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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# Change Test Run Layout

## 6.1 Changing Layout

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Changing Plate IDs</td>
<td>88</td>
</tr>
<tr>
<td>Changing Layout Contents</td>
<td>88</td>
</tr>
<tr>
<td>Changing Concentrations, Dilutions and Sample IDs</td>
<td>89</td>
</tr>
</tbody>
</table>

## 6.2 Manage Layouts

### 6.3 Assign a Saved Layout to a Test Run

### 6.4 Create and Edit Saved Layouts

### 6.5 Delete Layouts

### 6.6 Export and Import Layouts

## 7 Sign a Test Run

## 8 Support
1 Overview

1.1 Main Screen of the 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:

![Main Screen of MARS](image)

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, i.e. 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 3: Explore Your Data.

Under the main menu bar is the quick button bar which gains you access to common and often needed tasks.

The status bar at the bottom of the screen shows the reader type used to generate the data and the details of the user logged in with the data path showing where the data is stored.

To check the version number of the software and the modules used, select the menu item Help and select About MARS.

![Software Version](image)

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 control software screen you do not need to select a login user again. The software automatically starts with the same user as used in the control software. If more than one reader is installed on the computer, or if more than one copy of the software is needed then please read the chapter 1.3 Multiple Installations for more details.

If starting MARS without having the control software running, you will get the same login window as if you had started the control software:

<table>
<thead>
<tr>
<th>User</th>
<th>Password</th>
<th>User Path</th>
<th>Run Only</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADMIN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SIFIUS BLACK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USER</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When opening MARS from the desktop or program menu select the desired user and press run to start MARS.

If a user with the Run Only flag set is used, test run data and settings can not be changed with MARS.

More details about the functions of the login window can be found in the help of the control software or by pressing the help button of the login window.

Note: The login window of the readers NOVOstar and NEPHELOstar Galaxy has less options then the shown login window (the buttons Properties, Options, Password and Keys are not available).

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 selecting the Menu entry in the Test Run Menu.

Then the login window then appears and you can select the desired user.

1.3 Multiple Installations

It is possible to install the control software part of the reader more than once. For more details on how to do this see chapter 2 of software manual part I: Installation.

For each installation (called instances) of the control part there is a corresponding instance of MARS. Starting MARS from the control software automatically selects the same instance.

Beside instances you can start MARS more than once even with the same user if you start it directly and not from of the control software screen.

Note: The readers NOVOstar and NEPHELOstar Galaxy does not support this function!
# 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 the Open icon in the Quick Tool Bar or selecting the Menu Item in the test run Menu:

![Open Test Run](image)

Select a test run in the table by clicking on it with the mouse. More than one test run can be selected by holding down the Ctrl-Key whilst highlighting the desired test runs with the mouse and clicking on them.

The window provides you the following functions:

- **Open**: Opens all selected test runs and creates a node for each in the navigation tree
- **Copy**: Creates a copy of the selected test runs
- **Delete**: Deletes all selected test runs.
- **Export**: Export the selected test runs (for more details see the chapter 2.2 Import / Export Test Runs).
- **Import**: Import one or more test runs (for more details see the chapter 2.2 Import / Export Test Runs).
- **Merge Cycles**: Merge tool. Learn more about merging test runs in the chapter 2.3 Merging Test Runs

---

**Note:** This window opens automatically when you open MARS or change the user, but not if you select Open Last Test Run in the control software.

Select a test run in the table by clicking on it with the mouse. More than one test run can be selected by holding down the Ctrl-Key whilst highlighting the desired test runs with the mouse and clicking on them.

The test run window comes with several possibilities to sort, arrange and filter your test runs. This is explained in detail in the chapter 2.1 Group and Filter Test Runs.

The window provides you the following functions:
These functions are also available on the popup menu of the window. You can open the popup menu by pressing the right mouse button. The popup menu contains two further menu entries:

**Reset Test Run Settings and Changed Layout:** Select this menu entry to reset the settings and changed layout of the selected test run(s). See chapter 2.4 Test Run Settings and 6.1 Changing Layout.

**Assign Layout:** Select this menu entry to assign a saved layout to the test run. See chapter 6 Assign a Saved Layout to a Test Run.

The table contains the following columns:

**State**
The state field describes the history of a test run. The available states are defined below:

- **M:** The layout of the test run has been changed after the measurement. The M flag overwrites the flags O and C.
- **O:** The test run has been imported from an old database, meaning that no validation checks have been made in its' history.
- **C:** copied test run. If a test run with the M flag set is copied, the M flag is overwritten by the C flag.
- **X:** manipulations have been detected since the generation of the test run (manipulations done outside the evaluation software). X overwrites the other state contents.

If the state field is empty, the test run is still in its original state.

**Signed**
Indicates if the test run is signed ('yes') or not (empty). It is not possible to save further changes to this test run. See chapter 2.4 Test Run Settings.

**Test Name**
The test name is listed as it is defined in the test protocol.

**ID 1 / ID 2 / ID 3**
These are the plate identifiers that were created before the measurement.

**Date and Time**
The date and time that the measurement took place.

**Measurement Method**
The measured method (i.e. absorbance, fluorescence...)

**# Wells**
The plate format (number of wells) of the microplate.

**Test ID**
The unique number of the test run.

*Note:* The user can change the order of the columns so they may not appear in the same order as described (see also chapter 2.1 Group and Filter Test Runs).
2.1 Group and Filter Test Runs

The test runs’ table provides powerful functions to help order the stored test runs. The user can sort, group, use filtering and change the order of columns to help find data and to achieve their most useful view of the test run list.

If you change the settings of the test run table to your needs, MARS memorizes the settings when you close the software and will restore them when you open it again.

2.1.1 Sorting the Table

The table can be easily sorted by clicking on the column you want to sort by. By clicking once on a column header the list will be sorted in that column. This is indicated by a little arrow \( \uparrow \) in the column header. Clicking on the column header a second time, will sort the list in a descending order by that column (\( \downarrow \)).

It is possible to create a hierarchic list, sorting using more than one column. Just select the main column in the list to sort by and sort it as described above. To select the second sorting parameter press and hold the Shift-Key on the keyboard, click on the second column header you want as a next sort key and so on with each new sort key.

2.1.2 Grouping the Table

In the window above you see a grouped table. The grouping column is the Measurement Method column. As you see, the table contains blocks for each Measurement Method in the list. You can expand or close each block by clicking on the + or - button before each block header.

Grouping helps in ordering your test runs and makes finding a specific test run easy.

How to Group the List?

The list can be grouped using the mouse. Move the mouse cursor to the header of the column you want to use as a group criterion. Click the left mouse button and keep it pressed, then move the mouse cursor and highlighted column header to the grey area above the list releasing the left mouse button when the cursor is over the area, the data will then be sorted by the data in that column.

As with the hierarchic sorting you can also group hierarchy by selecting another column to drag to the group area.

2.1.3 Change the Position of a Column

If the default order of the columns is not suitable, you can change the position of each column manually. Click on the column header you want to move, and keep the mouse button down whilst dragging the column to the new position. When a position is reached where you can drop the column two green arrows are displayed, the column can then be dropped into one of these positions.
2.1.4 Filtering the Table

You can filter your list by nearly any filter criterion. The easiest way of defining a filter is to click on the small down arrow of the column header which appears if you move the mouse cursor over the header. A list with the whole content of the column will then be displayed:

<table>
<thead>
<tr>
<th>ID</th>
<th>ID 2</th>
<th>ID 3</th>
<th>Date</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI</td>
<td>Find Trim</td>
<td>QM 7.23777</td>
<td>2007-05-08</td>
<td>2:17:33 F</td>
</tr>
<tr>
<td>FI</td>
<td>Find Trim</td>
<td>QM 7.23777</td>
<td>2007-05-08</td>
<td>2:15:48 F</td>
</tr>
<tr>
<td>FI</td>
<td>Find Trim</td>
<td>QM 7.23777</td>
<td>2007-05-08</td>
<td>2:08:10 F</td>
</tr>
<tr>
<td>FI</td>
<td>Full Curve</td>
<td>QM 7.23777</td>
<td>2007-05-08</td>
<td>2:05:35 F</td>
</tr>
<tr>
<td>FI</td>
<td>Find Trim</td>
<td>QM 7.23777</td>
<td>2007-05-08</td>
<td>2:03:55 F</td>
</tr>
<tr>
<td>FI</td>
<td>Find Trim</td>
<td>QM 7.23777</td>
<td>2007-05-08</td>
<td>2:02:25 F</td>
</tr>
<tr>
<td>FI</td>
<td>Find Trim</td>
<td>QM 7.23777</td>
<td>2007-05-08</td>
<td>1:59:32 F</td>
</tr>
<tr>
<td>FI</td>
<td>Find Trim</td>
<td>QM 7.23777</td>
<td>2007-05-08</td>
<td>1:55:17 F</td>
</tr>
<tr>
<td>FI</td>
<td>Fluorescence Intensity</td>
<td>Kinetic with B</td>
<td>2007-02-26</td>
<td>2:23:49 F</td>
</tr>
<tr>
<td>M</td>
<td>Fluorescence Intensity</td>
<td>changed concentration</td>
<td>2007-02-26</td>
<td>1:55:17 F</td>
</tr>
<tr>
<td>M</td>
<td>Fluorescence Intensity</td>
<td>changed concentrations</td>
<td>413-0445</td>
<td>Fluorescence (FI), multichromatic</td>
</tr>
</tbody>
</table>

Select one or more contents by clicking the check box before the content title. To reset the filter for a single content just click the check box again. To reset the whole filter, just select the ‘All’ list entry from the drop down menu.

When a filter is defined, this is indicated by a new line which appears at the bottom of the list:

There the filter can be deleted by clicking the red button with the cross (x). If you want to discard the filter temporarily but leave the definition for later use, just uncheck the checkbox before the filter description by clicking on it, and to restore the filter again just click again in the check box.

It is possible to further customize the filter. Press the Custom list entry in the drop down menu to open the customize window. Try out this function to learn how powerful this is.
2.2 Import / Export Test Runs

2.2.1 Import Test Runs

Using the import function you can import test runs. Click on the Import button on the manage test runs window and a window opens where you can select a file with the test runs you want to import into the current users data:

Imported test runs will be added to the list and marked in grey.

2.2.2 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 the manage test runs window is open, just select the test run(s) you want to export in the list and click on the Export button on the top of the window.

If you want to export only the currently opened and active test run (the one which is selected in the navigation tree), press the export button on the Quick Button Bar:

A 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 .RUC. 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.
2.3 Merging Test Runs

2.3.1 What Means Merging Test Runs

The merging test runs function can be used to add the data of one test run to another test run, resulting in one new test run containing the data of both. There are two ways to merge test runs together:

- The kinetic cycles/intervals of two test runs can be merged so that the new number of cycles/intervals is the sum of the number of cycles/intervals of each.
- The wavelengths of two or more test runs can also be merged so that the number of wavelengths in the merged test run is the sum of the number of wavelengths of each.

To merge two test runs together highlight the two test runs in the test run table in the manage test runs window. The two merge buttons in the toolbar will be enabled if the selected test runs pass checks to establish if the data can be merged.

The data checks needed for merging test runs using either cycles or wavelengths are explained in the following two sections.

Note: Possible saved settings (including changed layouts) of the test runs to be merged will be lost!

2.3.2 Merge Cycles / Intervals

To merge two test runs by cycles/intervals press the Merge Cycles button on the top of the manage test runs toolbar.

The following conditions must be fulfilled to merge two test runs by cycles/intervals:

- The number of wavelengths used must be identical
- The sum of the resulting cycles/intervals must be less than 1000 (limitation of the data base).
- The measurement method must be identical
- The layout must be identical
- The measurement mode must be identical (fast kinetic [well mode] or slow kinetic [plate mode])
- The test runs must not contain spectrum data
- The test runs must not contain well scanning data

The cycle/interval times for the merged test runs of kinetic data are calculated as follows:

\[ t = (\text{time of last cycle/interval}) + (\text{Start time of second test run}) - (\text{Start time of first test run}). \]

2.3.3 Merge Wavelength

To merge the wavelengths of two test runs into one file press the Merge Wavelength button on the top of the manage test runs toolbar.

The following conditions must be fulfilled to merge two test runs by wavelength:

- The number of cycles/intervals must be identical
- The layout must be identical
- The measurement mode must be identical (fast kinetic [well mode] or slow kinetic [plate mode])
- The test runs must not contain spectrum data
- The measurement method could be identical - but it is not a requirement

Note: If test runs performed using different measurement methods are merged, the merged test run gains the settings of the first of the two test runs. For example, if you merge an absorbance test run with a fluorescence intensity test run, the merged test run data would be represented as an absorbance run - even though the fluorescence data covers a completely different range.
2.4 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. When you open a test run for the first time default settings will be 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 (i.e. from earlier opening the test run).
- If the test protocol with which the test run was created 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.
- The selected nodes in the navigation tree and the content filter tree (see chapter 3.2 Navigation Tree and 3.3 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.

If you change the 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 the save of the 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. Changing Test Run Layout) the changed layout will also be saved then.

**Note:** Run Only users can not save the changed settings and even can not change the layout of a test run.

To remove all performed settings (even an assigned template) you can select the menu entry of the test run menu, when the test run was opened: **Reset Test Run Settings** Ctrl+R. The test run settings will delete and the format will be reconfigured to a default setting using rule 3 of the list above.

In addition you can reset the settings of closed test runs, if you select the menu entry Reset Test Run Settings on the popup menu of the Manage Test Runs window after you’ve selected the test runs in the list.

Resetting the settings of a test run does not reset a changed layout. To reset both, the settings and the layout, select the menu entry Reset Layout in the Layout menu.

If you export or import a test run, the settings (including a changed layout) will be exported / imported as well.

**Note:** The settings and changed layout information of test runs created with the NEPHELOstar and NOVOstar reader series can not be retained.

After a test run has been signed (see chapter 7: Sign a Test Run) no further changes can be made to the settings. You can open the test run and change the settings online, but they will not be saved or exported.
3 Explore Data

After opening one or more test runs you can explore the data using the functions of the main window:

![Main Menu and Working Area](image)

The main menu top toolbar gains you access to the complete functionality of the software. There are shortcuts to important tasks available at the Quick Button Bar under the main menu toolbar. All of 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. There can be up to seven different pages in the working area where data can be inspected using different formats. Data can be obtained using the following tabs:

- **Microplate View:** Data is displayed in a grid according to the microplate format.
- **Table View:** Data is displayed in an Excel-like table format.
- **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).
- **Standards Curve:** The standards data are shown plotted in graphs with fitted curve(s).
- **Protocol Information:** Displays the settings used in the test run protocol.
- **21 CFR part 11:** Displays information relevant to fulfill the FDA 21 CFR part 11 compliance, including a full audit trail and signatures (if a test run is signed).

**Note:** this page does not appear if you have a NOVOstar or a NEPHELOstar Galaxy.
## 3.1 Main Menu

The main menu consists of the *Test Run*, *View*, *Calculations*, *Templates*, *Layouts* and *Help* menus:

### Test Run menu

- **Open Test Run**
  - Opens the manage test run window to select test runs for opening
- **Close Test Run**
  - Closes the test run
- **Export Test Run**
  - Export the test run currently opened and active (Import/Export)
- **Select/Deselect Node**
  - Selects or deselects a node in the navigation tree
- **Delete Node**
  - Deletes a node (is only possible for user generated nodes)
- **Save Test Run Settings**
  - Saves the settings of the test run (test run settings)
- **Reset Test Run Settings**
  - Resets all settings of the test run (test run settings)
- **Print page(s)**
  - Opens the print window to define the report to print (printing your data)
- **Sign Current Test Run**
  - Signs the current test run (sign a test run)
- **Change User**
  - Calls the login window to change the user (login)
- **Exit**
  - Ends the program

### View menu

- **Navigation Tree**
  - Change hiding/displaying the navigation tree
- **Color Mode Settings**
- **Zoom (%)**
  - Enter the zoom value for the microplate
- **Well Details**
- **Absorbance display mode**
  - Change absorbance display mode to either Transmission in %, Absorbance in OD (default) or Absorbance in mOD

### Calculations menu

- **Standard Curve Calculation Wizard**
- **New Calculation**
  - Select the accordant menu item to perform the desired calculation (see calculations)
- **Change Calculation**
  - Change a already performed calculation
- **Statistic over Selected Wells**
  - Perform a statistic over the selected wells
- **Define Kinetic Ranges**
  - Opens the define range window
**Templates menu**

Opens the manage template window.

Opens the manage template buttons window.

Create a template, using the settings of the current test run.

Assign a template to the current test run.

---

**Layouts menu**

Opens a window which displays the defined layout

Opens the manage layouts window.

Create a layout file, using the layout of the current test run.

Assign a layout file to the current test run.

Edit and change the layout of the current test run.

Reset the changed layout to the original layout of the measured protocol.

---

**Help menu**

Opens the help system

Contact possibilities to BMG LABTECH

Version numbers of the software and the modules
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).

The navigation tree 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 3.2.1 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 top most node is shown in bold.

Use this button to open or close the sub nodes of a node

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 (ائق) 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 again press Ctrl+N or select the menu item View->Navigation Tree.

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 the signal curve view, the standard curve 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:

<table>
<thead>
<tr>
<th>Row</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.917</td>
<td>1.502</td>
<td>0.773</td>
</tr>
<tr>
<td>B</td>
<td>2.916</td>
<td>1.498</td>
<td>0.771</td>
</tr>
<tr>
<td>C</td>
<td>2.920</td>
<td>1.502</td>
<td>0.770</td>
</tr>
<tr>
<td>D</td>
<td>2.916</td>
<td>1.498</td>
<td>0.771</td>
</tr>
</tbody>
</table>
There are two ways, to select a node in this mode:

1. If you click with the mouse on the box in the column Row of the node you want to select, a little button with an arrow appears beside the box. Click on that button and a list with colored boxes opens. The order of the color boxes in the list is equivalent to the order the selected data will be displayed in the microplate view or the table view. That means, green is always the first row/column, blue behind green and above red, yellow or pink - and so on. Select the color of the row, where you want your data to be displayed.

2. You can just double click on the node (or use the menu item Select/Deselected node either in the test run menu or in the popup 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 the color green and blue for other nodes, the red color will be used for this node. If no colors are left (default is five colors), the node will not be selected. You can change the number of selectable rows up to 10. How to do this, you can read in the section Display Options.

**Selecting data for curve charts**

If a chart is active such as the signal curve view, spectrum curve view or standard curve view), 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, i.e. if a test run has kinetic data then the signal curve view will be active, and only nodes with kinetic data will have a check box.

**Tree Popup Menu**

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

- **Close Test Run** (Ctrl+X): Closes the current test run (you will be asked if you want to save changed settings).
- **Export Test Run (Ctrl+E):** Exports the current test run (see chapter 2.2.2 Export Test Runs).
- **Select/Deselect Data Row (Ctrl+D):** Selects or deselects a node (if selectable). Alternatively you can double click on the node.
- **Delete** (Del): Deletes the node (added wavelengths or calculations which are not the basis of a calculation).
- **Change Calculation:** Opens a window to change calculations parameters (only available for calculation nodes).
- **Create Template...** (Ctrl+Alt+T): Creates a template with the current settings of the current test run.
- **Assign Template...** (Ctrl+A): Assigns a template to the current test run and overwrites its settings.
- **Sign Test Run...** (Ctrl+S): Signs the current test run.
- **Save Test Run Settings** (Ctrl+R): Saves the test run settings.
- **Reset Test Run Settings** (Ctrl+R): Resets the settings of the test run to the default values.

All the functions are also available in the main menu (see chapter 3.1 Main Menu).

*Note:* The popup menu shows only these functions, which are independent from the selected node or applicable on the selected node.

**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.
<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilutions</td>
<td>Represents the dilution factor for the wells (appears only if dilution factor &gt; 1 where defined)</td>
</tr>
<tr>
<td>Sample IDs</td>
<td>Represents the sample IDs of the samples (appears only if sample IDs where defined for the samples)</td>
</tr>
<tr>
<td>Injections</td>
<td>Parent node for all injection volume nodes (appears only if there have been injections in the test run)</td>
</tr>
<tr>
<td>Volume n</td>
<td>Sub nodes of Injections, for each defined injection volume (n is the number of the volume)</td>
</tr>
<tr>
<td>Data</td>
<td>Parent node for the raw data and the corrected raw data</td>
</tr>
<tr>
<td>Temperature</td>
<td>Represents the temperature during the test run measurement. (Appears only if temperature monitoring was set in the control software)</td>
</tr>
<tr>
<td>Raw Data</td>
<td>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.</td>
</tr>
<tr>
<td>Wavelength: ( \lambda/\text{filter} )</td>
<td>Always end node and possible sub 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 3.10.1 Adding a Discrete Wavelength). 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.</td>
</tr>
<tr>
<td>parallel (filter A / filter B)</td>
<td>Only for fluorescence polarization measurements. Represents the parallel measurement channel</td>
</tr>
<tr>
<td>perpendicular (filter A / filter B)</td>
<td>Only for fluorescence polarization measurements. Represents the perpendicular measurement channel</td>
</tr>
<tr>
<td>Spectrum</td>
<td>Appears only for test run with a measured spectrum. Represents the spectrum curves.</td>
</tr>
<tr>
<td>Spectrum blank corrected</td>
<td>Represents the blank corrected spectrum curves (if blanks are defined)</td>
</tr>
<tr>
<td>Blank corrected</td>
<td>Represents the blank corrected raw data (if blanks are defined)</td>
</tr>
<tr>
<td>Blank corrected (all groups)</td>
<td>Represents the data of performed corrections (see chapter 4.3 Corrections).</td>
</tr>
<tr>
<td>Neg. control correction</td>
<td></td>
</tr>
<tr>
<td>Neg. control correction (all groups)</td>
<td></td>
</tr>
<tr>
<td>FP calculations</td>
<td>Represents the calculations available for fluorescence polarization measurements (see chapter 4.5.1 FP Calculations).</td>
</tr>
<tr>
<td>TR FRET Calculations</td>
<td>Represents the ratio and the DeltaF calculation for time resolved fluorescence (dual emission) test runs (see chapter 4.5.2 TR-FRET Calculations).</td>
</tr>
<tr>
<td>Statistics</td>
<td>Represents the calculated statistic data for replicates (see chapter 4.4 Replicate Statistics) or for selected wells (see chapter 4.10 Statistic over Wells).</td>
</tr>
<tr>
<td>Moving average</td>
<td>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</td>
</tr>
<tr>
<td>Moving width: b Range n</td>
<td>used range is n. See chapter 4.6 Moving Average calculations</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Kinetic calculations</td>
<td>Represents the calculated data taken from a kinetic range. The range used for the calculation is displayed behind the calculation method (i.e. Slope of Range 1). See chapter 4.6 Kinetic Calculations.</td>
</tr>
<tr>
<td>Slope</td>
<td></td>
</tr>
<tr>
<td>Time to threshold</td>
<td></td>
</tr>
<tr>
<td>Time to max</td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
</tr>
<tr>
<td>Standard deviation n</td>
<td></td>
</tr>
<tr>
<td>% CV</td>
<td></td>
</tr>
<tr>
<td>% CV n</td>
<td></td>
</tr>
<tr>
<td>Standards calculations</td>
<td>Represents the recalculated concentrations taken from the standard curve fit results (see chapter 4.7 Standard Calculation / Curve Fitting).</td>
</tr>
<tr>
<td>Linear regression fit</td>
<td></td>
</tr>
<tr>
<td>4-Parameter fit</td>
<td></td>
</tr>
<tr>
<td>Cubic spline fit</td>
<td></td>
</tr>
<tr>
<td>Point to point fit</td>
<td></td>
</tr>
<tr>
<td>Segmental regression fit</td>
<td></td>
</tr>
<tr>
<td>2nd polynomial fit</td>
<td></td>
</tr>
<tr>
<td>3nd polynomial fit</td>
<td></td>
</tr>
<tr>
<td>Concentration calculations</td>
<td>Represents the result of performed calculations based on known and recalculated concentrations (only available if a standard fit was performed). See chapter 4.10 Concentration Calculations.</td>
</tr>
<tr>
<td>Difference</td>
<td></td>
</tr>
<tr>
<td>Ratio known/calc</td>
<td></td>
</tr>
<tr>
<td>Ratio calc/known</td>
<td></td>
</tr>
<tr>
<td>Percentage deviation</td>
<td></td>
</tr>
<tr>
<td>Calculations</td>
<td>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 4.8 Data Calculations).</td>
</tr>
<tr>
<td>data 1 / data 2</td>
<td></td>
</tr>
<tr>
<td>data 1 - data 2</td>
<td></td>
</tr>
<tr>
<td>data 1 * data 2</td>
<td></td>
</tr>
<tr>
<td>data 1 + data 2</td>
<td></td>
</tr>
<tr>
<td>Validations</td>
<td>Represents the result of a performed validation (see chapter 4.9 Validations).</td>
</tr>
<tr>
<td>good / bad</td>
<td></td>
</tr>
<tr>
<td>good / bad / unknown</td>
<td></td>
</tr>
<tr>
<td>Assay Quality</td>
<td>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)</td>
</tr>
<tr>
<td>Z' based on Cnt1 and Cnt2</td>
<td></td>
</tr>
<tr>
<td>Signal to blank (Cnt)</td>
<td></td>
</tr>
<tr>
<td>Signal to noise (Cnt)</td>
<td></td>
</tr>
<tr>
<td>Percentage calculation</td>
<td></td>
</tr>
</tbody>
</table>
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 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).

**Standards calculations:**
Wavelength: 485-P, 520-P (No. 1A)

**Linear regression fit**
Based on:
- Average
- Blank corrected
- Raw Data

Fit formula:
\[ Y = mx + b \]

**Parameter:**

<table>
<thead>
<tr>
<th>Group</th>
<th>m</th>
<th>b</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>299.2324</td>
<td>-5018.908</td>
<td>0.9983415</td>
</tr>
<tr>
<td>B</td>
<td>74.19454</td>
<td>-4966.302</td>
<td>0.9992626</td>
</tr>
<tr>
<td>C</td>
<td>255.4456</td>
<td>-2519.804</td>
<td>0.9979341</td>
</tr>
</tbody>
</table>

The type of data displayed depends on the type of data the selected node represents.

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 the case of the linear fit in the screenshot on the left it means: The linear fit was performed on a kinetic calculation (sum) of range 1.

The kinetic calculation was performed, based on blank corrected data, which again are 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 3.2.1 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) 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, 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 (top most nodes) represent the groups (only if groups are defined), otherwise the root node is visible, representing all wells.

Clicking on the check box shown before the node representing a well or group of wells will select them for use. The highlighted well (well A01 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 is the Microplate View page:

In this view, data are displayed according to the microplate layout defined. 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 3.16 Export Data) (you need to have installed a Microsoft Excel (minimum version Excel 97) on your PC).

**Popup Window**

The microplate view page has a popup menu that can be reached by pressing the right mouse button in the main window:

- **Toggle Use Well(s) / Don’t Use Well(s)**: Change the usage state of the well (see Toggle Wells)
- **Statistic over Selected Wells**: Perform a statistic over the selected wells
- **Copy to Clipboard**: Copy the microplate view graphic and values to the clipboard.
- **Export Data to Excel...**: 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:
This is useful for non colored printing reports, to see the description of the data according to the row in the microplate view.

### 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 tabs found at the top right border of the microplate grid.

Depending on the test runs' measurement method there can be up to three 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 (having cycles or intervals), the value for a selected cycle/interval can be displayed in each well (see section Kinetic Test Runs). The screen-shot at the top of this page shows the microplate view in value mode.

#### 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 (to limits define 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 ( ) on the right side of the grid. 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 depends on the minimum and maximum values of all displayed curves:

Spectrum mode

This mode is only available for measured absorbance spectra. It displays the spectrum curve for each well. If there are blanks defined in the layout of the test run you can select between the blank corrected spectrum curves or the raw spectrum curves with the drop down list above the grid (only visible in this mode, when blanks are defined). On the bottom of each well, a small spectrum bar is displayed that gives you an overview to the range of the measured spectrum. You can hide this bar using the display options window.

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 the color mode, 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 3.17 Well Scanning Data.

Kinetic Test Runs

You can display an overview of the kinetic curves for each well with the Kinetic View Mode (see chapter 3.4.1 View Modes). In the Value View Mode, and in the Color View Mode, you can select the cycle/interval you want to inspect using the kinetic drop down list at the top right of the microplate grid. Select the 'All cycles/Intervals' check box and in Color Mode a color bar representing each cycle/interval will be shown in each well.

Interval: 36 (22.40 s).
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 tab appears in the Quick Tool Bar when an absorbance test run is open allowing the user to select the most appropriate mode for the data to be expressed.

The mode can also be changed using the main menu (View->Absorbance display mode).

3.4.2 Selection 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 adjoining 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.

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 view mode:

<table>
<thead>
<tr>
<th>View Mode</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value mode</td>
<td>Opens a windows with detail information of the well (see chapter 3.4.3 Details of a Well below)</td>
</tr>
<tr>
<td>Color mode</td>
<td>Changes to the signal curve view page and displays the selected well(s) in the chart.</td>
</tr>
<tr>
<td>Kinetic curve mode</td>
<td>Changes to the spectrum curve view page and displays the selected well(s) in the chart.</td>
</tr>
<tr>
<td>Spectrum curve mode</td>
<td>Opens a window with a detailed view on the well scanning values of the well (see chapter 3.17 Well Scanning Data)</td>
</tr>
</tbody>
</table>

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 select the menu item View->Well Details...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 Starttime (plate mode tests only) and Duration are not available.
3.4.4 Zooming

When using the Microplate View, if there are many data values shown in one well or if using a microplate format with 384 or 1536 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 Toggle 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 popup menu, after selecting the wells in the microplate view.

Unused wells are displayed with diagonal grey stripes. Only the raw data values and the layout values are displayed (also in grey):

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>934</td>
<td>1178</td>
<td>1418</td>
<td>3350</td>
</tr>
<tr>
<td>S1</td>
<td></td>
<td>N</td>
<td>X1</td>
<td></td>
</tr>
<tr>
<td>20082</td>
<td>21165</td>
<td>21468</td>
<td>22151</td>
<td>23115</td>
</tr>
<tr>
<td>B</td>
<td>2081</td>
<td>3350</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td></td>
<td>N</td>
<td>X2</td>
<td></td>
</tr>
<tr>
<td>20681</td>
<td>22168</td>
<td>22354</td>
<td>22813</td>
<td>229372</td>
</tr>
<tr>
<td>B</td>
<td>24331</td>
<td>25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 three columns are predefined with the row and column name of the well and the content of the well. If there are groups defined in the layout, a fourth 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 3.4.5 Toggle Wells) the row of the well is shown with a light grey background and a dark grey text color.

You can order, filter or move each column in the table. Grouping is only possible with the Column Content and Group.

More information is given about common table functionality in the chapter 2.1 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 (Replicate 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 3.16 Export Data)

**Only used wells**  
If this check-box is unchecked, a row for each well in the microplate is displayed, even if it is not used in the layout of the test run.

**Detailed header**  
If this check-box is checked, the header of the data rows contains 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.

- **Select a cycle/interval group**  
This control appears only for kinetic test runs. With the drop down list, you can select which cycle/interval you want to display in the table. If at least one entry is checked, a column for each cycle/interval of the selected ranges is created (only if the selected data contains cycles/ intervals). The header of the column is expanded with the cycle/interval number and time. You can combine the selection of the ranges or you can select All Cycles/Intervals to display all cycles/intervals. To see only one cycle in the table, deselect all selected entries and select the cycle with the Cycle control on the right.

**Note:** If you have a lot of spectrum data (many wells and a large measured spectrum), the creation of the table with spectrum or blank corrected spectrum data may take some time (a progress bar will appear)!
3.6 Common Chart Functions

The subjects, described in this chapter apply to all three kinds of chart used in MARS:

The signal curves chart (available for kinetic test runs), the spectrum curves chart (only for absorbance spectrum measurements) and the standard fit curves chart (available after a performed standard calculation).

All these charts contain the following elements and functions:

- A chart with at least one Y-axis and one X-axis and selected curves in it
- A legend explaining the displayed curves:
- Two Control boxes to customize the legend:
- Crosshair functionality
- Zooming possibilities
- Two windows to change the settings of axis and curves.

Open windows for axes and curves settings

Ax x Settings... Curves Settings... Show Legend Custom Legend Positioning

Customize the chart legend

[Image of a signal curve chart with annotations for chart legend, Y axis, crosshair, and chart zooming controls.]

Cross hair controls

Show crosshair for active curve
Lock to data
Lock to curve
Time: 40 min 29 s
Fluorescence in RFU: 2514
3.6.1 Chart Axis and Curves

A chart consists of one X axis and one or more Y axis (i.e. 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 shown one on top of the other, the axis of each curve can be individually 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. The shapes of the data points belonging to the same Y axis are equal but the shapes of the data points of different Y axes are different (like the shapes of the two curves in the screen shot above).

To select a curve in the chart either click on the curve or click on the associated entry in the legend. 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 curve settings window (pressing the Curves Settings... Button, see chapter 3.8 Curve Settings Window).

You can define each axis according to the start and stop value, the increment value, the name and the scaling (logarithmic or linear), by opening the axis settings window to change these attributes (see chapter 3.7 Axis Settings Window).

The title of the chart can also be changed in the axis settings window.

3.6.2 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.

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).

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. The shapes of the data points belonging to the same Y axis are equal but the shapes of the data points of different Y axes are different (like the shapes of the two curves in the screen shot above).

To select a curve in the chart either click on the curve or click on the associated entry in the legend. 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 curve settings window (pressing the Curves Settings... Button, see chapter 3.8 Curve Settings Window).

You can define each axis according to the start and stop value, the increment value, the name and the scaling (logarithmic or linear), by opening the axis settings window to change these attributes (see chapter 3.7 Axis Settings Window).

The title of the chart can also be changed in the axis settings window.
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.

### 3.6.3 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** control 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 it with the mouse and dropping it at the desired position. 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.

### 3.6.4 Chart Popup Menu

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

<table>
<thead>
<tr>
<th>Menu Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoom to Normal Size</td>
<td>The menu items for zooming apply to the zoom functionality of the chart</td>
</tr>
<tr>
<td>Zoom In (+)</td>
<td>(see chapter 3.6.5 Zooming)</td>
</tr>
<tr>
<td>Zoom Out (-)</td>
<td>You can create a bitmap out of the chart and save it into a *.bmp file on</td>
</tr>
<tr>
<td></td>
<td>your file system. Depending on the usage of the created bitmap you can</td>
</tr>
<tr>
<td></td>
<td>create a normal or a high resolution bitmap.</td>
</tr>
<tr>
<td>Create Bitmap...</td>
<td></td>
</tr>
<tr>
<td>Create High Resolution Bitmap...</td>
<td></td>
</tr>
</tbody>
</table>

### 3.6.5 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 3.9 Signal Curves).

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.

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!
### 3.7 Axis Settings Window

The axis settings window lets you customize the settings of any chart axis. Click on the axis you want to change or on the **Axis Settings** Button above the chart. The axis settings window opens:

**Note:** Changes will be overtaken only, if you press the **Apply** button or if you change the axis group page.

![Axis Settings Window](image)

You can change the following attributes:

- **Chart Title**: Enter the title of the whole chart here.
- **Vertical Axis(Y) / Horizontal Axis(X)**: Select the group of axis you want to customize:
  - Vertical Axis(Y): all Y axis (one or more)
  - Horizontal Axis(X): the X axis (only one)
- **Select vertical axis**: This entry field is only shown if the Vertical Axis(Y) group was selected. Select the Y-Axis you want to customize.
- **Description**: Displayed title of the axis
- **visible**: Uncheck this control if you want to hide the axis.
- **Scale settings**: Controls in this group box define the scaling settings of the axis.
- **Autoscale**: 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 disabled then.
- **Start with 0**: Is only enabled, when *Autoscale* 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.

- **Maximum axis value**: If *Autoscale* is not checked, you can enter the desired maximum and minimum value of the axis here.
- **Minimum axis value**: Enter the increment value for the axis scaling here. The chart tries to use 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.

- **Desired increment**: 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.
3.8 Curve Settings Window

The curve settings window lets you customize the settings of any curve in the chart. Click on the Curve Settings Button above the chart. The curve settings window opens:

Select the curve or the group of curve you want to change:

If you want to make changes for a group of curves, select the Group of curves radio button. A chart may contain data curves and - if it is the standard curve chart - also calculated curves. Select if you want to do the changes on all curves, on the data curves only or on the calculated curves only by selecting the appropriate radio button in the Curve group box.

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.

**Note:** The selection of a curve group in the group box above the drop down list control is used as filter for the drop down list! If Data curves is activated, you see only data curves in the list.

The changes will be overtaken only, if you press the Apply button.

**Changeable settings:**

<table>
<thead>
<tr>
<th>Lines</th>
<th>Defines the look of the line between to data points.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible</td>
<td>Lines between data points are drawn as linear connection between two adjacent data points if this check box is checked.</td>
</tr>
<tr>
<td>Line style</td>
<td>Select the style of the line between the data points:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Points</th>
<th>Defines the look of the data points of the curve/data series (not enabled for calculated curves).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible</td>
<td>Data points are visible if this check box is checked.</td>
</tr>
<tr>
<td>Point shape</td>
<td>Select the shape of the data points of the curve/data series:</td>
</tr>
</tbody>
</table>

| Color | Define the color of the line and data points. If Default is checked, the color is selected automatically. |
3.9 Signal Curve

The signal curve chart if your test run contains kinetic data 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!

![Signal Curve Chart](image)

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 cycle/interval as a caption. If the range represents a baseline section of the kinetic (see chapter 4.3.3 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 a 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.

The popup menu for the signal curve chart is expanded to include special range functions (see also the section 3.9.1 Range Functions in the Chart):

- Add Range...
- Delete Active Range
- Zoom to Normal Size
- Zoom In (+)
- Zoom Out (-)
- Zoom in Active Range
- Create Normal Bitmap...
- Create High Resolution Bitmap...

**Note:** The **Mouse Zooming Active** can also be used to temporarily hide the ranges, for a better look of the data is needed.
3.9.1 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 popup 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 on the Quick tool bar. (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 right position.

### Changing Range Size

To change the size of the active range (changing the first or final cycle/intervals 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 cycle/interval 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 popup 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 cycle/interval to the last cycle/interval, 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 popup menu. A range that has been used to perform a calculation will not be deleted unless the calculation is deleted first.
3.10 Spectrum Curve

The spectrum curve chart will be available if your test run is an absorbance measurement. 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 grey by changing the corresponding setting in the display options window.

Note: If there is no well or node selected, the working area will appear 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 display options 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.

Note: At least one discrete wavelength from an absorbance spectrum will be needed to perform any further calculation, as calculations are not possible using the full spectrum data.
3.10.1 Adding a Discrete Wavelength

For measured spectrum data you must select one or more discrete wavelengths to create further calculations from the data. To add a new discrete wavelength to the test run, press the Add Wavelength button shown under the chart or open the popup menu and select the Add Wavelength item. You can add as many wavelengths as needed (The maximum number allowed = the total of all measured data points).

The following window will be shown:

At next free minimum Y value position: Searches for the minimum value of the active spectrum curve in the visible chart area (if the chart is zoomed, the minimum is taken within the zoomed area). If there is a discrete wavelength selected already at that point, the next higher minimum will be found and selected.

At next free maximum Y value position: (default if the crosshair is hidden) Searches for the maximum value of the active spectrum curve in the visible chart area (if the chart is zoomed, the maximum is taken within the zoomed area). If there is a discrete wavelength selected already at that point, the next lower maximum will be found and selected.

At Wavelength: (default if the crosshair is visible) Enter the lambda value of the wavelength needed. If the crosshair is visible, the X value of the crosshair can be used as default value.

3.10.2 Change the Lambda Value of a Discrete Wavelength.

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

- 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.

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).

3.10.3 Deleting a Discrete Wavelength

To delete a discrete wavelength, even after performing calculations, open the popup 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 popup or the Delete Node menu item in the test run menu).
3.11 Standard Curve

The standard curve chart option will be available if you have performed a standard 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 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 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 originally defined in the layout.

Check or uncheck the box of the group you want to see or hide.
3.11.1 Error Bars

Above the chart there is a further check box available: **Show Error Bars**.

If the boxed is checked the error bars will be displayed for the standards:

- 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 standard fit may not have a user generated replicate statistic in its base processes (see chapter 4: Perform Calculations).

3.11.2 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. 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 $r^2$. Its associated row is highlighted with a blue background.

To export all results to Excel, press the Button.
3.12 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.

- **Plate mode / Well mode / Endpoint settings**
  This gives the kinetic information of your test run, like number of flashes, number of measurement cycles, measurement interval time etc.

- **Optic settings**
  The optical properties, like filter names of excitation / emission filters 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.
3.13 21 CFR part 11

This page displays some information about the history of the test run.

**User settings**

This section shows the serial number of the reader used, the version of the control software and the firmware along with the user name.

**Trail settings**

The audit trail information of the test run is displayed here. It displays any modification or manipulation of the test run. If the test run is signed, the signature details are also displayed here.


**Note:** This page requires the FDA 21 CFR part 11 compliance support of the readers control software. The NOVOstar and the NEPHELOstar Galaxy products do not support the FDA 21 CFR part 11 compliance therefore this page is not available for these readers.
### 3.14 Color Settings

If the color mode in the microplate view is active or if well scanning window is open for well scan test runs, the color settings window will then be available.

Press the button on the right side of the microplate view or the Colors... button on the well scanning dialog to open the window:

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:

#### 3.14.1 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.

#### 3.14.2 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.

#### 3.14.3 Color Gradient

The measurement values will be displayed using different shades of colors or grey 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.

*Note:* To ensure that your changes are applied, you must press the Apply button.
3.15 Printing Your Data

To print data open the Define Print Pages window. Press the Print button on the Quick Tool Bar or select the menu item Test Run -> Print pages(s)... to open the window.

Printer Setup

In the Printer section of the window select the printer and the print orientation (landscape or portrait). To change the printer and the settings press the Printer Setup button.

The print orientation is predefined, depending on the page layout in MARS but can be changed.

Pages

Each available page is represented by a check box. If a page is not available its checkbox will be disabled or not visible (e.g. the spectrum check box is only visible for absorbance spectrum measurements).

Select the pages you want to print by clicking the according check boxes.

The microplate view can be printed on more than one page, if the size of the plate is bigger than 12 wells. The following table shows the possible number of pages depending on the size of the plate:

<table>
<thead>
<tr>
<th>No. of Wells</th>
<th>No. of pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;=12</td>
<td>one page</td>
</tr>
<tr>
<td>24 to 384</td>
<td>one page</td>
</tr>
<tr>
<td></td>
<td>four pages</td>
</tr>
<tr>
<td>1536</td>
<td>one page</td>
</tr>
<tr>
<td></td>
<td>four pages</td>
</tr>
<tr>
<td></td>
<td>16 pages</td>
</tr>
</tbody>
</table>

If the standard curve is enabled, you can decide whether to print the calculated fit parameters on the same page or to print the results on a separate page, click on the Fit results on separate page check box.

To print the selected pages, press the Print button.

Before sending the pages to the printer, the result can be viewed in a preview window. Press the Preview Button to open the preview window.
### 3.15.1 Print Preview

The preview window shows you the created print output that will be sent to the printer.

![Print Preview Window]

To navigate through the pages created, use the <Prev Page and Next Page> buttons. To send the output to the printer, press the Print button. The print window can be closed without printing by clicking on the Close button.
3.16 Export Data

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

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 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 the exported test run has more than one cycle/interval, you will be asked whether you want to export only the current cycle/interval or all cycles/epochs. If you decided to export all cycles/epochs, the data for each cycle/interval will appear on a new sheet in the Excel workbook.

If the test run generates more than 250 columns in the table view page, the data appear distributed on different sheets as some Excel versions are limited in the number of columns.
### 3.17 Exporting Fit Results

If the data exported from the microplate or table view contain the result of a standard fit calculation, a second 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, open the fit result window and press the button.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>User</strong>: USER</td>
<td>Path:C:\Program Files\BMG\Omega\User\Data\</td>
<td><strong>File Name</strong>: 422.xls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Test Name</strong>: FLUORESCENCE INTENSITY</td>
<td><strong>Date</strong>: 2007-02-26</td>
<td><strong>Time</strong>: 1:33:43 PM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ID1</strong>: Fluorescence Intensity</td>
<td><strong>ID2</strong>: 26.02.2007.13.33.339</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ID2</strong>: 413-0445</td>
<td><strong>Fluorescence (Ft)</strong>, multichromatic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Formal</strong>: Y = Bottom + (Top-Bottom) / (1 + 10^((log(EC50)-log(x))*Slope))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Well</th>
<th>Row</th>
<th>Well Col</th>
<th>Content</th>
<th>Group</th>
<th>Raw Data (485-P, 520-P)</th>
<th>Blank corrected raw data (485-P, 520-P)</th>
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<tr>
<td>A1</td>
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<td>A</td>
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<td>A</td>
<td>11973</td>
<td>11895</td>
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<td>Sample X14</td>
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<td>9794</td>
<td>9716</td>
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<td>A3</td>
<td>A</td>
<td>Sample X15</td>
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<td>4009</td>
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<tr>
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<tr>
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<td>Standard S1</td>
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<td>3655</td>
<td>3577</td>
</tr>
<tr>
<td>B4</td>
<td>B4</td>
<td>A</td>
<td>Standard S1</td>
<td>A</td>
<td>660</td>
<td>601</td>
</tr>
</tbody>
</table>

### Linear Regression
- **4-Parameter fit**

<table>
<thead>
<tr>
<th>Well</th>
<th>Group</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
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<td>A1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td></td>
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<tr>
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<td>B3</td>
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<td></td>
</tr>
<tr>
<td>B4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

48/92 0413F0016A 2008-03-31
3.18 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.

If more than one wavelength was measured (dual emission or multi chromatic test runs) you must choose which 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 and also exclude single scan points.

3.18.1 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 meanings:
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 centers lie 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.

**Description of the Window**

**Calculated Values:**

- **Result:** This value is the calculated average of all the scan points used. It will be displayed in the microplate view (value view mode) and used as the raw data value for further calculations.

- **Std. Dev:** The calculated standard deviation of the scan points used.

- **%CV:** The %CV is calculated by dividing the standard deviation of the scan points by the average of the scan points used, and then multiplying the answer by 100 to express the value as a percentage.

**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.

**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 3.14 Color Settings). The color legend shows the color gradient between the minimum value (Min:) and the maximum value (Max:) of a selected well.

**Change Scan Diameter**
Press **Scan Diameter Used** to change the diameter of the circle/square which defines the valid data points. The diameter used window will then open:

The **Scan Diameter Used** describes the diameter of a circle (for a round well shape) or of a square (for a square well) that defines the area within which 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. The used diameter window contains a slider control to change the diameter using the mouse to move the slider changing the diameter. Data points falling outside of the selected area will be displayed in a grey pattern indicating, that these points will not be used for calculating the average value of the well scanned.

**Note:** Changing the diameter will affect all wells used, not only the well altered in the detail window.

Alternatively the border in the image can be moved using with 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 it to the desired size before releasing the mouse button. Note that the new diameter will apply to all wells!

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.
3.19 View Microplate Layout

The microplate layout window can be accessed from the microplate view, showing the layout data of the plate. This is useful tool to get a quick view on the layout even if the microplate view is not the active page.

Click the layout button on the Quick Tool Bar to open the window.

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.

The size and the position of the window can also be changed and is stored during the whole program session.

Click the button to change the layout of the test run (see Changing Layout).
3.20 Display Options

The display options window lets you change the settings for three controls.

The window can be opened by selecting the menu item Display Options… in the menu View of the main menu.

If the reader used does not have a spectrometer, the Spectrum Curve Options will not be visible.

3.20.1 Navigation Tree Options

Autoselect 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 as long as 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 10.

3.20.2 View Options

Show text under quickstart buttons

If this control is checked (default), Text will be displayed under the buttons in the Quick Tool Bar

Select number 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 (Decimal separator =).

Print Options

Show test run number on printed output footer

If this control is checked (default), the test run number (internal id of the test run - PC-dependent) is printed on the footer of each print page.
3.20.3 Spectrum Curve Options

These options are only available if the used reader has a spectrometer installed for absorbance measurements.

- **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 line 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.
    - If the box is unchecked, a white background will be used.

- **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.


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 2.4 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:

<table>
<thead>
<tr>
<th>Standards calculations:</th>
<th>Linear regression fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength: 545-10, 590 (No. 24)</td>
<td>Based on:</td>
</tr>
<tr>
<td></td>
<td>Sum of Range</td>
</tr>
<tr>
<td></td>
<td>Average</td>
</tr>
<tr>
<td></td>
<td>Blank corrected</td>
</tr>
<tr>
<td></td>
<td>Raw Data</td>
</tr>
</tbody>
</table>

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 which 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 open the calculation window with the Calculation button on the Quick Tool Bar or select the required calculation method directly by selecting the corresponding menu item under the menu Calculations -> New Calculation, The calculation window will then open. If selecting the menu item under New Calculation, 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 4.11 Standard Calculation Wizard.

After selecting a number of 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 popup menu or by using the corresponding menu item under the calculations menu.

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 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. To change the input data, a new calculation would need to be preformed.

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 only for test runs containing kinetic data. At least one range should be defined to perform a kinetic calculation for that test run.

A range defines an extract of your kinetic data. A range can include the complete kinetic measurement data down to a single cycle/interval of a measurement.

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 kinetic calculation can then be selected and the input data for that calculation method defined. See the chapter 4.6 Kinetic Calculations, how to perform a kinetic calculation based on a range.
It is possible to define more than one kinetic calculation on the same range (e.g., both the slope of and the average may be needed from the same range).

Ranges are displayed in the signal 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.

It is possible to define a baseline range. It defines the cycles/intervals 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

After opening a kinetic test run for the first time, at least one range for the whole kinetic measurement will be created automatically.

If the test run contains injections, additional ranges will be defined: Creating one range before the injection and one range after the injection (including the injection cycle).

4.1.2 Define a Range

To define a new range use the range functionality options shown on the signal curve chart to add, move or resize a range or use the range menu:

Range Window

You can open the window with the Ranges button in the Quick Tool Bar or from the menu item Calculations -> Define Kinetic Ranges.

The window shows a list with all ranges and their start and stop cycles/intervals.

To select a range in the list to change the borders of this range, click in the cycle field you want to change and enter the new cycle value. 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 cycle/interval 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.

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 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 button in the window.

Depending on the test run the following calculations can be performed:

- Blank corrections
- Negative control corrections
- Baseline corrections
- Replicate statistics
- FP calculations
- TR-FRET calculations
- Moving average (curve smoothing for the signal curve)
- Kinetic calculations (Calculations based on ranges over cycles/intervals)
- Standard curve calculation (Curve fitting)
- Concentration calculations (Calculations based on known and calculated concentrations)
- Data calculations (Arithmetic operations between wavelength or output data of other calculations)
- Validations (Classify the data in good / bad / unknowns...)
- Assay Quality (Z’, signal to blank, signal to noise...)
- 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 popup 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.
4.3 Corrections

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

4.3.1 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 or a blank correction calculation already exists, the check box will be disabled. The blank correction calculates the average 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 of the blanks from each group and subtract the appropriate values from the corresponding groups. If you want to calculate the average of the blanks of all groups and subtract this value from all raw data, this can be done using: **blank correction (ignore groups)** option. This option is only made available if you have groups with blanks.

4.3.2 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 negative correction when you check the **negative control correction** check box. If no negative controls are available or a negative control correction calculation has already been performed, the check box will be disabled.

The negative control correction calculates the average 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 of the negative controls from each group and subtract the appropriate values from the corresponding groups. If you want to calculate the average of the negative controls of all groups and subtract this value from all raw data, this can be done using: **negative control correction (ignore groups)** option. This option is only made available if you have groups with negative controls.

4.3.3 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 this value from all the kinetic data of the selected input data.

**Select the 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 cycle/interval 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 baseline range is shown as a red border range. Change the borders of the range as described in the chapter 3.9.1 Range Functions in the Chart.

The calculation will automatically be updated then.
4.4 Replicate Statistics

The replicate statistics page is only enabled if the layout of the test run contains replicates.

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

Select the calculation method: Select the calculation method for the replicate statistic.

Available methods:

Average: Calculates the average of all replicates of the same content.

Standard deviation: Calculates the standard deviation based on samples (in this case replicates of the same content). 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:

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

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

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

Note: The standard deviation n is the recommended standard deviation method, as the measured data applies to an entire population and not just the samples.

%CV: Calculates the standard deviation of the replicates of the same content divided by the average of the replicates of the same content, 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 divided by the average of the replicates of the same content, and multiplies this number by 100 to express the result as a percentage.

Minimum: Finds the minimum value of the replicates of the same content.

Maximum: Finds the maximum value of the replicates of the same content.
4.5 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. Using these calculations, the two measurement channels will be compared against each other. The calculation operations available differ, depending on the measurement methods:

4.5.1 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.

**Select the 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).
    $$\frac{(\text{parallel} - \text{perpendicular})}{1000 \times (\text{parallel} + \text{perpendicular})}$$
  - **Anisotropy:** Calculates the anisotropy values from the two measured channels (parallel and perpendicular).
    $$\frac{(\text{parallel} - \text{perpendicular})}{1000 \times (\text{parallel} + 2 \times \text{perpendicular})}$$
  - **Intensity:** Calculates the intensity values from the two measured channels (parallel and perpendicular).
    $$\text{parallel} + 2 \times \text{perpendicular}$$
4.5.2 TR-FRET Calculations

Select the input data: Select the input data for the calculation. This can be the result of any calculation which obtains data from the two channels.

Calculations: Select the calculation you want to perform:

Available methods:

**Ratio**: Calculates the ratio between the two measured channels (channel A / channel B). 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 another content, you can change the content if you select another entry in the drop down list **negative control**. If the check box **Use average over all groups** is checked, groups will be ignored, when calculation the DeltaF value. The formula for DeltaF is:

\[
DeltaF = \frac{Ratio_{Signal} - Ratio_{neg}}{Ratio_{neg}} \times 100
\]

*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]).
4.6 Moving Average

The moving average calculation is a curve smoothing method for the signal curves of kinetic test runs.

Select the input data: Select the input data for the moving average calculation. This can be the raw data or the result of any calculation that obtains kinetic data.

Select range: The input data for a moving average calculation are always defined by the first cycle/interval and the last cycle/interval of a range. It is possible to have one or more ranges defined over your kinetic. 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 cycle/interval. Select a range for the calculation from this list. Only ranges with at least four cycles/intervals are shown, because the minimum number of cycles/intervals for the curve smoothing process is four. To view, create or change a range, press the Ranges button to open the range window.

Number of moving Cycles/Intervals: Enter the number of cycles/intervals used for the moving window over the signal curve (box car width). The number must be odd and minimum three. The maximum number is defined by the number of cycles/ranges in the selected range minus one.

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

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

The preview shows the signal curve and the smoothed curve of the first used well. The blue curve is the original signal curve, the red and thicker curve is the smoothed curve. If the measurement contains more then one wavelength data, each wavelength curve is displayed in the graph. In this case, the smoothed curve and the according signal curve have the same color but the smoothed curve is still the thicker one. Each wavelength curve has it's own color:

Move the slider on the left to change the width of the moving window (box car) and see how this affects the smoothing of the curve(s). If you've found the best width, press ok to take this value over 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 have to 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.

Select the 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.

Select range: The input data for a kinetic calculation are always defined by the first cycle/interval and the last cycle/interval of a range. It is possible to have one or more ranges defined over your kinetic. 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 cycle/interval. Select a range for the calculation from this list. To view, create or change a range, press the button to open the range window.

Select the 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 cycle/interval 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.

**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 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:

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

**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 n is the recommended standard deviation method, because the measured data applies to an entire population and not just samples.

**%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.

**%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.
4.9 Standard Calculations / Curve Fitting

The standard 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.

Select the input data: Select the input data for the standard calculation. This can be the result of any calculation generating end point data. If the test run is a kinetic measurement, a kinetic calculation will need to be performed first to reduce the kinetic data to one value for each well.

Select the calculation method: Select the curve fitting method for the standard calculation. Each curve fitting calculation 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 methods:

- **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):
  \[
  Y := mX + b.
  \]

- **4-Parameter fit**: Calculates the dose response curve for the standards. Result parameters are Bottom, Top, Slope, EC50 (IC50) for the formula:
  \[
  Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{(\log(\text{EC50}) - \log(\text{X}))} * \text{Slope})
  \]

- **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 segmental regression divides the standards into two segments and calculates a linear regression for each segment. The result of the fit is the result of each linear regression and the intersection point of the two lines.

- **2nd polynomial fit**: Calculates a quadratic polynomial curve according to the standards. The result is the parameters $b$ (offset), $c1$ (multiplier 1) and $c2$ (multiplier 2) for the fit formula:
  \[
  Y = b + c1X + c2X^2
  \]

- **3rd polynomial fit**: Calculates a third order polynomial curve according to the standards. The result is the parameters $b$ (offset), $c1$ (multiplier 1), $c2$ (multiplier 2) and $c3$ (multiplier 3) for the fit formula:
  \[
  Y = b + c1X + c2X^2 + c3X^3
  \]

**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.

**Note**: Using a logarithmic scaling can have influence to the fit result.
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.

4.9.1 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.

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.

<table>
<thead>
<tr>
<th>Text</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>n.a.</td>
<td>Not available - recalculation not possible (normally the fit itself was not possible)</td>
</tr>
<tr>
<td>&lt;&lt; std range</td>
<td>The calculated concentration value is under the defined limit for this calculation method. (see Limitations for Recalculated Concentrations table below)</td>
</tr>
<tr>
<td>&gt;&gt; std range</td>
<td>The calculated concentration value is above the defined limit for this calculation method. (see Limitations for Recalculated Concentrations table below)</td>
</tr>
<tr>
<td>&lt;&lt; Y range</td>
<td>The input value is either under the domain of the fit or under the defined limit for this calculation method. (see Limitations for Recalculated Concentrations table below)</td>
</tr>
<tr>
<td>&gt;&gt; Y range</td>
<td>The input value is either above the domain of the fit or above the defined limit for this calculation method. (see Limitations for Recalculated Concentrations table below)</td>
</tr>
<tr>
<td>ambiguous</td>
<td>The input value is ambiguous, that means that it fits to more than one concentration value.</td>
</tr>
</tbody>
</table>

Limitations for Recalculated Concentrations:

<table>
<thead>
<tr>
<th>Fit method</th>
<th>Input (y) minimum</th>
<th>Input (y) maximum</th>
<th>Concentration minimum</th>
<th>Concentration maximum</th>
<th>Min. no. of standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear regression</td>
<td>no limit</td>
<td>no limit</td>
<td>no limit</td>
<td>no limit</td>
<td>1</td>
</tr>
<tr>
<td>4-Parameter</td>
<td>Bottom</td>
<td>Top</td>
<td>minY*</td>
<td>maxX*</td>
<td>4</td>
</tr>
<tr>
<td>Cubic spline</td>
<td>min Y of calculated splines</td>
<td>max Y of calculated splines</td>
<td>minX*</td>
<td>maxX*</td>
<td>4</td>
</tr>
<tr>
<td>Point to point</td>
<td>minY*</td>
<td>maxY*</td>
<td>minX*</td>
<td>maxX*</td>
<td>2</td>
</tr>
<tr>
<td>Segmental regression</td>
<td>no limit</td>
<td>no limit</td>
<td>no limit</td>
<td>no limit</td>
<td>4</td>
</tr>
<tr>
<td>2nd polynomial</td>
<td>no limit</td>
<td>no limit</td>
<td>minX*</td>
<td>maxX*</td>
<td>3**</td>
</tr>
<tr>
<td>3nd polynomial</td>
<td>no limit</td>
<td>no limit</td>
<td>minX*</td>
<td>maxX*</td>
<td>4**</td>
</tr>
</tbody>
</table>

*) 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 (i.e from 3nd to 2nd if only 3 standards are available or from 2nd to linear regression if only 1 or 2 standards are available)
4.10 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.

Select the 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 ration between the known and the calculated concentration. The result will be multiplied with the entered ration multiplier.

**Ratio between calculated and known concentration:** Calculates the ration between the calculated and the known concentration. The result will be multiplied with the entered ration 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.
4.11 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.

Select the 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.

Select the 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).

Multiplier: Enter a multiplier for the calculation.

Select the calculation method: Select the calculation method for your data calculation.

Available methods: *minus*, *divided by*, *plus*, *multiplied by*. 
4.12 Assay Quality

The assay quality calculations page provides several methods to analyze your measurement.

Select the input data: Select the input data for the assay quality calculation. This can be the raw data or the result of any calculation.

Select the calculation method: Select the calculation method for your assay quality calculation.

Available methods:

**Z' (Z prime)**: Calculates the Z prime value based on the reference contents you define. Select a content out of the drop down list for each of the data on which the Z prime calculation is based on. The formula for the calculation is:

\[
Z' = 1 - \frac{3 \cdot \sigma_1 + 3 \cdot \sigma_2}{|\mu_1 - \mu_2|}
\]

\(\mu_1\) and \(\sigma_1\) Average and standard deviation of data 1
\(\mu_2\) and \(\sigma_2\) Average and standard deviation of data 2

**Signal to blank**: Calculates the Signal to blank ratio for each content. You can select the content which represents the blank value with the select blank drop down list. If the layout contains blank, it will be pre-selected. The formula for the calculation is:

\[
S / B = \frac{\mu_{\text{signal}}}{\mu_{\text{background}}}
\]

**Signal to noise**: Calculates the Signal to noise ratio for each content. 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:

\[
S / N = \frac{\mu_{\text{signal}} - \mu_{\text{background}}}{\sqrt{\sigma_{\text{signal}}^2 + \sigma_{\text{background}}^2}}
\]

**Percentage calculation**: Set the value of each content 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 fix number (like 0% = 0), you can enter the value in the related entry field. The formula for the calculation is:

\[
V_{0\%} = \frac{\text{Signal} - \mu_{0\%}}{\mu_{100\%} - \mu_{0\%}} \times 100
\]

\(\mu_{0\%}\) is the average of the values over the replicates of the 0% reference.
\(\mu_{100\%}\) is the average of the values over the replicates of the 100% reference.

**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!
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:

Select the 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** or **good / bad / unknown**.

Threshold (Used for the good / bad mode): Enter the threshold that divides the good from the bad data.

Lower Bound (Only for the good / bad / unknown mode): Enter the value for the lower boundary of your classification.

Upper Bound (Only for the good / bad / unknown mode): Enter the value for the upper boundary of your classification.

Displayed Text: Text is defined here that will be shown with the result of the classification.

- **Value <= Threshold (Lower Bound)**: Select the text displayed for the values under/equal to the threshold / lower bound out of the drop down list, or enter any text into the entry field.
- **Value >=(Threshold (Upper Bound)**: Select the displayed text for the values above the threshold / lower bound out of the drop down list, or enter any text into the entry field.
- **Value between Bounds** (only for the good / bad / unknown mode): Select the displayed text for the values between the lower bound and the upper bound out of the drop down list, or enter any text into the entry field.

4.14 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 popup menu or in the corresponding menu item under the calculations menu to open this window:

Select the input data: Select the input data for the well statistic. This can be the result of any calculation which outputs the data as numbers.

Select the calculation method: Select the calculation method for your well statistic.

Available methods: **Average, Standard deviation, Standard deviation n, %CV, %CV n, Minimum, Maximum**.

The available methods are the same as for the replicate statistics. For details on the methods, see the chapter 4.4 Replicate Statistics.

Press the OK - Button on the window to perform the calculation.
4.15 Standard Calculation Wizard

In most cases it is not necessary to perform each needed calculation step using the calculation window to perform a standard calculation as the standard calculation wizard can be used instead. Use the standard calculation wizard instead.

The wizard recommends the suitable calculation steps to apply to your standard calculation process.

Press the Wizard button on the Quick Tool Bar or select the menu item Calculations -> Standard Curve Calculation Wizard to open the wizard.

![Wizard Window](image)

4.15.1 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 maximum 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.

4.15.2 How the Wizard Works

The wizard recommends the calculations to perform 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 they want 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

- **Blank Correction**
  - **use blank corrected raw data**

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

- **Wavelength Calculation**

  - **Wavelength:**
    - A 405, 612 divided by
    - 595, empty
  - **Ratio multiplier:** 1

This option will appear only for end point test runs with two measured wavelengths. If you want to plot a standard curve 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: min, 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

- **Kinetic Calculation**

  - **Range 1**
    - **Range 1**
      - **start interval:** 1
      - **stop interval:** 4
      - **Injection at interval:** 5
  - **Range 2**
    - **Range 2**
      - **start interval:** 5
      - **stop interval:** 64
      - **Injection at interval:** 33.60

Select the calculation method: slope

Appears only for kinetic test runs. If the test run has an injection, you see two ranges in the group and the injection cycle / interval 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 cycle/interval of the measurement.

If there are two ranges, the first range includes the cycles/intervals before the injection, the second range the cycles/intervals 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 cycles/intervals 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 4.6 *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 method you want to perform using the method 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 4.7 *Standard Calculations*.

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, you can perform the required calculations step by step using the calculations window.
4.16 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:

   |   |   |   |   |   |   |   |   |   |
---|---|---|---|---|---|---|---|---|---|
A  |   |   |   |   |   |   |   |   |   |
   |   |   |   |   |   |   |   |   |
B  |   |   |   |   |   |   |   |   |   |
   |   |   |   |   |   |   |   |   |
C  |   |   |   |   |   |   |   |   |   |
   |   |   |   |   |   |   |   |   |
D  |   |   |   |   |   |   |   |   |   |
   |   |   |   |   |   |   |   |   |
E  |   |   |   |   |   |   |   |   |   |
   |   |   |   |   |   |   |   |   |
F  |   |   |   |   |   |   |   |   |   |
   |   |   |   |   |   |   |   |   |
G  |   |   |   |   |   |   |   |   |   |
   |   |   |   |   |   |   |   |   |
H  |   |   |   |   |   |   |   |   |   |
   |   |   |   |   |   |   |   |   |

   |   |   |   |   |   |   |   |   |   |
---|---|---|---|---|---|---|---|---|---|
   |   |   |   |   |   |   |   |   |

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 the layout has not been selected like this 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.

4.16.1 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 have to be applied by pressing OK. To save the changed layout permanent, you have to 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.
4.16.2 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.

4.16.3 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 whole time of the measurement. If this is not the case spread the range manually (see chapter 4.1: Ranges).

4.16.4 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 3.4: Microplate View).
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 Quick Tool Bar 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.

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.

Note: Templates are not the tool to transfer modified layouts from one test run to an 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 we 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 selecting the menu item Templates->Manage Templates....

The window will open and will show the following functions:

- A list of all defined templates.
- A description of a selected template
- Assign protocols to the template
- Remove assigned protocols from the template
- Export and Import templates
- Delete a template.

5.2.1 List of Templates

The window shows a table with all available templates listed.

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 Assign Protocols to Templates

After selecting a template, press the Assign To Protocol(s) Button, to open a window with a list of the protocols and assign one or more protocols to that test run.

At the top of the window you will see the name of the selected template.

The window shows a table with all available protocols in it. The table consists of four columns:

**Protocol name**: Shows the name of the test run protocol and how it was defined in the control software.

**Methods**: Shows the measurement method for that protocol.

**Type**: Shows if it is an end point or a kinetic test run protocol.

**Template**: Shows the name of an assigned template.

It is possible to select one or more protocol in the list. After selecting a protocol, you can immediately check if the template can be assigned to that protocol using the Verify Attributes table:

This table shows the two matching conditions for the last protocol in the list of selected protocols that must be fulfilled to assign the selected template to that protocol.

The last column of the table shows whether or not the conditions match, (yes) or no (a no with a red background)

If both criteria are fulfilled for all selected protocols, the Assign button is enabled and can be pressed to assign the template to the protocols.

If one of the selected protocols has already been assigned a template, the link to the old template will be replaced with the new template.
5.2.3 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.

5.2.4 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 Template....

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 is depending on the operating system! The shown window is the file window of Windows Vista.).

Enter a file name for the file and press Save. The generated file gets the extension .mtf. Files with this extension are recognized as exported templates by MARS.

To import templates, they must be exported by MARS (i.e. on another PC) and the file must have the extension .mtf.

Press the Import Template... 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.

5.2.5 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
- Select the menu item Templates->Create Template from Current Test Run... or select the menu item Create Template from the popup menu in the navigation tree.

On completing the above steps the create template window will then open.

5.3.1 Create template window

![Create Template from current Test Run](image)

**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. MARS will suggest a name for the template, based on the name of the test run protocol. You can use this name or change it.

**Template description**

This section describes the steps performed within the test run and that 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.

**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 button in the Quick tool bar (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 select the menu item Templates->Assign Template to Current Test Run... or the menu item Assign Template of the popup 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:

<table>
<thead>
<tr>
<th>Allowed methods</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence (dual emission)</td>
<td></td>
</tr>
<tr>
<td>Fluorescence (F1)</td>
<td></td>
</tr>
<tr>
<td>Fluorescence (F1), multichromatic</td>
<td></td>
</tr>
<tr>
<td>Time resolved Fluorescence (dual emission)</td>
<td></td>
</tr>
<tr>
<td>Time resolved Fluorescence (TRF)</td>
<td></td>
</tr>
</tbody>
</table>

**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 (yes) or not (no with a red background) with a template.

If both criteria are fulfilled the **Assign** button is enabled and can be pressed to assign the template 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.

**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 Quick Tool Bar 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 Quick Tool Bar. The template buttons created provide a 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 button on the Quick Tool Bar 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 Quick Button Bar. 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 pressing the button on the Quick Tool Bar:
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 Quick Button Bar.

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 Quick Tool Bar.

The new button will appear between the last user button created and the **Add Button** button in the Quick Tool Bar.

### 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 of 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 Creating 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.

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 grey 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 regimentations for transferring standard calculations to test runs with no standards are very small. Only the two conditions to assigning 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:** 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.
6 Change 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 can not 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, select the Change Test run Layout menu item in the Layout menu or press the Edit Layout button in the layout view window. 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.

To save the performed layout changes for the test run, you have to save the test run settings.

With the Load Layout button you can open a stored layout to use it for this test run. Only saved layouts that matches 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 these IDs are used to unique 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 the different ways to select wells, described below.

The first letter in the cell of a well indicates the content type (if less than 384 wells are displayed. Otherwise only the color of the label accords to the content):

<table>
<thead>
<tr>
<th>Letter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Sample The well's content has unknown concentration.</td>
</tr>
<tr>
<td>B</td>
<td>Blank The well contains water or buffer for measuring background.</td>
</tr>
<tr>
<td>S</td>
<td>Standard The well's content has a known concentration and can be used to formulate a standard curve.</td>
</tr>
<tr>
<td>N</td>
<td>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.</td>
</tr>
<tr>
<td>P</td>
<td>Control</td>
</tr>
</tbody>
</table>

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 less than 384 wells are displayed, the index is the number behind the content letter. Otherwise only the index is displayed in the cell.

**Replicates** is 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), activate the usage of groups with checking the **On** check box and 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 less than 384 wells are displayed. Otherwise only the background gets the according color of the group).

**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 can not define a content for unmeasured wells!
### 6.1.3 Changing Concentrations, Dilutions and Sample IDs

To change the known concentrations of standards 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.

<table>
<thead>
<tr>
<th>Well Row</th>
<th>Well Col</th>
<th>Content</th>
<th>Group</th>
<th>Concentrations</th>
<th>Dilutions</th>
<th>Sample IDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 1</td>
<td></td>
<td>Sample X125</td>
<td>A</td>
<td>1.000</td>
<td>Sample of Group A</td>
<td></td>
</tr>
<tr>
<td>A 2</td>
<td></td>
<td>Sample X14</td>
<td>A</td>
<td>10.000</td>
<td>Sample of Group A</td>
<td></td>
</tr>
<tr>
<td>A 3</td>
<td></td>
<td>Sample X15</td>
<td>A</td>
<td>20.000</td>
<td>Sample of Group A</td>
<td></td>
</tr>
<tr>
<td>A 4</td>
<td></td>
<td>Sample X16</td>
<td>A</td>
<td>30.000</td>
<td>Sample of Group A</td>
<td></td>
</tr>
<tr>
<td>A 5</td>
<td></td>
<td>Sample X17</td>
<td>A</td>
<td>40.000</td>
<td>Sample of Group A</td>
<td></td>
</tr>
<tr>
<td>A 6</td>
<td></td>
<td>Sample X19</td>
<td>A</td>
<td>50.000</td>
<td>Sample of Group A</td>
<td></td>
</tr>
<tr>
<td>A 7</td>
<td></td>
<td>Sample X19</td>
<td>A</td>
<td>60.000</td>
<td>Sample of Group A</td>
<td></td>
</tr>
<tr>
<td>A 8</td>
<td></td>
<td>Sample X20</td>
<td>A</td>
<td>70.000</td>
<td>Sample of Group A</td>
<td></td>
</tr>
<tr>
<td>A 9</td>
<td></td>
<td>Sample X21</td>
<td>A</td>
<td>80.000</td>
<td>Sample of Group A</td>
<td></td>
</tr>
<tr>
<td>A 10</td>
<td></td>
<td>Sample X22</td>
<td>A</td>
<td>90.000</td>
<td>Sample of Group A</td>
<td></td>
</tr>
<tr>
<td>A 11</td>
<td></td>
<td>Sample X23</td>
<td>A</td>
<td>100.000</td>
<td>Sample of Group A</td>
<td></td>
</tr>
<tr>
<td>A 12</td>
<td></td>
<td>Sample X24</td>
<td>A</td>
<td>110.000</td>
<td>Sample of Group A</td>
<td></td>
</tr>
<tr>
<td>B 2</td>
<td></td>
<td>Standard 51</td>
<td>A</td>
<td>200</td>
<td>Standard 1 of Group A</td>
<td></td>
</tr>
<tr>
<td>B 3</td>
<td></td>
<td>Standard 51</td>
<td>A</td>
<td>200</td>
<td>Standard 1 of Group A</td>
<td></td>
</tr>
<tr>
<td>B 4</td>
<td></td>
<td>Standard 51</td>
<td>A</td>
<td>200</td>
<td>Standard 1 of Group A</td>
<td></td>
</tr>
<tr>
<td>B 6</td>
<td></td>
<td>Negative Control</td>
<td>A</td>
<td>120.000</td>
<td>Negative Control Group A</td>
<td></td>
</tr>
</tbody>
</table>

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 2.1 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. Thus the dilution values can only be entered for all wells but not for blanks and standards.

The auto fill out 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 out 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.
6.2 Manage Layouts

The manage layouts functions provides you the possibility to create new layouts or layouts out of a test run, to change the layouts and to exchange the layouts by the export and import functionality. These layouts can be assigned to a test run, if the saved layout fits to the test run.

Select the Manage Layout menu item in the Layout menu 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. 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:

6.3 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 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 Load Layout button on the window. You see the same window as 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 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.

6.4 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 chapter 6.1: Changing Layout. 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.

6.5 Delete Layouts

Select one or more layouts in the list and press the Delete Layout(s) to delete the saved layouts.

6.6 Export and Import Layouts

You can export the saved layouts to exchange them with others. Select the layouts you want to export and press the Export Layout(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 Sign a Test Run

To ensure that your measured result and your performed evaluation on the test run cannot be changed or manipulated any more you can sign your test run.

The ability to sign a test run is also needed to fulfill the FDA 21 part 11 compliance. Read more about this in the software manual part IV: FDA 21 CFR part 11.

To sign a test run, you need a pair of RSA keys. How to get such keys is also described in the software manual part IV: FDA 21 CFR part 11.

Select the menu item Test Run -> Sign current test run... to sign the opened and selected test run. The authentication window will then appear:

After logging in, it is possible to enter a comment to the signature:

Press OK to finish the sign process.

Signed test runs are indicated with a blue sign text in the test run description area on top of the working area. An entry in the audit trail is generated and you can see the signature on the 21 CFR part 11 page. It is possible to add more than one signature to a test run (i.e. one for released and one for reviewed...). If a test run is signed, you cannot save any more changes made in the software for that test run. You can make a copy of the test run and change the copied test run because the signature will not be copied.

Note: The readers NOVOstar and NEPHELOstar Galaxy do not support this function!

8 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 using the following email addresses:

- Problems / questions regarding software: support@bmglabtech.com
- Problems / questions regarding the instruments: tech.service@bmglabtech.com

You can also use our technical request form (http://www.bmglabtech.com/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.