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Q-Rex Absolute Quantification HID Plug-in User Manual

For use with the Q-Rex Software v1.0 to calculate absolute concentration of targets by PCR

Sample to Insight

QIAGEN®

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Introduction

1 Introduction

Plug-ins for the Q-Rex Software grant additional analysis capabilities. Plug-ins for the Q-Rex Software cannot be used without the main software component. Familiarize yourself with the Q-Rex Software before installing and using plug-ins.

The Q-Rex Absolute Quantification HID Plug-in calculates the absolute concentration of targets in experimental samples using the amplification curves of samples of known concentration to establish a standard curve that correlates Cq value (y-axis) and target concentration (x-axis). The Q-Rex Absolute Quantification HID Plug-in has an automatic threshold function based on the defined standards that scans through all possible threshold levels until the best fit is found for the standard curve. The threshold can also be set manually.

1.1 Important Note

The *Q-Rex Absolute Quantification HID Plug-in User Manual* provides general information about the software settings. The recommended product-specific settings are described in the corresponding kit handbooks, available on individual product pages at **www.qiagen.com**. The currently available QIAGEN HID quantification products belong to the Investigator Quantiplex series.

1.2 About this user manual

This user manual provides information about the functions and features of the Q-Rex Absolute Quantification HID Plug-in. You will find general information about the functions and features of the Q-Rex Software in the *Q-Rex Software User Manual*.

Installing the Q-Rex Absolute Quantification HID Plug-in impacts only analysis aspects of the Q-Rex Software. This user manual describes changes to settings and functionalities necessary to perform the analyses enabled by the Q-Rex Absolute Quantification HID Plug-in. All other aspects of the Q-Rex Software remain unchanged, and therefore, instructions in the *Q-Rex Software User Manual* remain valid. Make sure to read the *Q-Rex Software User Manual* and pay particular attention to the listed limitations and warnings before working with the software.

Please refer to the *Rotor-Gene Q Manual* for complete information about the proper care, maintenance and use of the Rotor-Gene Q Instrument.

This user manual provides information about the Q-Rex Absolute Quantification HID Plug-in in the following sections:

- Introduction
- Set up an experiment
- Run an experiment
- Analyze an experiment
- Report and export results
- Troubleshooting

1.3 General information

1.3.1 Technical assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the Rotor-Gene Q, Q-Rex Software, the Q-Rex Absolute Quantification HID Plug-in or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**.

1.3.2 Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual. Please contact QIAGEN Technical Services.

1.3.3 Version management

This document is the *Q-Rex Absolute Quantification HID Plug-in User Manual*, which provides information about the Q-Rex Absolute Quantification HID Plug-in, version 1.0.

1.4 Getting help

Please refer to "Getting help" in the *Q-Rex Software User Manual* for a description of the available help function.

Working with absolute quantification HID experiments

2 Working with absolute quantification HID experiments

2.1 Set up an experiment

For all general information on how to set up a new experiment, refer to "Set up a new experiment" or "Set up an experiment via wizard" in the *Q-Rex Software User Manual*.

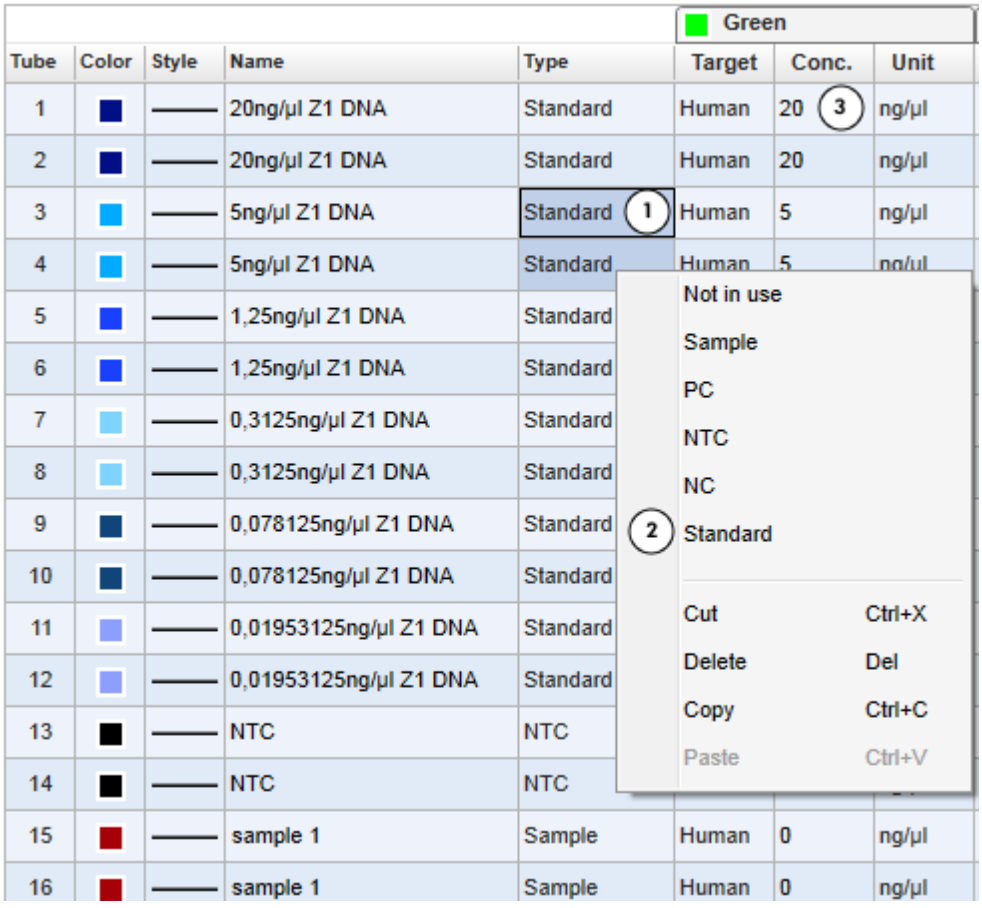
In absolute quantification human identification analysis, you must define standards and their concentrations to enable the calculation of target concentrations in experimental samples. Determine absolute concentrations of the standard samples by an independent method. A threshold is set during the analysis at the exponential phase of the amplification reaction for each standard to determine the Cq value corresponding to the concentration.

An experiment must meet the following requirements to perform an **Absolute Quantification HID** analysis:

- At least 2 tubes are assigned the sample type **Standard**.
- The standards have a defined valid concentration.
- Tubes containing standards must render a valid Cq value.

To define tubes as standards:

1. Click the **Sample Layout** step in the **Step Marker** of the **Experiment** environment.
2. Select the cells in the **Sample type** column (**1**) corresponding to the tubes.
3. Right-click the selected cells and select **Standard** in the context menu (**2**).
4. Enter a concentration value for each standard in the **Conc.** column (**3**).



2.2 Run an experiment

To run an experiment, see "Run an experiment" in the *Q-Rex Software User Manual*.

2.3 Analyze an experiment

The following sections describe using the Q-Rex Absolute Quantification HID Plug-in to determine absolute concentrations of samples:

Add an analysis

View plots

Define analysis parameters

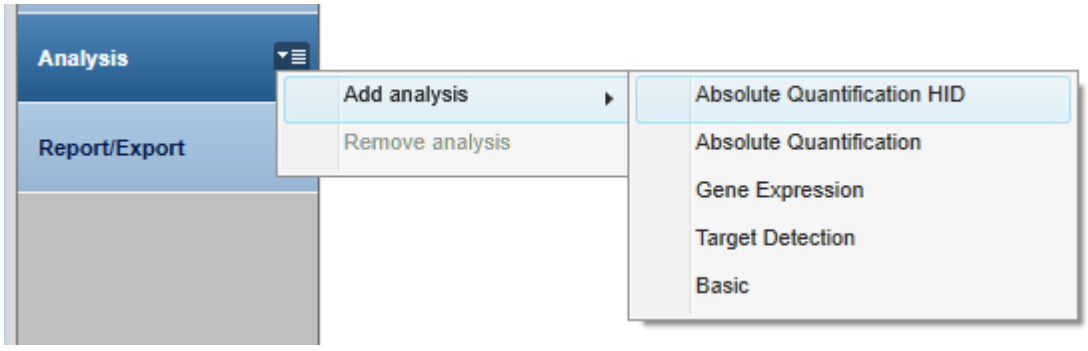
View results

For a description of general use concepts of the Q-Rex Software, see "Analyze an experiment" in the *Q-Rex Software User Manual*.

2.3.1 Add an analysis

For general information on how to add an analysis in the Q-Rex Software, see "Add an analysis" in the *Q-Rex Software User Manual*.

If the Q-Rex Absolute Quantification HID Plug-in was installed correctly, a menu item for the plug-in will appear in the list of available analyses:



2.3.2 View plots

In addition to the Raw Data and Normalized Data fluorescence plots, analysis with the Q-Rex Absolute Quantification HID Plug-in offers a Standard Curve plot.

For detailed information regarding the Standard Curve plot, refer to the "View standard curve plot" section.

For an overview of fluorescence plots in the Q-Rex Software, see "View fluorescence plots" in the *Q-Rex Software User Manual*.

2.3.2.1 View standard curve plot

The **Standard Curve** plot is displayed in the lower right area of the screen in the **Analysis** step.

The plot includes the following elements:

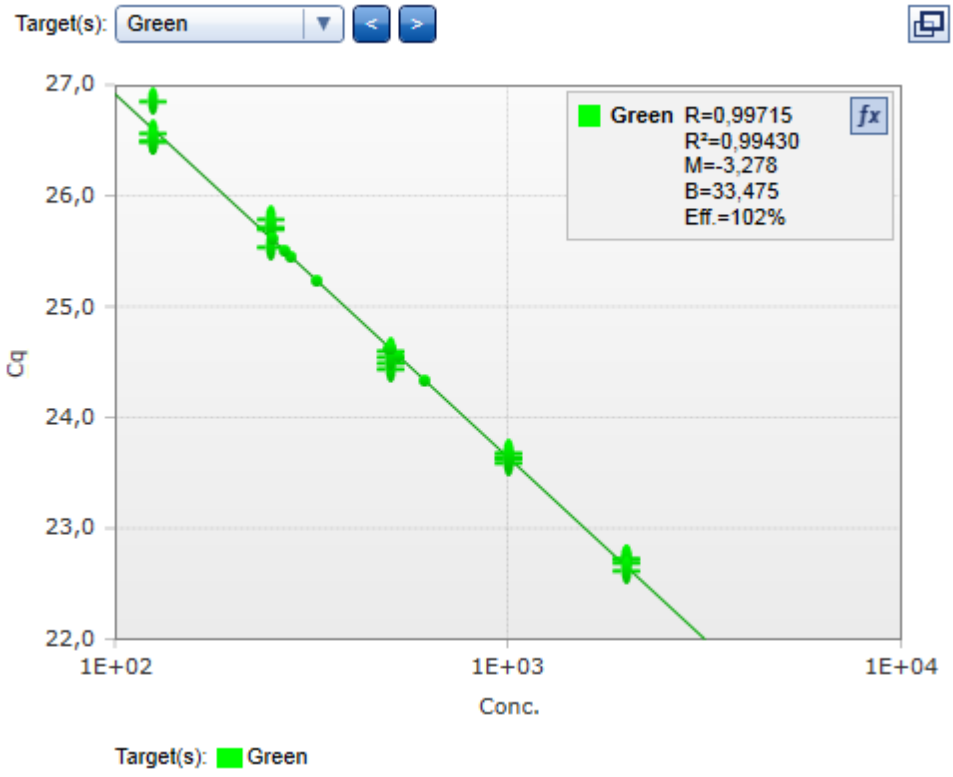
- A **Target Selector** (**1**)
- An icon to show the standard curve formula (**2**)
- A legend (**3**)

Use the **Target Selector** to display the standard curve of the analyzed targets. You can browse through a list of targets using the **Forward** and **Back** buttons.

Note: You can view multiple targets in the same standard curve plot window. The curves of the different targets are color-coded.

Crosses on the curve represent the samples defined as standards and dots are the data points of the experimental samples.

To display the standard curve formula, click the icon. The formula values appear in a layer over the plot.



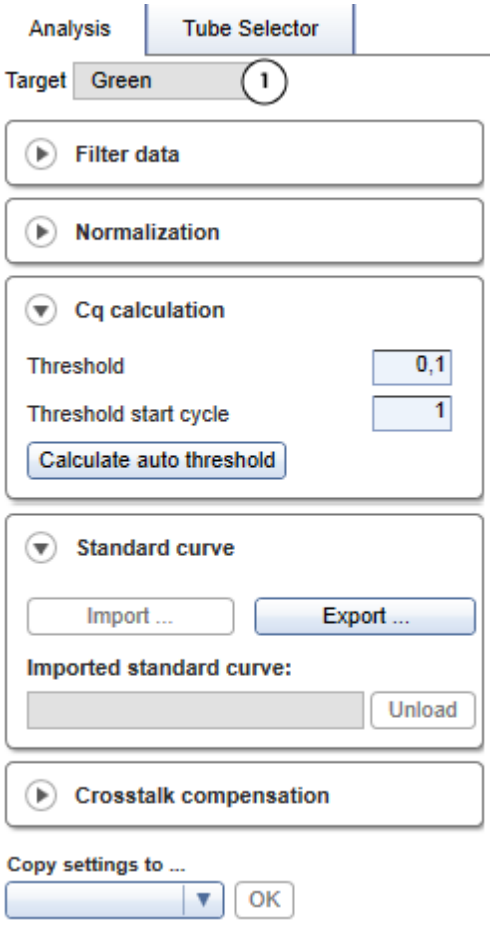
The following calculated values are displayed in the plot:

Calculated value	**Definition**
R-value	The correlation coefficient indicates the percentage of the data that are consistent with the statistical hypothesis.
R^2-value	The square of the correlation coefficient indicates how well the data points from the standards lie on a straight line. A value of 1.0 would be a perfect fit, a value of 0 would indicate a random distribution. If the value is much lower than 1.0, this might indicate problems with the standards.
Slope and intercept	Based on the linear formula y = MX + B, the slope (**M**) and the intercept (**B**) of the standard curve are automatically calculated and displayed.
Efficiency	The reaction efficiency of the run.

Note: Varying the threshold level in the standard curve causes the values to be dynamically recalculated. To change the threshold level, either click and drag the threshold line in the active plot or change the value in the **Analysis** tab of the **Drawer**.

2.3.3 Define analysis parameters

To define analysis parameters for each target, open the **Analysis** tab of the **Drawer** in the **Experiment** environment.



The active target for which the analysis parameters are defined is displayed at the top of the tab (**1**). This is the target selected in the active plot window. If multiple targets are selected in the active plot window, this field remains empty and analysis parameters cannot be edited.

Note: Reasonable default values are defined for most analysis parameters. If parameters must be defined, their entry fields are highlighted in yellow. Unless these required parameters are defined, the corresponding input fields appear as invalid and results cannot be displayed. If an invalid input field is hidden, the surrounding parameter group, the **Analysis** tab or even the **Drawer** itself are shown as invalid.

Note: More information on how to analyze data obtained using QIAGEN HID Kits can be found in the corresponding kit handbooks.

Define the following parameter groups for absolute quantification human identification analysis:

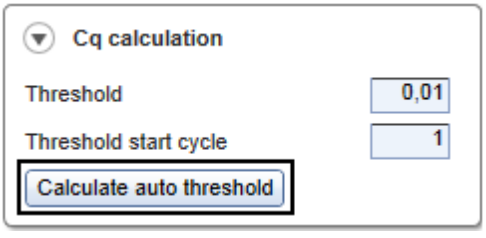
Filter data	See the *Q-Rex Software User Manual* for details.
Normalization	See the *Q-Rex Software User Manual* for details.
Cq calculation	See the "Define absolute quantification parameter" for details.
Optional: Melt peak analysis	Melt peak analysis option is only visible if melt data are available for the experiment. See the *Q-Rex Software User Manual* for details.

Optionally, the following additional analysis features can be used:

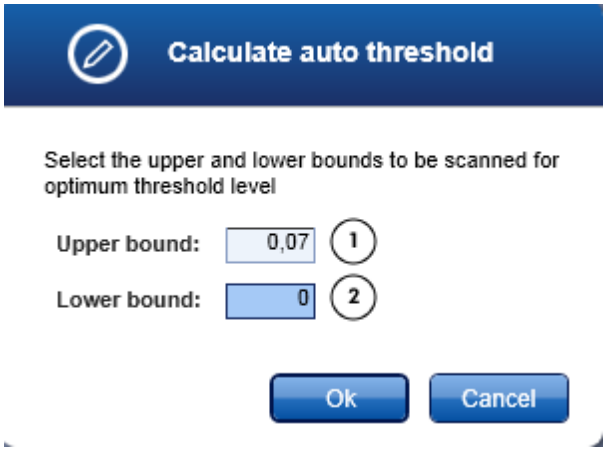
Standard curve	See the "Reuse of standard curves" for details.
Crosstalk compensation	See "Compensating crosstalk".
Copy settings to…	See "Copy analysis parameters".

2.3.3.1 Define absolute quantification parameters

In the **Analysis** tab of the **Drawer**, you can either manually define a Cq threshold or have the Q-Rex Absolute Quantification HID Plug-in to **Calculate auto threshold**, if standards have been used during the experiment.



The automatic threshold function scans a specified region of the standard plot to find a threshold setting that delivers optimal estimates of given concentrations. Click **Calculate auto threshold** and define the scanned region by entering an **Upper** (**1**) and **Lower** (**2**) bound.



Upon clicking **OK**, the Q-Rex Software scans the threshold level range to obtain the best fit curve through the samples defined as standards, (i.e., the R-value that most closely approximates 1.0). A new threshold is calculated and displayed in the **Analysis** parameters and **fluorescence plots**.

With the Q-Rex Absolute Quantification HID Plug-in version 1.0 it is possible to reuse standard curves from other experiments. This way concentrations can be calculated even if no standards have been used in the run. Detailed information on how to import or export standard curves can be found in the "Reuse of standard curves" chapter.

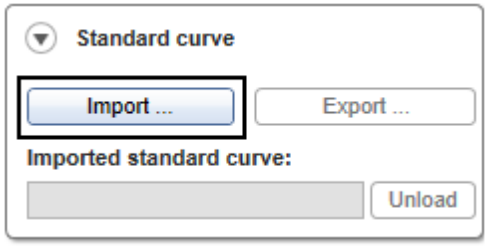
2.3.3.2 Reuse of standard curves

To import or export standard curves for each target, expand the **Standard Curve** section in the **Analysis** tab of the **Drawer**.

Import standard curves

If no standards have been used in the current experiment, a standard curve can be imported.

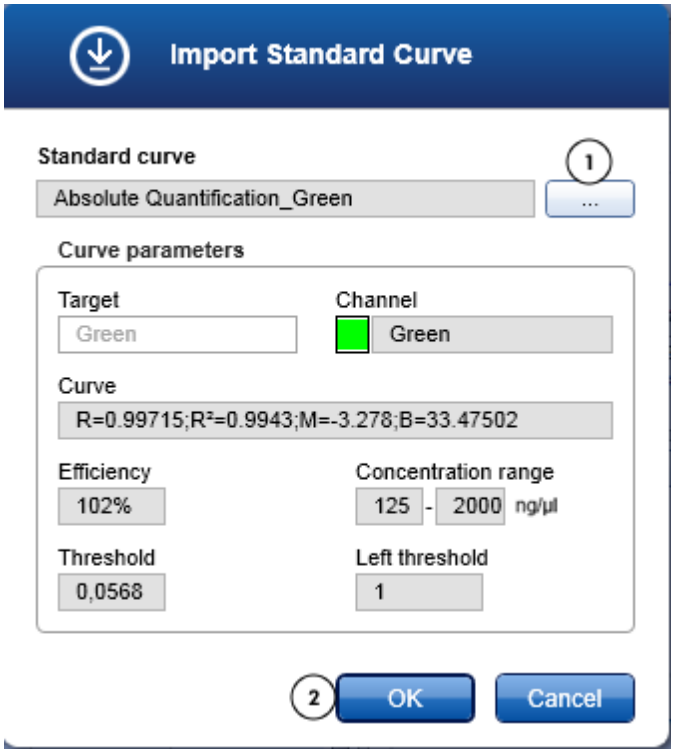
1. To import a standard curve from another experiment click on **Import**... to open the **Import Standard Curve** window.



Note: Standard curves can only be imported if no **Standards** have been used in the experiment. If the

Import... button appears de-activated and grayed out, go to the **Sample Layout** step and remove all **Standards** from the **Sample type** assignment.

2. Click on the browse button (**1**) to open a file dialog. In this dialog, you can select already exported standard curve files (*.qesc files) from your system. The curve parameters of the selected file will be shown in detail on the **Import Standard Curve** Screen as read-only fields.

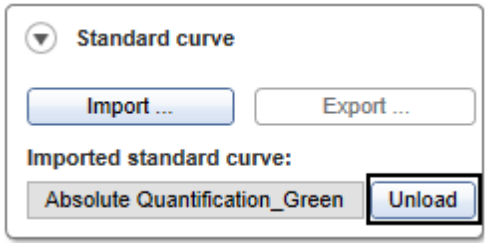


Target	Shows the target for which the standard curve was computed.
Channel	Shows the channel of the exported standard curve.
Curve	Displays the standard curve formula including R-value, R^2-value, slope and intercept. For further information on the values refer to "View standard curve plot".
Efficiency	Efficiency in percent.
Concentration range	The concentration range of the standards upon the standard curve has been computed.
Threshold	Used threshold during analysis of the target.
Left threshold	Used left threshold during analysis of the target.

3. After confirming the selection with a click on **OK** (**2**), the standard curve will be imported and will appear in the lower right section of the analysis view. Previously defined threshold and left threshold parameters will be overwritten with the corresponding threshold values from standard curve data. The thresholds will not be adaptable any longer and stand as read-only in the analysis view.

Unload standard curves

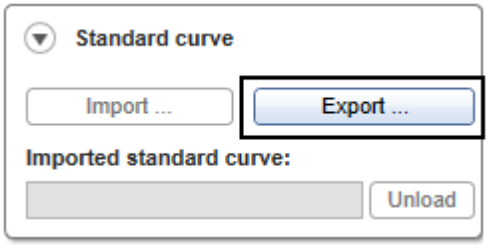
To unload the imported standard curve click on **Unload**. The imported standard curve will be removed from the experiment and the threshold will be removed. It can be calculated automatically or set manually again.



Export standard curves

To reuse a standard curve in another experiment, it has to be exported first.

1. Click on **Export**... to open the **Save Standard curve** dialog.



2. Adapt the default file name and location, if required. The standard curve will be saved and made available for being loaded in other experiments.

Note: It is not possible to export an imported standard curve. The **Export**... button is only active if standards have been run and a standard curve was created in the active experiment.

2.3.3.3 Compensating crosstalk

Using two or more acquisition channels in the same experiment can lead to observed crosstalk. Crosstalk occurs when fluorescent dyes and filters have overlapping wavelength ranges and the emission from one channel contributes to the acquisition of another channel.

In the Q-Rex Absolute Quantification HID Plug-in, you can compensate for this signal bleed from one channel into another. **Crosstalk compensation** allows you to remove this signal interference by:

Performing crosstalk compensation

Defining crosstalk compensation settings

Saving compensation factors

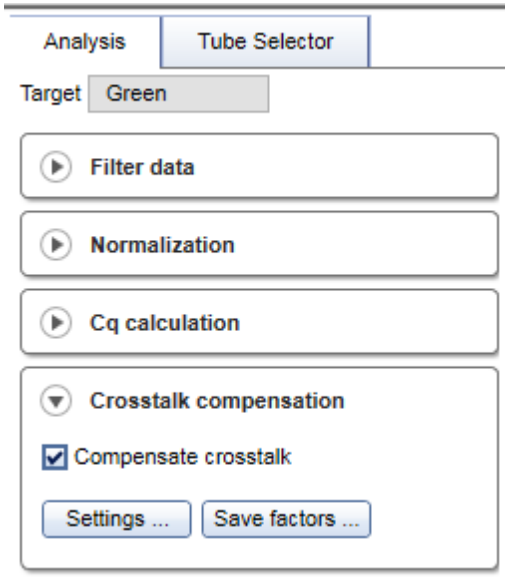
Loading compensation factors

Note: Crosstalk compensation cannot be a component of a template. Crosstalk compensation is added after execution and review of an experiment and only if it is required.

2.3.3.3.1 Performing crosstalk compensation

To set up crosstalk compensation, expand the **Crosstalk compensation** section in the **Analysis** tab of the **Drawer**.

Note: Crosstalk compensation cannot be a component of a template. Instead, it is added after execution and review of an experiment and only if it is required.



Click **Settings** to access the crosstalk compensation settings dialog. Define your crosstalk compensation and click **OK**.

Check the **Compensate crosstalk** checkbox to apply your defined crosstalk compensation settings. The compensation appears on the normalized data of the channels which were specified in the **Settings** dialog.

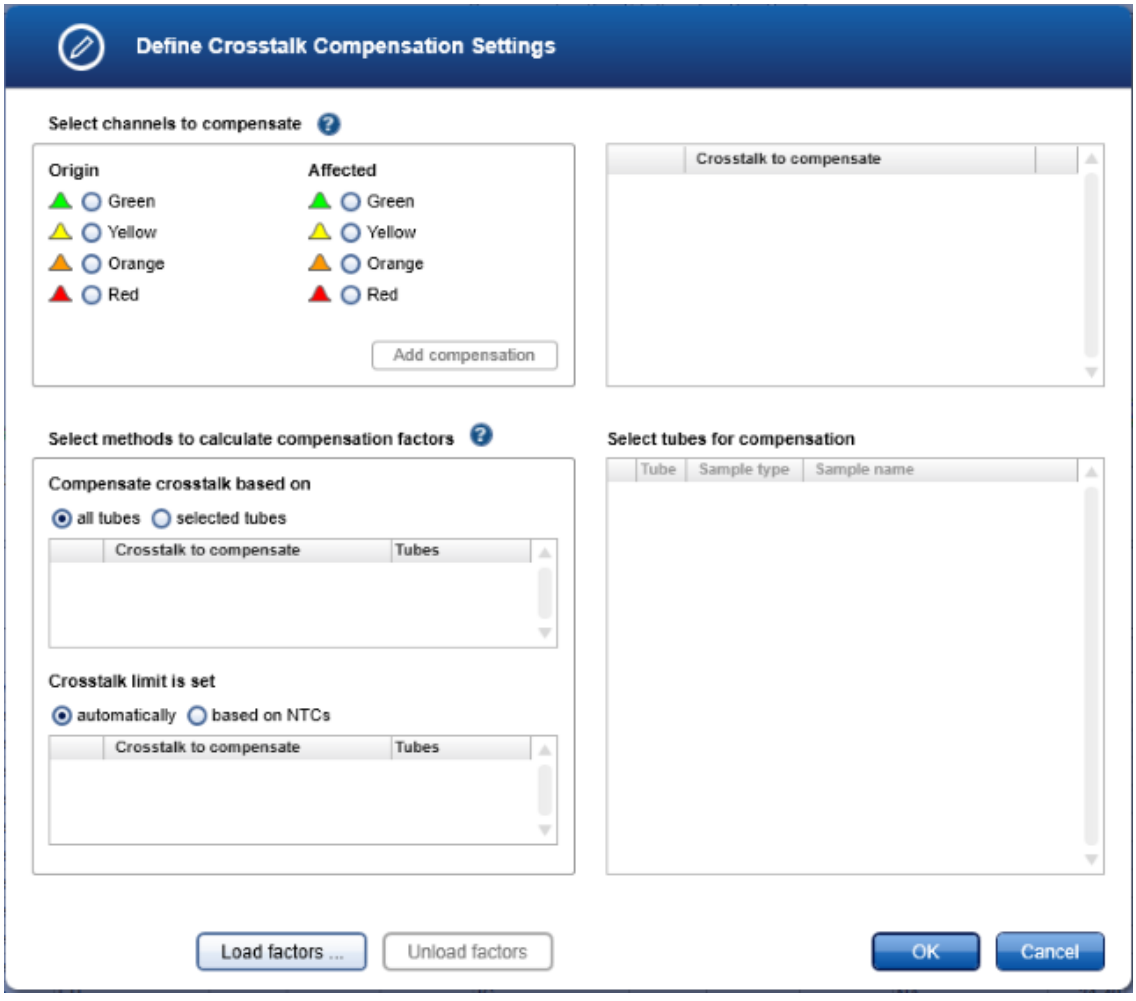
Remove the tick from the checkbox to remove the crosstalk compensation from the normalized data.

Note: Invalid crosstalk compensation settings disable the **Compensate crosstalk** checkbox and the **Settings** button is displayed as invalid.

Click **Save factors** to access the **Save Compensation Factors** dialog. You can save calculated crosstalk compensation factors to the file system and use these again in a different experiment.

2.3.3.3.2 Defining crosstalk compensation settings

Setup the compensation settings for your experiment in the **Define Crosstalk Compensation Settings** dialog.



You can load saved crosstalk compensation factors or define new settings. To define new settings:

1. Select the channels requiring crosstalk compensation. The **Select channels to compensate** section lists all channels in the experiment in two columns labeled **Origin** and **Affected**.

Select the channel causing the crosstalk from the **Origin** list and the channel affected by the crosstalk from the **Affected** list.

2. Add your selection to the **Crosstalk to compensate** section to the right by clicking **Add compensation**.

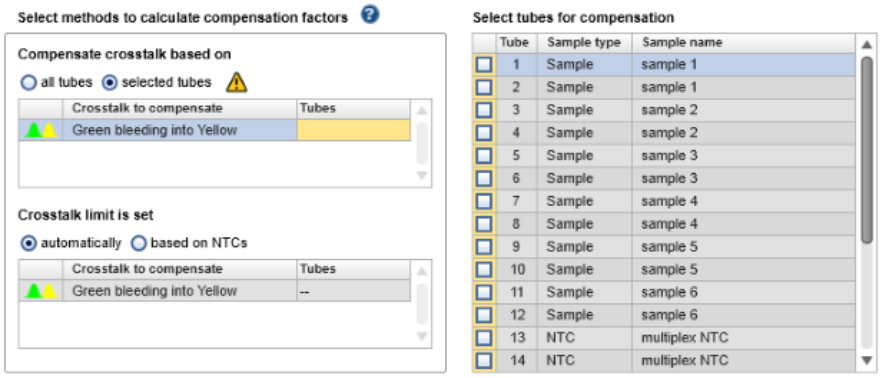
To remove a defined channel compensation, click  in the row of the defined crosstalk.

Note: Each combination of **Origin** and **Affected** channels can be added only once to the **Crosstalk to compensate** section. You cannot compensate channels with the same wavelength or channels that undergo data acquisition in different cycling steps.

3. Select the method to calculate compensation factors.

The factors for a compensation can be calculated based on **all tubes** or **selected tubes**.

all tubes	All tubes with Sample type other than **Not in use** are used for the calculation. This option is preselected by default and is best used in combination with the option to set the crosstalk limit **automatically** for an initial compensation in an experiment.
selected tubes	Only selected tubes are used to calculate compensation factors. You must define at least 1 tube with an expected signal in the **Origin** channel and no expected signal in the **Affected** channel. This is an advanced option and should be implemented only by experienced users. To select tubes, click the radio button **selected tubes** and a table of tubes in the experiment appears to the right.

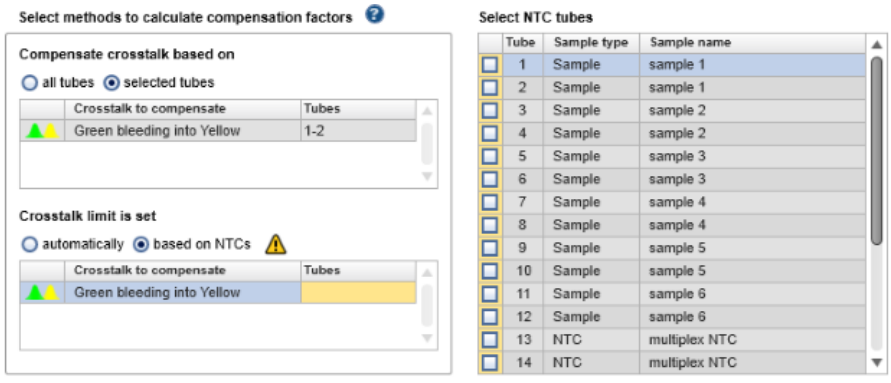


Check the checkbox of each tube to be used in the calculation. Each tube must have an expected signal in the **Origin** channel and no expected signal in the **Affected** channel.

4. Select the method to set the limit for the crosstalk compensation; that is, how far should the signals of affected tubes be compensated.

In the **Crosstalk limit is set** section, choose between setting the limit automatically or based on specific non-template controls (NTCs).

automatically	All tubes with Sample type other than **Not in use** are used to automatically calculate a compensation limit. This option is preselected by default and is best used in combination with the option to compensate crosstalk based on all tubes for an initial compensation in an experiment.
based on NTCs	Signals are compensated until the curve reaches that of crosstalk-free NTCs. This option is available if you choose to compensate crosstalk based on selected samples. Fluorescence signals are compensated until the fluorescence of the specified NTCs is reached. This is an advanced option and should be used only by experienced users. To select tubes for this option, click the radio button **based on NTCs** and a list of tubes in the experiment appears to the right.



In the table, check the checkbox of each tube to be used to set the limit. Each tube must have acquisitions in the **Origin** and **Affected** channels, but no template in either (NTC) and therefore no amplification signals.

Note: The options to compensate crosstalk based on all tubes and set the crosstalk limit **based on NTCs** cannot be used together.

5. Once you have defined the settings, click **OK** to return to the analysis to apply them.

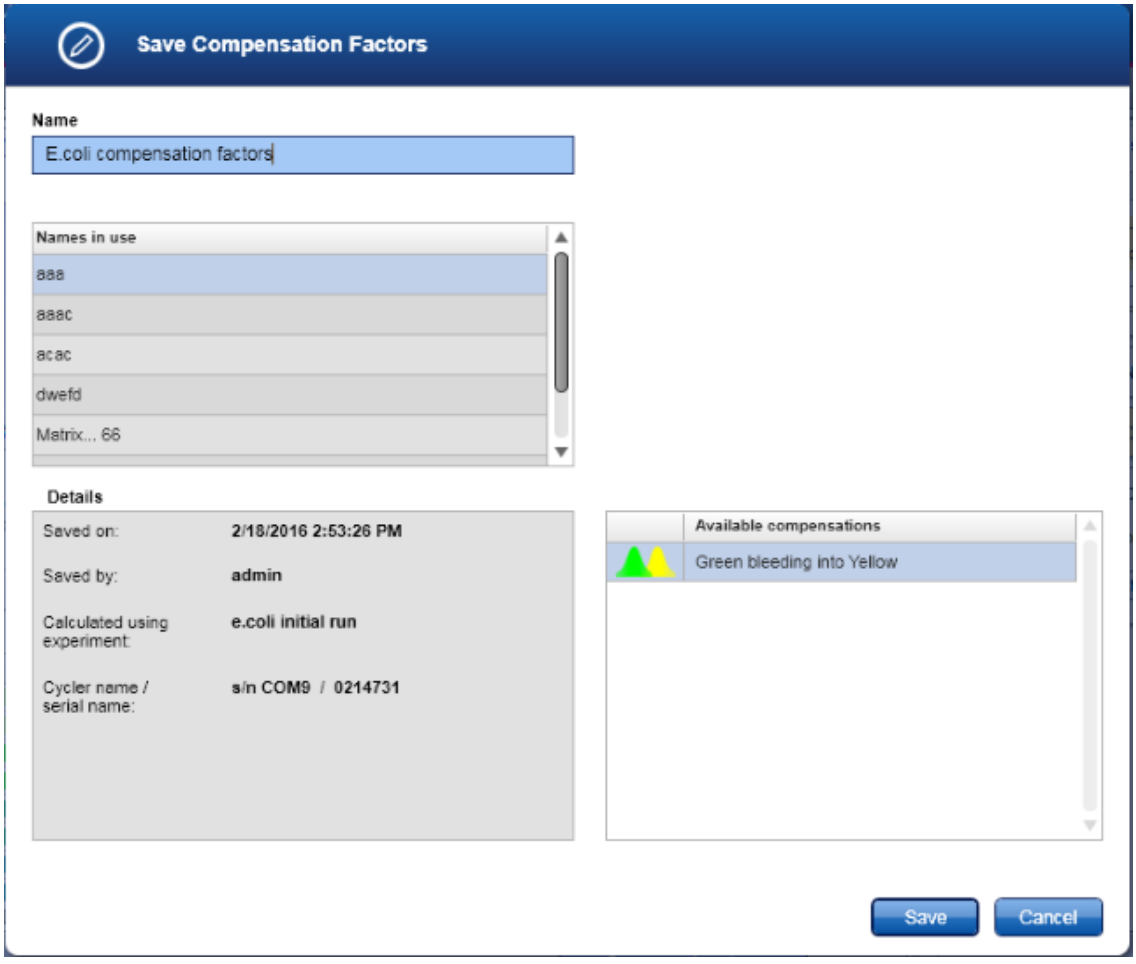
Alternatively, click **Cancel** to discard all entries made and exit the dialog.

2.3.3.3.3 Saving compensation factors

You can save calculated compensation factors to use them again in a different experiment. We recommend saving compensation factors only if they will be used for experiments run on the same instrument and with the same experimental setup.

To save your compensation factors:

1. Open the **Save Compensation Factors** dialog by clicking **Save factors** in **Crosstalk compensation** section of the **Analysis** tab of the **Drawer** (see Performing crosstalk compensation).



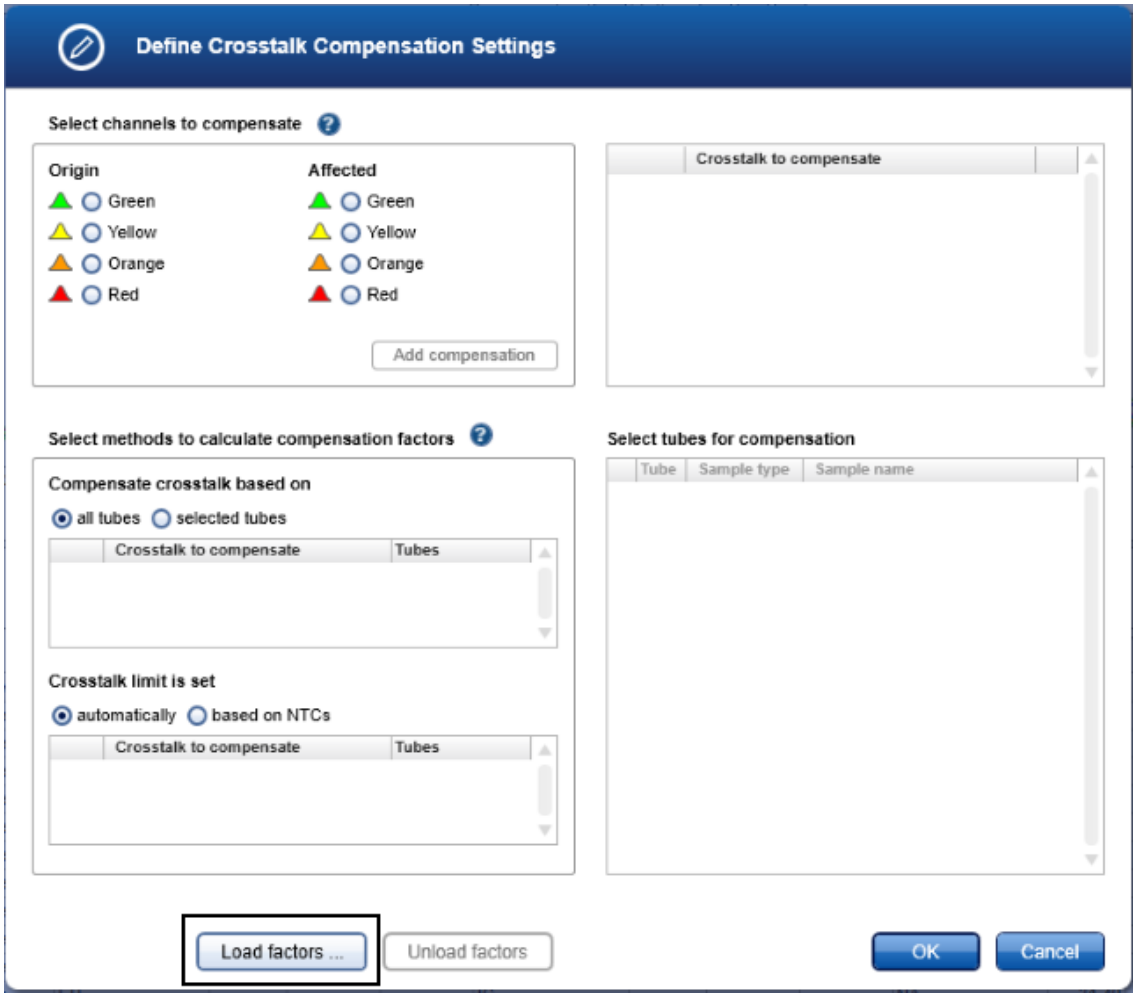
Available compensations	Lists all channels chosen to be compensated.
Details	Summarizes the experiment from which the compensation factors were calculated.
Names in use	Displays file names of compensation factors already saved and used on your system.

2. Click the **Name** field and enter a name for the compensation factors to be saved. If a 'saved factors' file already exist with the same name, a warning message is displayed.

3. Click **Save** to save the compensation factors and exit the dialog.

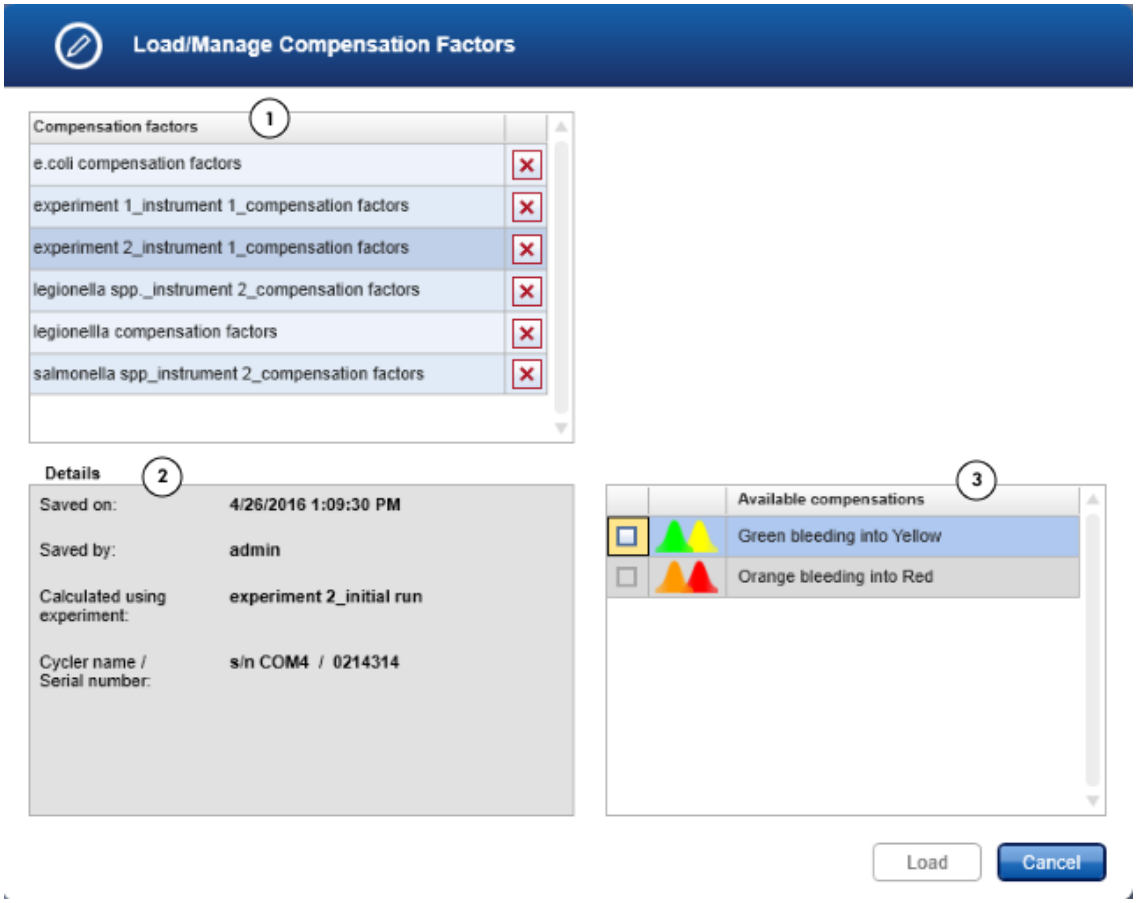
2.3.3.3.4 Loading compensation factors

To load compensation factors saved to your system, click **Load factors** in the **Define Crosstalk Compensation Settings** dialog.



The **Load/Manage Compensation Factors** dialog that appears lists all previously saved compensation factors. In this dialog, you can load compensation factors or remove unwanted compensation factor files from your system.

Note: We recommend using saved compensation factors only in experiments run on the same instrument and with the same experimental setup.



To remove compensation factor files, find the file in the **Compensation factors** field (**1**), click  and confirm the deletion of the compensation factors from your system.

To load saved compensation factors:

1. Select the file with the compensation factors you want to load from the **Compensation factors** field (**1**).
2. Check the information provided in the **Details** field (**2**) to make sure you have chosen the correct compensation factors.

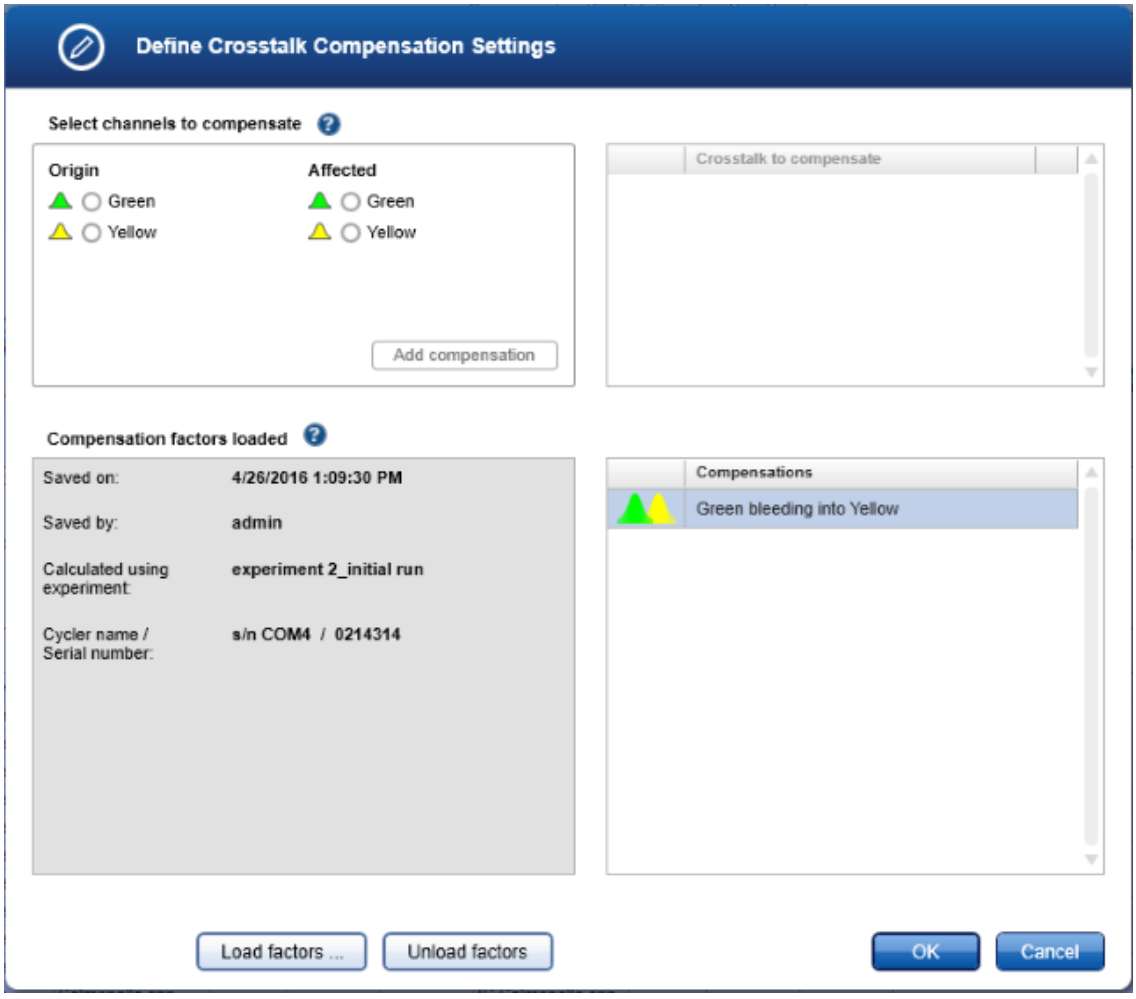
3. From the **Available compensations** table (**3**), select at least one compensation in the file to be loaded. Compensations which are not applicable to your experiment appear grayed out and cannot be selected.

4. Click **Load** and return to the **Define Crosstalk Compensation Settings** dialog. Details about the loaded compensation factors are displayed in this dialog.

5. Click **OK** to confirm your selection and return to the analysis.

Alternatively, you can load compensation factors from another file by clicking **Load** factors or unload the compensation factors by clicking **Unload** factors. All settings return to default and you can define new crosstalk compensation settings.

Click **Cancel** to return to the analysis step without saving defined compensation settings.



2.3.3.4 Copy analysis parameter

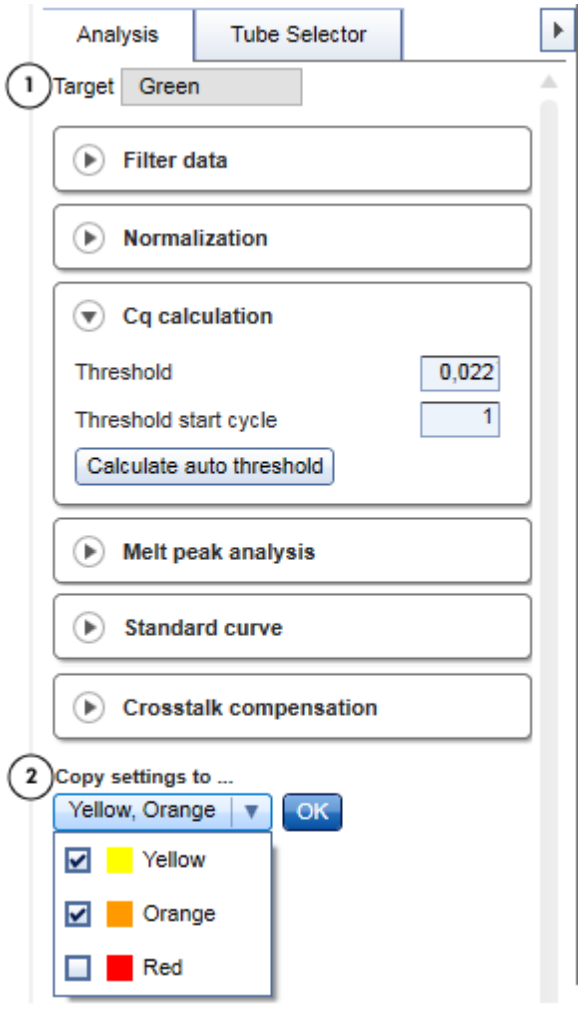
In **Absolute Quantification HID** analysis, parameters from one target can be copied to other targets using the **Copy settings to** (**2**) function of the **Analysis** tab in the **Drawer**.

To copy analysis parameters to one or more targets:

1. Define analysis parameters for the active target.

The active target for the analysis is visible at the top of the **Drawer** (**1**) and its editable analysis parameters are listed below. This is also the target selected in the active fluorescence plot.

2. The drop down menu under **Copy settings to** (**2**) lists all targets available except for the active target. Use the drop down menu to indicate the destination targets for the parameters.



3. Click **OK**. Filter data, normalization settings, thresholds, and melt settings (if available) from the active target are copied to all selected destination targets.

Note: If multiple targets are selected in the fluorescence plot, the **Target** field at the top of the **Drawer** remains empty, the analysis parameters cannot be edited, and the **Copy settings to** function is disabled.

2.3.4 View results

Once all required analysis parameters are defined, the **Results Table** in the lower half of the **Analysis** screen displays the results in 3 different views. Click the tabs at the top of the **Results Table** to access each view.

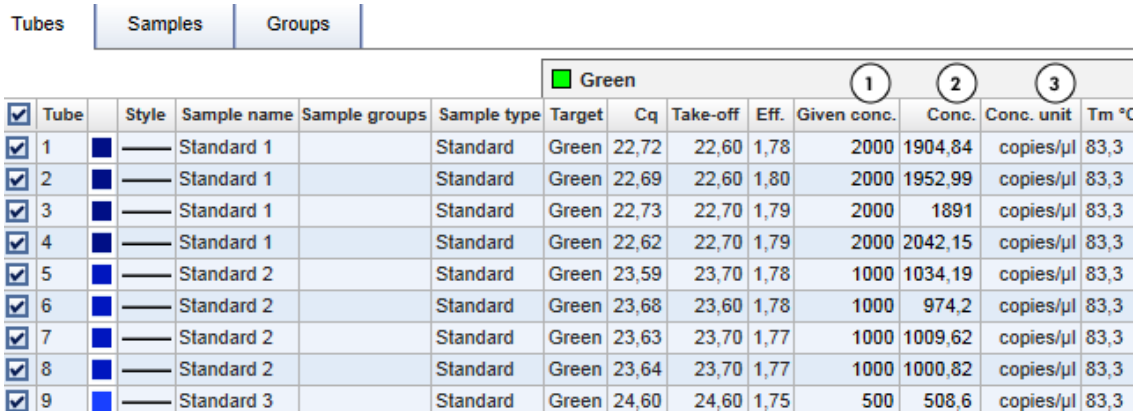
Tubes | Samples | Groups

						Green							
☑	Tube		Style	Sample name	Sample groups	Sample type	Target	Cq	Take-off	Eff.	Given conc.	Conc.	Conc. unit	Tm °C
☑	1		——	Standard 1		Standard	Green	22,72	22,60	1,78	2000	1904,84	copies/µl	83,3
☑	2		——	Standard 1		Standard	Green	22,69	22,60	1,80	2000	1952,99	copies/µl	83,3
☑	3		——	Standard 1		Standard	Green	22,73	22,70	1,79	2000	1891	copies/µl	83,3
☑	4		——	Standard 1		Standard	Green	22,62	22,70	1,79	2000	2042,15	copies/µl	83,3
☑	5		——	Standard 2		Standard	Green	23,59	23,70	1,78	1000	1034,19	copies/µl	83,3
☑	6		——	Standard 2		Standard	Green	23,68	23,60	1,78	1000	974,2	copies/µl	83,3
☑	7		——	Standard 2		Standard	Green	23,63	23,70	1,77	1000	1009,62	copies/µl	83,3
☑	8		——	Standard 2		Standard	Green	23,64	23,70	1,77	1000	1000,82	copies/µl	83,3
☑	9		——	Standard 3		Standard	Green	24,60	24,60	1,75	500	508,6	copies/µl	83,3

- **Tubes:** This view shows results for each tube in the experiment, with all acquisitions listed next to each other in a single row (see Tubes View).
- **Samples:** Reports results for all replicates of a sample. Typical results, such as Cq values, are reported as the arithmetic mean and the standard deviation of all sample replicates (see Samples View).
- **Groups:** Similar to the Samples view, the Groups view shows aggregated results. In this case, results are reported for sample groups (see Groups View).

2.3.4.1 Tubes View

The **Tubes View** of the **Results Table** shows results for each tube, laid out in a row.



Tubes | Samples | Groups

							Green				1	2	3	
☑	Tube		Style	Sample name	Sample groups	Sample type	Target	Cq	Take-off	Eff.	Given conc.	Conc.	Conc. unit	Tm °C
☑	1		——	Standard 1		Standard	Green	22,72	22,60	1,78	2000	1904,84	copies/µl	83,3
☑	2		——	Standard 1		Standard	Green	22,69	22,60	1,80	2000	1952,99	copies/µl	83,3
☑	3		——	Standard 1		Standard	Green	22,73	22,70	1,79	2000	1891	copies/µl	83,3
☑	4		——	Standard 1		Standard	Green	22,62	22,70	1,79	2000	2042,15	copies/µl	83,3
☑	5		——	Standard 2		Standard	Green	23,59	23,70	1,78	1000	1034,19	copies/µl	83,3
☑	6		——	Standard 2		Standard	Green	23,68	23,60	1,78	1000	974,2	copies/µl	83,3
☑	7		——	Standard 2		Standard	Green	23,63	23,70	1,77	1000	1009,62	copies/µl	83,3
☑	8		——	Standard 2		Standard	Green	23,64	23,70	1,77	1000	1000,82	copies/µl	83,3
☑	9		——	Standard 3		Standard	Green	24,60	24,60	1,75	500	508,6	copies/µl	83,3

Data are organized into the following columns:

Column Label	Description
–	The first column contains a check box to select or deselect a tube for analysis. The selection is synchronized with data in the **Tube Selector** and the **fluorescence plots** (see the *Q-Rex Software User Manual* for details).
Tube	Indicates the tube position in the rotor.
–	The third column displays the color used for the corresponding curve in a fluorescence plot.
Style	Indicates the line style used for the corresponding curve in a fluorescence plot.
Sample name	Lists the sample name.
Sample groups	**Optional:** If you defined sample groups, this column displays all groups to which a sample is assigned.
Sample type	Lists the assigned sample type (Sample, Standard, PC, NTC, NC, Not in use).
Target	Lists the target assigned to the tube for the specific acquisition.
Cq	Shows the Cq value calculated for the tube.
Take-off	Indicates the take-off point, i.e., the cycle where the run transitioned into the exponential phase.
Eff.	Displays the reaction efficiency of the tube.

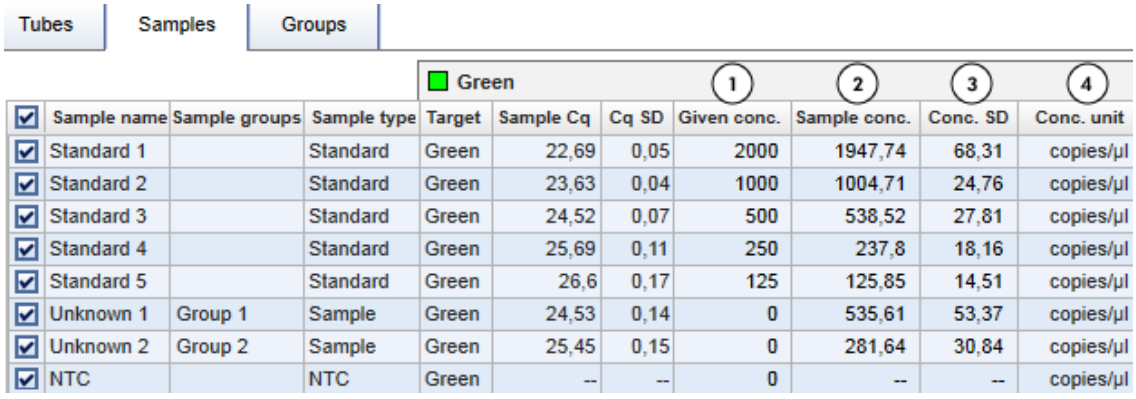
Tm°C	Optional: If the experiment contains melt data, this column displays the melting point of each sample.

With the Q-Rex Absolute Quantification HID Plug-in, 3 additional columns are displayed:

Column Label	Description
Given conc. (**1**)	Displays the concentration defined in the **Sample Layout** step.
Conc. (**2**)	Lists the calculated concentration value.
Conc. unit (**3**)	Displays the units for concentration values, as defined in the **Sample Layout** step.

2.3.4.2 Samples View

The **Samples View** of the **Results Table** shows results for technical replicates, laid out in a row. Results from tubes with the same name, acquisitions and targets are aggregated as a technical replicate.



Tubes | Samples | Groups

				Green			①	②	③	④
☑	Sample name	Sample groups	Sample type	Target	Sample Cq	Cq SD	Given conc.	Sample conc.	Conc. SD	Conc. unit
☑	Standard 1		Standard	Green	22,69	0,05	2000	1947,74	68,31	copies/µl
☑	Standard 2		Standard	Green	23,63	0,04	1000	1004,71	24,76	copies/µl
☑	Standard 3		Standard	Green	24,52	0,07	500	538,52	27,81	copies/µl
☑	Standard 4		Standard	Green	25,69	0,11	250	237,8	18,16	copies/µl
☑	Standard 5		Standard	Green	26,6	0,17	125	125,85	14,51	copies/µl
☑	Unknown 1	Group 1	Sample	Green	24,53	0,14	0	535,61	53,37	copies/µl
☑	Unknown 2	Group 2	Sample	Green	25,45	0,15	0	281,64	30,84	copies/µl
☑	NTC		NTC	Green	--	--	0	--	--	copies/µl

Data are organized into the following columns:

Column Label	Description
–	The first column contains a check box to select or deselect a sample with its technical replicates. The selection is synchronized with data in the **Tube Selector** and the **fluorescence plots** (see the *Q-Rex Software User Manual* for details).
Sample name	Lists the sample name.
Sample groups	Optional: If you defined sample groups, this column displays all groups to which a sample is assigned (see the *Q-Rex Software User Manual* for details).
Sample type	Lists the assigned sample type (Sample, Standard, PC, NTC or NC).
Target	Displays the target assigned to replicate tubes for the specific acquisition (see the *Q-Rex Software User Manual* for details).
Sample Cq	Lists the arithmetic mean of the Cq values of tubes with the same sample name and target.
Cq SD	Provides the standard deviation of the listed Cq values of tubes with the same sample name and target.

With the Q-Rex Absolute Quantification HID Plug-in, 4 additional columns are displayed:

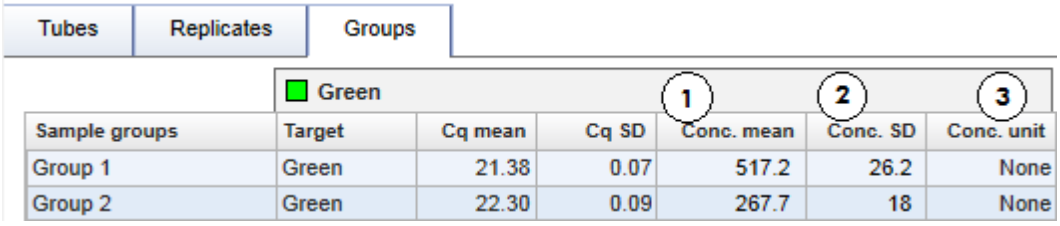
Column Label	Description
Given conc. (**1**)	Displays the concentration defined in the **Sample Layout** step.
Sample conc. (**2**)	Provides the arithmetic mean of the calculated concentration values of tubes with the same sample name and target.
Conc. SD (**3**)	Lists the standard deviation of the calculated concentration values of tubes with the same sample name and target.
Conc. unit (**4**)	Shows the units for concentration values, as defined in the **Sample Layout** step.

Note: A technical replicate will have multiple rows, if tubes of the sample have multiple target assignments.

Note: If a melt phase is part of the experiment, melt peak temperatures are only displayed in the Tubes View.

2.3.4.3 Groups View

The **Groups View** of the **Results Table** shows results for each defined sample group and acquisition, laid out in a row. Results are calculated from all tubes assigned to the same sample group.



Sample groups	Target	Cq mean	Cq SD	Conc. mean	Conc. SD	Conc. unit
Group 1	Green	21.38	0.07	517.2	26.2	None
Group 2	Green	22.30	0.09	267.7	18	None

Data are organized into the following columns:

Column Label	Description
Sample groups	Indicates the groups defined for the samples of the experiment (see the *Q-Rex Software User Manual* for details).
Target	Lists the target assigned to the sample group for the specific acquisition (see the *Q-Rex Software User Manual* for details).
Cq mean	Displays the arithmetic mean of the Cq values of all samples with the same sample group and target assignment.
Cq SD	Provides the standard deviation of the listed Cq values of all samples with the same sample group and target assignment.

With the Q-Rex Absolute Quantification HID Plug-in, 3 additional columns are displayed:

Column Label	Description
Conc. mean (**1**)	Displays the arithmetic mean of the calculated concentration values of all samples with the same sample group and target assignment.
Conc. SD (**2**)	Lists the standard deviation of the calculated concentration values of all samples with the same sample group and target assignment.
Conc. unit (**3**)	Shows the units for concentration values, as defined in the Sample Layout step.

Note: A sample group will have multiple rows, if samples of the group have multiple target assignments.

2.4 Report and export results

To create a report or to export results, see "Reports and exports" in the *Q-Rex Software User Manual*.

2.5 Troubleshooting

For information about error messages in the Q-Rex Software and troubleshooting, see "Troubleshooting" in the *Q-Rex Software User Manual*.

The Q-Rex Absolute Quantification HID Plug-in displays error messages and warnings when unexpected events or behaviors occur during use.
The following list includes the most common error messages or warnings that can occur while using the Q-Rex Absolute Quantification HID Plug-in and troubleshooting suggestions. These are specific to the Q-Rex Absolute Quantification HID Plug-in. For information about general error messages of the Q-Rex Software and troubleshooting, see "Troubleshooting" in the *Q-Rex Software User Manual*.

When contacting QIAGEN Technical Service for help, make sure to provide the Service Specialist:

- Steps and events leading to the error message.
- The Message ID. This number uniquely identifies the source of an error or warning and helps QIAGEN Technical Services to resolve the problem.

Error messages:

Message ID	Error text	Comments and suggestions
201857007	Minimal number of standards required.	The auto-find threshold feature requires that you have defined at least 2 selected standards. To use the automatic threshold calculation, at least two standards for the active target must be selected.

Warning messages:

Message ID	Warning text	Comments and suggestions
20000003	The same name is already saved for the compensation factors. Would you like to overwrite the existing file?	Use a different name to save the compensation factors. Otherwise, the existing file will be overwritten.
20000002	The selected compensation factors will be removed from your system and will no longer be available.	By confirming this warning, the compensation factors will be removed completely. Do not delete compensation factors that could be needed in the future.

2.6 Glossary

For definitions of general terms used in the Q-Rex Software, refer to the "Glossary" in the *Q-Rex Software User Manual*.

HID	Human identification is a field of investigation in forensic cases or paternity testing.

Appendices

3 Appendices

3.1 Appendix A – Limited License Agreement

QIAGEN's Q-Rex Absolute Quantification HID Plug-in Software License Agreement

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- delay,

- compensation due to defect,

- compensation for wasted expenses.

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