S2 A	S2 A	S2 C	S3 C	S4 C	S5 F	S6 F	S7 F		
	Standard Concentrations		100	100	100	100	50	25	12,5	200	200		
E	Layout		S3 A	S3 A	S3 A	S3 D	S4 D	S5 D					
	Standard Concentrations		50	50	50	50	25	12,5					
F	Layout		S4 A	S4 A	S4 A								
	Standard Concentrations		25	25	25								
G	Layout		S5 A	S5 A	S5 A								
	Standard Concentrations		12,5	12,5	12,5								
H	Layout												
	Standard Concentrations												

2. Trolox® or any other reference substance should be defined as standards in group A
3. The concentration of the Trolox® and of the samples should be typed in using the same unit, e.g. µM or mg/l depending on what is known about the sample.

If this layout has not been created before starting the measurement, the layout can be changed afterwards. This is necessary for using the templates. Please read more about *changing test run layouts* in chapter 6 *Change Test Run Layout*.

4.23.1 Changing the Layout for ORAC Test Runs

For using the easy ORAC evaluation template, all wells containing samples should be defined as standards in different groups setting the reference substance (Trolox®) into group A. The functions of the Change Test Run Layout window can be used as in the control software for the instrument.

After changing the layout, the concentrations of the standard and the samples have to be defined using the other sheet of the window Concentrations/Dilutions/Sample IDs.

The concentration unit of the Trolox® and the samples should be the same, e.g. µM or mg/l. After changing the layout and the concentrations the changes must be applied by pressing OK. To save the changed layout permanently, you must save the test run settings.

After changing the layout one of the ORAC templates can be applied and the calculations of the template will be performed automatically.

4.23.2 ORAC Templates

The ORAC templates available are:

- ORAC no injection
- ORAC 1 injection
- ORAC 2 injections

The assay can be performed either without using integrated pumps or with injection of only the ROS generator (e.g. AAPH – 1 injection) or with injection of the fluorophore (e.g. fluorescein) and the ROS generator (2 injections).

How to use templates is explained in chapter 5: *Using Templates*.

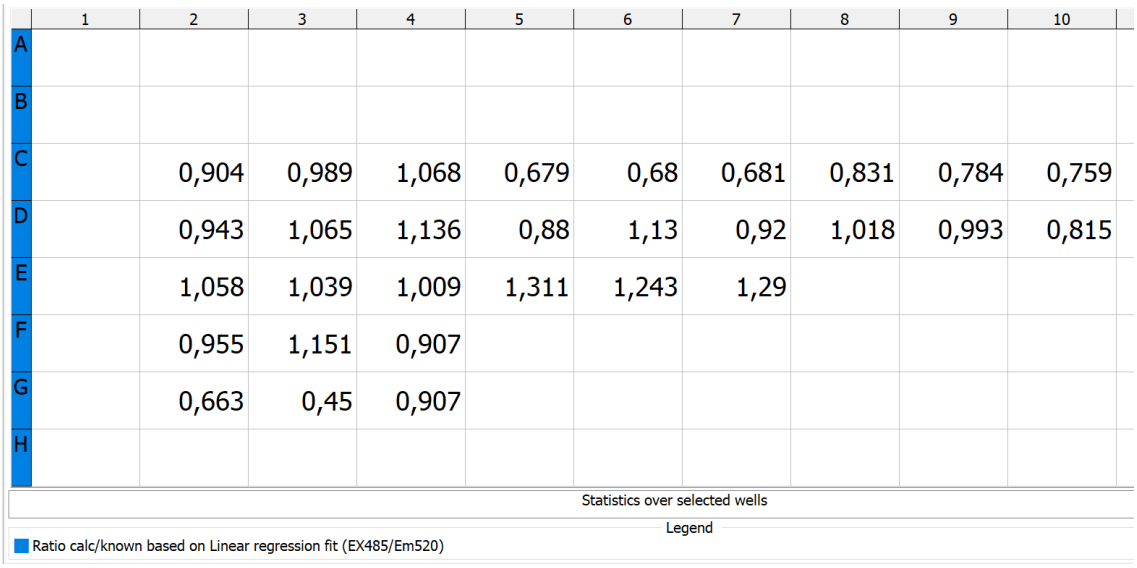
4.23.3 Optimized Settings for ORAC Measurements

The ORAC templates are created for standard measurement settings and should be adapted if different measurement settings are used (please use the appropriate template depending on the number of injections).

Check in the Signal Curve View if the predefined range is covering the complete time of the measurement. If this is not the case spread the range manually (see chapter 4.1: Ranges).

4.23.4 Trolox Equivalents (TE Values)

After applying the suitable ORAC template and confirming the range, the Trolox® equivalents of the Trolox® standards and all samples are automatically shown in the Microplate View.



The TE values for the reference substance Trolox® should be close to 1.00. The unit of the TE values is either per µmol, mg or ml sample depending on what is known about the sample.
The data node showing the TE result is called *Concentration calculations:* ***Ratio calc/known***. Next to that the *Raw data, Averages* or *Linear regression fit* results can be viewed, too (see chapter 3.3: *Microplate View*)

4.24 Robust Statistics

Robust statistics provides an alternative approach to standard statistical methods like average, standard deviation and percent coefficient of variation (%CV).
These estimators are not unduly affected by outliers in a sample population. You can also use the Remove Outliers function in MARS to find and remove outliers based on replicates. Sometimes outliers cannot be found easy and sometimes mask each other. Using the robust statistics instead of the standard statistical methods can help to get a better result in such cases.
If the robust statistics is used, equivalents for the mean (average), the standard deviation and the percent coefficient are used:

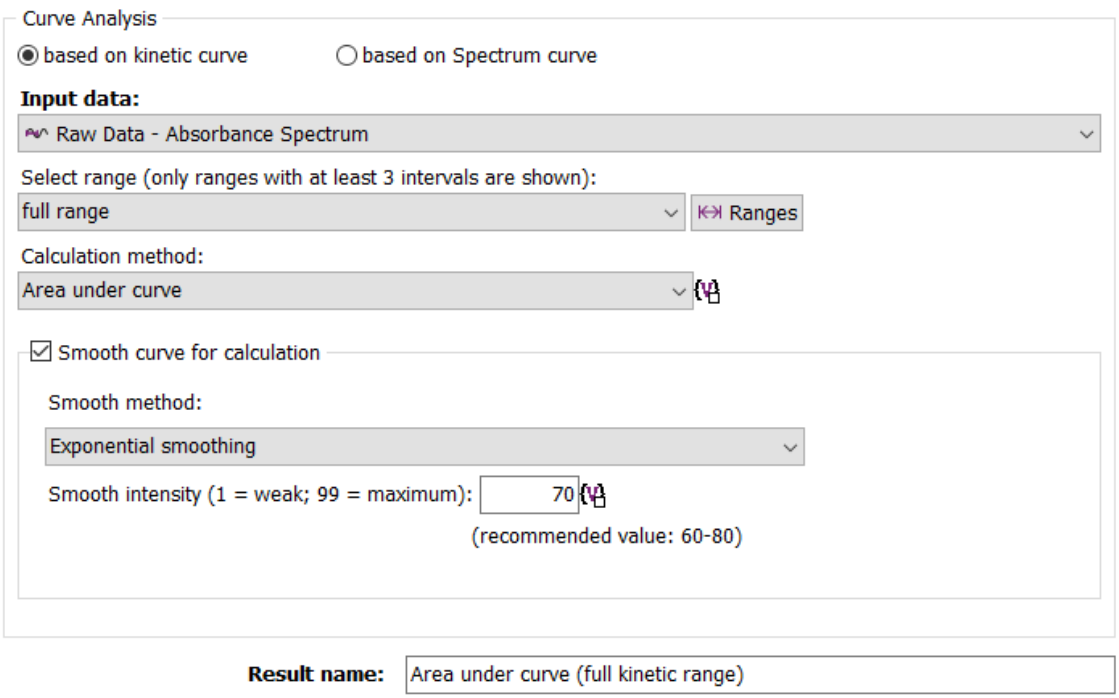
- *Median instead of mean/average.*
- *Robust standard deviation (rSD) instead of SD or SDn*: rSD = MAD x 1.4826 where MAD (median absolute deviation) is the median of the absolute Value of X_i- $Median_x$ (i iterates over all replicates of X).
- *Robust percentage coefficient of variation (%rCV) instead of %CV or %CVn*: %rCV = rSD/$Median_x$ * 100%

You can change the settings for robust statistics in the Calculation Settings dialog. The dialog can be opened with the button *Statistics and Error bar settings* in the *Common* group on the *Calculations* tab of the ribbon menu.
If robust statistics is used in MARS, all calculations based on average, SD, SDn, %CV and %CVn are recalculated by using the robust statistics equivalent. A manually performed average calculation is not affected by this control, but you can change this calculation manually into a median calculation.
Affected calculations are such as:

- Z'
- Signal to noise
- Signal to blank
- Percentage calculations
- All calculations using an average over replicates like the standard fit calculations.

4.25 Curve Analysis

The curve analysis $\frac{dy}{dx}$ calculation page provides the two main operations in calculus: Integration and differentiation.

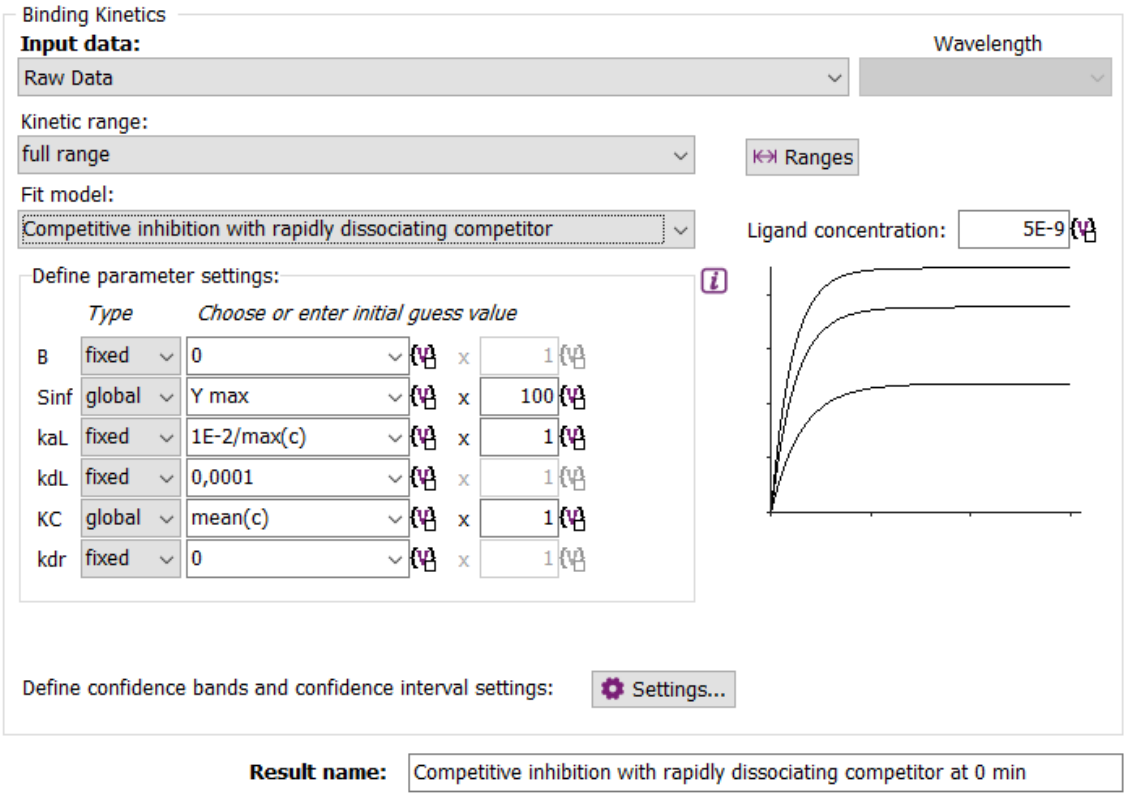


Input data: Select the input data for calculating the curve analysis. This can be the raw data or the result of any calculation that obtains kinetic data or spectra data.
If the input data has both, kinetic and spectra data, you can select if the analysis should be calculated based on the kinetic or based on the spectra curve with the radio control above the input data drop-down list: *based on kinetic curve / based on Spectrum curve*
Select range: The input data for a curve analysis calculation are always defined by the first time point and the last time point of a kinetic range or by the first wavelength and the last wavelength of a spectrum range. It is possible to have one or more ranges defined. The full range is always available and covers the total measurement. See the chapter 4.1: *Ranges*, how to define a range. All defined ranges are listed in the drop-down list with their start and stop time points or wavelength (depending on the selected input data). Select a range for the calculation from this list. Only ranges with at least three values are shown, because the minimum number of values for the curve analysis is three. To view, create or change a range, press the Ranges button to open the range window. You can also define an individual range or a content-based range, where the start and/or stop time point is not fix. Select the *Individual Range* or *Content-based ranges* in the drop-down list to use and define this type of range. Read more about individual and content based ranges in the chapter 4.1: *Ranges*.
Calculation method: Select the calculation method for your curve analysis.
Available methods:
Area under curve: Calculates the area under the curve in the selected range using the integration order one. The result is a single value for each curve that represents the area.
Differentiation: Calculates the differentiation curve. Select the differentiation order in the entry field below the drop-down list. You also can enter a moving interval for the differentiation. If you use a moving interval > 1, not only the dy/dx value of two data points are used to calculate the differentiation in one point, but also the values before and after the data point (moving interval = 5 means: two value pairs before and two value pairs after the current value are used).
Integration: Calculates the integration curve. Select the integration order in the entry field below the drop-down list.

Smooth curve for calculation: To get better results with noisy input data the curves can be smoothed before and after the differentiation/integration. Read more about the possible smooth methods in the chapter 4.7: *Curve Smoothing*.

4.26 Binding Kinetics Calculations

The binding kinetics calculation page is only enabled if it is a kinetic measurement and the layout of the test run contains standards. You can find a more detailed approach in the chapter Binding kinetics -Basics.



Input data: Select the input data for the calculation. This can be the result of any calculation that keeps the kinetic data.
Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry *All* in the list, the calculation will be performed for each wavelength.
Kinetic range: The input data for this calculation are always defined by the first iteration and the last iteration of a range. It is possible to have one or more ranges defined over your kinetic. The full range is always available and covers the total measurement. See in the chapter 4.1: *Ranges*, how to use and define a range. All defined ranges are listed in the drop-down list with their start and stop time points. Select a range for the calculation from this list. To view, create or change a range, press the Ranges button to open the range window. You can also define an individual range where the start and/or stop iteration is not fix. Select the *Individual Range* in the drop-down list to use and define this type of range. Read more about individual ranges in the chapter 4.1: *Ranges*.
Fit model: The binding kinetic calculation comes with six fit models. Details of the different fit models can be found in the chapter Binding kinetics -Basics.
Ligand concentration: Enter the ligand concentration for the two competitive inhibition fit models. The concentration unit must be the same as the entered concentration unit for the standards.
Define parameter settings: Each fit model comes with a number of fit parameters. To get a valid fit result, each parameter needs an initial guess value.

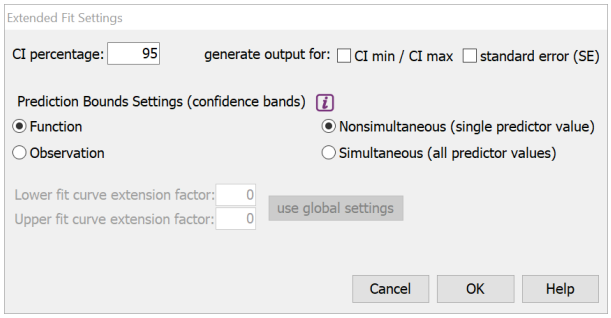
> Fit parameter type: If a parameter should be fix (it will not be fitted than) select fixed. If it should be fitted global (for all standards), select global and if it should be fitted individual for each standard, select free.

Weighted fit

Check this control if the weight of each standard should not be the same. You can give less weight to less precise standards and more weight to more precise standard. Select one of the offered weighting methods to define, how the weighting for each standard should be calculated.

Confidence Interval Settings

For all fit models, confidence intervals and confidence band are calculated automatically. Confidence intervals are calculated for each standard and for each fit result parameter which is not fix. The confidence band can be displayed in the binding kinetic curve chart.
To define or change the confidence bands and interval settings, click on the settings button to open the setting dialog for confidence bands and intervals:



CI percentage: Define the percentage value for the confidence interval calculation. A lower value results in a wider interval band.
generate output for...: Select *CI min / CI max* to generate result nodes for the upper and lower CI values for each fitted parameter. Select *standard error*, to generate a result node for the standard error (see Calculation Result below).
Prediction Bounds Settings: Select between the different settings:
Observation or Function Bounds: Provides a confidence interval for a new observation or for a new function (curve).In general, observation intervals are wider than function intervals, because of the additional uncertainty of predicting a new response value (the function plus random errors).
Non-simultaneous or Simultaneous Bounds: Provides a confidence interval using a single predictor value (non-simultaneous) or all predictor values (simultaneous). In general, simultaneous intervals are wider than non-simultaneous intervals, because of the additional uncertainty of bounding values for all predictors.

Note: The fit curve extension factors are not available for the binding kinetics calculation.

4.26.1 Calculation Result

In addition to the fit result curve, a result node for each available fit parameter will be created. If the check box *CI min / CI max* in the confidence interval settings dialog was selected, an additional node for the upper and lower value for each fit parameter will also be created.
After performing the calculation new nodes appear in the navigation tree. One for the fitted curve and one for each result parameter.
Drug binding is often characterized by the equilibrium dissociation constant KD which is the ratio of the dissociation rate k_d (please note the non-capital 'k') and the association rate k_a. Equilibrium binding assays can only determine K_D and not the two rates which contain useful information about the average drug residence time at a receptor for instance. However, kinetic binding assays can determine those rates and thus become an invaluable tool in drug screening and research.
Say ***L*** denotes an ensemble of ligand molecules that can bind to an ensemble of receptor molecules ***R*** to form a ligand-receptor complex/product LR. In chemical terms this can be written as:

$$\mathrm{L} + \mathrm{R} \underset{k_d}{\overset{k_a}{\rightleftarrows}} \mathrm{LR}$$

Herein k_a denotes the association and k_d the dissociation rate of the binding reaction. In a typical microplate-based experiment both the ligand and the receptor are each labelled with different dyes in order to form a FRET pair. When both interact with each other a FRET event occurs leading to increased signal in the acceptor channel. The rate c_{LR} at which the amount of the ligand-receptor complex *LR* changes, depends on the concentrations of the ligand, of the receptor and on the amount of *LR* already present a time t:

$$\dot{c_{LR}} = k_a \cdot c_L \cdot c_R - k_d \cdot c_{LR}$$

From this equation it becomes obvious that the association rate k_a is in units of $M^{-1}s^{-1}$ and the dissociation rate s^{-1} (if we take the SI units). The number of receptors that are unoccupied (c_R) at some time *t* might be expressed in terms of the total number c_{Rmax} of receptors and the number of currently occupied receptors c_{LR} (applying the law of mass conservation). Thus, the following differential equation is obtained:

$$\dot{c_{LR}} = k_a \cdot c_L \cdot \left(c_{R_{max}} - c_{LR}\right) - k_d \cdot c_{LR}$$

This equation can be solved for various scenarios, e.g. starting from a solution of ligand and receptor molecules in equilibrium ($\dot{c}_{LR}$=0) and then adding an excess of unlabelled ligand results in a continuous decrease of labelled *LR* complexes and thus in an exponentially decreasing signal (see dissociation model below). In another scenario a solution of pure ligand might be mixed with another solution of pure receptor leading to the association of ligands towards the receptors (see association model below). A mixture of both of the aforementioned scenarios, namely association first and then dissociation might be another scenario that can be derived (see association followed by dissociation model below).

In competitive binding experiments an inhibitor I (often referred to as competitor) is added to the reaction mixture which complicates the reaction equation:

$$\mathrm{L} + \mathrm{R} + \mathrm{I} \begin{cases} \overset{k_{a,L}}{\underset{k_{d,L}}{\rightleftarrows}} \mathrm{LR} \\ \overset{k_{a,I}}{\underset{k_{d,I}}{\rightleftarrows}} \mathrm{IR} \end{cases}$$

Herein $k_{a,I}$ and $k_{d,I}$ denote the association and dissociation rate of the inhibitor, respectively.

The reaction can then go along two pathways, either the association of ligand and receptor or the association of inhibitor and receptor. The corresponding differential equation becomes more complex:

$$\dot{c_{LR}} = c_{R_{max}} c_L k_{a,L} - c_{IR} c_L k_{a,L} - c_{LR}\left(c_L k_{a,L} + k_{d,L}\right)$$

MARS includes two models that are solutions to this differential equation (see *competitive binding model* and *competitive binding with rapid dissociating competitor* model).

The following sections describe the basic formulas of different kinetic binding models and how these models can be used in MARS to fit kinetic curves from binding experiments. In the following we use the letter *y* to denote the signal.

Dissociation model

Starting from an equilibrium state dissociation experiments are often performed by adding a vast amount of non-labelled ligand (blue circles in Figure 1) to a solution of labelled ligands (red circles) and labelled receptors (purple squares) being in equilibrium. In these kinds of assays a TR-FRET signal is recorded emerging from energy transfer between bound ligand and receptor-dye (green). Once the non-labelled ligand is added (in excess) it replaces the labelled ligand at the receptor resulting in a continuous decrease of the TR-FRET signal.

$$y(t) = B + (S_0 - B) \cdot e^{-k_d \cdot t}$$

B: Binding signal offset due to nonspecific binding (t→∞)

S_0: Binding signal at time t = 0

k_d: Dissociation rate constant of the ligand in s^{-1}

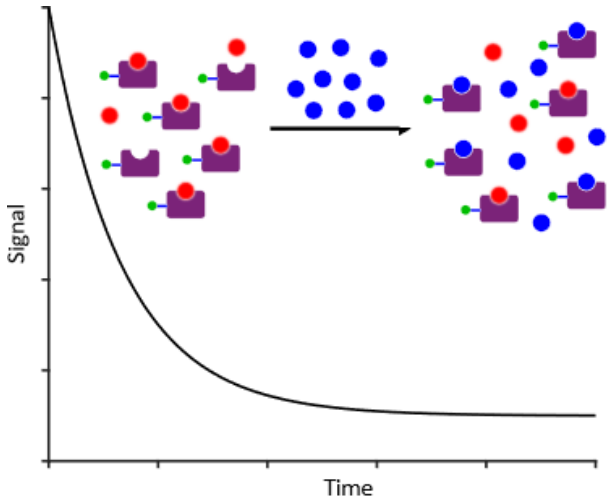


Figure 1: Typical curve shape for the dissociation model.

Association model (one or more concentrations of ligand)

In a typical association experiment, labelled ligands (red in Figure 2) are mixed with labelled receptors (purple squares) and the time course of association is measured by a TR-FRET signal increase. The signal increases until equilibrium is reached resulting in a plateau of the TR-FRET signal. The corresponding data can be fitted using the following equation:

$$y(t) = B + \frac{S_\infty \cdot c}{c + \frac{k_d}{k_a}} \cdot \left(1 - e^{-(c \cdot k_a + k_d) \cdot t}\right) \cdot e^{k_{dr} \cdot t}$$

B: Binding signal offset due to nonspecific binding
S_∞: Binding signal at (c→∞ and t→∞)

k_d: Dissociation rate constant of the ligand in s^{-1}

k_a: Association rate constant of the ligand in $M^{-1} \cdot s^{-1}$

c: Concentration of the ligand in M

k_{dr}: Signal drift term in s^{-1}. The drift might be caused by bleaching of the probe.

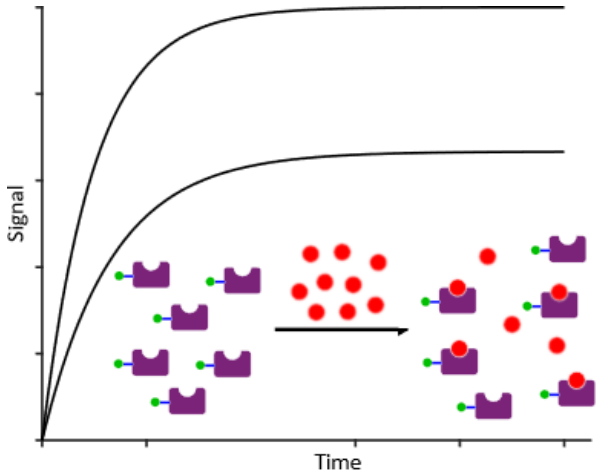


Figure 2: Typical curve shape for the association model with three ligand concentrations. The ordinate is the scale for signal y(t) and the abscissa corresponds to time t.

Please note that using only one ligand concentration results in ambiguous fit values for k_a and k_d. To get reliable values for k_a for one ligand concentration experiments, fix the dissociation constant k_d. k_d might be estimated from a dissociation experiment. Otherwise multiple concentrations need to be used in order to get reliable fit values for both k_a and k_d.

Association followed by dissociation model

An association followed by dissociation experiment is a mixture of an association experiment and a dissociation experiment (see Figure 3). First an association experiment is performed until labelled ligands (red circles) and labelled receptors (purples squares) have reached equilibrium (plateau of the TR-FRET signal). Then an excess of non-labelled ligand (blue circles) is added to the solution leading to a continuous replacement of labelled ligands with non-labelled ligands at the receptors. This results in an exponentially decreasing TR-FRET signal. The data can be fitted using the following equation:

$$y(t) = \begin{cases} B + \frac{S_\infty \cdot c}{c + \frac{k_d}{k_a}} \cdot \left(1 - e^{-(c \cdot k_a + k_d) \cdot t}\right) & for\ t < t_a \\ \left(B + \frac{S_\infty \cdot c}{c + \frac{k_d}{k_a}} \cdot \left(1 - e^{-(c \cdot k_a + k_d) \cdot t_a}\right)\right) \cdot e^{-k_d \cdot (t - t_a)} & for\ t \geq t_a \end{cases}$$

B: Binding signal offset due to nonspecific binding
S_∞: Binding signal at (c→∞ and t→∞)
k_d: Dissociation rate constant of the ligand in s^{-1}
k_a: Association rate constant of the ligand in $M^{-1} \cdot s^{-1}$
c: Concentration of the ligand in M
t_a: Time until association occurs in s.

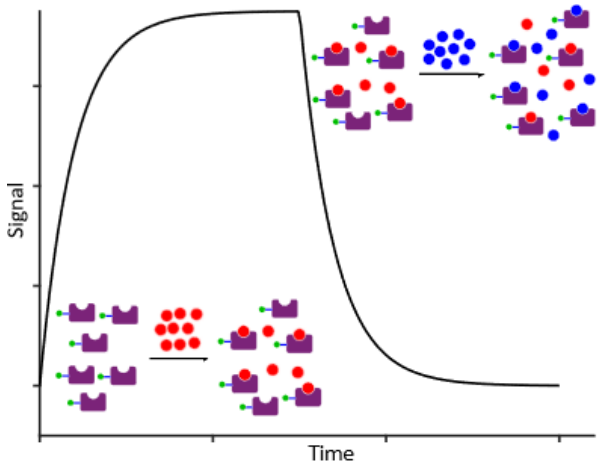


Figure 3: Typical curve shape for the association then dissociation model. The ordinate is the scale for signal y(t) and the abscissa corresponds to time t. The arrow indicates the time t_a until association occurs.

Please note that in contrast to sole association experiments an association followed by dissociation experiment can provide reliable fit values for k_a and k_d even for one ligand concentration.

Competitive binding model

In a typical competitive binding experiment labelled receptors are mixed with labelled ligands (red circles in Figure 4) of known kinetic properties (k_a and k_d are known) and a non-labelled inhibitor / competitor (compound of interest). Both types of molecules compete for space at the receptors resulting in binding curves as shown in Figure 4. These curves are fitted globally with the following model:

$$y(t) = \frac{S_\infty \cdot k_{a,L} \cdot c_L}{K_F - K_S} \left[\frac{k_{d,C} - K_F}{K_F \cdot K_S} + \frac{k_{d,C} - K_F}{K_F} \cdot e^{-K_F \cdot t} - \frac{k_{d,C} - K_S}{K_S} \cdot e^{-K_S \cdot t}\right] \cdot e^{k_{dr} \cdot t} + B \quad \text{with}$$

$$K_F = 0.5 \cdot \left[K_A + K_B + \sqrt{(K_A - K_B)^2 + 4 \cdot k_{a,L} \cdot k_{a,C} \cdot c_L \cdot c_C}\right] \quad \text{and}$$

$$K_S = 0.5 \cdot \left[K_A + K_B - \sqrt{(K_A - K_B)^2 + 4 \cdot k_{a,L} \cdot k_{a,C} \cdot c_L \cdot c_C}\right] \quad \text{with}$$

$$K_A = k_{a,L} \cdot c_L + k_{d,L}$$

$$K_B = k_{a,C} \cdot c_C + k_{d,C}$$

B: Binding signal offset due to nonspecific binding
S_∞: Maximum binding signal of the probe
$k_{a,L}$: Association rate constant of the ligand / probe $M^{-1} \cdot s^{-1}$
$k_{d,L}$: Dissociation rate constant of the ligand / probe in s^{-1}

$k_{a,C}$: Association rate constant of the competitor / inhibitor in $M^{-1} \cdot s^{-1}$

$k_{d,C}$: Dissociation rate constant of the competitor / inhibitor in s^{-1}

c_L: Concentration of the ligand / probe in M

c_C: Concentration of the competitor / inhibitor in M

k_{dr}: Signal drift term in s^{-1}. The drift might be caused by bleaching of the probe.

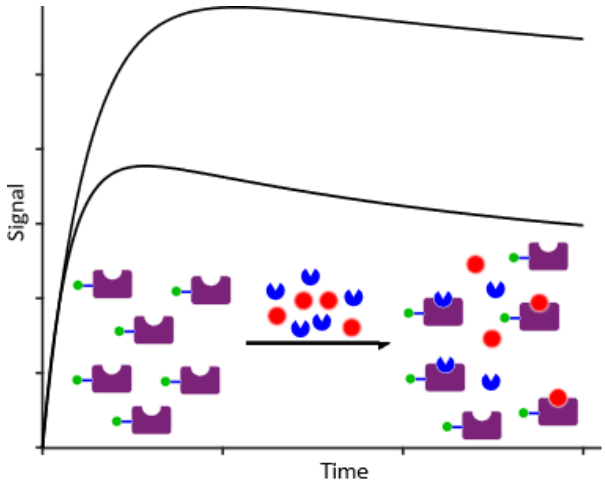


Figure 4:Typical curve shapes for the competitive binding model. The ordinate is the scale for signal y(t) and the abscissa corresponds to time t.

The competitive binding model is sometimes referred to as the Motulsky-Mahan model since Motulksy and Mahan first published this model in 1984.

Competitive binding model with rapid dissociating competitor

The competitive binding model with rapid dissociating competitor is similar to the competitive binding model. However, it was derived for a rapidly dissociating competitor at least providing reliable results (with sufficiently narrow confidence intervals) for the equilibrium dissociation constant of the competitor K_C:

$$y(t) = \left[\frac{S_\infty \cdot c_L \cdot k_{a,L} \cdot \left(1 - \frac{c_C}{K_C + c_C}\right)}{c_L \cdot k_{a,L} \cdot \left(1 - \frac{c_C}{K_C + c_C}\right) + k_{d,L}} \cdot \left(1 - e^{-\left(c_L \cdot k_{a,L} \cdot \left(1 - \frac{c_C}{K_C + c_C}\right) + k_{d,L}\right) \cdot t}\right) \right] \cdot e^{k_{dr} \cdot t} + B$$

B: Binding signal offset due to nonspecific binding

S_∞: Maximum binding signal of the probe

$k_{a,L}$: Association rate constant of the ligand / probe $M^{-1} \cdot s^{-1}$

$k_{d,L}$: Dissociation rate constant of the ligand / probe in s^{-1}

K_C: Equilibrium dissociation constant of the competitor / inhibitor in M

c_L: Concentration of the ligand / probe in M

c_C: Concentration of the competitor / inhibitor in M

k_{dr}: Signal drift term in s^{-1}. The drift might be caused by bleaching of the probe.

Figure 5 shows a typical competitive binding curve shape when the competitor (blue circles) and the labelled ligand (red circles) are simultaneously added to the receptors (purple squares) and the competitor rapidly dissociates from the receptor again.

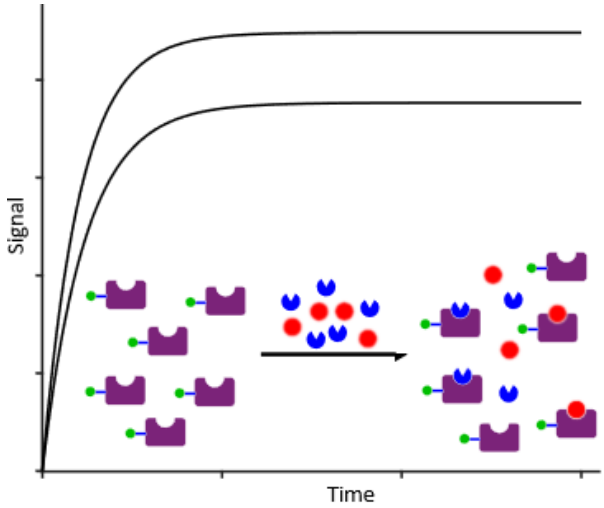


Figure 5: Typical curve shapes for the competitive binding model with rapidly dissociating competitor. The ordinate is the scale for signal y(t) and the abscissa corresponds to time t

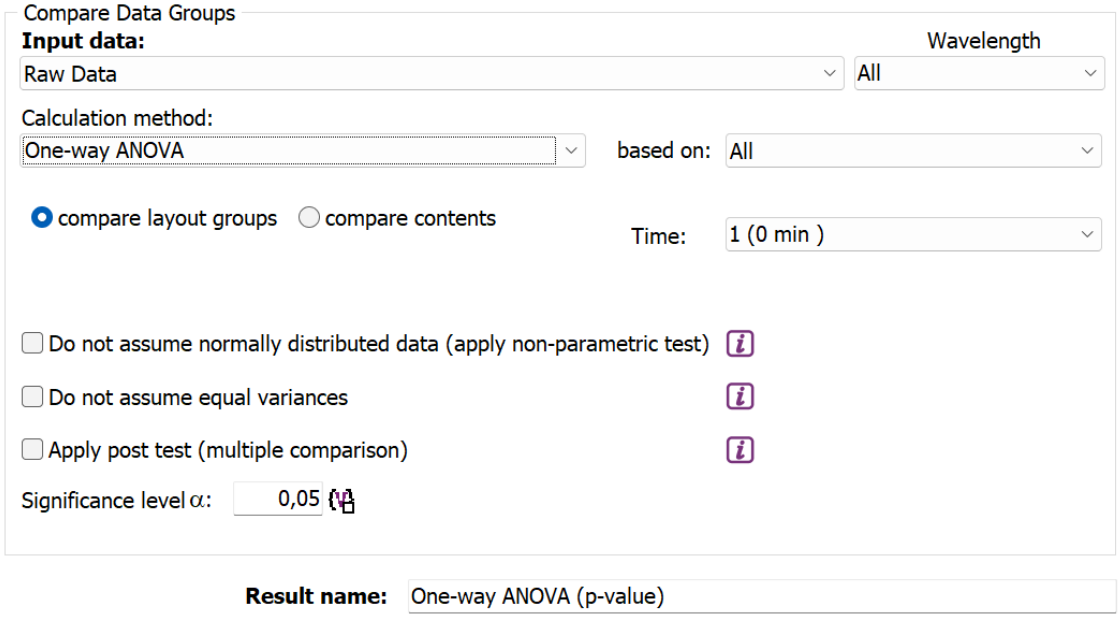
Note: Please note that MARS uses seconds as time unit internally. Thus, the initial guess values for rates and time values might need to be converted accordingly. Also note that concentration units should be kept consistent (e.g. the initial guess values for association rates and concentrations in the competitive binding models). The binding kinetic fitting feature is only available if standards are present in the layout.

4.26.2 Literature

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4.27 Compare Data Group Calculations

The compare data groups calculation page allows you to perform comparison of two or more data groups. These data groups can be defined by layout content or layout groups. For kinetic measurements, the groups can also be defined by loop data points (comparing measurements at a different time data for the same well).



Input data: Select the input data for the calculation. This can be the result of any calculation which outputs the data as numbers.

Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry *All* in the list, the calculation will be performed for each wavelength.
Calculation method: Select the calculation method for your calculation.
Available methods:
Unpaired t-Test: Compares two groups based on the layout (content or group).
One-way ANOVA: Compares three or more groups, based on the layout (content or group).
Paired t-Test: Compares data of two different loop data points (only available for kinetic measurements) or based on layout (content or group).
Repeated measures one-way ANOVA: Compares data of three or more different loop data points (only available for kinetic measurements) or based on layout (content or group).
based on: depending on the selected calculation method and the available data, you see one or two based on controls. Select the data groups to be compared with these controls for end point data. For paired t-Test and repeated measures one-way ANOVA, select the time point to be compared with the **Time** controls. The **based on** control let you select the content or layout group, for which the time points should be compared.

> **Note:** Please note that a paired t-test or repeated-measures ANOVA are mostly used for before-after-tests and shall not be used if independent data groups are to be compared. In this case it is more correct to choose an ordinary t-test or the ordinary one-way ANOVA.

4.27.1 Additional controls, depending on the selected calculation method and the available data:

compare layout groups / compare contents: If the layout contains layout groups and different contents with replicates, you must decide, whether the data should be compared based on the layout groups or based on layout contents.This is valid for unpaired t-Test and one-way ANOVA calculations.
Do not assume normally distributed data (apply non-parametric test): available for all calculations. One of the prerequisites for an ordinary t-test or an ordinary ANOVA is that the data within each group follow a normal / Gaussian distribution. If this is not met, please activate this option and a non-parametric method will be applied. ne might check this prerequisite by carefully looking at the subsequently generated box-plots, jitter-plots, scatterplots or violin-plots).
Do not assume equal variances: available for unpaired t-Test, one-way ANOVA and repeated-measures one-way ANOVA. One of the prerequisites for an ordinary t-test or an ordinary ANOVA is that the variances (scatter) of the different groups are (practically) identical. If this is not met, please check this option and it will be corrected or that (using Welch correction).
Apply post test (multiple comparison): available for one-way ANOVA and repeated-measures one-way ANOVA. By checking this option, additionally the mean / median of each data group will be compared to that of any other data group using a multiple-comparison method. Thus, one might be able to identify the groups that mainly contribute to a significant ANOVA or t-test result.
Significance level α: Enter the significance level (0-1) for the calculation.
The meaning and influence of the significance level and further explanation how to compare data base on t-Tests and ANOVA can be found in the chapter Data group comparison feature.

4.27.2 Calculation Result

The result of the comparison will be a plot for each group in a chart and a result node with the resulting *p*-value. Detailed tables with all comparison results can be opened by pressing the *Results* button above the chart. Details to the different plot types and the results tables can be found in the chapter Compare Data Plots.
.

4.27.3 Data group comparison feature

Introduction

Let us assume we were given the following layout:

	1	2
A	C1	S1
	91	97
B	C1	S1
	104	95
C	C1	S1
	94	98
D	C1	S1
	102	98
E	C1	S1
	150	134
F	C1	S1
	485	255

with data groups C1 and S1 each containing six independent replicates. C1 is the control group and S1 the group that was treated with some drug. Let us further assume that we want to check if the drug has a significant effect on the readout compared to the control group and thus has a significant effect on the system under study. This or similar questions are well-suited to be answered by a two-sample Student's t-test. Herein, the (absolute) difference between the group means is related to the standard deviation of this difference. If the corresponding ratio, let us call it *T*, is huge, the two means will likely not be the same. To be more exact, *T* is compared to a theoretically derived *t*-value depending on the confidence level α (α is by default set to 0.05) that needs to be entered by the user. If *T*>*t* then the two means will significantly differ, otherwise not. α is the probability that statistical significance is (falsely) declared, although there is none. Lowering α thus increases the confidence of the declaration and decreasing false positives, but also increases the chance of having false negatives, i.e., stating that there is no significant effect although there is one. As a result, choosing an appropriate α is a trade-off between false positives and false negatives. If the latter one is more severe, then one might go for a bigger α (e.g., α = 0.2). The above-mentioned *T*-value is typically converted into a *p*-value which then can be compared to α. If *p*<α, then the mean difference is statistically significant, otherwise not. MARS reports both, the *T*- and the *p*-value.

The Student's t-test assumes that the data within the groups follows a normal distribution. If this prerequisite is not true, one might use a non-parametric test such as Wilcoxon's rank sum test. This test and all other non-parametric tests rank the data and perform a statistical test on these ranks. Although non-parametric tests do not assume that the data come from a normal distribution, they assume that the data of the groups follow the same distribution (whatever the distribution might be).

Paired or in general repeated-measures analyses are often applied for before and after- tests. MARS offers a paired t-test e.g. to analyze cell-based assays in which the cells are measured before the addition of a drug and after its addition. In this case the before and after readout might correspond to two iterations of a kinetic (e.g. with an automatic or manual injection of the substance between iteration 1 and 2) or to different well groups (e.g. when the before treatment cells are in column A and the after treatment cells, that were pooled from the same stock but that have undergone the treatment, are in column B). In any case the paired t-test will check for a difference between the before and after group. The non-parametric pendant to the paired Student's t-test is the Wilcoxon signed rank-test.
If there are more than two datasets to be compared, the Student's t-test turns into an analysis of variance (ANOVA). Correspondingly, one can choose One-way ANOVA or Repeated-measures one-way ANOVA. ANOVA is a general statistical framework used to compare the means of three or more data groups. Let us assume we have four data groups (A, B, C and D, see Figure 1). For each data group we can estimate the variance, i.e., the average squared distance to the group mean (Figure 1 left). Averaging these variances over all data groups gives the so-called within-variance. We can also estimate a variance from the data group means (Figure 1 right), i.e., by calculating the squared distances of those means to the mean of the grand (dashed line in Figure 1). If the ratio of the latter mentioned variance to the formerly mentioned variance is much bigger than 1, we conclude that the variability between the group means is much bigger than the (random) variability within the data groups. Exactly this is the case in Figure 1. We thus conclude that at least one data group mean is significantly different from one of the others. But when is the ratio of variances far enough away from 1? This is decided based on an F-test. Like the Student's t-test, the F-test compares the calculated F-value (i.e., the ratio) with a theoretical value which (again) depends on the significance level α. A p-value is derived from the experimental F-value which helps to decide if at least one mean is significantly different from the rest (p<α) or not.

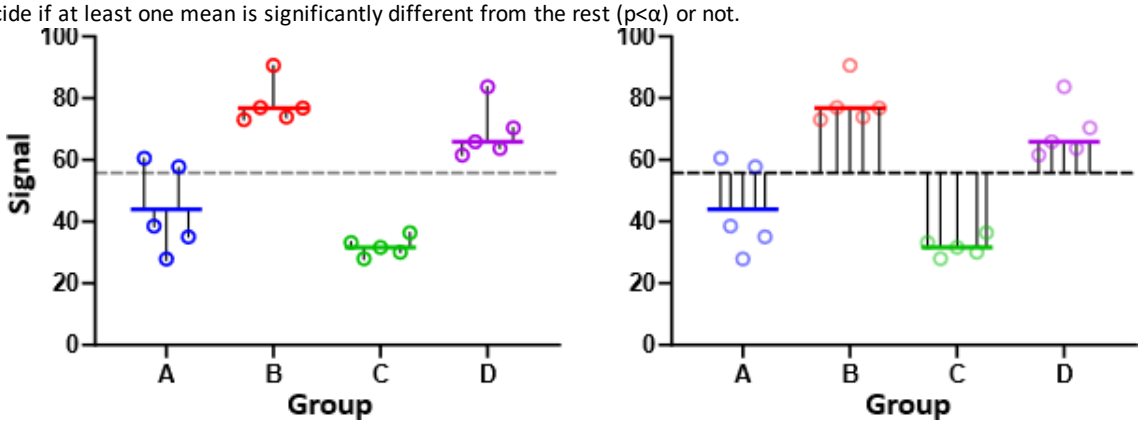


Figure 1: Principle of one-way ANOVA. One-way ANOVA compares the variance within the data groups (left) to the variance between the data groups (right). If the latter is much bigger than the former, one can conclude that at least one of the group-means (colored horizontal lines) is significantly different from the others. Otherwise, all data group means only randomly differ from the grand mean (dashed horizontal line).

If the normality assumption is not fulfilled, a non-parametric ANOVA can be performed which is based on an analysis of variance of the ranks. This test is referred to as Kruskal-Wallis-test. The non-parametric pendant to a rm-ANOVA is the so-called Friedman-test. All these tests are omnibus tests, i.e., they can detect that at least two groups are statistically significantly different, but they do not state which two (or more) are different. To elucidate this, one can apply a *post-hoc* test after the omnibus test. MARS can apply different *post-hoc* tests depending on the choice of the omnibus test. The appropriate *post-hoc* test is then automatically selected by MARS. The corresponding results of the omnibus test as well as of the *post-hoc* test (if applicable) will be reported in the *Results* tab. We will not address the details of all the data group comparison tests that are implemented but refer to dedicated literature (see Literature section). However, we provide an overview of all omnibus and *post-hoc* tests (multiple comparison tests) implemented in MARS and when exactly they will be applied (see Figure 2). Assuming the data within the data groups was normally distributed, the key criterion for choosing a Student's t-test or an ANOVA is the number of data groups that shall be compared. For n=2 (I. e. two data groups) either an unpaired t-test or a paired t-test. As noted above, the unpaired t-test shall be used in case of independent data groups, e. g. in case of different wells within the data groups. The paired t-test shall be used if the data groups are formed by two different iterations of the same well (one kinetic curve, or a part thereof, denotes one data group) and are dependent which is, for instance, true for "before and after injection" measurements. For n>2 (I. e. three or more data groups) either an ordinary *One-way ANOVA* can be performed or *repeated-measures ANOVA* being the multiple-groups pendant to the paired t-test. If the variances from the different data groups differ significantly, instead of an ordinary One-Way ANOVA a Welch's ANOVA is performed which takes the different group variances into account. If the variances of the pairwise differences of the data group values are not equal (which might often be the case for "before and after" experiments), MARS corrects the p-value of the test using the Geisser-Greenhouse correction.

If the data within the data groups is not assumed to be normally distributed MARS performs one of the abovementioned non-parametric tests (see also Figure 2). Please note that these types of tests do not need to take into account the equality of group variances. If desired, a *post-hoc* test can be performed after an omnibus test. These tests can uncover the data groups that are responsible for significant differences of the corresponding omnibus test.

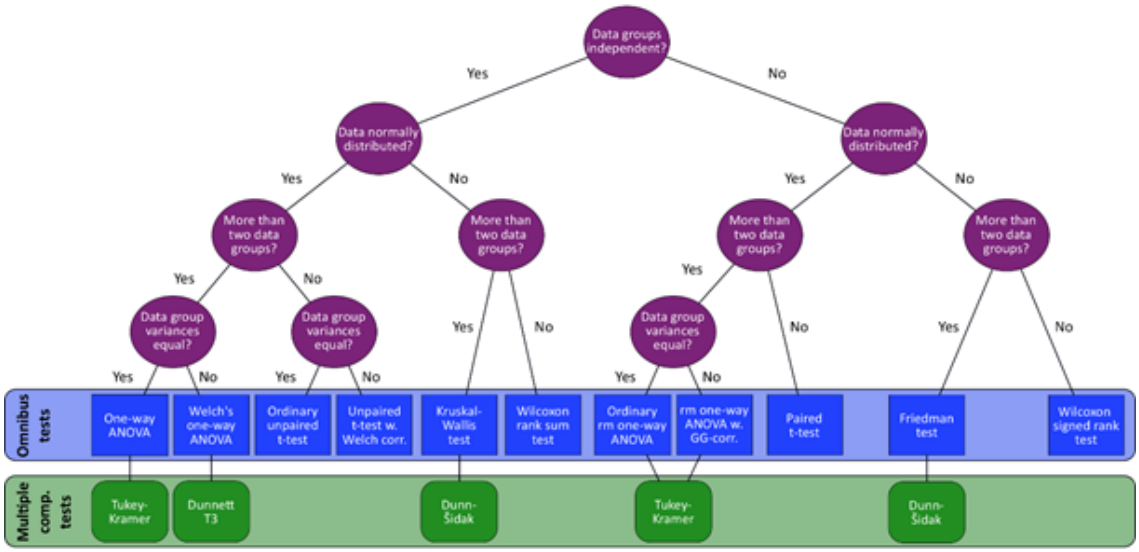


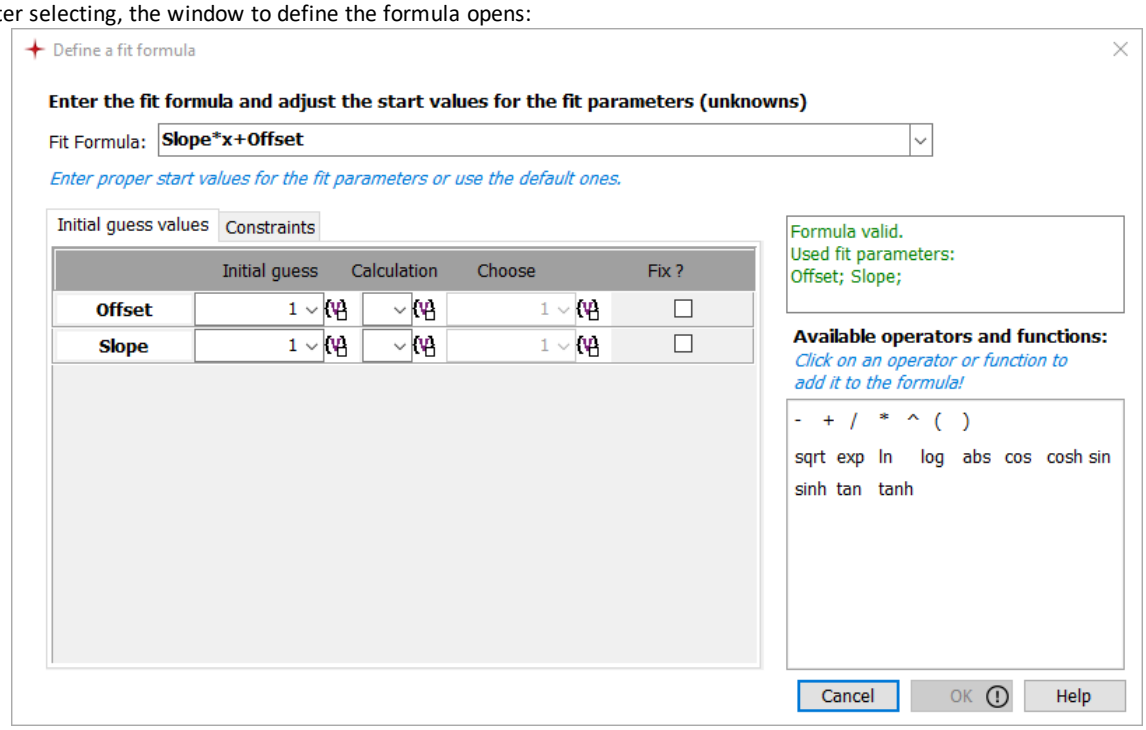
Figure 2: Flow-chart for the use of omnibus and multiple comparisons tests (*post-hoc* tests) implemented in MARS.

Literature

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- Welch, B. L. (1951, 12). On the Comparison of Several Mean Values: An Alternative Approach. Biometrika, pp. 330-336.
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4.28 User defined fit formulas

The standard curve fit calculation and the kinetic curve fit calculation offers a user definable fit formula. To use this fit formula, select the last entry *(User defined fit*) in the fit model pull down list.
After selecting, the window to define the formula opens:



Enter the formula into the *Fit Formula* entry field and press enter to apply the formula or select one of the predefined formulas using the pull-down button of the entry field. The formula will be checked for validity and parsed to find the containing parameters. If the formula is valid a table row is created for each fit parameter in the formula. Define the initial guess values for each fit parameter and - if desired - a lower and upper limit (constraints) for the parameter.
For details see section Initial guess values and constraints.

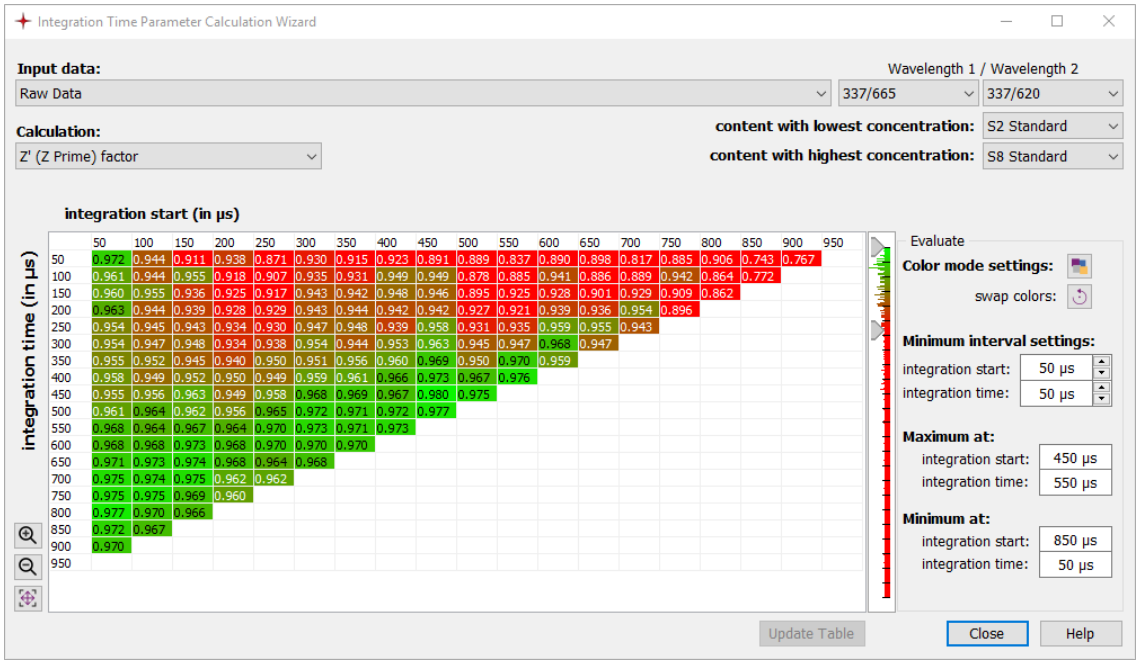
To add special operators to the formula, you can click on the operator or functions displayed in the *Available operators and functions* control or you can enter them directly in the formula edit field.
If you have entered the desired and valid fit formula, click *OK* to apply the formula to the calculation.
You can change the formula after you have closed this window, if you click on the *Edit Formula* button on the dialog where the calculation is defined.

4.28.1 Initial guess values and constraints

Initial guess values
A useful start value for the parameters (initial guess value) is important for the fit algorithm to find a satisfying result. The closer the start value is to the expected fit result for this parameter, the faster is the fit and the better is the fit result.
Initial guess: You can enter a value or select a predefined value using the pull-down button of the entry field.
Calculation: You can modify the initial guess value at runtime if you select a calculation and a second value entered or selected in the *Choose* column.
Choose: If a calculation is defined in the *Calculation* column, you can enter or select a value which will be used for the calculation of the initial guess value.
Fix ?: If you want to fix the parameter, check this box. It will not be fitted but used the defined initial guess value.
Constraints
Define the constraints for each parameter. If you define a constrain, the parameter will not be set below the lower limit or above the upper limit.
Lower Limit: Enter or select a lower limit for that parameter.
Upper Limit: Enter or select a upper limit for that parameter.

4.29 Integration Time Wizard

For data with a measured decay curve (only Alphascreen and TRF measurements) MARS offers a wizard to optimize the integration start and integration time parameters for further measurements.
If a test run with a measured decay curve is opened in MARS, an *Integration Time Wizard* menu in the *Data Reduction* group on the *Home* tab of the ribbon appears. After clicking this menu item, the Integration Time Parameter Calculation Wizard opens:



Input data: Select the input data for calculating the integration time matrix. This can be the raw data or the result of any calculation.
Wavelength 1 / Wavelength 2: If the test runs has more than one chromatic, select the two relevant chromatics for the selected calculation. If two different chromatics are selected, the ration of these two chromatics is used for the calculation.
content with lowest/highest concentration: Select the content with the lowest concentration and the content with the highest concentrations.
Calculation: Select the calculation method for your assay quality.
Available methods:
Assay Window: Calculates the ratio between the value of the lowest and highest concentrations content.
Z' (Z prime) factor: Only available, if replicates are defined in the layout. Calculates the Z prime value based on the value of the lowest and highest concentration contents. The formula for the calculation is:

$$Z' = 1 - \frac{(3 \cdot \sigma 1 + 3 \cdot \sigma 2)}{|\mu 1 - \mu 2|}$$

µ 1 is the average of the values over the replicates of the highest concentration content
σ 1 is the standard deviation over the replicates of the highest concentration content.
µ 2 is the average of the values over the replicates of the lowest concentration content
σ 2 is the standard deviation over the replicates of the lowest concentration content

R² of linear fit: Only available, if standards are defined in the layout. Calculates a linear fit based on the standards and uses the resulting r² value to fill the matrix.
LOD (limit of detection): Only available, if standards and blanks (more than one) are defined in the layout. Calculates a linear fit based on the standards to get the slope for the LOD formula:

3*SD(blank) / Slope.

SD(blank) is the standard deviation of the blank.

Define the desired input data and calculation and press the *Update Table* button to calculate the integration matrix. After the matrix is calculated, you can adjust the interval settings for the integration time and integration start range. To recalculate the matrix with the changed settings, press *Update Table* again.
Use the color slider on the right side of the table or the color settings button to adjust the color settings for the table.

4.30 Fit model comparison and fit quality metrics

Fitting experimental data can help verifying or falsifying a biochemical model (e.g., association binding versus competitive binding kinetics) and thus supports basic researchers understanding the underlying biochemical mechanisms. In this case it is helpful to distinguish if the data supports one model over the other. There are various metrics described in the literature that aid at judging the goodness of a fit where the R^2, also known as the coefficient of determination, is one of the most prominent ones. While the R^2 is good in distinguishing between a constant line model and a non-constant line model, it is

in general not suitable for non-linear fit models. However, other criteria such as the Akaike information criterion (AIC) or the Bayesian information criterion (BIC) have proven to be very powerful for nested and especially non-nested models. Comparing two nested models means that the simpler model can be derived from the more complex model by fixing one fit parameter (e.g. a 4P-fit is a 5P-fit with the symmetry parameter fixed to 1). Using the AIC, or even better the AIC corrected for small sample (AICc), one can find a good trade-off between goodness of fit (for least-square regression measured in terms of the residual sum-of-squares, SSE) and the model complexity (number of fit model parameters). The AICc will be output in MARS' Fit Results by default, together with the R^2. To compare two different fit models in MARS perform a standard curve fit with the first model and check the AICc in the fit results. Subsequently fit the second model to the data and check again the AICc in the fit results. For further analysis choose the model leading to a lower AICc.
If the number of data points exceeds the number of fit model parameters by two, the AICc is automatically calculated using the following equation:

$$AIC_c = N \cdot \log\left(\frac{SSE}{N}\right) + \frac{N+P}{1-(P+2)/N} + N \cdot \log(2\pi) + \sum_{i=1}^{N} \log(w_i)$$

Herein denotes N the number of data points, P the number of fit model parameters and wi weights which are ones in case of non-weighted fitting. As this is the default, the last term will mostly be zero. The first term is related to the goodness of fit and the second term is related to the fit model complexity. The third term is a constant for a given dataset and is often omitted by other software packages. As can be seen from the equation above, the number of data points N must not be equal to the number of fit model parameters P + 2 since the denominator in the second term becomes zero. In fact, the number of data points must additionally be larger than the number of fit model parameters since the SSE will be zero as the fit curve will then go through all the data points (due to mathematical reasons). In MARS, an AICc will be computed if N > P+2. One of two fit models is superior to the other if the corresponding AICc is smaller. One can also calculate the likelihood, that one model is correct compared to the other by taking the difference D1=AICc1- AICc2 between the AICc values (given AICc2 is the minimal AICc) and calculating the following expression:

$$p_1 = \frac{e^{-D_1/2}}{1 + e^{-D_1/2}}$$

This is the probability that fit model 1 is correct. This might be calculated using a user-defined formula in MARS. The above expression generalizes to the following expression in case of M fit models:

$$p_i = \frac{e^{-D_i/2}}{\sum_{j=1}^{M} e^{-D_j/2}}$$

with D_j= 〖AICc〗 _j-min(AICc).

> **Note:** Please note that the Standard Curve Wizard in MARS offers the ability to automatically choose a fit model (by clicking *Propose fit model*). The selection is based on the calculation of the AICc. It fits various models to the data and suggests the fit model whose AICc is minimal.

We have added multiple fit quality metrics that all have their strengths and weaknesses. The R^2 adjusted is similar to the R^2 but in contrast to this, the R^2 adjusted penalizes more complex models by incorporating the degress-of-freedom df (i.e. number of data points N minus number of fit parameters P). It is calculated as follows:

$$R^2_{adjusted} = 1 - \frac{SSE}{SST} \cdot \frac{N-1}{N-P}$$

where SST denotes the sum-of-squares total (squared deviation from the mean), SSE, N and P are the same as in the equation above. The term 1-SSE/SST corresponds to the well-known R^2. The denominator N-P corresponds to the degrees-of-freedom df. The smaller df (i.e. the more complex a fit model), the larger the right term in the equation and thus the smaller the R^2 adjusted. The term SSE/df is also known as the mean-squared error (MSE) and is itself often used as fit quality metric. Also, the square root form of MSE, the root mean squared error (RMSE) can be chosen as a fit quality metric output in MARS or might be applied for the fit model comparison in the standard curve wizard.

4.31 Literature

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4.32 Outlier identification methods

4.32.1 Introduction

For our purpose we will use the following definition of an outlier: An outlier is a data point that appears out-of-range compared to the other data points in the same data set. While this definition is rather general and non-mathematical there are various mathematical procedures described in the literature in order to identify outlying observations. They are mostly based on the assumption of normally distributed data (thus, these methods are sometimes referred to as parametric methods), i.e. data that follows a Gaussian distribution. However, there also exist methods to identify outliers which do not necessary require normally distributed data. In the following we will learn about the Grubbs-test, the Dixon-Q-test, the *generalized extreme Studentized deviate* (GESD)-test and one non-parametric outlier test, namely the Tukey-test. All these methods are implemented in MARS and might be used to remove one or multiple outliers in a data set as well remove outlying kinetic curves (more details, see below).

4.32.2 Parametric outlier identification methods

In what follows, parametric outlier identification methods will be explained.

Grubbs-test

The Grubbs-test is a very common and an easy to apply outlier detection method. It calculates a test statistic which is called the Grubbs-value (*GV*) in MARS which is based on the mean(y) and standard deviation s_y of the data set:

$$GV = \frac{|y_i - \bar{y}|}{s_y}$$

The further a data point y_i is away from the mean, the bigger the Grubbs-value. The calculated *GV* is compared to a tabulated Grubbs-value *g* in order to conclude if the corresponding data y_i is an outlier or not. The tabulated Grubbs-value is automatically calculated by MARS and depends on the number of data points and the confidence level used for the test (default confidence level is 95 %). If *GV>g*, the corresponding data point can be considered an outlier.
Please note that the Grubbs-test is rather useful for bigger sample sizes (i.e. with *N* > 30 data points). If *N* is smaller than 30 the Dixon-*Q*-test will be performed (by default). The Dixon-*Q*-test test will be discussed below.

Dixon- *Q*-test

The Dixon- *Q*-test is a very simple test that can be easily computed by hand. Therefore, the data is sorted with y_1 being the smallest and y_n being the largest value. Then a test statistic *Q* is calculated according to one of the following formulas:

$$Q = \begin{cases} 3 \le n \le 7: & \frac{y_2 - y_1}{y_n - y_1} \text{ or } \frac{y_n - y_{n-1}}{y_n - y_1} \\ 8 \le n \le 10: & \frac{y_2 - y_1}{y_{n-1} - y_1} \text{ or } \frac{y_n - y_{n-1}}{y_n - y_2} \\ 11 \le n \le 13: & \frac{y_3 - y_1}{y_{n-1} - y_1} \text{ or } \frac{y_n - y_{n-2}}{y_n - y_2} \\ 14 \le n \le 30: & \frac{y_3 - y_1}{y_{n-2} - y_1} \text{ or } \frac{y_n - y_{n-2}}{y_n - y_3} \end{cases}$$

The first formula (in front of the *or*) calculates the *Q*-value for a suspicious value at the lower end while the second formula (behind the or) calculates the *Q*-value for a suspicious value at the upper end. Please note that the Dixon-*Q*-test only applies to the range of 3 to 30 data points. Above 30 it is recommended to perform the Grubbs-test or the GESD-test.

GESD-test

The GESD-test is like the Grubbs-test. In fact, the Grubbs-test is a special case of the GESD-test. Unlike the Grubbs-test, the GESD was designed to remove multiple outliers from a data set. Only a pre-defined number *r* of suspected outliers needs to be entered by the user and the algorithm calculates r different Grubbs-values GV_j. In each of the *r* iterations the value y_j that is farthest away from the mean(y) will be removed and the Grubbs-test will be performed using the residual (*n-j*)-values. Finally, the number of outliers in the data set will be determined by the (last) index $j \leq r$ for which $GV_j > g_j$ where g_j denotes the critical Grubbs-value for *n-j*+1 data points.

Please note that information about the maximum number of suspected outliers goes into the GESD-test. This makes it more suitable to identify multiple outliers that might otherwise mask each other and might not be detected when applying the Grubbs-test sequentially.

Please also note that the GESD-test is recommended for larger samples sizes (i.e. for *n*>30) as is the Grubbs-test.

4.32.3 Non-Parametric outlier identification methods

Tukey-test

MARS includes another outlier test which stems from a "Box-Plot rule" by Tukey. Thus, we call this test the Tukey-test. For this test the data is sorted and the first Q_1 and third Q_3 quartile (i.e. the number of data points below Q_1 account for 25 % of the data and all below Q_3 account for 75 % of the data) is estimated. The so-called interquartile range, i.e. $IQR=Q_3-Q_1$, contains 50 % of the data and is some sort of spread metric for a given data set. The "Box-Plot rule" by Tukey says that any value *x* outside the two fences $Q_1-1.5 \cdot IQR$ and $Q_3+1.5 \cdot IQR$ indicates an outlying observation:

$$x \text{ is an outlier if } \begin{cases} x < Q_1 - 1.5 \cdot IQR & \text{or} \\ x > Q_3 + 1.5 \cdot IQR \end{cases}$$

Figure 1 shows the boxplot for a data set of 25 datapoints with one outlying observation (orange square). The shaded box represents the *IQR* and the whiskers (fences) represent the minimum and maximum of the data set without the outlier, respectively. The line inside the box represents the median of the data set.

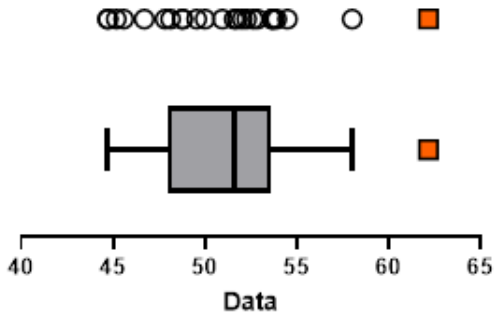


Figure 1: Data set with 25 data points and one outlying observation (square, top). The corresponding Tukey box-plot of this data is shown at the bottom.

In order to use the Tukey-test in MARS, the number of replicates must be at least five. Please note that the value 1.5 can be edited in MARS under *Formats and Settings* → *General* → *Calculations*.

The Tukey-test is the default method if (at least five) replicate kinetic curves shall be compared based on their distances to the average curve. These distances are calculated based on the so-called Minkowski distance:

$$d(y,m) = \left(\sum_{i=1}^{\#\,cylces} |y_i - m_i|^p \right)^{1/p}$$

Herein, *y* denotes one of the replicate kinetic curves and m denotes the average curve that is obtained by averaging all replicate curves. *i* denotes the i^{th}-cylce and *p* the exponent for the distance calculation. For *p*=1 (default), the distance is often referred to as Manhattan distance. For *p*=2, the distance is often referred to as Euclidean distance. This exponent can be edited in MARS under Formats and *Settings* → *General* → *Calculations*. For five replicate curves this results in the calculation of five distances that are used as input to the Tukey-test. If one curve is far away from the average curve, their *d* – value is pretty high compared to the other curves and will thus be identified as an outlier (see also Figure 2).

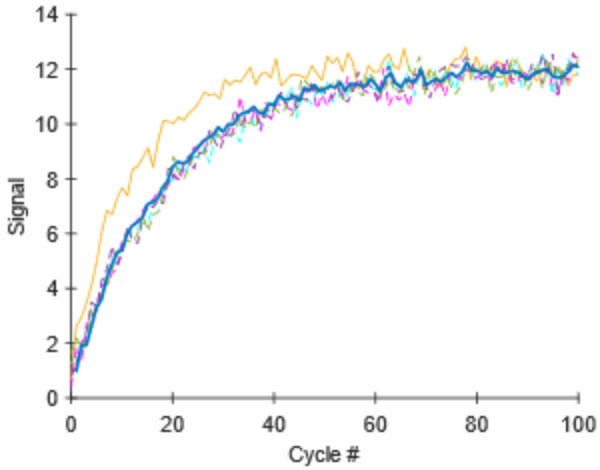


Figure 2: One curve (orange) out of five kinetic curves is far away from the average curve (blue solid line) leading to a big distance value d compared to the other curves. This curve will be identified as an outlier by the Tukey-test.

If one or more curves contain spikes, it might be worth thinking about performing a *curve smoothing (e. g. MAD smoothing)* first and perform the outlier identification after wards.

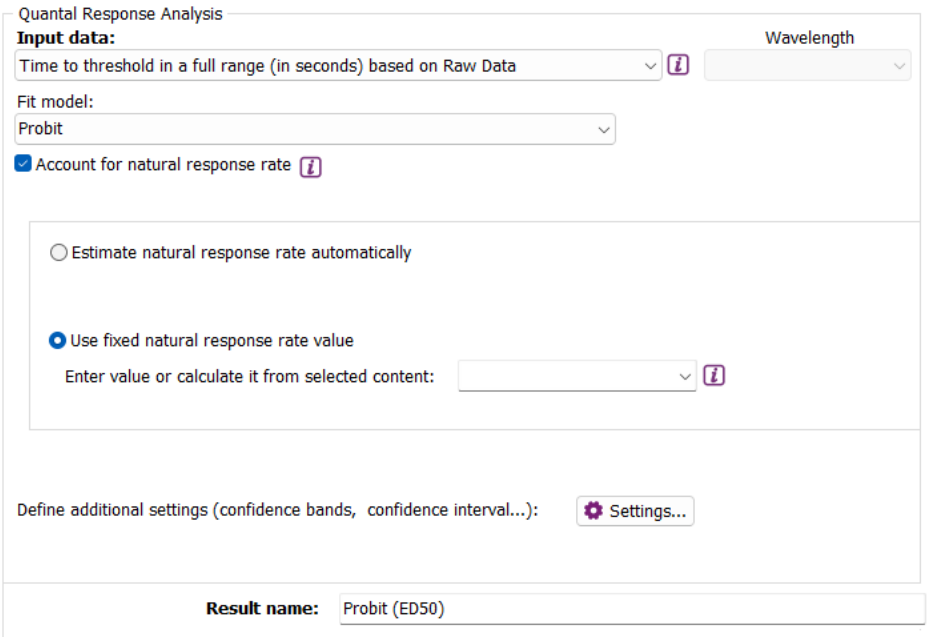
Note: Please note that the Grubbs-test, the Dixon-Q-test and the Tukey-test might be applied sequentially. In this case the test is performed a first time, calculating the required test statistic and removing all the identified outliers. It is applied a second time, calculating all the required test statistics from the residual data and removing all identified outliers. This procedure is continued until no more outliers are identified. If you think that outliers could potentially mask each other it might be better to perform the GESD-test.

4.33 Literature

- Dixon, W. J. (1953). Processing Data For Outliers. Biometrics, S. 74-89.
- Grubbs, F. E. (1950). Sample Criteria for Testing Outlying Observations. The Annals of Mathematical Statistics, S. 27-58.
- Grubbs, F. E. (1969). Procedures for Detecting Outlying Observations in Samples. Technometrics, S. 1-21.
- Iglewicz, B., & Hoaglin, D. C. (1993). How to Detect and Handle Outliers. ASQC Quality Press.
- Rorabacher, D. B. (1991). Statistical Treatment for Rejection of Deviant Values Critical Values of Dixon Q Parameter and Related Subrange Ratios at the 95 percent Confidence Level. Analytical Chemistry.
- Tukey, J. W. (1977). Exploratory data analysis. Addison-Wesley Pub. Co.

4.34 Quantal Response Analysis

The quantal response analysis page is only enabled if the layout of the test run contains standards. In addition, the input data for the quantal response analysis must be binary (0 and 1). You can get binary data with the Validation calculation or with the Kinetic Calculation Time To Threshold. A detailed description of the quantal response analysis can be found in the chapter 4.34: Quantal Response Analysis.



Input data: Select the input data for the quantal response analysis. Only data with binary result data are available in this list.
Wavelength: If the selected input data has more than one measured wavelength, it is possible to select the wavelength on which the calculation should be performed. If you select the entry *All* in the list, the calculation will be performed for each wavelength.
Fit model: Select the fitting model for the quantal response analysis. Each fitting model has a set of parameters that describes the fit result.
Available models:
Probit
Logit
Spearman-Kärber
Reed-Muench
Dragstedt-Behrens
Most Probable Number (MPN): for more details on this fit models read the chapter Quantal Response.

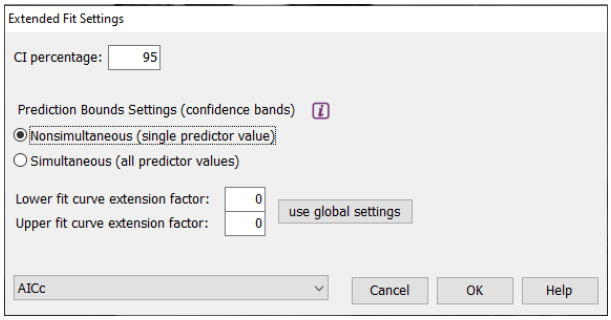
Apply trimmed Spearman-Kärber analysis (only if the selected fit model is Spearman-Kärber):

Account for natural response rate: Sometimes quantal dose-response curves do not approach zero at low doses, e.g. one may find nonzero responses in the (neg.) control group (natural response rate). Tick the checkbox to account for that.
If 'account for natural response rate' is checked, you can define further settings with the following controls :
Estimate natural response rate automatically: The natural response rate will be estimated automatically.
Use fixed natural response rate value *(only if the selected model is Probit or Logit)*: Use a fix response rate based on the entered value or the selected content:

> **Enter value or calculate it from selected content:** Select a content for the baseline or enter a baseline value between 0 and 1

Confidence Interval Settings and additional fit Settings

For all fit models, confidence intervals and confidence band are calculated automatically. Confidence intervals are calculated for each standard and for each fit result parameter. The confidence band can be displayed in the quantal response curve chart The confidence values for the fit result parameters are displayed as two additional columns in the fit result table.
To define or change the confidence bands and interval settings, click on the settings button to open the setting dialog for confidence bands and intervals:



CI percentage: Define the percentage value for the confidence interval calculation. A lower value results in a wider interval band.
Prediction Bounds Settings: Select between the different settings:
Non-simultaneous or Simultaneous Bounds: Provides a confidence interval using a single predictor value (non-simultaneous) or all predictor values (simultaneous). In general, simultaneous intervals are wider than non-simultaneous intervals, because of the additional uncertainty of bounding values for all predictors.
Use the ***Lower fit curve extension factor*** and the ***Upper fit curve extension factor*** to define how far the fit curve is drawn below the smallest standard and above the highest standard. Press ***use global settings*** to reset the values to the global extension factors (defined in the Calculation Settings Dialog).

Output fit quality metric:

In addition to the fit quality metrics R^2 and AICc (Akaike's information criterion corrected) that are always output, you can define further quality metrics to be added to the output in the fit results.
See the whole list of possible metrics:

Shortcut	Quality Metric
AICc	Akaike's information criterion, corrected for small sample sizes
AIC	Akaike's information criterion
BIC	Bayesian information criterion
McFadden R^2	McFadden R^2 is a modification of R^2 that adjusts for the number of predictors in a model.
Nagelkerke R^2	Nagelkerke R^2 is a modification of McFadden R^2 that scales it to a maximum value of 1.
CHI^2	Chi^2 is another fit quality metric.
CHI^2 pValue	The p-value is a measure of the probability that an observed difference between two variables could have occurred by chance . In the context of Chi^2 test, it is used to determine whether an observed distribution differs from an expected distribution .
Model deviance	Deviance is a goodness-of-fit statistic for a statistical model. It is a generalization of the idea of using the sum of squares of residuals (SSR) in ordinary least squares to cases where model-fitting is achieved by maximum likelihood
Model significance	Model significance refers to the statistical significance of a regression model's independent variables.

4.34.1 Fit Result

After performing a parallel line analysis, the fit result can then be inspected on the quantal response curve chart.
If a fit fails for a group of standards of the test run, the hint *Calculation of fit parameters not possible* appears on the detailed window for the fit result and on the fit result window.

4.34.2 Introduction

Quantal responses typically evolve as a result of quantal dose-response assays. In contrast to conventional dose-response assays, quantal dose-response assays return discrete data that are typically in binary format (e.g. dead/alive, 0/1 etc.) and not (quasi-) continuous as in the first case. Therefore, they require more of a special statistical treatment. Here we describe nonparametric and parametric methods used to analyze these quantal dose-response assays, namely the Spearman-Kärber, Reed-Muench and Dragstedt-Behrens method as nonparametric methods and logit and probit regression as parametric analysis methods all returning at least the effective dose 50 (ED50). All these methods are available in MARS. The core difference between non-parametric and parametric methods is that the former ones make no assumptions about the underlying distribution of the data, while the latter ones do. Spoken in practical terms one will obtain a fit curve for the parametric methods but not for the non-parametric methods. Both, the parametric and non-parametric methods have their advantages and disadvantages.
In a typical quantal response assay the biological system of interest (e.g. cells) is treated with two or more doses of a preparation/substance of interest and the response to each treatment is either present (1) or absent (0). These assays apply multiple replicates per dose and the (binarized) output e.g. might look like this:

		1	2	3	4	5	6	7	8	9	10	11	12
A	Standard Concentrations	100E-12	1E-9	10E-9	100E-9	1E-6	10E-6	100E-6	1E-3	100E-12	1E-9	10E-9	100E-9
	Binary output	0	0	0	0	1	1	1	1	1	0	0	1
B	Standard Concentrations	1E-6	10E-6	100E-6	1E-3	100E-12	1E-9	10E-9	100E-9	1E-6	10E-6	100E-6	1E-3
	Binary output	1	0	1	1	0	0	0	0	1	1	1	1
C	Standard Concentrations	100E-12	1E-9	10E-9	100E-9	1E-6	10E-6	100E-6	1E-3	100E-12	1E-9	10E-9	100E-9
	Binary output	0	0	0	0	1	1	1	1	0	1	0	0
D	Standard Concentrations	1E-6	10E-6	100E-6	1E-3	100E-12	1E-9	10E-9	100E-9	1E-6	10E-6	100E-6	1E-3
	Binary output	1	1	1	1	0	1	0	1	0	1	1	1
E	Standard Concentrations	100E-12	1E-9	10E-9	100E-9	1E-6	10E-6	100E-6	1E-3	100E-12	1E-9	10E-9	100E-9
	Binary output	0	0	0	1	0	0	1	1	0	0	0	0
F	Standard Concentrations	1E-6	10E-6	100E-6	1E-3	100E-12	1E-9	10E-9	100E-9	1E-6	10E-6	100E-6	1E-3
	Binary output	1	1	1	1	0	0	0	0	1	0	1	1
G	Standard Concentrations	100E-12	1E-9	10E-9	100E-9	1E-6	10E-6	100E-6	1E-3	100E-12	1E-9	10E-9	100E-9
	Binary output	0	0	0	0	1	1	1	1	0	1	0	0
H	Standard Concentrations	1E-6	10E-6	100E-6	1E-3	100E-12	1E-9	10E-9	100E-9	1E-6	10E-6	100E-6	1E-3
	Binary output	1	1	1	1	0	0	1	1	1	1	1	1

The binary output (consisting of zeros and ones only) can be created in MARS as part of the Time to threshold calculation or the Validations calculation. Only when this binary output is explicitly created with one of the two calculation methods, will the Quantal Response Analysis feature in MARS be accessible in the Calculations menu. The small numbers in the first rows of the microplate image correspond to the doses (Standard Concentrations) and the binary values (second row) are the results of one of the aforementioned calculations in MARS. But what happens behind the scenes in MARS when executing the Quantal Response Analysis feature? This will be discussed in more detail below.

4.34.3 Spearman-Kärber, Dragstedt-Behrens and Reed-Muench

These three non-parametric methods were developed and used at times when computers were not available, yet. These methods are fairly simple and can be even done by hand with moderate effort. They still have their right to exist as they often still return a meaningful median effective dose (ED50) when the parametric methods (see section below) might fail. This mostly has to do with the iterative character of the parametric methods. The calculation of the non-parametric methods start as follows.

1. MARS calculates the total number n of responses per dose.

2. Calculate the number of "positive" responses (e.g. those with ones) r.

3. Calculate the empirical response rate for all k doses $p_i = r/n_i$ given the number of responding replicates r_i at dose x_i and the total number of replicates n_i tested at that dose.

4. Plotting all p_i versus their corresponding doses x_i gives the (empirical) quantal dose-response curve.

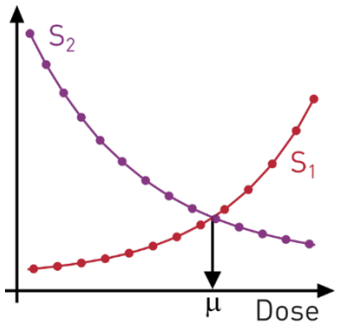
Please note that the x_i are typically defined as log doses. From here, the three methods perform different calculations. Let's first start with the calculations of the **Spearman-Kärber** method [1], [2]:

5. Calculate the median effective dose $\mu = \sum_{i=1}^{k} (p_{i+1} - p_i)(x_i + x_{i+1})/2$, given that all doses are (almost) equidistant and given p_1=0 and p_k=1, i.e. no replicates responds at the lowest dose x_1 and all replicates respond at the highest dose x_k. MARS has ways to deal with the situation if the latter conditions

are not met, e.g. by taking into account a baseline response using Abbott's correction, introducing a pseudo dose $x_0=2x_1-x_2$ with $p_0=0$ and/or $x_{(k+1)}=2x_k-x_{(k-1)}$ with $p_{(k+1)}=1$ (see [1]) or by applying the so-called trimmed Spearman-Kärber analysis (see [2]). The latter method being state-of-the-art as this also includes the original approach simply by setting the trim-value to zero.

The **Reed-Muench** method [3]:

5. Calculate: $S_1(r,x_i)=\sum_{j=1}^{i} r_j$ and $S_2(n-r,x_i)=\sum_{j=i}^{k}(n_j-r_j)$ for all doses x_i. Plotting these sums as a function of the doses, reveals a graph like this:



The intersection of the resulting two curves corresponds to the median effective dose μ. The intersection point is typically found by interpolation. Given it lies between doses x_i and x_(i+1) one can then calculate the median effective dose by:

$$\mu = x_i + \frac{d(S_2 - S_1)}{n_i - r_i + r_{i+1}}$$

Where $d = x_{(i+1)}-x_i$

The **Dragstedt-Behrens** method [4], [5]:

5. Given S_1 and S_2 from the Reed-Muench method, the Dragstedt-Behrens method calculates $P_i^*=\frac{S_1}{S_1+S_2}$ for each dose x_i and interpolates between the doses x_i and $x_{(i+1)}$ for which $P_i^*<0.5$ and $P_{i+1}^*>0.5$ (if not at one dose P_i^* is exactly 0.5).

While the Spearman-Kärber method additionally provides confidence intervals for the median effective dose, the Reed-Muench and Dragstedt-Behrens don't, or at least only under very limited circumstances (e.g. huge number of replicates etc., see [4] for more details).

Note: The x_i values are mostly logarithmized doses. Thus, we talked about doses instead of doses. MARS takes care of the appropriate logarithmization internally.

4.34.4 Logit and probit regression

These two methods are parametric methods meaning they make assumptions of the underlying distribution of the data. Looking at point 4 in the previous section, we calculated the ratio of the number of positive replicates r_i at dose x_i to the total number of replicates tested at that dose: $p_i=r_i/p_i$ for all doses. These ratios are essentially (empirical) probabilities stating how many replicates will respond on average to the given dose. The underlying probability mass function for some r, given the total number of replicates n per dose is a binomial:

$$P(r)=\frac{n!}{r!\,(n-r)!}P^r(1-P)^{n-r}$$

In a similar way that conventional least-square regression assumes the data to be normally distributed, for quantal response assays the assumption is rather that the data is binomially distributed. So conventional least-square regression does not apply in these cases. However, iteratively reweighted least-square (IRLS) or maximum-likelihood are often applied for this purpose. But how does the functional form of the quantal dose response curve look like? Here is where the logit and probit model differ. The logit model assumes a logistic model:

$$f(x)=\frac{1}{1+\exp\left(-(b_1+b_2x)\right)}$$

And the probit model assume the following model:

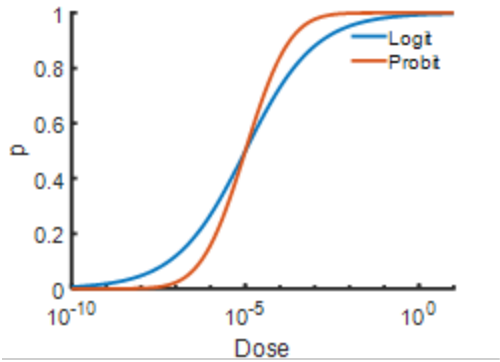
$$f(x)=\Phi(b_1+b_2x)$$

Where *x* is typically the logarithm of the dose, Φ the cumulative standard normal distribution function and b_1 and b_2 are model parameters that are iteratively optimized during the fit. These are related to the median effective dose 50 (ED50) by $log_{10}ED_{50}=b_1/b_2$ given the doses are log doses (which is often the case).

Please note that the expression $b_1+b_2 x$ in the brackets of the model functions is a linear function of *x* and so logit and probit regression are typically treated within the framework of generalized linear models (GLM). That's also how MARS performs the corresponding analyzes.

4.34.5 Natural response rate

Both *f(x)* (see former section) go from zero to one (see figure below).



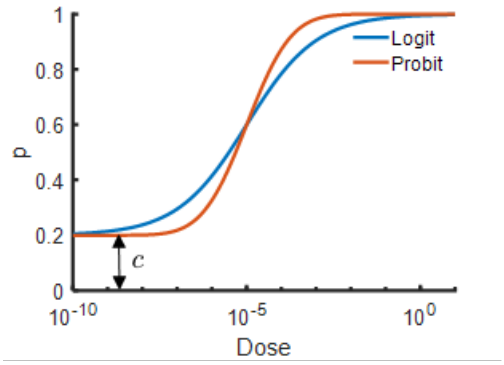
However, under experimental conditions there are sometimes responses, even without having applied any preparation. That means that even responses occur in negative controls (zero-dose). In these cases the aforementioned two models need to be adapted, i.e. for the logit model:

$$f(x) = c + \frac{1-c}{1+\exp\left(-(b_1+b_2 x)\right)}$$

And for the probit model:

$$f(x) = c + (1-c)\Phi(b_1+b_2 x)$$

Where c denotes the natural response rate that lies between 0 and 1 (see figure below).



Often *c* is simply calculated from the response rate *pNC* of the negative control group, or *c* is already known from previous experiments. Alternatively, *c* might be estimated as a third fit parameter within the fitting procedure. All these cases can be handled in MARS by checking the corresponding natural response rate option.

If either the Spearman-Kärber or the Dragstedt-Behrens or the Reed-Muench method are selected together with the Account for natural response option, then MARS performs a so-called Abbott-correction of the p_i-values:

$$p_i^* = \frac{p_i - c}{1-c}$$

Subsequently, MARS uses these corrected p_i-values for the corresponding analysis. If, however, the logit or probit model are selected, together with the aforementioned natural response rate option, MARS either inserts the (fix) c-value into the corresponding model equation (given the Use fix natural response rate value option was selected) or estimates this natural response rate during the fitting procedure (given the Estimate natural response rate automatically option was selected).

4.34.6 Most Probable Number (MPN)

The most probable number (MPN) analysis is used to analyze the number of bacteria in a water sample to reveal the bacterial density (i.e. in counts per volume). In practice it can be difficult to count the bacteria directly. It is thus common to set-up a dilution series of the bacterial sample with multiple replicates at each dilution step in dedicated microplate wells. The actual measurement then reveals whether there was growth in the wells [+] or not [-] (see figure below).

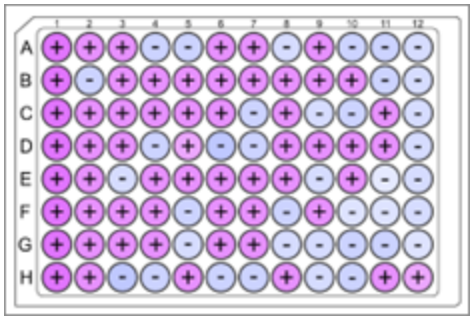


Figure 1: In assays estimating the MPN, the output is of quantal type with either wells being positively (+) and negatively (-) populated.

The analysis thus deals with quantal data and is thus found in MARS under the Quantal Response Analysis calculation. The assumption of the MPN analysis is that whenever there is one or more bacteria in a dilution, it exhibits growth. Another assumption is that the bacteria are randomly and homogeneously distributed in the sample (an assumption which might be violated to some extent in practice).

Let us assume we have a liquid of volume V having a (yet unknown) bacterial density of $\delta = {}^{N}/_{V}$. From this, we take a sample of volume v. The probability that our sample does not contain any bacterium, i.e. it is sterile, is:

$$p = \left(1 - \frac{v}{V}\right)^{N}$$

Where $\frac{v}{V}$ denotes the probability to have at least one bacterium in the sample. If v were equal to V it would be merely impossible to not have any bacterium in the sample (i.e. p=0). Typically, v is small compared to V and $\left(1 - \frac{v}{V}\right)^{N}$ can be approximated by an exponential:

$$p = \left(1 - \frac{v}{V}\right)^{N} \approx e^{-v\frac{N}{V}} = e^{-v\delta}$$

The probability of a sample being fertile is 1-p.
Let's assume we took n samples each of volume v. The probability of observing s sterile and n-s fertile samples is:

$$P(s|\delta) = \binom{n}{s} p^{s}(1-p)^{n-s} = \binom{n}{s} e^{-sv\delta}\left(1 - e^{-v\delta}\right)^{n-s}$$

This probability is a function of the bacterial density δ that has a maximum at the most probable number of δ. We can find δ by maximizing the log-likelihood of this function (while ignoring the binomial coefficient $\binom{n}{s}$):

$$\frac{(n-s)ve^{-v\delta}}{1 - e^{-v\delta}} = sv$$

However, as we have multiple dilutions with multiple replicates (since the likelihood of all samples being fertile or all samples being sterile is reasonably high when only working with a single dilution level) we come-up with a more general equation:

$$\frac{(n_1 - s_1)v_1 e^{-v_1\delta}}{1 - e^{-v_1\delta}} + \frac{(n_2 - s_2)v_2 e^{-v_2\delta}}{1 - e^{-v_2\delta}} + \cdots = s_1 v_1 + s_2 v_2 + \cdots$$

This is the equation whose root needs to be found to get the most likely value of the most probable number δ.

4.34.7 References

[1] M. A. Hamilton, R. C. Russo and R. V. Thurston, "Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays," Environmental Science & Technology, p. 714–719, 1977.

[2] D. J. Finney, Statistical Method in Biological Assay, Hafner, 1952.

[3] A. Carter, "USING THE SPEARMAN-KARBER METHOD TO ESTIMATE THE ED50".

[4] D. J. Finney, Probit Analysis, Cambridge University Press, 1971.

[5] L. Reed and H. Muench, "A simple method of estimating fifty per cent endpoints.," The American Journal of Hygiene., pp. 493-497, 1938.

[6] C. Spearman, "The Method of “Right and Wrong Cases” (Constant Stimuli) without Gauss’s Formula.," British Journal of Psychology, p. 227–242, 1908.

[7] G. Kärber, "Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche," Archiv für experimentelle Pathologie und Pharmakologie, pp. 480-483, 1931.

[8] B. Behrens, "Zur Auswertung der Digitalisblätter im Froschversuch," Naunyn-Schmiedebergs Archiv für experimentelle Pathologie und Pharmakologie, p. 237–256, 1929.

[9] C. A. Dragstedt and V. Lang, "RESPIRATORY STIMULANTS IN ACUTE COCAINE POISONING IN RABBITS," Journal of Pharmacology and Experimental Therapeutics, pp. 215-222, 1928.

5 Using Templates

Templates in MARS are a powerful tool to transfer settings, performed calculations and even the result of a standard curve fit to other test runs.
The templates are based on the settings of a test run as they contain all the information needed for the transfer.
In combination with test run protocols, you can get a quick result and report of your performed test without any manual action in MARS needed (see section ***Why Assign Templates to Protocols?*** below)
Individual buttons can be created for up to six templates, giving the possibility to change quickly between different views for one test run.
The software comes with a set of predefined templates matching to the predefined protocols for the readers control software. It is possible to use these templates to see how they work.
If you want to use your own templates, you must start by creating a template.
The next step could either be assigning the template to another test run or to a protocol (read more about assigning a template to a protocol in the chapter 5.2: *Manage Templates*).
If you want to use the template often, you can create a button for that template in the *Predefined Templates* group on the *Home* tab and on the *Templates* tab of the ribbon and give it a name you like. Details are described in the chapter 5.5: *Template Buttons*.
If you have a test run with standards and you want to use these standards to calculate the concentration values of samples in a different test run, you can do this using a template. Read how this works in the chapter 5.6: *Transfer of Standard Fit Results*.
If you have a test run with blanks and you want to use these blank values to calculate blank corrected values in a different test run without measured blanks, you can do this using a template. Read how this works in the chapter 5.7: *Transfer of Blank Values*.
You will soon have a large number of templates. To keep track of your templates, delete or exchange them for others (export / import templates), use the manage templates window.
To parametrize the template, you can use variables. You can decide if the value for the variable will be asked, each time the template is used, or if a manually entered default value is used. Read more about variables in chapter 4.2.1: *Define and Use Variables*.

> **Note:** Templates are not the tool to transfer modified layouts from one test run to one other. Use the Manage Layout functions to exchange layouts between test runs.

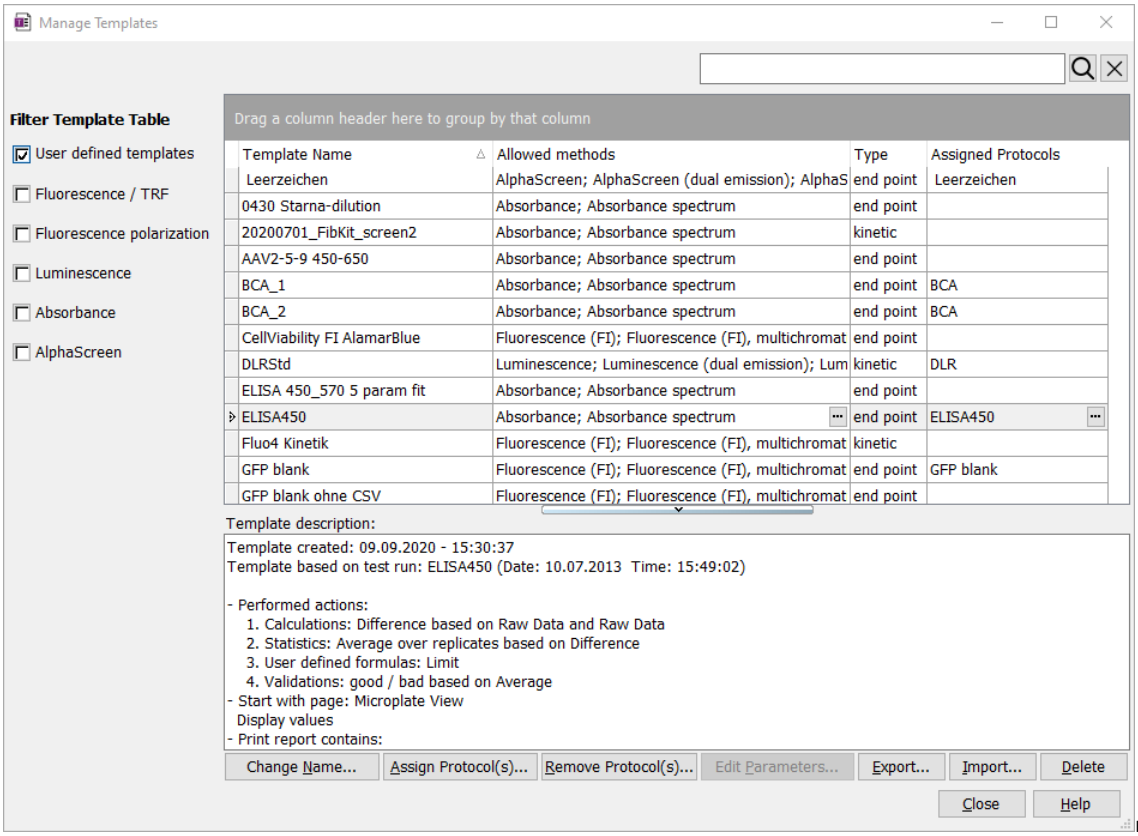
5.1 Why Assign Templates to Protocols?

In many cases the templates will be used more than once and are often applied to test runs, based on the same test run protocol defined with the BMG LABTECH control software for the reader.
If you do not want to assign a new template to each new test run, and equally do not want to add the calculations manually each time you can overcome this by assigning a template to a test protocol.
Assign the template to the test run protocol and for these test runs the template will automatically be assigned to any new performed test run based on that protocol. When the new test run is opened for the first time in MARS the settings defined in the template will be activated.
It is also possible to assign more than one protocol to a template.
To manage the templates and the assigned protocols use the Manage Templates window.

5.2 Manage Templates

To see the existing templates, open the Manage Templates window. This can be done by clicking *Templates* in the *Manage Templates* group on the *Templates* tab of the ribbon.
The window will open and will show the following functions:

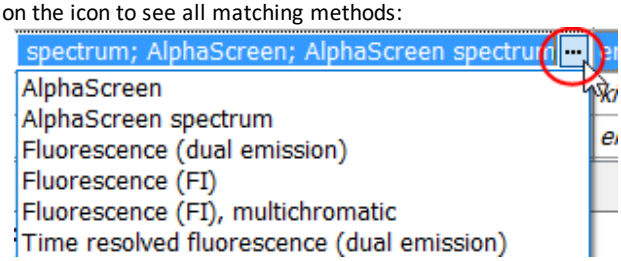
- A list of all defined templates.
- A description of a selected template
- Assigned protocols to the template
- Function to assign and remove protocols from the template
- Function to export and Import templates
- Function to delete a template
- Filter controls to filter the list of displayed templates
- Search field to search for templates in the list



mana

5.2.1 List of Templates

The window shows a table with all available templates listed. Use the filter controls on the left side to see only templates matching the selected measurement method.
The table contains four columns:
Table name: Shows the name of the template.
Allowed methods: The first matching condition: Shows the measurement methods that match the template. The column with the allowed methods can contain a small icon with three dots. This icon appears if the list of matching methods is longer than the column. Click on the icon to see all matching methods:



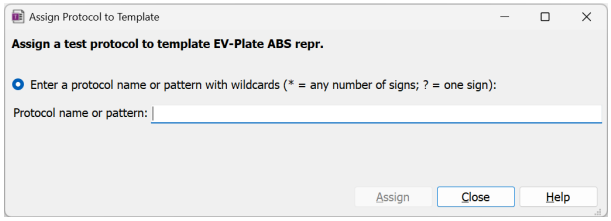
Type: The second matching condition: Shows if it is an end point or a kinetic test run template.
Assigned Protocols: Shows the names of the protocols, assigned to the template. This column can also contain the icon with the three dots. Click on it to open a list showing all assigned protocols.
A template can be selected from the list.
The **template description** contains a description of the performed calculations and the settings that will be set if the template is assigned to a test run.

5.2.2 Change Template Name

To change the name of an existing template, select the template and click on *Change Name...*. A small window opens to enter the new template name.

5.2.3 Assign Protocols to Templates

After selecting a template, press the *Assign To Protocol(s)* button, to enter a protocol name, or a pattern with wild cards. If the protocol name matches the pattern, the template will be assigned to the test run, based on that protocol. Possible wild cards are * (replaces any number of signs) and ? (replaces one sign). This gives you the possibility to assign a template to all test runs created with different protocols, containing the same pattern in their name.



5.2.4 Removing Assigned Protocols from the Template

Select a template in the list of templates using the *Manage Templates* window. If there is at least one protocol in the assigned protocols column, the *Remove Protocol(s)* button can be used to eliminate the template link from the protocols.

5.2.5 Edit Parameters

If the selected template contains user defined variables, the *Edit Parameters* button is enabled. Clicking the button opens a dialog, where the variable in the template can be change (default values, minimum or maximum values...). An explanation of all different kind of variables and how to use them in calculations and templates can be found in chapter 4.2.1: *Define and Use Variables*.

5.2.6 Export and Import Templates

To export templates, select the templates you want to export from the list of templates in the *Manage Templates* window and press *Export...*.
A file window will open to let you select a file destination for the saved file containing the exported templates (the look and feel of the file window depends on the operating system!).
Enter a file name for the file and press *Save*. The generated file gets the extension .mtf. Files with this extension are recognized by MARS as exported templates.
To import templates, they must be exported by MARS (e.g. on another PC) and the file must have the extension .mtf.
Press the *Import...* button to open a file window similar to the one above. Select the location of the exported file and select the file. Press the *Open* button in the window to import the template(s) into the list.

5.2.7 Delete Templates

Select one or more templates from the list of templates shown in the manage templates window. To delete the selected templates, press the *Delete* button.

5.3 Create a Template

A new template can be created from the settings used in an open test run.
This template can then be assigned for use with other test runs that fulfill the two matching conditions:

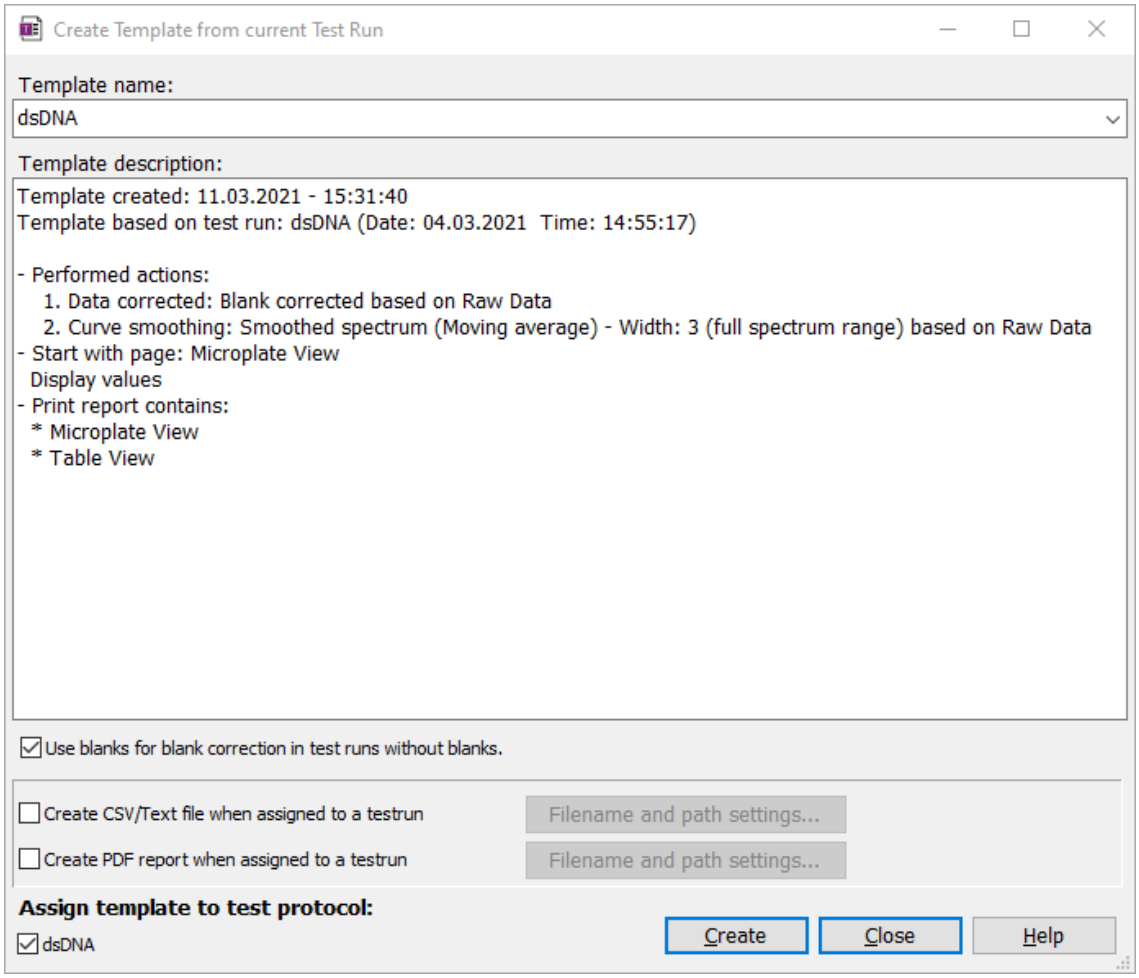
- The measurement method must be the same
- The read type must be the same. An endpoint template cannot be assigned to kinetic test run and vice versa.

To create a template for use with future test runs you must perform the test once and open it in MARS to create the template.
When creating a template, perform the following initial steps:

- Open the software and select a test run. The test run should be the same type as the test run/s you will assign the template to.
- Perform all the data analysis steps with the test run. When created, the template will then contain all the calculations, selections, view settings etc. of this test run
- Click *Create Template from Current Test Run*... in the *Use Templates* group on the *Templates* tab or select the menu item *Create Template* from the pop-up menu in the navigation tree.

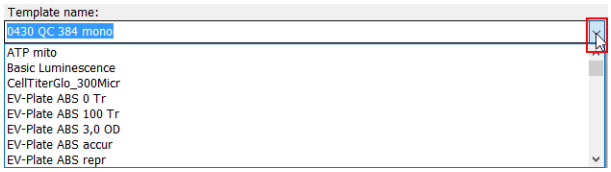
On completing the above steps the *Create Template* window will then open.

Create template window:



Template name

Enter the name of the template you want to create. The name must be unique as it will be used to find the template again. If you want to overwrite an existing template, you can select the template out of the drop-down list. To open the list, press the drop-down button:



Template description

This section describes the steps performed with the test run and what will be saved in the template. It is possible to expand the entries with your own explanation of the template. The description of the template will be displayed to allow the user to check the suitability of a template before assigning it to other test runs.

Use blanks for blank correction in test runs without blanks

If this control is checked, the average(s) of the blanks are stored in the template. If this template will be assigned to a test run with a layout with no blanks, a blank correction is performed based on the blank value saved in the template (blank transfer).

Create CSV/Text file / Create PDF report

If needed, a text (CSV) file and/or a PDF report file can be created based on the settings in the template, when the template is assigned to a test run. The settings for the file export or the PDF report can be defined by pressing the according *Filename and path settings...* button.

Assign Template to Test Protocol

A check box is shown at the bottom of the window along with the name of the test run protocol used to create the template. If you want to assign the new template directly to that protocol, leave the check box checked. If you don't want to assign the template to that protocol, uncheck the check box.

If you want to assign the template to another protocol, you can do this using the Manage Template window, after creating the template.

Press *Create* to generate the template. If you want to stop the creation of the template, press *Close*.

5.4 Assigning Templates

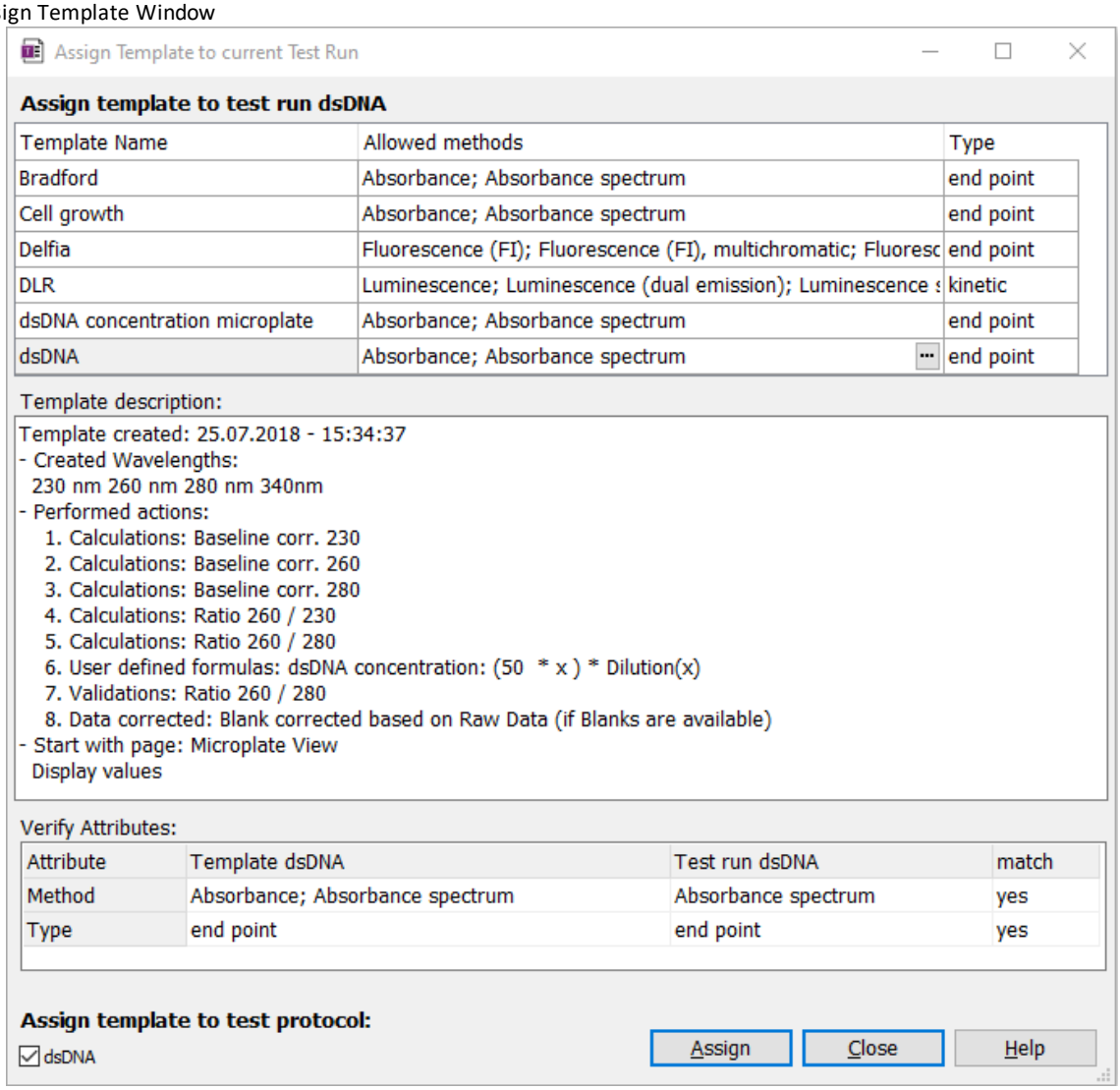
You can assign a template to a test run or to a protocol (see also chapter 5.1:*Why Assigning Templates to Protocols?*).
There are different ways to assign a template to different test runs or protocols:

5.4.1 Assign a Template to a Test Run

When assigning a template to a test run, the template will be used to create the settings for the test run. Templates can be applied to test runs manually if the test run is opened in MARS. The setting for that test run will also be saved automatically after successfully assigning a template to the run. The settings of the test run can still be changed after assigning a template. This is useful when using templates that perform only the first steps of your evaluation.
If a test run is signed (FDA 21 CFR part 11), you cannot assign a template to that test run.
There are four ways in which a template can be assigned to a test run:

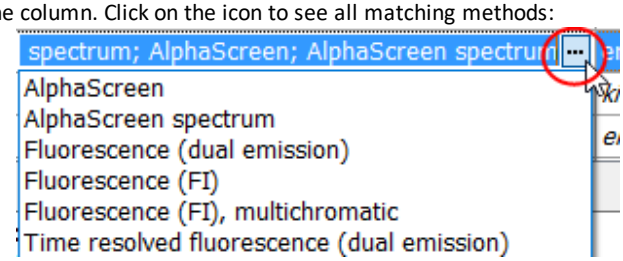
- When the test run is opened the first time in MARS and a template is assigned to the protocol of the test run.
- When you select the template, using the *Templates* control in the *Predefined Templates* group on the *Home* or the *Templates* tab (see Chapter 5.5: *Template Buttons*).
- When you have created a template button for that template and click on the button (see chapter 5.5: *Template Buttons*).
- When you click *Assign Template to Current Test Run...* in the *Use Templates* group on the *Templates* tab or the menu item *Assign Template* of the pop-up menu in the navigation tree and choose the template in the appearing assign template window.

Assign Template Window



On the top of the window the name of the current test run to which the template will be assigned is displayed.
Template list and template description
Click on a template in the table to view the description of the template in the template description area under the table.
The table has three columns:
Table name: Shows the name of the template.

Allowed methods: The first of the two matching conditions: Shows the measurement methods that match the template. This column shows the allowed methods and can contain a small icon with three dots. This icon appears if the list of matching methods is longer than the column. Click on the icon to see all matching methods:



Type: The second matching condition: Shows if it is an end point or a kinetic test run template.
The **template description** shows the steps the template will perform on the test run if you assign it.
Verify Attributes
This table shows the two matching conditions for the test run and the selected template that must be fulfilled to assign the template to that test run.
The last column of the table shows if the test run conditions match to the template:
yes: the combination is possible
allowed (with an orange background color): the combination is possible but with restrictions.
no (with a red background): the combination is not allowed.
If both criteria are fulfilled or at least allowed, the **Assign** button is enabled and can be pressed to assign the template to the test run.

> **Note:** If the template contains kinetic settings but the test run don't have kinetic data, the combination is allowed, but the kinetic settings will not be applied to the test run.

Assign template to test protocol
A check box is shown at the bottom of the window along with the name of the test run protocol used to create the template. If you want to assign the new template directly to that protocol, leave the check box checked. If you don't want to assign the template to that protocol, uncheck the check box.

Templates with variables
A template can contain variables. You can see if a template contains variables in the template description. Containing variables are shown with their variable name (like V1, V2...) and a description.
The variables in the template will be replaced by certain values if you assign the template to the test run. Depending on the kind of variable, the value will be asked to be entered or the defined default value is used.
Read more about variables in chapter 4.2.1: *Define and Use Variables*.

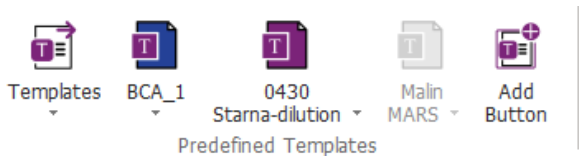
5.4.2 Assign a Template to a Protocol

There are three ways to assign a template to a protocol:

- When creating a template (see chapter 5.3: *Create a Template*).
- When assigning a template to a test run using the assign window (see Assign Template Window above).
- In the Manage Template window.

Read more about templates with protocols in the chapter 5: *Using Templates*.

5.5 Template Buttons



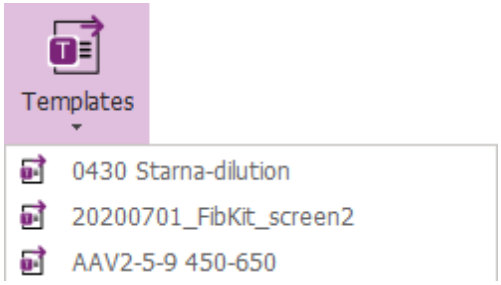
The *Predefined Templates* group on the *Home* tab and on the *Templates* tab comes with two default buttons for templates:

. This button gives quick access to all available templates for the current test run. See the section Templates Button below.

This button creates a new template button in the *Predefined Templates* group. The template buttons created provide quick and easy access to the templates most frequently used. See the section Add a User Template Button below.

5.5.1 Templates Button

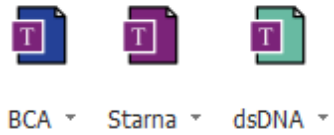
The *Templates* control in the *Predefined Templates* group on the *Home* tab and on the *Templates* tab shows a list of all templates available for use with the current test run. To open the list click on the button or on the small down arrow on the right side of the button:



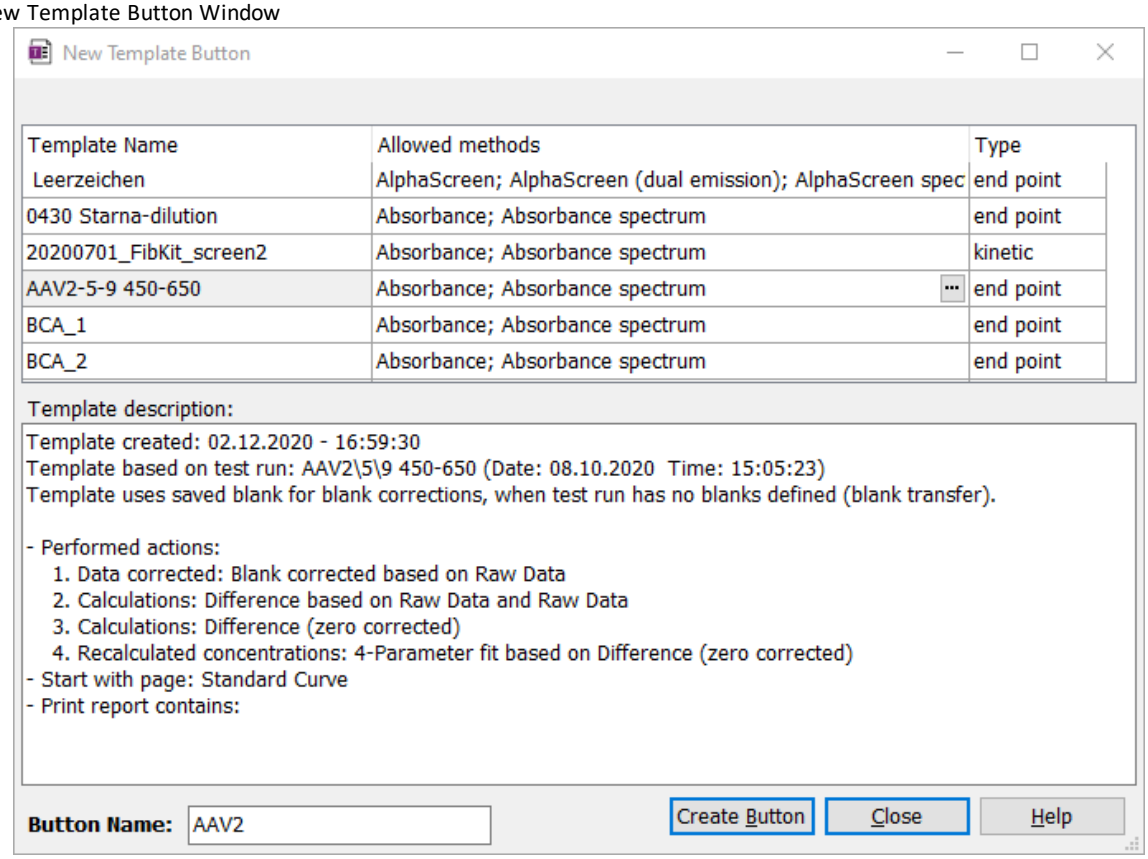
Click on a template in the list to assign that template to the current test run.
If no template is available for the current test run or if the current test run is signed the button is disabled.

5.5.2 Add a User Template Button

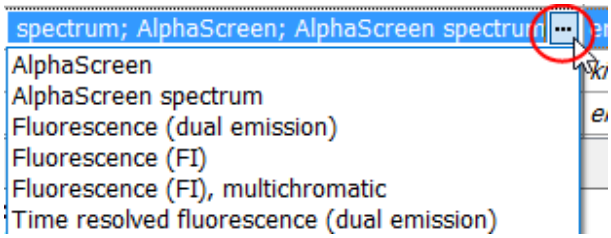
You can have up to six user template buttons in the *Predefined Templates* group. Each button has the same icon in a different color and an explaining text under the button:



The user button is enabled if the underlying template is assignable to the current test run. Otherwise the button is disabled. Pressing the button will assign the template to the current test run.
If there are less than six template-buttons (if you start the software first and you have never added a user button before, there will be no template buttons visible), a new button can be created by clicking *Add Button* in the *Predefined Templates* group:
New Template Button Window



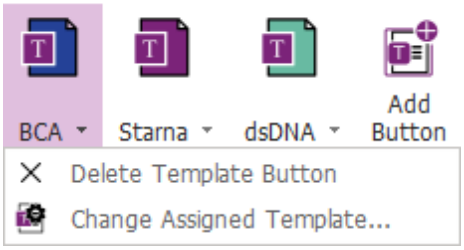
At the top of the window a table is shown with all available templates. The table consists of three columns:
Table name: Shows the name of the template.
Allowed methods: The first of the two matching conditions: Shows the measurement methods that match the template. This column shows the allowed methods and can contain a small icon with three dots. This icon appears if the list of matching methods is longer than the column. Click on the icon to see all matching methods:



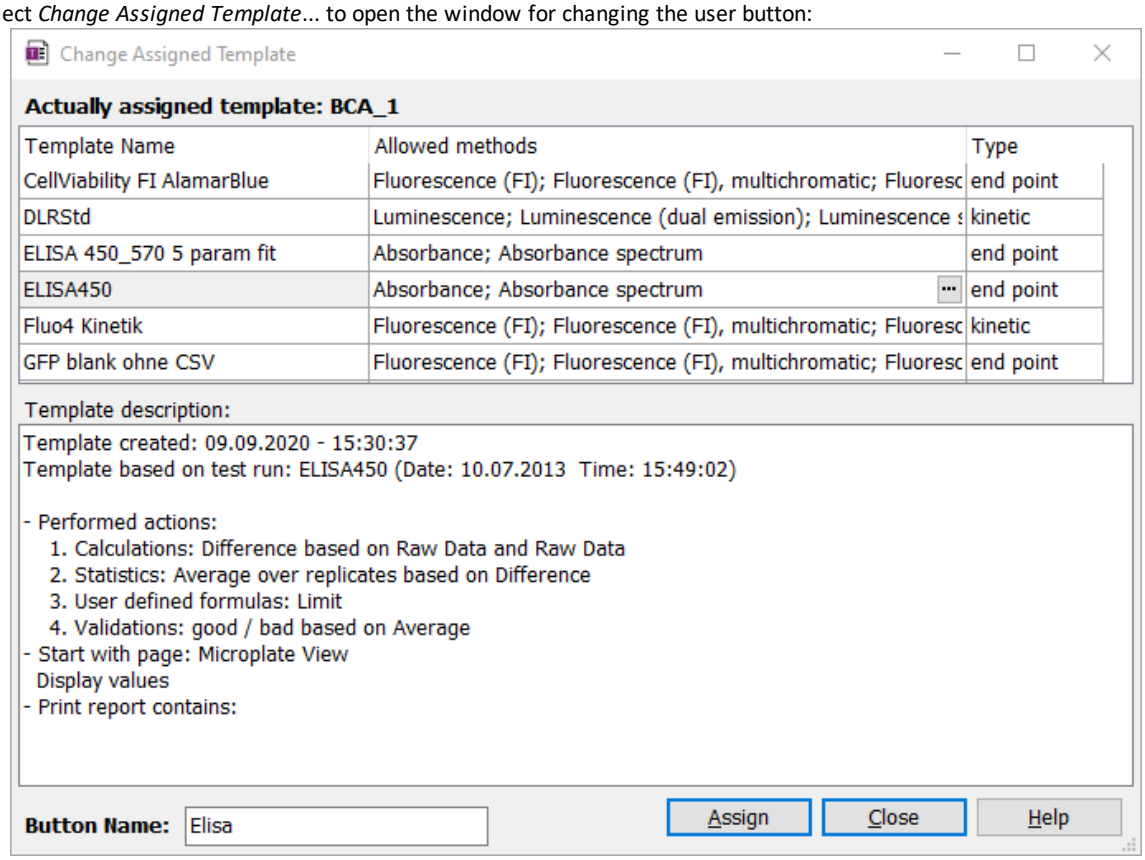
Type: The second matching condition: Shows if it is an end point or a kinetic test run template.
The **template description** shows the steps, the template will perform on the test run if you assign it.
At the bottom of the window you can enter a name for the button in the entry field **Button Name**. The entered name will appear under the button in the *Predefined Templates* group.
Select the template to be linked to the button in the table.
Click the *Create* button to add this button representing the selected template to the *Predefined Templates* group.
The new button will appear between the last user button created and the *Add Button* in the *Predefined Templates* group.

5.5.3 Changing and Deleting User Template Buttons

Templates associated with a user template button can be changed and user template buttons can be deleted.
Press the small down arrow on the right side of the button to see the two menu items with the mentioned functions.



Select *Delete Template Button* to delete the button.
Select *Change Assigned Template*... to open the window for changing the user button:

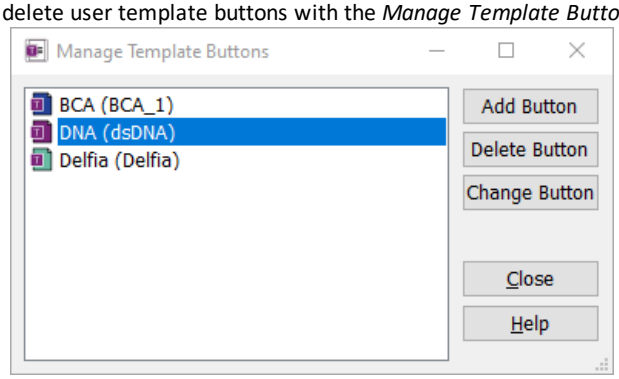


The window is the same as if a new template button is created (see New Template Button Window above). Additionally it shows the name of the assigned template at the top of the window. The assigned template is also selected in the template table.
The associated template and the name of the user template button can be changed.
Press *Assign* to accept the changes.

Note: If the user template button is disabled, changing or deleting the button as described in this chapter is not available. Use the Manage Template Button window instead.

5.5.4 Manage Template Buttons

You also can add, change and delete user template buttons with the *Manage Template Buttons* window:



Use this window to change or delete a template button if it is disabled.

5.6 Transfer of Standard Fit Results

If a test run is created without standards in the layout, it is possible to apply the standard curve fit of another test run to these samples to calculate the sample concentrations in the sample test run. Both the sample test run and the standards test run must be the same type to enable the transfer of the standard fit from the standards test run to the sample test runs:

- Run the measurement for the test run with the standards.
- Save the measurement values and open the test run in MARS.
- Perform one or more standard curve fits until you have the desired fit result.
- Delete all performed fits you do not want to have for your samples.
- Create a template from that test run (see chapter 5.3: *Create a Template*)
- Assign the template to the protocol for the test runs without standards if you want to have an automatic calculation of the samples.
- Run the measurement(s) for the test run(s) without the standards.
- Open the measured test run with MARS.

If the template was assigned to the protocol, the calculation will start automatically when the test run is opened. Otherwise the template with the standard calculation can be assigned after the test run has been opened.
Blanks will also be transferred to the test run, if the test run with the standards had blanks and in the layout of the test run are no blanks defined.
The calculation of the concentration values for each well used in the test run will then be done and the result can be viewed in the microplate or table view.
If the template contains more than one standard calculation, each of the calculations will be performed.
The standard curve of the fit used can also be inspected in the standard curve chart. The standards in the chart are shown in gray to indicate that they are not part of this test run.
The transferred standard calculation parameter cannot be changed anymore and single standards cannot be set to unused.
The regimentation for transferring standard calculations to test runs with no standards are very small. Only the two conditions to assign a template to a test run must be fulfilled:

- The measurement method must fit
- Both test runs must be either end point or kinetic test runs.

Note: To get a valid result you must ensure, that the measurement conditions are comparable. All parameters that have a direct influence to the measurement signal must be identical. See the table with parameters you have to check below.

Parameter	Measurement Method
used gain	all
used filter / top or bottom optic / used optic type / used optic module / used wavelength (absorbance spectrum)	all
used microplate (No. wells, plate type, plate color)	all
used volumes	all
No. of used flashes	TRF / HTRF

integration start time and integration time	TRF / HTRF, Alphascreen
excitation time	Alphascreen
measurement time	Luminescence
temperature	all
focal height (if adjustable - depends on the reader)	all
pathlength correction	Absorbance

Note: If you use a template with a standard calculation for a test run that contains standards, the standard calculation is performed based on the standards in that test run.

5.7 Transfer of Blank values

If a test run is created without blanks in the layout, it is possible to apply the blank values of another test run to this to calculate blank corrected values. It is done in the same way as the transfer of standard fit results:

- Run the measurement for the test run with the blanks.
- Save the measurement values and open the test run in MARS.
- Perform a blank correction and all other desired calculations until you have the desired result.
- Create a template from that test run (see chapter 5.3: *Create a Template*)
- Assign the template to the protocol for the test runs without blanks if you want to have an automatic calculation of blank corrected values.
- Run the measurement(s) for the test run(s) without the blanks.
- Open the measured test run with MARS.

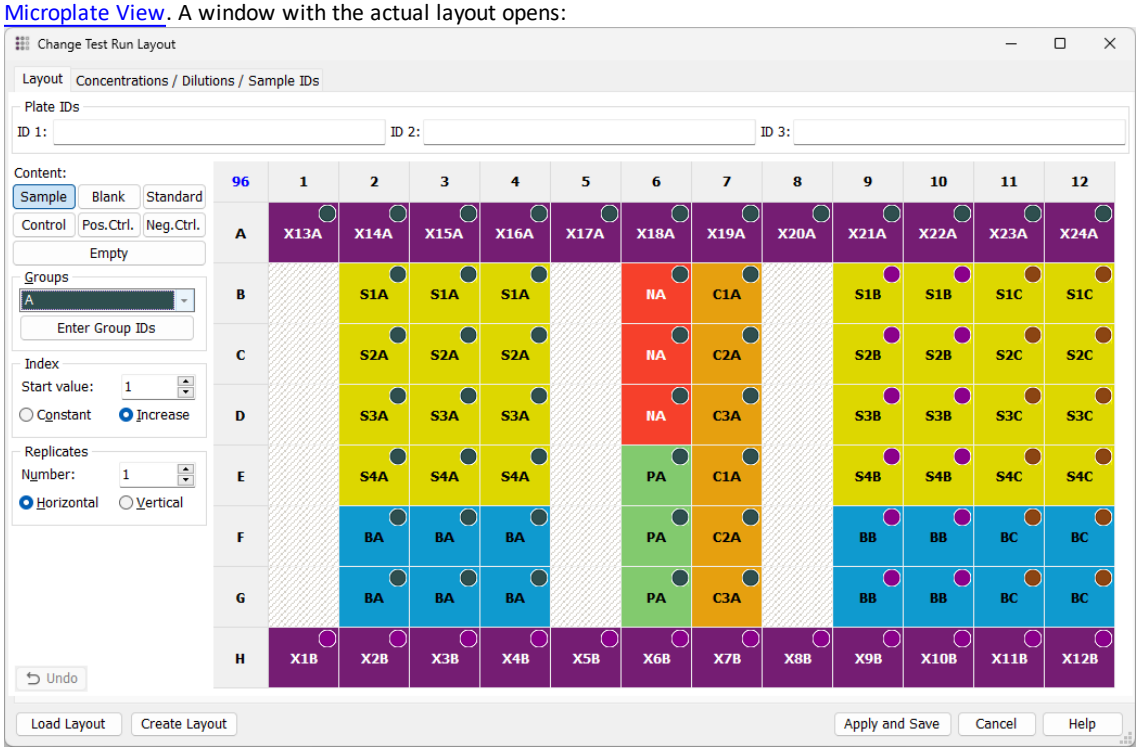
6 Test Run Layout

6.1 Changing Layout

The layout of a measured test run can be changed subsequently with MARS. You can change each parameter of the layout for the measured wells in the microplate: The layout content, the layout group, concentration / dilution values, sample IDs and the concentration unit. You also can change the plate IDs (1-3) of the test run.

> **Note:** Changing the layout of a test run may affect already calculated results or even delete a result that cannot be calculated any more.

The original layout of the test run (the layout of the measured protocol) is kept and the changed layout can be reset to the original layout at any time. Deleted results will not be restored after resetting the layout!
To change the layout of a loaded test run, click *Change Test run Layout* in the *Test Run Layout* group on the *Layout* tab or press the Edit Layout button in the layout view window. You also can click on the change layout icon on top of the Microplate View. A window with the actual layout opens:



How to change the single parameters of the layout is described in the next sections of this chapter.
After finishing the changing of the layout, press the *OK* button to apply the changes to the test run. A hint window will be displayed that describes the consequences for the actual performed calculations. Confirm this dialog to apply the changes.
After applying the changes, the settings of the test run with the changed layout will be saved automatically. With the Load Layout button you can open a stored layout to use it for this test run. Only saved layouts that match to the test run are provided. The condition when a layout matches to a test run is described in Manage Layouts.
To save the layout of the test run and use it with other test runs, press the Create Layout button.
Read more about saved layouts in Manage Layouts.
Press the Undo button to undo the last changes.

6.1.1 Changing Plate IDs

To change the plate IDs of the test run, enter the new values into the Plate ID fields ID 1, ID 2 and ID 3.

> **Note:** The plate IDs of a test run are used to identify a test run. Consider not to overwrite auto generated IDs if theses IDs are used as a unique identification of the test run!

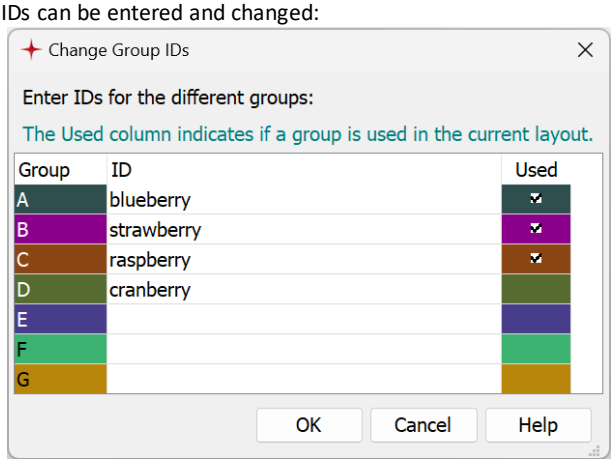
6.1.2 Changing Layout Contents

Changing the contents of a well works the same way as defining well contents for protocols in the control software. Select the content type first by clicking the appropriate content button (Sample, Blank, Standard...) and then use ways to select wells as described below.

The first letter in the cell of a well indicates the content type (when fewer than 384 wells are displayed. Otherwise only the color of the label indicates the content):

X	Sample	The well's content has unknown concentration.
B	Blank	The well contains water or buffer for measuring background.
S	Standard	The well's content has a known concentration and can be used to formulate a standard curve.
N P C	Negative Control, Positive Control, Control	The well's content has known concentrations, but will not be used for the standard curve calculation. It can be used for comparisons or for special calculations.

The **Index** is the reference number of the sample or the standard. The index box displays the number that will be used for the next well. If **increase** is selected, each well will be labeled successively. **Constant** will keep the same number in the case of continuous replicates.
If fewer than 384 wells are displayed, the index is the number behind the content letter. Otherwise only the index is displayed in the cell.
Replicates are the number of repeated samples or standards in a row. If you have duplicates on the microplate, you can select the number of replicates and whether they are labeled in the **horizontal** or **vertical** direction on the microplate.
If you want to use layout **Groups** (in MARS you can use up to 125 layout groups unlike in the control software where the limit is 26 layout groups), select the desired group in the drop-down list. The group will be shown in the layout grid using different background colors and by inserting the group letter at the end of the label for the well (if fewer than 384 wells are displayed. Otherwise only the background gets the according color of the group). In general, groups are represented by a group letter, starting with *A* for the first group and ending with *DU* for group number 125. To individualize the representation of the groups, you can enter Group IDs for each group. Press the Enter Group IDs button to open the window, where the group IDs can be entered and changed:

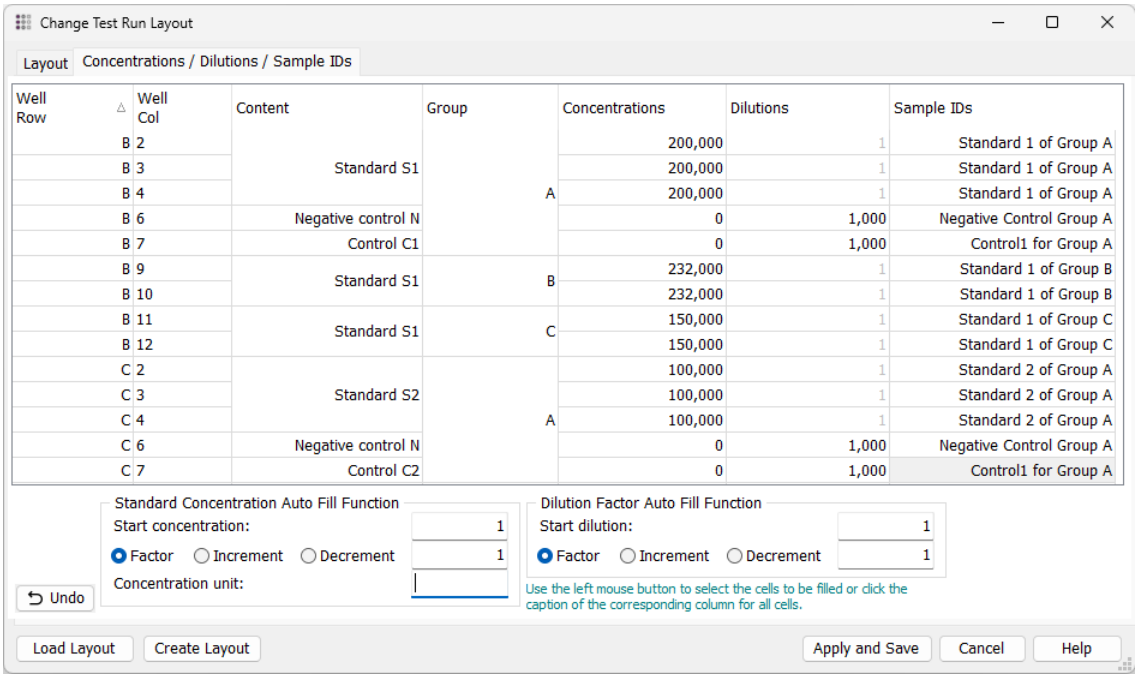


Methods of selecting wells to fill out the labels:
There are several ways to label the plate, after content, index and replicate settings are defined:
1. Double click on each well of that type.
2. If the wells of that content are in successive rows or columns, click on the first well with the left mouse key and drag across the wells containing the same content.
3. If a total row or column contains the same content, click the row letter or the column number and all wells of that row / column will be labeled.
4. To fill the entire microplate click on the format number (e.g. '96') in the top left corner.

Note: Unmeasured wells are disabled in the grid as you cannot define content for unmeasured wells!

6.1.3 Changing Concentrations, Dilutions and Sample IDs

To change the known concentrations of standards or controls and the used concentration unit, the dilution factor of samples or controls or the sample IDs of the contents, go to the Concentrations / Dilutions / Sample IDs sheet.
The sheet contains a table with all wells of the layout. You can enter the new concentration or dilution values and the sample ID.
For the concentrations and dilution values you can use the auto fill out function that works the same way as entering these values in the test definition of protocols in the control software.



Well Row	Well Col	Content	Group	Concentrations	Dilutions	Sample IDs
B	2			200,000	1	Standard 1 of Group A
B	3	Standard S1		200,000	1	Standard 1 of Group A
B	4		A	200,000	1	Standard 1 of Group A
B	6	Negative control N		0	1,000	Negative Control Group A
B	7	Control C1		0	1,000	Control1 for Group A
B	9	Standard S1	B	232,000	1	Standard 1 of Group B
B	10			232,000	1	Standard 1 of Group B
B	11	Standard S1	C	150,000	1	Standard 1 of Group C
B	12			150,000	1	Standard 1 of Group C
C	2			100,000	1	Standard 2 of Group A
C	3	Standard S2		100,000	1	Standard 2 of Group A
C	4		A	100,000	1	Standard 2 of Group A
C	6	Negative control N		0	1,000	Negative Control Group A
C	7	Control C2		0	1,000	Control1 for Group A

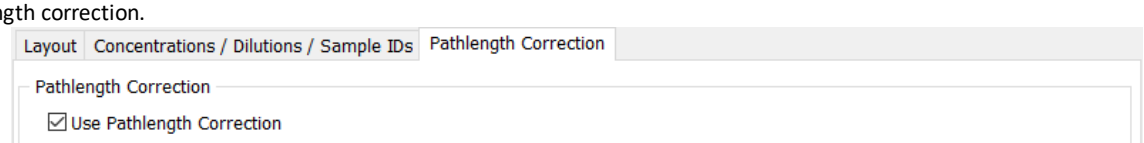
The table can be sorted, grouped and filtered by the columns *Well Row*, *Well Col*, *Content* and *Group* (the group column appears only if layout groups are used). More information is given about sorting, grouping and filtering in tables in the chapter 2.2: *Group and Filter Test Runs*, as it works the same way as for test runs.
Concentration values can only be entered for wells with content type standard, control, positive control and negative control. The dilution values can only be entered for all wells but not for blanks and standards.
The auto fill function can be used to define the concentrations and dilutions without entering them manually. The values will automatically be calculated using the given **Start** value and a number multiplied with (**Factor**), added to (**Increment**) or subtracted by (**Decrement**) the last calculated value.
Click with the left mouse button on the header of the according column to fill all wells. You can also select a specific set of successive wells by selecting them with the left mouse button down.
Enter the unit for the concentration values in **Concentration unit**.

If you enter concentration values, dilution values or sample IDs and the layout contains replicates, the entered values or IDs will be assigned to all replicates automatically. To enter different dilution values or sample IDs for each replicated well the options *Allow different dilution factors for replicates* and *Allow different sample IDs for replicates* must be activated. To activate these options, open the MARS Settings dialog (chapter 3.25.1) and check the appropriate check box controls.

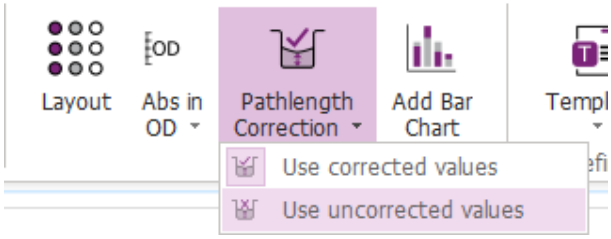
You can use the windows copy and paste function to exchange data (concentrations, dilutions and/or sample ids) between MARS and other programs like Excel. To copy data in the list, select them with the mouse and press the keys Ctrl+C or open the popup menu with the right mouse button and select *Copy*. To import data in the windows clipboard, select the first cell in the table, where the import should start and press the keys Ctrl+V or open the popup menu with the right mouse button and select *Paste*.

6.1.4 Changing Pathlength Correction Settings

If the measurement method of the test run is absorbance or absorbance spectrum, you can activate or deactivate the pathlength correction.



To activate the pathlength correction, check the according check box control. The absorbance values will be recalculated as values measured with a pathlength of 10mm. You can also activate or deactivate the pathlength correction if you select the *Pathlength Correction* icon on the *Display* group of the *Home* ribbon in Mars:

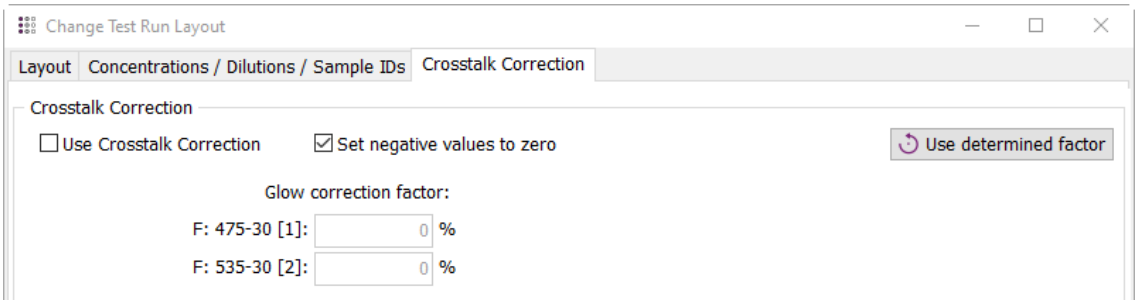


Note: For filter-based absorbance measurements and absorbance measurements not measured with the latest BMG readers, you also see a volume field to change the used volume in the well. To go back to the values defined when the test run was created/measured, click the *Use protocol settings* button.

6.1.5 Changing Crosstalk Correction Settings

If the measurement method of the test run is luminescence or AlphaScreen, you can change the settings for the crosstalk correction. To see and change the settings go to the Crosstalk Correction sheet.

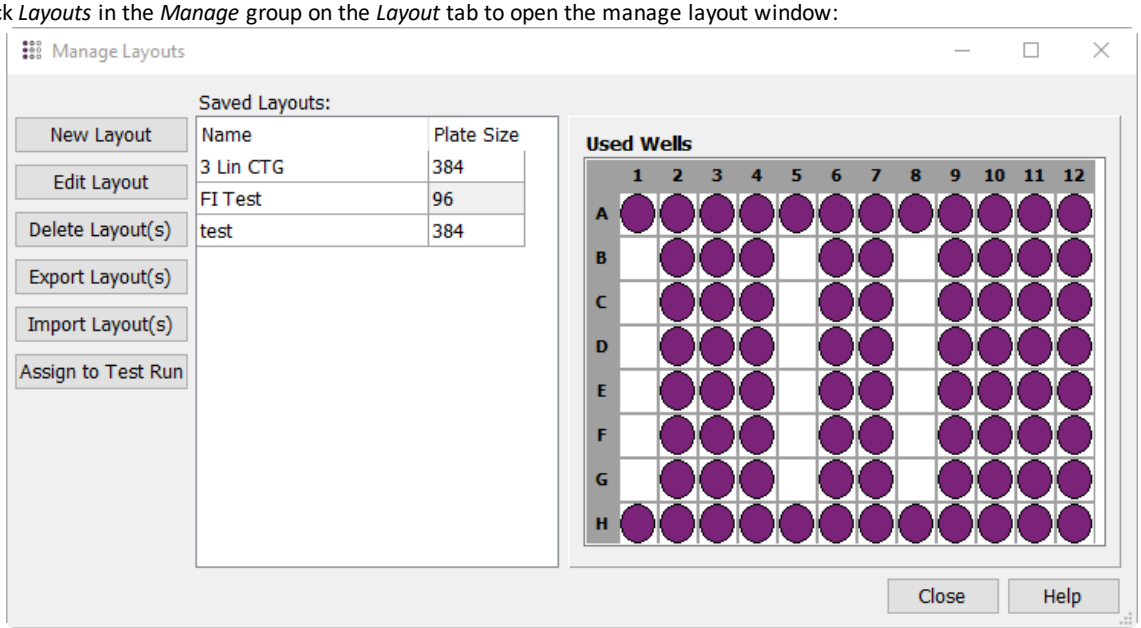
Note: This sheet is only available for test runs created with the reader families PHERAstar, CLARIOstar or VANTAstar.



To activate the crosstalk correction, check the according check box control. Enter the used glow correction factor for luminescence measurements. For AlphaScreen test runs you need to define the afterglow correction factor and the glow correction factor. For dual channel or multichromatic test runs, a factor for each channel and chromatic must be defined. The correction can lead to negative values. To prevent negative values, check the *Set negative values to zero* control. The raw data values will be recalculated using the entered correction factor.
To go back to the values defined when the test run was created/measured, click the *Use determined factor* button.

6.2 Manage Layouts

The *Manage Layouts* function provides you the possibility to create new layouts or layouts out of a test run, to change the layouts and to exchange the layouts using the export and import functionality. These layouts can be assigned to a test run, if the saved layout fits to the test run.
Click *Layouts* in the *Manage* group on the *Layout* tab to open the manage layout window:



The window contains a list with all saved layouts, containing the name of the layout and the size of the microplate. The dialog above shows the layout of a 96 well microplate. After selecting one layout in the list, the grid on the right side of the list shows the used wells in the save layout. The plate size and the used wells are important information as the decision if a saved layout can be assigned to a test run is based on this information.

6.2.1 Assign a Saved Layout to a Test Run

You can assign saved layouts to test runs. Therefore the two conditions must be fulfilled:
1. The size of the microplate of the test run and the saved layout must be identical.
2. The used wells in the saved layouts must be measured wells in the test run (there can be more measured wells in the test run but not in the saved layout).

There are four ways to assign a saved layout to a test run:
1. With the manage test run dialog above: Open the test run and then the manage layouts window. Select one layout in the list. If the layout can be assigned to the test run, the *Assign to test run* button is enabled and must be pressed to assign the layout.
2. With the menu item *Assign Layout to Test Run:* Open the test run and select the menu item. A window similar to the *Manage Layout* window shown above appears.
The list with the saved layouts contains only the assignable layouts. Select a layout and press *OK* to assign the layout to the test run.

3. With the change test run layout window: Press the 

button on the window. You see the same window as shown above. Select the desired layout and press *OK*. The layout will be displayed in the *Change Layout* window and overwrite the layout displayed before. Press *OK* on the *Change Layout* dialog to apply the new layout.
4. With the *Manage Test Run* window: Select one or more test runs of the same microplate size and open the popup menu by clicking the right mouse button. Select the menu item *Assign Layout.* Select the layout and press *OK.* The layout will be assigned to each selected test run if possible (see conditions above). If the layout cannot be assigned to one or more test runs, a message with a list containing the not assignable test runs appears.
If a test run is opened, you get a hint how the changed layout will affect your calculated results.

Note: Plate IDs are not part of the saved layouts and will not be changed after assigning a saved layout to a test run.

6.2.2 Create and Edit Saved Layouts

Press the *New Layout* button to create a new layout. After selecting the size of the microplate the new layout can be entered as described in . Press the *Save* button to save the new layout. You will be asked to enter a name for the new layout.
To view and change a saved layout, select the layout and press the *Edit Layout* button. Change the layout as described in chapter 6.1: *Changing Layout* and press *Save* to apply the changes.

6.2.3 Delete Layouts

Select one or more layouts in the list and press the *Delete Layout*(s) to delete the saved layouts.

6.2.4 Export and Import Layouts

You can export the saved layouts and exchange them with others. Select the layouts you want to export and press the *Export Layouts(s)* button. Select a directory to save the layouts. You can change the recommended filename. The file extension of the exported layout files is MLF.
To import layouts in a MLF file, press the *Import Layout(s)* button and select the directory with the MLF file. Select the file and press the *Import* button.

7 Support

If you have any problem / question regarding the software / the instruments, you should visit the support section on our web page (http://www.bmglabtech.com) or contact BMG LABTECH using the following email addresses:

- Problems / questions regarding software: support@bmglabtech.com
- Problems / questions regarding the instruments: techsupport@bmglabtech.com

You can also use our technical request form (https://www.bmglabtech.com/en/technical-support/).

Note: When you need support from BMG due to a software / firmware malfunction, you should send us the run log file together with the error description.