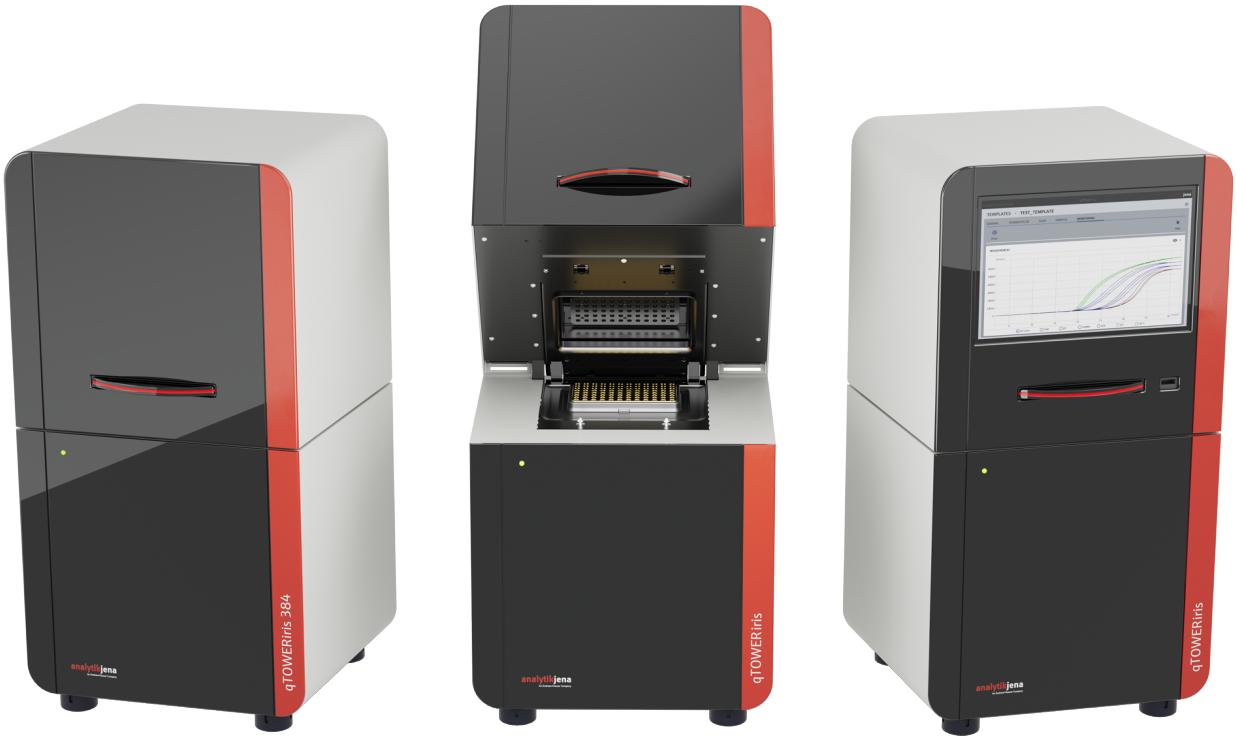
analytikjena
An Endress+Hauser Company

Operating Manual

qPCRsoft
Software for Real-Time Thermal Cycler



Manufacturer

Analytik Jena GmbH+Co. KG
Konrad-Zuse-Straße 1
07745 Jena / Germany
Phone: +49 3641 77 70
Fax: +49 3641 77 9279
Email: info@analytik-jena.com

Technical Service

Analytik Jena GmbH+Co. KG
Konrad-Zuse-Straße 1
07745 Jena / Germany
Phone: +49 3641 77 7407
Fax: +49 3641 77 9279
Email: service@analytik-jena.com

For a proper and safe use of this product follow the instructions. Keep the operating manual for future reference.

General Information

http://www.analytik-jena.com

Documentation Number

/

Edition

A (09/2023)

Technical Documentation

Analytik Jena GmbH+Co. KG

Table of contents

1 qPCRsoft product overview

The qPCRsoft software is used to control real-time PCR thermal cyclers and to create and analyze qPCR experiments.

Software version described

The information in this manual are based on qPCRsoft 5.0.

Supported devices

The qPCRsoft software allows controlling devices of the qTOWER iris series and analyzing their data.

qPCRsoft can be used to control up to 4 devices simultaneously with one PC. When controlling several devices at the same time, the PC will use one qPCRsoft program instance for each device.

Notes on this manual

This manual contains illustrations with sample layouts of thermal blocks with 96 wells. For thermal blocks with 384 wells, the layouts are expanded accordingly. All other functions of the software are the same.

This manual uses the following typographical marks:

- Software terms are marked in bold.
- Menu items are lined up separated by a vertical line "|", e.g. **File** | **Exit**.
- Within the project window, functions are presented on tabs which can contain further subtabs. In this case, tabs and subtabs are also lined up and separated by a vertical line, e.g. Project window **Settings** | **General**.
- Work steps for operating the software are highlighted by a "▸".

1.1 Installing qPCRsoft

Installing the program requires administrator rights for the operating system.

It is possible to control up to 4 qPCR thermal cyclers using one PC. One qPCRsoft program instance must be installed for each device. When starting a program instance, all qPCR thermal cyclers will be automatically scanned if they are connected to the PC or via Ethernet provided these devices are switched on. It is then possible to select one thermal cycler and control this device via the program instance. When controlling more than 2 devices from one PC, it is recommended to use the Ethernet interface to connect the devices.

System requirements for installation

For using qPCRsoft for controlling a real-time PCR device, your PC must meet the following minimum requirements:

Operating system	Windows 10
Processor	Dual core with at least 4 threads and 1.2 GHz
RAM	4 GB
Available hard disk space	At least 300 MB
Interfaces	At least USB 2.0 or Ethernet

Installation procedure

qPCRsoft is supplied on a CD or on a USB flash drive.

- ▸ Launch the "setup.exe" file in the installation and follow prompts of the installation wizard.
- ▸ Enable the language version for the installation.

✓ This is the language that will be used for the installation process. The software will be started using this as the default language. This setting can be changed later in the program.

- When installing the first program instance, select the installation path. All further instances will be stored in this path.
- Enter a name for the program instance.
  ✓ This is the name given to the subfolder for the instance in the installation path. Furthermore, the desktop icon for launching the instance will be given this name, provided you have selected to create a desktop shortcut.
- Proceed accordingly for all further installation requests.
  ✓ The program instance is installed. The PC's desktop will show the qPCRsoft icon.

Note

The program instance has only been installed correctly after it has been started once by a user with administrator rights. During this initial program launch, the user must enter a password for the program administrator.

Setting up an administrator account

It is required to assign an administrator password and to define an administrator for each program instance after installing the program. If you do not intend to use the user management, there is the option to disable the user management after logging in as an administrator.

- Launch a qPCRsoft program instance using its desktop icon.
- Open the window **Select working device** to select a device that is connected and switched on or to select a virtual device and click **Select** to confirm your choice.
- Open the window **Logging** to set the administrator password.
- Open the menu item **Extras** | **Options** on the **User management** tab to set up user accounts or to disable the user management.

Configuring dye modules

After launching the program for the first time, the dye modules that are installed on the device must be registered.

- Select the menu item **Extras** | **Edit color modules** and configure the dye modules available on the device.

Editing the qPCRsoft.ini file

For qPCR thermal cyclers connected via TCP/Ethernet, the network address for the program instance must be entered in the qPCRsoft.ini.

- Navigate to the qPCRsoft.ini file for the corresponding program instance in the installation folder and open this file using a text editor.
- Enter the TCP in the section **[KnownDevices]**. Every device must have a unique "KnownDevice" name and a number (see screen shot). The numbering starts with 0. Numbers must be unique, but should not be assigned as consecutive numbers.
- The section **[BackUp-KnownDevices]** is the backup area. This section can be used to enter devices that are currently not connected to the network whose addresses should be retained.

qPCRsoft.ini - Editor

Datei Bearbeiten Format Ansicht Hilfe

```
[System]
Mode=multi
Connection=TCP

[Device]
ID=3107S-0001
IP=localhost

[KnownDevices]
KnownDevice0=tcp://192.168.1.20#10001
KnownDevice1=tcp://192.168.1.24#10001
KnownDevice2=tcp://192.168.1.21#10001

[BackUp-KnownDevices]
KnownDevice0=tcp://172.16.55.200#10001
KnownDevice1=tcp://172.16.55.201#10001
KnownDevice2=tcp://172.16.55.202#10001
```

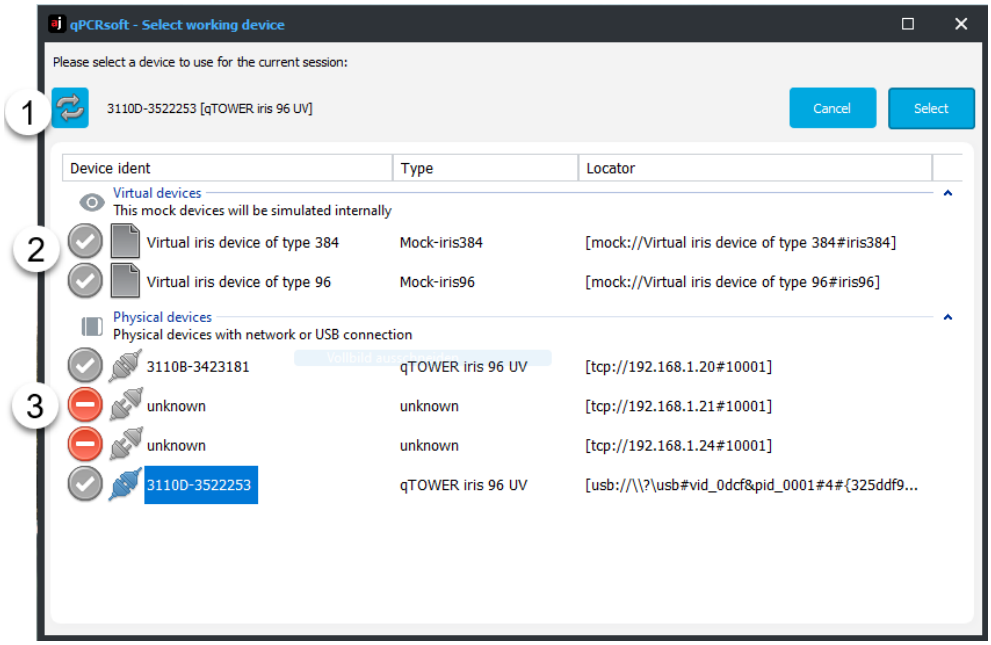
See also

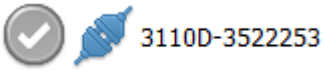
- General settings in the Options window [▶ 107]
- Configuring dye modules [▶ 110]

1.2 Start and exit qPCRsoft

Start window containing connected devices

After launching a qPCRsoft instance, the PC opens the window **Select working device** which can be used to select the connected devices.



No.	Description
1	This is the device that is currently connected to the program instance.
2	Device simulations selection When selecting a device from this area, the program instance will not be connected to a device. All of the functions of the software can be used, but no measurement can be carried out/simulated. Use this function to analyze qPCR projects or to create qPCR templates. Make sure to select the correct device type.
3	Selection of physical devices This section lists all devices which are currently connected directly to the PC via the USB interface or which are entered in the qPCRsoft.ini file as available devices on the network.  The device is switched on and can be connected to the program instance. The device's serial number is shown next to the icon to identify the device.  The device cannot be found, even though it is listed in the qPCRsoft.ini file because it is not switched on, for example. The word "unknown" is shown next to the icon.  (colored plug icon) This device has been selected for connection to a program instance.

Starting qPCRsoft

The maximum number of devices with their own program instances that can be controlled via one PC is 4. When controlling more than 2 devices from one PC, it is recommended to use the Ethernet interface to connect the devices.

- Switch on the qPCR thermal cycler.
- Use the desktop icon of the qPCRsoft instance to start the instance.
- Select the device in the **Select working device** window. All devices that can be selected are shown with their respective serial number.
- Click **Select**.
  - ✓ The qPCRsoft user interface is displayed.

If the user management has been installed, you will be prompted to enter a user name and a password. The program's user interface will only be accessible after successfully entering this data.

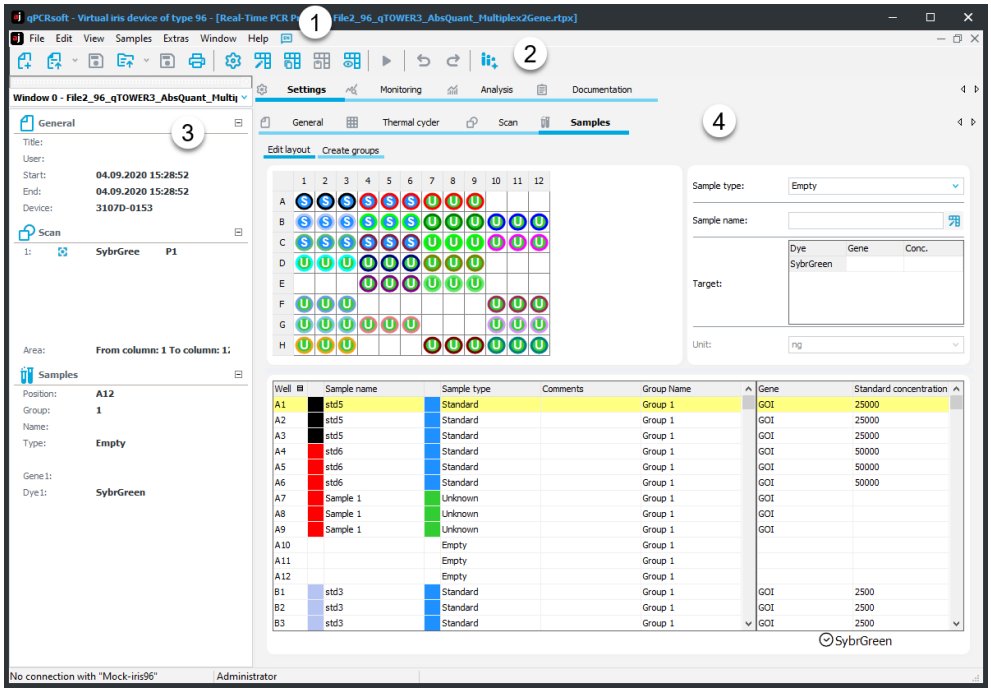
Exiting qPCRsoft

- Select the menu item **File** | **Exit** to exit.
  - ✓ If there are any open projects which have not been saved, a prompt will ask the user to save all changes to these projects before exiting.
- Use the menu item **File** | **Save project** to save projects.
- Select the menu item **File** | **Exit** once again.
  - ✓ qPCRsoft is exited.

1.3 qPCRsoft program layout

User interface

The user interface is opened after starting qPCRsoft.



No.	Element	Description
1	Menu bar	Most program functions are accessible via the menu items.
2	Toolbar	The icons on the toolbar are assigned to the most important program functions. These may change depending on the context. When hovering the mouse pointer over an icon, a tooltip will provide information on the function of this icon.
3	Project explorer	The project explorer is an important tool in qPCRsoft. This area contains the most important information about the current project.
4	Project interface	The project interface is used to edit the projects. This area is also used to configure all settings for starting and carrying out qPCR experiments and for analyzing these experiments.

Menu bar

The menu bar contains the following functions:

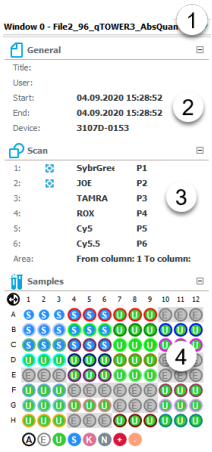
Menu	Description
File	Manage projects and templates Print Start multigene analysis/multiplate analysis Export data to a LIMS
Edit	Copy, paste and cut texts Undo and restore changes
View	Display and hide the project explorer
Extras	System functions Options for program-wide settings
Window	Arrange project windows on the project interface

Menu	Description
Help	Help function for qPCRsoft
	Information about the installed software
	Activation of the optional program module “21 CFR Part 11”

When selecting an analysis of a qPCR experiment, the corresponding menus will be shown. These menus and the associated icons are explained in this manual when describing the respective analyses.

Project explorer

The project explorer helps you when working on your projects. Its 3 sections contain important information about the active project.



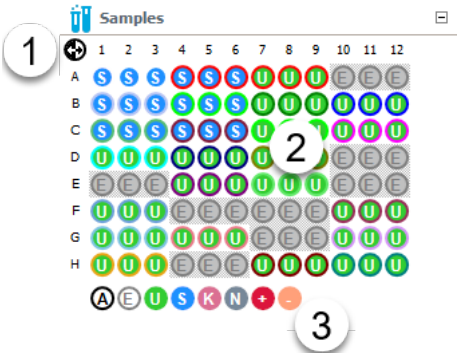
No.	Area	Description
1	Projects selection list	Selection of a project that is opened in qPCRsoft Selecting the project will activate that project and open it for editing.
2	**General**	Information on the title, operator, start and end time of the qPCR program execution and the device that is used
3	**Scan**	Overview of which colors and which layout areas were scanned
4	**Samples**	Brief information on sample layout Activation/deactivation of the display of the samples during the execution of the qPCR program Activation/deactivation of the samples during the analysis of the experiment Display of detailed information about a sample while the plate layout is processed

1.3.1 Project explorer Samples

The **Samples** section in the project explorer helps you to find your way around the sample layout. It provides a schematic representation of the sample layout, allows showing or hiding fluorescence curves during the execution of a qPCR program and deactivating outlier values in the analysis.

The layout is created in the project window **Settings** | **Samples**.

The section **Samples** of the project explorer shows the wells in different colors depending on the sample type. The color coding for the sample types and the fluorescence curves (colored rings around the sample type symbol) can be selected for the entire program in the menu item **Extras** | **Options** | **Colors**.



No.	Area	Description
1	Column/ row names	Clicking the names of the columns or rows will activate/deactivate the entire row for display or analysis.
2	Sample layout	Assigning samples to the thermal block
3	Sample types icons	Clicking the icons will activate/deactivate all wells of a selected sample type.

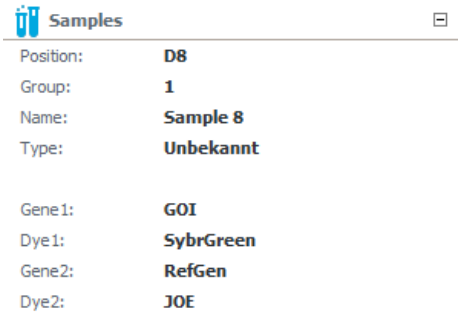
Displaying the sample type in the layout

The sample types are represented by letters in the layout.

Sample type	Icon	Description
Empty	**E**	Empty position in the layout (empty)
Unknown	**U**	Sample of unknown concentration or dilution
Standard	**S**	Sample of known concentration or dilution
NTC (No template control)	**N**	Complete reaction preparation without matrix strand
Calibrator	**C**	The target gene expression level of this sample is set as 1
Positive control	**+**	Positive control preparation for which a reaction product is expected
Negative control	**-**	Negative control preparation for which no reaction product is expected

Showing sample properties of a well

When hovering the mouse pointer above the sample layout in the project window **Settings** | **Samples**, the sample properties of the well above which the mouse pointer is placed will be displayed in the section **Samples**.



Enabling/disabling samples for display and analysis

The project window **Monitoring** allows displaying and hiding the fluorescence curves of individual wells during a qPCR program execution by enabling or disabling the display of the curves in the section **Samples**.

Activating /deactivating individual wells in the section **Samples** will include or exclude the results of the selected samples in the analysis in the project window. When selecting this feature, these values will not be deleted from the project and can be included in the calculations by reactivating them.

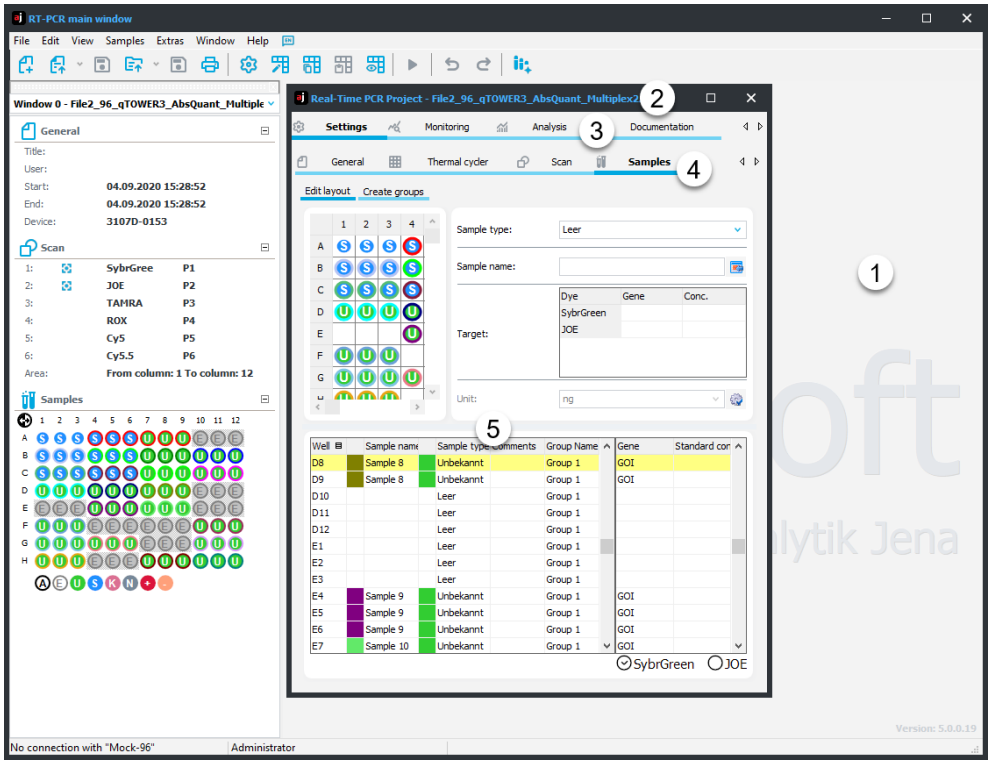
Active wells which will be included in the analysis are highlighted by a white sample type icon. Deactivated wells are displayed with a gray icon and their fluorescence curves are hidden. Empty wells are marked with an “E”, there is no fluorescence measurement for empty wells.

- Switching occurs by means of the mouse. The activation changes every time a well is clicked.
- For switching adjacent wells, hold the mouse button and move the pointer over the wells.
- Invert entire rows and columns by clicking on the letter A ... H for the row or the number 1 ... 12 for the column.
- Click the icon to invert the activations of the entire layout. Empty wells are not taken into account in this selection.
- To activate the entire layout except for the empty wells, click the Ⓐ icon under the diagram.
- To activate the wells of a selected sample type, click the corresponding icon under the layout. Hold the Ctrl key and click the desired sample types to activate multiple sample types.

1.3.2 Project window

After starting the program the project interface is initially empty. Only when a new project is created or a saved project and/or a template is loaded the project window opens.

A project for an experiment includes the settings and executions of the qPCR program, the definition of the sample layout and the analysis. All required settings and the fluorescence data that were determined are stored together and can be edited and managed in the project window.



No.	Element
1	Project interface Several project windows can be opened simultaneously on the project interface.
2	Project window
3	Tab bar with main topics
4	Tab bar with sub topics This bar is shown after selecting a main topic and organizes all further inputs and analyses.
5	Input and display area

Main topics

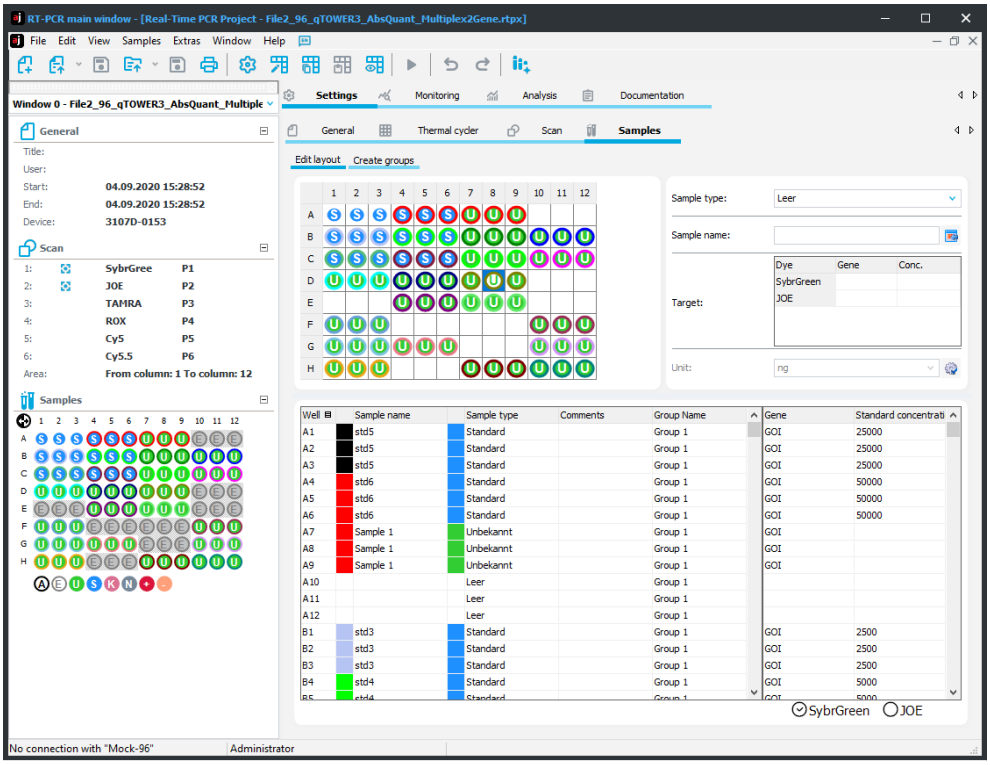
The project window contains 4 tabs with the main topics of the project.

Tab	Description
Settings	Settings for the qPCR program execution and for the sample layout
Monitoring	Start and monitoring of the qPCR program execution Determination of Ct values and of the melting temperatures T_m
Analysis	Evaluation algorithms for the analysis of collected data
Documentation	Input screen for MIQE-compliant documentation of qPCR experiments

The name of the selected tab is underlined in dark blue. After selecting a tab, a second or third row of tabs may appear for entering further parameters. Here, too, the name of the selected tab is underlined in dark blue. If the tabs do not all fit onto one screen, use the arrow keys on the right edge of the respective bar to scroll through the list of tabs. All tables and diagrams that cannot be fully displayed in the window have scroll bars to navigate through these elements.

Expanding the project window

When not working on multiple projects at the same time, for example for importing and exporting analysis parameters, it is possible to click on the square icon in the right corner of the window's title bar to expand the project window. The expanded window provides a clear view of the project. To activate a specific project in the project area, select it from the list in the project explorer.



Layout of the diagram and table areas

The tab **Settings** | **Samples** and the tabs **Analysis** allow enlarging and reducing the diagram and table area for a better view of the respective area.

- Move the mouse pointer to the place between the two sections until the mouse pointer turns into a double arrow.
- Keep the mouse button pressed to move the border of the section horizontally.

1.3.3 Help

To learn more about how to use qPCRsoft, open the menu item **Help** | **Content**.

The program will then display concise pieces of information on the icons in the toolbar after you move the mouse pointer above a particular icon.

1.3.4 Information on the software

Information on the software, such as the installed version, for example, can be found under the menu item **Help** | **Info**.

2 Projects and templates in qPCRsoft

The qPCRsoft software saves all experiments in project files. A project contains various information required for the execution of a qPCR experiment:

- Description of the experiment
- Temperature-time program (PCR protocol) of the thermal cycler
- Scan settings of the optical system
- Sample assignment of the thermal block with detailed information on each sample (sample layout)
- Measuring results and the corresponding analyses after the experiment has been performed

All basic information required for performing an experiment that is stored in the **Settings** project window, such as the description of the experiment, the PCR protocol, scan settings of the optical system and the plate assignment, can be saved as a template and used for standardized repetitions of the experiments.

2.1 Overview of file types in qPCRsoft

Different file types are created in qPCRsoft.

Templates/projects for 96-well thermal block

Extension	File type	Description
*.rtpx	real-time project file	Project file with settings for qPCR run, analysis and measurement results
*.rtsx	real-time settings file	File format for templates with settings for the qPCR run, without measurement results
*.mgax	real-time multigene assay file	Multigene or multiplate assay

Templates/projects for 384-well thermal block

Extension	File type	Description
*.rtpx384	real-time project file	Project file with settings for PCR run, analysis and measurement results
*.rtsx384	real-time settings file	File format for templates with settings for the qPCR run, without measurement results
*.mgax384	real-time multigene assay file	Multigene or multiplate assay

Other files

Extension	File type	Description
*.rta	real-time analysis file	Exported/imported analysis parameters of a project
*.rtprt	print report template	Print template

2.2 Managing projects and templates

Create new project

For a new project, open a new project window in the project interface of qPCRsoft.

- Click on the icon or select the menu item **File** | **New**.
  - ✓ A new project window opens. You can define the settings for a qPCR run and then start it, or save the settings as a template.

Create new project from a template

You can create a project from a template.

- Click on the icon or select the menu item **File** | **Open template...**.
- Select the template you want and click on **Open**.
  - ✓ A new project window opens with the default settings from the template. Add the missing entries for the experiment, e.g., the sample layout, and start the qPCR run.

Save a project

After a qPCR run, you can save the data as a project.

- Select the menu item **File**|**Save project as...**.
- In the **Save as** window, enter a name for the project and click **Save**.
  - ✓ The project is saved.

You can edit the analyses in an existing project and create further analyses. You can also save the changes.

- Click on the icon or select the menu item **File** | **Save project**.
  - ✓ The changes are saved.

You cannot start another qPCR run in an existing project. In this case, save a template from the project and create a new project from that template.

Save and export projects automatically

In the **Options**|**General** window, you can define the following options for automatically saving projects and automatically exporting data:

- Save automatically to a predefined folder after the end of the qPCR run
- Prompt to save the qPCR run before or after it is started
- Automatic CSV export of raw data, amplifications and melting curves
- Automatic CSV export of Ct values

Open project

You can open a project for viewing, edit its analyses or save its settings for the qPCR run as a template.

- Click on the icon or select the menu item **File** | **Open project...**.
- In the **Open** window, select the file and click **Open**.
  - ✓ The Workspace project window appears with the data of the selected project.

Create template

You can save the settings for the qPCR run and the sample layout in a project window as a template for further analyses.

- Select the menu item **File**|**Save template as...**.
- In the **Save as** window, enter a name for the template and click **Save**.
  - ✓ The settings for the qPCR run are saved as a template.

You can edit the settings in an existing template and save the changes.

- Click on the icon or select the menu item **File** | **Save template**.

✓ The changes are saved.

Open automatically saved backup file

You can also define whether a running qPCR run should be saved in a "Last Run" backup file. If the qPCR run is aborted unexpectedly, all measurement data up to the point the run is aborted is saved in this file. It is therefore still possible to analyze this data. The backup file is overwritten during the next qPCR run. You must enable saving of the backup file in the **Options** | **General** window.

- Select the menu item **File** | **Open automatically saved project...**.
  - ✓ The recovered data is displayed in a new project window.
- Save the data as a project with a different name.
  - ✓ The measurement data up to the point the qPCR run was aborted is saved.

See also

General settings in the Options window [▶ 107]

2.3 Transferring templates from a LIMS

qPCRsoft can be configured by another program, e.g., a LIMS (Laboratory Information Management System). To do this, the LIMS must generate a file that is read by qPCRsoft. The structure of the transfer file can be provided by Analytik Jena if needed. qPCRsoft uses the transfer file to create a template, with which a qPCR run can be started immediately.

The different export functions of qPCRsoft can be used to transfer the results of the qPCR run to the LIMS, depending on which data the LIMS expects.

Open LIMS transfer file

- Select the menu item **File** | **Import LIMS...**.
- In the **Open** window, select the TRF file and click **Open**.
  - ✓ The template created from the transfer file opens in the user interface. You can start a PCR run using this template.

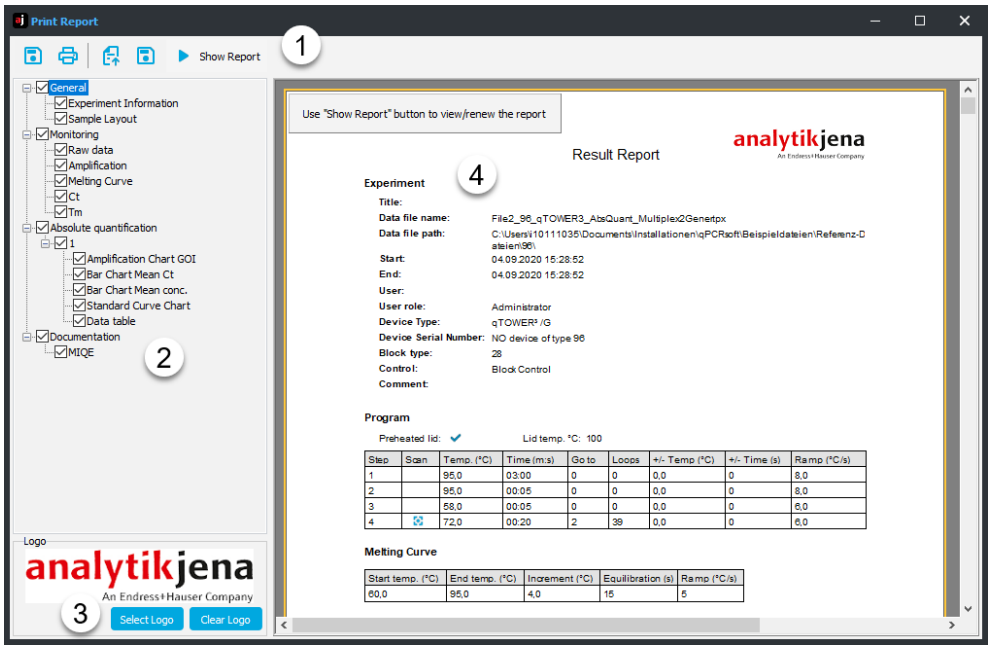
2.4 Importing and exporting analysis parameters

In a template you can only save the settings for the qPCR run and the sample layout. There is no option to save analysis settings. If you want to apply the analyses of one project to another project, you need to export and import the analyses between the projects.

- Activate the project whose analysis parameters you want to use in other projects.
- Select the menu item **File** | **Export analyses...**.
- In the **Save as** window, enter a name and click Save.
  - ✓ The analysis parameters are saved.
- Activate the project window into which you want to import the analysis parameters.
- Select the menu item **File** | **Import analyses...**.
- In the **Open** window, select the file with the analysis parameters and click **Open**.
  - ✓ The analyses are applied to the project in the active window.

2.5 Printing a project

The project report can be printed or saved as a PDF file. In the **Print Report** window, select the elements for the report and start the printout.



No.	Element	Description
1	Toolbar	Functions in the **Print Report** window
2	Project tree	Select the elements for the printout, arranged according to the main functions of the project window
3	Select logo	Select your company logo for the header of the printout The AJ logo is set by default.
4	Preview	Print preview of the selected elements

Toolbar of the Print preview window

If you move the mouse pointer over an icon, a tooltip is displayed showing the function.

Icon	Description
	Save report as PDF
	Print report
	Open print template
	Save print template
Show Report	Refresh print preview

Print/save report of a project

- In the qPCRsoft toolbar, click on the icon or select the menu item **File** | **Print**.
- In the project tree, activate the elements that you want to print or save in the report.
- Optionally, select a logo.
- Click on **Show Report** and check the content of the report.
- To print, click on the icon. In the **Print** window, check the print settings and click **OK**.

- To save as a PDF file, click on the icon. Choose a file name and click **Save**.
  - ✓ The report is printed or saved as a PDF file.

Select logo for the report

The logo appears in the printout and in the PDF file on the top right of each page. You can replace the default AJ logo with your own. The following image formats are permitted: GIF, JPEG, PNG, BMP, ICO, EMF, WMF, TIF.

- Click on **Select Logo** and select the logo in the **Open** window.
  - ✓ The logo is inserted on the report pages.

You can check the new report pages in the preview by clicking **Show Report**. To restore the AJ logo, click the **Reset Logo** button.

Use print templates

You can create print templates and use them as defaults for a printout. For example, you can specify a logo in the printout without having to select it again for each print job.

- In the **Print preview** window, activate all settings in the project tree under **General**, **Monitoring** and **Documentation** that you want to use in every report.
- If necessary, select the logo.
- Click on the icon, choose a file name in the **Save as** window and save the print template.
  - ✓ The print template is saved as an RTPRT file.
- To use a template, click on the icon and select the template in the **Open** window.
  - ✓ The print template is loaded and the preset elements are activated in the project tree. You can now activate the analyses for printing and change further settings.

3 Settings for a qPCR experiment

At the start of a qPCR experiment, you can either create a new project or open a template.

All necessary functions for creating a new project are summarized in another tab level in the project window under the **Settings** tab.

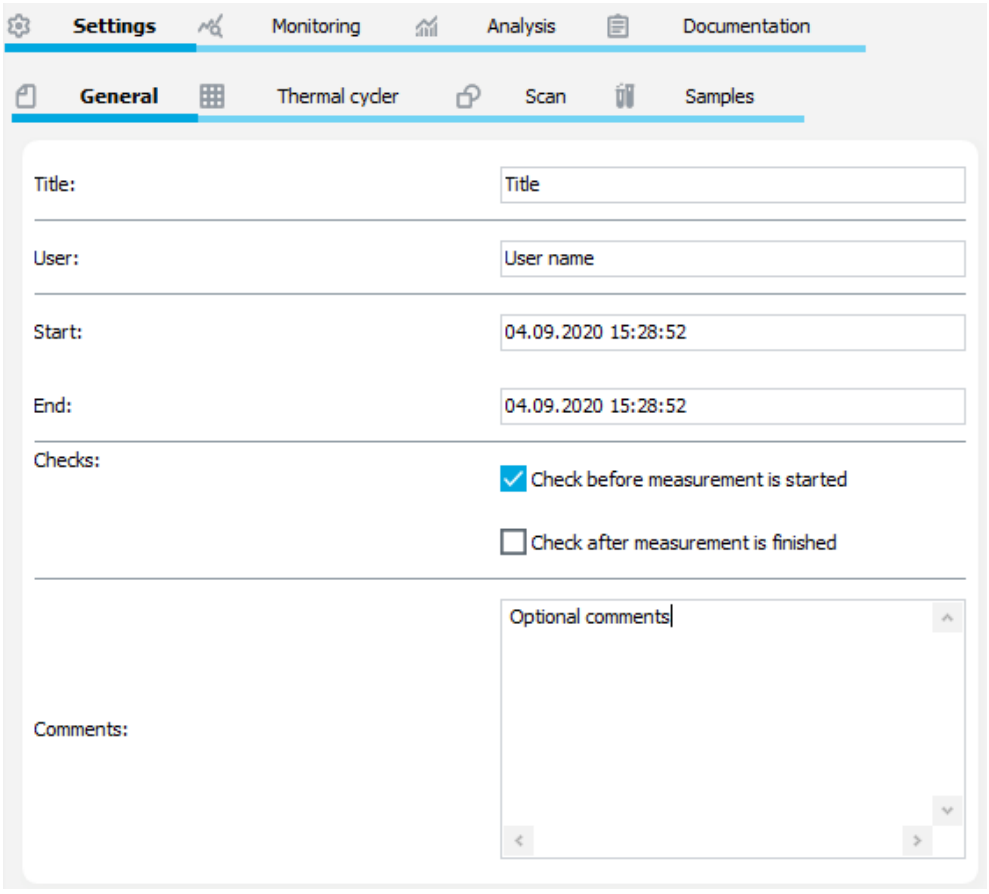
Tab	Description
General	General information and comments on the qPCR experiment
Thermal cycler	Programming of PCR protocols
Scan	Definition of the colors to be measured and settings for the measuring parameters
Samples	Sample table with detailed information on each sample and grouping of experiments in the layout

See also

- Managing projects and templates [▶ 18]
- Project window [▶ 14]

3.1 General information on the project

For every project general information can be saved. Details are entered in the **Settings General** project window.



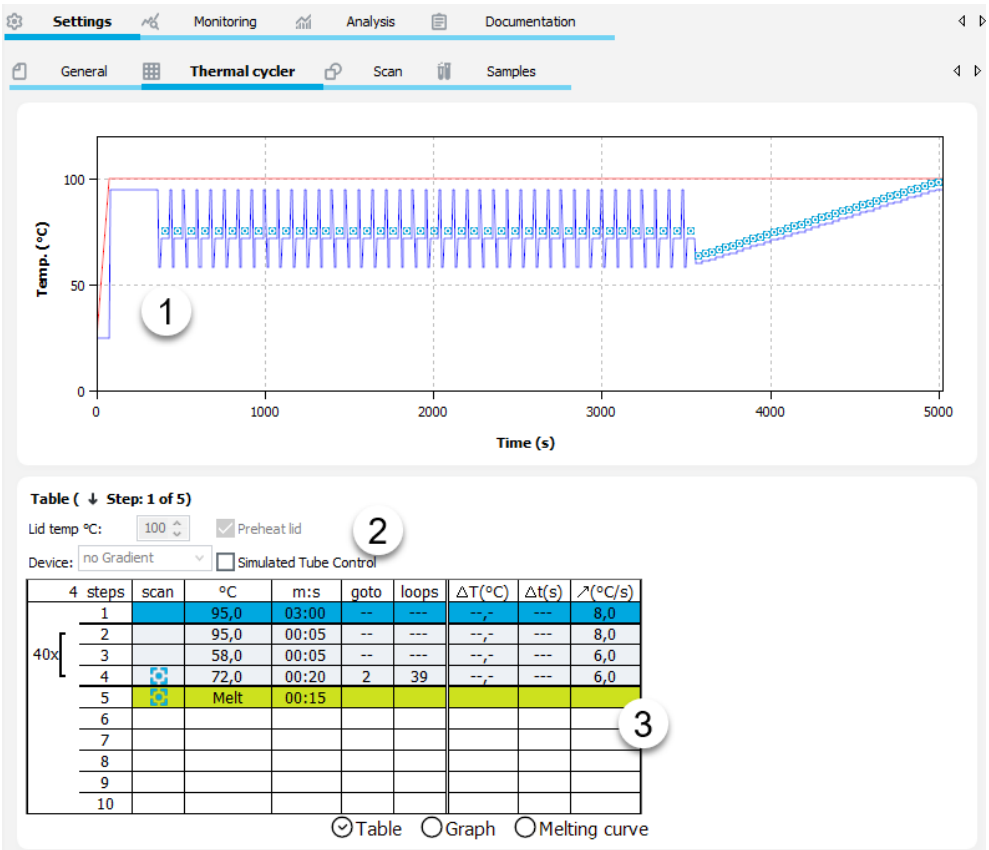
Option	Description
Title	Analysis title
User	User If you are using a user management option, the user name you signed in with will be entered automatically.
Start **End**	Start and end of the qPCR run This data is entered automatically during the qPCR run and cannot be edited.
Checks	Check of the optical fibers **Check before measurement is started** The functionality of the fiber is checked before the experiment is started. If a fiber is defective, the experiment is not started. **Check after measurement is finished** The fibers are checked after the experiment has ended. Values generated with defective fibers are not analyzed.
Comments	Optional comments on the qPCR experiment

For text input, you can use the usual commands for copying, cutting and pasting text. These commands can be found in the **Edit** menu.

3.2 qPCR program

You program the PCR protocol for a qPCR experiment in the **Settings** | **Thermal cycler** project window.

Thermal cycler project window



No.	Element	Description
1	Program preview	Graph of the programmed qPCR protocol
2	Program header	Options for lid heating, temperature control and selection of the device type
3	qPCR protocol	Area for programming the qPCR protocol with further display options **Table** Numerical programming **Graph** Graphical programming **Gradient** (only for devices with gradient block) Programming of a temperature gradient **Melting curve** Programming of the melting curve

Cycler menu and icons

When you select the **Thermal cycler** tab, the **Cycler** menu appears in the menu bar and further icons in the toolbar. Use the menu items and icons to edit the qPCR protocol steps. Select a step by clicking on its line. The line of the step turns blue.

Icon	Cycler menu	Description
	**Insert empty step**	Insert a new step before the selected step
	**Delete step**	Delete selected step
	**Cut out step**	Cut selected step
	**Copy step**	Copy selected step to the clipboard
	**Insert empty step**	Delete the copied step from the clipboard after the selected step

3.2.1 Entering options for lid heating and temperature control in the program header

The options for lid heating and temperature control set in the program header of the qPCR program apply to the entire qPCR program.

- Enter the following parameters in the program header:

Option	Description
Lid temp. °C	Set the lid temperature Default: 100 °C
Preheat lid	Activate lid preheating
Simulated Tube Control	Activate temperature control according to calculated sample temperature
Device	Device selection You can also create the qPCR program for a device other than the one initialized in the program. In this case, the maximum heating and cooling rates are preset by the selected device.

The temperature of the heated lid should generally be slightly above the maximum block temperature to prevent liquids from evaporating from the reaction preparation and condensing at the walls or the seal of the reaction cups. If the preheating function is enabled, the device will first heat the heated lid to the set temperature while the sample block temperature is kept constant at 25 °C. After a subsequent equilibration phase of 40 s during which the device will produce homogeneous temperature conditions

throughout the entire block, the PCR program is started and the sample block is heated. If the difference of the temperature of the block and the temperature of the heated lid exceeds 75 °C, the heated lid will be deactivated automatically, to extend the service life of the Peltier elements. At such low block temperatures, it is no longer expected that the sample will condense at the vessel's lid.

If the **Simulated Tube Control** option is enabled, the temperature in the sample is pre-calculated with the measured block temperature and the temperature is regulated to the sample temperature. This method is particularly recommended for fast programs. If the option is disabled, the block temperature is used for regulation. Particularly if the heating and cooling rates are high and the hold times are short the actual sample temperature can differ from the desired temperature.

3.2.2 Creating or editing a new temperature program

The temperature program for the thermal cycler is programmed in the **Settings** | **Thermal cycler** project window.

Insert and delete program steps

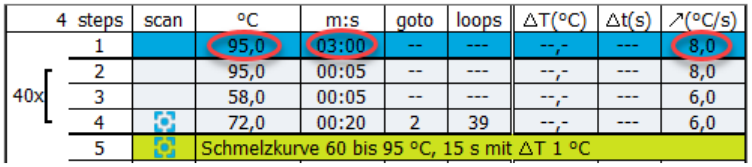
The **Cycler** menu and icons in the toolbar are used to manage program steps.

- Insert program steps using the keyboard or clicking the mouse at the end of a program: Press the cursor key **[↓]** in the last program line or click on the next empty line with the mouse.
  - ✓ The new step is added at the end of the program.
- Insert a step in the program: Select the step with the mouse and click the icon.
  - ✓ The new step is inserted above the selected step.
- Delete program step: Select the step with the mouse and click the icon.
  - ✓ The step is removed from the program.

Edit target temperatures, hold times and heating and cooling rates

- Click in the individual cells of the program step so that the numbers are highlighted dark blue and make the following entries:

Table column	Description
°C	Target temperature for the thermal block
m:s	Hold time for the temperature step in the format mm:ss The hold time begins as soon as the target temperature in the block is attained.
↗(°C/s)	Heating/cooling rate for the selected step The maximum heating/cooling rates of the selected device are preset.



	4 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
	1		95,0	03:00	--	---	--,-	---	8,0
40x	2		95,0	00:05	--	---	--,-	---	8,0
	3		58,0	00:05	--	---	--,-	---	6,0
	4		72,0	00:20	2	39	--,-	---	6,0
	5		Schmelzkurve 60 bis 95 °C, 15 s mit ΔT 1 °C						

Programming loops

A typical PCR program is made up of repetitive steps for denaturation, annealing and extension. It is possible to program loops for repeating steps; these loops are defined by a target step for returning to the beginning of the loop (**goto**) and the number of iterations (**loops**).

- In the last step of a loop, enter the step number to which the program shall go back in the **goto** field.
- Enter the number of loops in the **loops** column.

✓ Steps within the loop are indicated by a bracket on the left side of the table.

Note

The total number of cycles is determined from the number of programmed repetitions (**loops**) plus 1, as the corresponding sequence of steps prior to reaching the loop has already cycled once.

	4 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
	1		95,0	03:00	--	---	--,-	---	8,0
40x	2		95,0	00:05	--	---	--,-	---	8,0
	3		58,0	00:05	--	---	--,-	---	6,0
	4	⊙	72,0	00:20	2	39	--,-	---	6,0
	5	⊙	Schmelzkurve 60 bis 95 °C, 15 s mit ΔT 1 °C						

Optionally change target temperature and hold time

By programming increments and decrements, you can optionally change the target temperature and the hold time of a step in a loop by a defined amount from cycle to cycle.

▶ Click in the individual cells of the programming step and make the following entries:

Column	Description
ΔT(°C)	Optional increment/decrement for the target temperature If this is a step within a loop, the block temperature is increased or decreased by this value with each iteration. A positive value is used for an increment (rise) while a negative value is used for a decrement (fall). If the user does not enter any value, the target temperature remains the same for each iteration.
Δt(s)	Optional increment for the hold time If this is a step within a loop, the hold time is increased by this value with each iteration. If the user does not enter any value, the hold time remains the same for each iteration.

	4 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
	1		95,0	03:00	--	---	--,-	---	8,0
40x	2		95,0	00:05	--	---	--,-	---	8,0
	3		58,0	00:05	--	---	--,-	---	6,0
	4	⊙	72,0	00:20	2	39	-1,0	5	6,0
	5	⊙	Schmelzkurve 60 bis 95 °C, 15 s mit ΔT 1 °C						

Fluorescence measurement

For the qPCR program, you must perform a fluorescence measurement in a program step.

▶ Click in the **scan** column in the program step.

✓ The activated fluorescence measurement is indicated by the ⊙ icon.

You define the parameters for the fluorescence measurement under **Settings** | **Scan**.

	4 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
	1		95,0	03:00	--	---	--,-	---	8,0
40x	2		95,0	00:05	--	---	--,-	---	8,0
	3		58,0	00:05	--	---	--,-	---	6,0
	4	⊙	72,0	00:20	2	39	--,-	---	6,0
	5	⊙	Schmelzkurve 60 bis 95 °C, 15 s mit ΔT 1 °C						

Further actions

Optionally, you can add a melting curve to a qPCR program or enter a temperature gradient for gradient-capable thermal blocks.

See also

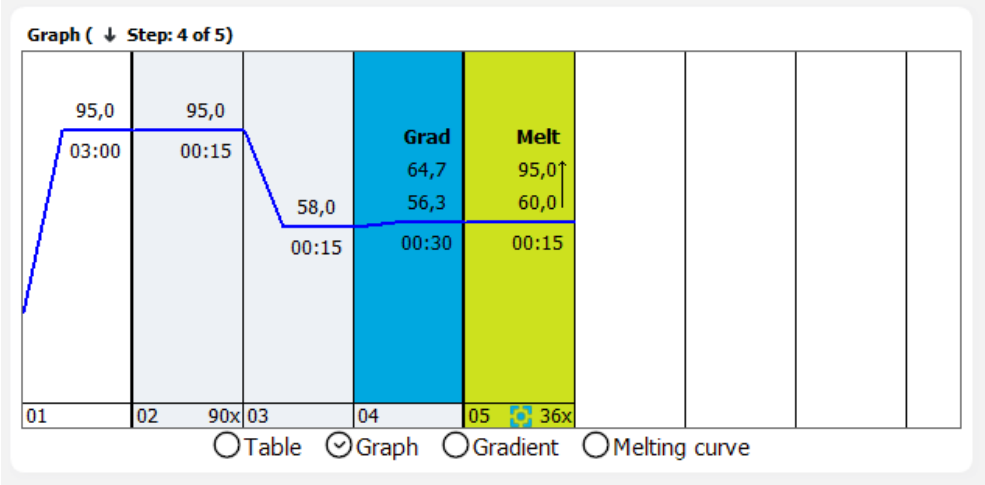
- Programming melting curves [▶ 29]
- Programming gradients [▶ 27]

3.2.3 Graphical display of the qPCR program

The qPCR run is displayed in a temperature-time chart in the top part of the **Settings** | **Thermal cycler** project window. The blue line shows the temperature curve of the thermal block. The red line shows the temperature curve of the heated lid. The icon indicates the step where the fluorescence measurement takes place.

Graph view

In the bottom part of the window, the **Graph** view shows the temperature curve of the program steps.



The steps with a gray background are part of a loop. A melting curve step is shown in green. The blue curve shows the temperature curve. In each step, the target temperature is displayed above the curve and the hold time below the curve. The bottom line of the graph shows the step number, the icon for the fluorescence measurement and the number of cycles of a loop.

Edit qPCR program in the graph

You can amend the qPCR program in the **Graph** view to a limited extent, however editing the program in the **Table** view is more convenient.

- Click on the number of a parameter, e.g., on a target temperature, and enter the parameter.
- Click on the curve in a step and move the curve up or down by holding down the mouse button. This changes the target temperature in the step.

In the **Graph** view, you cannot insert or delete steps in the temperature program and you cannot define loops.

3.2.4 Programming gradients

You can only use the gradient function when you are using a gradient-enabled thermal block/thermal cycler.

A temperature gradient is used to set a temperature profile which defines how the temperature in the thermal block changes over the course of the program step. The temperature gradient is always passed along the long side of the sample block to ensure that as many different temperatures as possible can be monitored.

The gradient runs from column to column, i.e. horizontally from left to right. The highest temperature may be in the first or last column. All samples in one column have the same temperature. However, the temperatures differ from column to column.

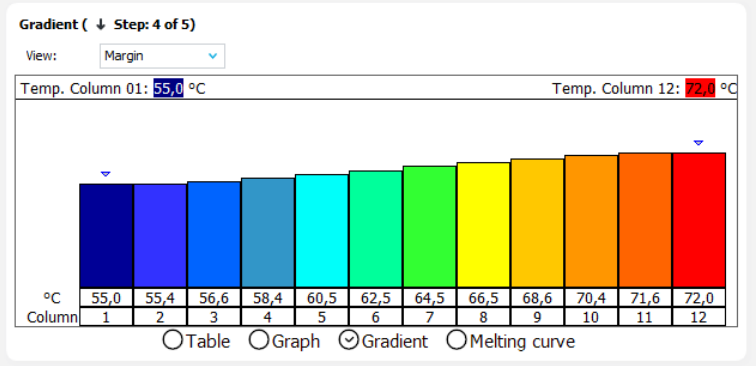
You can use the gradient function to find the ideal annealing temperature for new primer pairs, for example. Spread each of the replicates along the long sides of the sample block to determine the block temperature that will yield best results.

You program a temperature step with a gradient in the **Settings** | **Thermal cycler** project window in the **Gradient** view.

Temperature gradient with 2 limit temperatures

With this gradient, the change of the block temperature is defined via the temperature of the edge columns.

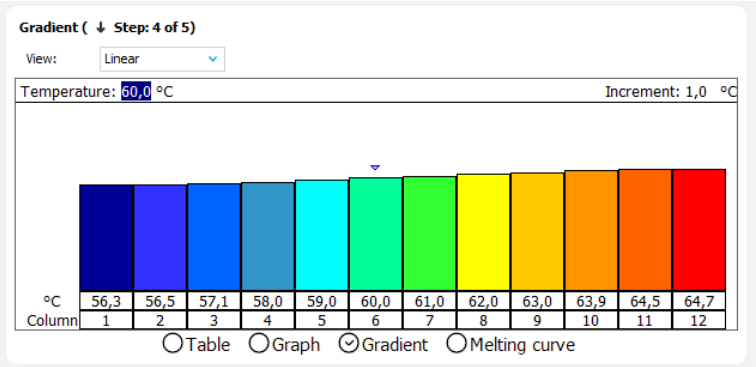
- In the **Table** view, select the program step in the program table and switch to the **Gradient** view.
- Select the **Margin** option from the **View** list.
- Enter the temperature for the left column in the **Temp. Column 1** field
- Enter the temperature for the right column in the **Temp. Column 12** field (96-block) or **Temp. Column 24** field (384-block) and confirm with the Enter key.
  - ✓ The temperature distribution in the block is calculated and displayed in the graph.



Linear gradient

For a linear gradient, the temperature is defined for the center of the thermal block, on a 96-block model that would be column 6, for example. The temperature is than decreased by a decrement from column to column on one side of the block and increased by an increment on the other side.

- In the **Table** view, select the program step in the program table and switch to the **Gradient** view.
- Select the **linear** option from the **View** list.
- Enter the temperature of the middle block column in the **Temperature** field and the temperature change in the **Increment** field and confirm with the Enter key.
  When entering a positive increment, the temperature is lowest in the left column 1 and highest in column 12 for the 96-well block (column 24 if using the 384-well block). When entering a negative increment with a minus sign, the temperature is highest in column 1 and lowest in column 12 (24).
  - ✓ The temperature distribution in the block is calculated and displayed in the graph.



Display in the program table

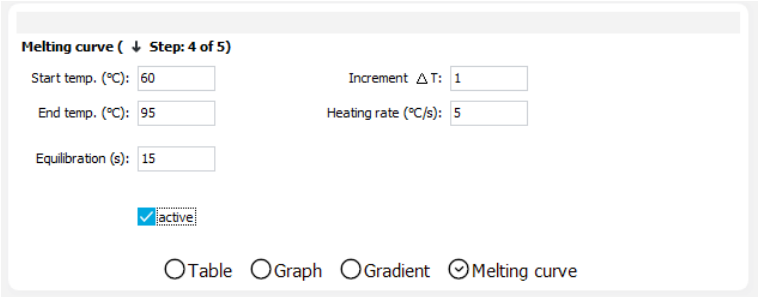
For a temperature gradient, the two temperature values of the left and right edge columns of the block are displayed in the **°C** column separated by a hyphen. You can also enter the two temperatures directly in the field and therefore program a gradient with 2 limit temperatures.

	4 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
	1		62,0-75,0	03:00	--	---	--,-	---	8,0
40x	2		95,0	00:05	--	---	--,-	---	8,0
	3		58,0	00:05	--	---	--,-	---	6,0
	4		62,0-72,0	00:20	2	39	--,-	---	6,0
	5		Schmelzkurve 60 bis 95 °C, 15 s mit ΔT 1 °C						
	6								
	7								
	8								
	9								
	10								

3.2.5 Programming melting curves

For experiments with intercalating dyes, it is recommended to check the specificity of the products by measuring a melting curve. You can program the corresponding step in the project window under **Settings** | **Thermal cycler** in the melting curve view.

Melting curve view

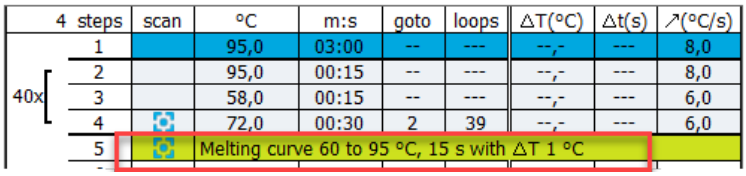


Option	Description
Start temp. (°C)	Start temperature of the melting curve
End temp. (°C)	End temperature of the melting curve
Equilibration (s)	Time in which the sample is adjusted to a temperature before the fluorescence measurement is recorded
Increment ΔT	Difference between two consecutive temperature steps in °C
Heating rate (°C/s)	Heating rate of the block
active	Add melting curve to the qPCR protocol

Enable melting curve

Amplification is required for programming the melting curve. If you want to record a melting curve without previous amplification, you must enter an additional step in the qPCR protocol before the melting curve step, in which the block is brought to the starting temperature of the melting curve and a fluorescence measurement is activated. It is not possible to record a melting curve without at least one preceding step.

- In the lower part of the **Thermal cycler** tab, select the **Melting curve** view.
- Set the parameters according to the description above.
- Enable the **active** option.
  - ✓ The melting curve is added to the qPCR program. The optical measurement is performed at each temperature step of the melting curve.

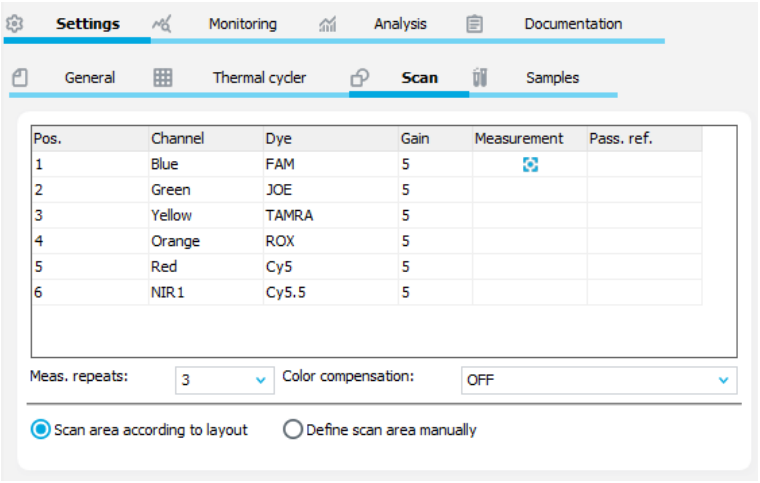


3.3 Fluorescence measurement – Settings | Scan project window

The product amplification is measured in the qPCR by the increase in fluorescence. Depending on the device configuration, up to 6 color channels with different excitation and detection wavelengths can be used for the fluorescence measurement. The parameters of the fluorescence measurement apply for all samples in the layout on which a measurement is to be performed.

You program the fluorescence measurement in the **Settings** | **Scan** project window.

Settings | Scan project window



The table contains the parameters of the scan properties. The specifications on position, channel and the selection list of available dyes cannot be changed in this table as they depend on the color modules installed in the device. You have to configure the installed color modules in the **Edit color modules** window.

Option	Description
Pos.	Color module position in the device
Channel	Color channel description
Dye	Selection list of dyes available for the color channel
Gain	Signal intensity The signal intensity can be adjusted in steps between 0 and 10. The higher the value, the higher the fluorescence signal in the color channel. Default value: 5
Measure	Scan in the color channel An activated scan is indicated by the icon.
Passive reference	Measurement of a reference dye **Note**: The LED technology used does not require a passive reference.

Other options in the Scan tab

Option	Description
Meas. repeats	Number of scan repetitions The individual scans are averaged and the measured value is taken from this average. This improves the signal-to-noise ratio. However, too many repetitions lead to unnecessarily long measurement times. Possible values: 1 to 16, default value: 3
Color compensation	Selection list for color compensations
Scan area according to layout	Scan area according to where samples are located on the **Samples** tab The scan is performed in all occupied wells. No scan is registered in the empty wells.
Define scan area manually	Select scan area manually on the **Scan** tab

Scan menu and icon

When you select the **Scan** tab, the **Scan** menu appears in the menu bar and an icon for editing the color compositions appears in the toolbar.

Icon	Scan menu	Description
	**Edit color compensations**	Define color compensation individually

See also

- Configuring dye modules [▶ 110]
- Using color compensation [▶ 32]

3.3.1 Adjusting the fluorescence measurement (scan)

The product amplification is measured in the qPCR by the increase in fluorescence. The following measurement parameters must be defined for that purpose:

- Color channels and dyes used for the scan
- Temperature step in the PCR protocol at which a scan takes place
- Area of the microplate that is scanned

For each color channel you want to scan with, set the parameters in the **Settings** | **Scan** project window. The number of selected color channels/dyes has no influence on the duration of the measurement. The measurement is performed with all selected color channels simultaneously.

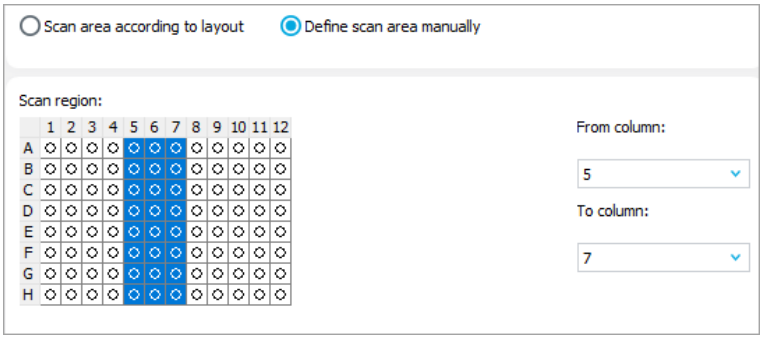
- In the color channel line, select the dye to be measured from the list.
- Set the signal intensity in the **Gain** column.
- Activate the fluorescence measurement in the channel by clicking in the **Measure** column. The activated measurement is indicated by the  icon.
- If necessary, activate the reference dye measurement by placing a tick ✔ in the **Pass. ref.** column.
- Enter the number of repetitions of the fluorescence measurements in the **Measurement repetitions color compensation** column.
  The default setting is 3 measurement repetitions. An increased number of repeat measurements reduces the measurement value distribution but also creates longer scanning times and thus longer qPCR run times.
- Select one of the options for defining the scan area (manually or according to layout).

✓ The fluorescence parameters for the scan in the qPCR program are now set. For the melting curve, the scan is performed at each temperature step.

3.3.2 Defining the scan area of the fluorescence measurement manually

The scan area is normally defined automatically according to the plate layout in the sample table. However, you can also define it manually. In this case, the scan area of the sample block is always defined column by column. The scan area must consist of connected columns.

- In the **Settings** | **Scan** project window, activate the **Define scan area manually** option.
  A graphic of the sample block layout appears.
- Enter the start and end columns of the area to be scanned in the **From column** and **To column** fields.
- Alternatively, select the columns in the measuring area using the mouse. To do this, click on a column and move over the scan area with the mouse button pressed down.
  ✓ Active columns are highlighted blue in the diagram.



3.3.3 Using color compensation

If several fluorescent dyes are used in one sample (multiplexing), spectral crosstalk of the fluorescence can occur, which can be corrected by color compensation.

Color compensation options

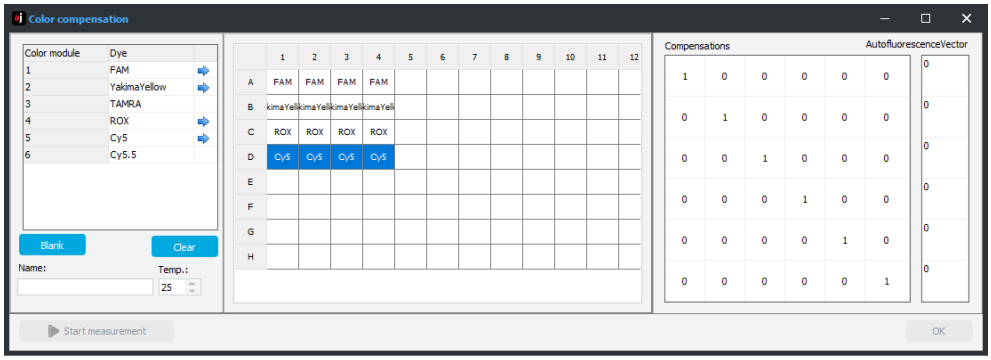
You can select the appropriate color compensation in the **Settings** | **Scan** project window from the **Color Compensation** list.

Option	Description
Off	The default setting for the color compensation is **Off**, because for the most frequent applications (only one active measuring channel or dyes with a large spectral distance, such as FAM and ROX) color compensation is not required.
Standard	With this color compensation, a compensation matrix is applied to the measurement data, which facilitates sufficient crosstalk compensation in all colors with a gain setting of "5". With the color compensations **Standard 1** and **Standard 2**, you have 2 different matrices to choose from. The suitability of the standard color compensation must be tested in an experiment.
Selection	Custom color compensations In order to use your own color compensations, choose the **Selection** option in the list. A window opens, in which color compensations that have already been recorded can be opened and used again. Only the color compensation data matching the settings in the **Scan** tab are displayed in black. Any color compensation data that is not valid appears in red and cannot be selected.

Create your own color compensation

A measurement is required for spectral calibration. The dyes for which color compensation is required must be enabled for the measurement and be present as individual samples in solution. For example, the sensors used in the subsequent PCR experiment can be used for the calibration measurements. For the calibration measurements the dye concentration should be approx. 0.1 µM.

- Select the dyes for which the calibration measurement is to be performed, using the icon in the table in the **Scan** tab.
- Click on the icon in the toolbar.
  The **Edit color compensations** window is displayed with the plate diagram.
- In the plate diagram, mark the wells containing the calibration samples for each dye individually. Tap the blue arrow next to the dye to assign that dye to the well.
- For an exact calibration measurement, it is advisable to create at least a triple replicate of each dye.
- It is also recommended to use blanks, i.e. samples that do not contain any dyes. Blanks can either be your NTC samples or wells filled with buffer solution.
- Enter a name for the new color compensation in the **Name** field.
- If necessary, enter a block temperature in the **Temp.** field or set it using the buttons. If the value "25" is set, no temperature control will take place.
- Tap **Start measurement** and start the calibration measurement.
  - ✓ The calibration measurement is carried out. When completed successfully, the new color compensation will be available on the Scan page.



Edit color compensation manually

If the color compensation for dye compensation is known from other experiments, you can enter the values manually in the compensation matrix or the autofluorescence vector.

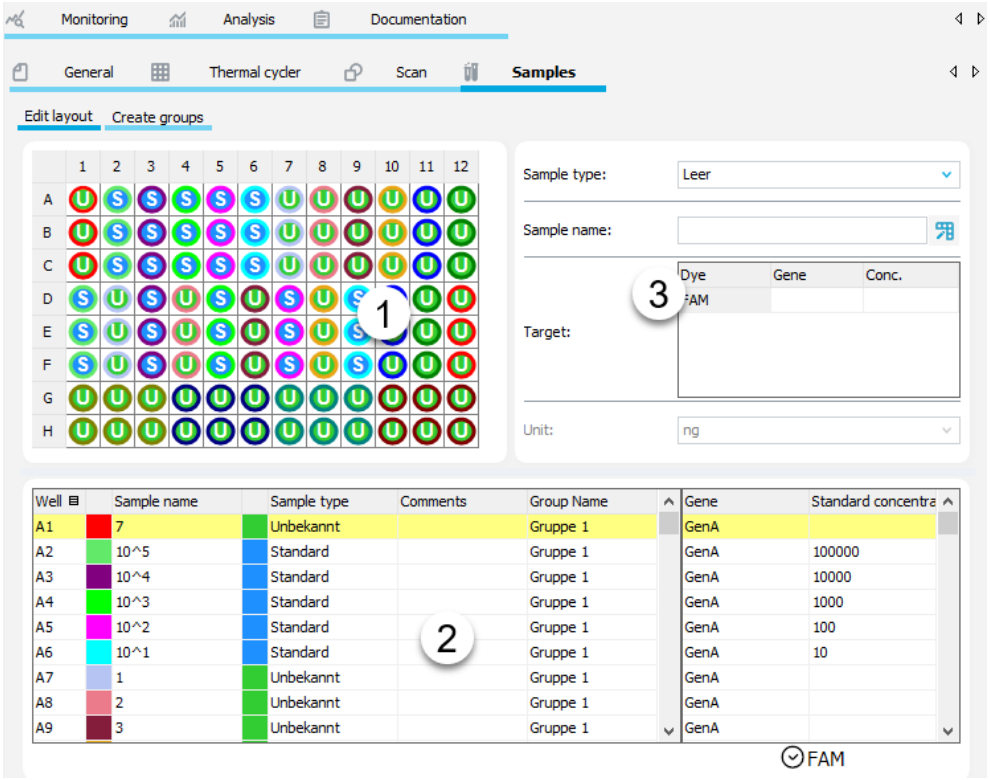
3.4 Sample layout

In the sample layout, you define the properties of the samples and their position in the sample block. Each sample can be described using its properties, such as name, gene, type, concentration, and dye.

If you place different experimental preparations in a sample layout, you can group them together.

You define the sample layout in the **Settings** | **Samples** | **Edit layout** project window.

Settings | Samples project window



No.	Element	Description
1	Layout view	Graphical display of the well assignment on the sample block
2	Sample table	Summary of the information on each sample
3	Input area	Input area for the sample properties: ■ Sample name ■ Sample type ■ Concentration of standard samples ■ Allocation of the dye and the analyzed gene

Samples menu and icons

When you select the Samples tab, the Samples menu appears and further icons are displayed in the toolbar. Use the menu items and icons to edit the sample layout.

Icon	Menu Samples	Description
	**Edit layout**	Edit layout
	**Copy layout**	Copy a selected area in the layout
	**Paste layout**	Paste a copied area in the layout
	**Layout preview**	Open a detailed view of the layout

3.4.1 Sample properties, sample types, replicates

You define the sample properties in the wells of the layout in the **Settings | Samples** project window. The samples are color-coded according to the sample type. You define the color coding of the wells under the **Extras | Options** menu item on the **Colors** tab. You must assign the following sample properties:

- Sample type
- Sample name
- Dye

- Gene
- Concentration of standards
- Unit for quantitative analyses

Sample types

In the layout, the selected sample types are highlighted with a symbol. The color coding of the symbol is also preset in the **Options** | **Colors** window and can be edited there.

Sample type	Icon	Description
Empty		Empty position in the layout
Unknown	U	Sample of unknown concentration or dilution
Standard	S	Sample of known concentration or dilution
NTC (No Template Control)	N	Complete reaction preparation without matrix strand
Calibrator	C	The target gene expression level of this sample is set as 1
Positive control	+	Positive control preparation for which a reaction product is expected
Negative control	-	Negative control preparation for which no reaction product is expected

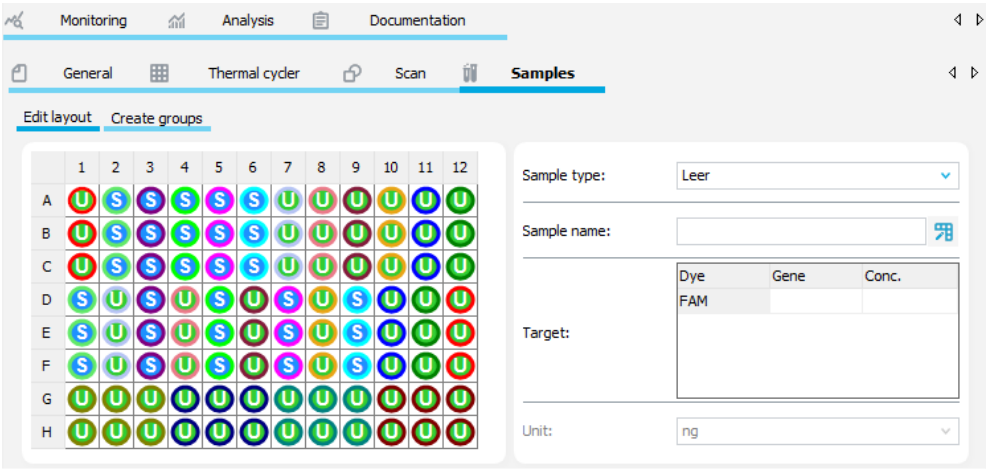
Replicates

Samples with identical sample properties (sample name, sample type, same gene-dye-assignments) are viewed as replicates. The individual values of these samples are averaged and their mean value is used for the remaining calculations.

With a multiplex assay, samples can have the same sample name and sample type; however, they differ as far as the gene-dye-assignment is concerned. These samples are identified as associated samples due to the same name. The analysis, however, is performed individually.

3.4.2 Entering sample properties in the layout diagram

The layout diagram shows the sample assignment of the thermal block. Occupied wells are shown with a colored sample type symbol. The colored ring around the well corresponds to the color of the fluorescence curve in the graph during monitoring and in the analyses. You can select the sample positions (wells) in the layout diagram and then assign the necessary properties to them in the lists and the target table to the right of the layout.



Assign sample properties to the wells

- Select wells in the layout:
  - Select individual wells by clicking with the mouse.

- For adjacent fields, move over the fields with the left mouse button pressed down.
  Move over non-connected fields by clicking the mouse while holding down the Ctrl key.
- Select rows or columns by clicking on the corresponding row or column name. To select all sample positions in the layout click the upper left button between A and 1.

▶ Enter the following sample properties in the area to the right of the layout diagram:

Option	Description
Sample name	Enter the name of the sample
Sample type	Select the sample type
Target table / **Gene** column	Enter the gene to be analyzed in the dye line or select it from the list
Target table / **Conc.** column	For standards Enter the concentration of the gene to be analyzed

▶ Press the icon or the ENTER key.
  ✓ The sample properties are assigned to the selected wells.

▶ Assign the **Blank** sample type to the wells: Select the wells and press the DEL key.

Note: The entries for the selected area will only be applied by the program after they have been assigned. Unassigned entries or changes are lost.

Edit properties or transfer properties to other wells

▶ Double-click on a well.
  ✓ The sample properties of the well are displayed in the input area.

▶ Edit the properties and transfer them to the well by clicking on the icon again.

▶ Select other wells and transfer the properties to this well by clicking on the icon.
  ✓ The sample properties are assigned to the selected wells.

Assign gene names subsequently

You can assign names to one or more samples at a later date.

▶ In the input area next to the sample layout, enter the gene names for the different dyes in the table.

▶ Select one or more samples in the sample layout.

▶ Right-click on one of the selected samples to open the context menu and select **Assign genes**.
  ✓ The selected samples are assigned the previously entered gene names.

Delete gene names from the table list

All gene names that have been assigned in the **qPCRsoft** program are listed in the **Gene** table column. You can delete entries that are no longer needed from the list for better clarity.

▶ Select a gene name in the list and press the F5 function key or the Del key.
  ✓ The selected name is deleted from the list.

Functions in the context menu

If you right-click on a well in the layout diagram, a context menu opens and you can apply the following functions to the well:

Function	Description
Assign IPC	For endpoint analysis Define a sample that does not contain an internal positive control (IPC-)

Function	Description
IPC- remove	Delete IPC- from a sample
Assign genes	Assign gene names subsequently
Assign colors	Define color of the fluorescence curve in the graph

See also

Editing the colors of the fluorescence curves [▶ 52]

3.4.3 Editing sample properties in the sample table

You can edit the settings made in the layout diagram in the sample table in the bottom part of the **Settings** | **Samples** | **Edit layout** project window.

Contents of the sample table

Column	Description
Well	Name of the well in the layout By clicking on the and icons you can sort the display in the table by layout rows or layout columns.
Swatch	The color field after the **Well** column shows the color of the fluorescence curve of the well.
Sample name	Sample description
Swatch	The color field in front of the **Sample type** column indicates the sample type.
Comments	Optional comment on the sample
Group Name	Assigned group If no groups are created in the layout, the name "Group 1" is displayed by default.
Gene	Analyzed gene in the sample
Standard concentration	Concentration of a sample of the **Standard** type

Customize table display

You can customize the table display and move or hide columns. The two columns **Gene** and **Standard concentration** are fixed and cannot be moved or hidden.

- To show or hide table columns, right-click on the table header and select all required columns in the context menu.
- To change the order of the table columns, click on the column header and move the column to the desired position with the mouse button pressed.

  ✓ The table is customized accordingly.

Edit table

You can enter properties directly into the sample table.

- Click on a well in the layout diagram or click directly in the field of the sample table.

  ✓ The table row of the well turns yellow.
- Enter the properties **Sample name**, **Comments** and **Standard concentration** directly in the field.
- Select the **Sample type** property from the list in the field.
- Enter the **Gene** property directly in the field or select it from the list. The list contains all gene names used in saved projects and templates.

Copy, cut, delete samples in the table

You can copy successive samples in the table and paste them elsewhere in the same table or in the sample table of another project. To copy and paste samples, you must **hold down the Ctrl key** during the entire process.

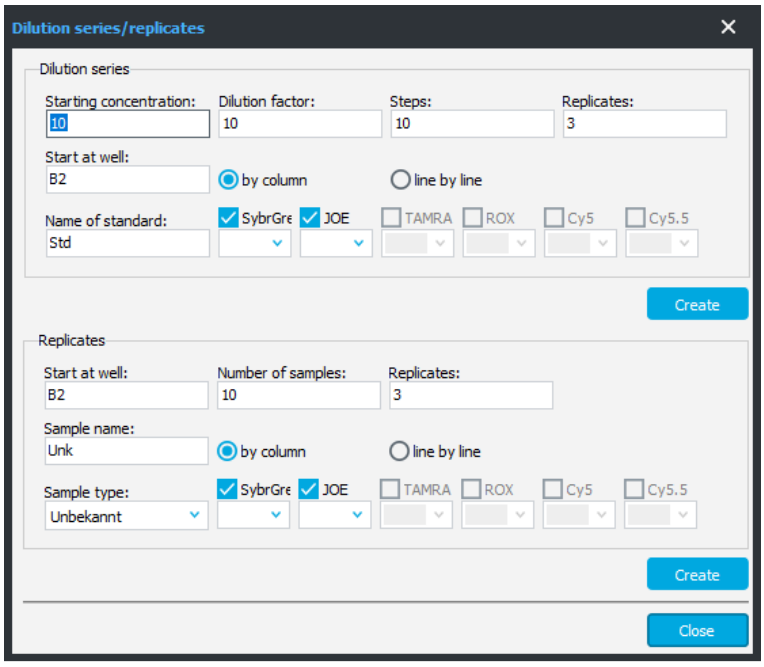
- Press and hold the Ctrl key.
- Click on the first table row to be copied and, keeping the mouse button pressed, select the table rows.
  - ✓ The table rows are shown in blue.
- Right-click to open the context menu and use the **Copy** command to copy the contents to the clipboard.
- Click on the table row below which you want to insert the content.
  The content can also be pasted into the sample table of another project.
- Hold down the Ctrl key and right-click to open the context menu, then use the **Paste** command to insert the content.
  - ✓ The samples are inserted into the table from the selected row. When you copy samples within the same table, you create replicates. Samples with the same sample properties (sample type, sample name, etc.) are considered replicates.

In the same way, you can also cut or delete samples via the context menu after selecting rows.

3.4.4 Creating automatic dilution series and replicates in the layout

If you use dilution series for standards or measure replicates in the experiment, you can create them automatically for the sample layout in the **Dilution series/replicates** window.

Dilution series/replicates window



Create dilution series and replicates

- Select the start well for the standards in the layout diagram and click on the icon in the toolbar.
- Enter the parameters for the dilution series or creation of the replicates (see below).
- Click the button **Create**.

✓ The standards/replicates are inserted into the layout and displayed in the layout diagram and in the sample table.

Input for dilution series

Option	Description
Starting concentration	Highest concentration in the dilution series (1st standard)
Dilution factor	Factor by which the concentration is diluted from step to step
Steps	Number of dilution steps/standards
Replicates	Number of repetitions per standard with the same concentration and dye/gene selection The mean value is calculated from the values of the replicates and used for further analysis.
Start at well	The wells are filled one after the other starting with this well. For this purpose, select the direction in which the wells are filled: **by column** or **line by line**. The well selected in the layout is preset. The entry can be edited.
Name of standard	Name of the standard The number 1 is appended to the name. With each dilution step, the number is increased by 1 (e.g. Std1, Std2, Std3, etc.)
Dyes/genes	All dyes can be activated for which fluorescence measurements have been activated in the **Settings** | **Scan** project window. A gene must be selected or entered for each activated dye.

Input for replicates

Option	Description
Start at well	The wells are filled one after the other starting with this well. For this purpose, select the direction in which the wells are filled: **by column** or **line by line**. The well selected in the layout is preset. The entry can be edited.
Number of samples	Number of samples
Replicates	Number of repetitions per sample The mean value is calculated from the values of the replicates of a sample and used for further analysis.
Sample name	Name of the samples The number 1 is appended to the name. With each new sample, the number is increased by 1 (e.g. Sample1, Sample2, Sample3, etc.).
Sample type	Selection of sample type
Dyes/genes	All dyes can be activated for which fluorescence measurements have been activated in the **Settings** | **Scan** project window. A gene must be selected or entered for each activated dye.

3.4.5 Exporting and importing sample layout in Excel

You can export a sample layout to Excel and import it back, for example to edit the sample names in Excel.

- Right-click on the sample table in the **Settings** | **Samples** | **Edit layout** project window.
- In the context menu, select **Export table as Excel file (*.xls)**.
- In the **Save as** window, choose a name and click the **Save** button.
  ✓ The layout is saved as an XLS file. You can now open and edit it in Excel.

- To import, right-click on the sample table, and in the context menu select **Import table from Excel file (*.xls)**.
- In the **Open** window, select the XLS file of the layout and click **Open**.
  - ✓ The layout is imported into the project.

3.4.6 Swapping sample layouts between projects

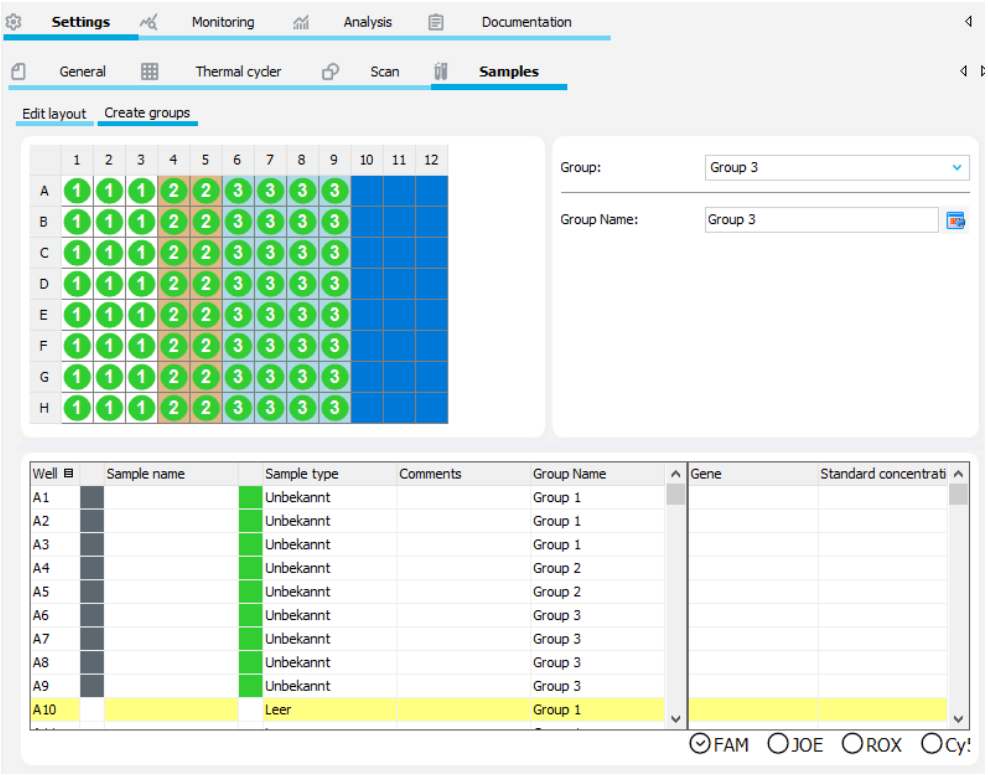
You can copy the sample layout of one project or parts of it to another project.

- Open the source and target projects in the project interface.
- Use the mouse to select the area to be copied in the sample layout of the original project.
- Click on the icon in the toolbar or select the menu item **Samples** | **Copy layout**.
- Activate the target project, click on the well located at the top right of the area to be pasted and then click on the icon or select the **Samples**|**Paste layout** menu item.
  - ✓ The copied areas of the original project are pasted at the selected position of the target project.

3.4.7 Creating groups

Several experiments can be performed on one microplate at the same time. The samples that are part of one experiment are combined in a group. A group contains a number of reaction mixtures that will be evaluated together later on. You can define a maximum of 12 such groups.

- In the **Settings**|**Samples** project window, select the **Create groups** tab.
  - ✓ The sample layout, the **User Group** list and the **Group Name** field are displayed in the top part of the window. In the layout, all samples are by default initially assigned to group 1 and identified with "1".
- Enter a name for group 1 in the **Group Name** field.
- Click on the icon or press the Enter key to apply the properties.
- In the layout, select the samples belonging to the next experiment.
  Select adjacent samples by holding down the mouse button. Select separate samples by clicking with the left mouse button while holding down the Ctrl key.
- Select the next group in the **Group** list, enter a new group name and accept it by clicking on the icon or pressing the Enter key.
- Continue in this way until all the different experiments have been created on the plate.
  - ✓ The samples that belong together (experiments) are marked with the same group number and color in the layout. The names are displayed in the sample table in the **Group Name** column.

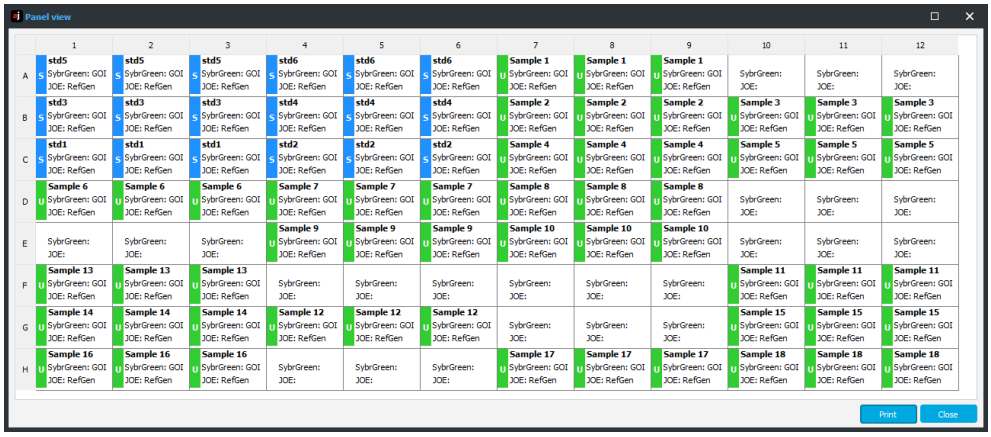


3.4.8 Showing an overview of the sample layout

The layout preview provides a complete overview of the assignment in the sample layout with samples and the corresponding information that has been saved for the samples.

Open layout preview

- Open the layout preview with the icon in the toolbar or the **Samples** | **Layout preview** menu item.
  - ✓ The layout preview is displayed in the **Panel view** window.



The layout preview contains the following information:

- Position on the PCR plate
- Gene
- Sample type by means of color marking at the edge
- Underlined in color to indicate group affiliation

If you move the cursor to a position in the layout, all known settings for this position, such as sample names, sample type and group and all genes and dyes to be measured for the sample as well as the concentration in the case of standards are displayed in detail.

Print layout preview

The table can be printed and used, for example, as a template for pipetting the samples or for documenting the experiment.

- Click on **Print** in the **Panel view** window.
- In the **Print** - **Layout** window, configure the printer and start the printout by clicking **Print**.
  - ✓ The table is sent to a printer.

3.4.9 Overview of the functions for editing a sample layout

Action	Where	Function
Left-click on well	Sample schema	Select well
Double-click on well	Sample schema	Show the values assigned to the well in the input area next to the schema
Left-click + drag	Sample schema	Select connected wells
Ctrl + left-click	Sample schema	Additionally select this well
Ctrl + left-click + drag	Sample schema	Select additional connected wells
Right-click on selected wells	Sample schema	Opens context menu: ■ Definition of wells without internal positive control (IPC-) ■ Assign genes: The gene names displayed in the input area are assigned to the selected wells. ■ Assign fluorescence curve colors
Enter key	Keyboard	Assign properties in the input area to a well, equivalent to icon
Del key	Keyboard	Delete the contents of the selected wells and set the wells' status to "empty"
F5 key	Keyboard/"Gene" edit field	Delete the selected gene from the list of genes
Left-click on Well table header	Table	Change sort order: row-by-row, column-by-column
Right-click on table header	Table	Open context menu to select columns to be displayed
Left-click + drag on table header	Table	Change order of columns
Left-click on table cell	Table	Make input/selection in the selected cell
Right-click on table	Table	Context menu for Excel export/import of layout table
Ctrl + right-click (+drag) on table rows (hold Ctrl key down)	Table	Context menu for copying, cutting, pasting or deleting the contents of the selected table rows
icon	Input area	Create dilution series and replicates in the layout

Action	Where	Function
Double-click on the color cell in the table row	Table	Open the **Color** window for selecting the color of the fluorescence curves
Shift key and double-click on color cell	Table	Reset color setting
Ctrl + double-click on the color cell	Table	Open the **Edit color** window

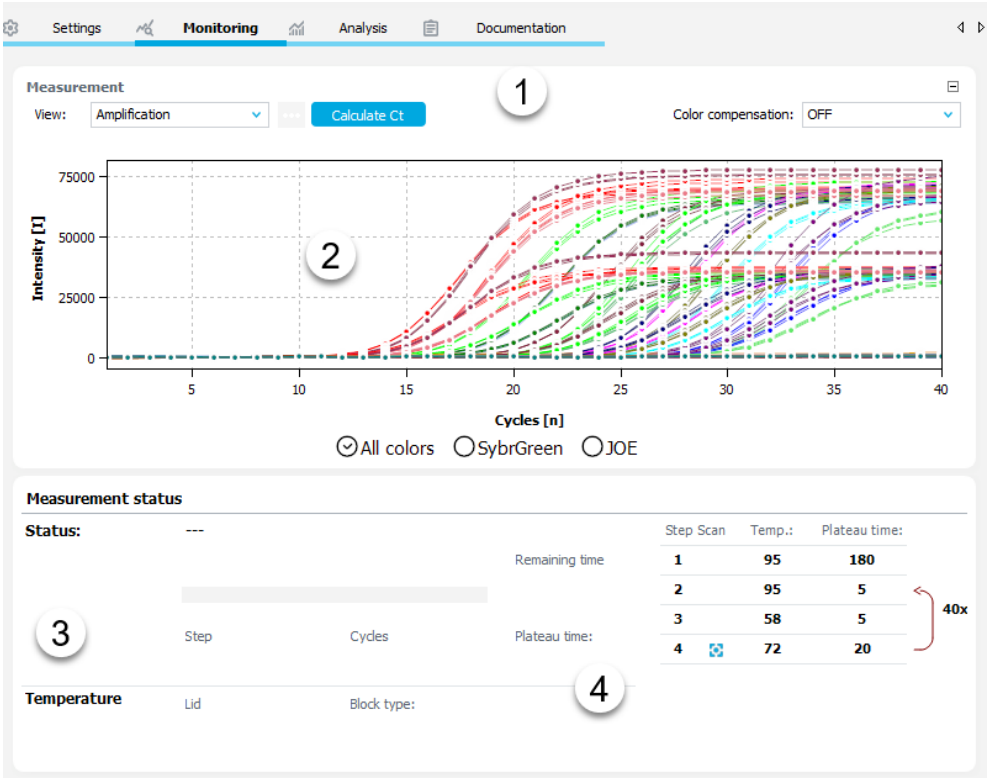
4 Monitoring

All functions required for starting and monitoring a qPCR run are combined in the **Monitoring** project window.

After saving the project with a completed qPCR run, you cannot start a new qPCR run for reasons of data integrity. If you want to start another qPCR run with the same settings, you must create a template from the project and use that template.

If a project was not saved after a completed qPCR run, you can start another qPCR run in it. This will overwrite the fluorescence data of the previous run.

Settings | Monitoring project window



No.	Element	Description
1	**View/ Calculate Ct/ Calculate Tm**	View selection: **Raw data**, **Amplification** (smoothed and baseline-corrected fluorescence curves) and **Melting curve** Calculation of Ct values and melting temperature
2	Fluorescence curves	Graphical representation of the fluorescence curves The fluorescence intensities are plotted against the cycles. Depending on the option selected, fluorescence curves are displayed sorted by dye or together.
3	Status of the qPCR run	Progress indicator during the qPCR run
4	Temperature program	Temperature program of the experiment

Monitoring menu and icons

When you select the **Monitoring** tab, the **Monitoring** menu appears in the menu bar and further icons in the toolbar. You use the menu items and icons to start the qPCR run and make default settings for the calculation of the Ct values and melting curves.

Icon	Monitoring menu	Description
	**Start measurement**	Start qPCR run
	**Stop measurement**	Stop qPCR run
	**View options**	Options for displaying fluorescence curves, baseline correction and threshold setting
		Determine threshold automatically

4.1 Starting and monitoring a qPCR run

Start qPCR run

- Enter parameters for the qPCR run in the **Settings** project window or open a template with entered parameters.
- Fill microtiter plate or tubes and seal with optical foil or lids for the qPCR.
- Load the sample block according to the sample layout and close the lid of the device.
- Switch to the **Monitoring** tab in the project window.
- Click on the ▶ symbol or select the menu item **Monitoring** | **Start measurement**.
  - ✓ The qPCR run starts.

You can follow the course of the fluorescence curves (intensity/cycles) in the graph of the **Monitoring** tab. The remaining time, the current temperatures of the thermal block and heated lid, and the temperature step just passed are displayed below the graph. In the temperature profile, a bar marks the current step.

Stop qPCR run

You can stop a qPCR run.

- Click on the ■ icon or select the menu item **Monitoring** | **Stop measurement**.
  - ✓ The qPCR run is stopped and not continued. The fluorescence data recorded so far is saved and can be analyzed.

Activate and deactivate the display of fluorescence curves

You can activate or deactivate the display of selected fluorescence curves in the Project Explorer in the **Samples** area, e.g., to observe the curves of a selected sample type during the qPCR run. Deactivating only affects the display. Fluorescence curves are recorded for all samples defined in the layout. Fluorescence values are not recorded at wells whose sample type is **Empty**.

Status indicators during the qPCR run

During the qPCR run, icons are displayed to indicate the status of the qPCR run.

Icon	Device status
	qPCR run is starting
	Measurement is being prepared
	System test in progress
	Fiber optics are being tested

Icon	Device status
	Reference measurement in progress
	Measurement in progress
	Measurement data is processed and made available for further analysis
	qPCR run completed successfully
	Error during the qPCR run
	qPCR run aborted by user

See also

Project explorer Samples [▶ 12]

4.2 Displaying amplification curves and calculating Ct values

Amplification is documented in the **Monitoring** project window by fluorescence measurements during the PCR run.

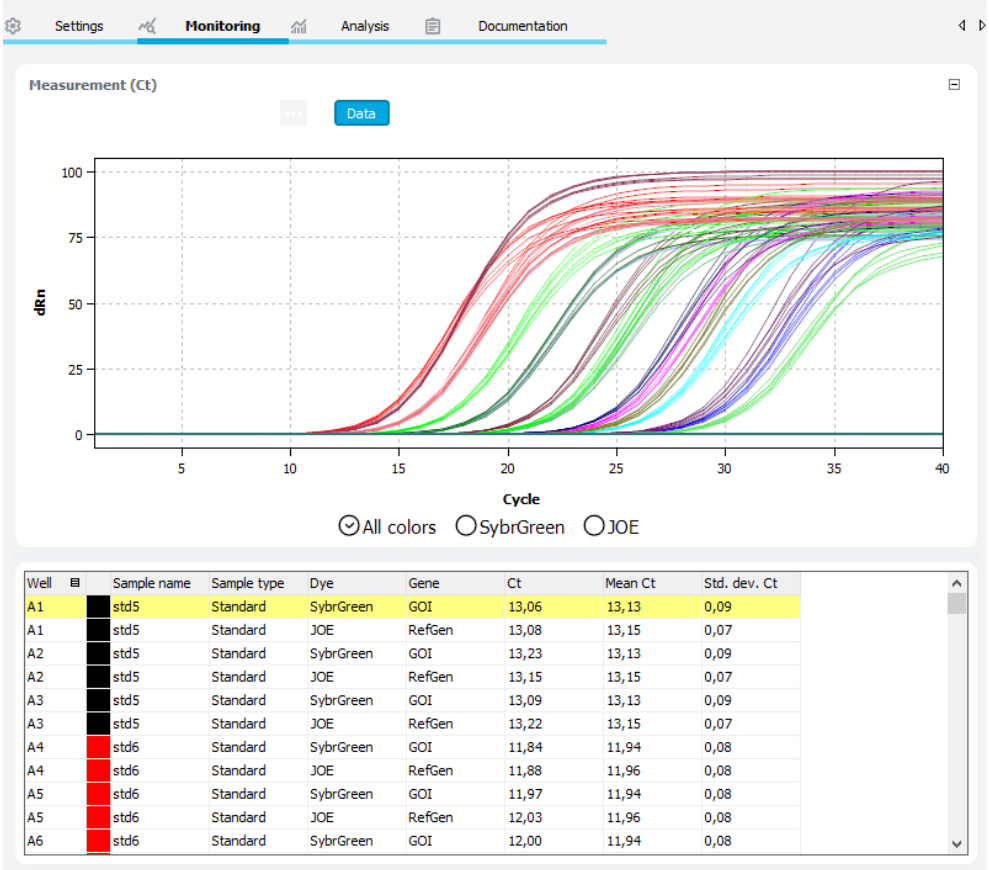
Fluorescence curve display

You can display the mathematically processed amplification curves or the raw data curve. For mathematical processing, click on the icon in the toolbar in the **View options** window to change settings.

- In the **Monitoring** project window, select the option **Amplification** or **Raw data** in the **View** list.
  - ✓ In the graph, the fluorescence intensity [dRn] is plotted against the number of cycles in relative units. The color of the curve displayed in each case corresponds to the color assigned to each well in the sample table.
- Select the fluorescence curves for the individual dyes with the options below the graph. The fluorescence curves of all dyes are displayed after selecting the **All colors** option.

After clicking on the ••• button above the curves, you can change the curve scaling and the parameters of the baseline correction.

Right-click on the graph to open a context menu to export the fluorescence data to a CSV file and to copy the graph to the clipboard.



Well		Sample name	Sample type	Dye	Gene	Ct	Mean Ct	Std. dev. Ct
A1		std5	Standard	SybrGreen	GOI	13,06	13,13	0,09
A1		std5	Standard	JOE	RefGen	13,08	13,15	0,07
A2		std5	Standard	SybrGreen	GOI	13,23	13,13	0,09
A2		std5	Standard	JOE	RefGen	13,15	13,15	0,07
A3		std5	Standard	SybrGreen	GOI	13,09	13,13	0,09
A3		std5	Standard	JOE	RefGen	13,22	13,15	0,07
A4		std6	Standard	SybrGreen	GOI	11,84	11,94	0,08
A4		std6	Standard	JOE	RefGen	11,88	11,96	0,08
A5		std6	Standard	SybrGreen	GOI	11,97	11,94	0,08
A5		std6	Standard	JOE	RefGen	12,03	11,96	0,08
A6		std6	Standard	SybrGreen	GOI	12,00	11,94	0,08

Calculate Ct values

After the qPCR run, the Ct values can be calculated directly from the amplification curves without having to create an analysis such as an absolute quantification.

- In the **Monitoring** project window, select the option **Amplification** or **Raw data** in the **View** list and click **Calculate Ct**.
- Optionally, set the threshold value for the individual dyes manually in the **Threshold** field above the graph, or calculate the threshold automatically by clicking on the icon.
  When calculating automatically, the settings under the menu item **Extras** | **Options** | **Analysis** are used.
  - ✓ The amplification curves are normalized and displayed individually or together on the list sheets for the dyes. The samples table below shows the Ct values of the individual samples and the mean values of the replicates.
- Click on **Data** to return to the previous view of the fluorescence curves.

Right-click on the sample table to open a context menu to export the data to a CSV file or an XLS file.

Show results for the calculation of Ct values

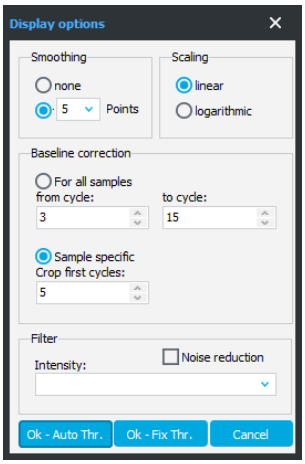
The sample table with the results is displayed in the bottom part of the project window.

Column	Description
Well	Position of the sample in the sample layout Click on the **Well** column title to arrange the table row by row or column by column according to the layout.
Graph color	Each sample is automatically assigned a color, and the corresponding fluorescence curve is displayed in that color. You can change the curve color by double-clicking or by holding down the Ctrl key and double-clicking.

Column	Description
Sample type	Sample type entered in the sample layout
Dye	Dye used for fluorescence measurement
Gene	Name of the gene measured in the sample
Ct	Ct value of the sample
Mean Ct	Mean Ct value of replicates
Std. dev. Ct	Standard deviation of the Ct values between replicates

Options for the analysis of amplification curves

In the **Display options** window, you can change the settings for the display and mathematical processing of the fluorescence curves. The window is displayed when you click on the icon in the toolbar or select the menu item **Monitoring** | **View options**.



Option	Description
Smoothing	Adjustment of the smoothing constraint for the fluorescence curves **none** No smoothing. **Points** Smoothing over the selected number of points.
Scaling	Select the scaling of the fluorescence curves (linear or logarithmic)
Filter	Select a digital filter for smoothing the fluorescence curves, can be adjusted in the levels **slight**, **medium** and **strong**
Noise reduction	Set noisy curves, which are not recognized by the software as amplification curves, to 0 and do not determine any Ct values
Ok - Auto Thr.	The threshold for a current project is recalculated according to the changes in this window. All other settings are also adopted and applied to the fluorescence curves.
Ok - Fix Thr.	The threshold set in the current project is retained regardless of changes in this window. All other settings are adopted and applied to the fluorescence curves.

See also

- Exporting fluorescence data [▶ 51]
- Exporting results tables [▶ 52]

4.3 Showing melting curves and calculating the melting temperature Tm

You can follow the course of the melting curves in the **Monitoring** project window.

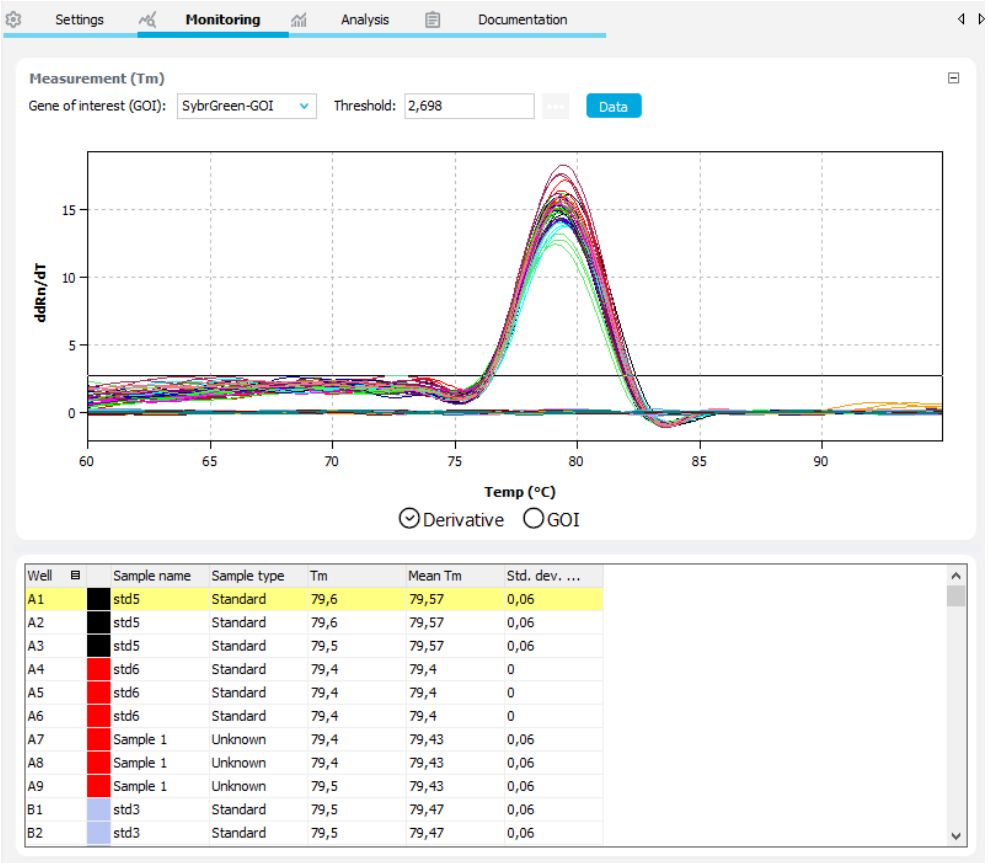
Show melting curves and fluorescence curves

- In the **Ct and Tm calculation in monitoring** project window, select the option **Melting curve** in the **View** list and click **Calculate Tm**.

The melting temperature T_m is determined from the peak maximum of the first derivative of the melting curves. The derivatives are displayed in the graph area in the **Derivative** view.

The melting curves are plotted against temperature in the graph area, either normalized to the highest fluorescence value or to the setpoint 100. These settings can be changed in the **View Options** window, which contains the same settings as the options window for the melting curve analyses.

For multiplex experiments, you can select the target gene/dye combination for display in the GOI list.



Well		Sample name	Sample type	Tm	Mean Tm	Std. dev. ...
A1		std5	Standard	79,6	79,57	0,06
A2		std5	Standard	79,6	79,57	0,06
A3		std5	Standard	79,5	79,57	0,06
A4		std6	Standard	79,4	79,4	0
A5		std6	Standard	79,4	79,4	0
A6		std6	Standard	79,4	79,4	0
A7		Sample 1	Unknown	79,4	79,43	0,06
A8		Sample 1	Unknown	79,4	79,43	0,06
A9		Sample 1	Unknown	79,5	79,43	0,06
B1		std3	Standard	79,5	79,47	0,06
B2		std3	Standard	79,5	79,47	0,06

Calculate Tm

After the qPCR run, the T_m values can be calculated from the melting curve without explicitly creating a melting curve analysis.

- In the **Monitoring** project window, select the **Melting curve** option in the **View** list and click **Calculate Tm**.
- If necessary, change the view and mathematical processing of the curves by clicking on  .
- Select the gene to be examined in the **Gene of Interest (GOI)** list.
  - ✓ Taking into account the parameters set under the menu item **Extras | Options | Analysis**, the melting temperature is calculated and the diagram and the sample table with the results are displayed.

- Optionally, set a threshold value on the **Derivative** tab to distinguish significant peaks from noise.
- Click on **Data** to return to the previous view of the fluorescence curves.

Right-click on the sample table to open a context menu to export the data to a CSV file or an XLS file.

Show results of the melting temperature calculation

The sample table in the bottom part of the **Monitoring** project window contains the calculated melting temperatures.

Column	Description
Well	Position of the sample in the sample layout Click on the **Well** column title to arrange the table row by row or column by column according to the layout.
Graph color	Each sample is automatically assigned a color, and the corresponding fluorescence curve is displayed in that color. You can change the curve color by double-clicking or by holding down the Ctrl key and double-clicking.
Sample name	Name entered in the sample layout
Sample type	Sample type entered in the sample layout
Tm	Melting temperature of the sample
Mean Tm	Mean melting temperature of the replicates
Std. dev. mean Tm	Standard deviation of the mean melting temperature of replicates

See also

Melting curve analysis options [▶ 78]

5 General functions for fluorescence curves and results table

In the **Monitoring** project window and in the tabs of the **Analysis** project window, the graphs of the fluorescence curves are displayed in the top part of the window and result tables, based on the sample layout, are displayed in the bottom part. The display of graphs and tables can be customized by the user. The contents of graphs and tables are exported to different formats via context menus.

5.1 Exporting fluorescence data

The data from the fluorescence measurement can be exported as a CSV file. In addition, the graphs of the fluorescence curves can be copied to the clipboard as a hardcopy and thereby made available to other programs.

You export the processed fluorescence data from the graphs of the tabs in the **Analysis** project window. You can export the raw data from the **Monitoring** project window.

Export a graph to the clipboard

- Right-click on the graph.
- Select the **Copy chart** function in the context menu.
  - ✓ The graph of the fluorescence curves is copied to the clipboard and can be used in other applications, e.g. in a Word file.

Export fluorescence data as CSV file

- Right-click on the graph.
- Select the **Save chart** function in the context menu.
- In the **Save as** window, choose the file name and confirm with **Save**.
  - ✓ The fluorescence values are saved in a CSV file.

Export fluorescence data automatically

You can save the raw data automatically at the end of a qPCR run. You can configure the settings under the menu item **Extras** | **Options** | **General**.

5.2 Customizing the results tables

The results tables of the analyses are located in the bottom part of the tabs of the **Analysis** project window and in the **Monitoring** project window. The results table contains different data sets depending on the selected analysis method. The selection and view of the displayed columns can be customized for each table.

- Right-click on a column heading to open the context menu, where you can enable or disable individual columns for display.
- To change the order of the columns, click on a column header and drag the column to the desired position while keeping the mouse pressed.
- To change the column width, move the cursor to the separator between two column headers. When the cursor has changed to a double arrow, click and holding down the mouse button and move the separator line to the desired column width.
- To sort the data of a column in ascending or descending order, click on the column header.

- Change the colors of a selected fluorescence curve by double-clicking on the color cell in the table row. Undo the color change by holding down the Shift key and double-clicking on the color cell.
- Hold down the Ctrl key and double-click to open the **Edit colors** window to assign a color to several wells. This window works the same way as for the settings of the colors via the sample layout in the **Settings** | **Samples** project window.
- Click on the **Well** column to switch between column-by-column and row-by-row display of the results. The column-by-column and row-by-row display is based on the arrangement of the samples in the layout.

See also

Entering sample properties in the layout diagram [▶ 35]

5.3 Exporting results tables

The sample tables with the results in the **Monitoring** project window and in the tabs of the **Analysis** project window can be exported to XLS and CSV files via a context menu. User-defined customizations are taken into account during the export. The following options are available:

Option	Description
Save table as Excel-File (*.xls)	Export results as an XLS file
Save table as Excel-File (*.xls) and run Excel	Export results as an XLS file and open the exported file in Excel
Save table as CSV-File (*.csv)	Export the results as a CSV file

- Right-click on the graph.
- Select an option in the context menu.
- In the **Save as** window, enter the file name and confirm with **Save**.
  - ✓ The content of the results table and the parameters of the qPCR protocol are saved in the selected format.

Export Ct values automatically

You can automatically export the Ct values to a CSV file at the end of a qPCR run. You can configure the settings for this under the menu item **Extras** | **Options** | **General**.

5.4 Editing the colors of the fluorescence curves

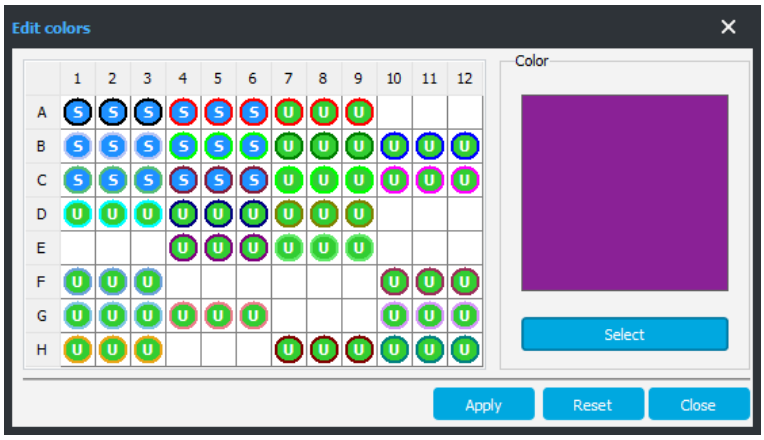
You select the color scheme of the fluorescence curves for the whole program in the **Options** | **Colors** window. You can choose between schemes for sample types, replicates or wells. For sample types, samples of the same type are indicated with one color. If replicates are selected, the curves of the replicates of a sample all have the same color. When coded by wells, each curve has a different color.

You can change the color of individual wells to highlight them in the graph. You configure the settings for this either in the sample layout of the **Settings** | **Samples** project window or in the sample tables in the bottom part of the project window.

Setting in the sample layout

In the layout diagram, the curve colors are shown as a colored ring around the sample type symbol of the wells. You can change the colors of one or more wells at the same time in the **Edit colors** window.

- Right-click on the sample diagram and select **Assign colors** from the context menu.
  - ✓ The **Edit colors** window appears.



- Click the **Select** button, choose a color in the **Color** window and confirm with **OK**.
- In the sample layout of the **Edit colors** window, select all wells that you want to have this color and click the **Apply** button.
- If necessary, assign curve colors to other wells.
- To undo the color change, select the affected wells and click the **Reset** button.
- Close the **Edit colors** window by clicking on the button.
  - ✓ The curve color of the sample (ring) is updated.

Setting in the sample table

The curve color is displayed in the sample table in the column before the sample name. You can also change the curve colors via these color cells.

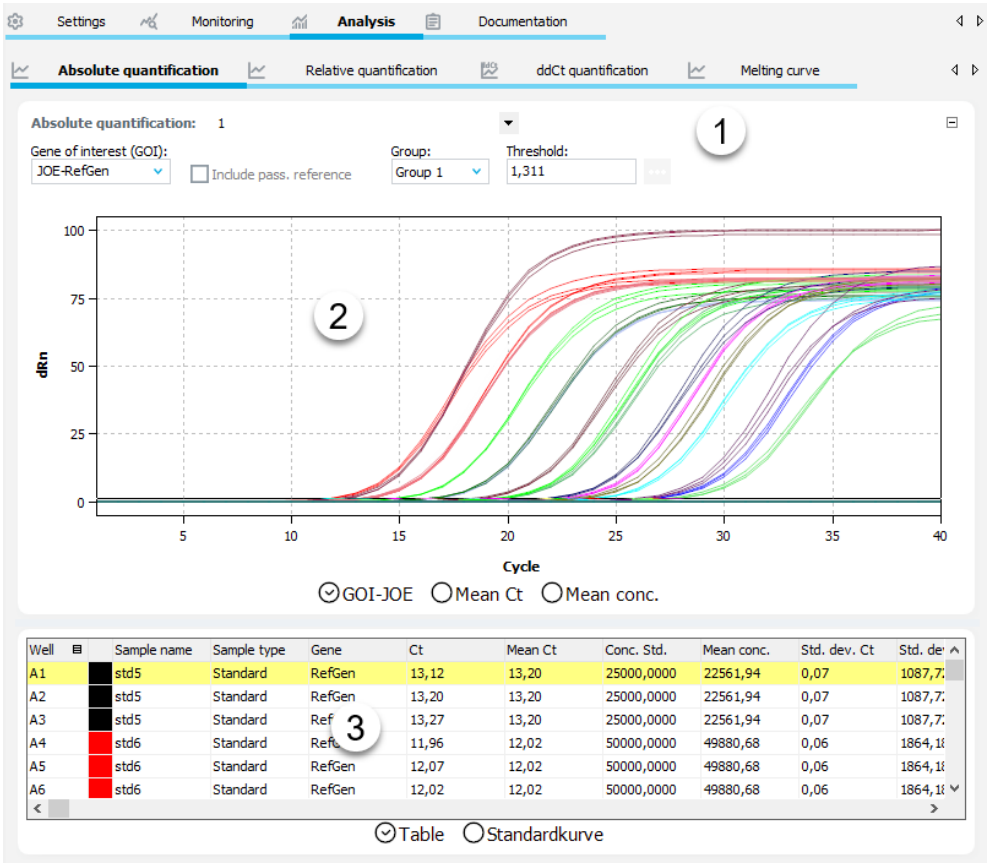
- Double-click on the color cell and choose the new color in the **Color** window, then confirm with **OK**.
  - ✓ The new color is assigned to the sample.
- To undo the color change, hold down the Shift key and double-click on the color cell again.
  - ✓ After a confirmation prompt, the color change is undone.
- To change the color in several wells, click on any color cell while holding down the Ctrl key. This **Edit colors** window appears.
- Continue as described above.

6 Absolute quantification

The absolute quantification is used to determine absolute copy numbers in samples based on the comparison with standards of known concentrations. This analysis is performed in the **Analysis Absolute quantification** project window. To do this, create a separate analysis in the project window for each gene of interest. The analyses are saved with the project and can be viewed and edited again at a later time.

6.1 Project window and menu for absolute quantification

Analysis | Absolute quantification project window



No.	Element	Description
1	Parameter settings	Selection of analysis parameters for the absolute quantification
2	Graph area	Display of fluorescence curves, mean Ct values, and mean concentration for a selected dye (gene)
3	Table area	Display of the sample table with the results and the standard curve of the dye (gene)

AbsQuant menu and icons

When you select the **Absolute quantification** tab, the **AbsQuant** menu appears in the menu bar and further icons appear in the toolbar for special functions for the absolute quantification.

Icon	AbsQuant menu	Description
	**Add absolute quantification**	Insert an analysis in the project window
	**Delete absolute quantification**	Remove the current analysis from the project window
	**Options absolute quantification**	Settings for the display of fluorescence curves and for the calculation of results
	**Autom. threshold**	Automatically determine the threshold for the calculation of Ct values
	**Import standard curve**	Import standard curve into the current analysis from a saved project or from the same project

6.2 Creating or deleting an analysis for an absolute quantification

Create analyses

In order to be able to perform an analysis, you must first create it in the project window with a name.

- Open the **Analysis** | **Absolute quantification** project window.
- Click on the  icon in the toolbar or select the menu item **AbsQuant** | **Add absolute quantification**.
- Enter the name for the analysis in the input window and confirm with **OK**.
  - ✓ The new analysis is created in the project. You can now select a GOI and edit the other analysis parameters. The fluorescence curves of the GOI (dye) are displayed in the graph area and the results are displayed in the sample table below it.

Remove analysis

You can remove an analysis that is no longer required.

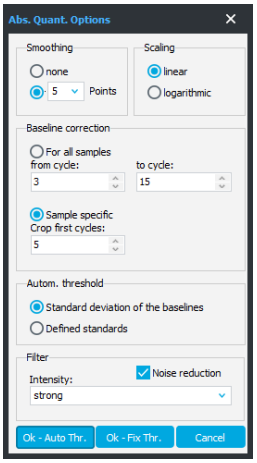
- Select the name of the analysis in the list.
- Click on the  icon in the toolbar or select the menu item **AbsQuant** | **Delete absolute quantification**.
  - ✓ The analysis is removed after a confirmation prompt.

6.3 Options for the absolute quantification

In the options for the absolute quantification, you configure the settings for the mathematical treatment of the fluorescence curves and constraints for the automatic threshold calculation.

The **Options absolute quantification** window is displayed when you click on the  icon in the toolbar or select the menu item **AbsQuant** | **Options absolute quantification**.

Abs. quantification options window



Option	Description
Smoothing	Adjustment of the smoothing constraint for the fluorescence curves **none** No smoothing. **Points** Smoothing over the selected number of points.
Scaling	Select the scaling of the fluorescence curves (linear or logarithmic)
Baseline correction	Select the baseline correction **For all samples** This correction determines the baseline for each sample in the same range. The lower and upper range limits must be set in the **from cycle** and **until cycle** fields. **Sample specific** This correction should be chosen if the curves have very different Ct values. The lower range limit for determining the baseline is set for all samples in the **Crop first cycles** field. The upper range limit is determined separately for each sample using an algorithm. **Note**: The type of baseline correction can only be set in this dialog box. The range limits for the correction can be adjusted in the project window.
Autom. threshold	**Standard deviation of the baselines** Calculation of the threshold as x-fold deviation of the standard deviation of the baselines (factor can be adjusted in the menu item **Extras** | **Options** | **Analysis**) **Defined standards** Selection of standards with the aim of obtaining the maximum value for the coefficient of determination R^2
Filter	Select a digital filter for smoothing the fluorescence curves, can be adjusted in the levels **slight**, **medium** and **strong**
Noise reduction	Set noisy curves, which are not recognized by the software as amplification curves, to 0 and do not determine any Ct values
Ok - Auto Thr.	The threshold for a current project is recalculated according to the changes in this window. All other settings are also adopted and applied to the fluorescence curves.
Ok - Fix Thr.	The threshold set in the current project is retained regardless of changes in this window. All other settings are adopted and applied to the fluorescence curves.

6.4 Editing parameters for the absolute quantification

Configure the parameters for the absolute quantification of an experiment in the fields and lists above the graph.

Option	Description
Selection list	Selection of an analysis created for the experiment
Gene of interest (GOI)	Selection list of target gene/dye combinations The fluorescence curves and standard curves are displayed according to the selection.
Include pass. reference	Can only be selected if a dye has been defined as a passive reference in the **Settings** | **Scan** project window. If this option is enabled, the fluorescence of the dye that has been set as a passive reference is used for standardization.
User Group	If several experiments (groups) have been created in the sample layout, the group of the experiment you want to analyze must be selected here.
Threshold	Set threshold value manually The threshold value must be between 1 and 100, depending on the standardized representation of the fluorescence curves (dRn).
•••	Select scaling and baseline setting of the fluorescence curve

Set the threshold value

To determine Ct values for the analysis a threshold value must be determined for each experiment.

There are several ways to set the threshold value:

- In the general options, click on the icon in the toolbar to set the options for determining the threshold automatically.
- Enter the threshold manually in the input field above the graph.
- In the graph, move the black threshold line vertically by holding down the mouse button. At the same time, the Ct values in the sample table are updated.
  Note: Due to the further spread of the early exponential range of the amplification, a logarithmic representation is better suited for setting the threshold manually in the display range than a linear representation.
- To calculate automatically, click on the icon in the toolbar. For the automatic calculation, the factor set under the menu item **Extras** | **Options** | **Analysis** and the settings from the options window (icon) are used.
  ✓ The resulting threshold value is updated and displayed in the **Threshold** input field for both manual determination and automatic calculation.

6.5 Displaying fluorescence curves for the absolute quantification

In the display area the measured data, standardized to the value 100 for the highest fluorescence intensity, are plotted against the cycle for the target gene selected. When selecting another target gene/dye combination in the GOI list, its fluorescence curves are displayed.

The fluorescence data is displayed as a linear or logarithmic representation depending on the selected display option. For both view types a brief information is shown as soon as the mouse pointer is positioned on one of the curves.

Toggle graph

- Click on the ••• icon above the graph.
- Select the option **Scaling logarithmic** or **linear**.
- Click next to the selection window.
  - ✓ The graph is rescaled. Due to the further spread of the early exponential range of the amplification, the logarithmic representation is recommended for setting the threshold manually.

See also

Exporting fluorescence data [▶ 51]

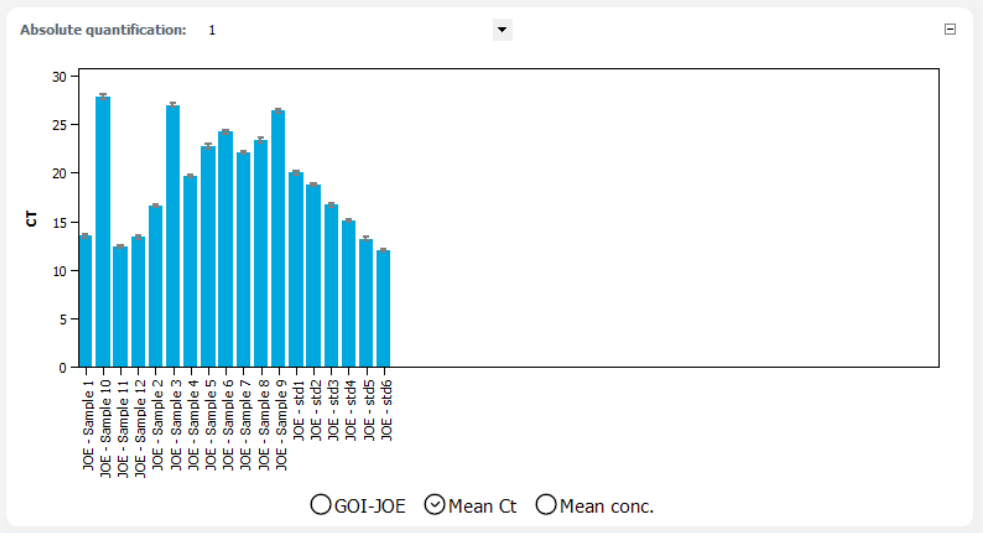
6.6 Showing mean Ct values and concentrations as bar graphs

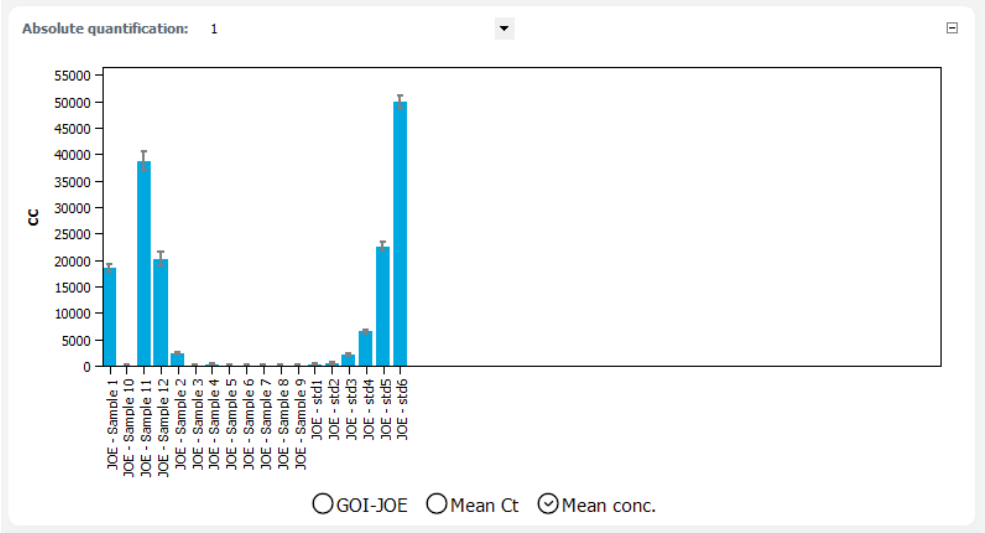
The Ct values and the concentrations of the samples are displayed as bar graphs in the corresponding views in the graph area of the **Analysis** | **Absolute quantification** project window.

Each sample is symbolized by a bar with the sample name at the bottom. The order of the samples in the diagram is alphabetical according to the sample name. The height of the bar corresponds to the mean Ct value or the mean concentration of the replicates of a sample. The range of the standard deviation is shown as a gray error bar at the end of the bar.

If you move the cursor over a bar, brief information about the position of the replicates in the sample layout, the mean Ct value, and the mean concentration is displayed.

If there is a large number of samples and not all sample bars are displayed at the same time, you can move the diagram horizontally by clicking on the graph and holding down the mouse button.





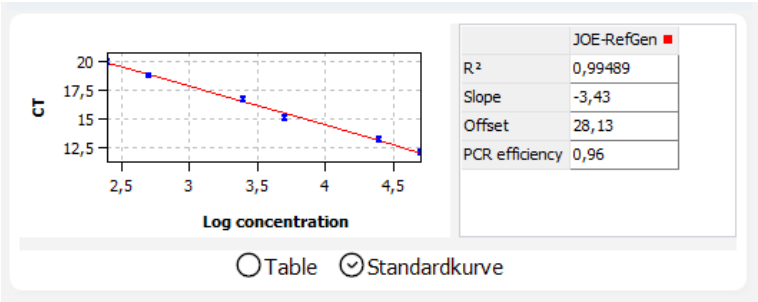
6.7 Displaying the standard curve for the absolute quantification

The standard curve of the experiment for the selected GOI is located in the bottom part of the **Analysis** | **Absolute quantification** project window.

For the display of the standard curve the Ct values of the standard samples are graphically plotted against the logarithm of their concentration. The respective data points have an error bar indicating the size of the standard deviation between replicates. For each data point, brief information with the sample name and mean Ct value of the replicates is displayed when hovering over with the cursor. In the value range on the right, the following data is displayed:

- Coefficient of determination R^2 of the straight-line equation
- Gradient of the standard line
- Intersection of the straight line with the y-axis at x=0 (offset)
- PCR efficiency

The standard curve and the values are automatically calculated and updated if the settings are changed.



6.8 Importing standard curves into an experiment for absolute quantification

You can measure standards in the experiment for absolute quantification and have the regression coefficients of the standard curve calculated from them or import standard curves from other experiments. These can be experiments from other projects or from another group in the same project.

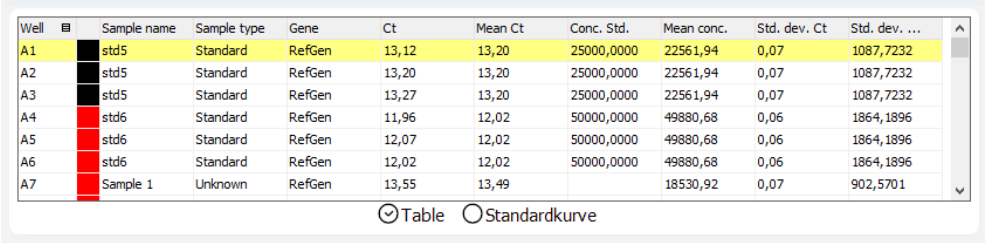
- Click on the  icon in the toolbar or select the menu item **AbsQuant** | **Import standard curve**.
- Select the import function in the **Import standard curve** window and edit the necessary parameters.
  - ✓ The standard curve is imported into the experiment and used to calculate the concentration.

The following options are available in the**Import standard curve** window:

Option	Description
import from this run	Import standard curve from the currently open project If several standard curves are saved in a project then all curves are displayed and a selection is possible.
import from saved run	Import standard curve from a saved project If several standard curves have been saved, select the curve from the list.
manual input	Coefficients of the standard curve are entered manually Enter the gradient and the axis intercept for the equation: Ct = gradient * log(conc) + axis intercept.
Delete external standards	Delete imported or entered standard curves The analyses are reset accordingly.

6.9 Displaying results of an absolute quantification

The sample table with the results is displayed in the bottom part of the **Analysis** project window in the **Table** view.



Well	Sample name	Sample type	Gene	Ct	Mean Ct	Conc. Std.	Mean conc.	Std. dev. Ct	Std. dev. ...
A1	std5	Standard	RefGen	13,12	13,20	25000,0000	22561,94	0,07	1087,7232
A2	std5	Standard	RefGen	13,20	13,20	25000,0000	22561,94	0,07	1087,7232
A3	std5	Standard	RefGen	13,27	13,20	25000,0000	22561,94	0,07	1087,7232
A4	std6	Standard	RefGen	11,96	12,02	50000,0000	49880,68	0,06	1864,1896
A5	std6	Standard	RefGen	12,07	12,02	50000,0000	49880,68	0,06	1864,1896
A6	std6	Standard	RefGen	12,02	12,02	50000,0000	49880,68	0,06	1864,1896
A7	Sample 1	Unknown	RefGen	13,55	13,49		18530,92	0,07	902,5701

Table Standardkurve

Column	Description
Well	Position of the sample in the sample layout Click on the **Well** column title to arrange the table row by row or column by column according to the layout.
Graph color	Each sample is automatically assigned a color, and the corresponding fluorescence curve is displayed in that color. You can change the curve color by double-clicking or by holding down the Ctrl key and double-clicking.
Sample name	Name entered in the sample layout
Sample type	Sample type entered in the sample layout
Group	Assignment of the sample to an experimental group
Gene	Name of the gene measured in the sample
Ct	Ct value of the sample
Mean Ct	Mean Ct value of replicates
Conc. Std.	Concentration of the standard sample

Column	Description
Mean conc.	Mean concentration of replicates The concentration is calculated with the mean Ct value from the standard curve.
Std. dev. Ct	Standard deviation of the Ct values between replicates
%CV Ct	Coefficient of variation of Ct values between replicates
Std. dev. mean. conc.	Standard deviation of the mean concentration

You can customize the display of the columns in the results table according to your requirements by right-clicking on the table header and activating the column display in the context menu. To export the data of the results table configured in this way to an Excel or CSV file, right-click on the tables and select the corresponding command in the context menu.

See also

- Exporting results tables [▶ 52]
- Customizing the results tables [▶ 51]

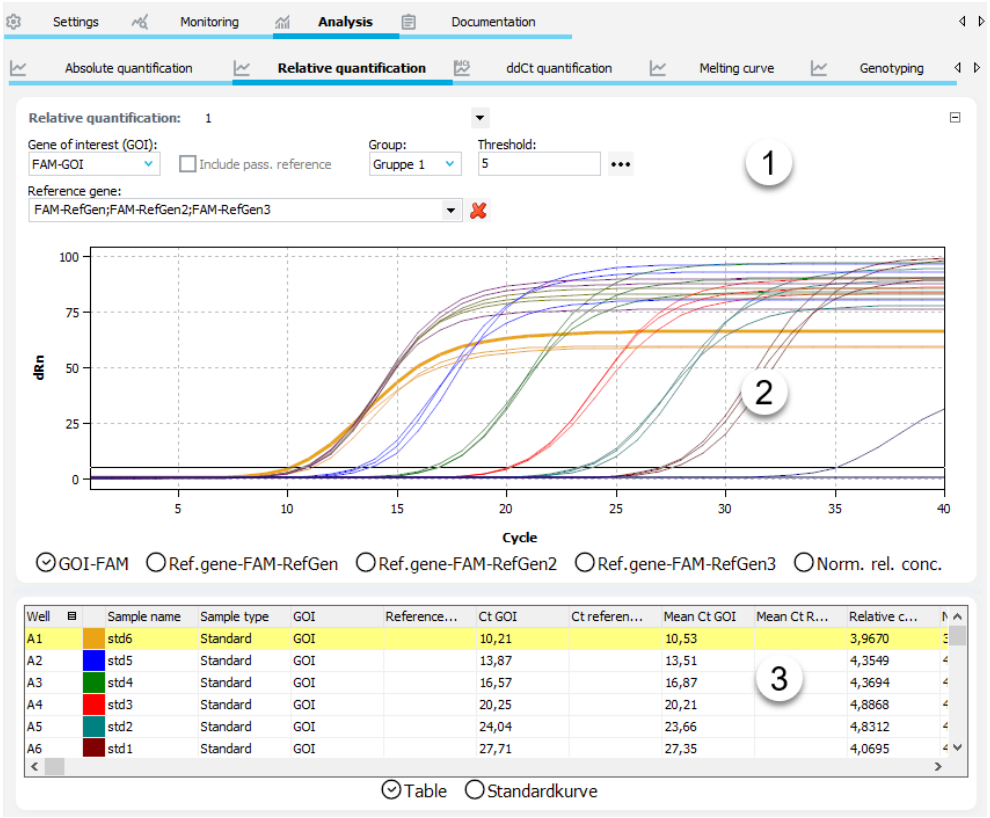
7 Relative quantification

Relative quantification is used to determine the relative expression ratio of the target gene (GOI) to one or more reference genes (usually housekeeping genes). If one of the samples is defined as calibrator, the expression ratio for this sample is set to 1 and the expression ratios of all other samples are stated relative to it. Relative quantification requires standard series for both the target gene and the reference gene, from which two calibration lines are calculated.

This relative analysis is performed in the **Analysis Relative quantification** project window. You create a separate analysis for each combination of GOI/reference gene. The analyses are saved with the project and can be viewed and edited again at a later time.

7.1 Project window and menu for relative quantification

Analysis | Relative quantification project window



No.	Element	Description
1	Parameter settings	Selection of analysis parameters for the relative quantification
2	Graph area	Display of the fluorescence curves of the GOI and the reference genes and the bar diagram of the normalized relative concentration of the samples
3	Table area	Display of the sample table with the results and the standard curves of the GOI and the reference genes

RelQuant menu and icons

When you select the **Relative quantification** tab, the **RelQuant** menu appears in the menu bar and further icons in the toolbar.

Icon	RelQuant menu	Description
	**Add relative quantification**	Insert an analysis in the project window
	**Delete relative quantification**	Remove the current analysis from the project window
	**Options relative quantification**	Settings for the display of fluorescence curves and for the calculation of results
	**Autom. threshold**	Automatically determine the threshold for the calculation of Ct values
	**Import standard curve**	Import standard curve into the current analysis from a saved project or from the same project

7.2 Creating or deleting an analysis for a relative quantification

Create analyses

In order to be able to perform an analysis, you must first create it in the project window with a name.

- Open the **Analysis** | **Relative quantification** project window.
- Click on the  icon in the toolbar or select the menu item **RelQuant** | **Add relative quantification**.
- Enter the name for the analysis in the input window and confirm with **OK**.
  - ✓ The new analysis is created in the project. You can now select a GOI and the reference genes and edit the other analysis parameters.

Remove analysis

You can remove an analysis that is no longer required.

- Select the name of the analysis in the list.
- Click on the  icon in the toolbar or select the menu item **RelQuant** | **Delete relative quantification**.
  - ✓ The analysis is removed.

7.3 Options for the relative quantification

In the options for the relative quantification, you configure the settings for the mathematical treatment of the fluorescence curves and constraints for the automatic threshold calculation.

The **Rel. quant. options** window is displayed when you click on the  icon in the toolbar or select the menu item **RelQuant** | **Options relative quantification**.

The following settings are available:

Option	Description
Smoothing	Adjustment of the smoothing constraint for the fluorescence curves **none** No smoothing. **Points** Smoothing over the selected number of points.
Scaling	Select the scaling of the fluorescence curves (linear or logarithmic)

Option	Description
Baseline correction	Select the baseline correction **For all samples** This correction determines the baseline for each sample in the same range. The lower and upper range limits must be set in the **from cycle** and **until cycle** fields. **Sample specific** This correction should be chosen if the curves have very different Ct values. The lower range limit for determining the baseline is set for all samples in the **Crop first cycles** field. The upper range limit is determined separately for each sample using an algorithm. **Note**: The type of baseline correction can only be set in this dialog box. The range limits for the correction can be adjusted in the project window.
Autom. threshold	**Standard deviation of the baselines** Calculation of the threshold as x-fold deviation of the standard deviation of the baselines (factor can be adjusted in the menu item **Extras** | **Options** | **Analysis**) **Defined standards** Selection of standards with the aim of obtaining the maximum value for the coefficient of determination R^2
Filter	Select a digital filter for smoothing the fluorescence curves, can be adjusted in the levels **slight**, **medium** and **strong**
Noise reduction	Set noisy curves, which are not recognized by the software as amplification curves, to 0 and do not determine any Ct values

7.4 Editing parameters for the relative quantification

Configure the parameters for the relative quantification of an experiment in the fields and lists above the graph.

Option	Description
Selection list	Selection of an analysis created for the experiment
Gene of interest (GOI)	Selection list of target gene/dye combinations The fluorescence curves and standard curves are displayed according to the selection.
Reference gene	Reference gene selection list You can select several reference genes at a time. Another tab is displayed in the graph area for each reference gene. Click on the  icon to remove all selected reference genes from the analysis.
Include pass. reference	Can only be selected if a dye has been defined as a passive reference in the **Settings** | **Scan** project window. If this option is enabled, the fluorescence of the dye that has been set as a passive reference is used for standardization.
User Group	If several experiments (groups) have been created in the sample layout, the group of the experiment you want to analyze must be selected here.
Threshold	Set threshold value manually The threshold value must be between 1 and 100, depending on the standardized representation of the fluorescence curves (dRn).

Option	Description
•••	Select scaling and baseline setting of the fluorescence curve

Set the threshold value

To determine Ct values for the analysis a threshold value must be determined for each experiment.

There are several ways to set the threshold value:

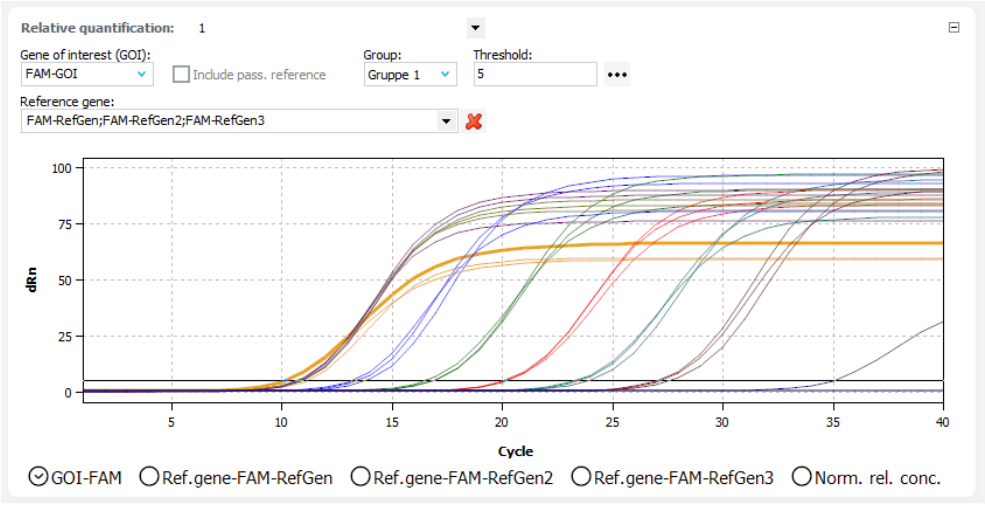
- In the general options, click on the icon in the toolbar to set the options for determining the threshold automatically.
- Enter the threshold manually in the input field above the graph.
- In the graph, move the black threshold line vertically by holding down the mouse button. At the same time, the Ct values in the sample table are updated.
  Note: Due to the further spread of the early exponential range of the amplification, a logarithmic representation is better suited for setting the threshold manually in the display range than a linear representation.
- To calculate automatically, click on the icon in the toolbar. For the automatic calculation, the factor set under the menu item **Extras** | **Options** | **Analysis** and the settings from the options window (icon) are used.
  ✓ The resulting threshold value is updated and displayed in the **Threshold** input field for both manual determination and automatic calculation.

7.5 Displaying fluorescence curves for the relative quantification

In the display area the measured data, standardized to the value 100 for the highest fluorescence intensity, are plotted against the cycle for the target gene selected. The target gene/dye combinations and reference gene/dye are each assigned a list sheet.

When selecting another target gene/dye combination in the GOI list, its fluorescence curves are displayed.

The fluorescence data is displayed as a linear or logarithmic representation depending on the selected display option. For both view types a brief information is shown as soon as the mouse pointer is positioned on one of the curves. Due to the further spread of the early exponential range of the fluorescence curve, the logarithmic representation is recommended for setting the threshold manually.



Toggle scaling

You can choose between linear or logarithmic scaling of the fluorescence curves.

- Click on the ••• icon above the graph.
- Select the option **Scaling logarithmic** or **linear**.
- Click next to the selection window.
  - ✓ The graph is rescaled.

Export fluorescence data

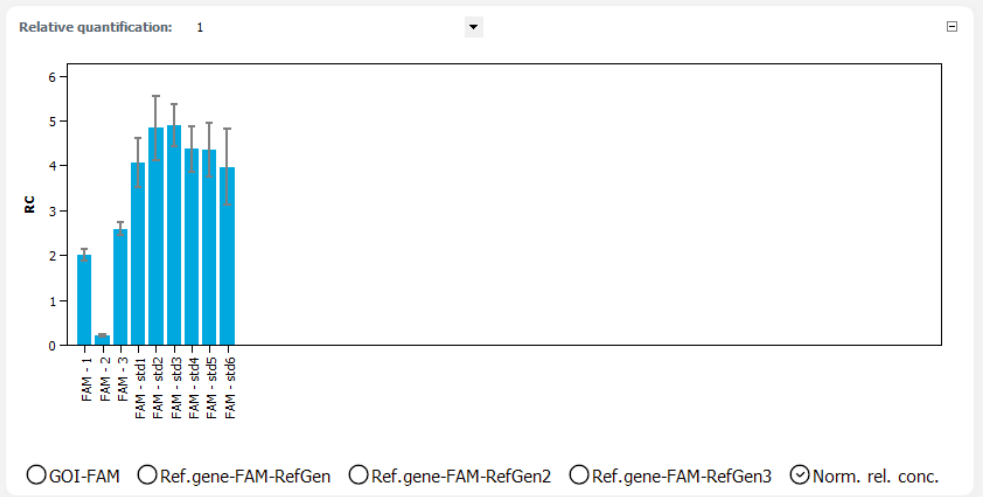
Right-click on a fluorescence graph to open the context menu to export the fluorescence data of the samples to a CSV file or save the graph as a hardcopy to the clipboard.

See also

Exporting fluorescence data [▶ 51]

7.6 Showing normalized relative concentrations as bar graphs

The normalized relative concentration of the samples is displayed as a bar chart on the **Norm. rel. conc.** tab in the graph area of the **Analysis** | **Relative quantification** project window.



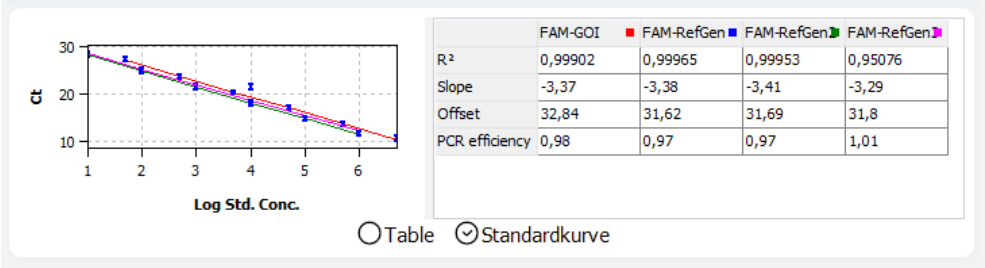
Each sample is symbolized by a bar with the sample name at the bottom. The order of the samples in the diagram is alphabetical according to the sample name. The height of the bar corresponds to the normalized relative concentration of the replicates of a sample. The range of the standard deviation is shown as a gray error bar at the end of the bar.

If you move the cursor over a bar, brief information about the position of the sample in the sample layout, the mean value, and the standard deviation is displayed.

If there is a large number of samples and not all sample bars are displayed at the same time, you can move the diagram horizontally by clicking on the graph and holding down the mouse button.

7.7 Displaying standard curve for the relative quantification

The standard curves of the experiment for the selected GOI are displayed in the bottom area on the Standard Curve tab.



For the display of the standard curve, the Ct values of the standard samples are graphically plotted against the logarithm of their concentration. The respective data points have an error bar indicating the size of the standard deviation between replicates. For each data point, brief information with the sample name and mean Ct value of the replicates is displayed when hovering over with the cursor. In the value table on the right the following calculated data is displayed:

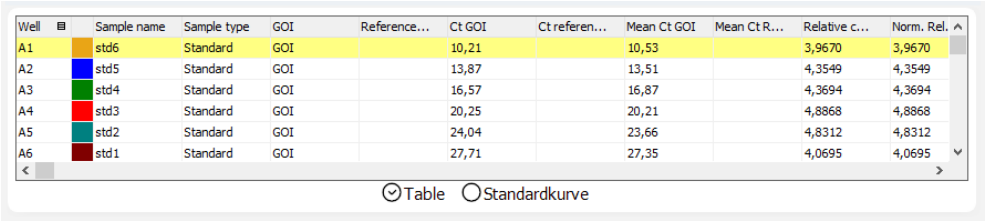
- Coefficient of determination R^2 of the straight-line equation
- Gradient of the standard line
- Intersection of the straight line with the y-axis at x=0 (offset)
- PCR efficiency

Each curve is displayed with an individual color. The color code is indicated in the header of the value table as a small colored square.

The standard curves and the values are automatically calculated and updated if the settings are changed.

7.8 Displaying results of a relative quantification

The sample table with the results is displayed in the bottom part of the **Analysis** project window in the **Table** view.



Column	Description
Well	Position of the sample in the sample layout Click on the **Well** column title to arrange the table row by row or column by column according to the layout.
Graph color	Each sample is automatically assigned a color, and the corresponding fluorescence curve is displayed in that color. You can change the curve color by double-clicking or by holding down the Ctrl key and double-clicking.
Sample name	Name entered in the sample layout
Sample type	Sample type entered in the sample layout
Group	Assignment of the sample to an experimental group
GOI	Target gene (gene of interest)
Reference gene	Reference gene
Ct GOI	Ct value of target gene
Ct reference gene	Ct value of reference gene
Mean Ct GOI	Mean Ct value of replicates of the target gene

Column	Description
Mean Ct Ref.gene	Mean Ct value of replicates of the reference gene
Conc. Std. GOI	Concentration of the standard for the target gene
Conc. Std. Ref.gene	Concentration of the standard for the reference gene
Mean conc. GOI	Concentration for the target gene determined from the standard curve using the mean Ct value
Mean conc. Ref.gene	Concentration for the reference gene determined from the standard curve using the mean Ct value
Std. dev. Ct GOI	Standard deviation of the Ct values between replicates of the target gene
Std. dev. RQ Ref.gene	Standard deviation of the Ct values between replicates of the reference gene
%CV Ct GOI	Coefficient of variation of the Ct values between replicates of the target gene
%CV Ct Ref.gene	Coefficient of variation of the Ct values between replicates of the reference gene
Relative conc.	Relative (x-fold) ratio of the expression of the target gene compared to the reference gene
Norm. Rel. conc.	Relative (x-fold) ratio of the expression of the target gene compared to the reference gene, normalized to the expression of the calibrator (if defined)
Std. dev. Relative conc.	Standard deviation of the relative concentration
Std. dev. Norm. Rel. conc.	Standard deviation of the normalized relative concentration

You can customize the display of the columns in the results table according to your requirements by right-clicking on the table header and activating the column display in the context menu. To export the data of the results table configured in this way to an Excel or CSV file, right-click on the tables and select the corresponding command in the context menu.

See also

- Exporting results tables [▶ 52]
- Customizing the results tables [▶ 51]

7.9 Importing standard curves for the relative quantification

You can measure standards in the experiment for the relative quantification and have the regression coefficients of the standard curve calculated from them or import standard curves from other experiments. The procedure for importing standard curves is the same as for absolute quantification.

See also

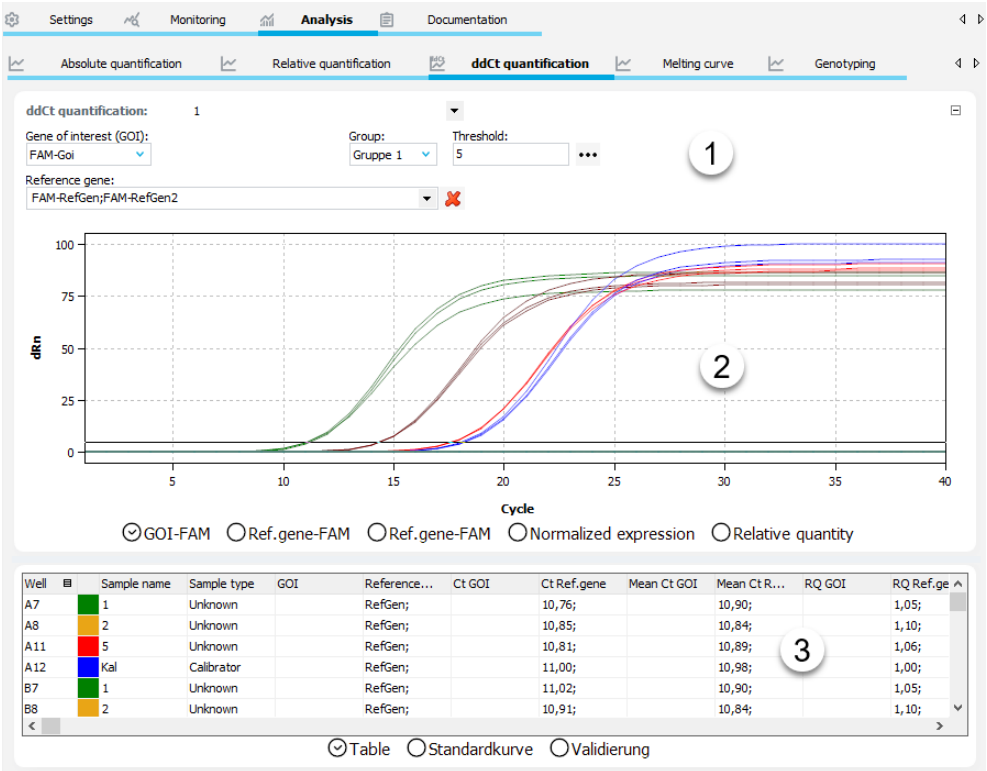
- Importing standard curves into an experiment for absolute quantification [▶ 59]

8 DeltaDeltaCt quantification (ddCt quantification)

The ddCt method is used to determine the relative expression ratio of the target gene to one or more reference genes (usually housekeeping genes). One of the samples must be defined as the calibrator. The expression level of the calibrator sample is set to one and the expression levels of the other samples are stated relative to this. To perform the ddCt method, it is not necessary to include standard series. A standard dilution series only needs to be defined if the ddCt method is to be validated in the same PCR run.

8.1 Project window and menu for ddCt quantification

Analysis | ddCt quantification project window



No.	Element	Description
1	Parameter settings	Selection of analysis parameters for the ddCt quantification
2	Graph area	Fluorescence curves of the GOI and the reference gene Bar graphs of normalized expression and relative quantity
3	Table area	Sample table with results, standard curve and validation

DeltaDeltaCt menu and icons

When you select the **ddCt quantification** tab, the **DeltaDeltaCt** menu appears in the menu bar and further icons appear in the toolbar for special functions for the ddCt quantification.

Icon	DeltaDeltaCt menu	Description
	**add ddCt quantification**	Insert an analysis in the project window

Icon	DeltaDeltaCt menu	Description
	**delete ddCt quantification**	Remove the current analysis from the project window
	**Options ddCt quantification**	Settings for the display of fluorescence curves and for the calculation of results
	**Autom. threshold**	Automatically determine the threshold for the calculation of Ct values

8.2 Creating or deleting an analysis for a ddCt quantification

Create analyses

In order to be able to perform an analysis, you must first create it in the project window with a name.

- Open the **Analysis** | **ddCt quantification** project window.
- Click on the  icon in the toolbar or select the menu item **DeltaDeltaCt** | **add ddCt quantification**.
- Enter the name for the analysis in the input window and confirm with **OK**.
  - ✓ The new analysis is created in the project. You can now select a GOI and the reference genes and edit the other analysis parameters.

Remove analysis

You can remove an analysis that is no longer required.

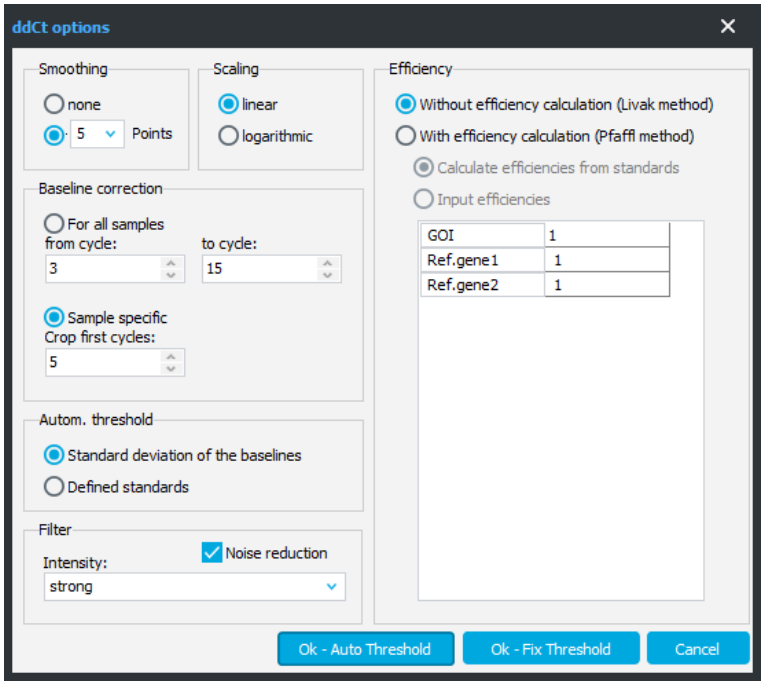
- Select the name of the analysis in the list.
- Click on the  icon in the toolbar or select the menu item **DeltaDeltaCt** | **delete ddCt quantification**.
  - ✓ The analysis is removed.

8.3 Options for a ddCt quantification

In the options for the ddCt quantification, you configure the settings for the mathematical treatment of the fluorescence curves and constraints for the automatic threshold calculation. In addition, you select the calculation mode for the Normalized Expression (NE) here.

The **ddCt options** window is displayed when you click on the  icon in the toolbar or select the menu item **DeltaDeltaCt** | **Options ddCt quantification**.

ddCt options window



Option	Description
Smoothing	Adjustment of the smoothing constraint for the fluorescence curves **none** No smoothing. **Points** Smoothing over the selected number of points.
Scaling	Select the scaling of the fluorescence curves (linear or logarithmic)
Baseline correction	Select the baseline correction **For all samples** This correction determines the baseline for each sample in the same range. The lower and upper range limits must be set in the **from cycle** and **until cycle** fields. **Sample specific** This correction should be chosen if the curves have very different Ct values. The lower range limit for determining the baseline is set for all samples in the **Crop first cycles** field. The upper range limit is determined separately for each sample using an algorithm. **Note**: The type of baseline correction can only be set in this dialog box. The range limits for the correction can be adjusted in the project window.
Autom. threshold	**Standard deviation of the baselines** Calculation of the threshold as x-fold deviation of the standard deviation of the baselines (factor can be adjusted in the menu item **Extras** | **Options** | **Analysis**) **Defined standards** Selection of standards with the aim of obtaining the maximum value for the coefficient of determination R^2
Filter	Select a digital filter for smoothing the fluorescence curves, can be adjusted in the levels **slight**, **medium** and **strong**
Noise reduction	Set noisy curves, which are not recognized by the software as amplification curves, to 0 and do not determine any Ct values
Efficiency	Selection of calculation rules for the normalized expression **Without efficiency calculation (Livak method)**

Option	Description
	With efficiency calculation (Pfaffl method)
Ok - Auto Thr.	The threshold for a current project is recalculated according to the changes in this window. All other settings are also adopted and applied to the fluorescence curves.
Ok - Fix Thr.	The threshold set in the current project is retained regardless of changes in this window. All other settings are adopted and applied to the fluorescence curves.

Calculation rule for the normalized expression

Two options are available to calculate the Normalized Expression NE:

- Without the PCR efficiency (Livak method)
- With the PCR efficiencies of GOI and reference genes (Pfaffl method)

To calculate the Normalized Expression NE, one sample must be defined as a calibrator.

In general, preference should be given to the Pfaffl method, since the basic assumption of the Livak method of equal efficiency in the amplification of the target gene and the reference gene rarely applies in practice and the calculation can thus lead to distorted values.

Liviak method

The Livak method assumes that the PCR efficiencies of the target gene (GOI) and reference gene (RefGene) are equal. In this case:

$$NE = 2^{-\Delta\Delta Ct}$$

where $\Delta\Delta Ct = \Delta Ct(Calibrator) - \Delta Ct(Sample)$

and $\Delta Ct(Sample) = Ct(GOI, Sample) - Ct(RefGene, Sample)$

$\Delta Ct(Calibrator) = Ct(GOI, Calibrator) - Ct(RefGene, Calibrator)$

Pfaffl method

In the Pfaffl method, the efficiencies determined for the target gene (GOI) and the reference gene (RefGene) are used in the calculation. The efficiencies (E(GOI) and E(RefGene)) can be calculated from dilution series or given to the software. Then:

$$NE = \frac{[1 + E(GOI)]^{\Delta Ct(GOI)}}{[1 + E(RefGene)]^{\Delta Ct(RefGene)}}$$

where $\Delta Ct(GOI) = Ct(GOI, Calibrator) - Ct(GOI, Sample)$

and $\Delta Ct(RefGene) = Ct(RefGene, Calibrator) - Ct(RefGene, Sample)$

8.4 Editing parameters for the relative ddCt quantification

Configure the parameters for the ddCt quantification of an experiment in the fields and lists above the graph.

Option	Description
Selection list	Selection of an analysis created for the experiment
Gene of interest (GOI)	Selection list of target gene/dye combinations The fluorescence curves and standard curves are displayed according to the selection.
Reference gene	Reference gene selection list

Option	Description
	You can select several reference genes at a time. Another tab is displayed in the graph area for each reference gene. Click on the icon to remove all selected reference genes from the analysis.
User Group	If several experiments (groups) have been created in the sample layout, the group of the experiment you want to analyze must be selected here.
Threshold	Set threshold value manually The threshold value must be between 1 and 100, depending on the standardized representation of the fluorescence curves (dRn).
•••	Select scaling and baseline setting of the fluorescence curve

Set the threshold value

To determine Ct values for the analysis a threshold value must be determined for each experiment.

There are several ways to set the threshold value:

- In the general options, click on the icon in the toolbar to set the options for determining the threshold automatically.
- Enter the threshold manually in the input field above the graph.
- In the graph, move the black threshold line vertically by holding down the mouse button. At the same time, the Ct values in the sample table are updated.
  Note: Due to the further spread of the early exponential range of the amplification, a logarithmic representation is better suited for setting the threshold manually in the display range than a linear representation.
- To calculate automatically, click on the icon in the toolbar. For the automatic calculation, the factor set under the menu item **Extras** | **Options** | **Analysis** and the settings from the options window (icon) are used.
  ✓ The resulting threshold value is updated and displayed in the **Threshold** input field for both manual determination and automatic calculation.

8.5 Displaying fluorescence curves for the ddCt quantification

In the display area the measured data, standardized to the value 100 for the highest fluorescence intensity, are plotted against the cycle for the target gene selected. The target gene/dye combinations and reference gene/dye are each assigned a list sheet.

When selecting another target gene/dye combination in the GOI list, its fluorescence curves are displayed.

The fluorescence data is displayed as a linear or logarithmic representation depending on the selected display option. For both view types a brief information is shown as soon as the mouse pointer is positioned on one of the curves. Due to the further spread of the early exponential range of the fluorescence curve, the logarithmic representation is recommended for setting the threshold manually.

Toggle scaling

You can choose between linear or logarithmic scaling of the fluorescence curves.

- Click on the ••• icon above the graph.
- Select the option **Scaling logarithmic** or **linear**.
- Click next to the selection window.

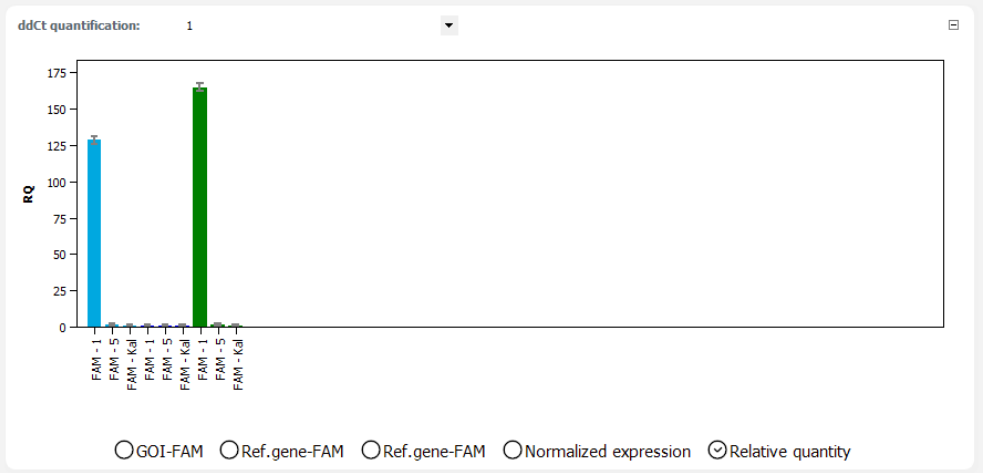
✓ The graph is rescaled.

Export fluorescence data

Right-click on a fluorescence graph to open the context menu to export the fluorescence data of the samples to a CSV file or save the graph as a hardcopy to the clipboard.

8.6 Displaying normalized relative expression and relative quantity as a bar graph

The normalized relative expression and relative quantity of the samples are displayed as bar graphs on the tabs in the graph area of the **Analysis** | **ddCt quantification** project window.



Each sample is symbolized by a bar with the sample name at the bottom. The order of the samples in the diagram is alphabetical according to the sample name. The height of the bar corresponds to the calculated normalized relative expression or the relative quantity of replicates of a sample. The range of the standard deviation is shown as a gray error bar at the end of the bar.

If you move the cursor over a bar, brief information about the position of the sample in the sample layout, the mean value, and the standard deviation is displayed.

If there is a large number of samples and not all sample bars are displayed at the same time, you can move the diagram horizontally by clicking on the graph and holding down the mouse button.

8.7 Displaying standard curves and validation curves for the ddCt quantification

For the calculation of the ddCt values, it is not necessary to determine a validation curve, although this can be used to check the quality of the data. Prerequisite for creating a validation curve is measuring a standard series with different dilution levels of target gene and reference gene. The validation curves and standard curves resulting from the standard series are displayed in the bottom part of the **Analysis** | **ddCt quantification** project window. In both curve displays, the data points have an error bar corresponding to the size of the standard deviation between replicates. When you hover over a data point with the mouse, brief information is displayed with the sample name and mean Ct value of the replicates.

Validation curves

In the display of the validation curves, the expression ratio between target gene and reference gene is shown graphically. For this purpose the mean Ct value of the target gene is subtracted from the mean Ct value of the reference gene for the respective dilution level and the resulting dCt(V) value plotted against the concentration logarithm. In the value range on the right the following calculated data is displayed:

- Coefficient of determination R^2 of the linear approximation
- Gradient of the approximation line
- Intersection of the straight line with the y-axis at x=0 (offset)

The gradient of the line should not exceed a value of ± 0.1. The assumption then applies that the efficiencies of the amplification of the target gene and reference gene are effectively equal and the calculation of the ddCt values produces valid data.

The validation curves and the values are automatically calculated and updated if the settings are changed.

Standard curves

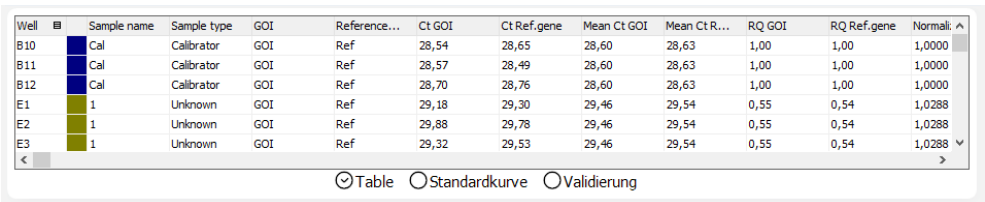
For the display of the standard curves, the Ct values of the standard samples are graphically plotted against the logarithm of their concentration. The following calculated data is displayed in the value table:

- Coefficient of determination R^2 of the straight-line equation
- Gradient of the standard line
- Intersection of the straight line with the y-axis at x=0 (offset)
- PCR efficiency

Each curve is displayed with an individual color. The color code is indicated in the header of the value table as a small colored square. The standard curves and the values are automatically calculated and updated if the settings are changed.

8.8 Displaying results of a ddCt quantification

The sample table with the results is displayed in the bottom part of the **Analysis** project window in the **Table** view.



Column	Description
Well	Position of the sample in the sample layout

Column	Description
	Click on the **Well** column title to arrange the table row by row or column by column according to the layout.
Graph color	Each sample is automatically assigned a color, and the corresponding fluorescence curve is displayed in that color. You can change the curve color by double-clicking or by holding down the Ctrl key and double-clicking.
Sample name	Name entered in the sample layout
Sample type	Sample type entered in the sample layout
Group	Assignment of the sample to an experimental group
GOI	Target gene (gene of interest)
Reference gene	Reference gene
Ct GOI	Ct value of target gene
Ct Ref.gene	Ct value of reference gene
Mean Ct GOI	Mean Ct value of replicates of the target gene
Mean Ct Ref.gene	Mean Ct value of replicates of the reference gene
Std. dev. Ct GOI	Standard deviation of the Ct values between replicates of the target gene
Std. dev. RQ Ref.gene	Standard deviation of the Ct values between replicates of the reference gene
%CV Ct GOI	Coefficient of variation of the Ct values between replicates of the target gene
%CV Ct Ref.gene	Coefficient of variation of the Ct values between replicates of the reference gene
dCt GOI	Delta Ct value of replicates of the target gene
dCt Ref.gene	Delta Ct value of replicates of the reference gene
RQ GOI	Calculated relative quantity for replicates of the target gene in the original sample
Mean RQ Ref.gene	Calculated relative quantity for replicates of the reference gene in the original sample
Mean RQ Ref.gene	Mean calculated relative quantity for replicates of the reference gene in the original sample
Norm. expression	Normalized relative (x-fold) ratio of the expression of the target gene in the sample compared to the calibrator

You can customize the display of the columns in the results table according to your requirements by right-clicking on the table header and activating the column display in the context menu. To export the data of the results table configured in this way to an Excel or CSV file, right-click on the tables and select the corresponding command in the context menu.

9 Melting curve analysis

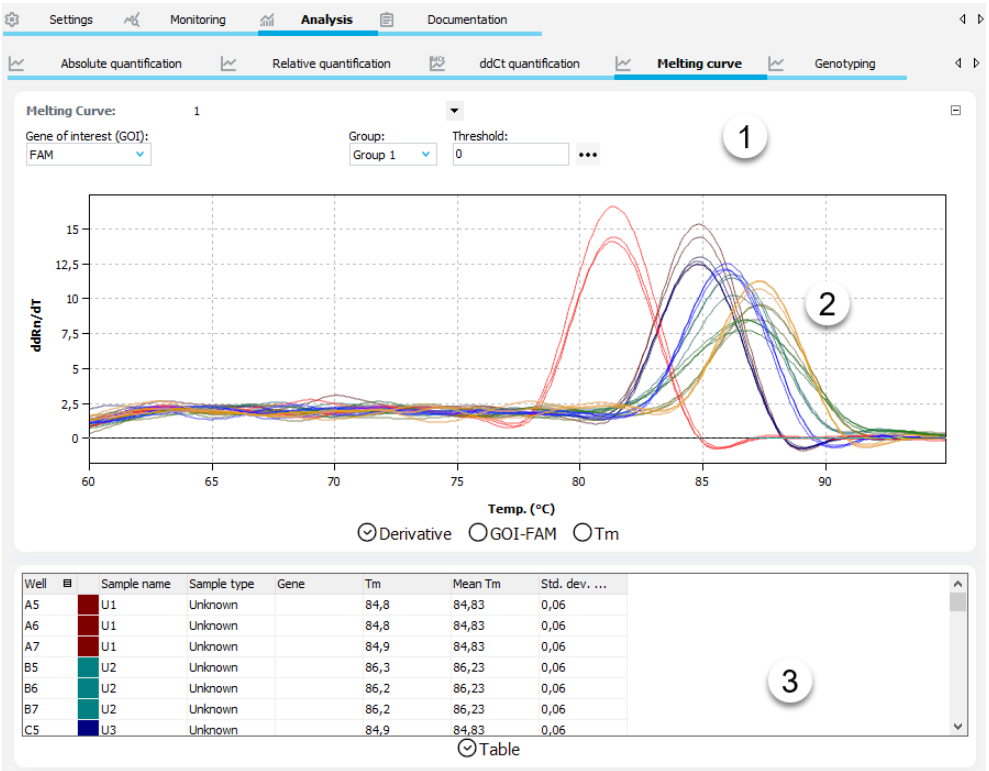
In the melting curve analysis the temperature in the reaction preparation is gradually increased until a denaturation of the PCR product results. The dissociation of the fragment into individual strands results in the release of the intercalating dye. The concurrent reduction of the fluorescence intensity is measured by the device and logged. By generating the first derivation of the fluorescence curve you obtain a peak that describes the melting point and approximately also the concentration of the PCR fragment. Using the melting curve analysis it is possible to differentiate whether the reaction has resulted in the generation of a specific PCR product or whether unspecific by-products, such as primer dimers, have been formed.

See also

Project window and menu for the melting curve analysis [▶ 77]

9.1 Project window and menu for the melting curve analysis

Analysis | Melting curve project window



No.	Element	Description
1	Parameter settings	Selection of analysis parameters for the melting curve analysis
2	Graph area	Derivation and fluorescence curves of the GOI Bar graph of the melting temperature of the samples
3	Table area	Sample table with the results

MeltCurve menu and icons

When you select the **Melting curve** tab, the **MeltCurve** menu appears in the menu bar and further icons appear in the toolbar with special functions for the melting curve analysis.

Icon	MeltCurve menu	Description
	**Add melting curve**	Insert an analysis in the project window
	**Delete melting curve**	Remove the current analysis from the project window
	**Options melting curve**	Settings for the display of fluorescence curves and for the calculation of results
	**Autom. threshold**	Automatically determine the threshold for the calculation of the melting temperature

9.2 Creating or deleting an analysis for a melting curve analysis

Create analyses

In order to be able to perform an analysis, you must first create it in the project window with a name.

- Open the **Analysis** | **Melting curve** project window.
- Click on the  icon in the toolbar or select the menu item **MeltCurve** | **Add melting curve**.
- Enter the name for the analysis in the input window and confirm with **OK**.
  - ✓ The new analysis is created in the project. You can now select a GOI and the reference genes and edit the other analysis parameters.

Remove analysis

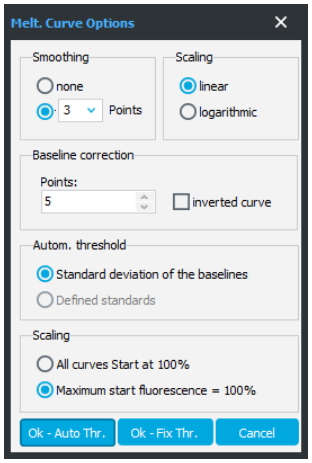
You can remove an analysis that is no longer required.

- Select the name of the analysis in the list.
- Click on the  icon in the toolbar or select the menu item **MeltCurve** | **Delete melting curve**.
  - ✓ The analysis is removed after a confirmation prompt.

9.3 Melting curve analysis options

In the options for the melting curve analysis, you configure the settings for the mathematical treatment of the fluorescence curves and constraints for the automatic threshold calculation.

The Melt. Curve Options window is displayed when you click on the  icon in the toolbar or select the menu item Melting curve| Options melting curve.



Option	Description
Smoothing	Adjustment of the smoothing constraint for the fluorescence curves **none** No smoothing. **Points** Smoothing over the selected number of points.
Scaling	Select the scaling of the fluorescence curves (linear or logarithmic)
Baseline correction	**Points** Info still missing here
inverted curve	To analyze fluorescence data from protein stability measurements, you can invert the melting curves.
Autom. threshold	The threshold only takes effect in the derivation. Only curves whose maximum dRn/dT is greater than the threshold are analyzed. **Standard deviation of the baselines** Calculation of the threshold as x-fold deviation of the standard deviation of the baselines (factor can be adjusted in the menu item **Extras** | **Options** | **Analysis**) **Defined standards** Selection of standards with the aim of obtaining the maximum value for the coefficient of determination R^2
Scaling	**All curves Start at 100%** All fluorescence curves are normalized so that the starting point begins at 100%. **Maximum start fluorescence = 100%** The highest starting fluorescence is set equal to 100%. All further curves are calculated accordingly.
Ok - Auto Thr.	The threshold for a current project is recalculated according to the changes in this window. All other settings are also adopted and applied to the fluorescence curves.
Ok - Fix Thr.	The threshold set in the current project is retained regardless of changes in this window. All other settings are adopted and applied to the fluorescence curves.

9.4 Editing parameters for the melting curve analysis

Configure the parameters for the melting curve analysis of an experiment in the fields and lists above the graph.

Option	Description
Gene of interest (GOI)	Selection list of target gene/dye combinations Generally, an intercalating dye must be selected for the target gene for the melting curve analysis. The fluorescence curves are displayed according to the selection.
User Group	If several experiments (groups) have been created in the sample layout, the group of the experiment you want to analyze must be selected here.
Threshold	Set threshold value manually The threshold only takes effect in the **Derivative** view. Only curves whose maximum dRn/dT is greater than the threshold are analyzed.

Set the threshold value

With the threshold value for the derivatives of the melting curves, you can exclude samples from the analysis whose maximum peak is smaller than the specified threshold value.

There are several ways to set the threshold value:

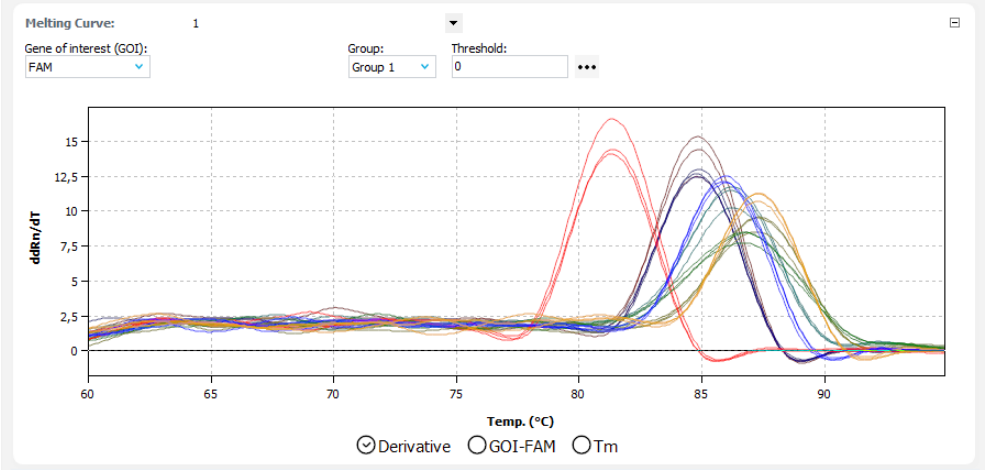
- In the general options, click on the icon in the toolbar to set the options for determining the threshold automatically.
- Enter the threshold manually in the input field above the graph.
- In the graphical representation of the derivative, move the black threshold line up or down by holding down the mouse button.
- To calculate automatically, click on the icon in the toolbar. For the automatic calculation, the factor set under the menu item **Extras** | **Options** | **Analysis** and the settings from the **Melt. Curve Options** window (icon) are used.
  - ✓ The resulting threshold value is updated and displayed in the **Threshold** input field for both manual determination and automatic calculation.

9.5 Display fluorescence curves and melting curve

Display melting curves

The melting temperature T_m is determined from the peak maximum of the first derivative of the melting curves. The graphs are displayed in the display area in the **Derivative** view.

To analyze fluorescence values from protein stability measurements, you can invert the melting curves using the **inverted curve** option in the **Melt. Curve Options** window.

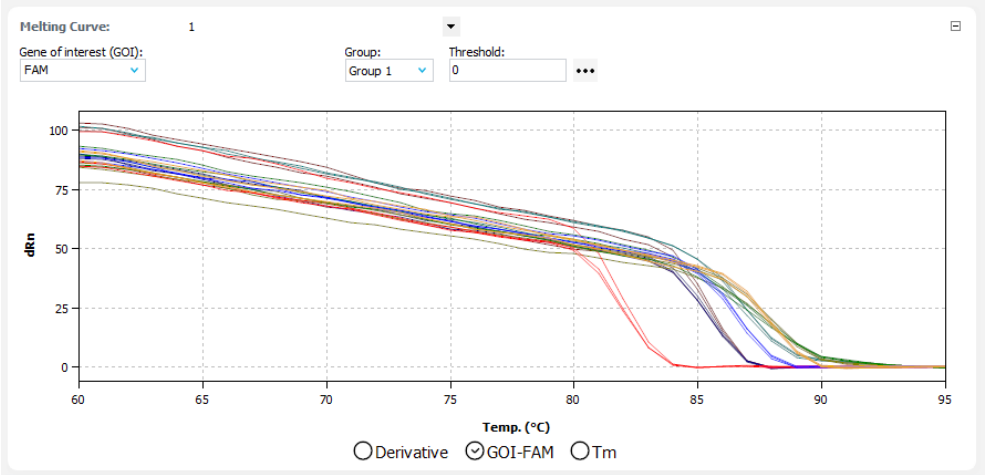


Display fluorescence curves

In the display area, the fluorescence values are plotted against temperature either normalized to the highest fluorescence value or to the setpoint 100, depending on the settings in the window.

For multiplex experiments, you can select the target gene/dye combination for display in the GOI list.

The fluorescence data is displayed as a linear or logarithmic representation depending on the selected display option. If you move the cursor over a curve, brief information about the sample is displayed.



Toggle scaling

You can choose between linear or logarithmic scaling of the fluorescence curves.

- Click on the ••• icon above the graph.
- Select the option **Scaling logarithmic** or **linear**.
- Click next to the selection window.
  - ✓ The graph is rescaled.

Export fluorescence data

Right-click on a fluorescence graph to open the context menu to export the fluorescence data of the samples to a CSV file or save the graph as a hardcopy to the clipboard.

See also

Exporting fluorescence data [▸ 51]

9.6 Displaying mean melting temperatures as bar graphs

The melting temperatures of the samples are displayed as bar graphs in the Tm view in the graph area of the **Analysis** | **Melting curve** project window.

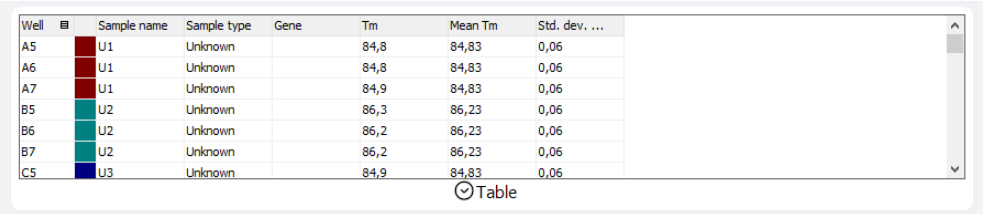
Each sample is symbolized by a bar with the sample name at the bottom. The order of the samples in the diagram is alphabetical according to the sample name. The height of the bar corresponds to the mean melting temperature of the replicates of a sample. The range of the standard deviation is shown as a gray error bar at the end of the bar.

If you move the cursor over a bar, brief information about the position of the replicates in the sample layout, the mean melting temperature, and the standard deviation is displayed.

If there is a large number of samples and not all sample bars are displayed at the same time, you can move the diagram horizontally by clicking on the graph and holding down the mouse button.

9.7 Displaying results of a melting curve analysis

The sample table with the results is displayed in the bottom part of the **Analysis** project window in the **Table** view.



Column	Description
Well	Position of the sample in the sample layout Click on the **Well** column title to arrange the table row by row or column by column according to the layout.
Graph color	Each sample is automatically assigned a color, and the corresponding fluorescence curve is displayed in that color. You can change the curve color by double-clicking or by holding down the Ctrl key and double-clicking.
Sample name	Name entered in the sample layout

Column	Description
Sample type	Sample type entered in the sample layout
Group	Assignment of the sample to an experimental group
Gene	Name of the gene measured in the sample
Tm	Melting temperature of the sample
Mean Tm	Mean melting temperature of the replicates
Std. dev. mean Tm	Standard deviation of the mean melting temperature of replicates

You can customize the display of the columns in the results table according to your requirements by right-clicking on the table header and activating the column display in the context menu. To export the data of the results table configured in this way to an Excel or CSV file, right-click on the tables and select the corresponding command in the context menu.

See also

- Exporting results tables [▶ 52]
- Customizing the results tables [▶ 51]

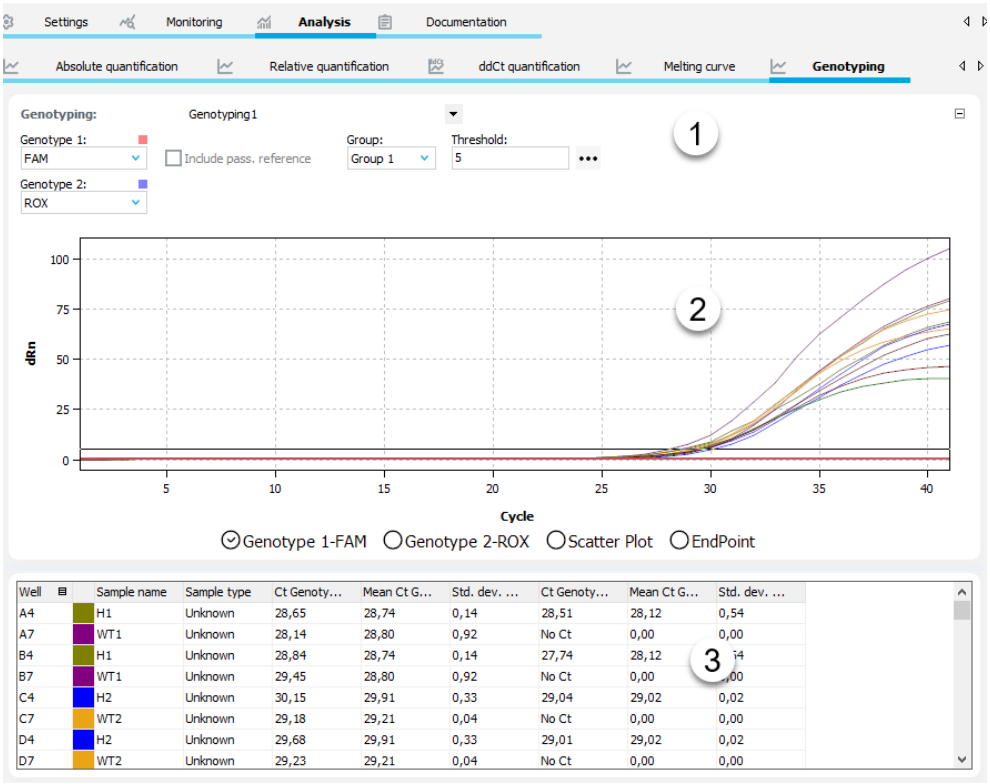
10 Genotyping

Genotyping is used to determine differences in sequences between a sample and a standard. The standard is defined as a reference sequence (genotype 1), while the genetic state of the sample is determined by the experiment.

Genotyping reveals which alleles an individual has inherited from their parents.

10.1 Project window and menu for genotyping

Analysis | Genotyping project window



No.	Element	Description
1	Parameter settings	Selection of analysis parameters for genotyping
2	Graph area	Fluorescence curves for genotype 1 and genotype 2 Bar charts and scatter plot of the results
3	Table area	Sample table with the results

Genotyping menu and icons

When you select the **Genotyping** tab, the **Genotyping** menu appears in the menu bar and further icons appear in the toolbar with special functions for genotyping.

Icon	Genotyping menu	Description
	Add Genotyping	Insert an analysis in the project window
	Delete Genotyping	Remove the current analysis from the project window

Icon	Genotyping menu	Description
	Options Genotyping	Settings for the display of fluorescence curves and for the calculation of results
	Autom. threshold	Automatically determine the threshold for the calculation of Ct values

10.2 Creating or deleting an analysis for genotyping

Create analyses

In order to be able to perform an analysis, you must first create it in the project window with a name.

- Open the **Analysis** | **Genotyping** project window.
- Click on the icon in the toolbar or select the menu item **Genotyping** | **Add Genotyping**.
- Enter the name for the analysis in the input window and confirm with **OK**.
  - ✓ The new analysis is created in the project. You can now select a GOI and the reference genes and edit the other analysis parameters.

Remove analysis

You can remove an analysis that is no longer required.

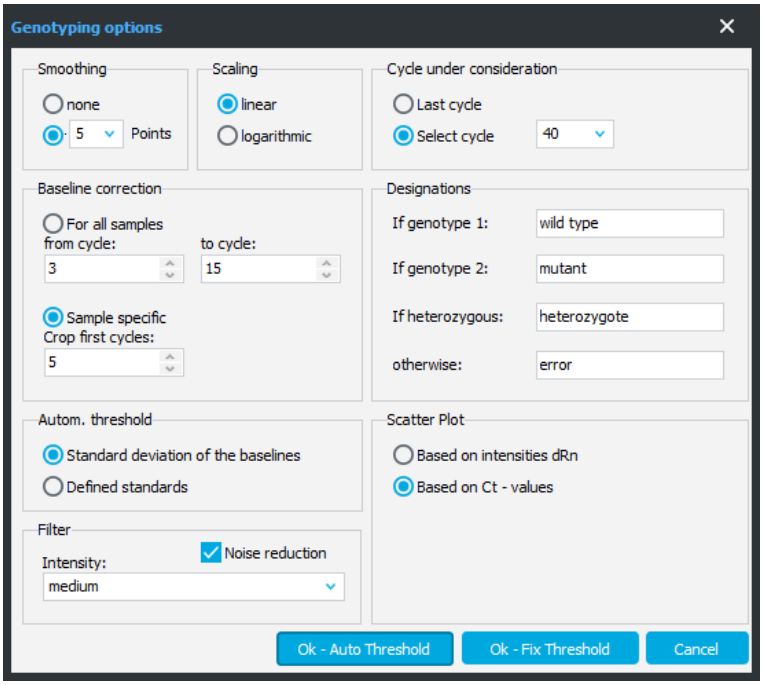
- Select the name of the analysis in the list.
- Click on the icon in the toolbar or select the menu item **Genotyping** | **Delete Genotyping**.
  - ✓ The analysis is removed.

10.3 Options for genotyping

In the options for genotyping, you configure the settings for the mathematical treatment of the fluorescence curves and constraints for the automatic threshold calculation. In addition, you select the calculation mode for the Normalized Expression (NE) here.

The **Genotyping options** window is displayed when you click on the icon in the toolbar or select the menu item **Genotyping** | **Options Genotyping**.

Genotyping options window



Option	Description
Smoothing	Adjustment of the smoothing constraint for the fluorescence curves **none** No smoothing. **Points** Smoothing over the selected number of points.
Scaling	Select the scaling of the fluorescence curves (linear or logarithmic)
Baseline correction	Select the baseline correction **For all samples** This correction determines the baseline for each sample in the same range. The lower and upper range limits must be set in the **from cycle** and **until cycle** fields. **Sample specific** This correction should be chosen if the curves have very different Ct values. The lower range limit for determining the baseline is set for all samples in the **Crop first cycles** field. The upper range limit is determined separately for each sample using an algorithm. **Note**: The type of baseline correction can only be set in this dialog box. The range limits for the correction can be adjusted in the project window.
Autom. threshold	**Standard deviation of the baselines** Calculation of the threshold as x-fold deviation of the standard deviation of the baselines (factor can be adjusted in the menu item **Extras** | **Options** | **Analysis**) **Defined standards** Selection of standards with the aim of obtaining the maximum value for the coefficient of determination R^2
Filter	Select a digital filter for smoothing the fluorescence curves, can be adjusted in the levels **slight**, **medium** and **strong**
Noise reduction	Set noisy curves, which are not recognized by the software as amplification curves, to 0 and do not determine any Ct values
Cycle under consideration	Set the cycle used for the analysis

Option	Description
	Last cycle Preferred setting (endpoint) **Select cycle** Set another cycle of the qPCR run in the list
Designations	Option to enter own names for the categories **Genotype 1**, **Genotype 2**, **heterozygous** or **otherwise**
Scatter Plot	Generate the scatter plot using the fluorescence intensities at the cycle under consideration or based on Ct values
Ok - Auto Thr.	The threshold for a current project is recalculated according to the changes in this window. All other settings are also adopted and applied to the fluorescence curves.
Ok - Fix Thr.	The threshold set in the current project is retained regardless of changes in this window. All other settings are adopted and applied to the fluorescence curves.

10.4 Editing parameters for genotyping

Configure the parameters for genotyping of an experiment in the fields and lists above the graph.

Option	Description
Selection list	Selection of an analysis created for the experiment
Genotype 1	Selection list of target gene/dye combinations of the wild type
Genotype 2	Selection list of target gene/dye combinations of the mutant
Include pass. reference	Can only be selected if a dye has been defined as a passive reference in the **Settings** | **Scan** project window. If this option is enabled, the fluorescence of the dye that has been set as a passive reference is used for standardization.
User Group	If several experiments (groups) have been created in the sample layout, the group of the experiment you want to analyze must be selected here.
Threshold	Set threshold value manually The threshold value must be between 1 and 100, depending on the standardized representation of the fluorescence curves (dRn).
•••	Select scaling and baseline setting of the fluorescence curve

Set the threshold value

To determine Ct values for the analysis a threshold value must be determined for each experiment.

There are several ways to set the threshold value:

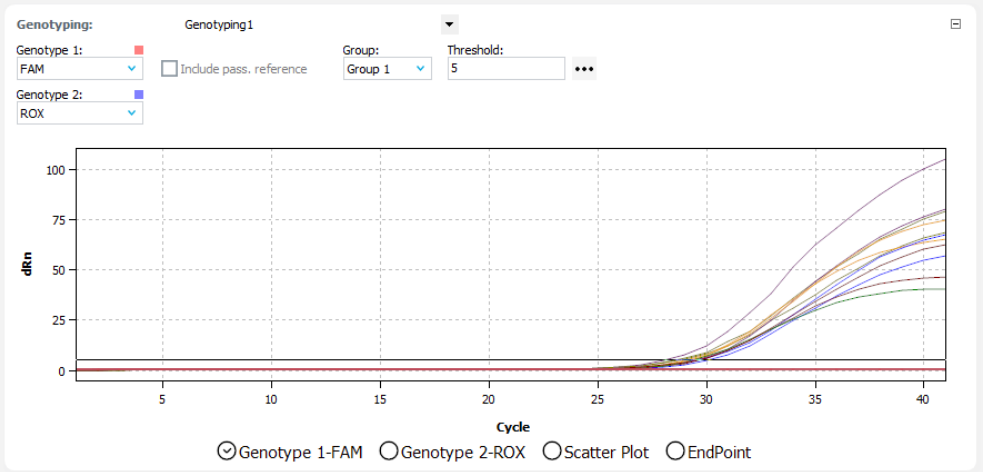
- In the general options, click on the icon in the toolbar to set the options for determining the threshold automatically.
- Enter the threshold manually in the input field above the graph.
- In the graph, move the black threshold line vertically by holding down the mouse button. At the same time, the Ct values in the sample table are updated.
  Note: Due to the further spread of the early exponential range of the amplification, a logarithmic representation is better suited for setting the threshold manually in the display range than a linear representation.

- To calculate automatically, click on the icon in the toolbar. For the automatic calculation, the factor set under the menu item **Extras** | **Options** | **Analysis** and the settings from the options window (icon) are used.
  - ✓ The resulting threshold value is updated and displayed in the **Threshold** input field for both manual determination and automatic calculation.

10.5 Displaying fluorescence curves for genotyping

In the graph area, the fluorescence curves of the wild type/dye and mutant/dye combinations are each displayed in a view.

The fluorescence data is displayed as a linear or logarithmic representation depending on the selected display option. For both view types a brief information is shown as soon as the mouse pointer is positioned on one of the curves. Due to the further spread of the early exponential range of the fluorescence curve, the logarithmic representation is recommended for setting the threshold manually.



Toggle scaling

You can choose between linear or logarithmic scaling of the fluorescence curves.

- Click on the icon above the graph.
- Select the option **Scaling logarithmic** or **linear**.
- Click next to the selection window.
  - ✓ The graph is rescaled.

Export fluorescence data

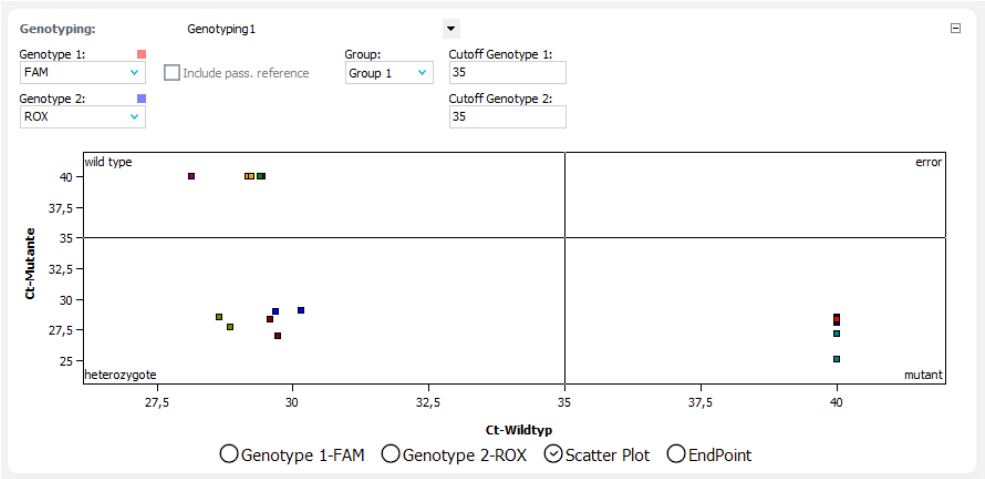
Right-click on a fluorescence graph to open the context menu to export the fluorescence data of the samples to a CSV file or save the graph as a hardcopy to the clipboard.

10.6 Displaying scatterplot and bar chart for genotyping

Show scatter plot

The genotyping scatter plot is displayed in the **Scatter Plot** view. The scatter plot is divided into four quadrants for wild type, mutant, heterozygote and error. The samples are each assigned to one of the quadrants based on the measured relative fluorescence or Ct values of the two dyes.

The cut-off values are the threshold values above which the question about a sample's response is answered with "yes". The respective cut-off for the assignment of samples is indicated in the scatter plot by two black lines. By keeping the mouse button pressed down, you can move the position of the lines and thus change the cut-off. Alternatively, enter the cut-off values for Genotype 1 and Genotype 2 in the analysis parameters above the scatter plot.



Show bar chart

The measured relative fluorescences are displayed as bars in the **Endpoint** view.

For 96-well thermal blocks, the X-axis is scaled from A to H according to the rows of the block. The first 12 samples correspond to positions A1 ... A12 of the block, the next 12 samples to positions B1 ... B12 and so on. You can change the cut-off values in the graph by moving the horizontal lines with the mouse button pressed down, or enter them in the fields above the graph.

10.7 Displaying the results of genotyping

The results table of the genotyping summarizes all data for the samples. The columns shown in the results table differ depending on the tab selected in the graph area. For the fluorescence curves, there is a summary table in which the fluorescence data of both dyes are processed. If the fluorescence intensity at the endpoint is analyzed, the sample tables of scatter plot and bar chart contain the same data.

Column	Description
Well	Position of the sample in the sample layout

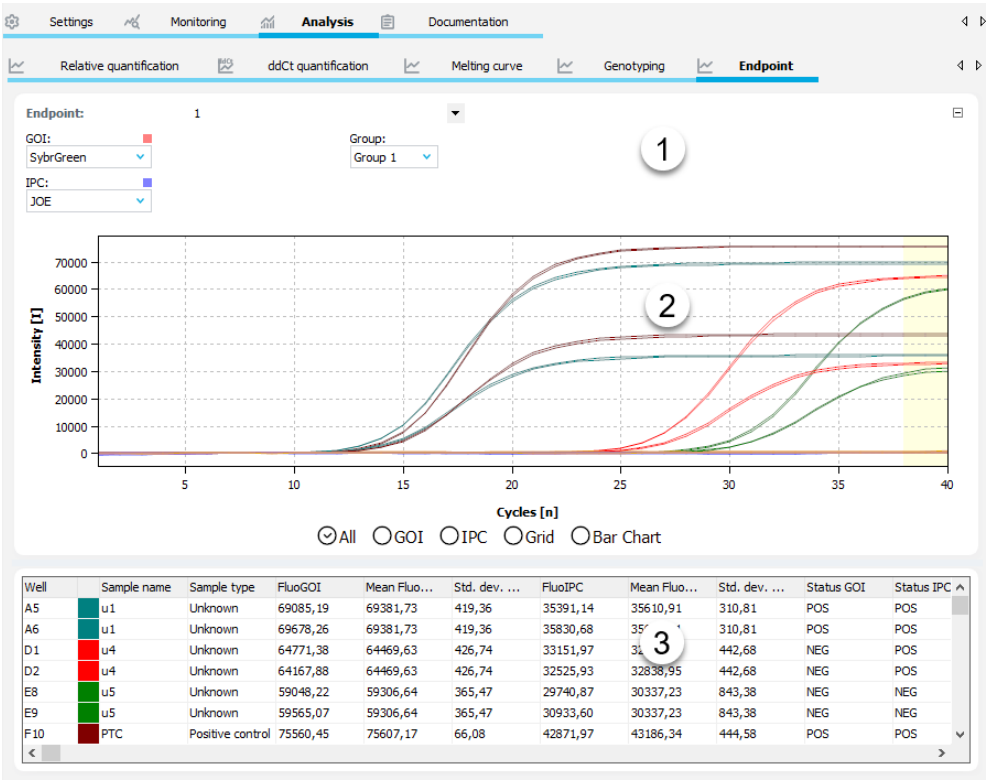
Column	Description
	Click on the **Well** column title to arrange the table row by row or column by column according to the layout.
Sample name	Name entered in the sample layout
Sample type	Sample type entered in the sample layout
Group	Assignment of the sample to an experimental group
Ct Genotype 1	Ct value of Genotype 1
Mean Ct Genotype 1	Mean Ct value of replicates of the Genotype 1
Std. dev. Genotype 1	Standard deviation of the Ct values between replicates of the Genotype 1
Ct Genotype 2	Ct value of Genotype 2
Mean Ct Genotype 2	Mean Ct value of replicates of the Genotype 2
Std. dev. Genotype 2	Standard deviation of the Ct values between replicates of the Genotype 2
Genotype	Assignment of sample according to Genotype 1, Genotype 2, heterozygouse or error "?"
Reaction Genotype 1	Yes or No, depending on endpoint fluorescence or Ct value
Reaction Genotype 2	Yes or No, depending on endpoint fluorescence or Ct value
Genotype replicates	Assignment of the replicates of a sample according to Genotype 1, Genotype 2, heterozygote or error
dRn Genotype 1	Normalized fluorescence intensity of the Genotype 1 response
Mean dRn Genotype 1	Normalized fluorescence intensity between replicates of the Genotype 1 response
Std. dev. dRn Genotype 1	Standard deviation of the normalized fluorescence intensity between replicates of the Genotype 1 response
dRn Genotype 2	Normalized fluorescence intensity of the Genotype 2 response
Mean dRn Genotype 2	Normalized fluorescence intensity between replicates of the Genotype 2 response
Std. dev. dRn Genotype 2	Standard deviation of the normalized fluorescence intensity between replicates of the Genotype 2 response

11 POS/NEG analysis in the endpoint

The positive/negative analysis (POS/NEG analysis) is used to determine whether certain targets are present in a sample or not. The experiments can be set up as singleplex or multiplex, whereby the fluorescence of the samples is analyzed in the endpoint, i.e. after amplification. The position of the endpoint in relation to the cycles, as well as the number of cycles to be included can be defined. In the analysis, a cut-off value is calculated using the endpoint fluorescence of the NTC samples, which is used to discriminate between positive and negative for each individual sample. The analysis takes into account internal positive controls (IPC) that can be added to each sample to avoid false-negative results and that lead to an increase in the certainty of the result.

11.1 Project window for a POS/NEG analysis

Analysis | Endpoint project window



No.	Element	Description
1	Parameter settings	Selection of analysis parameters for the POS/NEG analysis
2	Graph area	Fluorescence curves of the gene/dye combinations and the internal positive control IPC Display of results on sample layout Bar charts of the results
3	Table area	Sample table with the results

Endpoint menu and icons

When you select the **Endpoint** tab, the **Endpoint** menu appears in the menu bar and further icons appear in the toolbar for special functions for the POS/NEG analysis.

Icon	Endpoint menu	Description
	Add Endpoint	Insert an analysis in the project window
	Delete Endpoint	Remove the current analysis from the project window
	Endpoint options	Settings for the display of fluorescence curves and for the calculation of results
	Auto Threshold/CutOff	Automatically determine the cut-off value for the POS/NEG analysis

11.2 Creating or deleting an analysis for a POS/NEG analysis

Create analyses

In order to be able to perform an analysis, you must first create it in the project window with a name.

- Open the **Analysis** | **Endpoint** project window.
- Click on the icon in the toolbar or select the menu item **Endpoint** | **Add Endpoint**.
- Enter the name for the analysis in the input window and confirm with **OK**.
  - ✓ The new analysis is created in the project. You can now select a GOI and the reference genes and edit the other analysis parameters.

Remove analysis

You can remove an analysis that is no longer required.

- Select the name of the analysis in the list.
- Click on the icon in the toolbar or select the menu item **Endpoint** | **Delete Endpoint**.
  - ✓ The analysis is removed.

11.3 Options for a POS/NEG analysis

In the options for the POS/NEG analysis, you configure the settings for the mathematical treatment of the fluorescence curves and constraints for the automatic threshold calculation.

The **Endpoint options** window is displayed when you click on the icon in the toolbar or select the menu item **Endpoint** | **Endpoint options**.

Autosampler adjustment window Endpoint options

Option	Description
Smoothing	Adjustment of the smoothing constraint for the fluorescence curves **none** No smoothing. **Points** Smoothing over the selected number of points.
Scaling	Select the scaling of the fluorescence curves (linear or logarithmic)
Baseline correction	Select the baseline correction

Option	Description
	For all samples This correction determines the baseline for each sample in the same range. The lower and upper range limits must be set in the **from cycle** and **until cycle** fields. **Sample specific** This correction should be chosen if the curves have very different Ct values. The lower range limit for determining the baseline is set for all samples in the **Crop first cycles** field. The upper range limit is determined separately for each sample using an algorithm. **Note**: The type of baseline correction can only be set in this dialog box. The range limits for the correction can be adjusted in the project window.
Endpoint	**last ... Cycles** Use the mean of the last number of cycles as the endpoint fluorescence **from cycle/until cycle** / Use the mean of the endpoint fluorescences in the selected range for the analysis By default, the mean of the last 2 cycles is used for the analysis.
CutOff calculation	**with negative control or NTC** The cut-off value is calculated from the mean fluorescence of the NTC samples plus the amount given in percent from the difference between the maximum sample fluorescence and the fluorescence of the NTC samples, each in the endpoint. **with internal positive control (IPC) and NTC** Cut-off values are calculated independently for NTC and IPC. The standard deviation of the fluorescence of the NTC samples, and the samples without added internal positive control (IPC) is multiplied by a tabulated factor T, which results from the desired confidence interval and the number of samples. **Use CutOffs from Table** Enter cut-offs into the table manually
Ok - Auto CutOff	The cut-off value for a current project is recalculated according to the changes in this window. All other settings are also adopted and applied to the fluorescence curves.
Ok - Fix CutOff	The cut-off value set in the current project is retained regardless of changes in this window. All other settings are adopted and applied to the fluorescence curves.

If no IPC samples are defined in the plate layout, the option with IPC and NTC is not available. IPC samples can be defined in the **Settings** | **Samples** project window by selecting the wells in the plate view, right-clicking on the selection and assigning the **IPC** property in the context menu.

See also

Entering sample properties in the layout diagram [▶ 35]

11.4 Editing parameters for the POS/NEG analysis

Configure the parameters for the POS/NEG analysis of an experiment in the fields and lists above the graph.

Option	Description
Selection list	Selection of an analysis created for the experiment
Gene of interest (GOI)	Selection list of target gene/dye combinations
IPC	Selection of the dye used to detect the internal positive control
User Group	If several experiments (groups) have been created in the sample layout, the group of the experiment you want to analyze must be selected here.
CutOff	Endpoint fluorescence at which a sample is considered positive

Selection of cycles for the endpoint analysis

By default, the mean of the fluorescence data of the last two cycles is used as the endpoint fluorescence. You can also define several cycles at the end of the PCR run or a range of cycles within the PCR run for the calculation.

You configure the settings after clicking on in the **Endpoint options** window. The selected ranges are highlighted in yellow in the fluorescence curve graphs.

Set cut-off values

You can set the cut-off values for GOI and IPC manually or have them calculated automatically, whereby various methods can be set in the **Endpoint options** window. After creating an analysis and each time the cut-off value or other options are changed, the analysis results are recalculated and the graphical and tabular displays are refreshed.

Manually

- Enter cut-off values directly in the Cut-off input field in the parameters above the graph or in the **Endpoint options** window.
- Move the cut-off line in the graph of the fluorescence curves by holding down the mouse button.

Auto scaling

- Click on the icon or select the menu item **Endpoint** | **Auto Threshold/CutOff**.
  - ✓ The cut-off value is determined according to the settings in the **Options Endpoint** window.

11.5 Evaluation of the results of the POS/NEG analysis

The evaluation of single samples and replicates (POS, NEG, ???, CHECK) is based on the following relationships:

Without IPC

Endpoint fluorescence of the single sample GOI	Result
> Cut-off (GOI)	POS (positive)
≤ Cut-off (GOI)	NEG (negative)

With IPC

Endpoint fluorescence of the single sample GOI	Endpoint fluorescence of the single sample IPC	Result
> Cut-off (GOI)	> Cut-off (IPC)	POS (positive)
≤ Cut-off (GOI)	> Cut-off (IPC)	NEG (negative)
> Cut-off (GOI)	≤ Cut-off (IPC)	??? (uncertain)
≤ Cut-off (GOI)	≤ Cut-off (IPC)	??? (uncertain)

Evaluation when using replicates

Samples present as replicates (same sample names) are only assessed as clearly POS or NEG if all replicates of the sample are POS or NEG. If this is not the case, the result is given as CHECK. It is possible to deactivate any outlier samples via the project explorer.

Results of the individual replicates	Result of sample
all POS	POS (positive)
all NEG	NEG (negative)
other	CHECK (check, eliminate outliers if necessary)

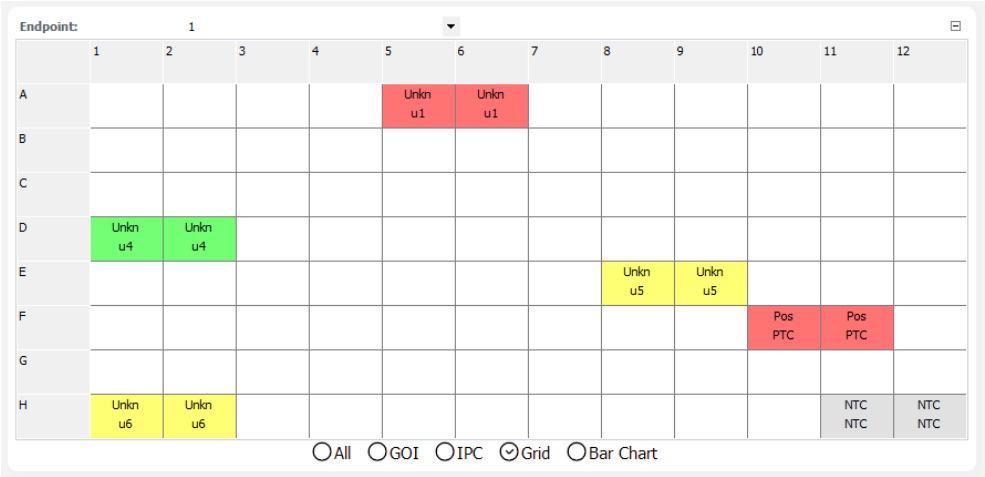
See also

Project explorer Samples [▶ 12]

11.6 Displaying results in the sample layout and as bar chart

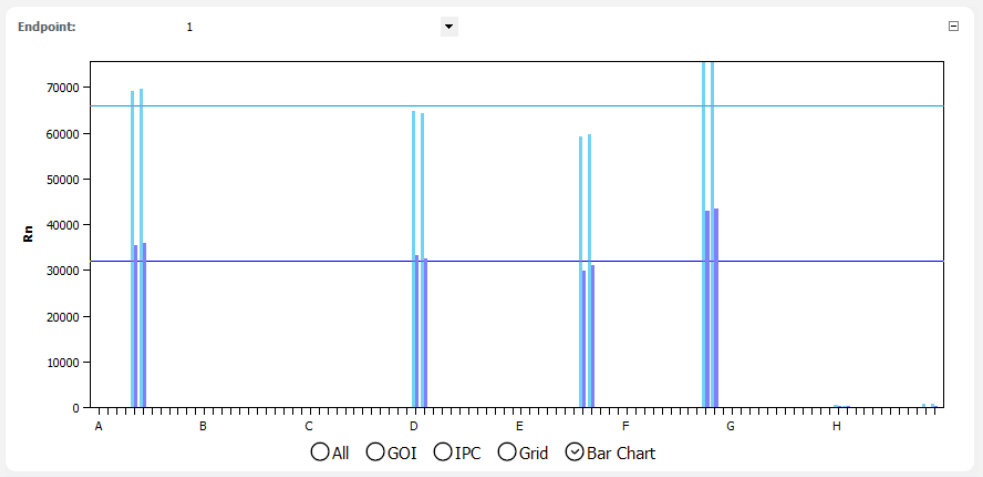
Results in the sample layout

The **Grid** view gives you a quick overview of the results of the individual wells. You can define the colors with which positive, negative, uncertain or IPC samples are displayed, under **Extras** | **Options** | **Colors**. If you place the cursor on a well, the sample name and the endpoint fluorescence values for GOI and IPC, if applicable, are displayed.



Bar chart

The bar chart shows the endpoint fluorescence of GOI and IPC for all wells together and the current cut-off values as a horizontal line. The light blue lines are for the GOI and the dark blue lines are for the IPC. You cannot change the cut-off lines in this view. If you place the cursor on a bar, the sample name and the endpoint fluorescence values for GOI and IPC, if applicable, are displayed.



11.7 Results of a POS/NEG analysis

The sample table with the results is displayed in the bottom part of the project window.

Well	Sample name	Sample type	FluoGOI	Mean Fluo...	Std. dev. ...	FluoIPC	Mean Fluo...	Std. dev. ...	Status GOI	Status IPC
A5	u1	Unknown	69085,19	69381,73	419,36	35391,14	35610,91	310,81	POS	POS
A6	u1	Unknown	69678,26	69381,73	419,36	35830,68	35610,91	310,81	POS	POS
D1	u4	Unknown	64771,38	64469,63	426,74	33151,97	32838,95	442,68	NEG	POS
D2	u4	Unknown	64167,88	64469,63	426,74	32525,93	32838,95	442,68	NEG	POS
E8	u5	Unknown	59048,22	59306,64	365,47	29740,87	30337,23	843,38	NEG	NEG
E9	u5	Unknown	59565,07	59306,64	365,47	30933,60	30337,23	843,38	NEG	NEG
F10	PTC	Positive control	75560,45	75607,17	66,08	42871,97	43186,34	444,58	POS	POS

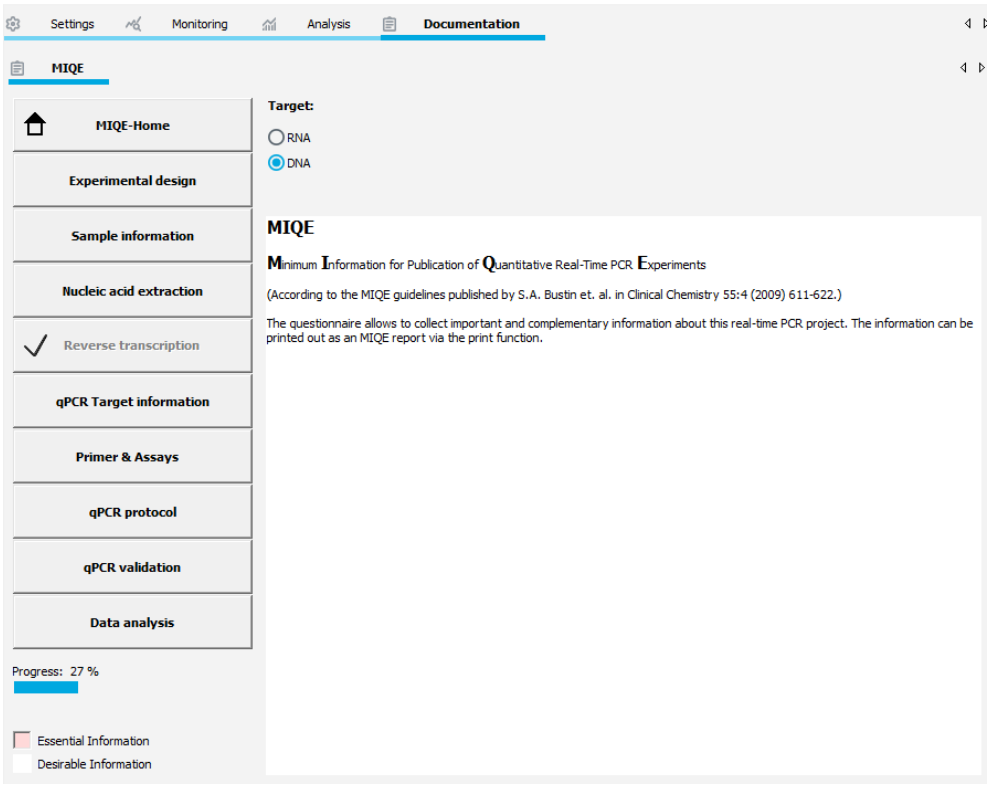
Column	Description
Well	Position of the sample in the sample layout Click on the **Well** column title to arrange the table row by row or column by column according to the layout.
Graph color	Each sample is automatically assigned a color, and the corresponding fluorescence curve is displayed in that color. You can change the curve color by double-clicking or by holding down the Ctrl key and double-clicking.
Sample type	Sample type entered in the sample layout
FluoGOI	Endpoint fluorescence of the target gene
Mean FluoGOI	Mean endpoint fluorescence of replicates of the target gene
Std. dev. FluoGOI	Standard deviation of endpoint fluorescence of replicates of the target gene
FluoIPC	Endpoint fluorescence of the IPC
Mean FluoIPC	Mean endpoint fluorescence of replicates of the IPC
Std. dev. FluoIPC	Standard deviation of endpoint fluorescence of replicates of the IPC
Status GOI	**POS**, if **FluoGOI> CutOff**, otherwise **NEG** (for each well)
Status IPC	**POS**, if **FluoIPC > CutOff**, otherwise **NEG** (for each well)
Result samples	Evaluation **POS/NEG/???** for each well
Result replicates	Evaluation **POS/NEG/CHECK** of the replicates

12 MIQE documentation

In 2009, an international group of experts led by Prof. Steven Bustin developed guidelines for the publication of qPCR data (Bustin et al. 2009, Clinical Chemistry 55:4, 611-622). The underlying aim is to avoid the publication of incomplete or erroneous qPCR data and to ensure the comparability and reproducibility of experiments. The corresponding guidelines set out requirements regarding the minimum information content necessary for the publication of data. The guidelines have become known under the abbreviation "MIQE" (Minimum Information for Publication of Quantitative Real-Time PCR Experiments).

Notes for completing the MIQE documentation

- MIQE consists of a questionnaire on a total of nine different topics related to qPCR experiments. In the **qPCRsoft** project window, a button has been created for each topic, which can be used to access the corresponding catalog of questions for that topic. In addition, there is a **MIQE-Home** button that you can use to return to the MIQE main menu from any point.
- Essentially, first you should define by selecting the corresponding option whether DNA or RNA was used as starting material in the experiments. If the **DNA** option is chosen, the question catalog on reverse transcription does not have to be completed and the corresponding button is not available.
- After clicking on a button, the corresponding question catalog is opened. The number of questions differs between the respective topics. The user should answer as many questions as possible.
- Some of the answers are taken from the currently open or active project if the corresponding information is available.
- The software has a progress bar that shows as a percentage how many questions have been answered. The MIQE question catalog distinguishes between important questions, which should definitely be answered, and supplementary questions. The input fields for important questions are highlighted in light red in each topic, while supplementary questions are shown in white. The progress bar only takes account of the answered important questions. The total number of questions differs depending on whether DNA or RNA was selected as the starting material. The software cannot assess the quality of the answers. It is the responsibility of the user to complete the question catalog in full and with the necessary diligence.
- It is possible to import MIQE data from other projects. Click on the  icon in the toolbar or select the menu command **MIQE** | **Import MIQE documentation** to open a dialog window. After selecting the corresponding project, saved MIQE data is imported into the current project.
- The question catalog can be printed out via the menu item **File** | **Print**. To do this, check the **MIQE** option in the project tree of the **Print** window.


Settings
Monitoring
Analysis
Documentation
MIQE
MIQE-Home
Experimental design
Sample information
Nucleic acid extraction
Reverse transcription
qPCR Target information
Primer & Assays
qPCR protocol
qPCR validation
Data analysis
Progress: 27 %
Essential Information
Desirable Information
Target:
RNA
DNA
MIQE
Minimum Information for Publication of Quantitative Real-Time PCR Experiments
(According to the MIQE guidelines published by S.A. Bustin et. al. in Clinical Chemistry 55:4 (2009) 611-622.)
The questionnaire allows to collect important and complementary information about this real-time PCR project. The information can be printed out as an MIQE report via the print function.

13 Multigene/multiplate assay

The multigene/multiplate assay ensures the analysis of qPCR data of several target genes at the same time, or the analysis of data from several project files if, for example, several PCR plates were used for the experiment. The multigene/multiplate assay is carried out as a dialog in its own user interface, independent of the qPCRsoft program. The basis of the multigene/multiplate assay is project files saved by qPCRsoft. A ddCt assay must be created in the respective projects in order to be able to analyze them in the multigene/multiplate assay.

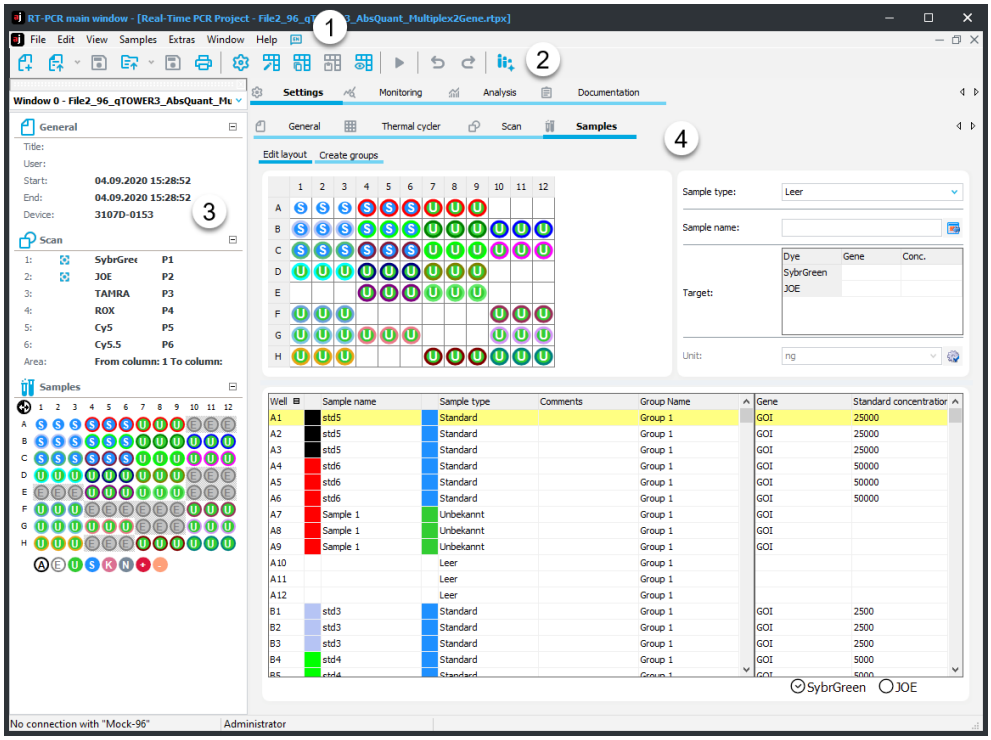
Start a multigene/multiplate assay

- In the qPCRsoft toolbar, click on the  icon or select the menu item File | Multi Gene Assay - Multi Plate Assay.

  ✓ The MultiGene user interface appears.

You can load an already saved analysis or start a new analysis by loading the project files.

MultiGene user interface



No.	Element	Description
1	Menu bar	Menu items for opening, editing, saving and printing the multigene/multiplate assay and Help
2	Toolbar	Icons for managing the analysis
3	Project list	Manage the projects of the assay
4	Sample layout	Activate and deactivate individual samples for the assay Definition of the Inter-Plate Standard (IPS)
5	Tab **Projects**	Plots of the fluorescence curves and the results of the ddCt analysis of the project selected in the project list
6	Tab **Analysis**	Display of multigene/multiplate assay as bar graphs and table

13.1 File management for multigene/multiplate assay

Click on the icon to show the **MultiGene** user interface, which initially contains no data. Only one assay can be carried out at a time in **MultiGene**. For a further assay, an already existing assay must first be closed.

Create new multigene/multiplate assay

- Click on the icon in the toolbar or select the menu item **File** | **New MultiGeneAssay**.
  - ✓ A new assay is created. A multigene/multiplate assay that is already open is closed. If changes have been made that have not yet been saved, you will be prompted to save them. In the next step of the assay, you have to import the project files with ddCt analyses from**qPCRsoft**.

Open saved multigene/multiplate assay

- Click on the icon in the toolbar or select the menu item **File** | **Open MultiGeneAssay...**.
- In the **Open** window, select the saved file and confirm with **OK**.
  - ✓ The multigene/multiplate assay with project list, sample layout, measurement results and analyses is displayed.

Note: If the file type "*.mgax" is linked to **qPCRsoft** under **Extras** | **Options** | **File**, then **MultiGene** opens automatically after you double-click the selected file.

Save a multigene/multiplate assay

The multigene/multiplate assay is saved with all added project files and analyses.

- Select the menu item **File** | **MultiGeneAssay save as...**.
- In the **Save as** window, enter a file a name and click the **Save** button.
- Save changes in a saved assay by clicking on the icon or selecting the menu item **File** | **Save MultiGeneAssay**.
  - ✓ The assay is saved.

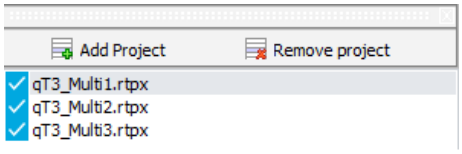
Print a multigene/multiplate assay

You can print the results of the multigene/multiplate assay.

- Open the **Analysis** tab.
- Click on the icon in the toolbar or select the menu item **File** | **Print MultiGeneAssay...**.
- Use the **Options** to choose the printer parameters with use **Print** to start the printout.
  - ✓ The protocol of the assay results is printed on the selected printer.

13.2 Selecting project files for a multigene/multiplate assay

When a new multigene/multiplate assay is started, the user interface is initially empty. In the next step you have to load the project files with a ddCt assay from qPCRsoft to analyze them in **MultiGene**. The projects used are displayed in the project list.



Load project

- Click the button **Add Project**.
- Select one or more project files in the **Open** window and load them into the workspace by clicking the **Open** button.
  - ✓ The projects appear in the project list. The fluorescence curves and the results of the ddCt assay of a project selected in the list are displayed on the **Projects** tab.

Remove projects

- Select the project in the list and click the **Remove project** button.
  - ✓ The project is deleted from the project list and is no longer part of the assay. When the assay is saved, this project is no longer included in the MGAX file.

Deactivate a project

Instead of permanently removing the project from the assay, you can also deactivate it.

- Clear the checkbox in front of the project name.
  - ✓ The project is not taken into account in the assay, but is included in the MGAX file after saving.

13.3 Activating samples and selecting interplate standards

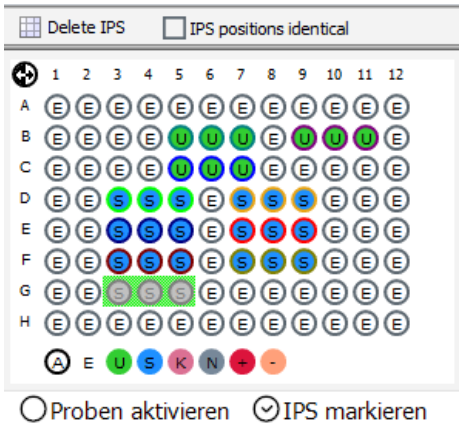
Activate/deactivate samples

You can activate and deactivate samples for the individual projects in **MultiGene** in the sample layout in the same way as in the **Samples** project explorer in qPCRsoft. The sample types are selected on the **MultiGene** sample layout with the same colors and symbols as in the project explorer of qPCRsoft.

- Select the **Projects** tab.
- Select the project in the project list.
  - ✓ The fluorescence curves and analysis results of the project are displayed on the **Projects** tab.
- Select the **Activate samples** view under the sample layout.
- Deactivate the outliers as in the **Samples** project explorer.
  - ✓ The deactivated samples are no longer taken into account in further assay.

Select interplate standards

In the multigene/multiplate assay, interplate standards (IPS) are included in each sample layout and the deviations between them are determined and calculated.



- Select the **Select IPS** view under the sample layout.
- Use the mouse to select the layout area that contains the IPS samples.

✓ The IPS samples are displayed in gray against a green background. For all other samples, only the sample type symbol is displayed. Empty wells are labeled with an "E".

- If the IPS samples are in the same position on all plates, activate the **IPS positions identical** option.
  ✓ The selection is applied to all loaded projects.
- To delete the IPS in all loaded projects, click on the **Delete IPS** button.

13.4 Setting threshold and PCR efficiencies for the multigene/multiplate assay

All measured values and settings are applied from the loaded project files. In **MultiGene**, the threshold value for each dye and the PCR efficiency can be set again. None of the other settings can be changed. This is only possible in the respective individual projects in **qPCRsoft**.

Edit threshold value

The threshold value can be edited for each dye of a loaded project.

- Select the **Projects** tab.
- Select the project in the project list by clicking it with the mouse.
- Select the dye on the **Projects** tab in the **Dye** list.
- If necessary, select the experiment group in the **Group** list.
- Edit the threshold in the **Threshold** field.
- Alternatively, in the graph of the fluorescence curves, move the black threshold line using the cursor.
  ✓ When the threshold is changed, the values in the results table are recalculated.

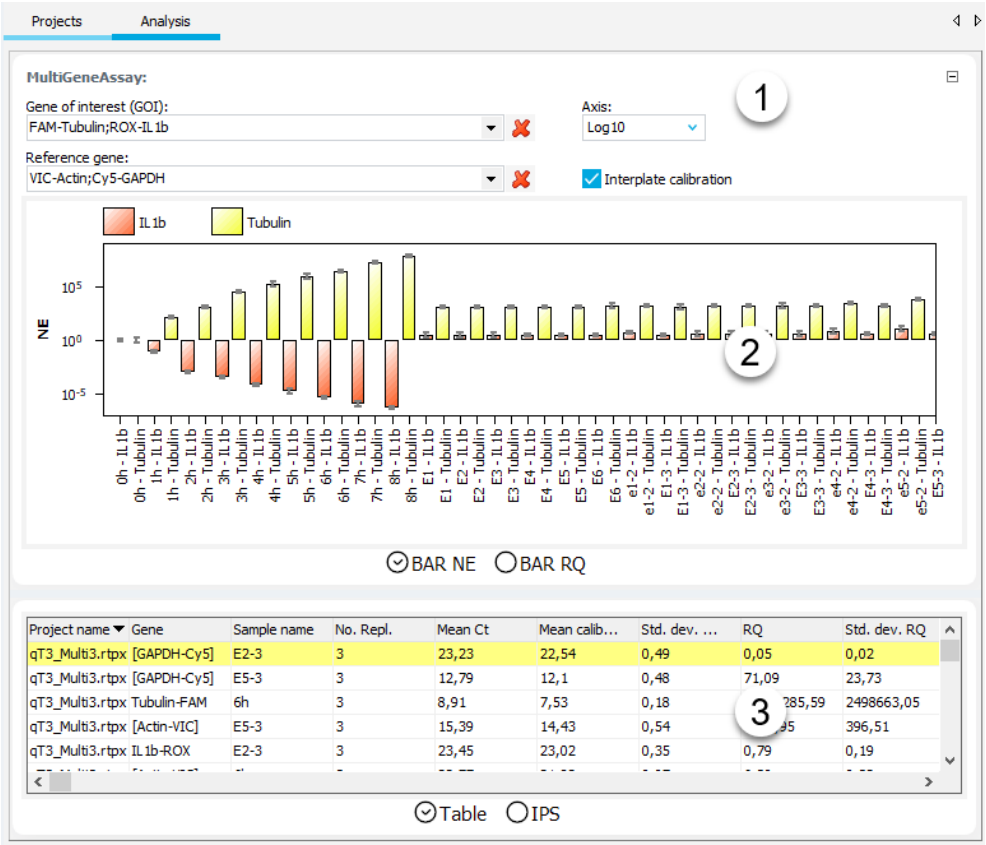
Edit PCR efficiency

The PCR efficiency is taken from the loaded project files. For the analysis, the PCR efficiency can be adjusted for the genes under consideration.

- Select the **Analysis** tab.
- In the toolbar, click on the  icon or select the menu item **Extras** | **Options**.
- In the **Options** window for the individual genes, edit the PCR efficiency and confirm with **OK**.
  ✓ The analysis results are recalculated with the edited PCR efficiencies.

13.5 Analysis of the multigene/multiplate assay

The results of the multigene/multiplate assay are displayed on the **Analysis** tab in the display area.



No.	Element	Description
1	Parameter settings	Selection of the analysis parameters
2	Graph area	Bar graphs of normalized expression and relative quantity
3	Table area	Sample table with the results

13.5.1 Editing parameters for the multigene/multiplate assay

Set the multigene/multiplate assay parameters on the **Analysis** tab in the fields and lists above the graph.

Option	Description
Gene of interest (GOI)	Selection list of target gene/dye combinations The fluorescence curves and standard curves are displayed according to the selection.
Reference gene	Reference gene selection list You can select several reference genes at a time. Another tab is displayed in the graph area for each reference gene. Click on the icon to remove all selected reference genes from the analysis.
Scaling	Select the scaling of the Y-axis
Interplate calibration	If interplate calibration is activated, the defined IPS samples of all plates are calculated* together and the corrected mean Ct values are calculated from the mean Ct values of the replicates (see results table). The corrected mean Ct values are then used in the calculation of the relative quantity as well as the normalized expression. If interplate calibration is deactivated, the corrected mean Ct values are equal to the mean Ct values.

*Correction calculation

$$Ct_{i,p}^{corr} = Ct_{i,p}^{meas} - \overline{Ct}_{p}^{IPC} + \frac{1}{N}\sum_{p=1}^{N} Ct_{p}^{IPC}$$

with

$Ct_{i,p}^{corr}$ – *corrected Ct – value for replicate i on plate p*

$Ct_{i,p}^{meas}$ – *measured Ct – value for replicate i on plate p*

$\overline{Ct}_{p}^{IPC}$ – *mean value of Ct – values of IPS – samples on plate p*

$\frac{1}{N}\sum_{p=1}^{N} Ct_{p}^{IPC}$ – *mean value of Ct – values of all IPS – samples on all N plates*

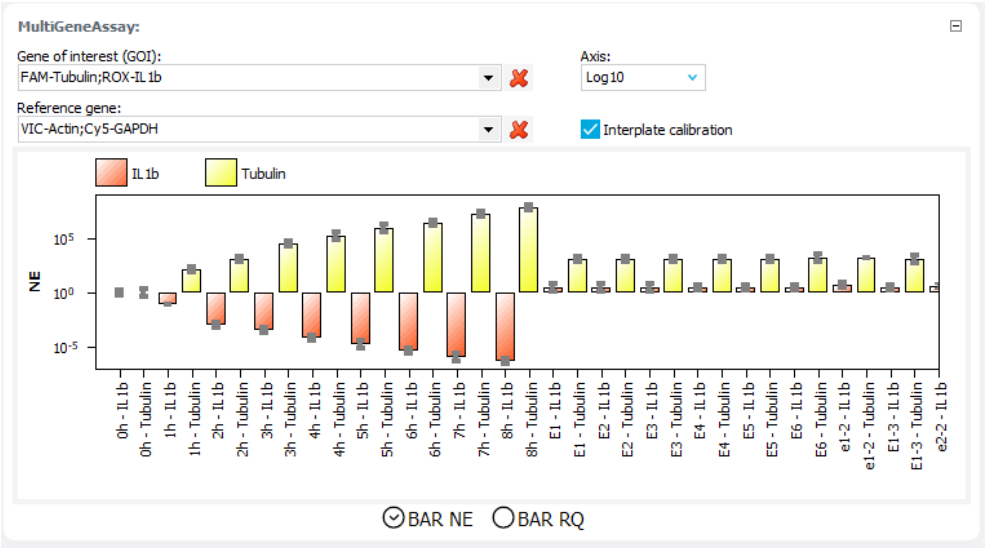
13.5.2 Display of results of the multigene/multiplate assay

Bar charts

The results of the multigene/multiplate assay are displayed in the form of bar charts. Since not all bars can be displayed in the chart window at the same time if there is a large number of samples, the window content can be shifted horizontally by left-clicking on the chart area and dragging the mouse to the left or right. Scrolling the mouse wheel compresses or widens the width of the display. Alternatively, you can use the arrow keys [↑] and [↓]. The respective sample name is given below each bar. Right-click on the graph to open a context menu with options to sort the results in the X-axis by gene or by sample name. Furthermore, in this context menu you can export the displayed values as a CSV file or copy the graph to the clipboard for use in other applications.

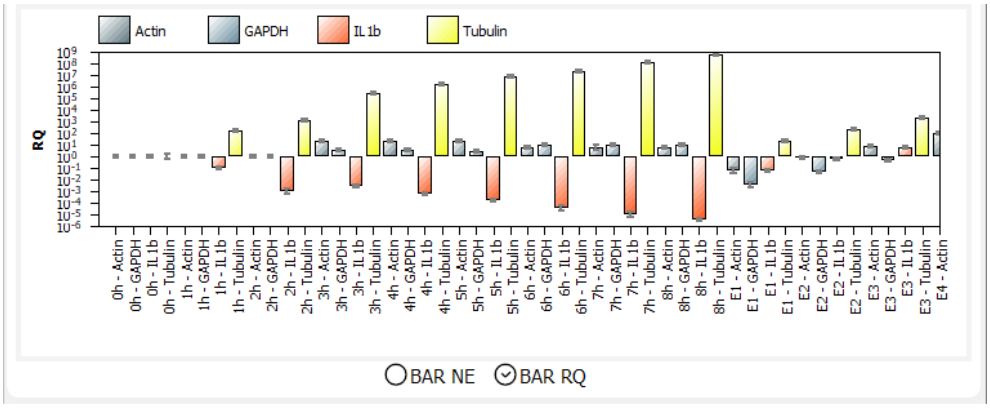
Bar NE view

In the **BAR NE** view, the expression of the selected target genes is plotted, normalized to the expression of the reference genes. The height of the bars is determined by the calculated normalized expression of the replicates. For each bar, brief information about the sample name, the mean value and the calculated standard deviation is displayed when you hover over it with the cursor. The standard deviation of the normalized expression is displayed in the form of an error bar. When you hover over a bar with the mouse, brief information is displayed with the sample name, the mean value, and the standard deviation.



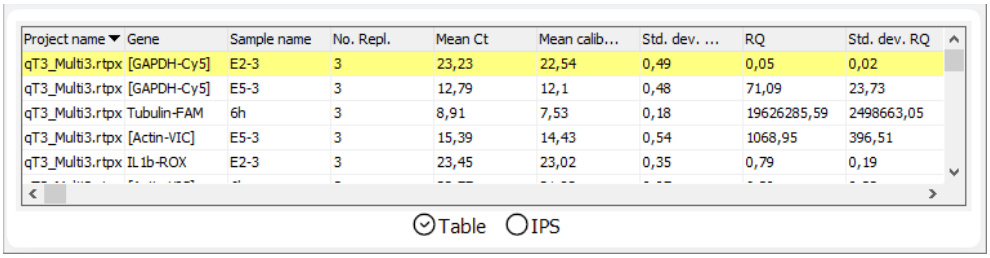
Bar RQ view

The **BAR RQ** view displays the relative quantity for target and reference genes. The height of the bar corresponds to the relative quantity of the replicates. The error bar marks the size of the standard deviation.



Results table

The results table for the multigene/multiplate assay is shown in the **Table** view.

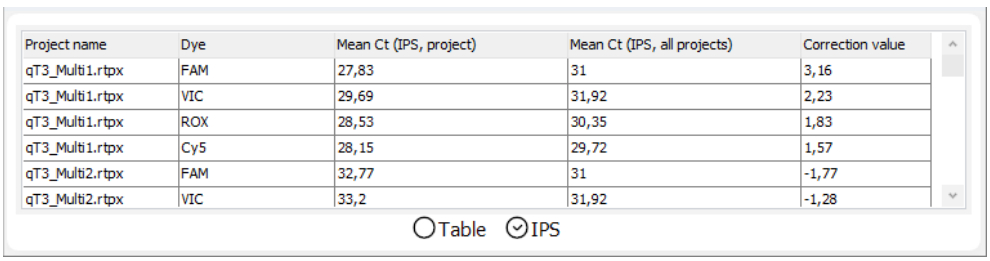


Project name	Gene	Sample name	No. Repl.	Mean Ct	Mean calib...	Std. dev. ...	RQ	Std. dev. RQ
qT3_Multi3.rtpx	[GAPDH-Cy5]	E2-3	3	23,23	22,54	0,49	0,05	0,02
qT3_Multi3.rtpx	[GAPDH-Cy5]	E5-3	3	12,79	12,1	0,48	71,09	23,73
qT3_Multi3.rtpx	Tubulin-FAM	6h	3	8,91	7,53	0,18	19626285,59	2498663,05
qT3_Multi3.rtpx	[Actin-VIC]	E5-3	3	15,39	14,43	0,54	1068,95	396,51
qT3_Multi3.rtpx	IL1b-ROX	E2-3	3	23,45	23,02	0,35	0,79	0,19

Column	Description
Project name	Name of the project
Gene	Name of the gene measured in the sample
Sample name	Name entered in the sample layout
No. Repl.	Number of repetitions of a sample
Mean Ct	Mean Ct value of replicates
Mean calib. Ct	Mean Ct value of the replicates of a sample corrected with the help of the IPS
Std. dev. Calib. Ct	Standard deviation of the corrected mean Ct value of the replicates of a sample
RQ	Relative quantity of the gene for replicates in the original sample
Std. dev. RQ	Standard deviation of the relative quantity of the gene for replicates in the original sample
Norm.exp	Normalized expression of the sample
Std. dev. norm. expression	Standard deviation of the normalized expression of the sample

Interplate standards

The IPS view summarizes the data of the interplate standards.



Project name	Dye	Mean Ct (IPS, project)	Mean Ct (IPS, all projects)	Correction value
qT3_Multi1.rtpx	FAM	27,83	31	3,16
qT3_Multi1.rtpx	VIC	29,69	31,92	2,23
qT3_Multi1.rtpx	ROX	28,53	30,35	1,83
qT3_Multi1.rtpx	Cy5	28,15	29,72	1,57
qT3_Multi2.rtpx	FAM	32,77	31	-1,77
qT3_Multi2.rtpx	VIC	33,2	31,92	-1,28

Column	Description
Project name	Name of the project
Dye	Dye used to determine the Ct value of the IPS sample

Column	Description
Mean Ct (IPS, project)	Mean Ct-value of the IPS samples in the project (dye-dependent)
Mean Ct (IPS, all projects)	Mean Ct value of the IPS samples in all projects (dye-dependent)
Correction value	Ct correction value applicable to all samples of the named project (first column) and the dye (second column)

14 Functions of the menu Extras

14.1 General settings in the Options window

The **Options** window is used to make changes to all settings that apply to the entire program.

Opening the Options window

To use most of the functions in the **Options** window, you must be logged into the program as an administrator.

- Close all project windows in qPCRsoft.
- Select the menu item **Extras** | **Options**.
  - ✓ The **Options** window appears.
- Change the respective settings in the individual tabs and click OK.
  - ✓ The settings are applied across the entire program.

General tab

The **General** tab is used to define the options for saving and exporting the project data.

Option	Description
Auto save folder (project)	For saving the projects (result data) automatically after completing the execution of a PCR program, enter the name of the path here, under which the projects shall be saved.
Automatically (in the auto save folder)	Projects will be saved automatically to the folder specified above after the PCR program was executed. There are the following options for generating file names: **[DATE]_XXX** The file name is generated from the date and a sequential number. **[Name]_XXX** The file name is generated from a freely selectable name (entered in the input field) and a sequential number. There are the following output formats: **RTProject extended file (*.rtpx)** Save results data in a qPCRsoft project **RT result bin file (*.ajpcrresbin)** Save results data in a bin file **RT result xml file (*.ajpcrresxml)** Save results data in an XML file
Manually after the qPCR run	After the qPCR program was executed, the system will open the window **Save project** for entering the project's file name.
Manually at the start of the qPCR run	When the qPCR program execution is started, the window **Save project** is opened. The execution of the qPCR program can only start after the file name is entered and confirmed.
Save backup file "Last_Run.rtpx" (in the auto save folder)	Data of a currently executed qPCR protocol can be saved in a backup file. If the execution of this qPCR program is interrupted prematurely, all fluorescence measurements taken up to this point are recorded in this file. The backup file is saved in the folder **Auto save folder (project)** and will be overwritten each time a new qPCR program execution is started.
autom. raw-data .csv export at the end of the run	After the execution of a qPCR program, 2 files (amplification and raw data) and, if necessary, the melting curve are exported to a CSV file for each dye.

Option	Description
	The file names for raw data and amplifications are composed of the following values: Template_name_Type_Date_Time_Dye.csv (Example: Kit_template_AD_2023-09-21_1154_FAM.csv) For melting curves, the dye name is omitted in the file name: Template_name_Type_Date_Time.csv (Example: Kit_template_MD_2023-09-21_1154.csv) The value "Type" is used to describe the exported fluorescence data: ■ AD = Amplification data ■ MD = Melting curve data ■ RD = Raw data
autom. ct-data .csv export at the end of the run	After a qPCR program has been executed, all determined Ct values are exported to a CSV file. The file name is composed of the following values: Template_name_Type_Date_Time.csv (Example: SyGreen-Assay_Ct_2023-10-23_1501.csv)
Folder for automatic export	If you have enabled the automatic CSV export of the raw data or Ct values, please enter the path to save the export files here.

Data format tab

Use the tab **Data format** to specify the decimal separator and the number of decimal places for the displayed values.

Language tab

Use the tab **Language** to select the language for the program interface.

Measurement tab

Use the tab **Measurement** to set the basic options for fluorescence measurement and block temperature control.

Option	Description
Sensitivity	Basic sensitivity of the detection system This setting affects all dyes and should only be changed if particularly weak or intense samples are to be measured. Default setting: 5
Measurement repetitions color compensation	Number of measurement iterations for recording the color compensation
Display negative values due to color compensation	If enabled, the system will also display negative values as a result of the color compensation, otherwise, the system would produce the output "0" instead.
Simulated Tube Control	If enabled, the temperature in the sample is pre-calculated with the measured block temperature and the temperature is controlled to the sample temperature. This method is particularly recommended for fast protocols and high sample volumes. If disabled, the block temperature is controlled according to the selected temperature program. Particularly if the heating and cooling rates are high and the hold times are short the actual sample temperature can differ from the desired temperature.

Analysis tab

The tab **Analysis** allows entering a factor for the quantitative evaluations (**Quantification factor**), for the melting curve analysis (**Melting factor**) and for genotyping (**Genotyping factor**) which is used for the automatic calculation of the threshold.

After enabling the option **Fix scaling to 100%**, the scaling of the Y-axis in all diagrams representing normalized fluorescence values (fluorescence, dRn) is set to 100%. There is no automatic scaling if the displayed curves are less than 100%. This makes it easier to analyze minor fluorescence.

Device tab

The **Device** tab is used to enable the recording of device communication data which are used for diagnosing errors. In case of problems, the AJ Service department may ask you to record this data and to send it to the Service department.

Option	Description
Log application (recommended)	Log file of qPCRsoft By default, this recording function with the selection **Info** is always enabled. This log file is small and can be transmitted quickly, if required.
Log device traffic	Communication between qPCRsoft and the device This log file can become very large and should therefore only be generated on request.
Log device fiber check results	If the fiber test is activated before or after the execution of the qPCR program in the project settings, the measured values of the fiber test are recorded.

File tab

The tab **File** is used to activate this kind of file types for which, when selected, the file explorer of the operating system qPCRsoft is automatically started and the file is opened.

User management tab

Use the tab **User management** to enable the use of the user administration. This function requires administrator rights.

When the option **User login required** is disabled, the user will not be prompted to enter their login credentials when the program is started. The functions for setting up the user management are not available.

Colors tab

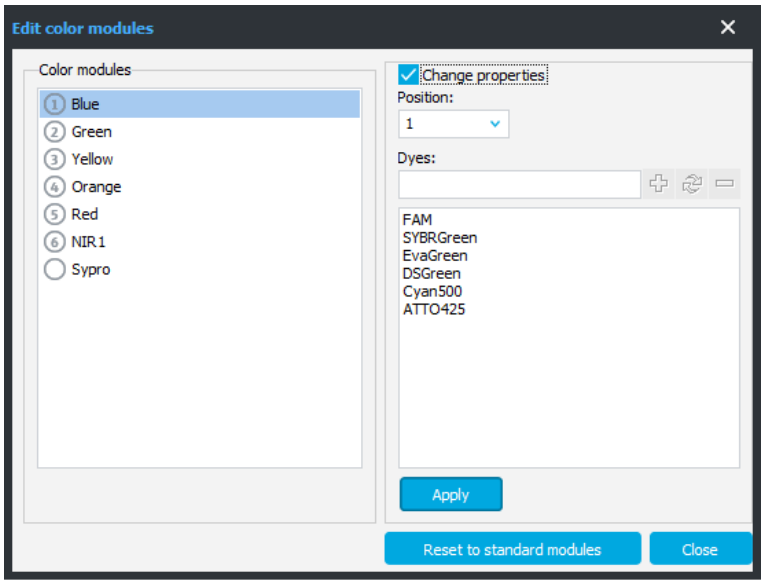
The tab **Colors** allows defining the following color settings:

- Display color for the sample type and replicates in the plate layout
- Color of the fluorescence curves by sample type, well or replicate
- Colors for marking positive and negative evaluations

Depending on the selected option for the color of the sample type, replicate or well curve, the corresponding color scheme is applied to the fluorescence curves display.

14.2 Configuring dye modules

After inserting the dye modules into the device's measuring head, the dye modules must be specified in the software's **Edit color modules** window.



Specifying the installed dye modules

- Select the menu item **Extras | Edit color modules**.
  - ✓ The **Edit color modules** window appears.
- Select the module that is installed in the device from the list of modules.
- Enable the option **Properties** and select the position at which the module has been installed into the device.
- If required, add further dye names if these are not included in the list.
- Click the button **Apply** to apply the settings to the module.
- Proceed likewise for the other dye modules that are installed.
  - ✓ The modules that have been installed to the device are now available in qPCR-soft.

Defining new dye modules or deleting existing dye modules

If your dye module is not featured in the list, you must create a new one. Dye modules that are not installed can be deleted from the list.

- Adding a new dye module to the list: Click the button **Add**. Use the field **Module Code** in the input window to enter the new module's code. Confirm with **OK**.
  - ✓ The new module is now available in the list.
- For removing a dye module from the list: Click on the dye module in the list. Click the button **Remove**.
  - ✓ The dye module is deleted from the list.

Assigning dyes

It is possible to assign additional dyes to the dye modules. A dye can only be assigned to one module at a time. If you intend to measure it with a different module, you must first remove it from the initial module.

- Highlight the dye module in the list and activate the option **Properties**.
- Use the input field **Dyes** to enter the name of the dye that is detected with the module.
- Click the **+** button.

✓ The dye is added to the list below.

- To remove a dye, highlight the dye in the list and click the **–** button.
  ✓ The dye is removed from the list.
- Click the button **Apply**.
  ✓ The changes to the properties are assigned to the dye module.

14.3 Changing the device selection

The connected device is selected when the program instance is started. It is possible to connect the program instance to another device later without having to quit the program.

- Switch on the qPCR thermal cycler.
- Select the menu item **Extras** | **Device selection**.
- Select the device in the window **Select working device** and click **Select**.
  ✓ The selected device is connected to the program instance.

14.4 Initializing the device

The initialization of a device sets the device back to its original state. It is only necessary to initialize a device after an error has occurred.

- Select the menu item **Extras** | **Device initialization**.
  ✓ The original state is restored on the device. The device is ready for taking measurements again.

14.5 Connecting the device to a PC

When started, qPCRsoft is connected to a device that is switched on. Whether or not a device is connected is indicated in the bottom left corner of the status bar.

If after about 30 seconds no connection can be established, select the menu item **Extras** | **Device ident** to solve the problem.

15 User management

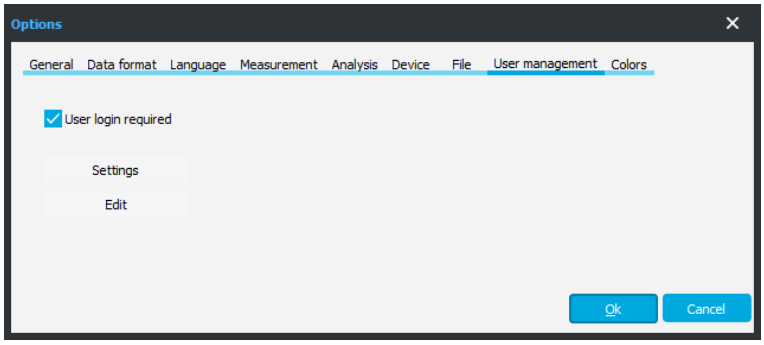
Note on general data security

Any project, template, analysis and communication files generated by qPCRsoft can only be read and amended with qPCRsoft because of the encryption used.

Activating the user management

Activate the user management under the menu item **Extras | Options | User Management**.

Option	Description
User login required	If activated, the user management becomes effective the next time the program is started. Logging in to the program is then only possible with a valid user profile. **Note**: The first time the program is started after installation, an administrator with access to the user management is created.
Configuration	Settings for passwords, logging in and logging out
Edit	Manage user profiles



15.1 Basic settings for passwords, logging in and logging out

To access the basic settings that apply to all users, select the menu item **Extras | Options** and click the **Settings** button on the **User management** tab.

You can make the following settings in user management:

- Number of login attempts
  If the number of allowed login attempts to a user account is exceeded, i.e., if the attempts fail, the user account is deactivated. It can then only be reactivated by the administrator.
- Minimum length of the user name and the password
- Required characters in the password
- Warning prior to password expiring
  The password expiration is set in the user profile.
- Logout if inactive
  After the specified time has expired without mouse or keyboard activity, the program interface will be locked and the login window is displayed. The user first has to enter the password before the interface can be used again. If the button in the login window is clicked, the program is closed. It is not possible to change the user at this point. Automatic logout does not occur if a qPCR run is active.

15.2 User profiles and preset user groups

Users are managed in the **User profiles** window with the overview of all user profiles created. Select the menu item **Extras** | **Options** and click the **Edit** button on the **User management** tab.

There are the following functions:

Function	Description
Add	Create a new user profile
Edit	Edit an existing user profile
Remove	Delete a user profile that is no longer required

By default, these functions are only available to users belonging to the **Administrator** group. However, they can also be assigned to a **Supervisor** by editing the user rights.

Add/edit user profile

- Create new user profile: In the **User profiles** window, click on the **Add** button.
- Edit existing user profile: Select the user profile in the list and click the **Edit** button.
  - ✓ The window for editing the user profile appears.
- Edit the user profile data on the **General** and **Password** tabs.
- Optionally enable access to further functions outside the selected user group.
- Confirm the setting by clicking on **Ok**.
  - ✓ The user profile is displayed in the User Profiles window.

Data of a user profile

In the **User profile** | **General** window, enter the user name and select the user group.

Option	Description
User name and password different	Login name at program start
Full Name	Actual name (optional)
Description	Further description (optional)
User Group	Assign a user group
Edit user group access	Individually adjust the rights of the user within the user group

In the **User profile** | **Password** window, configure the settings for the password and deactivate the user profile.

Option	Description
User can change password / **Confirm password**	Enter password and repeat
User must change password on new login	If activated, users must change their password at first login.
User can change password	Users are allowed to change their own password.
Password never expires	The password is valid without a time limit. If deactivated, enter the expiration date.
User is disabled	The user profile was automatically locked after multiple failed login attempts or by an authorized user. The time when the user was locked is displayed.

Option	Description
	Enter the number of possible login attempts in the **Options** | **User management** | **Settings** window.
User is locked	The user profile has been locked by an authorized user. The user name no longer appears in the login dialog, however, the user remains created. The time when the user was deactivated is displayed.
User can sign electronically	The user is allowed to sign a project electronically. This right is only available if the add-on module 21 CFR Part 11 is enabled.

User groups

The following user groups are implemented in **qPCRsoft**:

User group	Rights
Administrator	■ This user group has unlimited rights to all program functions ■ Administrators can create, delete, lock and unlock users and assign rights to them ■ Can deactivate user management in the **Extras** | **Options** | **User management** window
Supervisor	■ Has rights the same as the administrator, but cannot create and manage users ■ The administrator can block certain rights for each user logged in as supervisor.
Operator	■ Can start a qPCR experiment and calculate Ct values and melting temperatures in the **Monitoring** project window The following rights cannot be assigned to an operator: ■ Create and manage users ■ Create and save templates ■ Save projects ■ Make changes in the project window on the tabs **General**, **Thermal cycler**, **Scan** and **Layout**

By selecting a user group you automatically assign a certain user role to the user and thus preset rights which you can additionally supplement or reduce using the **Edit user group access** function. This allows individual rights to be defined for each user. It is also possible to set up several administrators with different rights.

▶ In the **User profile** | **General** window, click the **Edit user group access** button.

✓ The window containing the rights of the selected user appears.

The following rights settings are possible:

- If a checkbox is ticked, this right is granted to the user and the user can use the function.
- Checkboxes with a padlock symbol cannot be changed.
- The number of blocked rights is determined by the selected Administrator, Supervisor or Operator user group and increases in this order. This means that an operator has fewer rights than a supervisor or administrator from the outset and can never be assigned all rights.
- An administrator has all rights in the program and these rights can only be restricted by removing the checkmarks. The right of an administrator to manage and create users cannot be locked, otherwise user management would no longer be possible.

15.3 Changing a password

If changing the password is permitted in the user profile, a supervisor or operator can open the profile in user management and change the password. They have no access to any other settings.

- Select the menu item **Extras** | **Options** | **User management**.
- Click the button **Edit**.
  - ✓ The **User profiles** window appears.
- Select your own user profile in the list and click on the Edit button.
- Enter the new password in the **User profile** | **Password** window and confirm.
- Click **Ok** to confirm the settings and close all windows.
  - ✓ The password has been changed.