July 2021

QIAquant™ 384 Software User Manual

Software for real-time PCR thermal cycler

For Molecular Biology Application. Not for use in diagnostic procedures.

Sample to Insight

QIAGEN

Contents

1 Introduction

The QIAquant 384 Software can be used to create and perform PCR and real-time PCR experiments. This section describes the basic setup and layout of the operating elements of the software.

For short instructions that offer a quick start to this software, see Appendix A, page 155.

Described software version

This description is based on the QIAquant 384 Software version 1.0.3.

Supported devices

The program supports the device control and data analysis of the QIAquant 384 devices.

File formats

The QIAquant 384 Software uses file formats for projects, templates and multi-gene analyses with the extensions QRTP384, QRTS384, or QMGA384. When saving, you can select in which format the files are to be saved.

1.1 How to use this user manual

The following symbols and conventions are used to facilitate orientation in the manual:

- In the description of the operating procedures, menu commands, dialog boxes, buttons, options, etc. are highlighted in bold.

Menu commands of a command sequence are separated by ">", e.g., **File** > **Open Project**.

Buttons are written in bold, e.g., **Save**.

1.1.1 Technical assistance

At QIAGEN® we pride ourselves on the quality and availability of our technical support. Our Technical Services department is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any

questions, or if you experience any difficulties regarding QIAquant 384 Software or QIAGEN products in general, do not hesitate to contact us.

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

For technical assistance or more information, please visit our Technical Support Center at **support.qiagen.com**.

1.1.2 Policy statement

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

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

1.2 Installation of the QIAquant 384 Software

Administrator rights on the operating system are required for installing the program.

System requirements for installing the QIAquant 384 Software

To use the QIAquant 384 Software to control your real-time PCR device, your PC must meet the following minimum requirements:

Operating system	Windows 10
Processor	Min. Intel Core i3, >1 GHz
RAM	1 GB
Available hard disk space	Min. 300 MB
Interfaces	Min. USB 2.0

Installation procedure

QIAquant 384 Software is delivered on CD-ROM.

1. Insert the CD in the CD-ROM drive. Normally, the installation's start window opens automatically.
   If this is not the case, run the **setup.exe** file on the CD.
   A selection dialog window appears, for installing the device driver, user management, or for viewing the PDF files of the manuals.
2. Click **Install**.
   The installation routine begins.
3. Follow the further instructions of the installation program.
4. Switch on the device at the power switch. Start the QIAquant 384 Software.

   Note: The software will only be installed correctly if it has been run once with administrator privilege. A password for the program administrator must be entered during this first run.

Set up administrator

After the software installation, an administrator password must be assigned, and an administrator must be specified.

1. Start the QIAquant 384 Software.
2. Enter and confirm the password for the administrator in the **Login** window.
3. Set up the user profiles (see "User Management", page 143).
   If user management is not necessary, disable user management via **Extras** > **Options/Usermanagement**.

1.3 Starting and exiting QIAquant 384 Software

Starting QIAquant 384 Software

4. To start the QIAquant 384 Software, click the **Start** button on the Windows desktop. Then go to the **Programs** folder and click **QIAquant 384 Software**.

   (Alternatively, you may click the **QIAquant 384 Software** icon on the Windows desktop.)

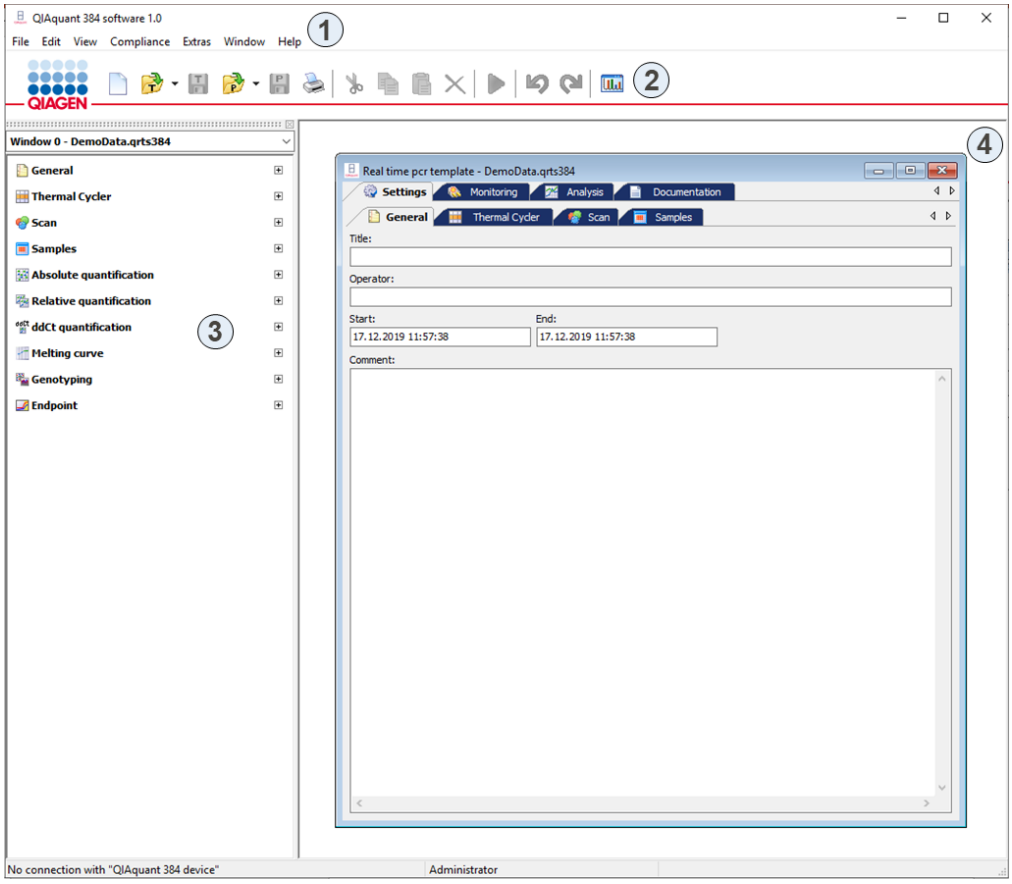
   You will be prompted to enter your username and password.

   Note: An administrator and password must be defined during the first program start. Only the administrator can set up further user accounts or disable the user management.

Exiting QIAquant 384 Software

1. To exit the QIAquant 384 Software application, activate the **File** > **Exit** menu command.
2. The program will display a message if any projects that have not yet been saved are still open at this point.
3. If you want to save these projects, click **Yes**. Save the projects in the **Save as** standard window
4. Then call up the **File** > **Exit** menu command again to exit QIAquant 384 Software.

1.4 The main window in QIAquant 384 Software



After starting the QIAquant 384 Software, the main window opens. It has the following sections:

Menu bar (1)	Contains menu commands for opening, editing and saving projects, managing user profiles, setting basic software options, etc.
Toolbar (2)	Commands for editing projects are arranged in the toolbar. The commands offered in the toolbar may change according to context.
Project explorer (3)	A drop-down menu provides a quick overview of the most important information on the current open project.
Project interface (4)	Used to process projects. As soon as a new project is created or an existing project is loaded, a window opens where all relevant settings for the respective project can be made.

1.4.1 Menu commands overview

The menu bar is context-sensitive and is automatically adapted to the program tasks. Menu items that are not necessary for the current work interface are automatically hidden. The following menu commands are available in the QIAquant 384 Software:

Menu	Function	Description
File	New	Opens a new project
	Open template	Opens a template
	New from library	Opens the QIAGEN kits template library
	Open project	Opens a project
	Open autom. saved project	Opens an automatically saved project
	Save template	Saves a template file in the QIAquant 384 Software standard folder
	Save template as	Saves a template file in any user-selected folder
	Save project	Saves a project file in the QIAquant 384 Software standard folder
	Save project as	Saves a project file in any user-selected folder
	Import analyses	Opens an analysis file
	Export analyses	Saves an analysis file
	Import LIMS	Imports a transfer file, with which the software of another program can be configured, e.g., LIMS
	MultiGene	Starts the MultiGene analysis, for analyzing experiments that comprise multiple PCR plates and multiple genes
	Close	Closes a template or a project
	Close all	Closes all open projects or templates
	Print	Prints a project
	Exit	Closes the software

Menu	Function	Description
Edit	Undo	Reverses the last text modification (up to 10 steps)
	Redo	Restores the last deleted text item (up to 10 steps)
	Cut	Cuts a marked text area
	Copy	Copies an active and/or marked text area
	Paste	Pastes a text area copied to the clipboard
	Delete	Deletes an active and/or marked text area
	Mark all	Marks a complete text area
	User management	Opens the window for creating user profiles and changing the password (only available if the user management function is activated)
Compliance	Show audit trail	Displays the available audit trail
	Show log file	Displays available log file
	Show signatures	Displays available signatures
	Sign digitally	Creates a new digital signature
View	Project explorer	Toggles on/off the project explorer view in the main window
	Toolbar	Toggles on/off the toolbar view in the main window
Scan	Set color compensation	Opens the window for creating files for spectral color compensation
Extras	Device initialization	Resets the connected device to the initial state
	Device identification	Activates the connected device
	Transport lock	Prepares the device for transport
	Options	Opens the window for general basic software settings
Window	Tile horz	Arranges project windows horizontally
	Tile vert	Arranges project windows vertically
	Cascade	Arranges project windows in a cascaded fashion
	Info	Displays software information
Cycler	Add empty step	Adds a new step
	Delete step	Deletes a step
	Cut step	Cuts a step and copies it onto the clipboard
	Copy step	Copies the parameters in one step onto the clipboard
	Paste step	Inserts a copied step
Scan	Edit color compensation	Opens the window for creating files for spectral color compensation

Menu	Function	Description
Samples	Edit layout	Edits the sample table
	Copy layout	Copies the area of the sample table
	Paste layout	Inserts the copied area of the sample table
	Preview layout	Shows a detailed view of the plate assignment
Monitoring	Start qPCR run	Starts the PCR run
	Stop qPCR run	Stops the PCR run
	Pause qPCR run	Pauses the PCR run
	Display options	Displays options for the product accumulation curves
	qBase export	Exports measured values as a CSV or an XLS file in qBase format (available after C_t calculations)
AbsQuant	Add abs. quantification	Creates new evaluation
	Delete abs. quantification	Deletes evaluation
	Options abs. quantification	Opens a window for basic evaluation settings
	Automa. threshold	Automatically determines the fluorescence threshold for detecting C_t values
	Import standard curve	Import a saved standard curve
	qBase export	Exports measured values as a CSV or an XLS file in qBase format
RelQuant	Add rel. quantification	Creates a new evaluation
	Delete rel. quantification	Delete evaluation
	Options rel. quantification	Opens a window for basic evaluation settings
	Automa. threshold/cut off	Automatically determines the fluorescence threshold for detecting C_t values
	Import standard curve	Imports a saved standard curve
DeltaDeltaCt	Add ΔΔCt quantification	Creates new evaluation
	Delete ΔΔCt quantification	Deletes evaluation
	Options ΔΔCt. quantification	Opens a window for basic evaluation settings
	Automa. threshold	Automatically determines the fluorescence threshold for detecting C_t values
Melting Curve	Add melting curve	Creates a new evaluation
	Delete melting curve	Deletes evaluation
	Options melting curve	Opens a window for basic evaluation settings
	Automa. threshold	Determines threshold automatically
Genotyping	Add genotyping	Creates new evaluation

Menu	Function	Description
	Delete genotyping	Deletes evaluation
	Options genotyping	Opens a window for basic evaluation settings
	Autom. threshold\cut off	Automatically determines the fluorescence threshold for detecting C_t values
Endpoint	Add endpoint	Creates a new evaluation
	Delete endpoint	Deletes evaluation
	Options endpoint	Opens a window for basic evaluation settings
	Auto threshold\cut off	Automatically determines the cut-off fluorescence value for decision POS/NEG
MIQE	Import MIQE documentation	Imports MIQE information from another project

1.4.2 Overview of the tools in the toolbar

The buttons in the toolbar are context sensitive. The program automatically adjusts the toolbar to the window content and adds buttons if this is required and useful for the current project window view. Buttons that are not accessible for the current contents of the workspace are hidden.

You can display or hide the toolbar via the **View** > **Toolbar** menu command.

Button	Command	Function
General		
	New	Opens a new project
	Open template	Opens a template
	Save template	Saves a template
	Open project	Opens a project
	Save project	Saves a project
	Print project	Prints a project
	Undo	Reverses the last modification
	Redo	Restores the last deleted modification

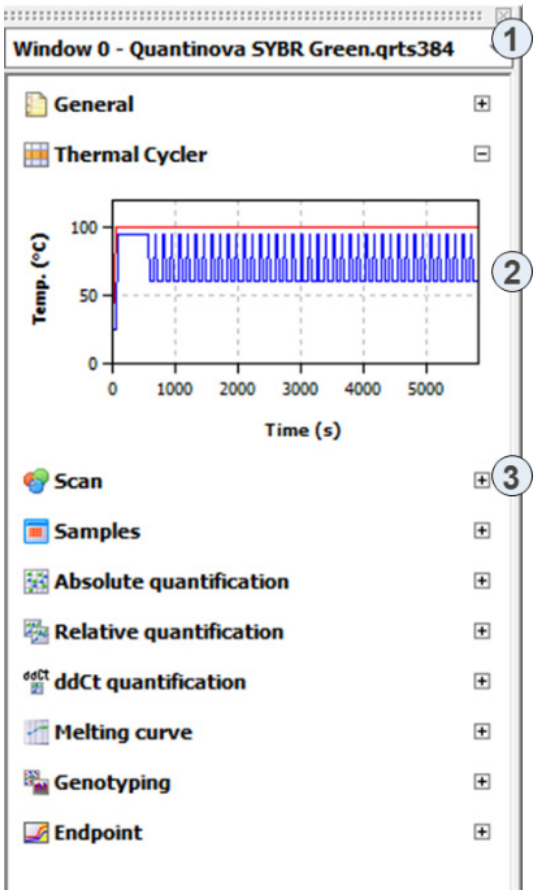
Button	Command	Function
	Cut	Cuts the marked area
	Copy	Copies the active and/or marked area
	Paste	Pastes the copied area onto the place where the cursor is located
	Delete	Deletes the active and/or marked area
	MultiGene	Starts the MultiGene and multiplate analysis
PCR protocol		
	Add empty step	Adds a new step
	Delete step	Deletes a step
	Activate melting curve	Adds a step for melting curve determination
	Cut step	Cuts a step and copies it into the clipboard
	Copy step	Copies the parameters in one step into the clipboard
	Paste step	Pastes the copied step
Color		
	Edit color compensation	Opens the window for creating files for spectral color compensation
Samples		
	Edit layout	Assigns changes that were made to the sample table
	Copy layout	Copies an area in the sample table
	Paste layout	Pastes the area that was copied from the sample table
	Preview layout	Displays a complete view of the plate assignment
MIQE		
	Import MIQE documentation	Imports MIQE information from another project
Monitoring		
	Start PCR protocol	Starts the PCR run

Button	Command	Function
	Stop PCR protocol	Ends the PCR run
	Pause PCR protocol	Breaks the PCR run
	Options	Displays options of the product accumulation curves
	qBase export	Exports measured values as a CSV or an XLS file in qBase format
Evaluation/absolute quantification		
	New	Creates a new evaluation
	Delete	Deletes an evaluation
	Options	Opens a window for basic evaluation settings
	Automatic threshold	Automatically determines the fluorescence threshold value for C_t value determination
	Import standard curve	Imports a saved standard curve
	qBase Export	Exports measured values as a CSV or an XLS file in qBase format
Evaluation/relative quantification		
	New	Creates a new evaluation
	Delete	Deletes an evaluation
	Options	Opens a window for basic evaluation settings
	Automatic threshold	Automatically determines the fluorescence threshold value for C_t value determination
	Import standard curve	Imports a saved standard curve
Evaluation/ΔΔCt analysis		
	New	Creates a new evaluation
	Delete	Deletes an evaluation
	Options	Opens a window for basic evaluation settings
	Automatic threshold	Automatically determines the fluorescence threshold value for C_t value determination

Button	Command	Function
Evaluation/Melting curve		
	New	Creates a new evaluation
	Delete	Deletes the current evaluation
	Options	Opens a window for basic settings for the evaluation
	Automatic threshold	Automatically determines the threshold
Evaluation/Genotyping		
	New	Creates a new evaluation
	Delete	Deletes the current evaluation
	Options	Opens a window for basic settings for the evaluation
	Auto threshold/cut off	Automatically determines the threshold
Evaluation/Endpoint		
	New	Creates a new evaluation
	Delete	Deletes the current evaluation
	Options	Opens a window for basic settings for the evaluation
	Auto threshold/cut off	Automatically determines the cut-off fluorescence value for decision POS/NEG

1.4.3 Project explorer components

Different menus (2) in the project explorer offer a quick overview of the currently processed project. Individual projects can be selected via a selection list (1). The information on the individual menus can be displayed or hidden via the **[+]** and **[–]** (3) buttons.

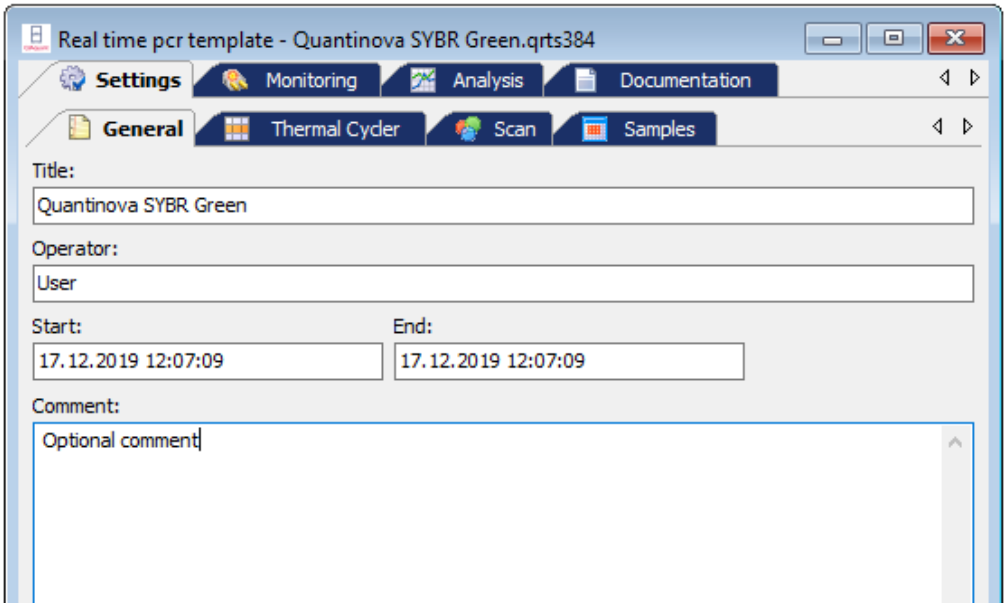


Menu	Information
General	Project title, user, date, time, and device
Thermocycler	Graphic display of the history of the PCR program in the active project
Scan	Overview of the colors and areas of the PCR plate that are being scanned
Samples	Displays a short info text on the plate layout. Used for activation or deactivation of samples during measurement and evaluation (see "Display options for monitoring", page 59, and "Activating/deactivating samples for analysis", page 71). The edit mode for the plate layout displays detailed information on the selected well.
Absolute quantification	Graphic display C_t against log concentration
Relative quantification	Graphic display C_t against log concentration
ΔΔCt	Graphical display dCt(V) compared to log concentration
Melting curve	Graphical display of melting curve compared to temperature
Genotyping	Graphical display of dRn Genotype 1 compared to dRn Genotype 2 as a scatter plot or bar graph
Endpoint	Graphical display of endpoint fluorescence as a bar chart for the GOI and the IPC

You can display or hide the project explorer via the **View** > **Project explorer** menu command.

1.4.4 Project interface with project window

The project window opens when a new project is created or when a saved project and/or a template is loaded. The QIAquant 384 Software comes with standard QIAGEN real-time PCR kits templates preinstalled for convenience.



In the project window, all parameters, measuring data, and evaluations for one PCR plate are combined. The basic functions are arranged on the 4 main tabs at the top:

Tab	Function
Settings	Contains all functions required for defining real-time PCR runs
Monitoring	Contains different tools for monitoring real-time PCR runs
Analysis	Includes the evaluation algorithms implemented in the software for analyzing acquired data
Documentation	Opens the input mask for MIQE-compliant documentation of real-time PCR experiments

These 4 tabs are always visible. The view of the project window changes depending on the selection of a function tab. To indicate the tab the descriptions refer to, the tabs and list sheets are listed in the order in which they were activated and divided by a slash, e.g., **Settings/Thermocycler/Table**.

1.4.5 Version number of the software

The **Help** > **Info** menu command opens the window with information on the version number of the software program.

2 Managing Projects and Templates

The QIAquant 384 Software saves all experiments in the project files. A project contains different information required to perform a real-time PCR experiment:

- Description of the experiment
- PCR protocol
- Scan settings of the optical system
- Plate assignment with detailed information on each sample
- Measuring results and the corresponding evaluation after the experiment has been performed

All basic information required for performing an experiment that is stored in the project window on the **Settings** tab (e.g., the description of the experiment, the PCR protocol, scan settings of the optical system, and the plate assignment) can be saved as a template.

The following file extensions are used in the software:

Extension	File type
QRTP384	Real-time project file
QRTS384	Real-time settings file
QMGA384	Real-time multigene analysis file
QRTA384	Real-time analysis file
QRTF	LIMS transfer file

2.1 Creating a new project or opening a project

A project is always indicated in a project window in a section of the project interface of the main window.

Creating a new project

To create a new project, select  or the **File** > **New** menu command.

A new project with standard presets is created in the project window.

Creating a new project based on a template

A new project can be opened with a saved template:

1. Click  or select the **File** > **Open template** menu command.
2. In the standard window, select the desired template to open files, and confirm the selection with **OK**.
3. A new project with the parameter settings of the template is created in the project window.

Opening a saved project

1. Click  or select the **File** > **Open project** menu command.
2. In the standard window, select the desired project to open files, and confirm the selection with **OK**.

   The project with the parameter settings, measurement results, and evaluations is created in the project window.

Opening an automatically saved project

The QIAquant 384 Software programs enables you to automatically save the last completed real-time PCR run to a folder of your choice, thus preventing data loss due to unexpected terminations of a PCR run.

1. Recover the terminated measurement with the **File** > **Open autom. saved project** menu command.
2. Save the file as a project file under a different name.
3. To change the storage location of the file, proceed as follows:
   3a. Select **Extras** > **Options** to open the window of the same name.
   3b. Open the **General** tab.
   3c. Click **[...]** and select a storage location.

Viewing projects

You can open several projects at a time. Each project is displayed in its own project window. With the commands from the **Window** menu, the project windows can be arranged:

Command	Description
Tile horz	In the horizontal layout, the project windows are shown below each other. If there are more than 4 project windows, the windows are arranged in 2 columns.
Tile vert	In the vertical layout, the project windows are shown next to each other. If there are more than 4 project windows, the windows are arranged in 2 columns.
Cascade	In the cascading layout, the project windows are placed on top of each other with an offset.

Changes can only be made in the respective active window.

2.2 Saving a template

All basic information required for performing an experiment that is stored in the project window on the **Settings** tab (e.g., the description of the experiment, the PCR protocol, scan settings of the optical system and the plate assignment) can be saved as a template.

1. Select the **File** > **Save template as** command.
2. To save files, enter the name of the template in the standard window, and save the template with **OK**.

   The changes in a template can be saved with the **File** > **Save template** menu command.

   Optionally, you can click  in the toolbar.

2.3 Saving a project

You can save the project with all parameters of the PCR run, the fluorescence curves, and evaluations.

1. Select the **File** > **Save project as** command.
2. To save files, enter the name of the template in the standard window, and save the template with **OK**.

The changes in a project can be saved with the **File** > **Save project** menu command. Optionally, you can click  in the toolbar.

In the **Options/General** window (**Extras** > **Options**) you can define the default folder in which the file will be saved. The results of the PCR run are saved automatically under a file name with the date and time of the save.

2.4 Importing/Exporting analyses

Settings for the data evaluations of a project can be saved (exported) and later imported to an open project. The evaluations are applied to the open project when they are imported.

1. Select the **File** > **Export analyses** menu command.
2. To save files, enter the name of the analysis, and save the files with **OK**.
   Analyses are saved with the extension **QRTA384**.
3. Select the **File** > **Import analyses** menu command.
4. Select the name of the analysis in the default window for opening files, and import the analysis into the current project by clicking **OK**.

2.5 Creating a project template from a transfer file

Select **File** > **Import LIMS** to create a project template from a transfer file and start a PCR run (see "Appendix C: Creating a Project Template from the Transfer File (LIMS)", page 163).

2.6 Performing a Multigene/Multiplate Analysis

Selecting **File** > **MultiGene** allows the analysis of experiments that require multiple PCR runs and that comprise multiple genes (see "Multigene-/Multiplate-Analysis", page 127).

2.7 Closing project windows

The **File** > **Close** menu command closes the active project window. To close all project windows, select **File** > **Close all**. If any unsaved changes have been made in project windows, a confirmation prompt appears.

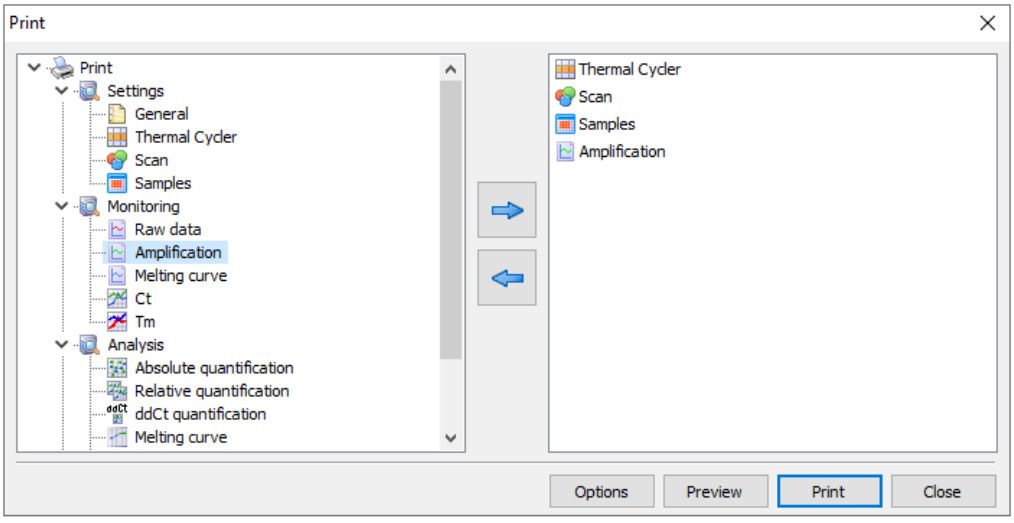
2.8 Printing

You can specify the desired contents for printing a project in a selection list:

1. Select **File** > **Print**.
2. Manage the print output using the displayed lists. Select the desired information in the list on the left and click  to transfer it to the print list on the right. To remove undesired information from the print list, click  .

3. Click **Print** to start the printout.

   Select **Options** to configure the printout, or **Preview** to display a page view of the print image.



The individual print modules are sorted into the **Settings**, **Monitoring**, and **Analysis** subgroups in the **Print** window.

3 Settings for a Real-Time PCR Experiment

If you want to start a new project, create a new project, or open a template:

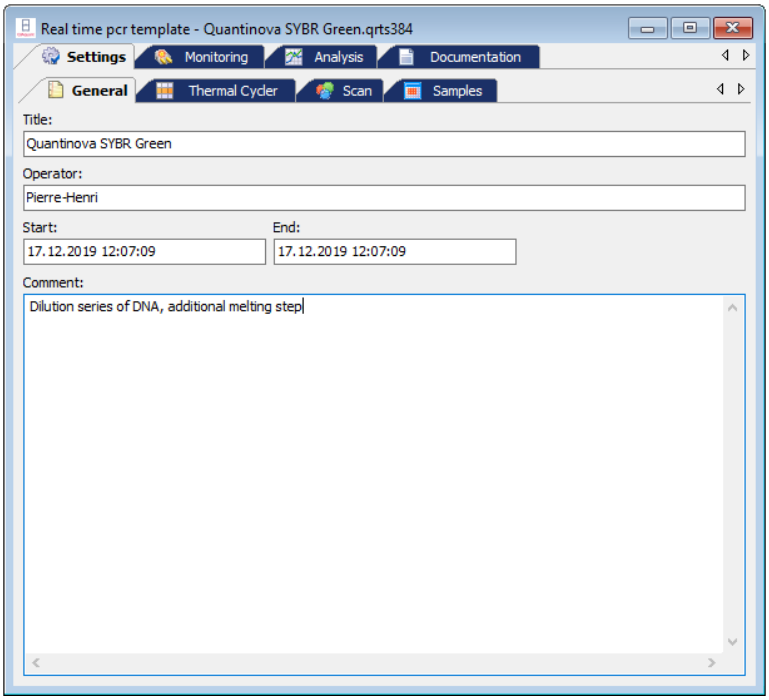
- Create an empty project with a click  in the toolbar.
- Optionally, open a template with  to use the previously stored parameters for the new project or to modify them.

All functions necessary to create a new project are combined under the **Settings** tab. Additional tabs on the second level are assigned to the **Settings** tab:

Tab	Function
General	Allows you to enter general information and remarks
Thermocycler	Used for programming PCR protocols
Scan	Defines the colors to be measured and the settings for the measuring parameters
Samples	Opens the sample table in which detailed information on each sample can be saved and groups of experiments defined

3.1 Entering general information on the project

You can save general information on each project. The entries can be made on the **General** tab:

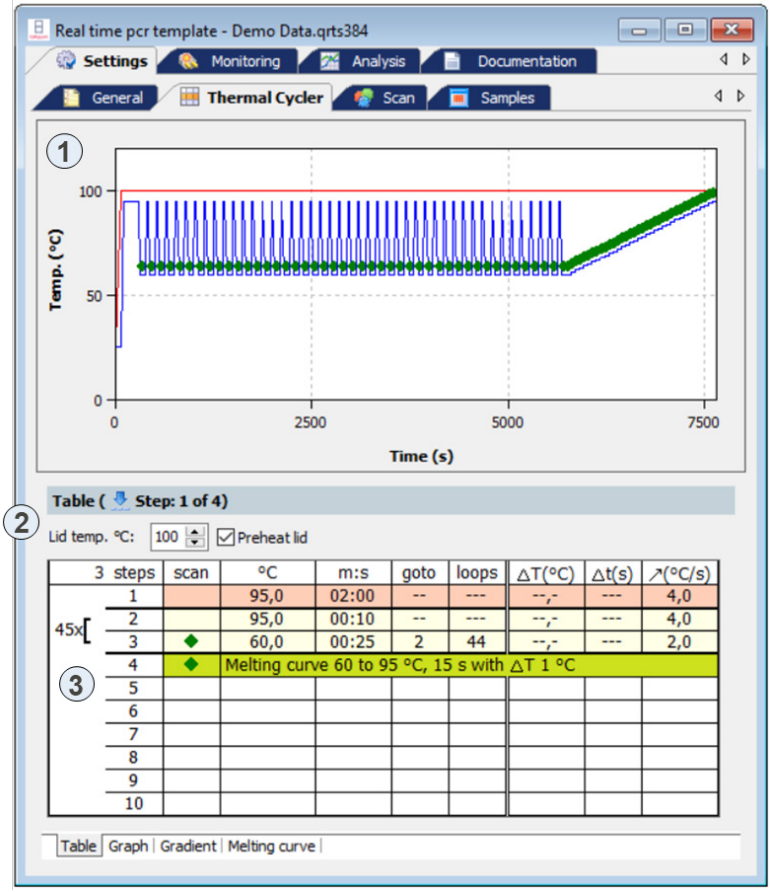


Command	Description
Title	Analysis title
Operator	User If you are using a user management option, the user name you signed in with will be entered automatically.
Start/End	Date and time the project started and ended
Comment	Comment input

Note: The **General** tab supports commands for **Copy**, **Cut**, and **Paste**, found in the **Edit** menu.

3.2 Creating a PCR protocol

A PCR protocol must be programmed for each real-time PCR experiment. All necessary functions are combined in the **Settings/Thermocycler** project window. The screen is divided into 3 sections: graphical program preview (1), program header (2), and program table (3).



The program preview illustrates the history of the PCR protocol. The program header defines the general conditions for the PCR protocol, such as the programmed lid temperature and the lid-heating mode. The programming table offers a clear representation of the individual steps of the program.

The **Thermocycler** tab contains 4 list sheets. The tabs for switching between the list sheets are located at the bottom of the window.

List sheet	Function
Table	Contains a table for programming PCR programs
Graph	Offers the option for graphical programming of PCR programs
Melting curve	Is used to enter parameters for measuring a melting curve
Gradient	Enables set up of PCR programs using the gradient function

Editing a PCR protocol

Programs can be edited in the tabular or graphical representation.

You can switch between **Table** and **Graph** screens. To edit one step (the current active step is highlighted), you can use the corresponding function from the **Cycler** menu in the menu bar or the corresponding symbol from the toolbar:

Symbol	Cycler >	Description
	Add empty step	Inserts a new step behind the active step
	Delete step	Deletes the active step
	Cut step	Cuts the active step
	Copy step	Copies the active step
	Paste step	Inserts a copied step behind the active step

3.2.1 Entering information in the program header

The program header contains several general program settings:

- Options for lid heating
- Temperature control method
- Stand-by mode activation after the PCR is complete

Option/List	Description
Lid temp.	Sets the lid temperature. The temperature of the heating lid should generally be slightly above the maximum block temperature to prevent liquids from evaporating from the reaction mixture and condensing at the walls or lid of the reaction cups.
Preheat lid	If activated, this option preheats the lid to the set **Lid temperature** before the actual PCR program starts. This is the recommended default setting to ensure the formation of a homogeneously tempered air cushion between the sample containers. This leads to an improved temperature uniformity between the samples. While the lid is being heated, the block is kept at a constant 25°C. If the option is deactivated, the PCR program starts while the lid is still being heated. Adjustable lid temperature: 30–110°C **Note**: After the heating lid has reached the target temperature, this is followed by a 40-second equilibration before the program is started.

3.2.2 Overview on the PCR protocol

The PCR program is entered into the program table of the **Table** list sheet. One row of the table contains the parameters of a temperature step.

You can navigate in the program table by using the mouse or the 4 arrow keys (← → ↑ ↓) on the keyboard. Each entry is confirmed with the **Enter** key or the → arrow key. The cursor jumps into the corresponding field in the adjoining column. If the cursor is in the last row, an additional temperature step is inserted with ↓. Click on the next empty row to insert an additional temperature step.

	4 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
	1		95,0	02:00	--	---	--,-	---	3,0
40x	2		95,0	00:05	--	---	--,-	---	3,0
	3		58,0	00:05	--	---	--,-	---	2,0
	4	◆	72,0	00:15	2	39	--,-	---	3,0
	5	◆	Melting curve 60 to 95 °C, 15 s with ΔT 1 °C						
	6								
	7								
	8								
	9								
	10								

The following values are entered into the table or calculated from the default values:

Value	Description
Scan	Measures the sample fluorescence during this step, if marked
Steps	Number of the step in the temperature program Is automatically numbered consecutively
°C	Enter the target temperature of the step in °C
m:s	Enter the hold time of the target temperature (minutes:seconds)
goto/loop	Define the loop with the number of repetitions for a cycle
ΔT(°C)	Enter the increment or decrement of the target temperature within the PCR run
Δt(s)	Enter the increment or decrement of the hold time within the PCR run
↗(°C/s)	Enter the heating or cooling rate to reach the target temperature in the temperature step

3.2.3 Inserting a new temperature step/deleting a temperature step

- An additional temperature step can be inserted with one of the following functions:
  - Set the cursor in the last row of the temperature program and press the ↓ arrow key.
  - Select **Cycler** > **Add empty step.**
  - Click  .
  - Click on the next row under the program.

The total number of steps and the current processed step are displayed in the protocol header.

- To delete a program step, move the cursor to the program row and click  .
  Optionally, select **Cycler** > **Delete step**.

3.2.4 Entering the target temperature, hold time and heating/cooling rates

- Enter the target temperature of the temperature step into the **°C** column.
- Into the **m:s** column, enter the hold time in the "minutes:seconds" format (e.g., hold time of a duration of 1 min 20 s should be entered as "1:20").
- For special applications, it may be necessary to adjust the heating and cooling rates.
  Enter the average heating and cooling rate for each step in the **↗(°C/s)** column.

Note: The value in the ↗**(°C/s)** column defines the speed at which the target temperature is reached. If the temperature is be heated (or cooled) at a speed of 3°C per second between step 2 and step 3, the value 3.0 must be entered for step 3.

Note: If the speed is to be modified for the whole program, the heating or cooling rates must be adjusted in each individual step, as shown below.

	4 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
	1		95,0	02:00	--	---	--,-	---	4,0
40x	2		95,0	00:05	--	---	--,-	---	4,0
	3		58,0	00:05	--	---	--,-	---	2,0
	4	◆	72,0	00:15	2	39	--,-	---	4,0
	5	◆	Melting curve 60 to 95 °C, 15 s with ΔT 1 °C						
	6								
	7								
	8								
	9								
	10								

3.2.5 Defining loops

Program sequences that are repeated regularly can be summarized in loops. Generally, a loop is then defined by a target step for the return (**goto**) and the number or repetitions (**loops**).

	4 steps	scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
	1		95,0	02:00	--	---	--,-	---	4,0
40x	2		95,0	00:05	--	---	--,-	---	4,0
	3		58,0	00:05	--	---	--,-	---	2,0
	4	◆	72,0	00:15	2	39	--,-	---	4,0
	5	◆	Melting curve 60 to 95 °C, 15 s with ΔT 1 °C						
	6								
	7								
	8								
	9								
	10								

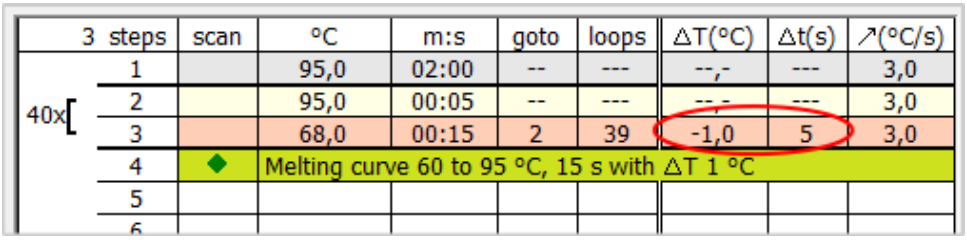
4. Place the cursor on the last step of the future loop (step 4 in the example above).
5. Enter the number of the target step into the **goto** column ("2" in the example above).
6. In the **loops** column, enter the number of repetitions ("39" in the example above).

After you enter the target step and the repetitions, the programmed loop will be displayed as a bracket on the left side of the table.

Note: The total number of loops displayed in the bracket is determined from the number of programmed repetitions plus 1, as the corresponding sequence of steps prior to reaching the loop has already cycled once.

3.2.6 Entering increments/decrements for temperature and hold time

By programming increments/decrements, the temperature or hold time can be modified by a specific amount from one cycle to the other within a loop. This technology is, for instance, used for the touchdown PCR.



3 steps		scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
40x[	1		95,0	02:00	--	---	--,-	---	3,0
	2		95,0	00:05	--	---	--,-	---	3,0
	3		68,0	00:15	2	39	-1,0	5	3,0
	4	◆	Melting curve 60 to 95 °C, 15 s with ΔT 1 °C						
	5								
	6								

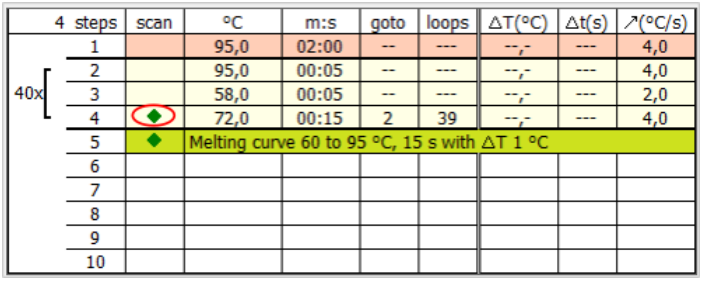
- Enter the desired changes for the temperature step whose values you wish to modify within the loop. Use the [–] sign to specify a decrement, i.e., temperature or hold time are reduced from cycle to cycle by the specified amount. No sign or [+] mark an increment, with the result that the parameter increases by the specified amount from cycle to cycle.
- To modify the target temperature in steps, enter the changes in the **ΔT(°C)** column.
- To modify the hold temperature in steps, enter the changes in the **Δt(s)** column.

Note: The modified step must be within a loop. Otherwise, the entries in the columns **ΔT(°C)** and **Δt(s)** have no effect.

Note: The extension of the hold time of one step affects the total run time of a protocol. A program with many cycles and with significant hold-time increases will take substantially longer than a comparable program without a programmed extension.

3.2.7 Arranging a fluorescence measurement

- To define the measurement of the sample fluorescence in a temperature step of the PCR protocol, click in the **Scan** column of the temperature step. A green diamond (◆) indicates that the measurements is active.



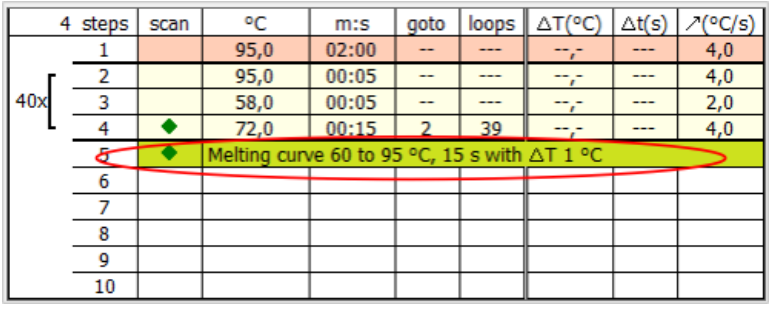
4 steps		scan	°C	m:s	goto	loops	ΔT(°C)	Δt(s)	↗(°C/s)
40x[	1		95,0	02:00	--	---	--,-	---	4,0
	2		95,0	00:05	--	---	--,-	---	4,0
	3		58,0	00:05	--	---	--,-	---	2,0
	4	◆	72,0	00:15	2	39	--,-	---	4,0
	5	◆	Melting curve 60 to 95 °C, 15 s with ΔT 1 °C						
	6								
	7								
	8								
	9								
	10								

Define the parameters for the fluorescence measurement on the **Scan** tab (see "Defining the parameters for the fluorescence measurement", page 34).

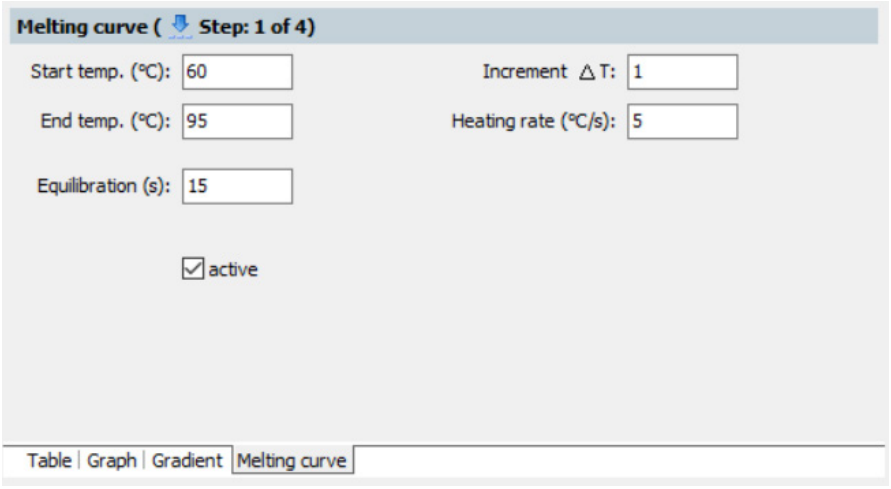
Note: If a step for melting curve determination is added, the scanning process is automatically activated for this step. For all other steps of the PCR protocol, the allocation must be made manually.

3.2.8 Adding a melting curve analysis

For experiments with intercalating dyes, we recommend to check the specificity of the products by measuring a melting curve. The device can be programmed to add the corresponding step in the PCR protocol. Activate the option **active** on the **Melting curve** tab.



- A melting curve can be added to the program table by checking the **active** box in the **Melting curve** tab.
  The melting curve is added to the last temperature step in the table.
- To remove a melting curve from the program table, uncheck the **active** box in the **Melting curve** tab.
- Set the individual parameters of the melting curve step on the **Melting curve** tab:



The following parameters can be modified:

Parameter	Description
Start temp. (°C)	Start temperature of the melting curve
End temp. (°C)	End temperature of the melting curve
Equilibration (s)	Time for equilibration of the sample in a temperature before a measurement is performed
Increment ΔT	Difference between 2 adjoining temperature steps, in °C
Heating rate (°C/s)	Heating rate of the block
active	Add a melting curve at the end of the PCR protocol

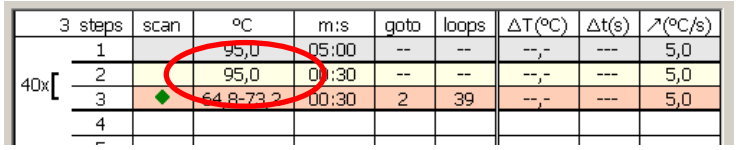
The fluorescence measurement is automatically defined when the melting curve is recorded.

3.2.9 Programming the temperature gradient

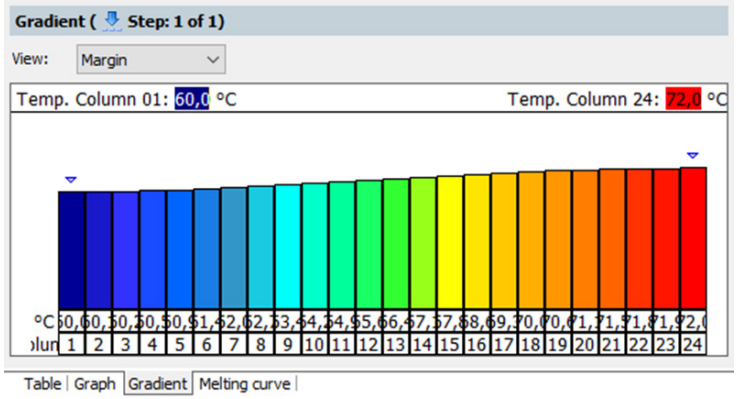
Gradients can be programmed over the whole temperature range of the thermoblock between 4.0°C and 99°C. The gradient range can be ≤40°C.

Temperature gradient with margin rows

- Gradients are defined in the program table by entering 2 temperature values separated by a dash. The first value entered corresponds to the temperature in column 1 (left block side), the second value to the temperature in column 24 (right block side).



The progression of the temperature gradient can be reviewed on the **Gradient** tab. The temperatures in the individual columns of the block are summarized in a table below the bar graph.

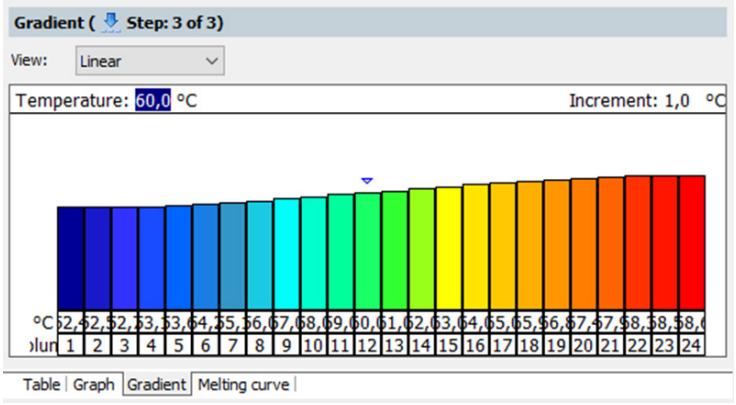


- Adjust the gradient progression by entering temperature values for the first (**Temp. Row 1**) and last (**Temp. Row 24**) column of the block.
  The values displayed in the table are then updated.
- Alternatively, you can click on the top end of column 1 or column 24 and drag the cursor to change the height of the column. Moving the columns changes the respective temperature value accordingly.

Programming a linear gradient

In addition to the option of defining a gradient by entering temperature values for columns 1 and 24, the gradient can also be programmed starting with an annealing temperature in the center of the block using fixed temperature increments.

- On the **Gradient** tab, from the **View** list, select the **Linear** option.
- In **Annealing temp.**, enter the temperature for column 12. In **Increment**, enter the desired temperature change from column to column.
  The values displayed in the table are updated after the values have been entered.
- Another way to adjust the gradient is by clicking on the top end of column 12 and dragging the cursor to change the height of the column. Moving the column changes the annealing temperature accordingly.

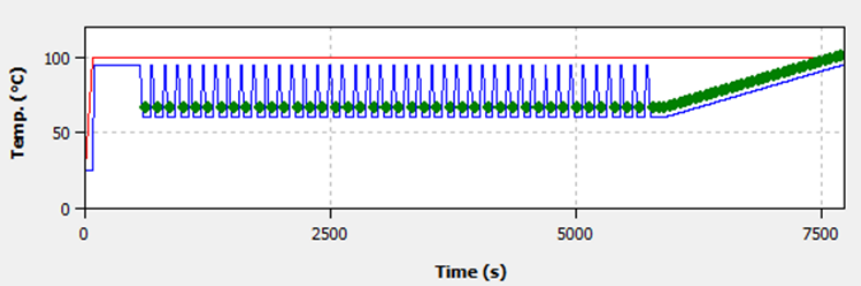


Programming a linear gradient

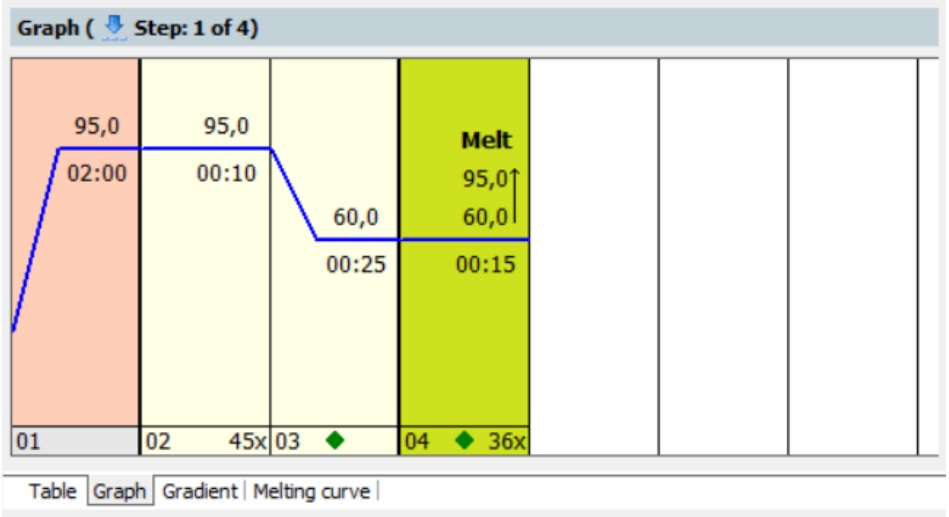
3.2.10 Graphical display and programming the PCR protocols

The **Settings/Thermocycler** project window graphically displays the history of a programmed PCR protocol in the **Table** and **Graph** screens. It represents the temperature curve of the block (blue line)

and the heating lid (red line) over time. The green diamond marks steps during which the fluorescence is measured.



Programs are generally created in the table view, which allows new steps to be programmed quickly and provides a summarized overall survey of the protocol structure. Some programming options are only available in the table view. The graphical programming mode additionally offers a schematic representation of the temperature profile and the option to adjust protocols quickly. In the **Settings/Thermocycler** project window, the table view and the graphical mode can be switched via the **Table** or **Graph** sheets.



The graphical programming is generally performed in the same way as in the programming table.

- By selecting a step (clicking on it), it becomes active and is highlighted light red.
- The bottom part of the display shows the number of the corresponding protocol step.
  Next to it, the number of repetitions in loops (right) and scanning processes (middle) can be programmed in this field. The number of repetitions is indicated as a figure (e.g., 40x) and can be edited by clicking on it.

Planned measurements are displayed by means of a green diamond in the middle of the field and can also be selected or deselected by clicking on it.

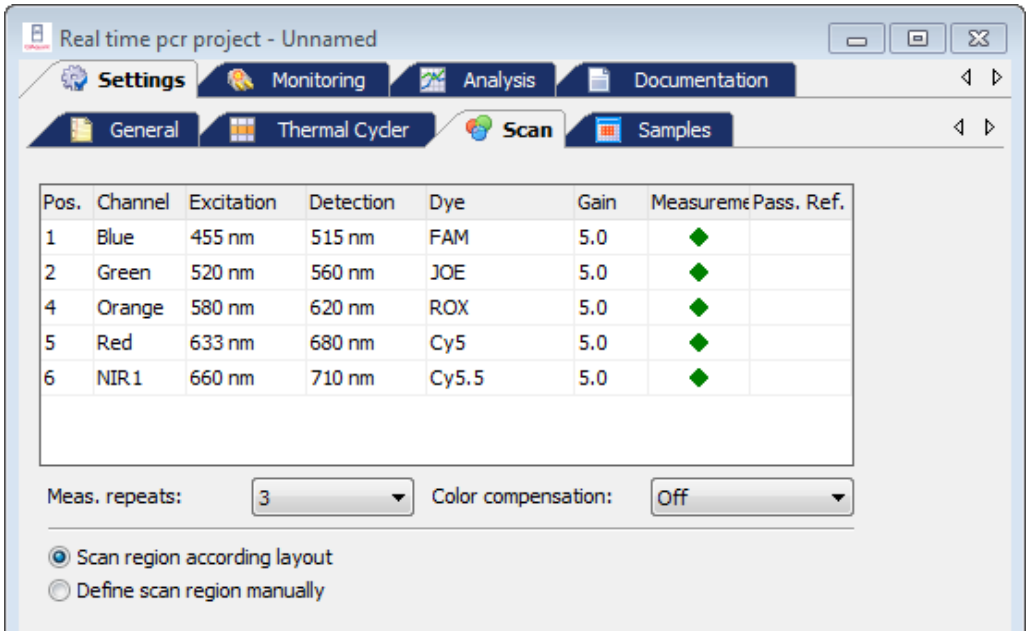
- Temperatures and hold times are indicated as numerical values above or below the blue line that displays the corresponding temperature level at the individual steps. The values can be modified by clicking on them. Melting curve steps are marked with the addition **Melt**. Additionally, an upward-pointing arrow is displayed with the melting curve steps (↑).
  Note: The number of repetitions in melting curve steps cannot be modified.
- The temperature value at each step can be modified using the mouse. For this purpose, click and drag to move the blue line of the temperature curve up or down.

3.3 Defining the parameters for the fluorescence measurement

The PCR amplification is measured by the increase in fluorescence in the real-time PCR. The following measurement parameters must be defined for that purpose:

- Dyes to be measured
- Temperature step of the PCR protocol during which a measurement is to take place
- The area on the PCR plate that is to be scanned

The colors to be measured are defined in the **Settings/Scan** project window:



Up to 5 color channels with different excitation and detection wavelengths can be used for the fluorescence measurement. The parameters of the fluorescence measurement apply for all layout samples on which a measurement is to be performed.

The excitation and detection wavelength information is only displayed in the scan menu when the QIAquant 384 instrument is connected to the computer and switched on.

The **Scan** tab contains a table with different parameters for defining the scan properties:

Table parameters	Description
Pos.	Color module position in the device
Channel	Color channel description
Excitation/Detection	Excitation and detection wavelength of the color channel
Dye	Defines the dye to be measured for the corresponding channel in the table by means of a selection list
Gain	Regulation of 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 corresponding channel. Default value: 5.
Measurement	Activates dye measurement. An active measurement is indicated with a green diamond (◆).
Pass. ref.	The LED technology of the device does not require a passive reference. If you wish to measure a reference dye anyway, place a checkmark in this column.

The information on position, excitation, channel, and detection of the available dyes cannot be modified in this table. Following lists and options are available on **Scan** tab:

Option	Description
Meas. repeats	Enter the number of repetitions of the fluorescence measurement. Possible values: 1–16.
Color compensation	Activate spectral compensation (see "Color compensation", page 37).
Scan region according to layout	Sample measurement according to the layout of the samples on the **Samples** tab (see "Editing the sample table", page 40).
Define scan region manually	Sample measurement according to manual settings (see "Manually defining the scan region", page 36).

Set the following parameters for each channel you wish to make a measurement for:

1. Select the dye to be measured in the **Dye** column. Click in the cell and mark the dye in the list that opens.

   Note: The number of measured dyes does not have an influence on the scan time.

2. Set the signal quality in the **Gain** column.
   The default setting is **5**.
3. Activate the fluorescence measurement in the channel in the **Measurement** column by placing a green diamond (◆).
   Channels that are not marked with a diamond will not be measured.
4. If necessary, activate reference dye measurement by placing a [✓] in the **Pass. ref.** column.
5. Enter the number of repetitions for the fluorescence measurements in the **Meas. repeats** field (default setting: **3**).

   Note: An increased number of repeat measurements reduces the measurement value distribution but also creates longer scanning times and thus longer protocolling times.
6. Select one of the options for the **scan region** (**manually or according to layout**).

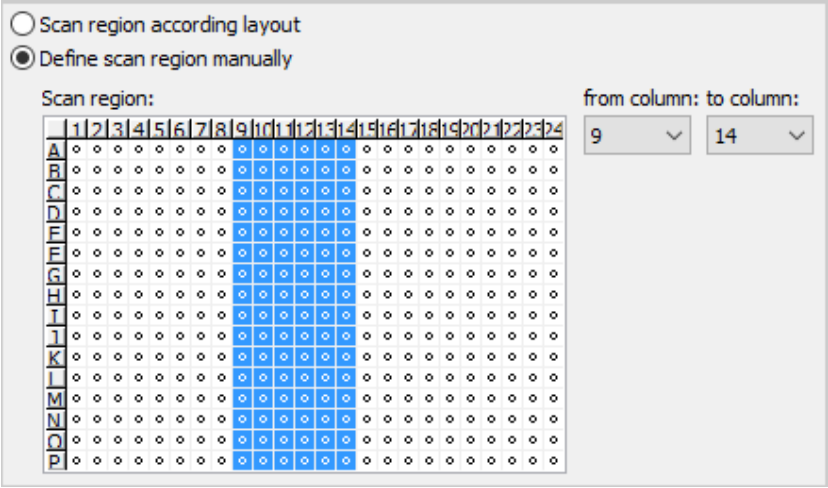
3.3.1 Manually defining the scan region

The scan region can be defined according to the plate layout in the sample table (see "Editing the sample table", page 40) or manually. The scan region for the thermocycler is always defined per column. It must always consist of connected columns.

1. For a manual sample selection, select the **Define scan region manually** option on the **Settings/Scan** tab.
2. A graphical representation of the sample block is opened.
3. Enter the first and last column of the region to be scanned into the **From column** and **To column** fields.

   Optionally, you can use the mouse to select the columns. To select an individual column, click directly into that column. If you wish to select several columns, click and drag the cursor over the corresponding area.

   Active columns are highlighted in blue in the diagram.



3.3.2 Color compensation

If you are using several dyes per reaction mixture, the result may be fluorescence crosstalk. This means that a second dye is excited and measured next to the desired dye at the same time. To subtract the fluorescence quotient of the second dye, you can use the color compensation function on the **Settings/Scan** project tab.

QIAquant 384 Software offers 2 different options for color compensation on measurement data:

1. Using the default color compensation **Standard**
2. Recording and selection of custom color compensation

Color compensation: Off

The default setting for the color compensation is **Off**, because color compensation is not required for the most common applications (only one active measuring channel or dyes that are spectrally widely spaced, such as FAM and ROX).

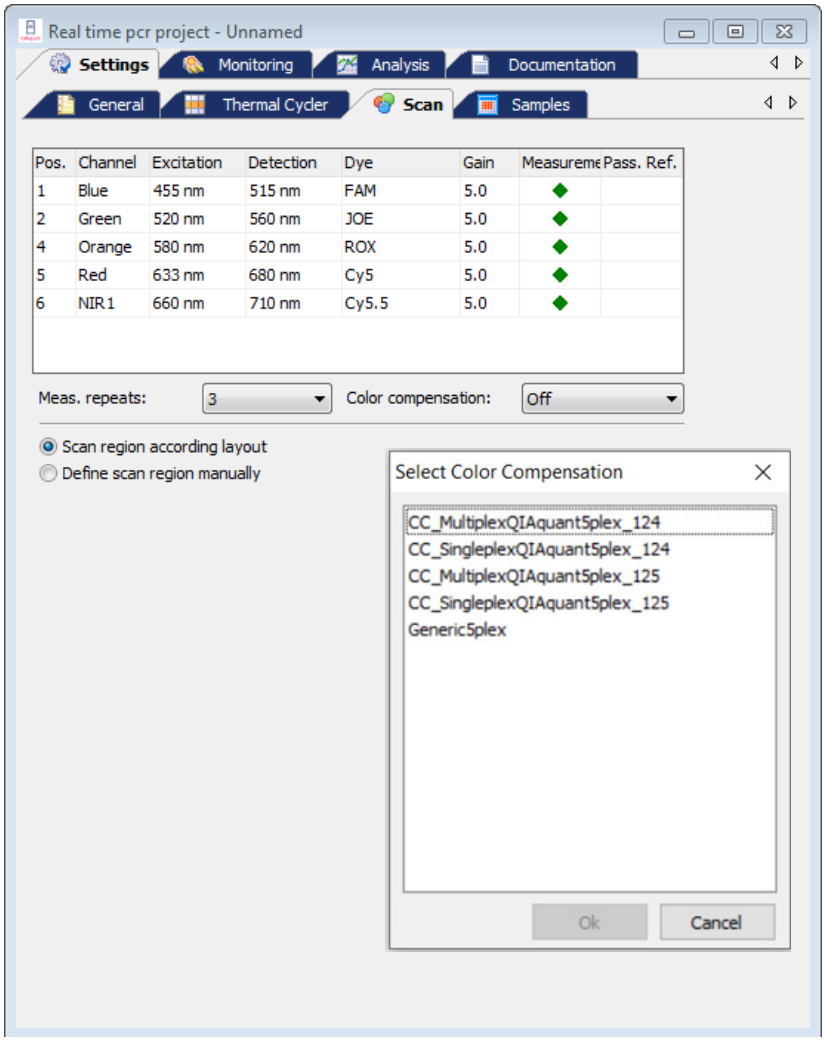
Color compensation: Standard

Using the default color compensation, a compensation matrix is applied to the measurement data. This permits sufficient compensation of the crosstalk in all colors, in the case of the gain setting **5**.

To use default color compensation, select **Standard** from the **Color compensation** list.

Color compensation: Selection

A different color compensation setting can be selected; for instance, using a compensating matrix recorded with the used dyes (see "Spectral calibration" below). Select **Select** from the **Color compensation** list. A window appears in which color compensations that have already been recorded can be opened and used again. In the window, compensation data that meet the settings on the card **Scan** appear in black; invalid compensation data appear in red and cannot be selected.

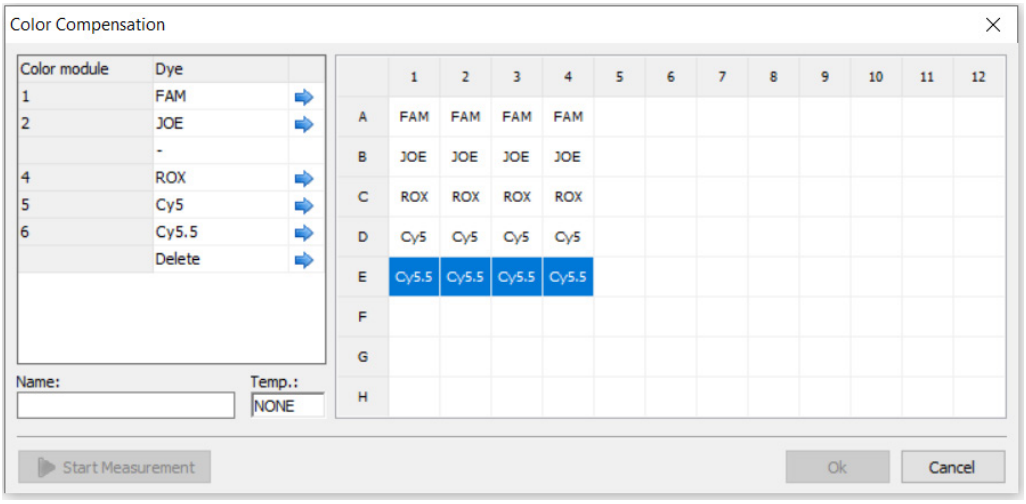


Window for selection of color compensation data

Select a color compensation setting for the current project, and then press **OK**.

Spectral calibration

Select  or **Scan** > **Edit color compensation** to create a new color compensation setting by measurement. This process is called spectral calibration. A new window opens in which all the required settings can be made. The window is divided in a selection list for dyes and a plate diagram.



Color compensation for spectral calibration window

To record the calibration data, the dyes required for color compensation must be available individually in solution. The dye concentration for the calibration measurement should be approximately 0.1 µM/l.

In the displayed plate diagram, the wells that contain the calibration samples are marked individually for each dye. Click the blue arrow to assign the dye in each sample to the marked well. The dyes offered for selection are those that were selected in the **Settings/Scan** project tab.

For an exact calibration measurement, we recommend that each dye is measured at least in triplicate. In addition, the temperature at which the calibration measurement for each dye is performed should be identical. This should be the same temperature at which the fluorescence measurements are performed during the PCR run.

Start the calibration measurement by clicking  **Start measurement**.

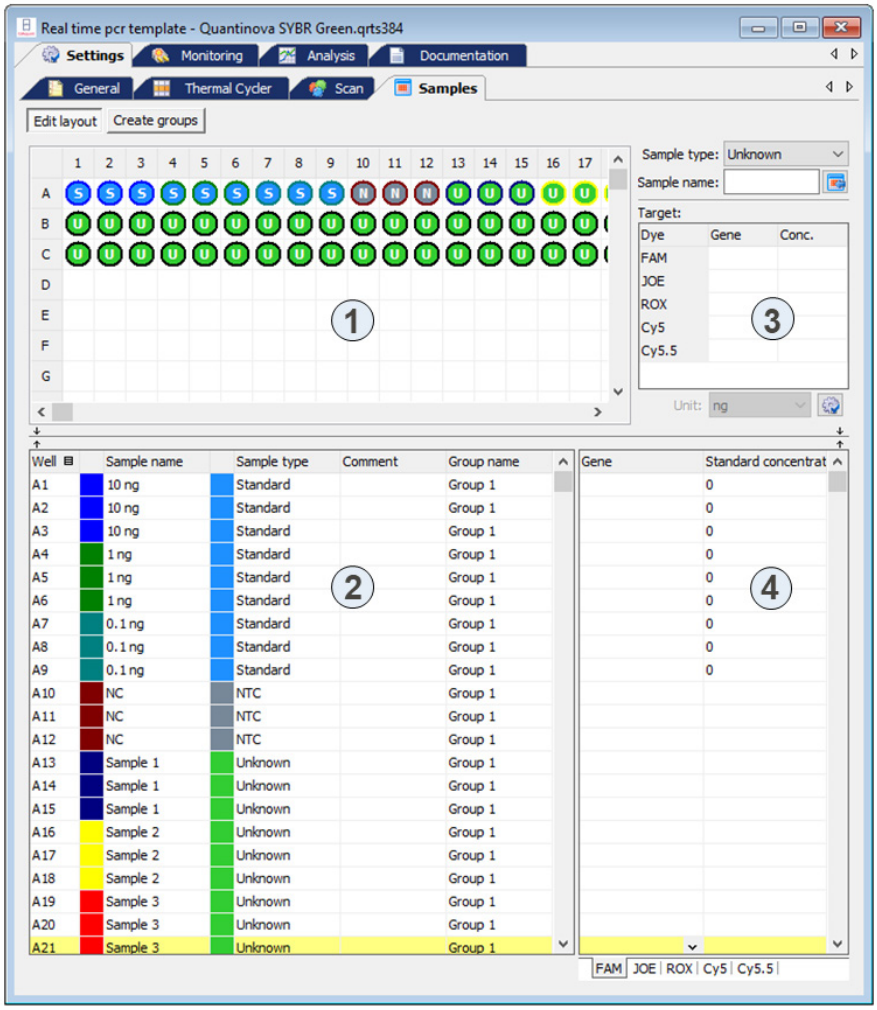
Note: The selection of available dyes cannot be modified in this table. Modifications can only be made on the **Scan** tab.

The new color compensation needs to be assigned a description in the name field. It is included in the selection list after pressing **OK** and will be displayed in the corresponding window. Templates that are no longer in use can be deleted with **Delete**.

3.4 Editing the sample table

The sample table defines which sample is in which position of the block. These details are required for using the evaluation functions of QIAquant 384 Software. Here, a sample can be described by means of its properties, such as name, gene, type, concentration, and dye. Furthermore, samples from different experimental approaches can be combined in groups.

The necessary entries can be made on the **Samples** tab after pressing the **Edit layout** button. The corresponding window is divided into different sections:

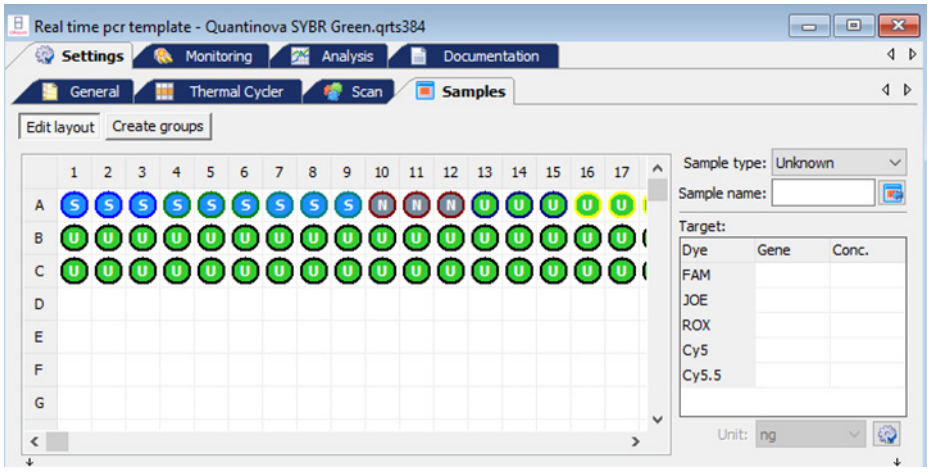


Range	Function
Layout view (1)	Graphical display of the well assignment on the microplate
Sample table (2)	Summary of the information on each sample
Edit area (3)	Edit area for the sample properties: • Sample name • Sample type • Concentration of standard samples • Allocation of the dye and the analyzed gene
Dyes (4)	Dyes and assigned genes for each sample

Note: The sample table can also be edited after the real-time PCR run has been completed.

3.4.1 Entering sample properties in the layout

You can define the properties for the samples in the wells on the **Settings/Samples** project tab in the layout view and the edit area next to it.



Note: The color code of the inner circle that defines the different sample types and the color code of the outer ring that defines replicates can be modified in menu **Extras** > **Options** > **colors**.

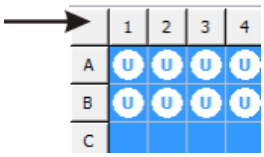
The following sample types can be defined:

Sample type	Symbol	Definition
Empty		Describes an empty position in the PCR plate
Unknown	U	Sample of unknown concentration or dilution (measuring sample)
Standard	S	Sample of known concentration or dilution
Calibrator	K	Sample whose target gene expression level is set to 1
No template control (NTC)	N	Complete reaction mixture but without template strand
Positive control	+	Positive control assay for which a reaction product can be expected
Negative control	–	Negative control assay for which no reaction product is to be expected

Samples with identical sample properties (sample name, sample type, same gene-dye assignments) are viewed as replicates. For a better overview, replicates are indicated by the same color code of the outer ring. The individual values of these samples are averaged, and their mean value is used for the remaining calculations.

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

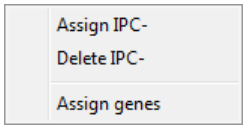
- Mark the sample position to be edited by clicking on the layout. Click and drag the cursor to select adjacent positions. To select nonadjacent positions, click each position while pressing the **Ctrl** key.
  To mark rows or columns, click the corresponding row or column name A–P or 1–24.
  You can mark all sample positions in the layout by clicking the gray button at the top left of the layout (see arrow below).



- Enter the following sample parameters in the adjacent edit area:

Parameter	Description
Sample name	Sample description
Sample type	Selection of the sample type (see top of table)
Target table	
Gene column	In the dye row, enter the gene to be analyzed
Conc. column	For standards Enter the concentration of the gene to be analyzed

- You can assign sample properties to the marked positions by clicking  or pressing **Enter**.
  To clear one or more wells, press the **Del** key.
  Note: The entries for the selected area will only be applied by the program after they have been assigned. Entries or modifications that are not assigned will be lost.
- To display defined sample properties in the edit area, double-click on a well.
  You can edit the information and assign them to the well again by clicking  or by pressing **Enter**.
  To assign these sample properties to other wells, mark the desired wells and then click  or press **Enter**.
- By using the context menu, it is possible to assign gene names to the selected wells afterwards without changing the other properties that had already been assigned. The context menu can also be used to assign and to remove sample wells that do not contain an internal positive control (IPC-). The context menu is displayed by right-clicking the PCR plate scheme.

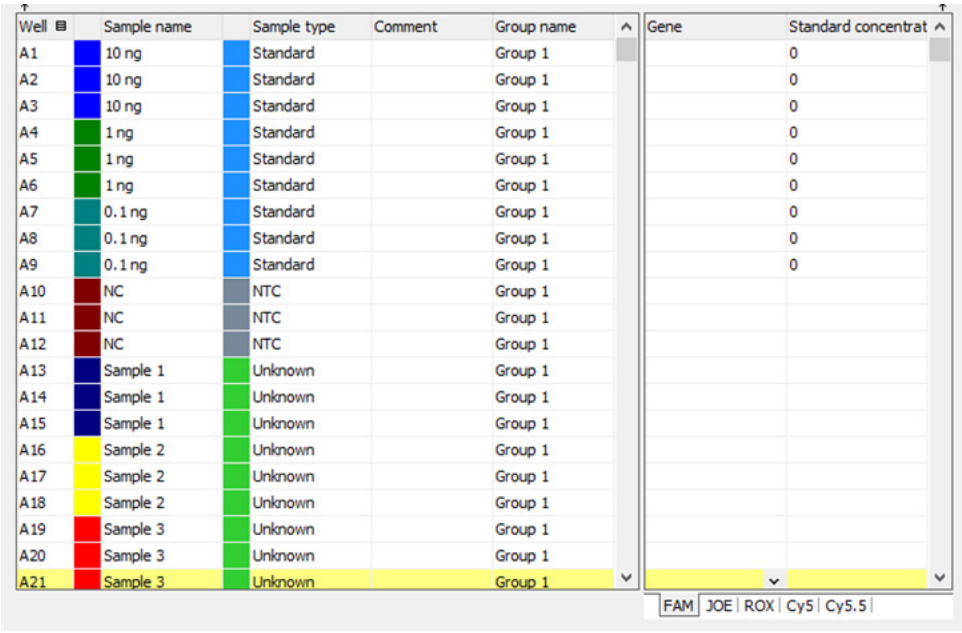


The entry of a sample layout is described by means of an example for a singleplex assay and a multiplex assay (sees “Entering a sample layout for a singleplex assay”, page 48, and “Entering a sample layout for a multiplex assay”, page 46).

3.4.2 Entering sample properties into the sample table

You can also make entries in the sample table itself.

- Select the desired position in the layout view or a field directly in the sample table.
  The corresponding row is then highlighted yellow.
- Enter descriptions and/or values directly in the designated cells.
  The sample table is edited cell by cell. Multiple selections and the associated assignment of parameters to several cells or rows at a time are not possible.



Well	Sample name	Sample type	Comment	Group name	Gene	Standard concentrat
A1	10 ng	Standard		Group 1		0
A2	10 ng	Standard		Group 1		0
A3	10 ng	Standard		Group 1		0
A4	1 ng	Standard		Group 1		0
A5	1 ng	Standard		Group 1		0
A6	1 ng	Standard		Group 1		0
A7	0.1 ng	Standard		Group 1		0
A8	0.1 ng	Standard		Group 1		0
A9	0.1 ng	Standard		Group 1		0
A10	NC	NTC		Group 1		
A11	NC	NTC		Group 1		
A12	NC	NTC		Group 1		
A13	Sample 1	Unknown		Group 1		
A14	Sample 1	Unknown		Group 1		
A15	Sample 1	Unknown		Group 1		
A16	Sample 2	Unknown		Group 1		
A17	Sample 2	Unknown		Group 1		
A18	Sample 2	Unknown		Group 1		
A19	Sample 3	Unknown		Group 1		
A20	Sample 3	Unknown		Group 1		
A21	Sample 3	Unknown		Group 1		

FAM | JOE | ROX | Cy5 | Cy5.5

The genes and, in the case of standard samples, their concentration are summarized separately by dye in the second part of the sample table. A list sheet is assigned to each dye. It is thus possible to use different standard concentrations for each gene. The number of displayed sheets depends on which dyes have been activated in the **Settings/Scan** project tab for this measurement.

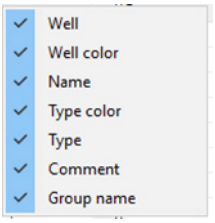
- Enter for each gene the desired standard concentration.

Selecting the table view

Depending on the selected window size and the number of dyes to be measured, 2 buttons with left and right arrows appear. They can be used to move between different tabs.

The number of columns that are displayed in the sample table can be user defined:

- Right-click on a column header to display and mark the desired columns in the context menu.
- The order of the columns can be changed by clicking on the table header and dragging with the mouse.



Selection field for defining the columns displayed in the sample table.

Selecting curve colors

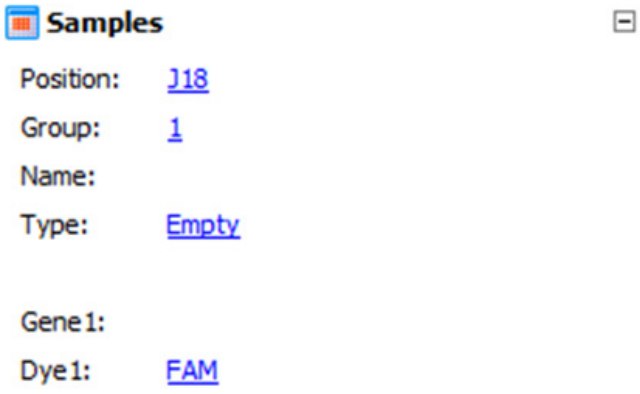
Note: General color settings can be made in the **Options/Color** window (**Extras** > **Options**). This is where the colors for wells, replicates, and sample types are determined.

In the graphic representation, the amplification curve is highlighted in the color shown in the second column of the sample table. You can change the color individually:

- Double-click on the color cell to be changed in the sample table, and select the desired color in the **Color** window.
- To reset the color of a well, press the **Shift** key (⇧) and double-click on the color cell at the same time. The color is reset to the default setting in the **Options/Color** window.
- To allocate the same color to multiple wells, press the **Ctrl** key and double-click on the color cell of a well.
  Mark the applicable wells in the plate layout in the **Edit colors** window and select the shared color. Click **Accept** to assign the color to the wells. Click **Reset** to reset the color changes in the marked wells to the default settings in the **Options/Color** window.

Displaying sample properties in the project explorer

Moving the mouse pointer over a well on the **Sample** project tab in the layout view will display the properties of the well in the **Samples** menu item in the project explorer.



3.4.3 Entering a sample layout for a multiplex assay

The following example shows the definition of 4 samples and standards with 3 repeat measurements each in the layout. The GAPDH gene is analyzed with the FAM dye and the c-Myc gene with the VIC dye. The 2 dyes are selected on the **Settings/Scan** project tab and activated for measurement. The indicated sample names and standard concentrations serve as examples only.

1. Activate the **Edit layout** button.

Emptying sample layout

2. Empty the plate layout to make sure that no unintentional entries remain:
   - Mark the complete plate by clicking on the gray button in the top left of the layout.
     In **Sample type**, select **Empty**.
   - Click  or press **Enter**.

Defining samples

3. Mark the 3 wells A1–A3.
4. Input the following settings:

Parameter	Entered value
Sample name	Sample 1
Sample type	Unknown
FAM	GAPDH
VIC	c-Myc

Note: The genes are allocated to the corresponding dye by entering the name of the gene or by selecting it from the displayed list in the **Target** table.

5. Click  or press **Enter** to assign the sample properties to the 3 wells.
6. Repeat steps 3–5 for the other samples.
   Use the following parameters:

Wells	Sample name	Sample type	FAM	VIC
A4–A6	Sample 2	Unknown	GAPDH	c-Myc
B1–B3	Sample 3	Unknown	GAPDH	c-Myc
B4–B6	Sample 4	Unknown	GAPDH	c-Myc

Defining the standard samples

7. Mark the 3 wells C1–C3.
8. Make the following settings:

Parameter	Entered value
Sample name	Std 1
Sample type	Standard

9. Make the following entries in the **Target** table:

Dye	Gene	Conc.
FAM	GAPDH	100
VIC	c-Myc	50

Note: The **Conc.** column in the **Target** table is only available for the **Standard** sample type.

10. From the **Unit** list, choose a concentration or mass unit. You can select from the following units:
    - ng
    - ng/µl
    - ng/ml
    - pg/µl
    - copies
    - copies/µl
    - copies/ml
    - mg/ml
    - IU/µl
    - IU/ml
    - %
11. Click  or press **Enter** to assign the sample properties to the 3 wells.
12. Repeat steps 7–10 for the other 3 standards.
    Use the following parameters:

		Conc.	
Wells	**Sample name**	**FAM**	**VIC**
C4–C6	Std 2	50	5
D1–D3	Std 3	10	1
D4–D6	Std 4	0.1	0.05

- As sample type, select **Standard**.
- Assign the genes to the dyes as described in step 6.
  - The plate layout for a multiplex assay is complete.

3.4.4 Entering a sample layout for a singleplex assay

The following example shows the definition of 4 samples and 4 standards with 3 repeat measurements each in the layout. The GAPDH and c-Myc genes are analyzed with the FAM dye with the help of 2 sensors. The FAM dye was selected on the **Settings/Scan** project tab and activated for the measurement. The indicated sample names and standard concentrations serve as examples only.

1. Activate the **Edit layout** button.

Emptying sample layout

2. Empty the plate layout to make sure that no unintentional entries remain:
   - Mark the complete plate by clicking the gray button at the top left of the layout.
   - In the **Sample type** list, select **Empty**.
   - Click  or press **Enter**.

Defining samples

3. Mark the 3 wells A1–A3.
4. Make the following settings:

Parameter	Entered value
Sample name	Sample 1
Sample type	Unknown
FAM	GAPDH

5. Click  or press **Enter** to assign the sample properties to the 3 wells.
6. Mark the 3 wells A4–A6.
7. Make the following settings:

Parameter	Entered value
Sample name	Sample 1
Sample type	Unknown
FAM	c-Myc

8. Click  or press **Enter**., to assign the sample properties to the 3 wells.
9. Repeat steps 3–8 for the other 3 samples.
   Use the following parameters:

Wells	Sample name	Sample type	Gene/FAM
B1–B3	Sample 2	Unknown	GAPDH
B4–B6	Sample 2	Unknown	c-Myc
C1–C3	Sample 3	Unknown	GAPDH
C4–C6	Sample 3	Unknown	c-Myc
D1–D3	Sample 4	Unknown	GAPDH
D4–D6	Sample 4	Unknown	c-Myc

Defining the standard samples

10. Mark the 3 wells E1–E3.
11. Make the following settings:

Parameter	Entered value
Sample name	Std1
Sample type	Standard

12. Make the following entries in the **Target** table:

Dye	Gene	Conc.
FAM	GAPDH	100

Note: The **Conc.** column in the **Target** table is only available for the **Standard** sample type.

13. From the **Unit** list, choose a concentration or mass unit. You can select from the following units: ng, ng/µl, ng/ml, pg/µl, copies, copies/µl, copies/ml, mg/ml, IU/µl, IU/ml, or %.
14. Click  or press **Enter** to assign the sample properties to the 3 wells.
15. Repeat steps 10–13 for the other standards.
    Use the following parameters:

Wells	Sample name	Gene	Conc.
E4–E6	Std. 1	c-Myc	100
F1–F3	Std. 2	GAPDH	75
F4–F6	Std. 2	c-Myc	75
G1–G3	Std. 3	GAPDH	50
G4–G6	Std. 3	c-Myc	50
H1–H3	Std. 4	GAPDH	10
H4–H6	Std. 4	c-Myc	10

16. Click  or press **Enter** to assign the sample properties to the 3 values.

    Note: Connected samples must have the same sample name.

    - The plate layout for a singleplex assay is complete.

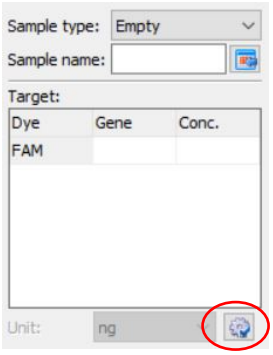
3.4.5 Automatic generation of dilution series/replicates

If dilution series or replicates are measured in an experiment, the layout creation can be automated.

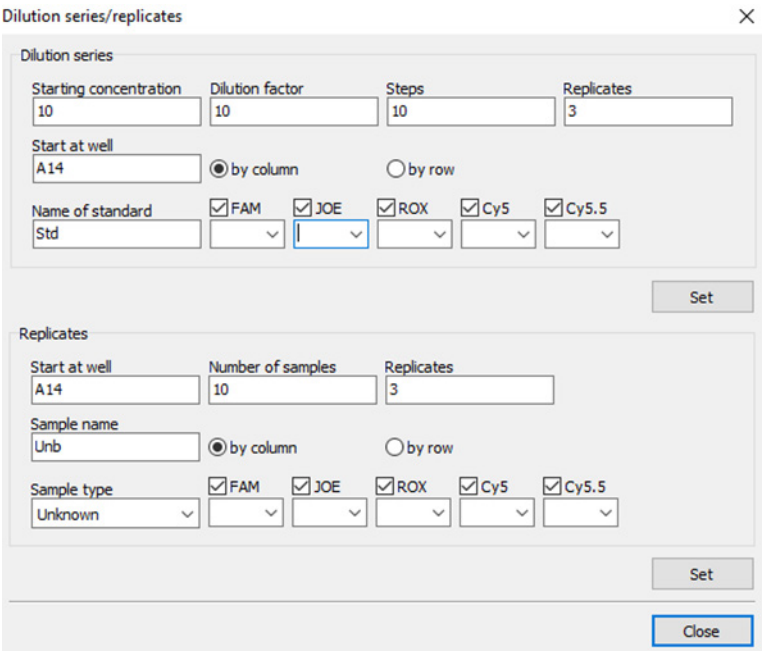
17. Mark the well at which the dilution series or the sample replicate sequence should start (**Start at well**) or mark an area on the PCR plate for the dilution series or replicates.

    If no area is preselected, the plate is automatically filled to the rim.

18. Click  on the **Settings/Samples** project tab in the layout view.



In the window **Dilution series/replicates**, parameters for automatic dilution series and replicates can be defined.



Creating a dilution series

1. Enter the starting concentration, dilution factor, and number of dilution steps and replicates.
2. Specify the start point (**Start at well**) and select whether the entry in the layout table should be done line by line or column by column.

For determination of the start point, the software automatically takes over the currently active position in the layout or the first position at the upper left from a group of selected wells. Alternatively, the start point can also be defined by manual entry in the corresponding field.

3. Enter a name for the standard sample (**Name of standard**) and assign dyes to be measured (multiple selections are allowed). The default name for each replicate goes up by increments of 1 (e.g., GAPDH1, GAPDH2, etc.).
4. Activate the dyes for which dilution series should be created. Select the names genes for the standards. A separate treatment of dyes (targets) is possible in such a way.
5. Click **Set**.
   - The dilution series is created automatically by the software, and the corresponding data is displayed in the layout and the sample table.

Creating replicates

1. Enter the start point (**Start at well**) and the **Number of samples** and the **Number of replicates**.

   For determination of the start point, the software automatically takes over the currently active position in the layout or the first position at the upper left from a group of selected wells. Alternatively, the start point can also be defined by manual entry in the corresponding field.
2. Enter a **Sample name** and select whether the entry in the layout table should be done line by line or column by column. The sample name for each replicate goes up by increments of 1 (e.g., Test1, Test2, etc.)
3. Specify the **Sample type**.
4. Activate the dyes for which replicates should be created. Select the names genes for the samples. A separate treatment of dyes (targets) is possible in such a way.
5. Click **Set**.
   - Replicates are created automatically by the software, and the corresponding data is displayed in the layout and the sample table.

3.4.6 Defining groups

Several experiments can be performed on a single 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.

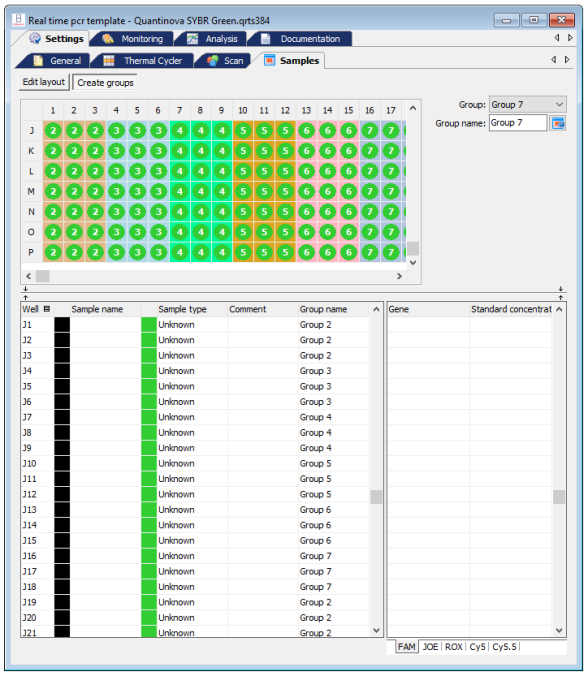
The groups are defined in the **Settings/Samples** project window.

1. In the **Settings/Samples** project window, click **Create groups**.

   The **Group** list and the **Group name** field are activated.
   In the layout, all samples are marked **1**. This means they have been assigned to group 1.
2. In the layout, select the samples that are part of one experiment. To select adjacent positions, click and drag the cursor oven an area. To select nonadjacent positions, click each position while pressing the **Ctrl** key.
3. Select the next group from the **Group** list.
4. Enter the description for the experiment in **Group name**. You may select any group name.
5. Click  or press **Enter** to assign the group properties to the samples.

   The samples that belong together are marked with the group number in the layout.

   The descriptions are displayed in the sample table in the **Group name** column.



3.4.7 Layout preview

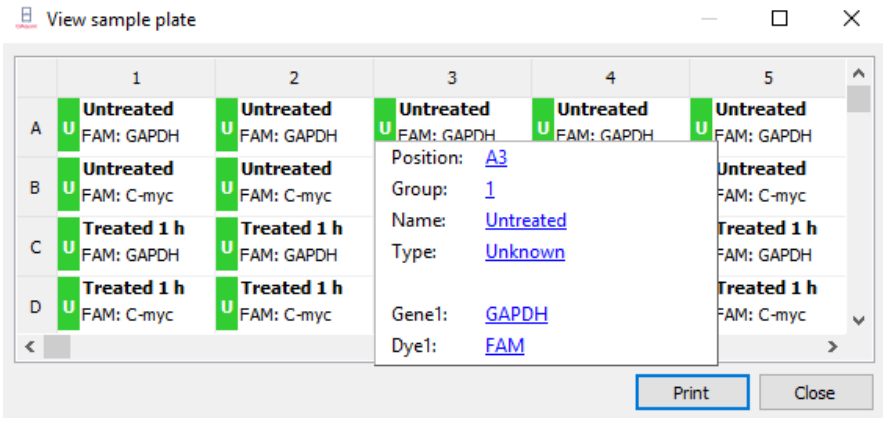
The layout preview provides a complete overview of the layout of the PCR plate with samples and the corresponding information that has been saved for the samples.

- Open the layout preview by clicking  in the toolbar or click **Samples** > **Preview layout**.
  The layout preview is displayed in the **View sample plate** window.

The layout preview provides an overview of the following properties:

- Position on the PCR plate
- Genes to be measured
- Sample type by means of color marking at the edge
- Group affiliation, indicated by colored underline

If you move the cursor to a specific position, all known settings for this position (e.g., 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.



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

- Click **Print** in the **View sample plate** window to print the table.

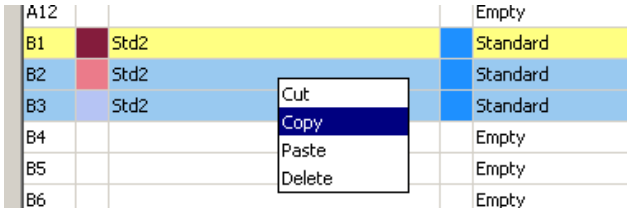
3.4.8 Copying the layout

The layout view or parts of the layout can be copied and inserted into another project.

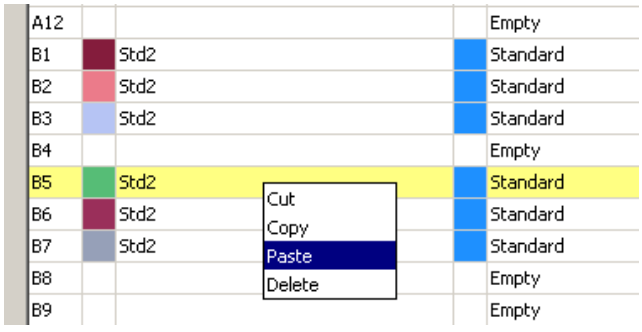
1. Click and drag to mark the area in the layout view to be copied.
2. Transfer the information to the clipboard by clicking  in the toolbar or click **Samples** > **Copy layout**.
3. Select the target project.
4. Paste the information by clicking  or use the **Samples** > **Paste layout** menu command.
   - The copied areas are inserted at the same position in the target project like their position in the source file.

The method described to edit the layout is related to the graphical presentation of the PCR plate in the upper part of the project window. If the layout table is to be edited by copy-paste, this can be done by right-clicking while keeping the Ctr-button pressed. In this way, areas within the same project can be copied.

1. Press and hold the **Ctrl** key down during the whole operation.
2. Mark the lines to be copied or cut by clicking and dragging up or down.
3. Right-click on the desired function



4. Click the line where the copied samples shall be inserted. Right-click to display Paste options.
   - In the example, the standard Std2 is replicated 5 times by its identical sample name.

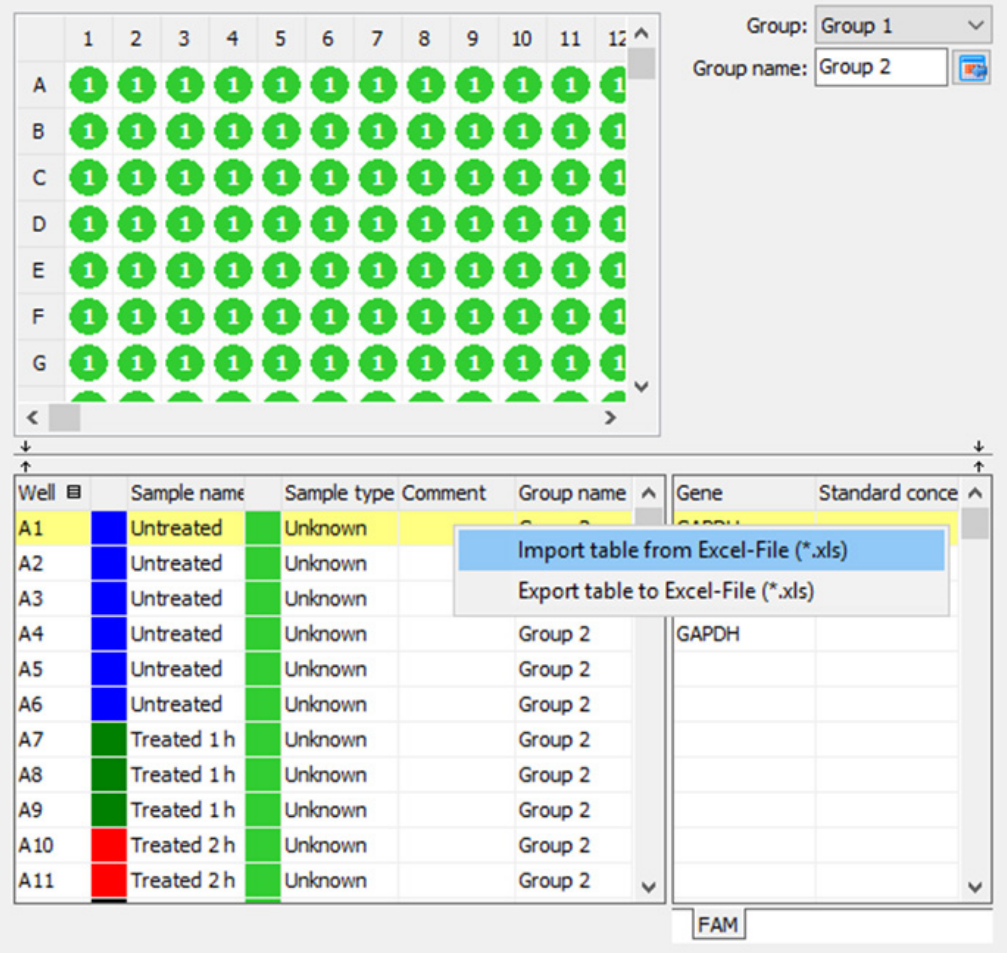


3.4.9 Exporting or importing the layout in Excel®

The layout can be exported or imported as an Excel file (*.xls). The exported data can be edited in Excel and then reimported.

- Right-click on the sample table.
  A context menu with the **Import table from Excel file (*.xls)** and **Export table to Excel file (*.xls)** menu commands opens.

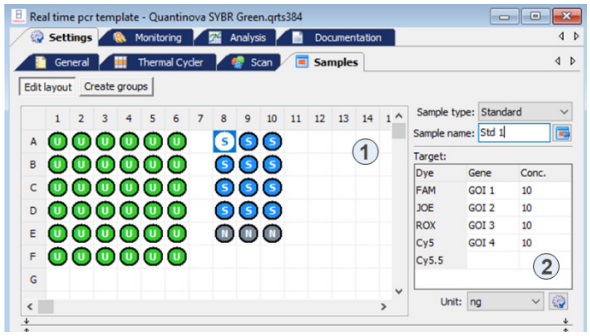
Select the desired menu command.



Excel export and import function for the sample layout

3.4.10 Functions for creating and editing a plate layout

Action	Where	Function
Click a well	PCR plate scheme	Selects this well
Double-click a well	PCR plate scheme	Shows the information assigned to this well in the edit fields
Click and drag	PCR plate scheme	Selects consecutive wells
Ctrl + click	PCR plate scheme	Selects this well additionally
Ctrl + click and drag	PCR plate scheme	Selects consecutive wells additionally
Right-click selected wells	PCR plate scheme	Opens context menu for: • Assigning and deleting wells that do not contain an internal positive control (IPC-, only relevant to end-point analysis) • Assigning genes afterwards (Gene names shown in the edit field will be assigned to selected wells.)
ENTER	Keyboard	Complies with function **assig** (symbol  placed near the edit field **Sample name** or in the toolbar)
DEL	Keyboard	Deletes information assigned to well and changes sample type to **Empty**
Function button F5	Keyboard/ edit field **Gene**	Removes the selected gene from the displayed list of genes
Click the table header, column **Well**	Table	Changes sort sequence from **by line** to **by column** and vice versa
Right-click table header	Table	Opens context menu for selection of colums to be displayed in the table
Right-click and drag table header	Table	Changes the sequence of the colums
Click a line	Table	Highlights this line and allows for data input or selection of sample types directly in the table
Right-click on a line	Table	Opens context menu for: • Exporting the layout table to XLS • Importing the layout table from XLS
Ctrl + right-click (+ drag) on a line of the table (keep **Ctrl** pressed)	Table	Opens context menu to copy, cut, paste, or delete the contents of the selected table lines
 below target table	Edit field	Opens dialog for automatically creating dilution series and replicates
Double-click on the color cell in the table row	Table	Opens the **Color** window for selecting the color of the amplification curve
Shift key and double-click on color cell	Table	Resets the color setting of the amplification curve to the default setting in the **Options/Color** window
Ctrl + double-click on color cell	Table	Opens the **Edit color** window. A selected color can be allocated to the amplification curves of multiple wells at the same time.



1: PCR plate scheme

2: Edit fields

4 Monitoring

All functions required for starting and monitoring a real-time PCR run are combined in the **Monitoring** project tab.

Note: After the PCR run is finished, you can either save the project or continue without saving. If you save the project, you cannot change the settings anymore and, for data integrity, can no longer start a new PCR run from that project. If you want to start a new PCR run with the same settings, you must first generate a template from the project and then open it.

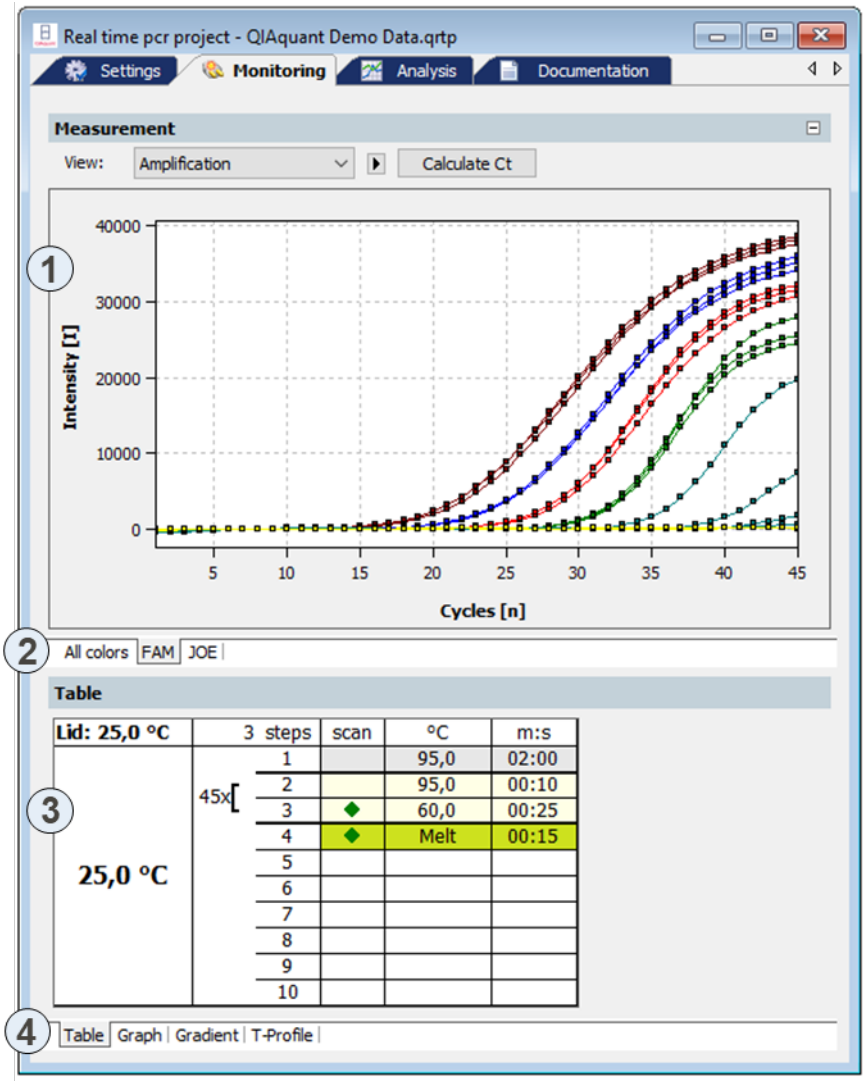
4.1 Starting the PCR protocol

Activate the **Monitoring** tab in the project window to display the symbols for starting the PCR and the **Monitoring** menu protocol defined in the **Settings** project window in the tool bar.

Symbol	Monitoring/... menu command	Description
	Start qPCR run	Start the PCR run
	Stop qPCR run	The PCR run is interrupted and will not be continued. The data recorded up to this point is saved and can be evaluated.
	Pause qPCR run	The PCR run is interrupted. The symbol flashes during the break. The PCR run can be continued by clicking  again.
	View options	Defines default settings for the Monitoring view.

4.2 Display options for monitoring

The **Monitoring** project window is divided into the following areas:



Note: The image shows the window with the lid and thermoblock temperatures turned off. The 25°C displayed represents room temperature. Lid temperature during the run is 105°C.

Area	Function
Measuring results (1)	Displays the measured fluorescence data. The fluorescence intensity is plotted against the cycle.
Colors tabs (2)	Switches between the fluorescence accumulation curves that were measured for individual dyes.
PCR protocol (3)	Displays the PCR protocol. The active step is indicated by a green arrow.
Protocol view tabs (4)	Switches between different views of the PCR protocol (tabular, graphic, temperature profile).

The fluorescence measurements are displayed in the top area. On the different list sheets, you can choose between the display of the measurement results of all dyes simultaneously or the display of the individual dyes.

In the **View** list, you can switch between the **Amplification**, **Melting curve**, and **Raw data** views. The C_t value can be calculated for the amplification and the melting temperature T_m for the melting curve.

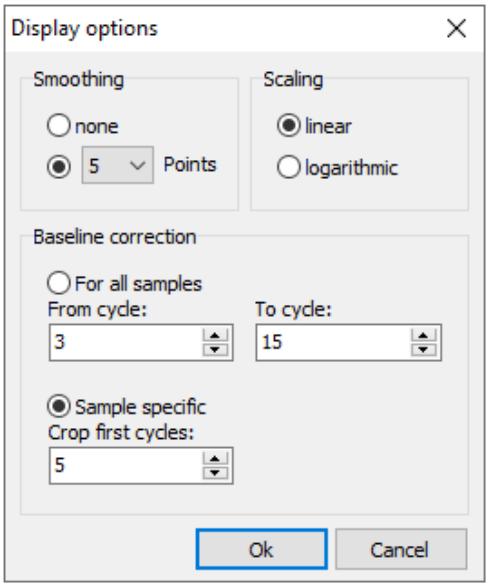
The current PCR protocol is displayed at the bottom of the table in the project window. The active step is marked with a green arrow during the PCR run. On the different sheets you can choose between a tabular or graphical view of the PCR protocol or the temperature profile. The view of the PCR protocol is described in the section "Monitoring the PCR run", page 63.

Note: For each amplification curve, a short information is displayed when the mouse pointer hovers on it.

4.2.1 Default settings for the Monitoring view

For all views in the **Monitoring** project window, it is generally possible to choose between linear and logarithmic scaling for the graphical display of the data. The setting for the baseline correction can also be changed.

- Select **Monitoring** > **Display options** or click  in the toolbar.



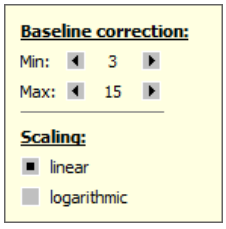
Parameter/Option	Description
Smoothing	Setting the smoothing conditions for the measured data
Scaling	Scaling options for the data (**linear** or **logarithmic**)
Baseline correction	There are 2 options for the baseline correction: **For all samples** If this option is selected, the baseline for every sample in the same range is determined. The upper and lower range limit must be set in the **From cycle** and **To cycle** fields. **Sample specific** Select this option if the curves have significantly different C_t values. The lower range limit for determining the baseline is set in the **Crop first cycles** field for all samples. The upper range limit is determined separately for each sample by an algorithm. **Note**: The type of baseline correction can only be set in this dialog. The range limits for the correction can also be adjusted in the project window.

4.2.2 Adjusting the view in the Monitoring project window

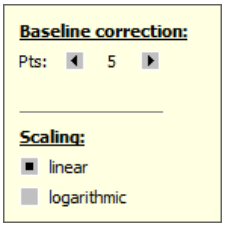
In the **Monitoring** project window, you can adjust the preset parameters (**Monitoring** > **Display options** menu command) for the display of the scaling as well as the range limits for the baseline correction.

1. Click the  button above the chart.

   A selection field for setting the display options and entering the baseline parameters opens.



For all samples baseline correction parameter



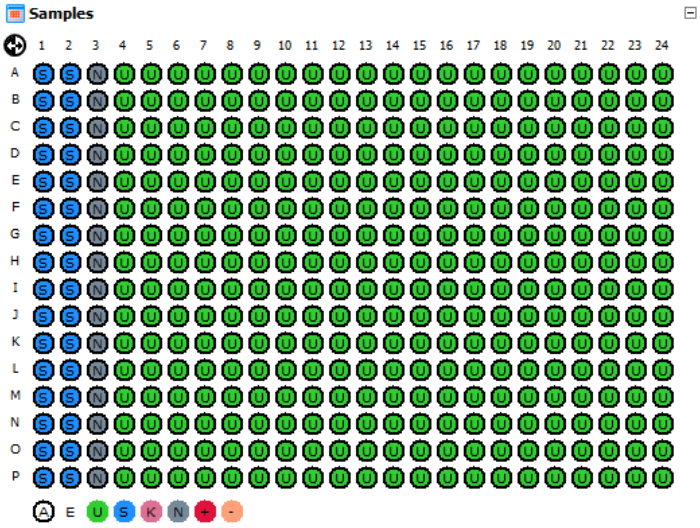
Sample-specific baseline correction parameter

2. Change the baseline correction limits and activate the **linear** or **logarithmic** option for the desired view of the fluorescence curves.

4.2.3 Displaying and hiding measurement results for individual wells

The sample view in the **Monitoring** project window is controlled via the **Samples** menu item in the project explorer. Measurement results in the individual wells can be hidden or shown.

Note: The selection in the project explorer only influences the display of the fluorescence data but not the measurement.



The color code for each sample type can be modified in menu **Extras/Options** > **colors**.

The marking of the sample assignment corresponds to the marking on the **Samples** project tab:

Sample type	Symbol	Definition
Empty	E	Describes an empty position on the PCR plate
Unknown	U	Sample of unknown concentration or dilution (measuring sample)
Standard	S	Sample of known concentration or dilution
Calibrator	K	Sample whose gene expression level is set as 1
No template control (NTC)	N	Complete reaction preparation but without matrix strand
Positive control	+	Positive control preparation for which a reaction product is expected
Negative control	–	Negative control preparation for which no reaction product is expected

Active wells (i.e., displayed wells) are marked with their sample type symbol. For deactivated wells, the position is gray and the fluorescence data is hidden. Empty wells are marked **E**. By default, measurement data for empty wells are not shown. For control, by activating empty wells, the measurement data can be displayed.

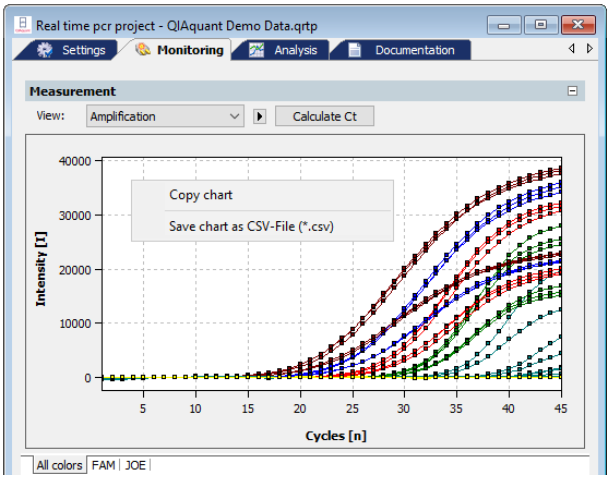
- Click with the mouse to toggle. The activation changes with each click on a well.
- You can select adjacent wells by clicking and dragging the cursor over the wells. To select nonadjacent positions, click each position while holding down the **Ctrl** key.
- Complete rows and columns can be inverted by clicking on the letter or number of rows A–H or columns 1–12.

- The activation status of the complete plate can be inverted by clicking , at the top left, between A and 1.
- To activate all wells, click the symbol below the chart.
- To activate only samples of a specific tpe, click the corresponding symbol below the chart. To activate multiple sample types at the same time, click each sample type while holding down the **Ctrl** key.

4.2.4 Exporting fluorescence data

The data from the fluorescence measurement can be exported as CSV files. In addition, the graphical display of the measurement results can be copied onto the clipboard as a hard copy and is hereby made available for other programs.

- Right-click on the graph.
- A selection window for export and hard copy appears.
- Click **Copy chart** to copy the chart to the clipboard.
- Select the **Save chart** option to export the fluorescence data.
  The **Save as** standard window opens.
  Enter a file name and confirm with **OK**.

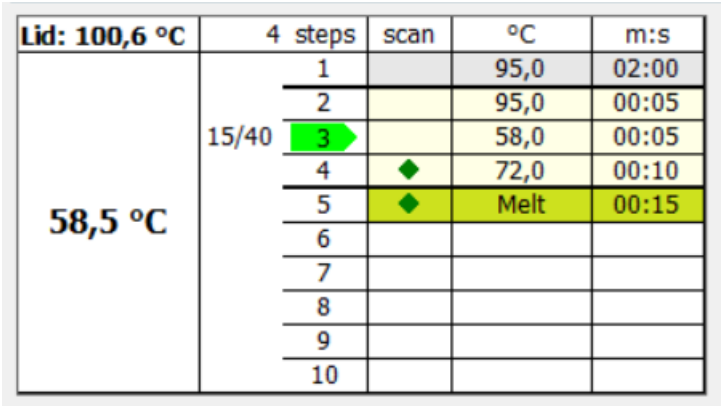


4.3 Monitoring the PCR run

The running PCR protocol is shown in the bottom part of the **Monitoring** project tab. It is generally possible to choose between the 4 different views (list sheets) **Graph**, **Table, Gradient**, and **T-Profile** via tabs.

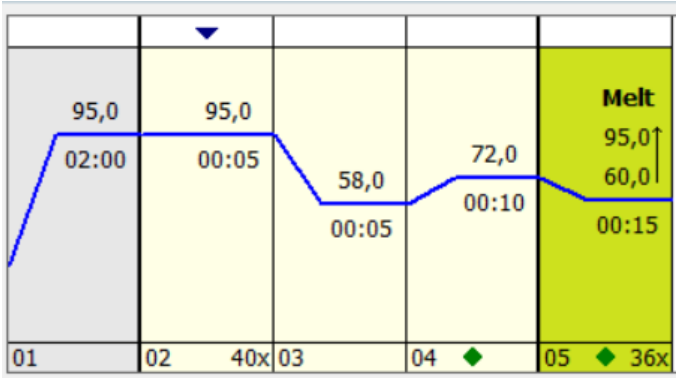
In addition to the display on the list sheets, a status bar shows further information on the protocol, such as the plateau time, the calculated remaining time, and (in programmed loops) the step number and the number of loops.

Table list sheet



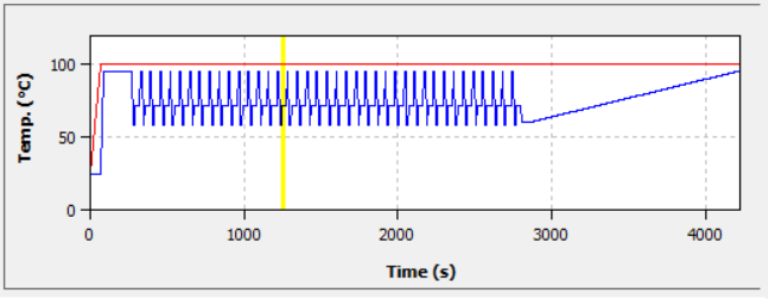
Element	Description
Lid	Current lid temperature
Temperature display	Current block temperature
Steps	Temperature steps in the PCR protocol. The active step is marked by a green arrow.
°C	Target temperature of the step

Graph list sheet



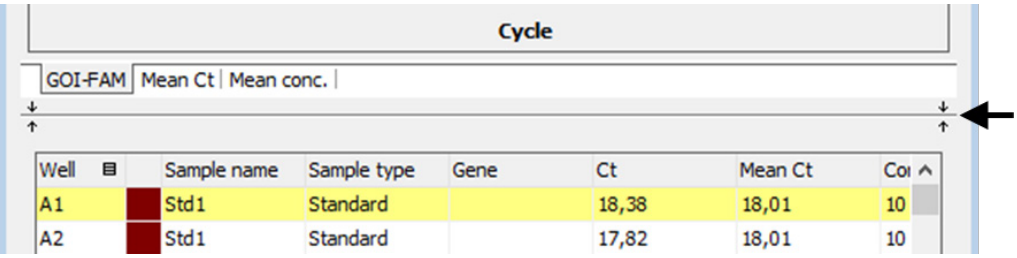
The **Graph** list sheet contains the same elements of the graphical representation of the PCR protocol as the **Settings/Thermocycler/Graph** project window. Once again, the active step is marked by a green arrow.

T-profile list sheet



In the representation of the temperature profile, a yellow progress bar indicates the step that is currently being performed.

The size of the area displayed for fluorescent curves and results table or standard curves can be adjusted by the slider control that is located between the 2 areas.



4.4 Displaying product accumulation curves and calculating C_t values

The product accumulation is documented by means of fluorescence measurements during the PCR run in the project window **Monitoring**.

Displaying the product accumulation curve

- Select the **Amplification** or **Raw data** option from the **View** list to display measurement curves for the product accumulation.
  In the chart, the fluorescence intensity I is plotted against the number of cycles in relative units. The color of the curve that is being displayed corresponds to the color assigned to each well in the sample table (**Settings/Sample** project window).

- Select the measurement results for the individual dyes via the corresponding list sheets.
  You have the option to display the measurement results of all dyes together (**all colors** list sheet) or only the results of an individual dye.

The display options for the product accumulation curves are described in the section "Display options for monitoring", page 59.

Calculating the C_t values

After the PCR run, the C_t values can be calculated directly from the amplification curves without having to create an analysis such as **Absolute quantification**.

- Select the **Amplification** or **Raw data** option from the **View** list and click **Calculate Ct**.
  The amplification curves are normalized and displayed individually or together on the list sheets for the dyes. The table below the displayed amplification curve window shows the C_t values of the individual samples and the mean values of the replicates.
  The threshold value for the individual dyes can be set on the applicable list sheet. The parameters that were set in the **Options/Analysis** window are factored in.
- After clicking on **Data**, you return to the display of the fluorescence intensities.

Export of data

The **Amplification data**, **Raw data**, and **Ct values** can be exported individually as *.csv file by right-clicking into the respective graph or table and selecting **Save Graph/Table as CSV file** (*.csv).

To export all data within one single *csv file, right-click into the table displaying the Ct values and select **Export LIMS as CSV-File** (*.csv).

4.5 Displaying melting curves and calculating melting temperatures T_m

Displaying melting curves

The course of the melting curve after the PCR can be monitored in the **Monitoring** project window.

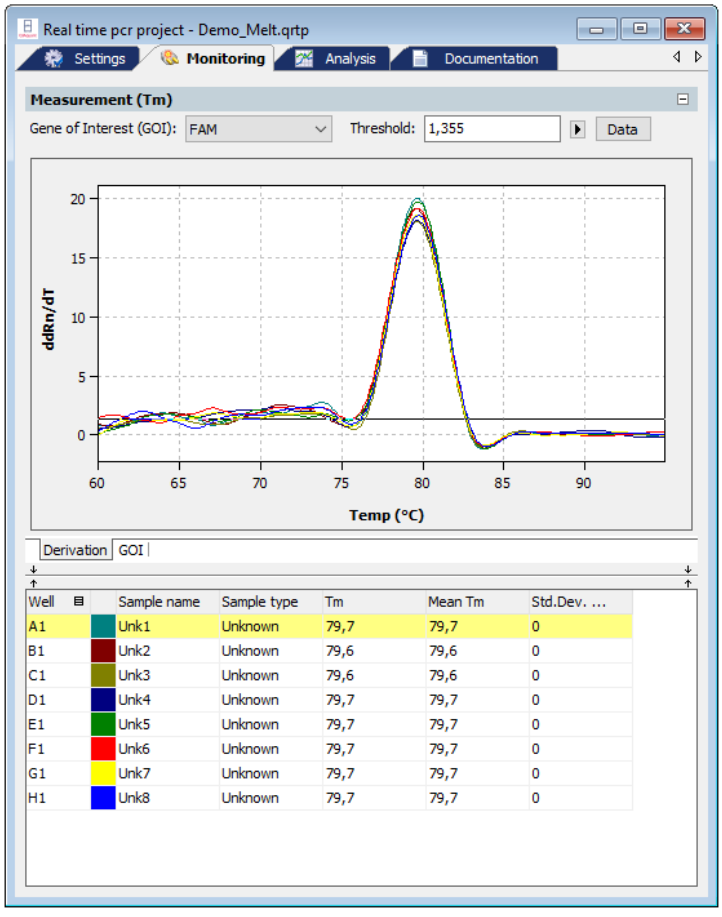
- To display the melting curves, select the **Melting curve** option from the **View** list.

The display options for the product accumulation curves are described in "Display options for monitoring", page 59.

Calculating the melting temperature T_m

For a PCR run with included melting curve, the melting temperatures can be calculated in the Monitoring window without having to create a **Melting curve** analysis.

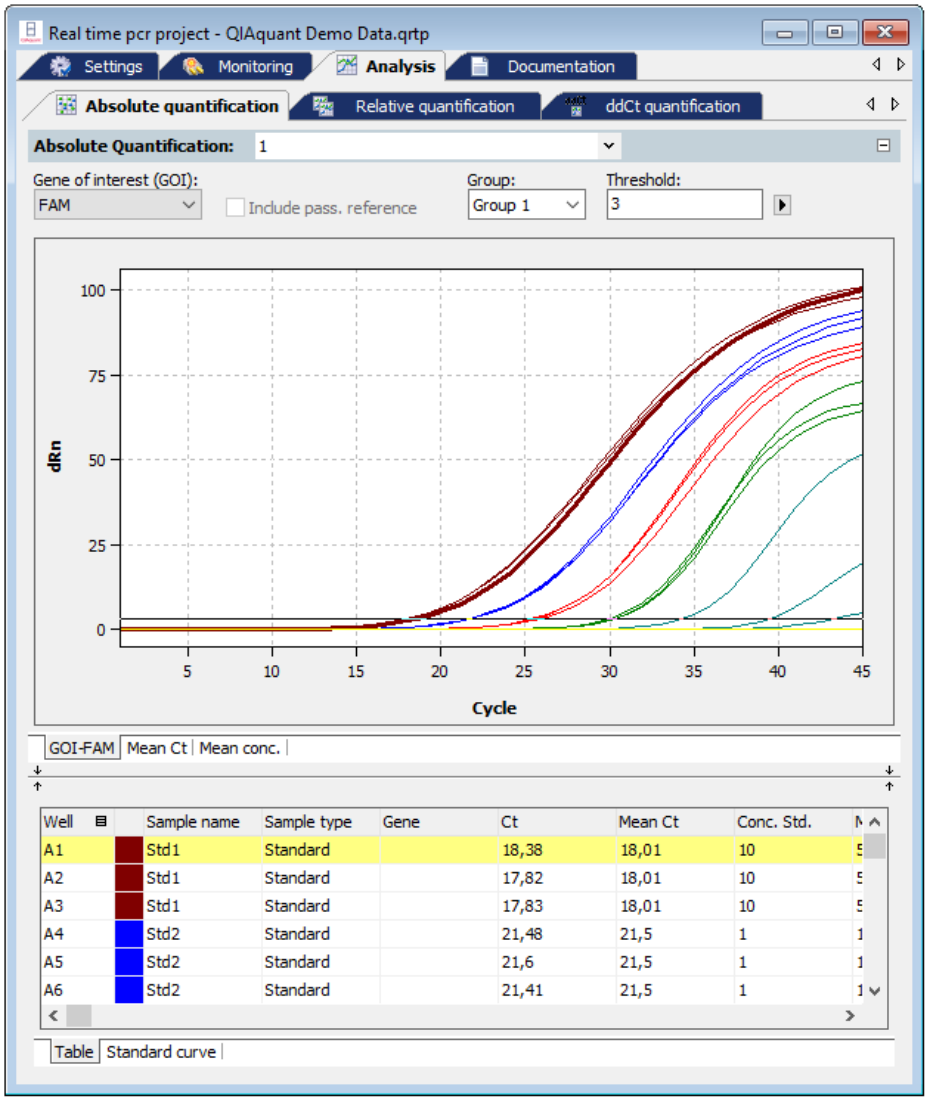
- In the **Monitoring** project window, select the **Melting curve** option in the **View** list.
- Click **Calculate Tm**.
- Select the gene to be examined in the **Gene of Interest (GOI)** list.
  The melting temperature is calculated, taking into account the parameters set in the **Options/Analysis** window, and the diagram and the results table are displayed.
  As an option, a threshold value can be set on the **Derivative** tab, with which significant peaks can be distinguished from insignificant ones.
- After clicking on **Data**, you return to the display of the fluorescence intensities.



5 Analysis

On the **Analysis** tab of the project window, the following methods are available for evaluating real-time PCR experiments on 6 cards:

- Absolute quantification
- Relative quantification
- ΔΔCt method
- Melting curve determination
- Genotyping
- POS/NEG analysis at the end point



The individual analysis methods can be accessed via the subordinated tabs. For each selection method, different analysiss can be created.

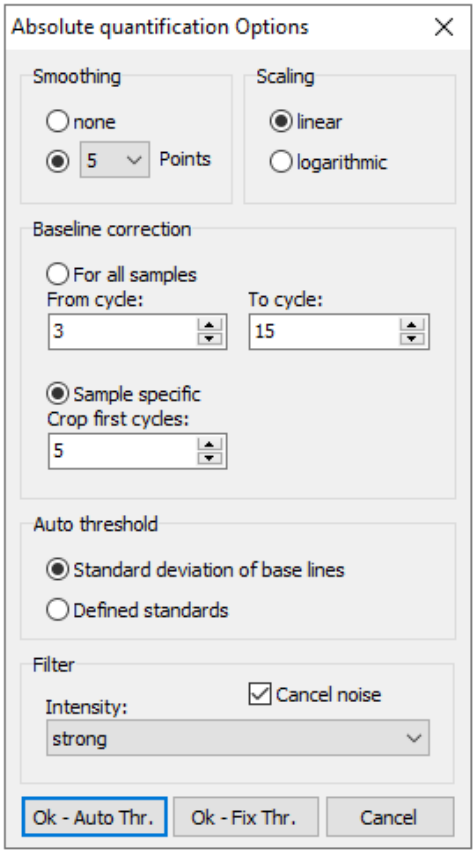
Toolbar and menu commands are adjusted to the requirements of the selected method tab.

5.1 General functions in the analysis project window

5.1.1 Making basic settings

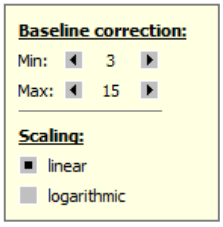
Presets can be made for some analysis parameters.

1. Click  in the toolbar.
2. Set up the following parameters:

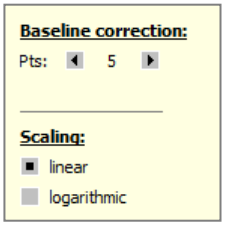


Option	Function
Smoothing	Smoothing the fluorescence curves on the basis of the calculated moving average over a range of 2–12 measuring points or representation without smoothing
Scaling	Linear or logarithmic representation of fluorescence curves
Baseline correction	At the correction of the base line, you can choose between 2 options: • For all samples: At this correction, the base line is determined for every sample in the same area. The lower and upper area limits have to be edited in the fields From cycle and To cycle. • Sample specific: This correction should be chosen if the curves have very different Ct values. The lower area limit for the determination of the base line will adjusted in the field Crop first cycles for all samples. The upper area limit is separately found out by an algorithm for each sample. **Note:** The manner of the base line correction can be selected only in this dialog. The area limits can, however, be adapted for the correction in the project window.
Autom. threshold	Calculation of the threshold as a deviation of x times of the standard deviation of the baselines (factor can be adjusted under **Extras/Options/Analysis** in the main menu) or based on defined standards, with the goal to get the maximum value for the coefficient of determination (R^2)
Filter	Digital filter for smoothing the fluorescence curves; adjustable in steps: slight, medium, and strong
Cancel noise	Curves with high background noise that are not interpreted as amplification curves by the software are set to 0, and no C_t values are calculated
Auto Threshold	The threshold line is calculated anew when changes to the basic settings are made
Fix Threshold	The set threshold line is maintained when changes to the basic settings are made

More setting options may be available, depending on the analysis method used. They are explained separately in the respective sections. All items displayed on the **Analysis** project tab can also be accessed quickly via the settings area of the baseline and displayed as linear or logarithmic representations. For this purpose, a selection for display options can be opened in the corresponding window via the right-arrow button ▶:



For all samples baseline correction parameter

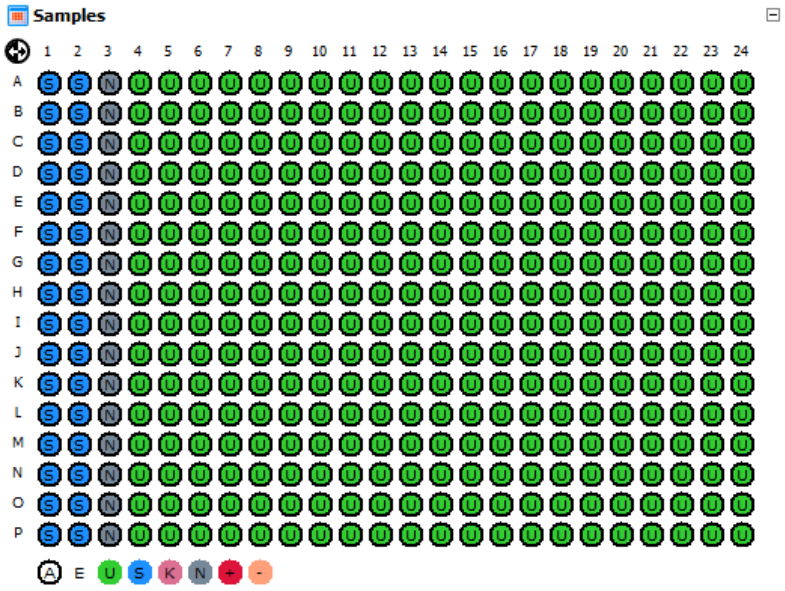


Sample specific baseline correction parameter

5.1.2 Activating/deactivating samples for analysis

Samples from individual wells can be activated or deactivated for analysis in the project explorer **Samples** menu item. This enables you, for instance, to exclude excessive values when calculating mean values.

Note: The selection in the project explorer only influences the analysis of the fluorescence data. Measured data will not be deleted.



The marking of the sample assignment corresponds to the marking on the **Samples** project tab. The color code for each sample type can be modified in menu **Extras/Options** > **colors**.

Sample type	Symbol	Definition
Empty	E	Describes an empty position on the PCR plate
Unknown	U	Sample of unknown concentration or dilution (measuring sample)
Standard	S	Sample of known concentration or dilution
Calibrator	K	Sample whose gene of interest expression level is set as 1
No template control (NTC)	N	Complete reaction preparation but without matrix strand
Positive control	+	Positive control preparation for which a reaction product is expected
Negative control	–	Negative control preparation for which no reaction product is expected

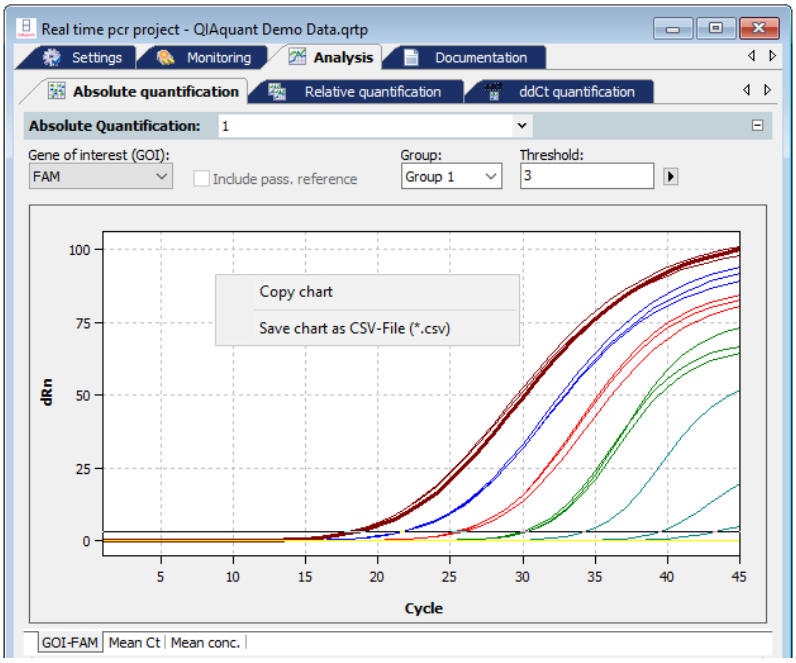
Active wells (i.e., wells included in the analysis) are marked with their sample type symbol. For deactivated wells, the position is gray and fluorescence data is hidden. Empty wells are marked **E**.

- Click with the mouse to toggle activation settings.
- You can select adjacent wells by clicking and dragging the cursor over the wells.
- Complete rows and columns can be inverted by clicking on the letter or number of the rows A–H or columns 1–12.
- The complete plate can be inverted by clicking on the top left between A and 1.
- To activate all wells, click below the chart.
- To activate only samples of a specific type, click the corresponding symbol below the chart. To activate multiple sample types at the same time, click the sample types while holding down the **Ctrl** key.

5.1.3 Exporting fluorescence data

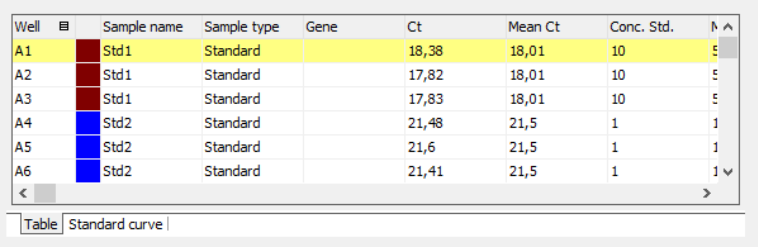
The data from the fluorescence measurement can be exported as CSV files. In addition, the graphical display of the measurement results can be copied to the clipboard as a hard copy and is hereby made available for other programs.

- Right-click on the graph.
- A selection window for export and hard copy appears.
- Click **Copy chart** to copy the chart to the clipboard.
- Select the **Save chart as CSV-File** option to export the fluorescence data.
  The **Save as** standard window opens.
  Enter a file name and confirm with **OK**.



5.1.4 Configure results table

For each analysis, the results are summarized in a table that is accessible by the **Table** tab.



Well	Sample name	Sample type	Gene	Ct	Mean Ct	Conc. Std.
A1	Std1	Standard		18,38	18,01	10
A2	Std1	Standard		17,82	18,01	10
A3	Std1	Standard		17,83	18,01	10
A4	Std2	Standard		21,48	21,5	1
A5	Std2	Standard		21,6	21,5	1
A6	Std2	Standard		21,41	21,5	1

Table | Standard curve

Depending on the analysis method, the results table contains different data sets; but for each table, the view and selection of columns to be displayed can be user defined:

- Right-click on a column header to display a box in which single columns can be selected or deselected to be shown in the table.
- Click and drag a column header to modify the arrangement the columns.
- Click the left or right border of a column header to modify the column width.
- Click a column header to arrange data ascending or descending, numerically or alphabetically.
- Change the colors of the amplification curves by double-clicking on the color cell in the table row, pressing the **Ctrl** key, and then double-clicking the **Edit color** window to set the color for multiple wells (see “Entering sample properties into the sample table”, page 44).
- Click the button on the **Well** column to switch the display of data between columnwise and row-wise representation of the results. The columnwise or row-wise representation is based on the arrangement of samples in the layout.

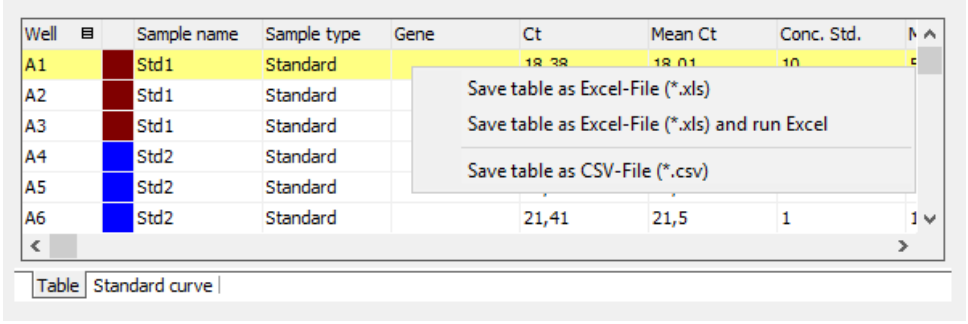
Symbol	Meaning
Well	Display of data by columns
Well	Display of data by rows

5.1.5 Export results

The results table can be exported as an XLS or a CSV file.

- Right-click on the results table. A context menu opens with the commands **Save Table as Excel-File (*.XLS)**, **Save Table as Excel-File (*.XLS) and run Excel** and **Save Table as CSV-File (*.CSV).**
- Select the corresponding command.
- The standard Windows dialog **Save as** opens. Enter a name for the file and click **OK**.

Note: User-defined configurations of the results table are included in the exported data set (see “Configure results table“, page 73).



5.2 Absolute quantification

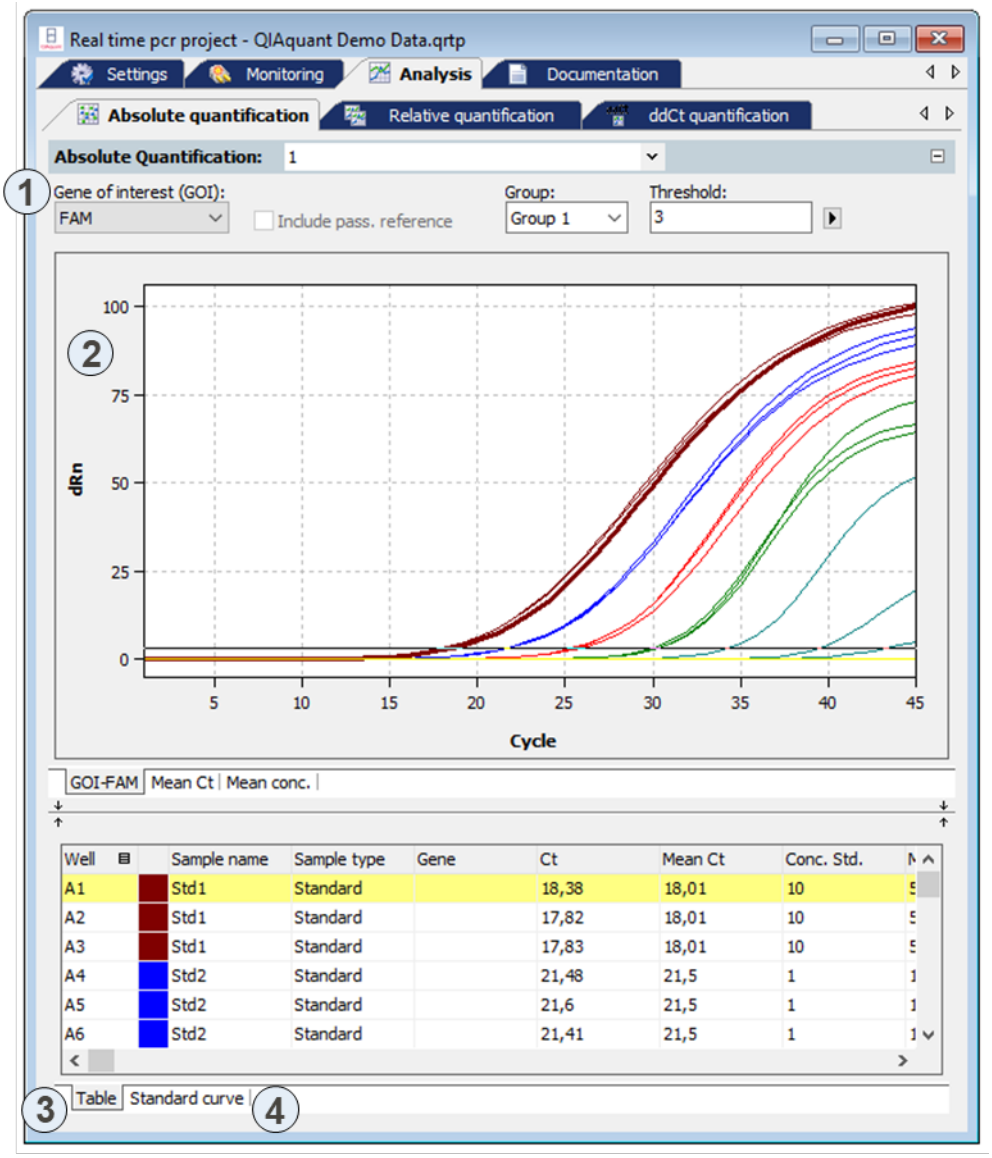
Absolute quantification is used to determine absolute copy numbers in samples, with the help of the comparison with standards with known concentrations.

5.2.1 Creating an analysis for an absolute quantification

1. Go to the **Analysis/Abs quant** project tab.
   If the tab is not visible, click the arrows in the tab bar. This will scroll the tabs.
2. Click in the toolbar or select **AbsQuant** > **Add abs. quantification**.
3. An input window appears. Enter the description for the current analysis.

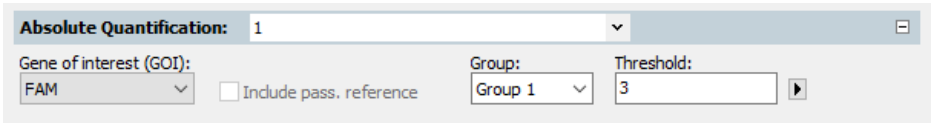
On the **AbsQuant** tab, the following information is activated:

- Parameter settings (1)
- Display of fluorescence spectra (2)
- Display of result table with measurement results (3)
- Display of the standard curves and the calculated coefficients (4)



Window for absolute quantification

5.2.2 Setting parameter for absolute quantification



Set the following parameters for the absolute quantification:

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 and regression curves for the concentration are displayed, according to the selection.
Include pass. reference	Only active if a dye has been defined as passive reference on the **Settings/Scan** project tab If this option is activated, the fluorescence of the dye that has been set as passive reference is used for standardization.
Group	If several experiments were carried out on the PCR plate, select the group of the experiment to be analyzed (see "Defining groups", page 52).
Threshold	Manually adjust threshold value. The threshold value must be between 1 and 100, depending on the standardized representation of the fluorescence curves (dRn). **Note**: The threshold value can be calculated automatically or set manually in the chart (see also "Setting the threshold value" below).
	Opens the selection window with display options (see "Displaying the fluorescence curves for absolute quantification", page 77).

Setting the threshold value

To determine C_t values for the analysis, a threshold value needs to be determined for each experiment first.

You have several options for setting the threshold value:

- In the general options (see "Making basic settings", page 69)
- Manually, in the parameters for the respective analysis (see table above)
- Graphically, in the fluorescence curves representation:
  In the chart, move the black threshold line up or down with the cursor. Press and hold the left mouse button while doing so. At the same time, the C_t values in the result table are updated.
  Note: Due to the further spread of the early exponential area of the product accumulation curves, a logarithmic representation is better suited for setting the threshold manually in the display range than a linear representation.

- By automatic calculation:
  The automatic calculation of the threshold value is activated by clicking  .
  Alternatively, you can call up the **AbsQuant** > **Autom. Threshold** menu command.

Whether you choose manual or automatic calculation, the resulting threshold value is updated and displayed synchronously in the corresponding **Threshold** input field.

Fix threshold

The threshold value becomes recalculated by the software each time that basic settings for the analysis are changed. For the analysis, the option **Fix Threshold** can be used so that the threshold value is retained even when the basic settings for the analysis are changed (see "Making basic settings", page 69).

5.2.3 Displaying the fluorescence curves for absolute quantification

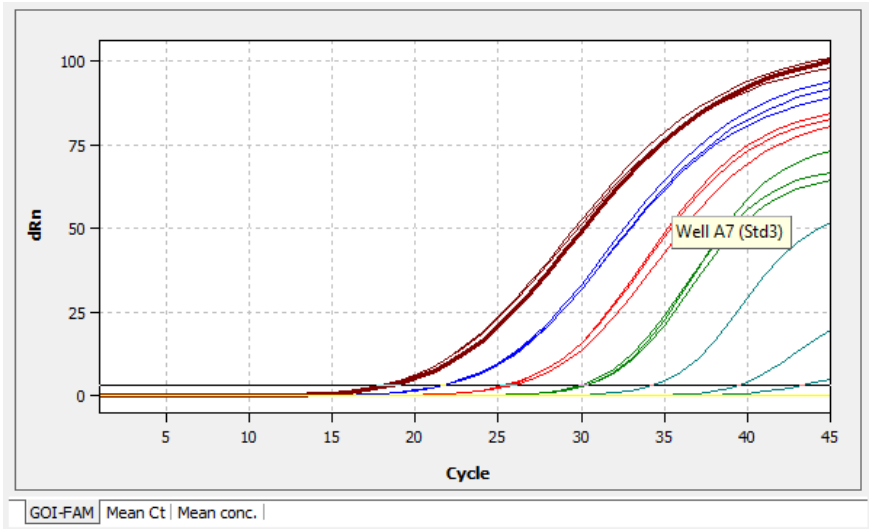
In the display range, the measured data – standardized to the value 100 for highest fluorescence intensity – is plotted against the cycle for the selected target gene.

The respective fluorescence curves are displayed by switching to another target gene/dye combination.

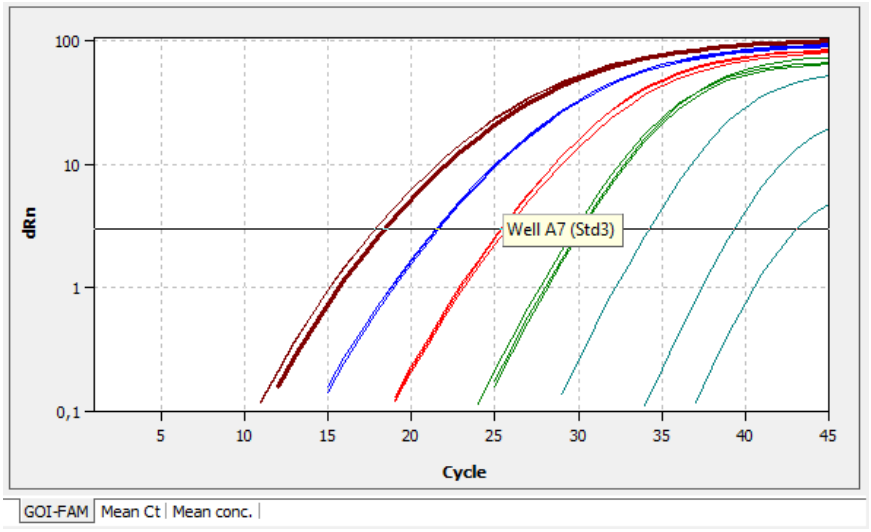
The fluorescence data is displayed as a linear or logarithmic representation depending on the selected display option. For both display options, the program shows brief information on the sample if the cursor is placed on one of the curves.

Switching the display options for the chart

1. Click  in the parameter bar.
   A selection window for the display options opens.
2. Select the **Scaling logarithmic** or **linear** option.
   Click next to the selection window. The changes are applied.



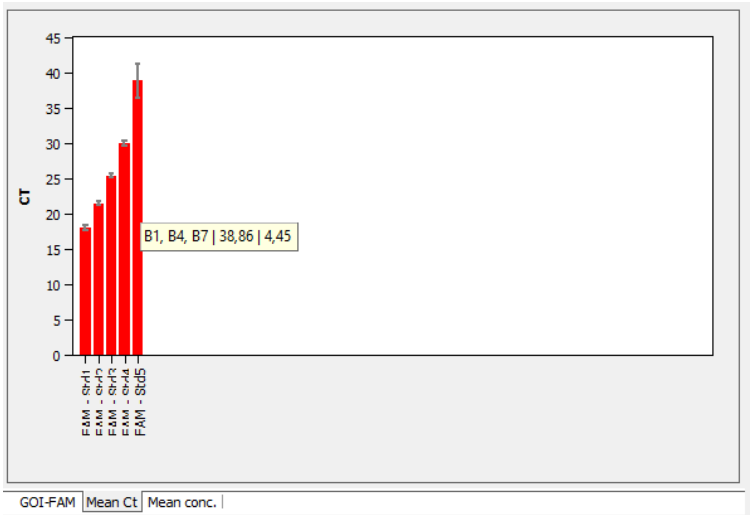
Linear representation of the fluorescence curve for the absolute quantification



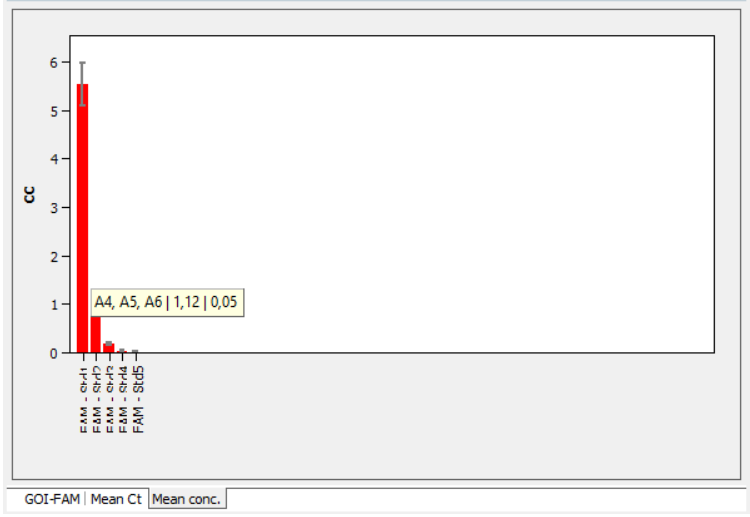
Logarithmic representation of the fluorescence curve with horizontal threshold line

5.2.4 Display of the mean C_t values or mean concentrations

After selecting the tab **Mean. Ct** or **Mean. conc.**, the display changes to a presentation of the results as bar chart. The respective sample name is given below each bar. The height of the bar is determined by the mean C_t-value or the calculated mean concentration of replicates. For each bar some short information about the position of the samples, the mean value and the calculated standard deviation is shown if the mouse pointer is placed on it. The size of the standard deviation is shown as error bar on top of each bar. Since for large numbers of samples not all bars can be displayed in the screen the diagram can be moved horizontally with pressed left mouse button and dragged to the desired position.



Bar chart for the display of mean C_t values of replicates



Bar chart for the display of mean concentrations of replicates

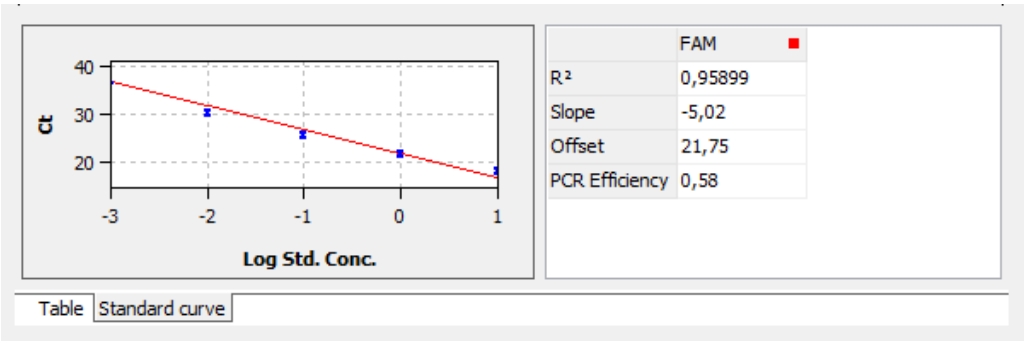
5.2.5 Displaying the standard curve and results of an absolute quantification

In the bottom **Standard curve** section of the **Analysis** project window, you can switch between the calculated standard curve and the result table via the **Curve** and **Table** list sheets.

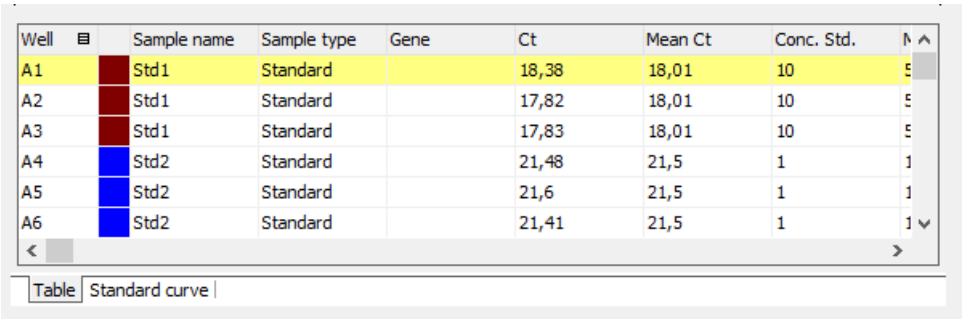
For the display of the standard curve, the C_t values of the standard samples are plotted graphically against the logarithm of their concentration. In the value range on the right, the following calculated data are displayed:

- The coefficient of determination (R^2) of the linear equation
- The slope coefficient
- The intersection of the curve with the y-axis at x=0 (offset)
- The PCR efficiency

The standard curve and the values are automatically calculated by the QIAquant 384 Software and are updated in case of setting modifications.



The result table for the absolute quantification contains all data and the associated measurement values for the samples.

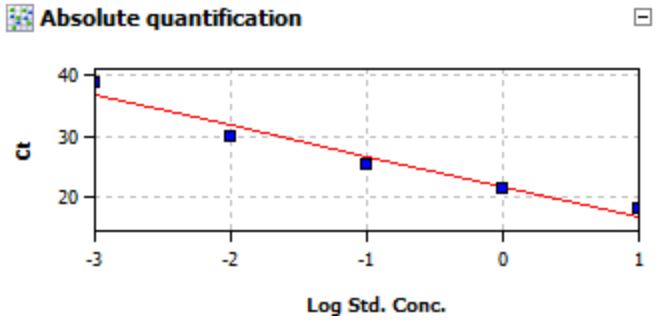


For absolute quantification, the results table contains the following information:

Column	Description
Well	Position of sample
Color of curve	Each sample is automatically assigned an unchangeable color, which is used to display the corresponding fluorescence curve
Sample name	Name of sample
Sample type	Type of sample
Group	Assignment of the sample to an experimental group
Gene	Name of gene measured in the sample
Ct	C_t value of sample
Mean. Ct	Mean C_t value of replicates
Conc. Std	Concentration of the standard sample
Mean conc.	Concentration determined from the standard curve on the basis of the mean C_t value
StdDev. Ct	Standard deviation of the C_t values between replicates
%CV Ct	Variation coefficient of the C_t values between replicates
StdDev. mean conc.	Standard deviation of the mean concentration

Display in the project explorer

A shortened representation of the standard curve that was calculated by the software is displayed in the project explorer under **Abs. quant.** The image displays the graphical plot of the C_t values of the standard samples against the logarithm of their concentration:

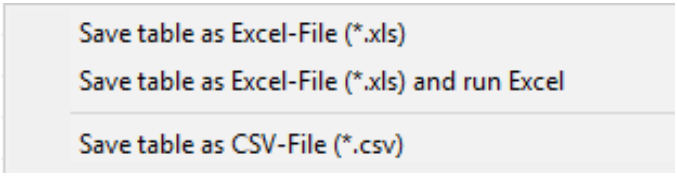


5.2.6 Exporting results of calculations

Exporting measurement data and results of calculations enables you to perform more extended data analysis by using dedicated software.

XLS and CSV

You can design the results table according to your requirements by setting type, sequence, and width of the columns to be displayed. You can also adjust the sort sequence of data in the column (alphabetical, numerical, by column, by row). The table configured this way can be exported as an XLS or a CSV file by right-clicking the table.



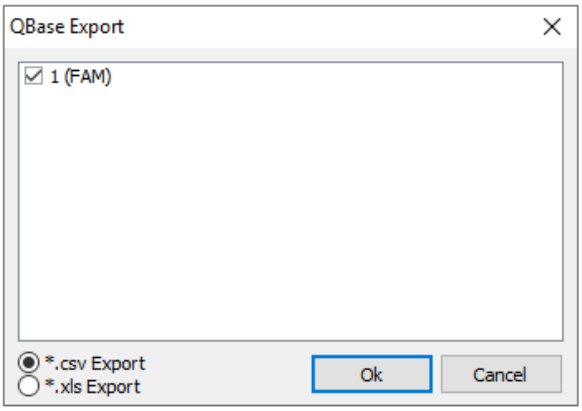
Export to qBASE+

qBASE+ is a software that allows for a more detailed statistical analysis of your qPCR data. For information, see **www.biogazelle.com**. Using the "Absolute Quantification" analysis module of the QIAquant 384 Software, you can export qPCR data to a file that can be read and analyzed by qBASE+.

1. To export qPCR data for use in qBASE+, click  in the toolbar.
   Alternatively you can select the menu **Absolute Quantification** > **qBASE Export**.
2. Choose the targets to be exported and the data format to be used (qBASE+ is able to read both formats) in the next dialogue.

The following information is sent to qBASE+:

- Well
- Sample type
- Sample name
- Gene name
- C_t value
- Concentrations of standards
- Sample active/inactive



Note: Only genes that were previously set as GOI are displayed in the export dialogue. Therefore, in multiplex assays for each GOI, i.e., each dye, an absolute quantification must be defined. To do this, repeat the steps explained in "Creating an analysis for an absolute quantification", page 74, topic 2 and 3, for each GOI.

5.2.7 Importing the standard curve

Next to the option to measure a standard curve in the experiment, the QIAquant 384 Software can also be used to determine the concentration of the samples based on the saved standard curve. You can use the import function for this purpose.

1. Use the  icon in the toolbar to open the **Import standard curve** window.

   Optionally, you can call up the **AbsQuant** > **Import standard curve** menu command.

   The mathematical equation upon which the standard curve is based, as well as the associated dye, are each displayed in the list fields of the window.
2. Select one of the import options from the **Import standard curve** window and make the corresponding entries:

Option	Meaning
Import from this run	Imports a standard curve from the current open project. If several standard curves are saved in one project, all curves are displayed and you can make a selection.
Import from saved run	Imports a standard curve from a saved project. If several standard curves have been saved, select the corresponding curve from the list.
Manual input	Standard curve coefficients are entered manually. Enter the gradient and the intercept for this equation: C_t = gradient * log(conc) + intercept.
Delete external standards	Deletes imported or entered standard curves so that they are no longer used for analysis.

5.2.8 Deleting the analysis of an absolute quantification

An analysis that is no longer required can be removed.

1. Activate the analysis by selecting its description in the evaluation list of the method tab.
2. Click  in the toolbar or select **Absolute quantification** > **Delete evaluation**.
   The analysis is removed.

5.3 Relative quantification

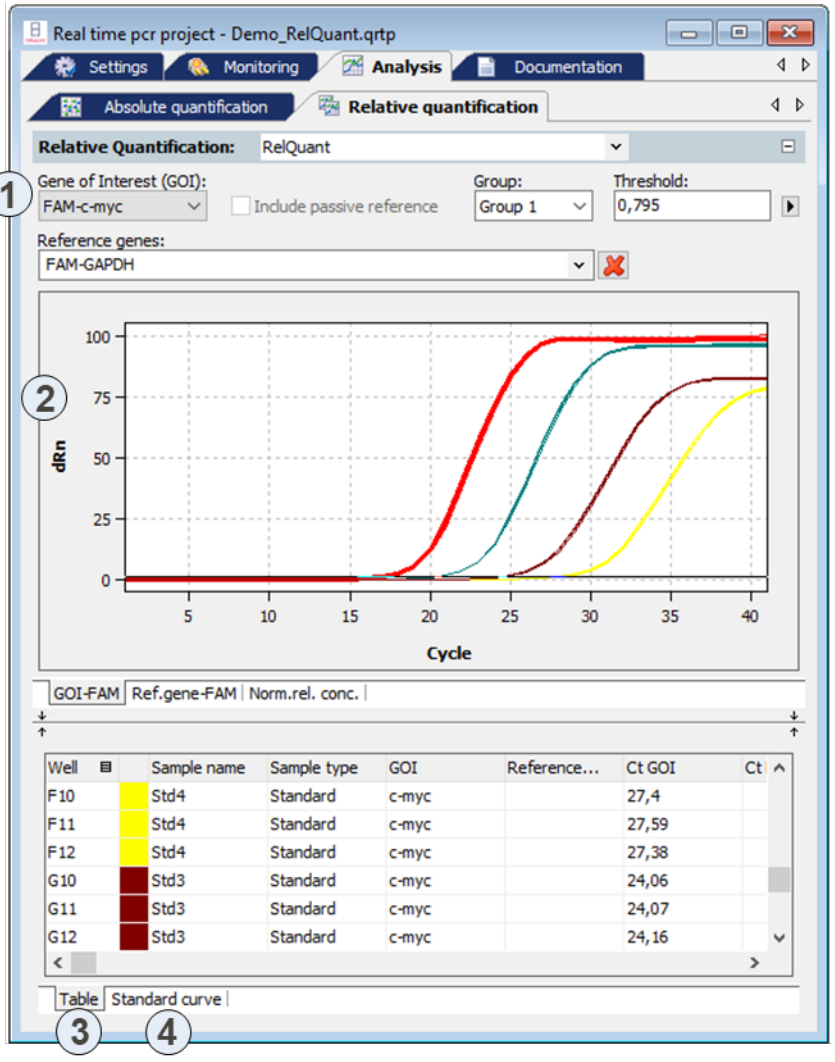
Relative quantification allows for determination of the relative expression level of a GOI in relation to one or more reference genes (often housekeeping genes). If one of the samples is denoted as the calibrator, the expression level of that sample is set to one, and the relative expression levels of all the other samples are given in relation to the calibrator sample. For relative quantification, standard dilution series are required for the GOI as well as for the reference genes.

5.3.1 Creating a new analysis for a relative quantification

1. Go to the **Analysis** > **RelQuant** project tab.
   If the tab is not visible, click the arrows  in the tab bar. This will scroll the tabs.
2. Click  in the toolbar or select **RelQuant** > **Add rel. quantification**.
3. An input window appears. Enter the description for the current analysis.

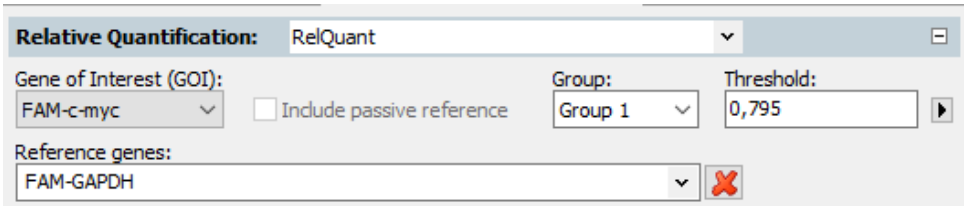
On the **Rel. Quant.** tab, the following information is activated:

- Parameter settings (1)
- Display of the fluorescence curves for the target gene and the reference gene (2)
- Display of the result table with the added values (3)
- Display of the standard curves for the target gene and the reference gene and the calculated coefficients (4)



Note: If multiple replicates have been used and one replicate or more of the reference gene does not show a Ct value, the calculation will be adjusted to use only the Ct values from the replicate(s) with a Ct value.

5.3.2 Parameter settings for relative quantification



The following parameters must be set for the relative quantification:

Option	Description
Selection list	Selection of an analysis created for the experiment
Gene of interest (GOI)	Selection list of target gene/dye combinations. Fluorescence and regression curves for the concentration are displayed according to the selection. Only one target gene at a time can be selected.
Reference genes	Reference gene selection list. In contrast to the target gene, several reference genes can be selected at the same time. Therefore, the number of list sheets that are in the display range grows with each reference gene. The icon is used to remove all reference gene settings from the analysis.
Include pass. reference	Only active if a dye has been defined as a passive reference on the **Settings/Scan** project tab. If this option is activated, the fluorescence of the dye that has been set as a passive reference is used for standardization.
Group	If several experiments were carried out on the PCR plate, select the group of the experiment to be analyzed. (see "Defining groups", page 52).
Threshold	Manually adjust threshold values. The threshold value must be between 1 and 100, depending on the standardized representation of the fluorescence curves (dRn). **Note**: The threshold value can be calculated automatically or set in the graph.
	Opens the selection window with display options

Setting the threshold value

To determine C_t values for the analysis, a threshold value needs to be determined for each experiment first. You have several options for setting the threshold value:

- In the general options (see "Making basic settings", page 69)
- Manually, in the parameters for the respective analysis (see table above)
- Graphically, in the fluorescence curves representation:
  In the chart, move the black threshold line up or down by clicking and dragging with the cursor. At the same time, the C_t values in the result table are updated.

Note: Due to the further spread of the early exponential area of the product accumulation curves, a logarithmic representation is better suited for setting the threshold manually in the display range than a linear representation.

- By having it calculated automatically:
  The automatic calculation of the threshold value is activated by clicking on  .
  Alternatively, you can call up the **RelQuant** > **Autom. Threshold** menu command.

Whether you choose manual or automatic calculation, the resulting threshold value is updated and displayed synchronously in the corresponding **Threshold input** field.

Fix threshold

The threshold value becomes recalculated by the software each time basic settings for the analysis are changed. For the analysis, the option **Fix Threshold** can be used so that the threshold value is retained if the basic settings for the analysis are changed (see "Making basic settings", page 69).

5.3.3 Displaying the fluorescence curves in the relative quantification

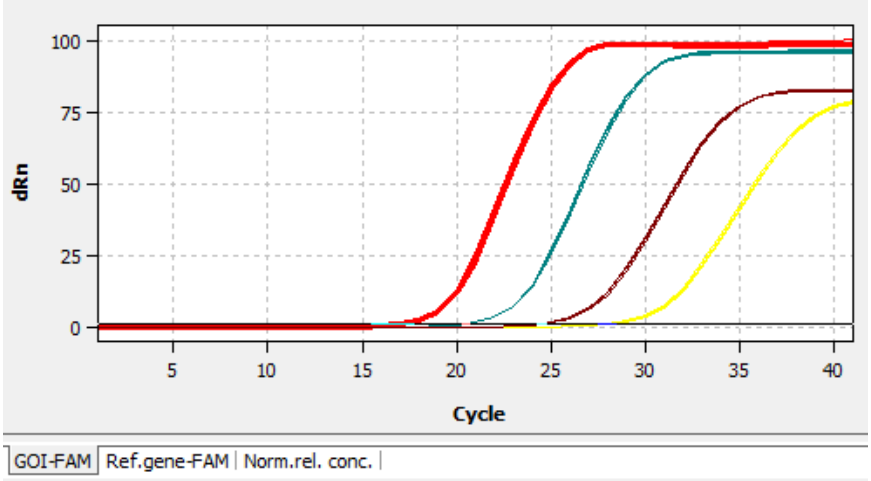
In the display range, the measured data – standardized to the value 100 for highest fluorescence intensity – is plotted against the cycle for the selected target gene. The target gene/dye and the reference gene/dye combinations are each assigned a list sheet that can be activated by clicking on the gene/dye tab on the bottom.

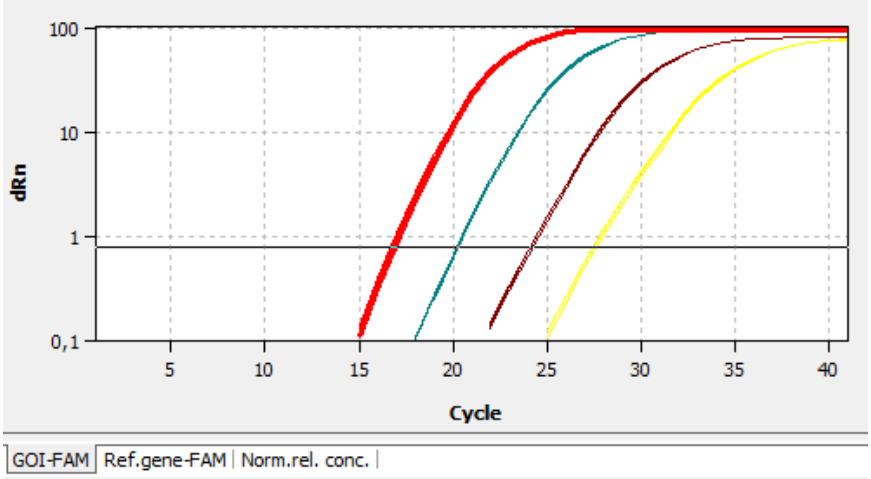
Since only one target gene/dye combination is permitted at a time, the fluorescence curves of the selected combination are displayed whenever a new combination is selected. The number of the available list sheets depends on the number of selected reference genes.

The fluorescence data is displayed as a linear or logarithmic representation, depending on the selected display option. For both display options, the program shows brief information on the sample if the cursor is placed on one of the curves.

Switching the display options for the chart

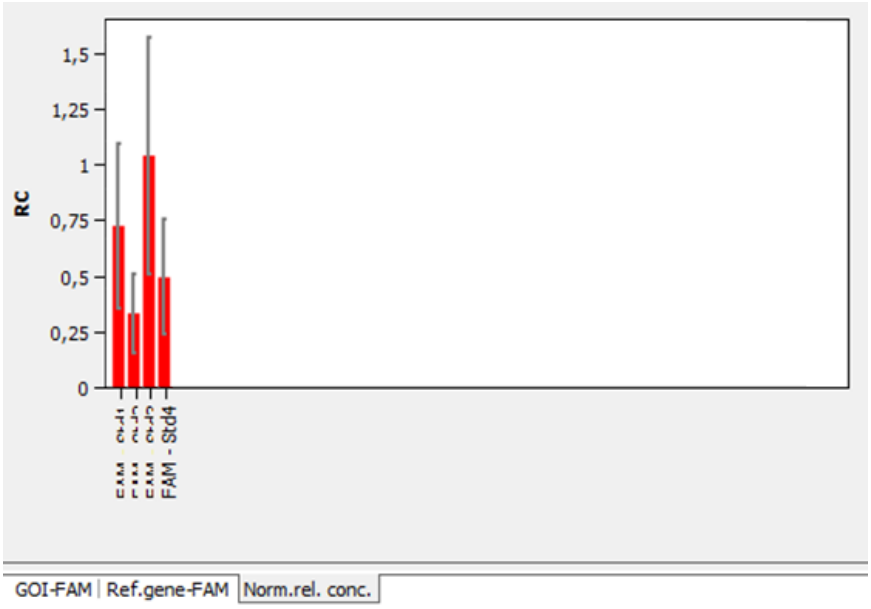
1. Click  on the parameter bar.
   A selection window for the display options opens.
2. Select the **Scaling logarithmic** or **linear** option.
   Click next to the selection window. The changes are applied.


100
75
50
25
0
dRn
5
10
15
20
25
30
35
40
Cycle
GOI-FAM
Ref.gene-FAM
Norm.rel. conc.


100
10
1
0,1
dRn
5
10
15
20
25
30
35
40
Cycle
GOI-FAM
Ref.gene-FAM
Norm.rel. conc.

5.3.4 Display of the normalized relative concentrations

After selecting the **Norm. rel. conc.** tab, the display changes to a bar chart presentation of the results. The respective sample names are given below each bar. The height of each bar is determined by the mean C_T-value or the calculated mean concentration of replicates. Hovering the mouse cursor on a bar displays some information about the position of the samples, the mean value, and the calculated standard deviation. The magnitudes of standard deviation are shown as an error bar on top of each bar. Because not all bars can be displayed in the screen if there is a large number of samples, the diagram can be moved horizontally by clicking and dragging with the mouse.



Bar chart for display of normalized relative concentrations

5.3.5 Displaying the standard curves and the results of a relative quantification

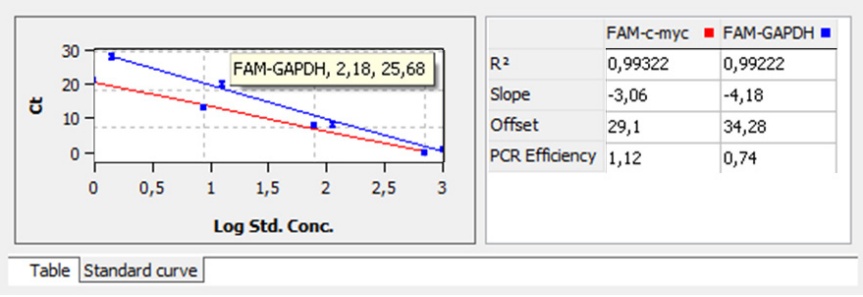
In the upper area of the project window, you can switch between the display of the calculated standard curves for the target gene and all selected reference genes and the result table for the relative quantification using the **Standard curve** and **Table** list sheets.

For the display of the standard curve, the C_T values of the standard samples are plotted graphically against the logarithm of their concentration. For the respective data points, errors bar are shown that indicate the standard deviation between replicates. For each data point, some short information

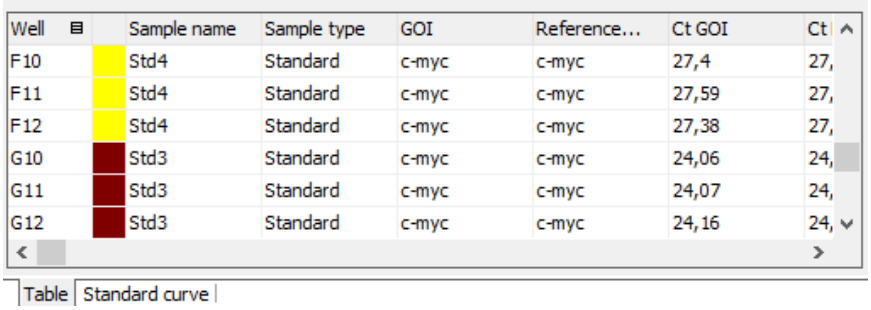
about the sample name and the size of the standard deviation of replicates is shown if the mouse cursor hovers on it. In the value range on the right, the following calculated data are displayed:

- the coefficients of determination (R^2) of the linear equation
- the standard curve gradients
- the intersections of the curves with the y-axis at x=0 (offset)
- the PCR efficiency

When more than one standard curve is displayed, each curve has its individual color. Accordingly, each table has a color code in the header that reflects the assignment to the respective standard curve. The standard curve and the values are automatically calculated by the QIAquant 384 Software and updated in case of settings modifications. According to the number of genes used, a scroll bar appears under the table. It can be used to navigate through the table's columns.



The result table for the relative quantification contains all data and the associated measurement values for the samples.

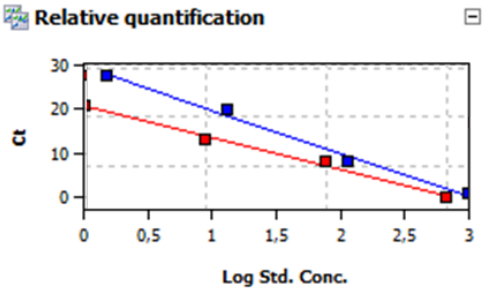


For the relative quantification, the results table contains the following information:

Column	Meaning
Well	Position of sample
Color of curve	Each sample is automatically assigned an unchangeable color, which is used to display the corresponding fluorescence curve
Sample name	Name of sample
Sample type	Type of sample
Group	Assignment of the sample to an experimental group
GOI	Gene of interest
Reference gene	Reference gene
C_t GOI	C_t value of gene of interest
C_t reference gene	C_t value of reference gene
Mean. C_t GOI	Mean C_t value of replicates of the gene of interest
Mean. C_t ref. gene	Mean C_t value of replicates of the reference gene
Conc. Std. GOI	Concentration of the standard for the gene of interest
Conc. Std Ref. Gene	Concentration of the standard for the reference gene
Mean Conc. GOI	Concentration for the gene of interest determined from the standard curve on the basis of the mean C_t value
Mean Conc. Ref. Gene	Concentration for the reference gene determined from the standard curve based on the mean C_t value
Std. Dev. C_t GOI	Standard deviation of the C_t values between replicates of the gene of interest
Std. Dev. Ref Gene	Standard deviation of the C_t values between replicates of the reference gene
%CV C_t GOI	Variation coefficient of the C_t values between replicates of the gene of interest
%CV C_t Ref Gen	Variation coefficient of the C_t values between replicates of the reference gene
Relative Conc.	Relative (x-fold) expression level of the gene of interest in relation to the reference gene
Norm. Rel. Conc.	Relative (x-fold) expression level of the gene of interest in relation to the reference gene, standardized to the expression of the calibrator (if defined)
Std. Dev. Relative Conc.	Standard deviation of the relative concentrations
Std. Dev. Norm. Rel. Conc.	Standard deviation of the normalized relative concentrations

Display in the project explorer

A shortened representation of the standard curves calculated by the software is displayed in the project explorer under **Relative quantification**. The image displays the graphical plot of the C_t values of the standard samples against the logarithm of their concentration.



5.3.6 Importing the standard curve for relative quantification

Next to the option to measure a standard curve in the experiment, the QIAquant 384 Software can also be used to determine the concentration of the samples based on the saved standard curve. You can use the import function for this purpose.

1. Use the  icon in the toolbar to open the **Import standard curve** window.
   Optionally, you can call up the **RelQuant** > **Import standard curve** menu command.
2. All additional setting are analogous to the settings for the absolute quantification (see “Importing the standard curve“, page 83).

5.3.7 Deleting the analysis of a relative quantification

An analysis that is no longer required can be removed.

1. Activate the analysis by selecting its description in the evaluation list of the method tab.
2. Click  in the toolbar or select **RelQuant** > **Delete rel. quantification**.
   The analysis is removed.

5.4 ΔΔCt method

The ΔΔCt method allows determination of the relative expression level of a GOI in relation to one or more reference genes (often housekeeping genes). One of the samples must be denoted as the calibrator. The expression level of the calibrator is set to one, and the relative expression levels of all the other samples are given in relation to the calibrator sample. In ΔΔCt method, there is no need to measure standard dilution series. However, if the ΔΔCt method shall be validated within the same PCR run, standard dilution series must be defined.

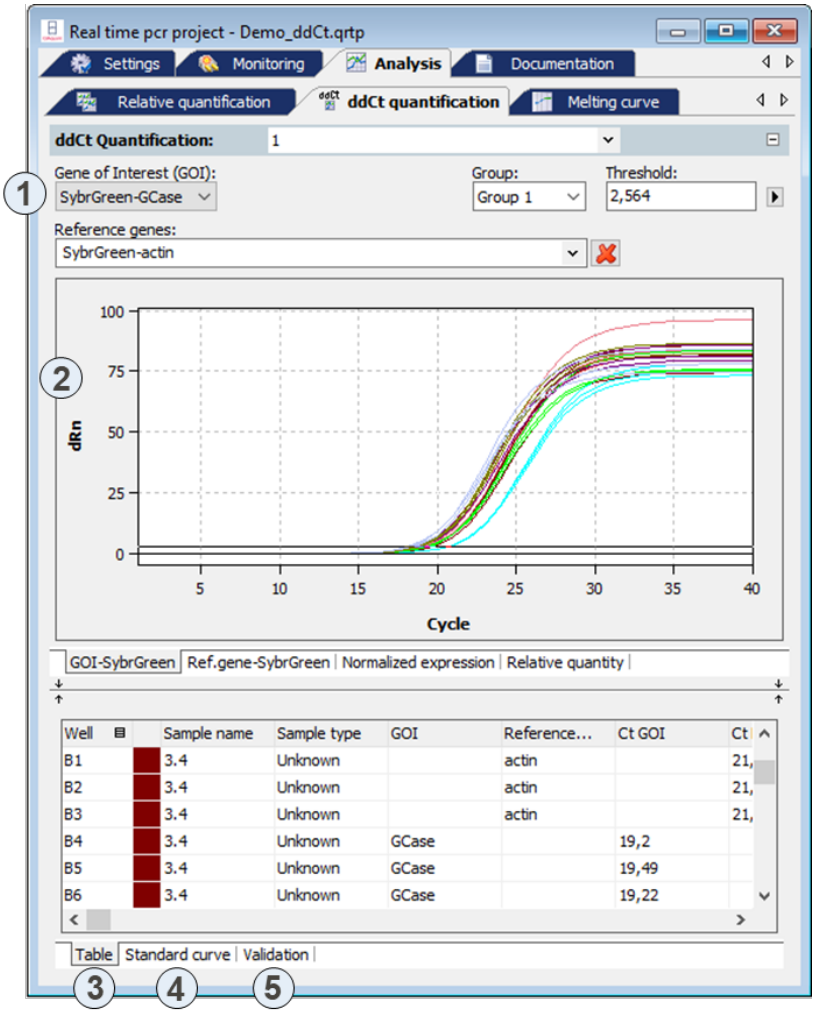
5.4.1 Creating a new analysis for a ΔΔCt method

1. Go to the **Analysis/ddCt quantification** project tab.
   If the tab is not visible, click the arrows  in the tab bar. This will scroll the tabs.
2. Click 

in the toolbar, or select **DeltaDeltaCt** > **Add ddCt quantification**.
3. An input window appears. Enter the description for the current analysis.

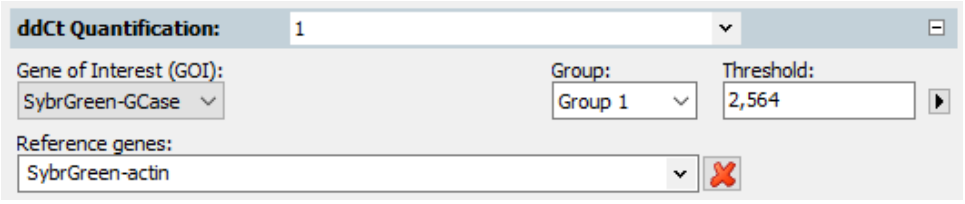
On the **DeltaDeltaCt** tab, the following information is activated:

- Parameter settings (1)
- Display of the fluorescence curves for the target gene and the reference gene (2)
- Display of the result table with the added values (3)
- If standards have been defined, display of the standard curve (4) and the validation curve (5) for the expression level of the GOI in relation to the reference gene and the calculated factors



Note: If multiple replicates have been used and one replicate or more of the reference gene doesn't show a Ct value, the calculation will be adjusted to use only the Ct values from the replicate(s) with a Ct value.

5.4.2 Parameter settings for the ΔΔCt method



The following parameters must be set for the ΔΔCt method:

Option	Description
Selection list	Selection of an analysis created for the experiment
Gene of interest (GOI)	Selection list of combinations of measured dyes and target genes to be quantified. Only one target gene at a time can be selected.
Reference genes	Selection list for the reference genes. In contrast to the target gene, several reference genes can be selected at the same time. The number of list sheets in the display range therefore grows with each reference gene. With the  symbol you can remove all selected reference genes from the selection.
Group	If several experiments were carried out on the PCR plate, select the group of the experiment to be analyzed. (see "Defining groups", page 52).
Threshold	Manually adjust threshold values. The threshold value must be between 1 and 100, depending on the standardized representation of the fluorescence curves (dRn). **Note**: The threshold value can be calculated automatically or set in the graph.
	Opens the selection window with display options

Setting the threshold value

To determine C_t values for the analysis, a threshold value needs to be determined for each experiment first.

You have several options for setting the threshold value:

- In the general options (see "Making basic settings", page 69).
- Manually in the parameters for the respective analysis (see table above)
- Graphically in the fluorescence curves representation:
  In the chart, move the black threshold line up or down with the cursor. Press and hold the left mouse button while doing so. At the same time, the C_t values in the result table are updated.
  Note: Due to the further spread of the early exponential area of the product accumulation curves, a logarithmic representation is better suited for setting the threshold manually in the display range than a linear representation.
- By having it calculated automatically:
  The automatic calculation of the threshold value is activated by clicking  or **DeltaDeltaCt** > **Autom. Threshold**.

Whether you choose manual or automatic calculation, the resulting threshold value is updated and displayed synchronously in the corresponding **Threshold iput** field.

Fix threshold

The threshold value is recalculated by the software each time that basic settings for the analysis are changed. For the analysis, the option **Fix Threshold** can be used so that the threshold value is retained if the basic settings for the analysis are changed (see "Making basic settings", page 69).

5.4.3 Displaying the fluorescence curves for the ΔΔCt method

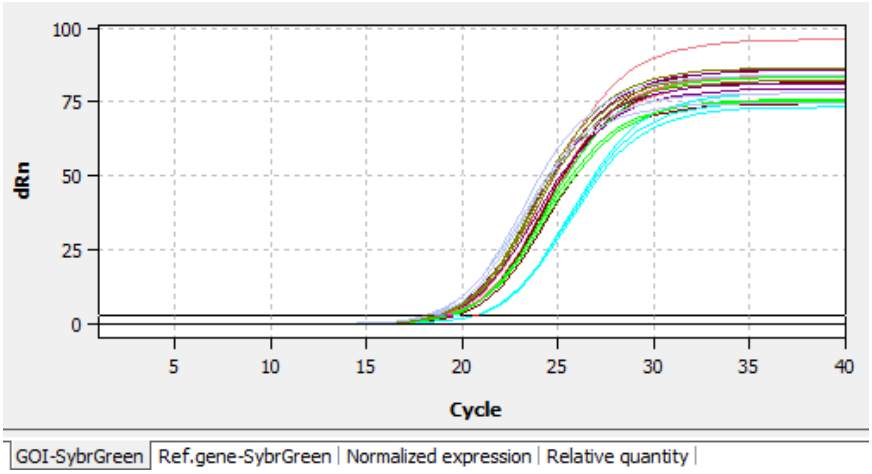
In the display range, the measured data – standardized to the value 100 for highest fluorescence intensity – is plotted against the cycle for the selected target gene. The gene/dye and the reference gene/dye combinations are each assigned a list sheet that can be activated by clicking on the gene/dye tab on the bottom.

In the analysis, you can always use only one target gene but several reference genes. The number of the available list sheets depends on the number of the selected genes.

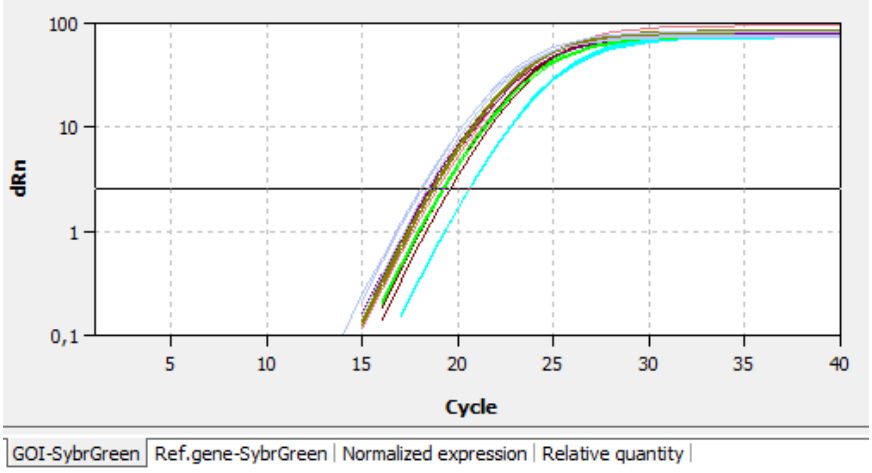
The fluorescence data is displayed as a linear or logarithmic representation, depending on the selected display option. For both display options, the program shows brief information on the sample if the cursor is placed on one of the curves.

Switching the display options for the chart

1. Click  in the parameter bar.
   A selection window for the display options opens.
2. Select the **Scaling logarithmic** or **linear** option.
   Click next to the selection window. The changes are applied.



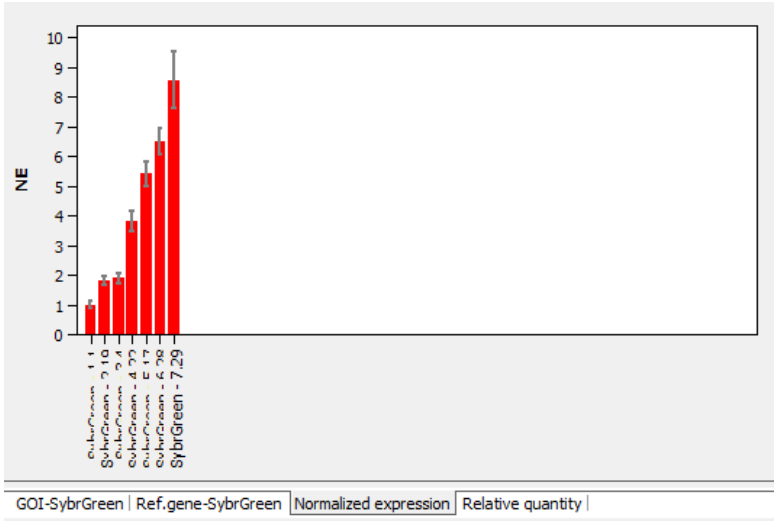
Linear representation of the fluorescence curve for the ΔΔCt method



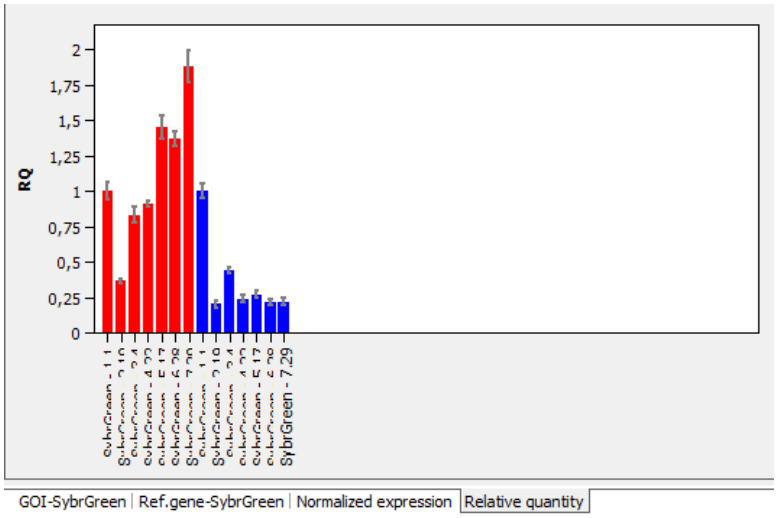
Logarithmic representation of the fluorescence curve for the ΔΔCt method

5.4.4 Display of the normalized relative expression or relative quantities

After selecting the **Normalized expression** or **Relative quantity** tab, the display changes to present the results as bar chart. The respective sample names are given below each bar. The height of each bar is determined by the calculated normalized expression or the calculated relative quantity of replicates. For each bar, some information about the position of the samples, the mean value, and the calculated standard deviation is shown when the mouse cursor hovers on it. The magnitudes of standard deviation are shown as an error bar on top of each bar. For the representation of the relative quantities, the results for the target gene and reference gene, the bars are colored differently. Because not all bars can be displayed on the screen when there is a large number of samples, the diagram can be moved horizontally by clicking and dragging with the mouse.



Bar chart for display of normalized expression of replicates



Bar chart for display of relative quantities of replicates

5.4.5 Setting the calculation mode for the standardized expression

The QIAquant 384 Software enables you to calculate the standardized expression (SE) with 2 different methods:

- Without PCR efficiency calculation (Livak method)
- With PCR efficiency calculation for GOI and reference genes (Pfaffl method)

To calculate the SE, one sample must be defined as the calibrator.

Calculating the SE without efficiency calculation (Livak method)

The Livak method assumes that the PCR efficiencies of the GOI and the reference gene (RefGene) are equal. The following applies:

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

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

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

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

Calculating the SE with efficiency calculation (Pfaffl method)

The Pfaffl method considers the efficiencies determined for the GOI and the reference gene (RefGene). The efficiencies (E(GOI) and E(RefGene)) can be calculated from dilution series or specified in the software. The following applies:

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

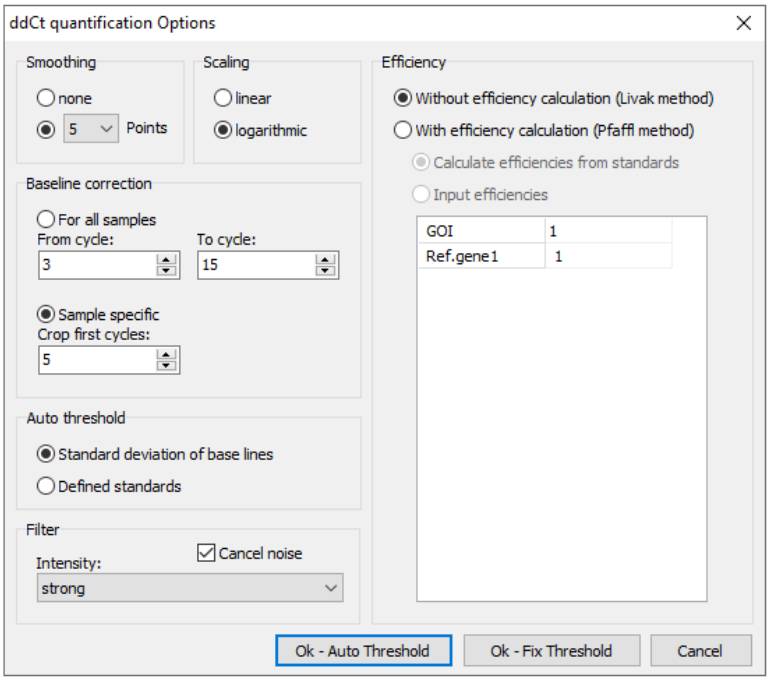
where $\Delta Ct(GOI) = Ct(GOI, Calibrator) - Ct(GOI, Sample)$

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

The Pfaffl method is generally preferred, because the basic assumption of the Livak method (equal efficiency when amplifying the GOI and the reference gene) is rarely the case in practice and the calculation can therefore lead to incorrect values.

The calculation method is selected in the **ddCt Options** window.

- Click  in the toolbar or select **DeltaDeltaCt** > **ddCt Quant. options**.
- Select the desired calculation method.
  For the Pfaffl method, the efficiency values can be determined automatically from the standard curves (dilution series) for the GOI and the reference gene (if standards have been defined), or the values can be entered manually in the respective fields.



ddCt Options window for presetting the analysis of the ΔΔCt method

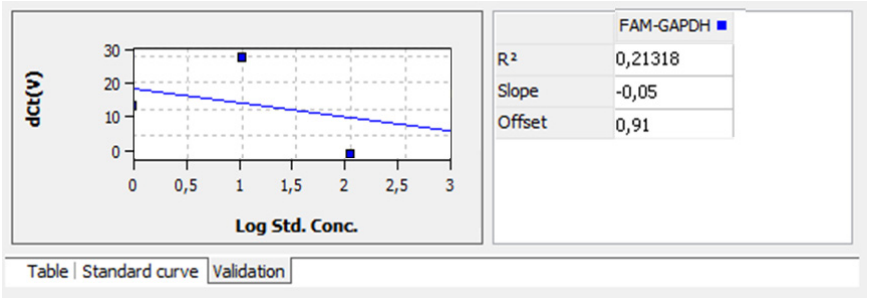
5.4.6 Displaying the validation curves and values

To calculate the $\Delta\Delta C_t$ values, there is no need to determine a validation curve. However, it might be useful to consult it when checking data quality. A prerequisite for creating a validation curve is the measurement of a standard series with different dilution levels of target gene and reference gene. If standard series have been measured for the target and reference gene, the expression ratio between target and reference gene is represented graphically in the **Validation curve** display. For this purpose, the mean C_t value of the target gene is subtracted from the mean C_t value of the reference gene for the corresponding dilution level, and the resulting dCt(V) value is plotted against the logarithm of the concentration.

In the value range on the right, the following calculated data is displayed:

- The coefficient of determination (R^2)
- The slope coefficient
- The intersection of the curve with the y-axis at x=0 (offset)

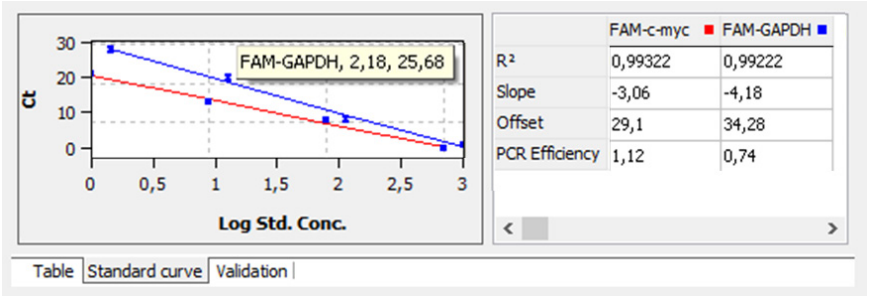
The gradient of the curve should not exceed ±0.1. The assumption then applies that the efficiencies of the amplification of the GOI and the reference gene are almost identical and the calculation of the ΔΔCt values produces valid data.

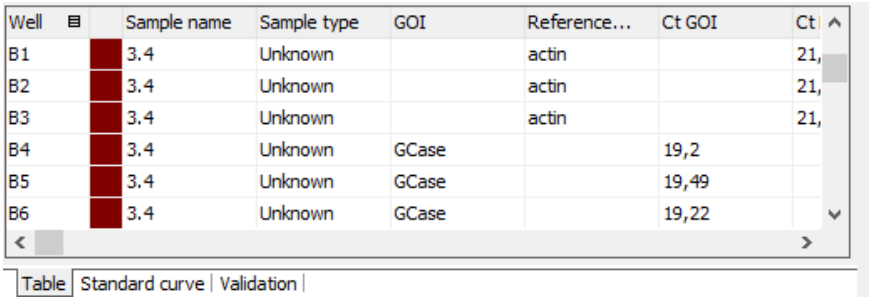


Validation curves view for the ΔΔCt method

You can switch between the ΔΔCt calculation views using the **Table**, **Standard curve**, and **Validation** tabs. The validation curves and the values are automatically calculated by the QIAquant 384 Software and updated if the settings change. For validation curves as well as for standard curves, the respective data point errors bar are shown that indicate the standard deviation between replicates. For each data point, some short information about the sample name and the mean C_t value of replicates is shown when the mouse cursor hovers on it.

When more than one standard curve is displayed, each curve has its individual color. Accordingly, each table has a color code in the header that reflects the assignment to the respective standard curve.



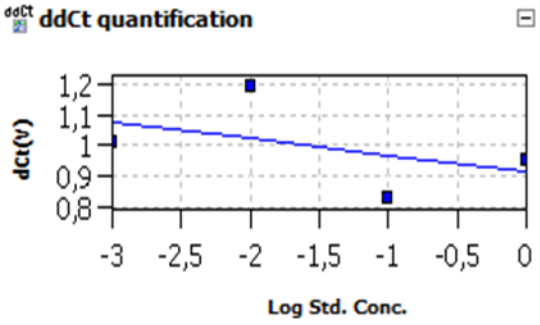


For the ΔΔCt quantification, the results table contains the following information:

Column	Meaning
Well	Position of sample
Color of curve	Each sample is automatically assigned an unchangeable color, which is used to display the corresponding fluorescence curve
Sample name	Name of sample
Sample type	Type of sample
Group	Assignment of the sample to an experimental group
GOI	Gene of interest
Reference gene	Reference gene
C_t GOI	C_t value of gene of interest
C_t Ref. gene	C_t value of reference gene
Mean C_t GOI	Mean C_t value of replicates of the gene of interest
Mean C_t ref. gene	Mean C_t value of replicates of the reference gene
Std. Dev. C_t GOI	Standard deviation of the C_t values between replicates of the gene of interest
Std. Dev. C_t ref. gene	Standard deviation of the C_t values between replicates of the reference gene
%CV C_t GOI	Variation coefficient of the C_t values between replicates of the gene of interest
%CV C_t ref. gene	Variation coefficient of the C_t values between replicates of the reference gene
dCt GOI	Delta C_t value for replicates of the gene of interest
dCt ref. gene	Delta C_t value for replicates of the reference gene
RQ GOI	Calculated relative amount for replicates of the gene of interest in the original sample
RQ ref. gene	Calculated relative amount for replicates of the reference gene in the original sample
Mean RQ ref. gene	Average calculated relative amount for replicates of the reference gene in the original sample
Norm. expression	Standardized relative (x-fold) expression level of the gene of interest in the sample, in relation to the calibrator

Display in the project explorer

A shortened representation of the validation curves calculated by the software is displayed in the project explorer under **DeltaDeltaCt**. The image displays the graphical plot of the dCt(V) values against the logarithm of the sample concentration:



5.4.7 Deleting a ΔΔCt method analysis

An analysis that is no longer required can be removed.

1. Activate the analysis by selecting its description in the evaluation list of the method tab.
2. Click  in the toolbar or select **DeltaDeltaCt** > **Delete ddCt quantification**.
   The analysis is removed.

5.5 Melting curve analysis

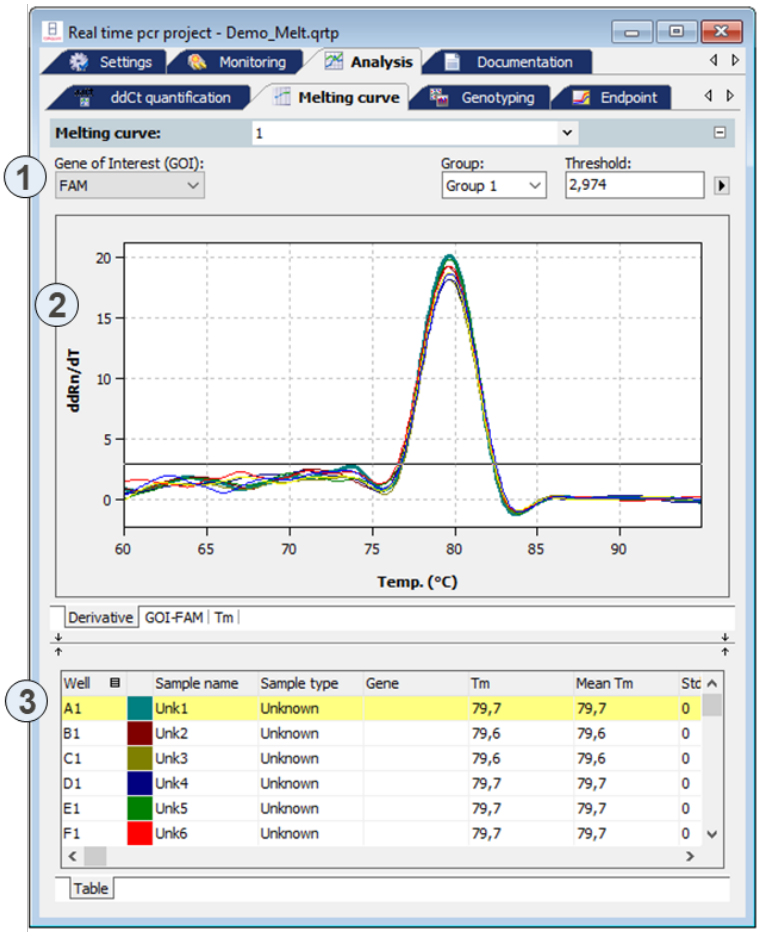
When performing a melting curve analysis, the temperature in the reaction mixture is increased successively until the PCR product is denatured. The dissociation of the fragment in single strands will result in the release of an intercalating dye. The associated reduction of the fluorescence intensity is measured and recorded by the device. By forming the first derivative of the fluorescence curve, you will get a peak that describes the melting point and the approximate concentration of the PCR fragment. Through melting curve analysis, you can differentiate whether the reaction has caused the formation of a specific PCR product or whether unspecific by-products, such as primer-dimers, were produced.

5.5.1 Creating a new melting curve analysis

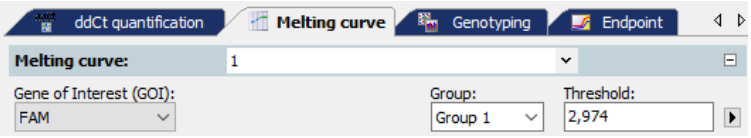
1. Go to the **Analysis** > **Melt. curve** project tab.
   If the tab is not visible, click the arrows in the tab bar. This will scroll the tabs.
2. Click in the toolbar or select **Melting curve** > **Add melting curve**.
3. An input window appears. Enter the description for the current analysis.

On the **MeltCurve** tab, the following information is activated:

- Parameter settings (1)
- Display of fluorescence values as a function of the temperature and/or its first derivative (2)
- Display of the result table (3)



5.5.2 Parameter settings for melting curve analysis



Set the following parameters for the melting curve quantification:

Option	Description
Selection list	Selection list of a selection created for the experiment
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. According to the selection, the fluorescence and regression curves for the concentration are displayed.
Group	If several experiments were carried out on the PCR plate, select the group of the experiment to be analyzed (see "Defining groups", page 52).
Threshold	Manually adjust threshold values. The threshold is now effective on the **Derivative** tab. Only curves whose maximum dRn/dT is greater than the threshold are analyzed. **Note**: The threshold value can be calculated automatically or set manually in the chart (see also "Setting the threshold value" below).
	Opens the selection window with display options

Setting the threshold value

For the correct analysis, a threshold value must be determined for the melting curves.

You have several options for setting the threshold value:

- In the general options (see "Making basic settings", page 69)
- Manually, in the parameters for the respective analysis (see table above)
- Graphically, in the representation of the fluorescence curves derivative:
  In the chart **Derivative**, move the black threshold line up or down by clicking and dragging with the mouse. At the same time, the T_m values in the result table are updated.
- Automatic calculation:
  The automatic calculation of the threshold value is activated by clicking  or selecting **Melting curve/Autom. Threshold**.

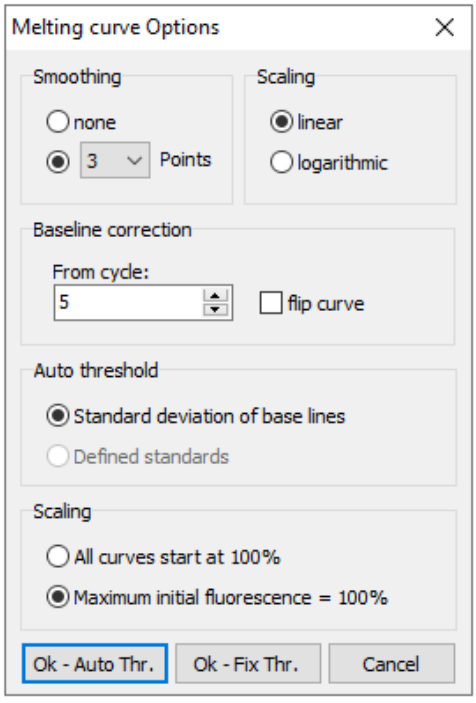
Whether you choose manual or automatic calculation, the resulting threshold value is updated and displayed synchronously in the corresponding **Threshold input** field.

Fix threshold

The threshold value becomes recalculated by the software each time basic settings for the analysis are changed. For the analysis, the option **Fix Threshold** can be used so that the threshold value is retained if the basic settings for the analysis are changed (see "Making basic settings", page 69).

5.5.3 Displaying fluorescence curves/melting curves

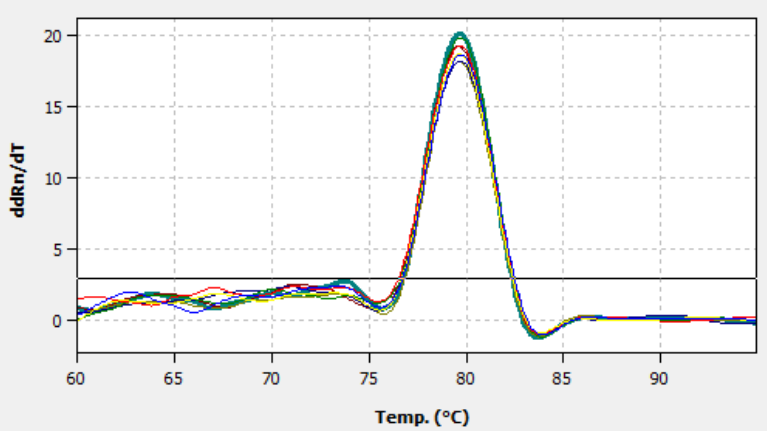
In the display area, the measured fluorescence curves are shown in relation to the temperature and are either standardized to the highest fluorescence value or both standardized to a target value of **100**, depending on the settings made in the **Melt. Curve Options** window. The data is displayed as a linear or logarithmic representation, depending on the selected display option.



The program shows brief information on the sample if the cursor is placed on one of the curves.

The melting temperature T_m is determined by forming the first derivative of the melting curves from the maxima of the forming peaks. To evaluate fluorescence data from protein stability

measurements, the melting curves can be reversed. The reversal of the melting curves can be activated via the **flip curve** option.



You can switch between the melting curves display and the derivatives via the tab at the bottom-left corner of the display range.

Switching the display options for the chart of fluorescence curves

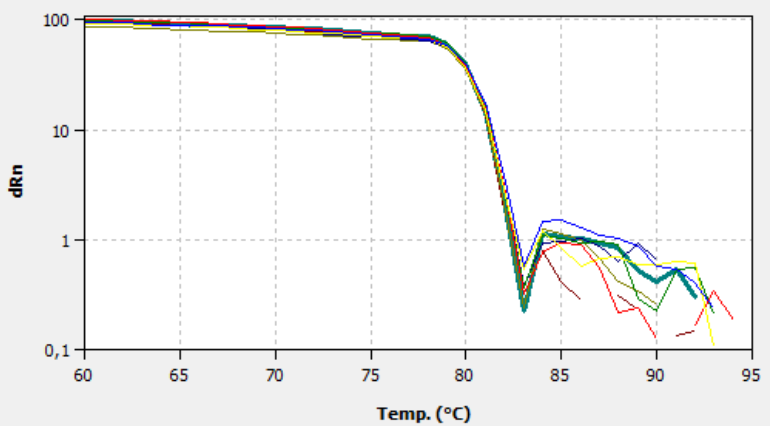
1. Click  in the parameter bar.

   A selection window for the display options opens.

2. Select the **Scaling logarithmic** or **linear** option.

   Click next to the selection window. The changes are applied.

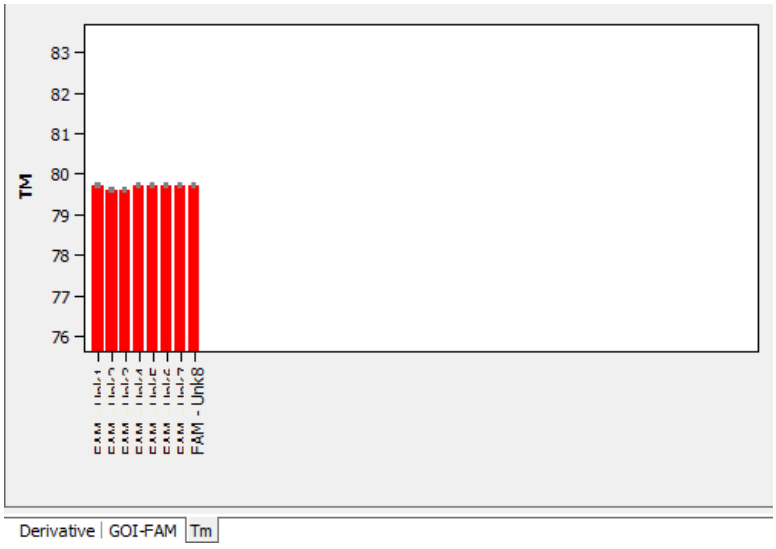
   The **Derivative** tab cannot be displayed logarithmically.



The linear display does not show a threshold line.

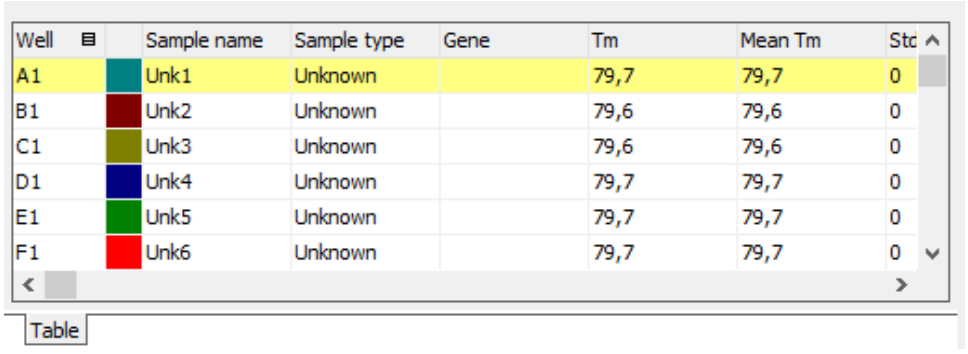
5.5.4 Display of melting temperatures

After selecting the tab T_m, the display changes to a presentation of the results as bar chart. The respective sample name is given below each bar. The height of the bar is determined by the measured melting temperature of replicates. For each bar, some short information about the position of the samples, the mean value, and the calculated standard deviation is shown when the mouse hovers on it. The magnitude of standard deviation is shown as an error bar on top of each bar. Because not all bars can be displayed in the screen when there is a large number of samples, the diagram can be moved horizontally by clicking and dragging with the mouse.



5.5.5 Displaying the result table for the melting curves

The result table for melting curves contains all data and the associated measurement values for the samples.



Well		Sample name	Sample type	Gene	Tm	Mean Tm	Stc
A1		Unk1	Unknown		79,7	79,7	0
B1		Unk2	Unknown		79,6	79,6	0
C1		Unk3	Unknown		79,6	79,6	0
D1		Unk4	Unknown		79,7	79,7	0
E1		Unk5	Unknown		79,7	79,7	0
F1		Unk6	Unknown		79,7	79,7	0

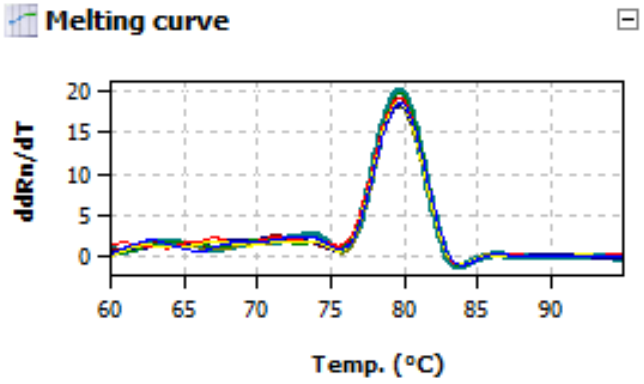
Table

For melting curves, the results table contains the following information:

Column	Meaning
Well	Position of sample
Color of curve	Each sample is automatically assigned an unchangeable color, which is used to display the corresponding fluorescence curve
Sample name	Name of sample
Sample type	Type of sample
Group	Assignment of the sample to an experimental group
Gene	Name of gene measured in the sample
T_m	Melting temperature of the sample
Mean T_m	Mean melting temperature of replicates
Std Dev Mean T_m	Standard deviation of mean melting temperature of replicates

Display in the project explorer

A shortened representation of the melting curves calculated by the software is displayed in the project explorer under **Melt Curve**. This representation shows the graphical plot of the first derivative of the fluorescence values against the temperature.



5.5.6 Deleting a melting curve analysis

A melting curve analysis that is no longer required can be removed.

1. Activate the analysis by selecting its description in the evaluation list of the method tab.
2. Click  or select **Melting curve** > **Delete melting curve**.
   The analysis is removed.

5.6 Genotyping

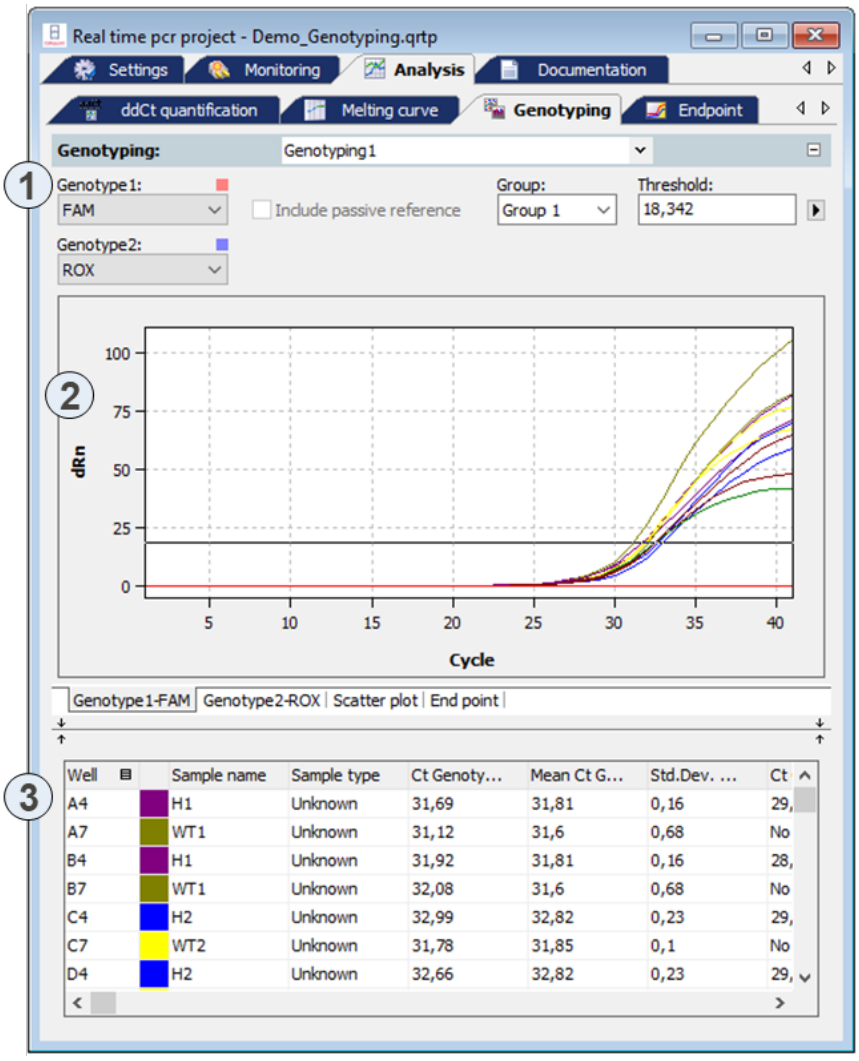
Genotyping determines sequence differences between a sample and a standard. The standard is defined as the reference sequence (genotype 1); the genetic condition of the sample is to be determined in the experiment. Genotyping shows which alleles an individual has inherited from its parents.

5.6.1 Creating a new analysis for genotyping

1. Go to the **Analysis** > **Genotyping** project tab.
   If the tab is not visible, click the arrows  in the tab bar. This will scroll the tabs.
2. Click  or select **Genotyping** > **Add genotyping**.
3. Enter the description for the current analysis in the input window.

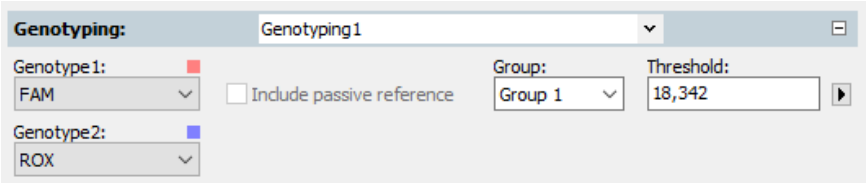
The following information is activated on the **Genotyping** tab:

- Parameter setting for defining the threshold and selecting the dye with which the wild type and the mutant were measured (1)
- Display of the fluorescence curves of wild type and mutant and/or display of the results as a scatter plot or bar graph (2)
- Display of the result table with the results (3)



Genotyping window

5.6.2 Parameter settings for genotyping

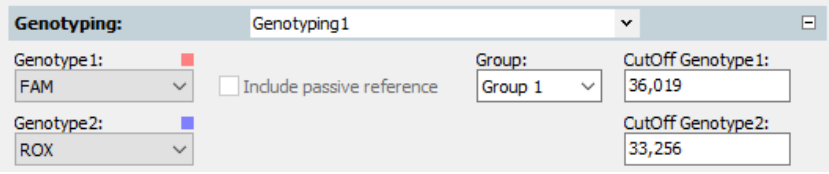


Parameter settings for genotyping for the fluorescence curve display

Set the following parameters for the absolute quantification:

Option	Description
Selection list	Selection of an analysis created for the experiment
Genotype 1	Selection list for the dye used to measure the genotype 1
Genotype 2	Selection list for the dye used to measure the genotype 2
Include passive reference	Only active if a dye has been defined as passive reference on the **Settings/Scan** project tab. If this option is activated, the fluorescence of the dye that has been set as a passive reference is used for standardization.
Group	If several experiments were carried out on the PCR plate, select the group of the experiment to be analyzed (see "Defining groups", page 52).
Threshold	Manually adjust threshold value. The threshold value must be between 1 and 100, depending on the standardized representation of the fluorescence curves (dRn). **Note**: The threshold value can be calculated automatically or set manually in the chart (see see also "Setting the threshold value" below).
▶	Opens the selection window with display options (see "Displaying the fluorescence curves for absolute quantification", page 77).

When changing to the scatter plot or endpoint bar graph, 2 fields are displayed for the **CutOff** value for the **Genotype 1** and the **Genotype 2** instead of the **Threshold** field:



Setting the threshold value

To determine C_t values for analysis, a threshold value must be determined for each experiment.

You have several options for setting the threshold value:

- In the general options (see "Making basic settings", page 69)
- Manually, in the parameters for the respective analysis (see table above)
- Graphically, in the fluorescence curves representation:
  In the chart, move the black threshold line up or down by clicking and dragging with the mouse. At the same time, the C_t values in the result table are updated.
  Note: The logarithmic view is more suitable than the linear view for placing the threshold manually in the display area, because of the wider spread of the early exponential area of the product accumulation curves.

- By having it calculated automatically:

  To activate the automatic calculation of the threshold value, click  or select **Genotyping** > **Autom. threshold**.

Whether you choose manual or automatic calculation, the resulting threshold value is updated and displayed synchronously in the **Threshold** input field.

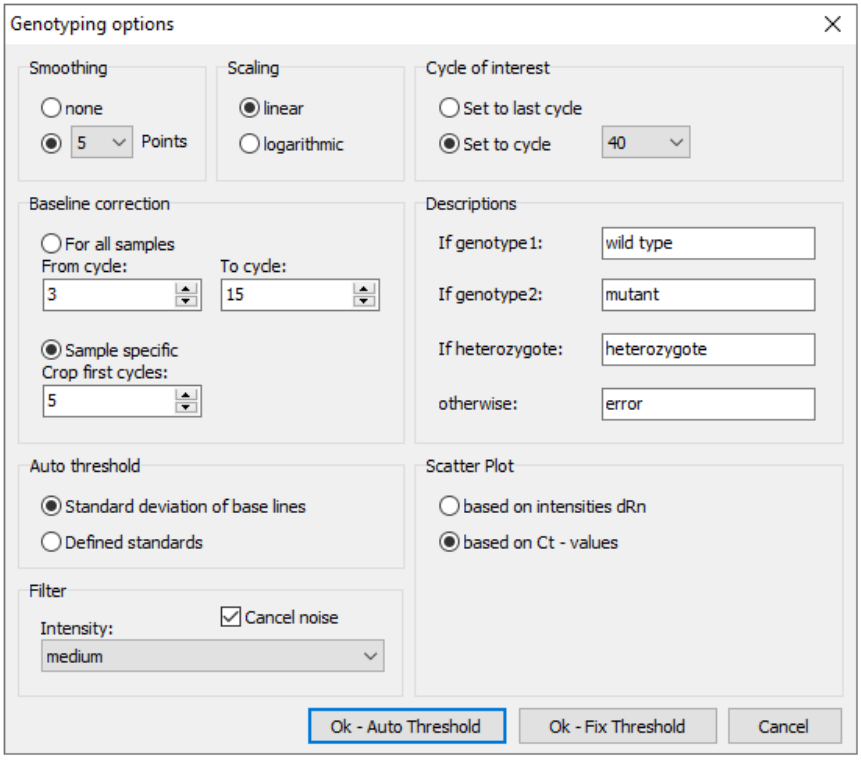
Fix threshold

The threshold value becomes recalculated by the software each time basic settings for the analysis are changed. For the analysis, the option **Fix Threshold** can be used, so that the threshold value is retained if the basic settings for the analysis are changed (see "Making basic settings", page 69).

5.6.3 Specifying genotyping options

Special analysis options are available for genotyping.

- Click  in the toolbar to open the **Genotyping Options** window.
  Alternatively, you can call up the **Genotyping/Genotyping Options** menu command.



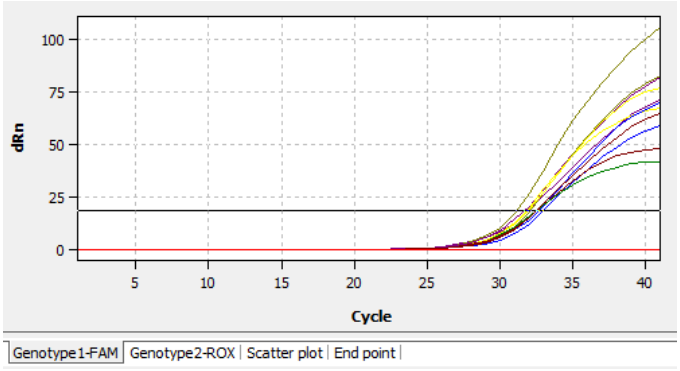
Option	Description
Cycle of interest	Select the cycle for analysis. This can preferably be the last cycle (end point) but also any other cycle of the PCR run. The respective cycle can be selected from a list.
Descriptions	Input fields for own names for the genotype1, genotype 2, heterozygote categories or otherwise
Scatter plot	Generation of scatter plot, based on fluorescence intensities of analyzed cycle and/or C_t values

5.6.4 Displaying the fluorescence curves, scatter plot and bar graph

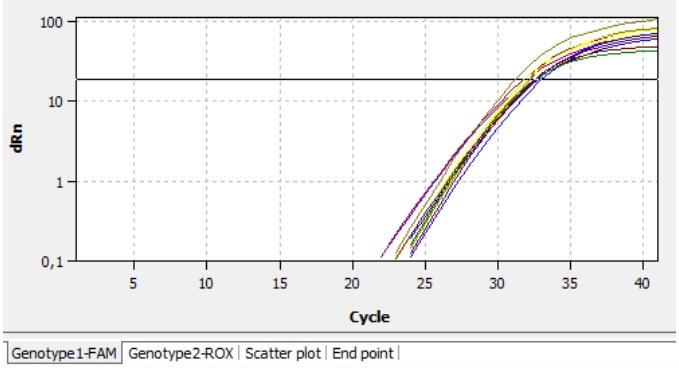
The respective combination of genotype 1 dye or genotype 2 dye displayed is shown on the tabs at the bottom-left corner of the area. The entry for the respective active combination on the tab is highlighted in white. The fluorescence data is displayed as a linear or logarithmic representation, depending 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.

1. Click the  button in the parameter bar. A selection window for the display options opens.
2. Select the **Scaling logarithmic** or **linear** option.

   Click next to the selection window. The changes are applied.



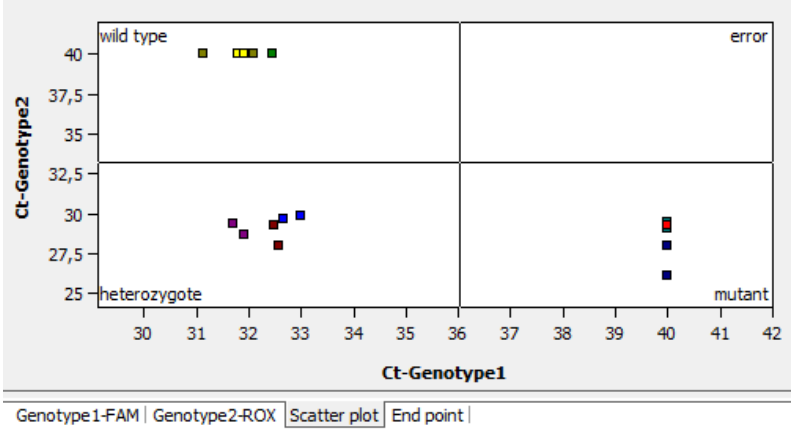
Linear representation of the fluorescence curve for genotyping



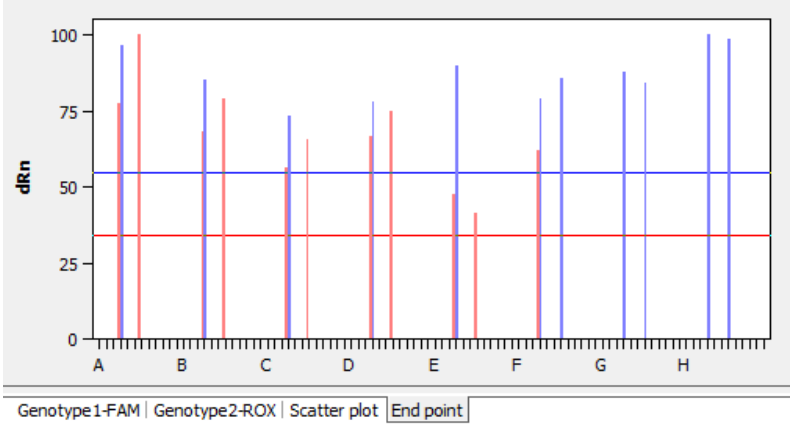
Logarithmic representation of the fluorescence curve with horizontal threshold line

To determine C_t values for the analysis, a threshold value must be determined for the fluorescence curves. This can be automated but can also be done manually in the display area (see “Parameter settings for genotyping“, page 111).

In addition to the fluorescence curves representation, the results can also be displayed as a scatter plot or bar graph. This can be selected via the respective tabs below the display area.



The scatter plot is divided into 4 quadrants for genotype1, genotype 2, heterozygote, and error. The samples are assigned to one of the quadrants based on the measured relative fluorescence or the C_t values of the 2 dyes. The respective cutoff values for the sample assignment are represented by 2 black lines in the scatter plot view. To change the position of the lines and thus change the cutoff value, click and drag the lines with the mouse. Alternatively, the respective cutoff values for genotype 1 and genotype 2 can be entered in their respective fields in the selection area (see “Parameter settings for genotyping“, page 111).



Bar chart for genotyping

The bar graph shows the measured relative fluorescence as bars. The x-axis is scaled from A–H based on the rows of the block, i.e., the first 12 samples correspond to positions A1–A12 in the block, the next 12 samples correspond to positions B1–B12, etc. The cutoff value is set by clicking and dragging the red or blue horizontal line up or down using the mouse, or by entering the respective cutoff values for mutant and wild type in their respective fields in the selection area of this view (see "Parameter settings for genotyping", page 111).

The cutoff values are thresholds after which the question of whether a sample shows a reaction is answered with "Yes" (see **Reaction genotype 1** and **Reaction genotype 2** table columns).

5.6.5 Display of the values for the genotyping analysis

The result table for the genotyping combines all data and corresponding measurements for the samples. The columns shown in the result table differ depending on the tab selected in the display area. The table for the fluorescence curves provides a summary that includes the measuring data of both dyes. If the fluorescence intensity at the end point is evaluated, the result table for the scatter plot is the same as the one for the bar graph. However, the data summarized in the result table for the fluorescence curves differ partly from the data in the scatter plot or bar graph analysis.

Well		Sample name	Sample type	Ct Genoty…	Mean Ct G…	Std.Dev. …	Ct
A4		H1	Unknown	31,69	31,81	0,16	29,
A7		WT1	Unknown	31,12	31,6	0,68	No
B4		H1	Unknown	31,92	31,81	0,16	28,
B7		WT1	Unknown	32,08	31,6	0,68	No
C4		H2	Unknown	32,99	32,82	0,23	29,
C7		WT2	Unknown	31,78	31,85	0,1	No
D4		H2	Unknown	32,66	32,82	0,23	29,

For the genotyping, the results table contains the following information:

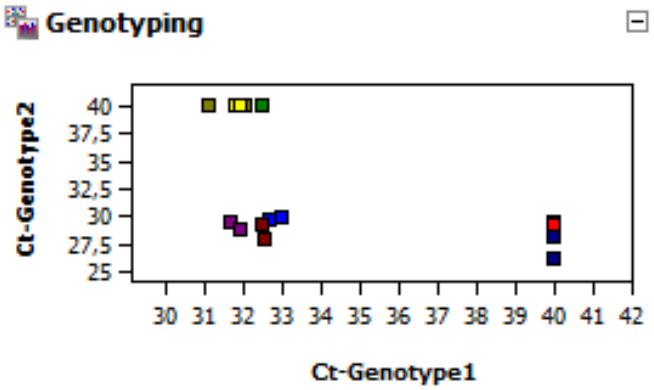
Column	Meaning
Well	Position of sample
Color of curve	Each sample is automatically assigned an unchangeable color, which is used to display the corresponding fluorescence curve
Sample name	Name of sample
Sample type	Type of sample
Group	Assignment of the sample to an experimental group
Ct Genotype 1	Ct value of genotype 1
Mean Ct Genotype 1	Mean Ct value of replicates of the genotype 1
Std. Dev. Ct 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. Ct Genotype 2	Standard deviation of the Ct values between replicates of the genotype 2
Genotype	Assign the sample according to genotype 1, genotype 2, heterozygote, or error
Reaction Genotype 1	Yes or no, depending on the endpoint fluorescence or Ct value
Reaction Genotype 2	Yes or no, depending on the endpoint fluorescence or Ct value
Genotype Replicates	Assign replicates according to genotype 1, genotype 2, heterozygote, or error ("?" symbol)
dRn Genotype 1	Standardized fluorescence intensity of the genotype 1reaction
Mean dRn Genotype 1	Standardized fluorescence intensity between replicates of the genotype 1 reaction
Std. Dev. dRn Genotype 1	Standard deviation of the standardized fluorescence intensity between replicates of the genotype 1reaction
dRn Genotype 2	Standardized fluorescence intensity of the genotype 2reaction
Mean dRn Genotype 2	Standardized fluorescence intensity between replicates of the genotype 2reaction
Std. Dev. dRn Genotype 2	Standard deviation of the standardized fluorescence intensity between replicates of the genotype 2 reaction

Individual columns can be shown or hidden by selection or deselection. The layout of the columns can also be freely modified. By clicking and dragging a column heading, depressed columns can be swapped. The display of the results in the table can thus be adjusted as desired.

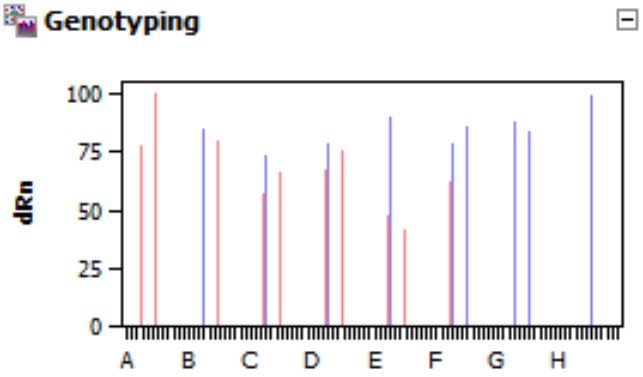
Display in the project explorer

Optionally, the scatter plot or the endpoint analysis calculated by the software can be displayed in short form in the project explorer under the point **Genotyping**. The scatter plot is divided into 4 quadrants for mutant, heterozygous, wild type, and error. The samples are assigned to a quadrant by the relative fluorescence or the C_t values determined for both fluorescent dyes. For the endpoint

analysis, the measured relative fluorescence is plotted as bar chart. The height of the bars defines the samples as genotype 1, genotype 2, heterozygous, or error.



Representation of results for genotyping as scatter plot.



Representation of results for genotyping as bar chart.

5.6.6 Deleting a genotyping

An analysis that is no longer required can be removed.

1. Activate the analysis by selecting its name in the evaluation list of the method tab.
2. Click  or select **Genotyping** > **Delete Genotyping**.
   The analysis is removed.

5.7 POS/NEG analysis at the end point

Positive/negative analysis at the end point of a qPCR is used to decide whether or not a target gene is present in the reaction mix. Such an analysis can be configured as a singleplex or multiplex experiment using fluorescence data at the end point of a PCR run, i.e., after amplification is finished. The position of the end point – with respect to the cycle number – as well as the cycles to be included can be set by you. Using the NTC samples, a cutoff value is calculated that discerns positive and negative for each individual sample. The software also accounts for internal positive controls (IPC) that can be added to each sample in order to avoid false negative results; this increases the confidence level of the experiment.

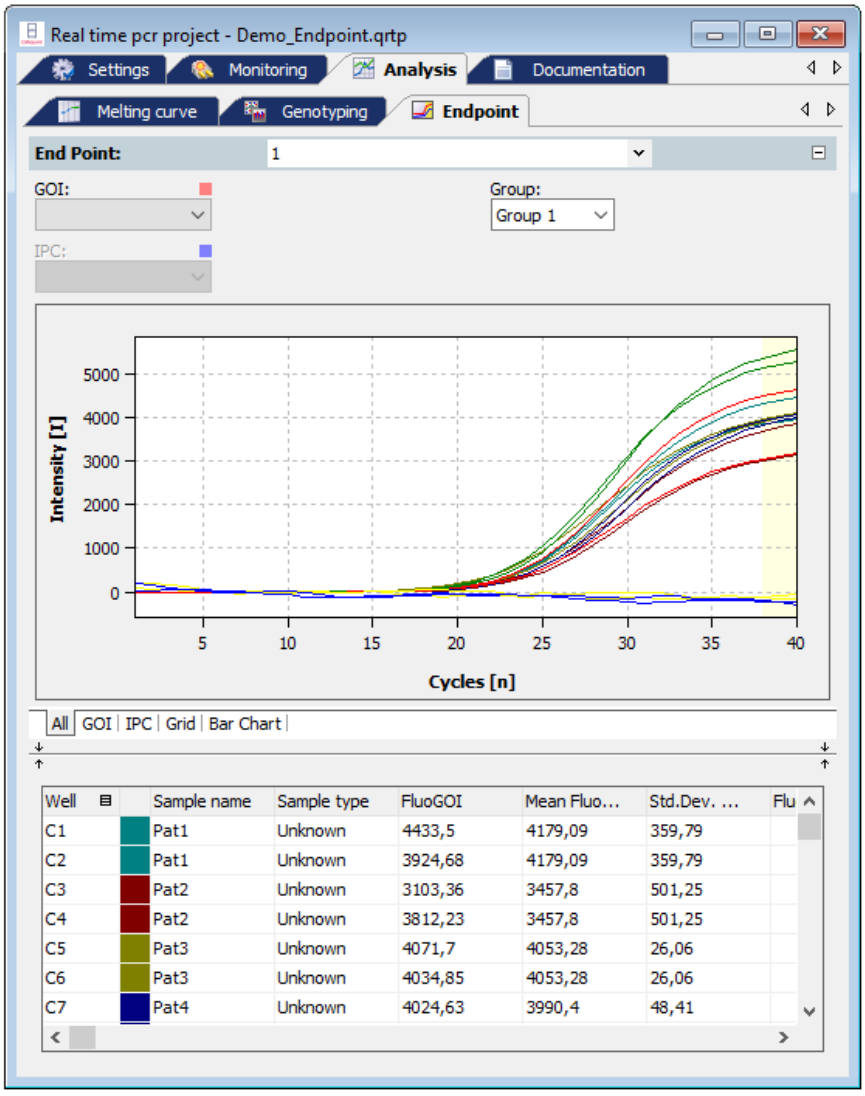
5.7.1 Starting endpoint analysis

1. Go to the **Analysis/Endpoint** project tab. If it is not visible, click the arrows in the tab bar. This will scroll the tabs.
2. Click  or select **Endpoint** > **Add Endpoint.**
3. An input window appears. Enter the description of the current analysis.

Note: An endpoint analysis can only be opened if the plate layout contains at least one NTC sample (see "Editing the sample table", page 40).

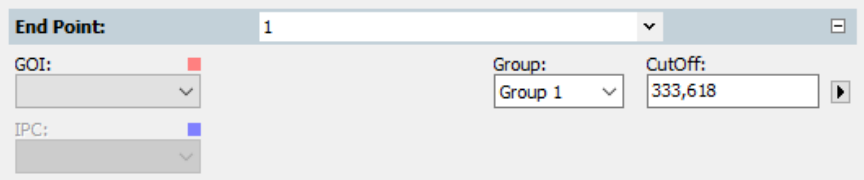
On the Endpoint tab, the following information is activated:

- Edit fields for parameter settings
- Amplification curves of the selected GOI and the IPC in a combined diagram
- Amplification curves of the selected GOI and the IPC in separate diagrams, which allow for setting the cutoff.
- Display of results in a PCR plate scheme
- Display of results in a bar chart
- Results table with measurement data and classification of the unknown samples as POS, NEG, ??? , CHECK



Well	Sample name	Sample type	FluoGOI	Mean Fluo...	Std.Dev. ...	Flu
C1	Pat1	Unknown	4433,5	4179,09	359,79	
C2	Pat1	Unknown	3924,68	4179,09	359,79	
C3	Pat2	Unknown	3103,36	3457,8	501,25	
C4	Pat2	Unknown	3812,23	3457,8	501,25	
C5	Pat3	Unknown	4071,7	4053,28	26,06	
C6	Pat3	Unknown	4034,85	4053,28	26,06	
C7	Pat4	Unknown	4024,63	3990,4	48,41	

5.7.2 Parameters settings for endpoint analysis



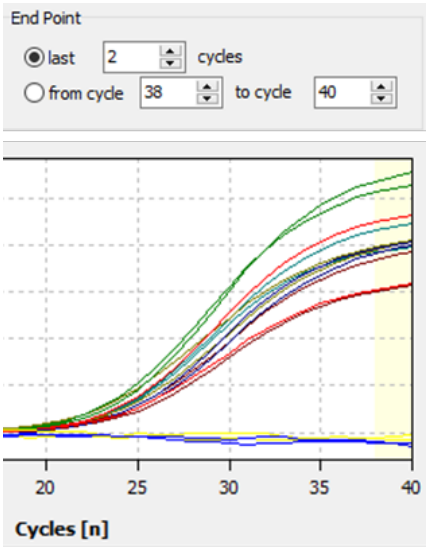
1. Set the following parameters for endpoint analysis:

Option	Meaning
Shortlist	Selection of an analysis created for this experiment
Gene of interest (GOI)	Selection list of the gene/dye combination of interest
Internal positive control (IPC)	Selection of the gene/dye combination that is used for IPC
Group	If several experiments were carried out on the plate, selection of experiments to be analyzed.
Cutoff	Cutoff defines the fluorescence value above which the sample is considered positive

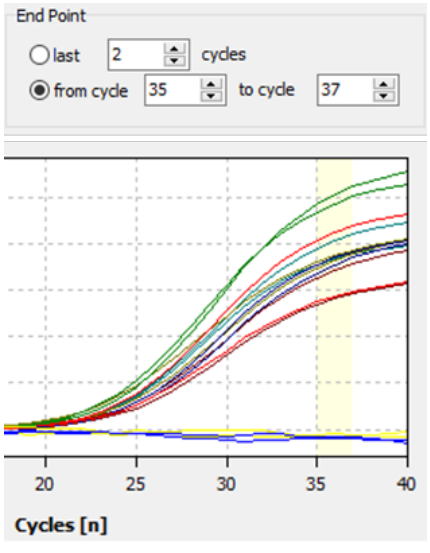
2. Define the cycle numbers that shall be considered as end point.

   By default, the mean fluorescence of the last 2 cycles is taken for calculations. You can define more than 2 cycles at the end or an interval of cycles of the qPCR run as source of endpoint fluorescence data.

   The selected cycles are highlighted in the chart by a yellowish bar.



Last 2 cycles selected



Cycles 35 to 37 selected

Setting cutoff values

Cutoff values for GOI and IPC can be set manually or calculated automatically. You can select different methods for automatic calculation in the options dialogue  for endpoint analysis.

Manual methods

- Enter a number for cutoff directly in the edit field **Cutoff** or in the options dialogue
- Shift the cutoff line using the mouse in the tabs GOI and IPC

Automated methods

- With negative control (NTC):
  The cutoff value is calculated from the mean fluorescence of the NTC samples plus the fraction (given in percent) from the difference between maximal sample fluorescence and mean fluorescence of NTC samples. Fluorescence data is taken from the specified endpoint cycles.
- With internal positive control IPC and NTC:
  Cutoff values for NTC and IPC are calculated separately. The standard deviation of the fluorescence of all NTC samples (or IPC samples, i.e., samples that do not contain an internal positive control) is multiplied with a factor T that is read from a table and is dependent from the desired confidence interval and the number of samples.
- Click  to start the automatic calculation of cutoffs according to the settings in the options. Alternatively, you can select **Endpoint** > **Auto Threshold CutOff** from the menu.

Note: If the plate layout does not contain samples that are labeled as IPC- , the option **with internal positive control and NTC** will not be available. On the tab **Settings/Samples**, selected wells can be labeled as IPC- by right-clicking the highlighted wells and selecting **Assign IPC-** from the context menu (see "Editing the sample table", page 40).

5.7.3 Displaying results in end point analysis

The results of the analysis (POS, NEG, ???, CHECK) are evaluated according to the following rules:

Without IPC

Fluorescence of a single GOI sample	Result
> Cutoff (GOI)	POS (positive)
≤ Cutoff (GOI)	NEG (negative)

With IPC

Fluorescence of a single GOI sample	Fluorescence of a single IPC sample	Result
>Cutoff (GOI)	>Cutoff (IPC)	POS (positive)
≤Cutoff (GOI)	>Cutoff (IPC)	NEG (negative)
>Cutoff (GOI)	≤Cutoff (IPC)	??? (problematic)
≤Cutoff (GOI)	≤Cutoff (IPC)	??? (problematic)

Analysis of replicates

Samples that are present in replicates are considered to be POS or NEG if all replicates of that sample are POS or NEG. If this is not the case, CHECK will be displayed. It is possible to manually deselect samples in the project explorer that are recognized as outliers.

Fluorescence of replicates	Result of sample
All POS	POS (positive)
All NEG	NEG (negative)
Otherwise	CHECK (eliminate outliers or repeat sample)

After creation of a new analysis, or after having changed options or cutoff values, the results are recalculated, and charts and table will be refreshed.

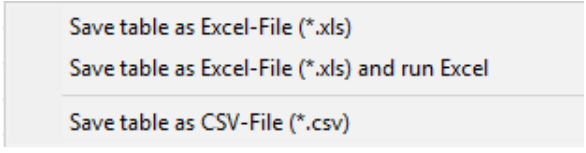
Results table

Well		Sample name	Sample type	Group	FluoGOI	Mean FluoGOI	Std.Dev. FluoGOI	FluoIPC	Mean FluoIPC	Std.Dev. FluoIPC	Status GOI	Status IPC	Result sa...	Result replicates
A4		H1	Unknown	Group 1	43894,45	41071,62	3992,08	17019,34	15944,93	1519,44	POS	POS	POS	POS
B4		H1	Unknown	Group 1	38248,79	41071,62	3992,08	14870,52	15944,93	1519,44	POS	POS	POS	POS
C4		H2	Unknown	Group 1	31714,67	34864,64	4454,74	12812,05	13328,08	729,78	POS	POS	POS	POS
D4		H2	Unknown	Group 1	38014,61	34864,64	4454,74	13844,11	13328,08	729,78	POS	POS	POS	POS
E4		H3	Unknown	Group 1	26587,02	30696,55	5811,76	15718,75	14758,92	1357,41	POS	POS	POS	POS
F4		H3	Unknown	Group 1	34806,09	30696,55	5811,76	13799,08	14758,92	1357,41	POS	POS	POS	POS
G4		NTC	NTC	Group 1	-199,7	-24,41	247,89	15239,33	16507,95	1794,1	NEG	POS	NEG	NEG
H4		NTC	NTC	Group 1	150,88	-24,41	247,89	17776,58	16507,95	1794,1	NEG	POS	NEG	NEG
A7		WT1	Unknown; IPC-	Group 1	55748,83	50011,56	8113,73	-238,58	-213,27	35,8	POS	NEG		
B7		WT1	Unknown; IPC-	Group 1	44274,29	50011,56	8113,73	-187,95	-213,27	35,8	POS	NEG		
C7		WT2	Unknown; IPC-	Group 1	36536,85	39388,36	4032,65	-246,75	-149,28	137,84	POS	NEG		
D7		WT2	Unknown; IPC-	Group 1	42239,87	39388,36	4032,65	-51,81	-149,28	137,84	POS	NEG		
F7		M2	Unknown	Group 1	-346,38	-411,01	91,4	15201,38	15105,65	135,39	NEG	POS	NEG	NEG
G7		M2	Unknown	Group 1	-475,65	-411,01	91,4	15009,91	15105,65	135,39	NEG	POS	NEG	NEG
H7		M3	Unknown	Group 1	-85,52	-85,52	0	17320,61	17320,61	0	NEG	POS	NEG	NEG

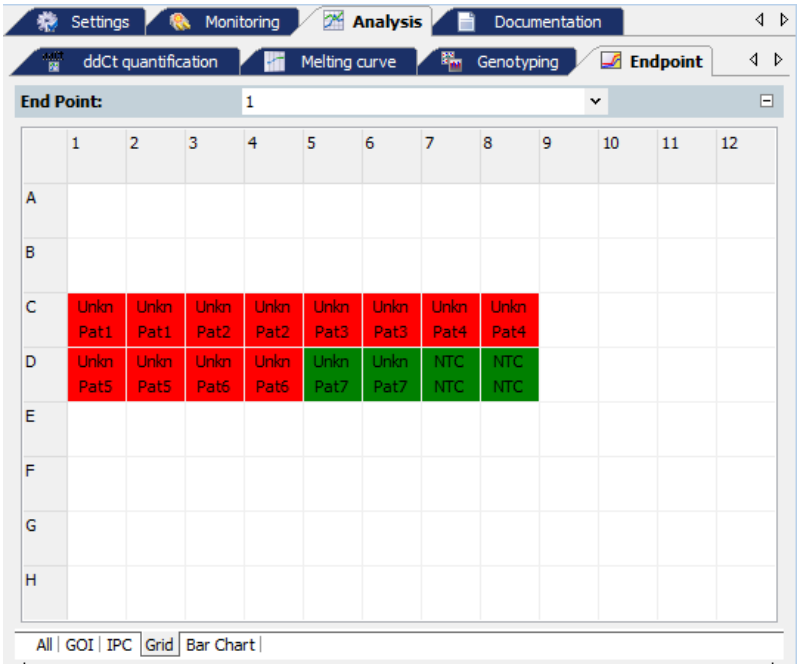
For the endpoint, the results table contains the following information:

Column	Meaning
Well	Position of sample
Color of curve	Each sample is automatically assigned an unchangeable color, which is used to display the corresponding fluorescence curve
Sample name	Name of sample
Sample type	Type of sample
FluoGOI	Fluorescence of the GOI samples at the endpoint
Mean FluoGOI	Mean fluorescence of the GOI replicates at the endpoint
Std. Dev. FluoGOI	Standard deviation of the GOI replicates at the endpoint
FluoIPC	Fluorescence of the IPC samples at the endpoint
Mean FluoIPC	Mean fluorescence of the IPC replicates at the endpoint
Std.Dev.FluoIPC	Standard deviation of the IPC replicates at the endpoint
Status GOI	POS if FluoGOI > CutOff, else NEG (for each sample well)
Status IPC	POS if FluoIPC > CutOff, else NEG (for each sample well)
Result sample	Rating POS/NEG/??? for each well
Result replicates	Rating POS/NEG/CHECK of the replicates

The results table displays all calculated numerical data and the analysis of single samples and replicates. You can design the results table according to your requirements by setting type, sequence, and width of the columns to be displayed. You can also adjust the sort sequence of data in the column (alphabetical, numerical, by column, by row). The table configured this way can be exported as XLS or CSV file by right-clicking the table.



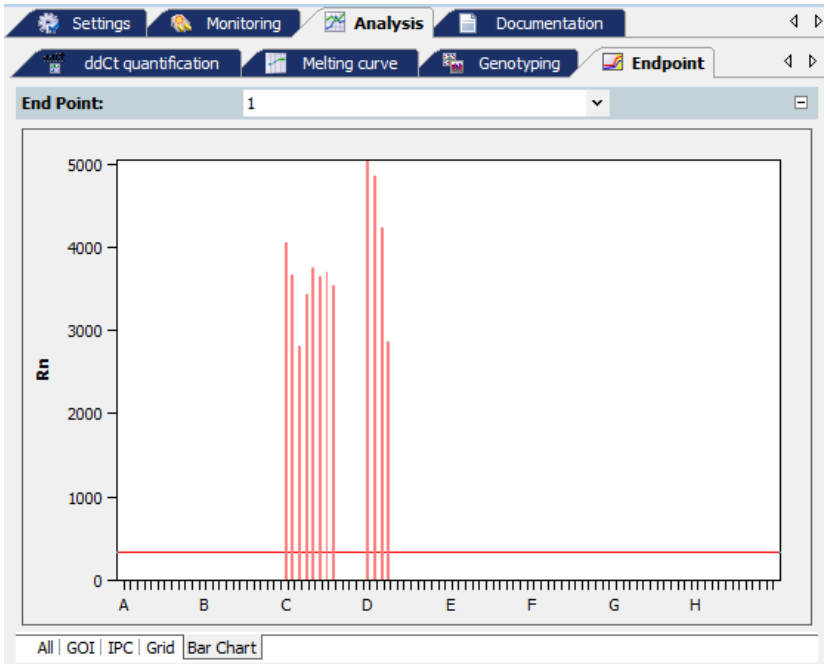
PCR plate scheme (grid)



For QIAquant 384, columns 1–24 and rows A–P are available using the scroll bars.

The PCR plate scheme offers a quick overview of the results obtained in each single well. If the mouse pointer hovers on a well, a hint box is displayed showing the sample name and the endpoint fluorescence of GOI and IPC for that well. You can change the colors that code for POS, NEG, and ??? in **Extras/Options/Colors.**

Bar chart



The bar chart displays the endpoint fluorescence of the GOI together with the IPC, as well as the corresponding cutoff values as horizontal lines. Red lines denote the GOI and blue ones the IPC. If the mouse pointer is moved across a bar, a hint box is displayed showing the sample name and the end point fluorescence of GOI and IPC for the corresponding well.

Note: Cutoff lines cannot be changed in the bar chart. To change cutoff lines, please switch to the **GOI** or **IPC** tabs.

6 Multigene-/Multiplate-Analysis

Multigene-/multiplate-analysis is used to analyze real-time PCR data of multiple target genes in parallel or to analyze data from multiple project files if, for instance, several PCR plates were used for an experiment. Multigene-/multiplate-analysis is executed as dialog in a separate window independent of the surface of the QIAquant 384 Software. Project files generated by the QIAquant 384 Software are used. Project files must include a $\Delta\Delta C_T$-analysis for multigene-/multiplate-analysis.

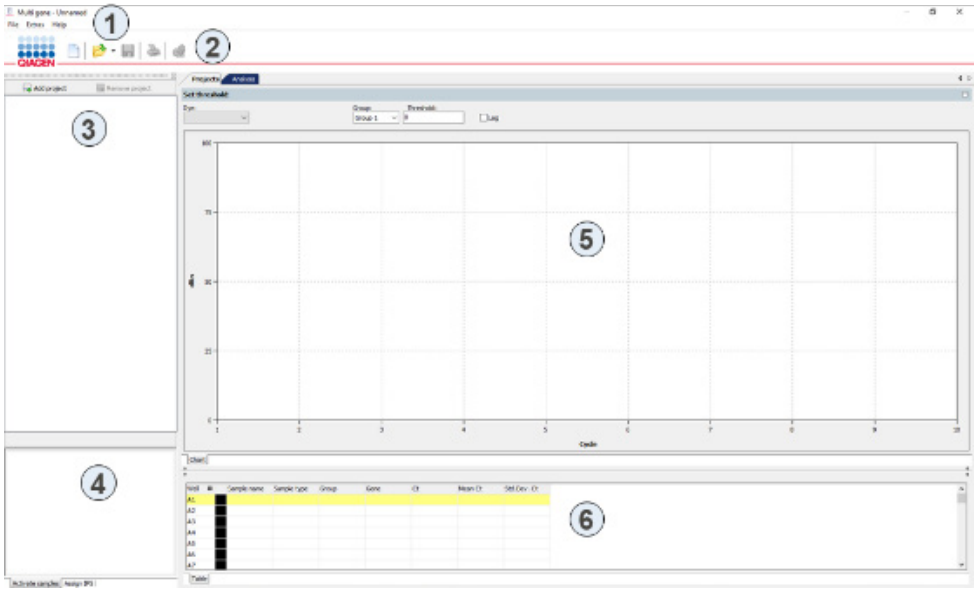
6.1 Start Multigene-/Multiplate-Analysis

Click  in the toolbar. A new window for multigene-/multiplate-analysis appears.

6.2 The window for multigene-/multiplate-analysis

The window for multigene-/multiplate-analysis is divided into the following sections:

Menu bar (1)	Contains the menu commands for, e.g., opening, editing, saving, and printing of multigene-/multiplate-analysis files, and a help function
Toolbar (2)	Commands for editing multigene-/multiplate-analysis files are arranged here
Project list (3)	Lists and administrates projects that are used for multigene-/multiplate-analysis
Samples (4)	Measurement data of samples can be selected/unselected (only data of selected samples are included in the analysis and used for results calculation) and the position of IPS can be defined
Data display (5)	Amplification curves of imported project files are shown, or the results of the multigene-/multiplate-analysis are plotted as bar chart
Results table (6)	Shows imported measurement data of activated project file or the results of multigene-/multiplate-

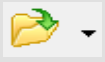


6.3 Overview of menu commands

In the multigene-/multiplate-analysis window, these commands are available in the **File** menu:

File >	Description
New Multigene-/Multiplate-Analysis	Opens a new multigene-/multiplate-analysis
Open Multigene-/Multiplate-Analysis	Opens a multigene-/multiplate-analysis
Save Multigene-/Multiplate-Analysis	Saves a multigene-/multiplate-analysis in the QIAquant 384 Software standard folder
Save Multigene-/Multiplate-Analysis as	Saves a multigene-/multiplate-analysis in any user-selected folder
Print Multigene-/Multiplate-Analysis	Prints the results of a multigene-/multiplate-analysis
Close	Closes the window for multigene-/multiplate-analysis

6.4 Overview of the tools in the toolbar

Button	Command	Function
	New	Opens a new multigene-/multiplate-analysis
	Open	Opens a multigene-/multiplate-analysis
	Save	Saves a multigene-/multiplate-analysis
	Print	Prints a multigene-/multiplate-analysis
	Options	Allows the input of efficiencies of PCR reactions for single genes of interest

6.5 Managing multigene-/multiplate-analysis files

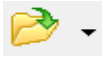
After clicking  , the **Multigene-/Multiplate-Analysis** dialog starts in a separate window and contains no data.

6.5.1 Start new multigene-/multiplate-analysis

To start a new multigene-/multiplate-analysis, click  or select **File** > **New**. An already-loaded multigene-/multiplate-analysis will be closed. If the analysis has been modified and the changes are not yet saved, a prompt opens.

Note: Only one multigene-/multiplate-analysis can be opened.

6.5.2 Open saved multigene-/multiplate-analysis

1. Click  or select **File** > **Open Multigene-/Multiplate-Analysis**.
2. In the standard window, select the desired file to open and confirm the selection with **OK**.

Note: If the file type is linked to the QIAquant 384 Software application in **Extras** > **Options**, double-clicking the file will automatically start the dialog for multigene-/multiplate-analysis.

The multigene-/multiplate-analysis – including the project list, sample layout, measurement data, and analysis – opens.

6.5.3 Save multigene-/multiplate-analysis

The multigene-/multiplate-analysis, including all loaded project files and analysis, is stored.

1. Select **File** > **Save Multigene-/Multiplate-Analysis**.
2. To save files, enter the name of the template in the standard window and click **OK**.

Changes in a multigene-/multiplate-analysis can be saved with **File** > **Save Multigene-/Multiplate-Analysis**. Alternatively, you can click  in the toolbar.

6.5.4 Close multigene-/multiplate-analysis

The **File** > **Close Multigene-/Multiplate-Analysis** menu command closes the Multigene-/Multiplate-Analysis window. If any unsaved changes have been made in analysis, a confirmation prompt appears.

6.5.5 Print multigene-/multiplate-analysis

The data and results of a multigene-/multiplate-analysis can be printed using the **Print** function. For this purpose, an analysis must be applied and, in the data display, the tab **Analysis** must be selected (see "The window for multigene-/multiplate-analysis", page 127). Start printing by selecting **File** > **Print Multigene-/Multiplate-Analysis** or click  in the toolbar.

6.6 Load project files

When a new multigene-/multiplate-analysis is started, a blank window is displayed initially. To perform an analysis project, files have to be loaded. In the project files, a $\Delta\Delta C_T$-analysis must be applied to evaluate the files by multigene-/multiplate-analysis.

1. To load a project file, click **Add project**.
2. In the standard window, select one or more stored project files and confirm your selection with **OK**. Loaded projects are listed in the project list.
   2a. To remove a project file, mark the file in the project list.
   2b. Click **Remove project**.

6.7 Activate/deactivate project files for multigene-/multiplate-analysis

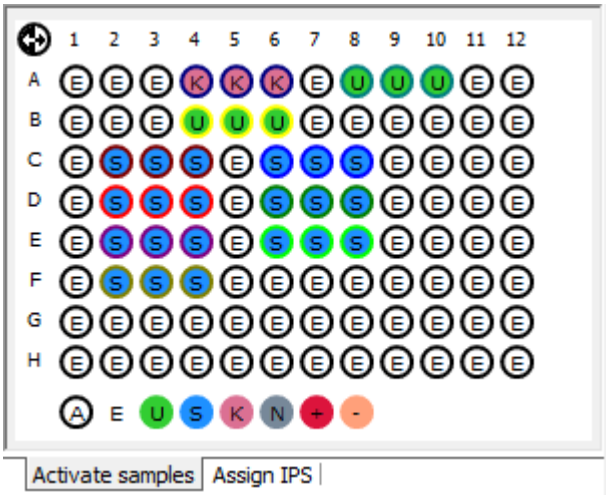
In the project list, a check box is shown next to each loaded project. By the check box, projects can be activated or deactivated. Only data from activated projects are used for analysis.

The data of deactivated project files remain loaded in the background and, after reactivation, are used for analysis again.

6.8 Activate/deactivate samples for multigene-/multiplate-analysis

In the data display for multigene-/multiplate-analysis, activate the **Projects** tab (see "The window for multigene-/multiplate-analysis", page 127). On the **Activate samples** tab of the data display, samples of single wells can be activated or deactivated for analysis. By deactivation of samples, outliers are eliminated and not included in the calculation of mean values.

Note: The sample selection only influences the analysis of data; no measurement data is deleted.



The sample layout is taken from the loaded project files. The color code for each sample type can be modified in menu **Extras/Options > Colors**.

Sample type	Symbol	Definition
Empty	E	Describes an empty position on the PCR plate
Unknown	U	Sample of unknown concentration or dilution (measuring sample)
Standard	S	Sample of known concentration or dilution
Calibrator	K	Sample whose gene of interest expression level is set as 1
No Template Control (NTC)	N	Complete reaction preparation but without matrix strand
Positive Control	+	Positive control preparation for which a reaction product is expected
Negative control	–	Negative control preparation for which no reaction product is expected

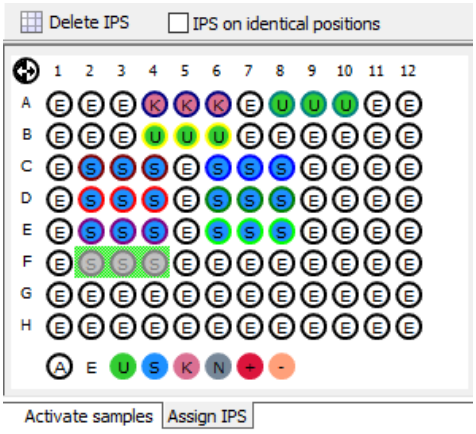
Active wells (i.e., displayed wells) are marked with their sample type symbol. For deactivated wells, the position is gray and the fluorescence data is hidden. Empty wells are marked **E**.

- Click with the mouse to switch. The activation changes with each click on a well.
- You can select adjacent wells by clicking and dragging the cursor over the wells. To select nonadjacent positions, hold the **Ctrl** key down while clicking each position.
- Invert entire rows and columns by clicking the letter or number of rows A–H or columns 1–12.
- Activation status of the complete plate can be changed by clicking , between A and 1.
- To activate all wells, click , found below the chart.
- To activate only samples of a specific type, click the corresponding symbol below the chart. To activate multiple sample types at the same time, hold the **Ctrl** key down while clicking the sample types.

6.9 Define interplate standards for multigene-/multiplate-analysis

Activate the **Projects** tab in the data display for multigene-/multiplate-analysis (see "The window for multigene-/multiplate-analysis", page 127). For multigene-/multiplate-analysis, interplate standards (IPS) are used on each PCR plate, and deviations among themselves are determined and calculated.

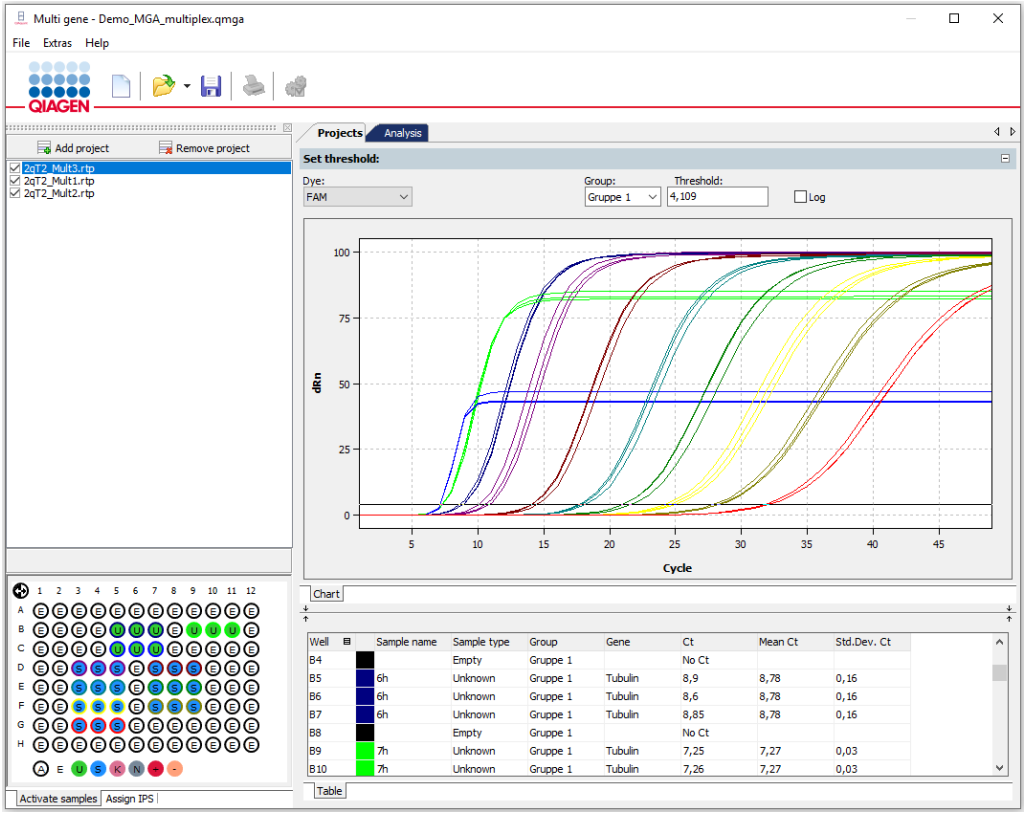
1. To define IPS, activate the **Assign IPS** tab in the sample display.



2. Select the position of IPS:
   - Click and drag the area that contains the IPS samples:
     Selected samples are displayed in gray against a green background. For unselected samples, only the sample type icon is displayed. Empty wells are marked with **E**. To select samples, see also "Activate/deactivate samples for multigene-/multiplate-analysis", page 130.
   - If the IPS position is the same on all plates, the current selection is transferred to all loaded projects by checking the IPS checkbox on identical positions.
   - Click **Delete IPS** to delete the IPS in all loaded projects.

6.10 Set threshold and PCR-efficiencies for multigene-/multiplate-analysis

After loading project files, the measurement data for the active project is shown in the display area. The active project is marked blue in the project list on the left. By clicking on the name in the project list, another project can be activated, thus switching between different projects.

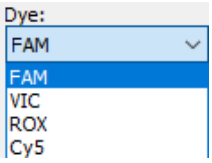


All measurement results and settings are taken from the loaded projects. In the dialog for multigene-/multiplate-analysis, most settings cannot be modified; this is only possible in the individual projects in the QIAquant 384 Software.

Edit threshold

On the **Projects** tab, the threshold for each dye can be adapted.

1. Activate the relevant project in the project list.
2. Select a dye from the list on **Projects**.



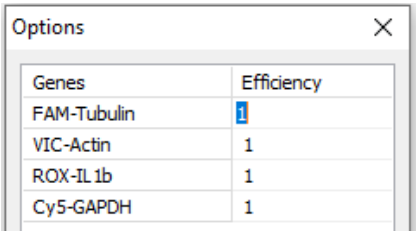
3. If in the project file, different groups are defined, select the right group from the list.
4. To modify the threshold value, 2 different options are available:
   - In the chart, click and drag the black threshold line up or down with the mouse.
   - Enter a value in the **Threshold** field.

With the modification of the threshold, the values in the result table displayed below are updated.

Edit PCR efficiency

Basically, PCR efficiencies are taken from the loaded project files, but it is possible to adjust the value for the considered genes.

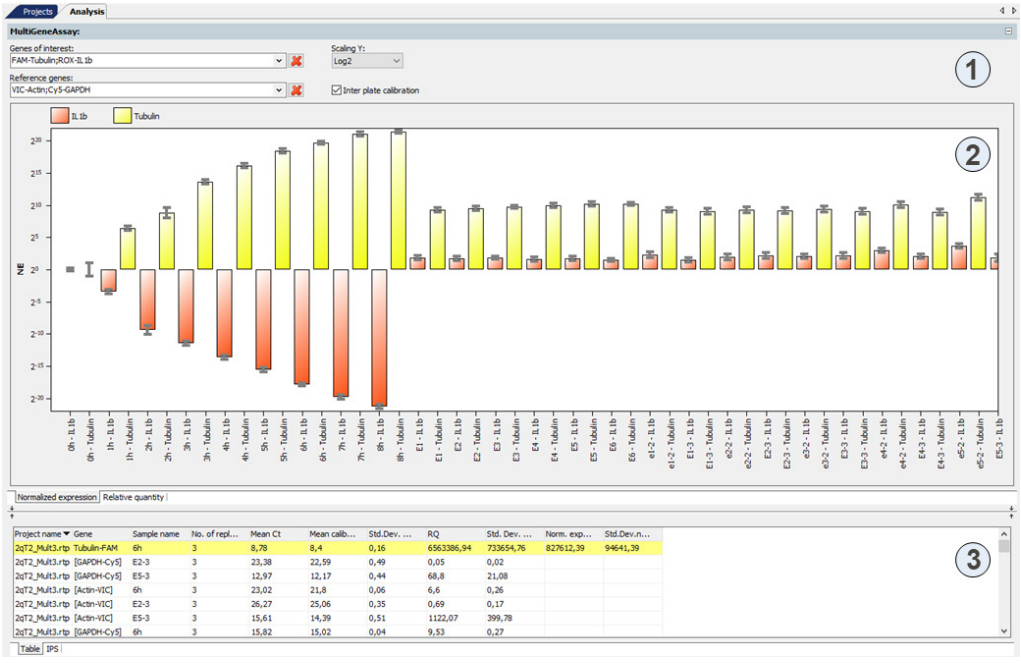
1. Activate the **Analysis** tab in the data display and click  in the toolbar.
2. Adjust for each gene the PCR efficiency in the **Options** window.



3. Enter a new value and confirm with **OK**.

6.11 Evaluation of the multigene-/multiplate-analysis

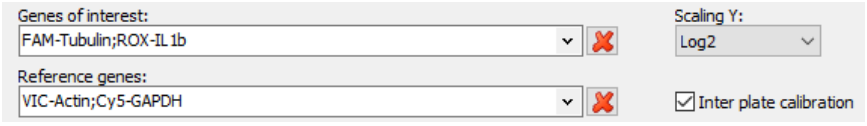
The results of the multigene-/multiplate-analysis are summarized under the **Analysis** tab.



The data display is divided into the following sections:

- Parameter settings (1)
- Graphical data display (2)
- Results table with measured values (3)

Parameter settings for multigene-/multiplate-analysis



Option	Description
Genes of Interest (GOI)	Selection list of target gene/dye-combinations. Multiple target genes can be selected. The symbol is used to remove all target genes from the evaluation
Reference genes	Selection list of reference gene/dye-combinations. Multiple reference genes can be selected. The symbol is used to remove all reference genes from the evaluation.
Scaling Y	Selection of the scaling of the Y-axis.
Interplate calibration	With activated interplate calibration from the selected IPS samples of all plates, a correction factor is determined; from the **mean C_t values** of replicates, the **corrected mean C_t values** are calculated (see results table). The **corrected mean C_t values** are then used to calculate relative quantity and normalized expression. If the interplate calibration is deactivated, **corrected mean C_t values** are equal to **mean C_t 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

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

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

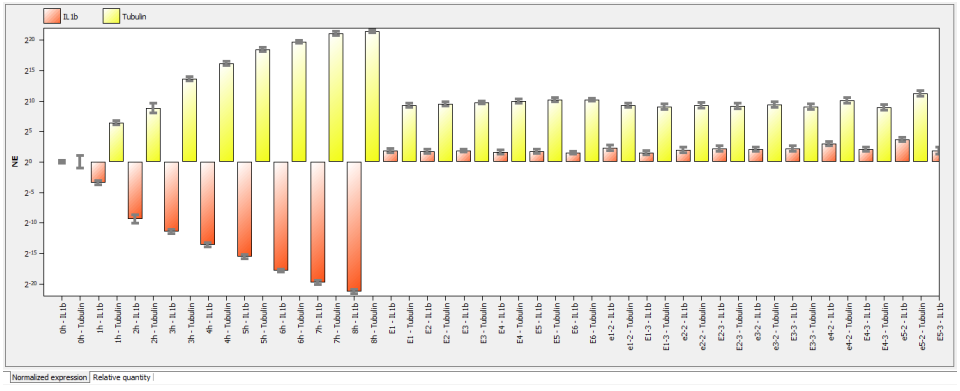
$\overline{Ct}_{p}^{IPC} - mean\ value\ of\ Ct - values\ of\ IPS - samples\ on\ plate\ p$

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

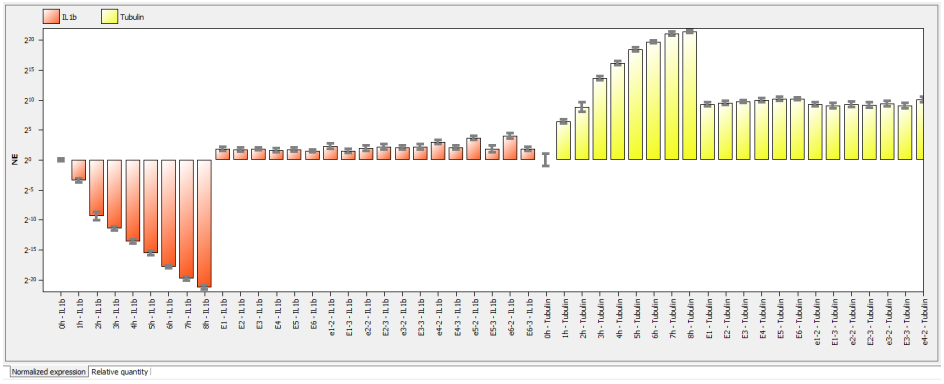
Results display for multigene-/multiplate-analysis

The results of the multigene-/multiplate-analysis are shown as bar charts. In the **Normalized Expression** tab, the expression of selected GOIs – normalized to the expression of the reference genes – is shown. In the second tab, **Relative Quantity**, the corresponding quantity for the GOIs and reference genes is displayed. The respective sample name is given below each bar. The height of the bar is determined by the calculated normalized expression of replicates. Hovering the mouse cursor on each bar displays some short information about the sample name, mean value, and the calculated standard deviation. The standard deviation for the normalized expression is shown as error bar on top of each bar. For large numbers of samples, click and drag the display horizontally to see all the bars. To narrow or broaden the width of the representation, scroll with the mouse wheel or use the ↑ and ↓ keys.

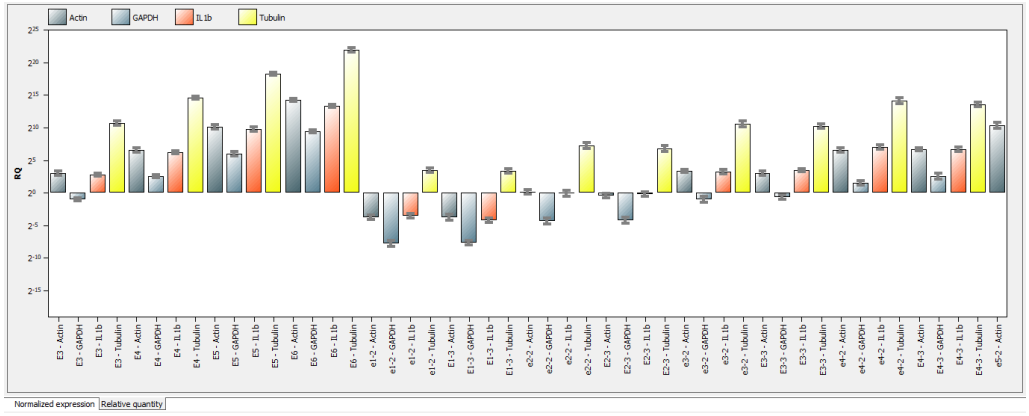
Right-clicking on the chart sorts the results by genes or samples names on the x-axis.



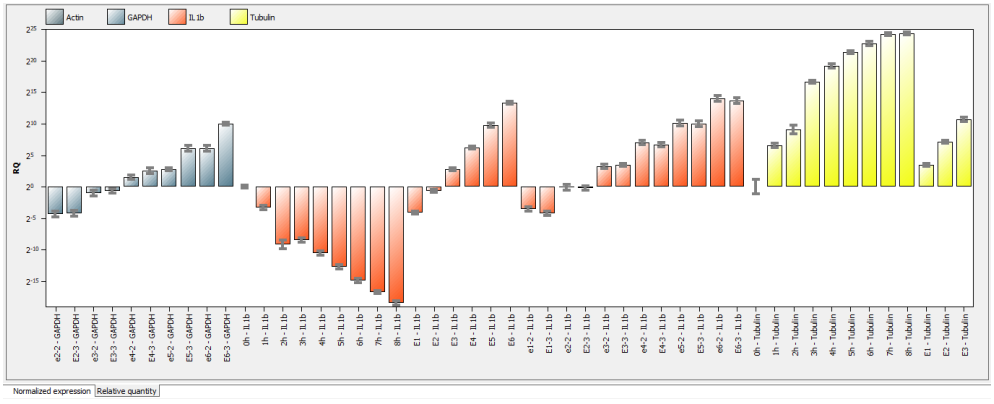
Representation of the normalized expression as bar chart sorted by sample name.



Representation of the normalized expression as bar chart sorted by gene name.

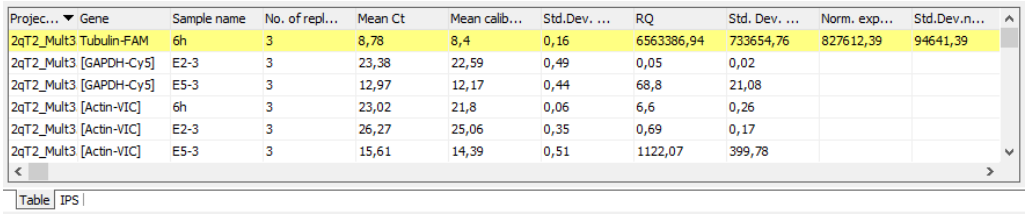


Representation of the relative quantity as bar chart sorted by sample name.



Representation of the relative quantity as bar chart sorted by gene name.

In the **Table** tab of the results table for multigene-/multiplate-analysis, all data and measurement values for the samples are summarized.



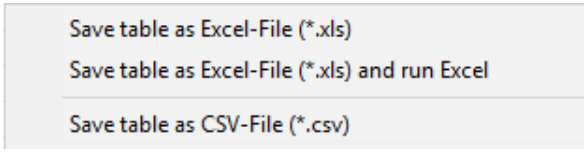
Projec...	Gene	Sample name	No. of repl...	Mean Ct	Mean calib...	Std.Dev. ...	RQ	Std. Dev. ...	Norm. exp...	Std.Dev.n...
2qT2_Mult3	Tubulin-FAM	6h	3	8,78	8,4	0,16	6563386,94	733654,76	827612,39	94641,39
2qT2_Mult3	[GAPDH-Cy5]	E2-3	3	23,38	22,59	0,49	0,05	0,02		
2qT2_Mult3	[GAPDH-Cy5]	E5-3	3	12,97	12,17	0,44	68,8	21,08		
2qT2_Mult3	[Actin-VIC]	6h	3	23,02	21,8	0,06	6,6	0,26		
2qT2_Mult3	[Actin-VIC]	E2-3	3	26,27	25,06	0,35	0,69	0,17		
2qT2_Mult3	[Actin-VIC]	E5-3	3	15,61	14,39	0,51	1122,07	399,78		

Table IPS

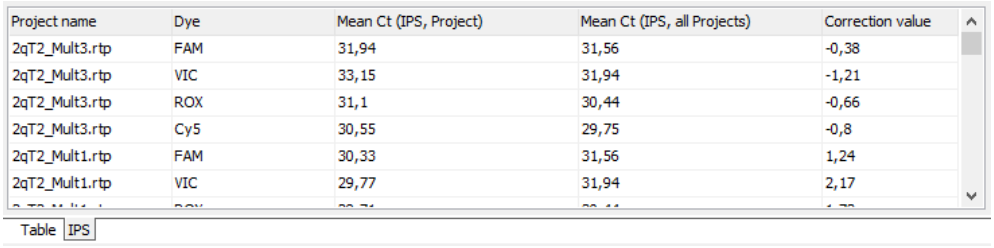
For the multigene-/multiplate-analysis, the results table contains the following information:

Column	Meaning
Project name	Name of the loaded project that contains the sample
Gene	Name of the investigated gene
Sample name	Name of the sample
No. of replicates	Number of replicates of the sample
Mean C_t	Mean C_t-value of the replicates of a sample
Mean Calib. C_t	Mean C_t-values of replicates of a sample calibrated by the IPS
Std. Dev. Mean Calib. C_t	Standard deviation of the mean calibrated C_t-values of replicates of a sample
RQ	Calculated relative quantity for replicates of the gen in the original sample
Std. Dev. RQ	Standard deviation of the calculated relative quantity for replicates of the gen in the original sample
Norm. Exp.	Normalized expression of the sample
Std. Dev. Norm. Exp.	Standard deviation of the normalized expression of the sample

You can design the results table according to your requirements by setting type, sequence, and width of the columns to be displayed. You can also adjust the sort sequence of data in the column (alphabetical, numerical, by column, by row). The table configured this way can be exported as XLS or CSV file by right-clicking the table.



All IPS data are summarized in the **IPS** tab of the results table for Multigene-/Multiplate-Analysis.



Project name	Dye	Mean Ct (IPS, Project)	Mean Ct (IPS, all Projects)	Correction value
2qT2_Mult3.rtp	FAM	31,94	31,56	-0,38
2qT2_Mult3.rtp	VIC	33,15	31,94	-1,21
2qT2_Mult3.rtp	ROX	31,1	30,44	-0,66
2qT2_Mult3.rtp	Cy5	30,55	29,75	-0,8
2qT2_Mult1.rtp	FAM	30,33	31,56	1,24
2qT2_Mult1.rtp	VIC	29,77	31,94	2,17

Table IPS

Column	Meaning
Project name	Name of the loaded project that contains the IPS sample
Dye	Dye that has been used to determine the C_t value of the IPS sample
Mean C_t (IPS, project)	Mean C_t value of IPS samples in the project (dependent upon the used dye)
Mean C_t (IPS, all projects)	Mean C_t value of IPS samples in all projects (dependent upon the used dye)
Correction value	C_t correction value that is applied for all samples of the project (column 1) and the dye (column 2)

7 MIQE Documentation

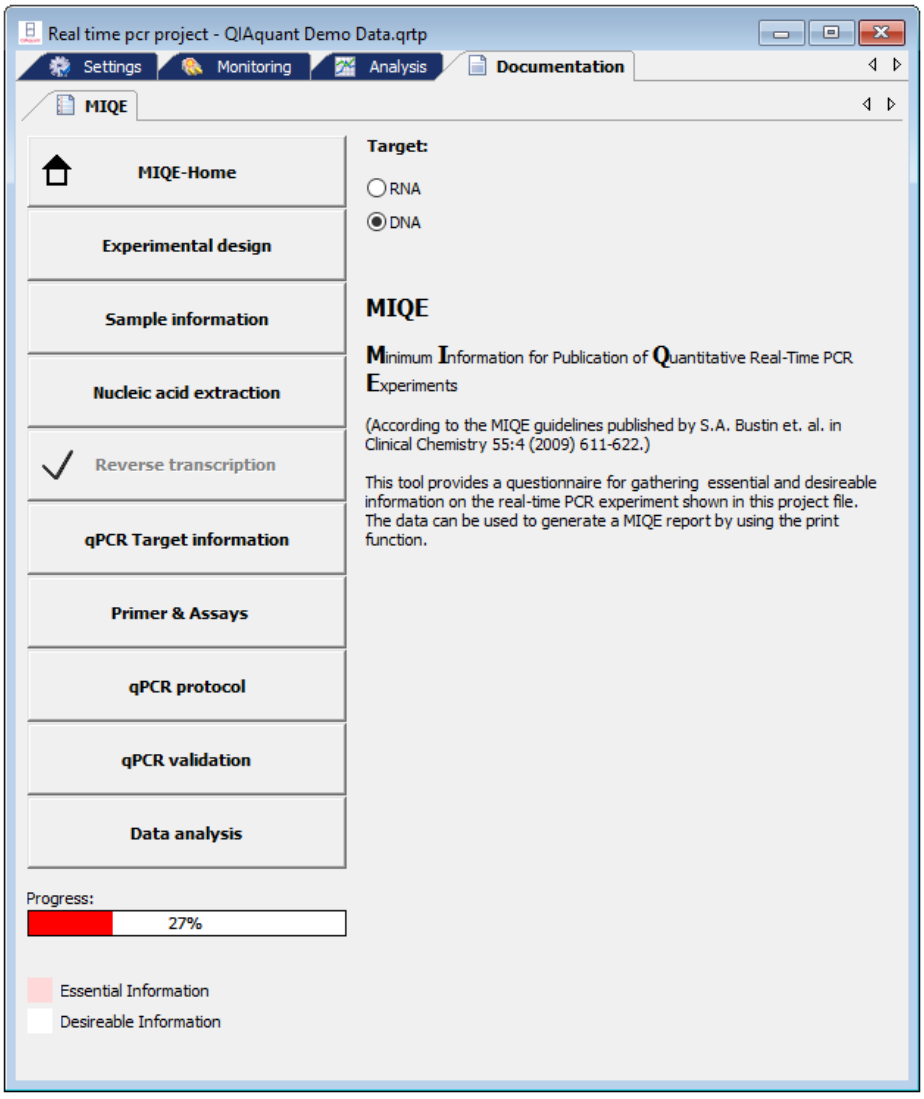
In 2009, an international group of experts led by Prof. Steven Bustin developed guidelines for the publication of real-time PCR data.* The aim was to prevent the publication of incomplete or incorrect real-time PCR data and to ensure comparability and reproducibility of experiments. The guidelines regulate the requirements regarding the minimum information necessary for the publication of data and have become known as MIQE (minimum information for publication of quantitative real-time PCR experiments).

Instructions for completing the MIQE documentation

1. MIQE consists of a list of questions to 9 different topics about real-time PCR experiments. In the QIAquant 384 Software, under the **MIQE** tab, buttons for each topic are present, providing access to the corresponding list of questions. Additionally **MIQE-Home** allows you to jump back to the main menu from any submenu.
2. In general, at first, by activating the corresponding radio button in the main screen, it should be defined whether DNA or RNA has been used as starting material. If the **DNA** option is activated, the questions for **Reverse Transcription** do not have to be answered, and the corresponding button becomes inactive.
3. After clicking on a button, the corresponding questionnaire opens. The number of questions differs between the topics. The user should answer as many questions as possible.
4. The answers to some questions are taken from the currently opened project and active project if the relevant information is available.
5. The completeness of the answers to the questions is presented by the software as a progress bar and is measured in %. The MIQE questionnaire distinguishes between essential and optional questions. The entry fields for essential questions are highlighted by a red-colored background, whereas optional questions have a white-colored background. For the progress bar, only the answered essential questions are ranked by the software. The total number of questions varies depending on whether DNA or RNA has been used as starting material. The software cannot evaluate the quality of the answers. It is up to the user to fully answer the questions and with due diligence.
6. The QIAquant 384 Software offers to import MIQE data from other projects. Clicking  in the toolbar or selecting **MIQE** > **Import MIQE Documentation** opens a dialog screen. After the appropriate project is selected, the saved MIQE data is imported into the current project.

* Bustin, S.A., et al. (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry **55**, 611–622.

7. The questionnaire can be printed by the **Print** function of the QIAquant 384 Software. Select the **MIQE** under **Documentation** (see "Printing", page 21) and click **Print**. It is always the complete questionnaire that is printed.



8 Functions in the **Extras** Menu

8.1 Device initialization

Device initialization sets the device to its original state and is only required when an error occurs.

- Call up the **Extras** > **Device initialization** command.

8.2 Connecting the device to the PC

The software QIAquant 384 Software automatically recognizes the connected instrument and whether it is switched on or not. The instrument may also be switched on and off during running QIAquant 384 Software. Whether an active connection is established will be indicated in the lower-left corner of the status line.

- If a connection cannot be established within 30 seconds, select **Extras** > **Device identification** to solve the problem

8.3 General settings in the QIAquant 384 Software

General settings for the QIAquant 384 Software program can be made in the **Options** window.

Note: For most of the settings in the **Options** window, you need to be logged into QIAquant 384 Software as an administrator.

1. Close all projects.
2. Select **Extras** > **Options** to open the window of the same name.
3. Make the following settings on the tabs:

Tab	Function
General	Define the default folder for saved files (see "Saving a project", page 20).
Data format	Select decimal separator for the data export and number of decimal places to be displayed for different variables.
Measurement	**Sensitivity** Set the basic sensitivity for optical measurements. **Meas. rep. color comp.** Enter the measurement repetitions for recording the color compensation. **Show negative values resulting from color compensation** If activated, negative values are also displayed in the sequence of the color compensation, otherwise the output is "0".

Tab	Function
Analysis	You can enter a factor for the quantitative evaluations (**Quant factor**), for the melting curve analysis (**Melting factor**), and for the genotyping (**Genotyping factor**) in the list fields. This factor will be used for the automatic calculation of the threshold.
Device	Enables you to define a file name into which the device communication data will be written. The recorded data is used for error diagnosis (see "Appendix B: How to Save Communication Data", page 162).
User management	Activate or deactivate the user management. If the user management is deactivated, no login prompt will appear at the program start. The functions for setting up the user management and for signing projects will not be available.
Colors	Allows selection of the color code of sample type and replicates and the display of curve color according to sample type, well, or replicates. The desired colors for wells, replicates, sample types, and the marking of positive and negative analyses can also be set.

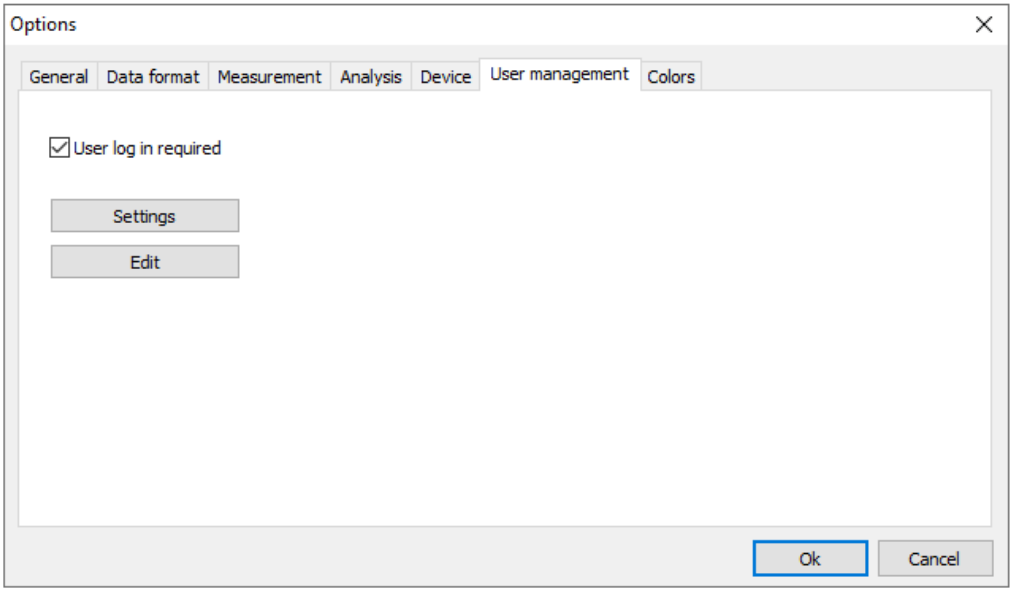
9 User Management

Note on the general data security

Due to the encryption used, the reading and changing of project, template, analysis, and communication files generated by QIAquant 384 Software is only possible with QIAquant 384 Software.

The user management will be enabled in **Options** > **User management**.

Option	Description
User login is required	If enabled, the user management is effective at the next program start. Logging on 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 administration is created.
Settings	Settings for passwords, login, and logout
Edit	Management of user profiles

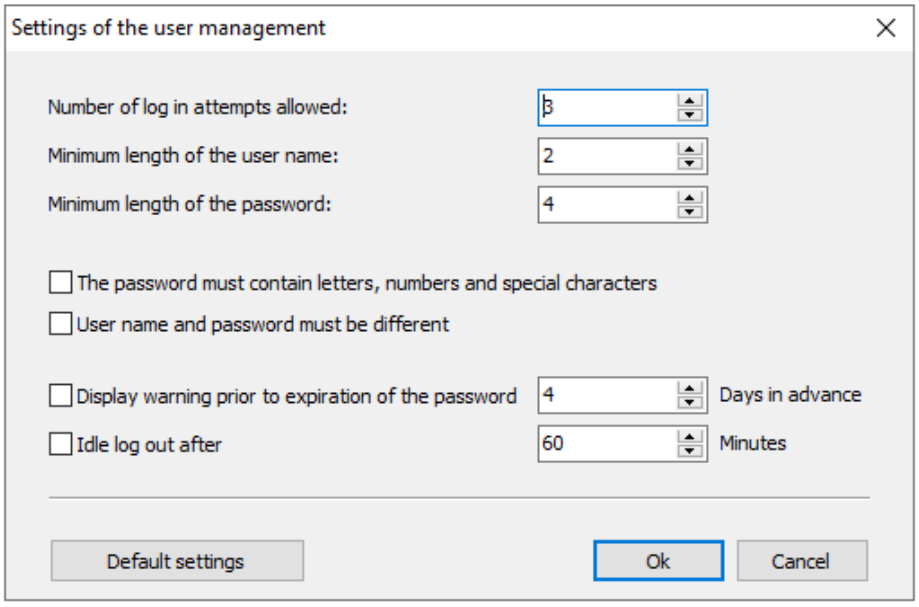


9.1 Basic settings for password, login and logout

To access the basic settings that apply to all users, select **Extras > Options**. On the **User management** tab, click **Settings**.

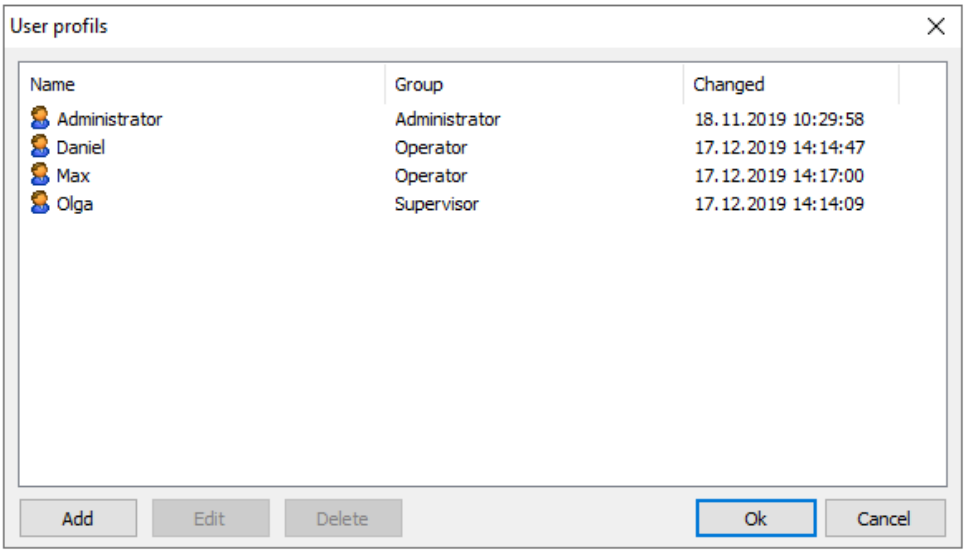
You can make the following settings in user management:

- 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 password
- Required characters in the password
- Warning prior to password expiration. The password expiration is set in the user profiles
- Logout in case of inactivity: 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 **Cancel** is selected in the login window, 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.



9.2 Managing user profiles/user groups

To access user management, select the **Extras** > **Options** menu command. On the **User management** tab, click **Edit**.



The following functions are available in the extended user management:

Function	Description
Add	Create a new user profile
Edit	Edit a selected user profile
Delete	Delete a selected user profile

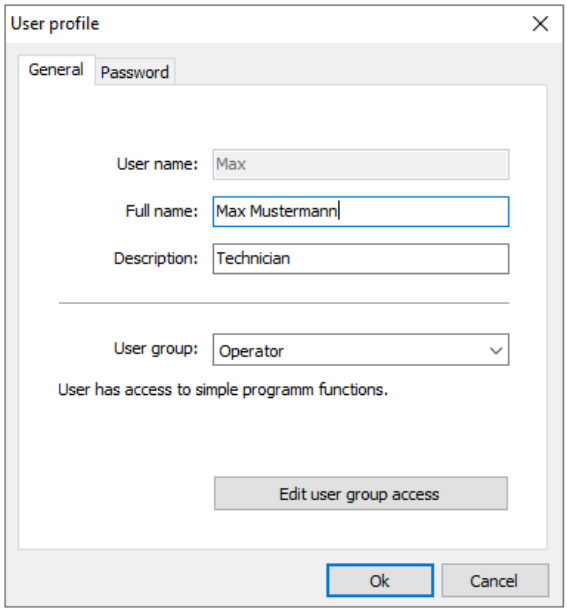
By default, these functions are only available to users belonging to the **Administrator** group. However, they can also be assigned to a **Supervisor** by editing the user rights.

Add/edit user profile

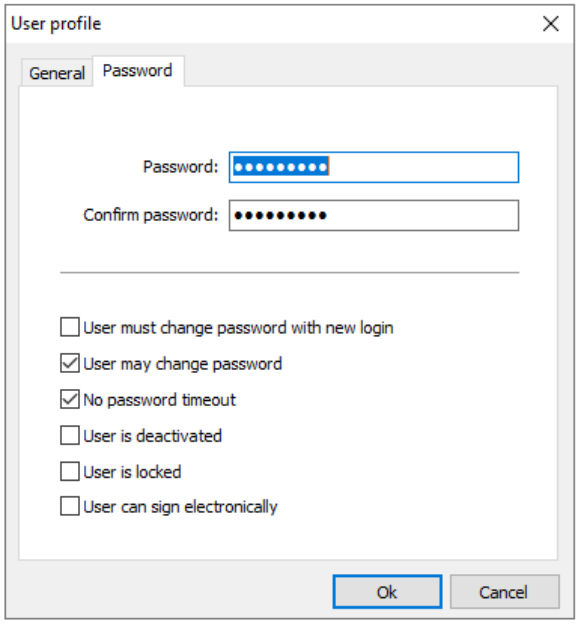
- To create a new user profile, click **Add**.
- To edit an existing user profile, mark the user profile in the list and click **Edit**.
  - The window for editing the user profile appears.

- Enter the following data:

Option	Description
General tab	
User name	Login name at program start
Full name	Actual name (optional)
Description	Further description (optional)
Password tab	
Password	Enter the password
Confirm the password	Repeat the password
User must change password with new login	If activated, users must change their password at first login
User may change password	Users can change their own password
No password timeout	The password is valid without a time limit. If deactivated, expiry date must be specified.
User is deactivated	The user profile was automatically locked after multiple failed login attempts or by an authorized user. Enter the number of possible login attempts in the settings (see "Basic settings for password, login and logout", page 144). The time when the user was locked is displayed.
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 in the system. The time when the user was deactivated is displayed.
User can sign electronically	The user is allowed to sign a project electronically (see "Digital signatures", page 153).



Settings for the user name and the assigned user group



Settings for the password and the validity of the user profile

User groups

The following user groups are available:

Administrator:

- This user group has unlimited rights to all program functions
- Administrators can create, delete, lock and unlock users, and assign rights to them
- They can change their own password and that of other users
- They can deactivate user management in **Extras** > **Options** > **User management**

Supervisor:

- This user group has the same rights as the administrator, but administrators can block certain rights for each user logged in as a supervisor

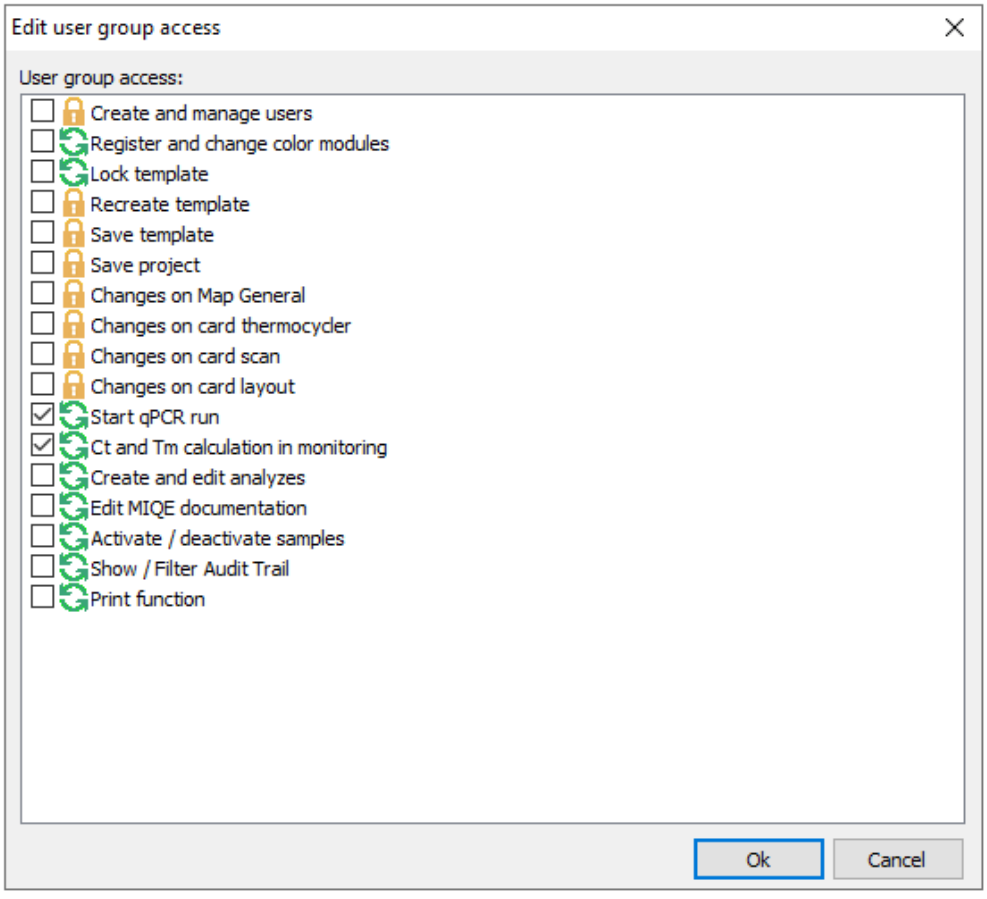
Operator:
The following rights cannot be assigned to an operator:

- Create and manage users
- Create and save templates
- Save projects
- Implement changes in the **Options** window on the **General**, **Thermal cycler**, **Scan**, and **Layout** tabs

With this selection you automatically assign a certain user role to the user and thus preset rights that you can additionally supplement or reduce using the **Edit user 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 **Edit user group access**.
The window of the same name containing the rights of the selected user appears.

- If a checkbox is activated by a checkmark, this right is granted to the user, and the user can use the function.
- Checkboxes with a lock symbol cannot be changed.
- The number of these locked rights is determined by the selected **Administrator**, **Supervisor**, or **Operator** 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.



9.3 Edit password

If users may change their password, they can open their user profile in the user management and change the password. Supervisors and operators can only do this in user management. They have no access to other functions in the user management.

1. Select **Extras** > **Options** > **User management.**
2. Click **Edit** to open the **User profiles** window.
3. Select your user profile and click **Edit**.
4. Change and confirm the password in the window **User profile** > **password**.
5. Confirm the settings and exit all windows with **OK**.

10 21 CFR Part 11 Module

The QIAquant 384 Software is compatible with the requirements of 21 CFR Part 11. The following functions are available:

- Audit trail in templates and projects, i.e., changes made to the project settings are continuously recorded
- Login monitoring, during which attempts to log into the program and also changes to the user settings are stored
- Editors for the evaluation of audit trails and log files using search functions, including a print function
- Creation and display of digital signatures in templates and projects with monitoring of their validity
- Automatic logout in case of inactivity, with the option of adjusting the time

10.1 Audit trail

The audit trail records changes to metadata in templates and projects. Metadata determines how the results are calculated and displayed from the raw data of a qPCR run, and thus influences the result of the experiment. The raw data, on the other hand, always remains unchanged, meaning it is not part of the audit trail. If settings are changed in the project or the template, the new metadata is appended as a block to the original data when the file is saved. This process takes place automatically in the background. If a project is frequently opened and edited, the scope of the audit trail can increase considerably. It is not possible to delete contents in the audit trail.

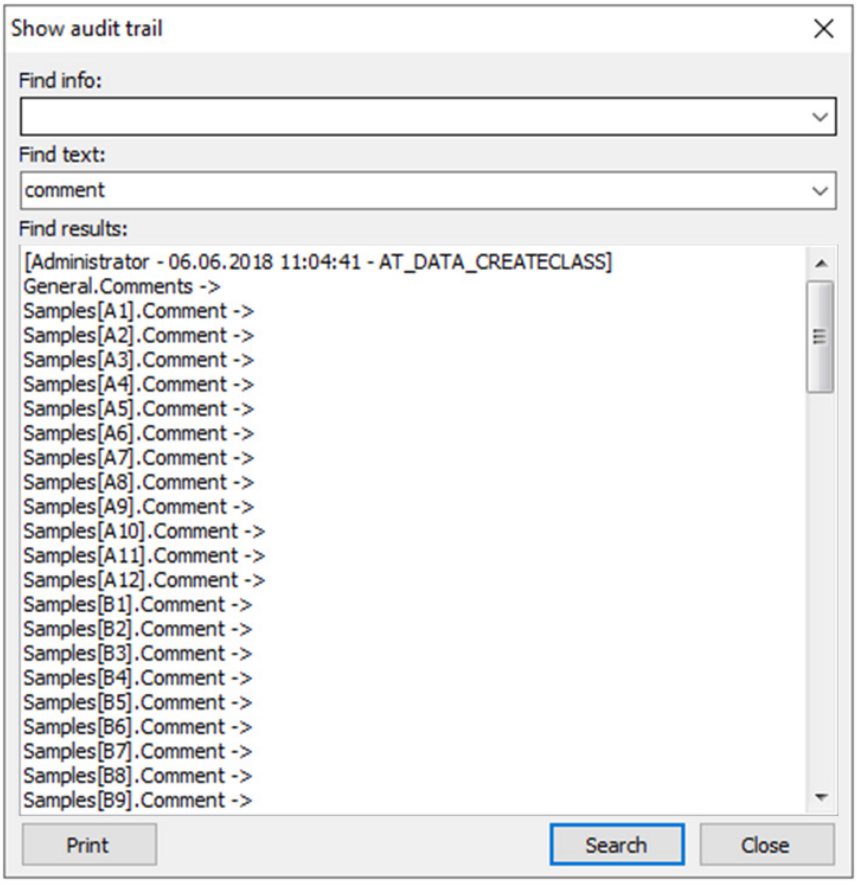
Display and evaluate audit trail

Search functions are available for evaluating the (in some cases, extensive) audit trail of a project or a template. These functions allow you to systematically search for and identify changes to metadata.

- Select the **Compliance** > **Show audit trail** menu command.
- To start the search, click **Search**.
- To print out the content that is displayed in the text field (the complete audit trail or reduced content because of a search), click **Print**.

- The following search options are available:
  - The **Find info** and **Find text** input fields are empty: The entire audit trail is displayed.
  - There is an entry in the **Find info** field: Search in the block headers, i.e., in the text in the square brackets. This makes it possible to track when the file was created, by whom, and how often it was edited.
  - There is an entry in the **Find text** field: Search in the audit trail. A search can be carried out for terms that characterize certain settings for the qPCR run, the display of results, the analyses, the layout and general information. An overview of the terms and their meaning can be found in Appendix D, page 164.

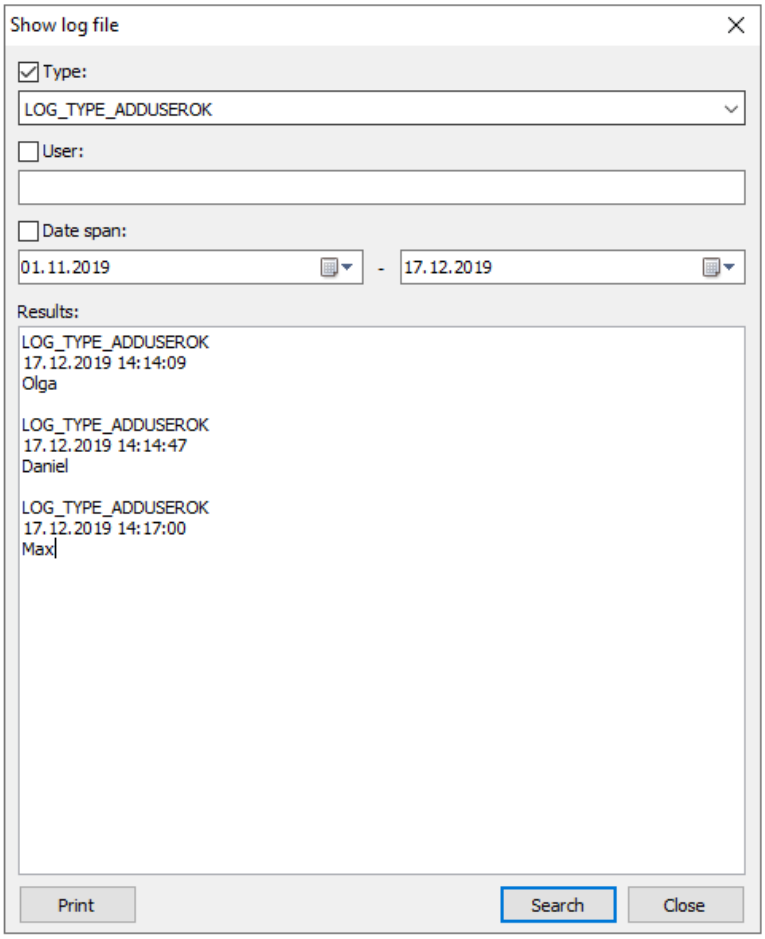
The input fields save search terms that have been previously used and make them available for reuse via the drop-down menu.



10.2 Login monitoring

All login processes in the QIAquant 384 Software are monitored and stored in encrypted form in a log file that is protected against manipulation by a checksum. This file also contains information about newly created users and digital signatures. The program records whether logins and signatures were successful or failed. In case of failed operations, the cause is also indicated (e.g., wrong password, wrong user name).

- Select the **Compliance > show log file** menu command.
- Limit the display by activating the checkboxes and the selection in the corresponding lists.
- To print the displayed results, click **Print**.



Log file with filter

10.3 Digital signatures

10.3.1 Signing a document

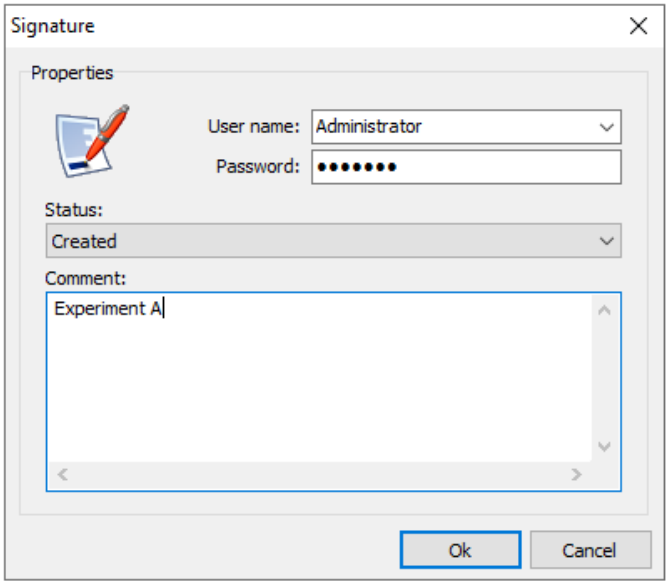
Registered users who also have the relevant authorization can digitally sign a template or a project (see “Managing user profiles/user groups“, page 145). The signature is equivalent to a handwritten signature because it can be clearly traced back to its creator.

Sign an active document

- Select the **Compliance** > **Sign digitally** menu command.
  The **Signature** window appears.
- Make the following settings:

Parameter	Description
User name	Select the registered users who are authorized to sign. The user logged in to the program is preset in the selection.
Password	Enter the user’s password.
Status	Select the signing status: **Created**, **Processed**, or **Approved.**
Comment	Add explanation to the signature (optional).

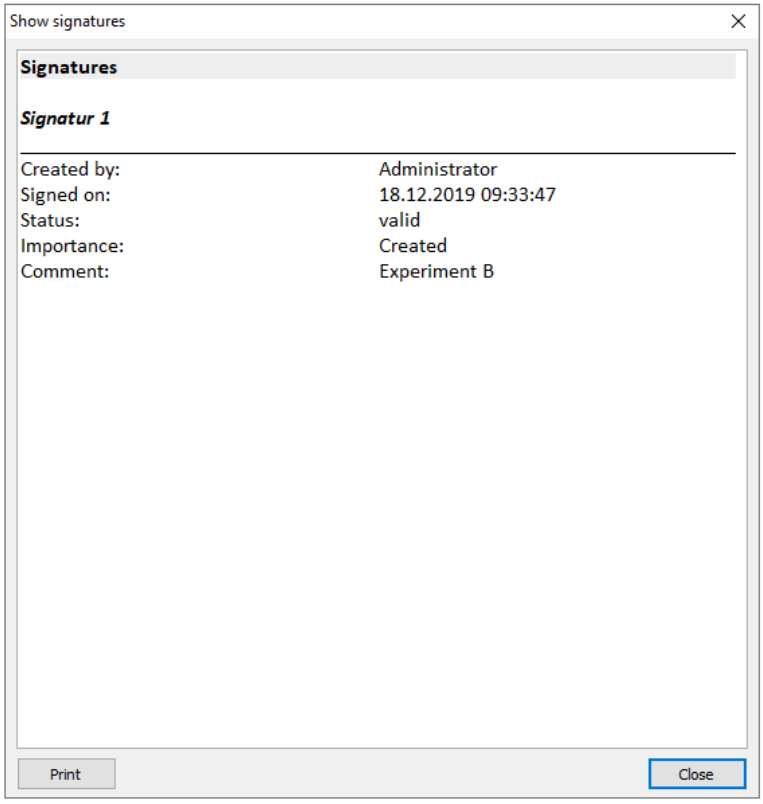
- Confirm your entries with **OK**.
  The digital signature is saved in the project or the template. Digital signatures are valid as long as no changes are made to the project or the template.



10.3.2 Displaying signatures

You can view and print out the signatures assigned to a project or a template.

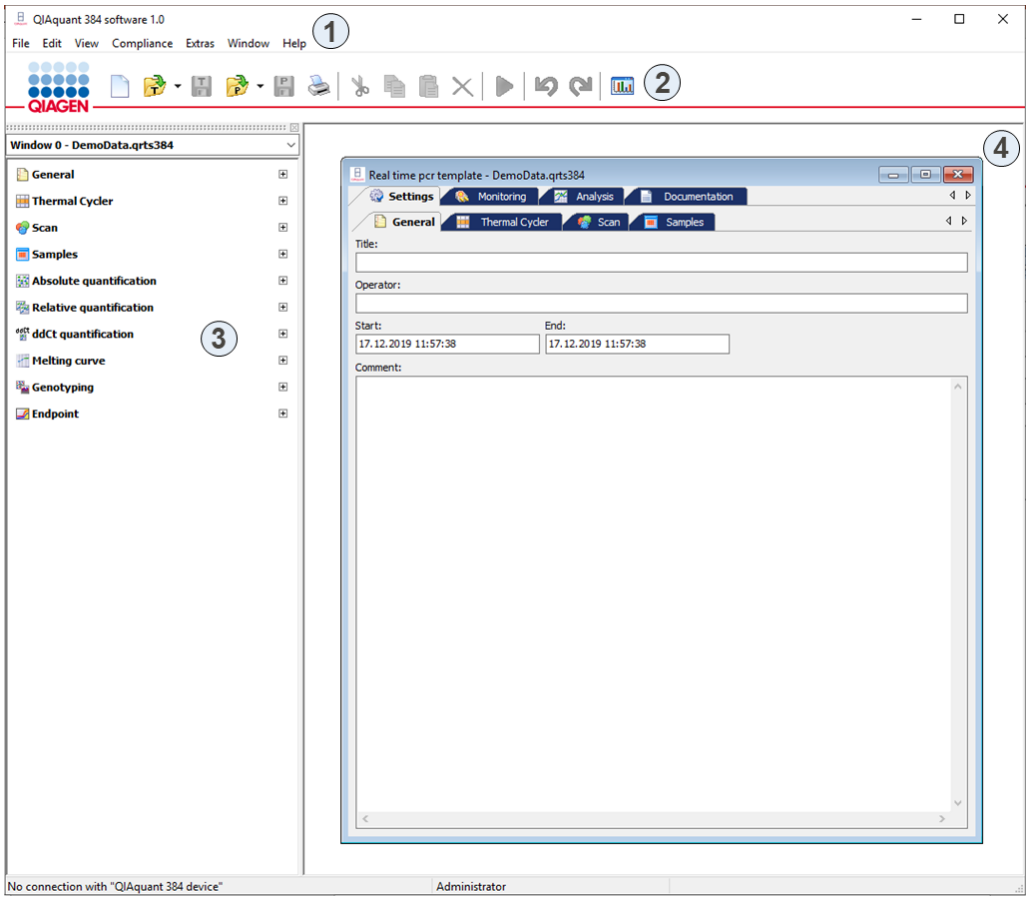
- Select the **Compliance** > **Show signatures** menu command.
  The **Show signatures** window appears. You can check the validity of the date and the creator of the signature.
- To print the displayed data, click **Print**.



Overview of the signatures of a template or a project

Appendix A: Short Instruction

The QIAquant 384 Software main window

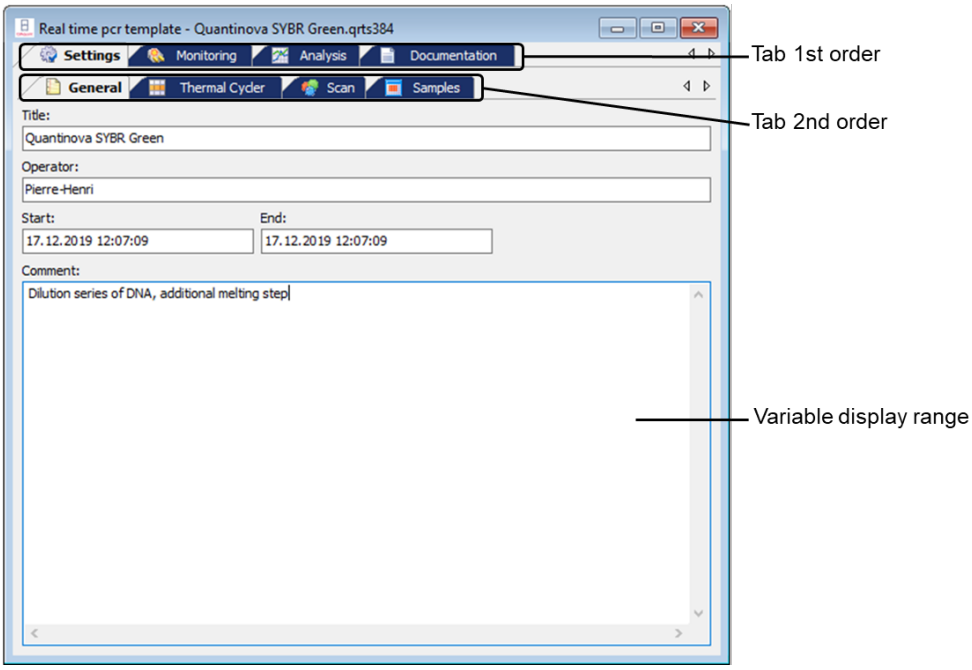


The main window has different areas. **1**: Menu bar, **2**: Toolbar, **3**: Project explorer, and **4**: Project interface.

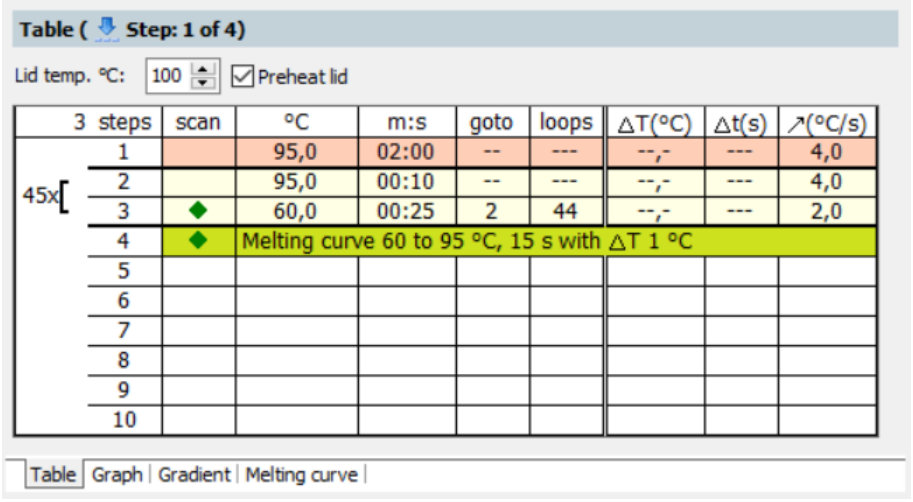
Project window: Settings

1. To create a new project, select **File** > **New** or press  in the toolbar.
   Select **File** > **Open project** or **File** > **Open template** to load saved files.

2. Open the **Settings** tab.



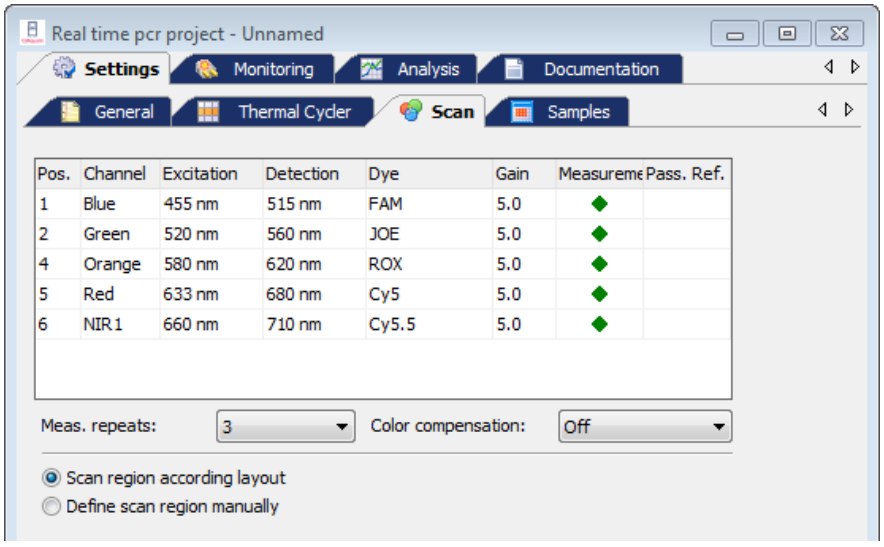
3. Enter general information for the project under the **General** tab.
4. Enter PCR program under **Thermal Cycler**.



- Set lid temperature and activate or deactivate the preheat mode.
  - Set temperature and time for each step. If desired, define gradient.
  - For loops set the step number in column **goto** the program jumps back to and in the column **loops** define the number of measurement repeats.

- If necessary define temperature or time increments or adjust the ramping rates.
- If necessary activate the melting step and edit parameters.
- In the column **scan** define at which step the fluorescence is measured.

5. Define the fluorescence measurement settings on the tab **Scan**:

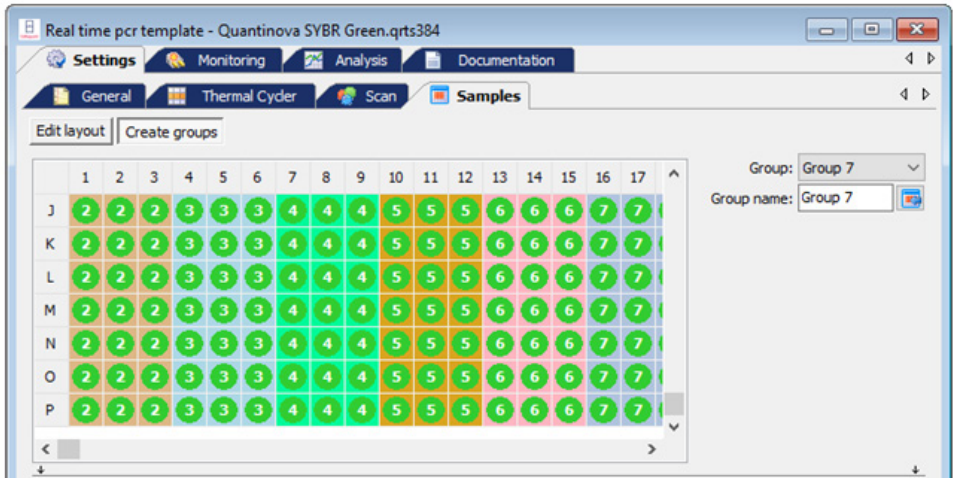


- Set hash key in the column **Measurement** for each channel to measure
- Select dye to measure in column **Dye** and set LED intensity in column **Gain**.
- For passive reference check mark in column **Pass. Ref.**
- Set number of measurement repeats and define region to scan.
- If necessary activate color compensation.

6. Define plate layout under the tab **Samples** (can be done during the run):

- Set sample name and sample type.
- Enter name for the gene to be measured in column **Gene**.
- For standards enter concentration in column **Conc.** and set unit
- Select position or region in the plate layout.
- Assign settings to the selected wells using Toolbar button  .

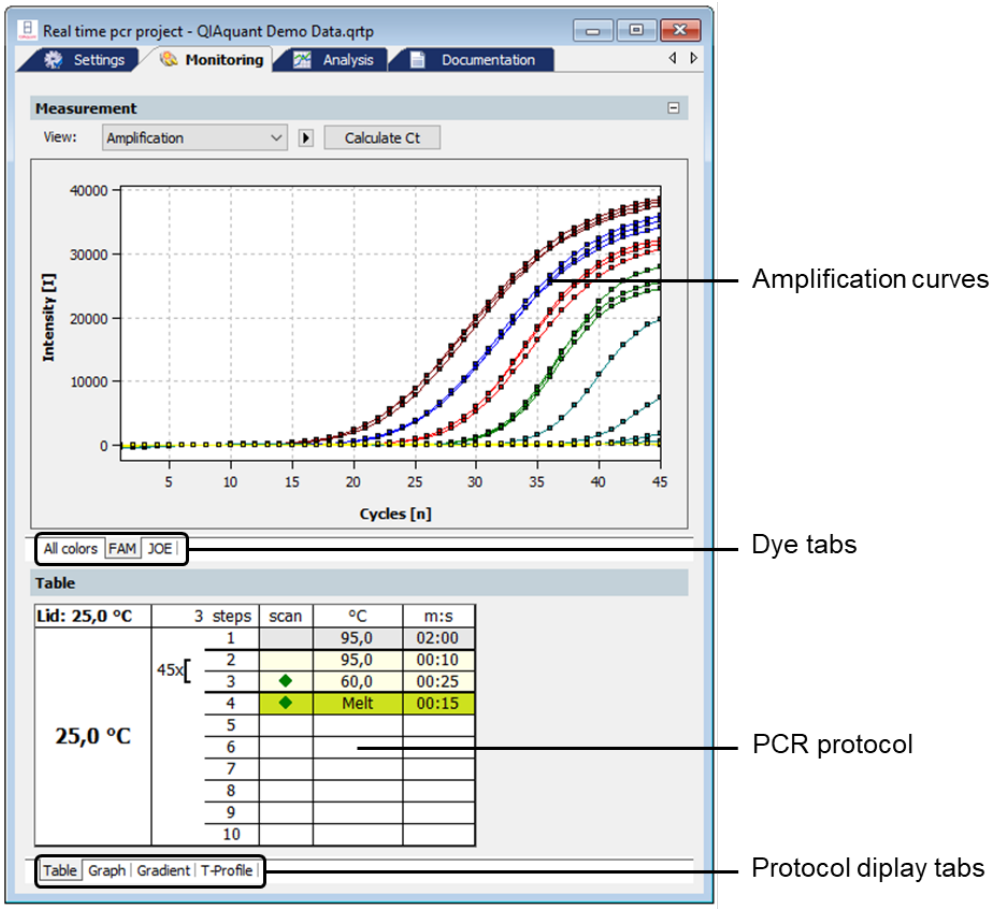
7. If the plate contains samples from different experiments that have to be analyzed separately create groups:



   - Select group and set group name.
   - Select position or region in the plate layout.
   - Assign settings to the selected wells using Toolbar button  .
8. The layout preview functions provide a comprehensive overview for the plate layout. To activate the layout preview use button  .

Project window: Monitoring

- Press button  to start the run
- In the **Monitoring** window the results of the run are displayed in real-time



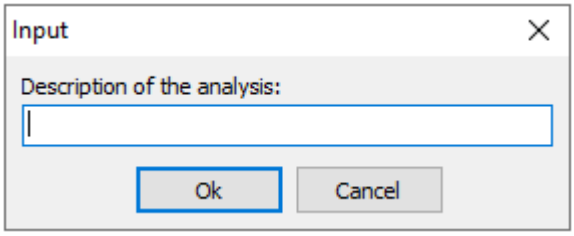
- For the results either the PCR accumulation curves or the melting curves can be displayed. Use selective list **View** to display the different results.
- Use dye tabs to select between the different dyes.
- The PCR protocol can be displayed tabular, graphical or as temperature profile.
- After the PCR run, C_t values and melting temperatures can be calculated for all samples without applying an evaluation. Click the **Calculate C_t** button in the amplification curve display or **Calculate T_m** in the melting curve display.

Project window: Analysis

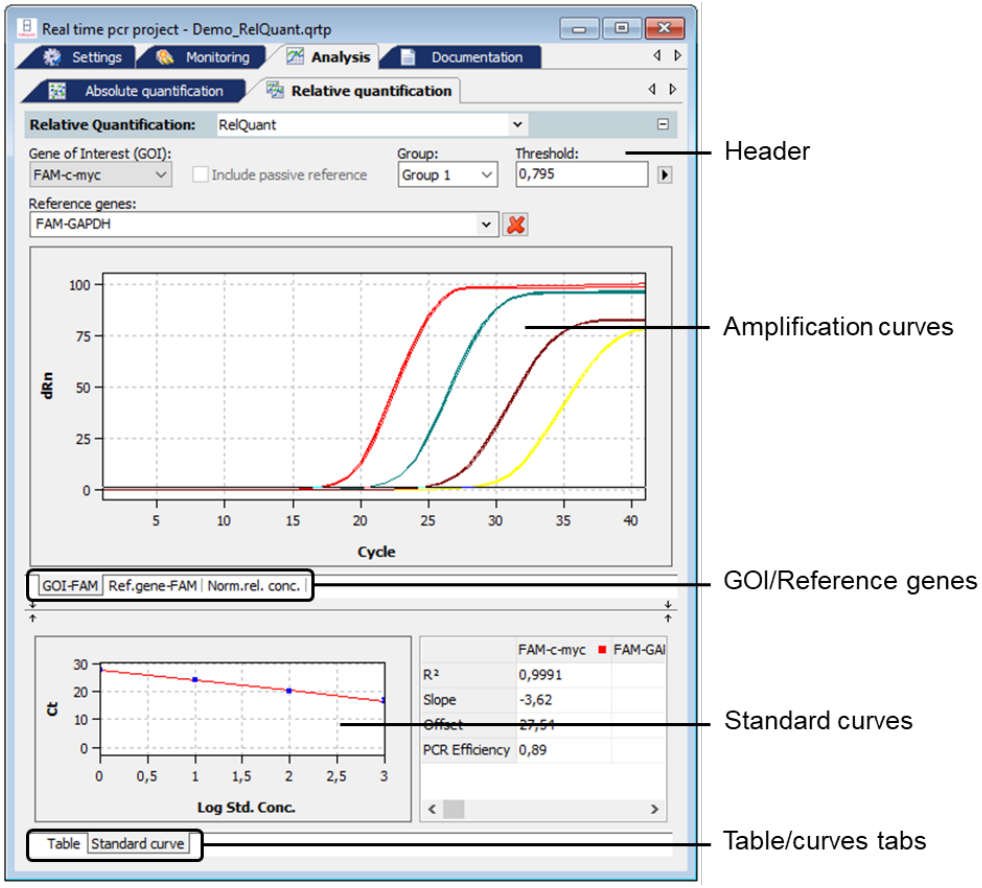
For analysis different analysis methods for absolute quantification, relative quantification, ΔΔCt-method, genotyping and melting curve analysis are available.

- To start an analysis select corresponding tab and press button **Add analysis** in the toolbar. This button is labeled with a [+] symbol (for example ).

- In the window that opens enter a name for the analysis:



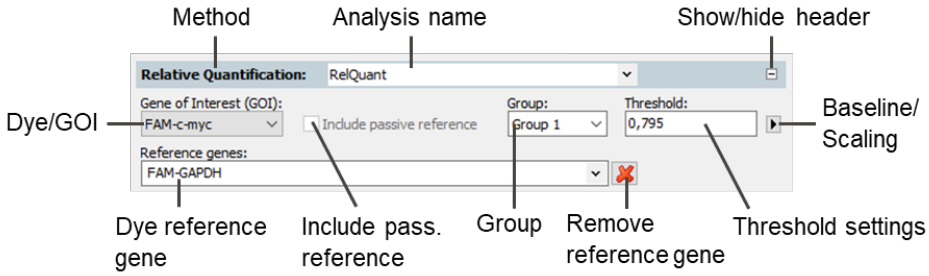
The analysis window is separated in a header for general settings, a graphical chart to display amplification curves and an area to display standard- or validation curves or the result table:



In the header various settings can be made that differ by the analysis methods:

- Select GOI.
- If applicable set passive reference (e.g., ROX) for normalization.

- Select between different experiments (groups).
- Select at least one reference gene to calculate a standard or validation curve.
- Set baseline correction.
- Set threshold (manually or automatically).
- Switch between linear and logarithmic display of the fluorescence intensities.

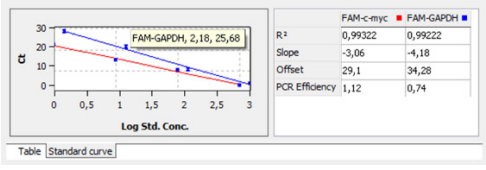


- In the amplification curves area the fluorescence intensity curves are shown:
- In the linear mode the base line correction can be checked.
- In the logarithmic mode the threshold line can be set manually. Move the threshold line up or down by using the mouse.
- Switch by the tabs between the different dyes.
- If the cursor is moved on a curve some short information for the sample is displayed.

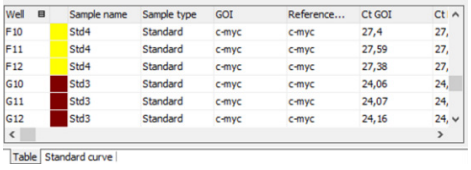
In the lower part of the analysis window the standard or validation curves are displayed:

- For the curves calculated values are displayed in a table.
- Use tabs to switch between the display of curves and of the result table.

Standard/Validation curves



Results table



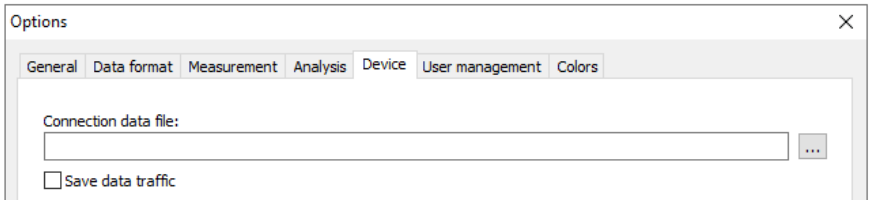
- Define columns to be displayed in the result table after right mouse click on a column header.
- Export data from the result table as *.csv files after right mouse click in the table.

Appendix B: How to Save Communication Data

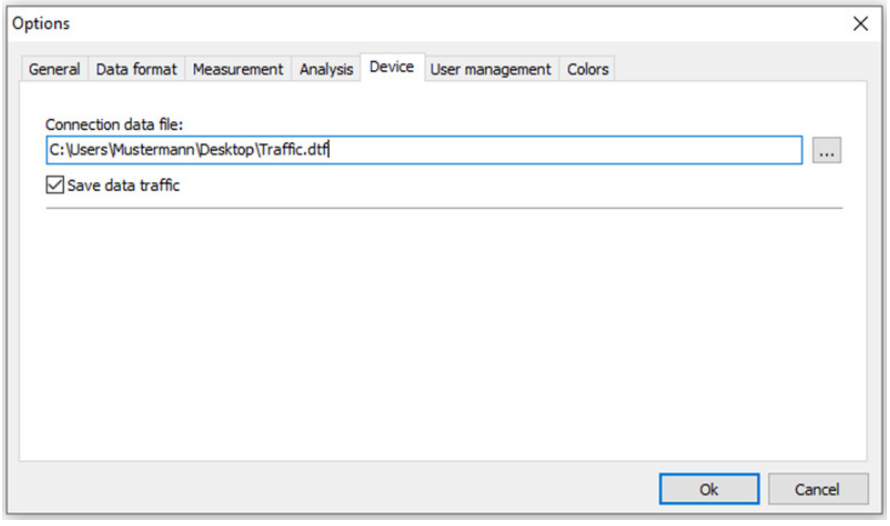
If you encounter problems with your device, you can record the communication data of the device and send it to QIAGEN Technical Service. This makes troubleshooting easier. For this purpose, recording must be activated and a file name for the memory of the data should be set.

Note: To activate the recording of the data traffic, you must have administrator rights.

1. Run the QIAquant 384 Software.
2. Select **Extras** > **Options** and open the **Device** tab.



3. Check the **Save data traffic** checkbox.
4. Click **[...]**. The **Open** dialog appears.
5. Type in a file name to which the communication data shall be sent. The extension of the traffic file is DTF.
6. Confirm the dialog by clicking **Open**.



During the next PCR run, the communication data between instrument and PC will be recorded in the file denoted. Send this file to QIAGEN Technical Service upon request.

Appendix C: Creating a Project Template from the Transfer File (LIMS)

The QIAquant 384 Software can be configured by another program (e.g., a laboratory information management system [LIMS]). In order to do this, the LIMS has to create a file that is imported by QIAquant 384 Software via the **File** > **Import LIMS** function. The so-called transfer file has a specific structure that can be provided by QIAGEN if required. The QIAquant 384 Software creates a template by using the transfer file, with which a PCR run can be started immediately.

The different export functions of QIAquant 384 Software can be used to transfer the results of the PCR run to the LIMS, depending on which data the LIMS expects.

Appendix D: Entries in the Audit Trail

Audit-Trail print out	Description
Administrator - 25.06.2018 08:47:00 - AT_DATA_CREATECLASS	Who created this project and when? Default Audit Trail data set is following.
Administrator - 04.07.2018 11:10:17 - AT_DATA_SAVEPROJECT	Who saved this project and when? Altered Audit Trail data set is following.
General	
DEVICETYPE	device type: 3=QIAquant 384
APPVERSION	version of QIAquant 384 Software used
FIRMWAREVERSION	firmware version of the connected device
DataUser.Name -> Administrator	logged in user
DataUser.Level	general user role (Administrator, Supervisor, Operator)
DataUser.LevelBits	granted rights of this user, coded in bits
DataUser.Password	password of the logged in user (not displayed here)
DataUser.Userdependent	not used
General.Title	title entered on the card General
General.Operator	operator
General.DateTime	date and time of project creation
General.Comments	comments entered on the card General
General.DeviceID	device identification data read from EEPROM
Thermal Cycler	

Audit-Trail print out	Description
CyclerProgram -> ProgData.BlockType=14;ProgData.LidTemp=100;ProgData.HotStart=True; ProgData.Control=10;ProgData.Standby=False;ProgData.BlockTemp=12;ProgData.MeasTime=10;MeltData.StartTemp=60; MeltData.EndTemp=95;MeltData.Gradient=1;MeltData.Time=16.128;MeltData.Ramp=5; MeltData.Equilibration=6;MeltData.Active=False;StepCount=4;Head.ProgramNumber=1; Head.Gradient=False;Head.ProgramPath=TOP;Head.ProgramName=PCR;Head.ProgramDate.Day=11; Head.ProgramDate.Month=7;Head.ProgramDate.Year=12;Step1.ScanFalse;Step1.Temp=95; Step1.Time=02:00;Step1.Goto=-;Step1.Loops=-;Step1.TempInc=-;Step1.TimeInc=-;Step1.Ramp=5;Step2.ScanFalse; Step2.Temp=95;Step2.Time=00:05;Step2.Goto=-;Step2.Loops=-;Step2.TempInc=-;Step2.TimeInc=-; Step2.Ramp=5;Step3.ScanFalse;Step3.Temp=58;Step3.Time=00:05;Step3.Goto=-;Step3.Loops=-; Step3.TempInc=-;Step3.TimeInc=-;Step3.Ramp=5;Step4.ScanTrue;Step4.Temp=72;Step4.Time=00:15; Step4.Goto=2;Step4.Loops=40;Step4.TempInc=-;Step4.TimeInc=-;Step4.Ramp=5;	program of the thermal cycler used for this experiment
Fluorescence Measurement	
ColorModule.Position -> 1	color module position 1
ColorModule.Code -> Blue.455.515.11.3	type of the color module used
ColorModule.Color -> FAM	dye selected
ColorModule.Gain -> 5	set gain
ColorModule.Meas -> -1	activated for measurement (-1) or not (0)
ColorModule.Refr -> 0	used as passive reference, Yes (-1), No (0)
ColorModule.Position -> 2	color module position 2
ColorModule.Code -> Green.520.560.11.2	type of the color module used
ColorModule.Color -> JOE	dye selected
ColorModule.Gain -> 5	set gain
ColorModule.Meas -> 0	activated for measurement (-1) or not (0)
ColorModule.Refr -> 0	used as passive reference, Yes (-1), No (0)
ColorModule.Position -> 3	color module position 3
ColorModule.Code -> Orange.580.620.11.2	type of the color module used

Audit-Trail print out	Description
ColorModule.Color -> ROX	dye selected
ColorModule.Gain -> 5	set gain
ColorModule.Meas -> 0	activated for measurement (-1) or not (0)
ColorModule.Refr -> 0	used as passive reference, Yes (-1), No (0)
ColorModule.Position -> 4	color module position 4
ColorModule.Code -> Red.633.680.11.1	type of the color module used
ColorModule.Color -> Cy5	dye selected
ColorModule.Gain -> 5	set gain
ColorModule.Meas -> 0	activated for measurement (-1) or not (0)
ColorModule.Refr -> 0	used as passive reference, Yes (-1), No (0)
ColorModule.Position -> 5	color module position 5
ColorModule.Code -> NIR1.660.710.11.1	type of the color module used
ColorModule.Color -> Cy5.5	dye selected
ColorModule.Gain -> 5	set gain
ColorModule.Meas -> 0	activated for measurement (-1) or not (0)
ColorModule.Refr -> 0	used as passive reference, Yes (-1), No (0)
Compensation.Name ->	name of the color compensation used
Compensation.Infos0.Position through Compensation.Infos5.Position	information about color modules, dyes and gains used for this color compensation, displayed for all 6 color module positions
Compensation.Infos0.Color through Compensation.Infos5.Color	
Compensation.Infos0.Gain through Compensation.Infos5.Gain	color module the following gene is measured with
Compensation.Matrix0, 0 through Compensation.Matrix5,5	elements of the color compensation matrix
Scan.Repetitions -> 3	number of measurement repeats
Scan.SpectralCompensation -> 0	type of the color compensation (off, standard, select)
Scan.FromCol -> 1	begin of the scanned area of the plate
Scan.ToCol -> 12	end of the scanned area of the plate
Scan.ColRangeType -> 1	select scanned area from layout (-1) or manually (0)
Layout	
Layout.Code -> Blue.470.520.11.3	color module the following gene is measured with
Layout.InfosA1.Gene through Layout.InfosH12.Gene	gene name of each well of the plate
Layout.InfosA1.Concentration through Layout.InfosH12.Concentration	standard concentration of each well of the plate
Units -> ng	concentration unit
SamplesA1.Name through SamplesH12.Name	sample name for all wells
SamplesA1.Typ through SamplesH12.Typ	sample type for all wells
SamplesA1.SubTyp through SamplesH12.SubTyp	sample sub type for all wells
SamplesA1.Comment through SamplesH12.Comment	comments for each well
SamplesA1.Active through SamplesH12.Active	Is this well activated?

Audit-Trail print out	Description
SamplesA1.Marked through SamplesH12.Marked	Is this well marked in the explorer?
GroupsA1 -> 0 through GroupsH12	To which group does the well belong?
GroupNames -> Group 1 through GroupNames -> Group 12	names of the 12 groups possible
LOCK -> 0	template locked? Yes (-1), No (0)
Calculate C_t	
CtAnalysis.Thresholds -> 0,794712458619839	threshold value the C_t values were calculated with
CtAnalysis.Smooth -> -1	smoothing On(-1), Off(0)
CtAnalysis.SmoothMode -> 5	number of points used for smoothing
CtAnalysis.Log -> 0	logarithmic chart On(-1), Off(0)
CtAnalysis.BaseLineRange0.Min -> 3	manually set lower boundary of the base line (cycle number)
CtAnalysis.BaseLineRange0.Max -> 15	manually set upper boundary of the base line (cycle number)
CtAnalysis.BaseLineRange1.Min -> 5	automatically set lower boundary of the base line (cycle number)
CtAnalysis.BaseLineRange1.Max -> -1	calculate automatically upper boundary of the base line range Yes (-1), No (0)
CtAnalysis.AutoBaseLine -> -1	automatic base line determination On (-1) or Off (0)
CtAnalysis.AutoThreshold -> 0	auto threshold On(-1), Off(0)
CtAnalysis.Filter -> -1	filter On(-1), Off(0)
CtAnalysis.FilterOptions -> 2	filter strength slight(0), medium(1) or strong(2)
CtAnalysis.FilterSmooth -> 0	smooth the filter data, Yes (-1), No (0)
Calculate T_m	
TmAnalysis.GOI.Color ->	melting curve GOI
TmAnalysis.GOI.Gene ->	melting curve of gene
TmAnalysis.Threshold -> 0	threshold value for determination of melting temperature
TmAnalysis.Smooth -> -1	smooth melting curve , Yes (-1), No (0)
TmAnalysis.SmoothMode -> 3	number of points used for smoothing
TmAnalysis.Log -> 0	logarithmic chart On(-1), Off(0)
TmAnalysis.BaseLineRange.Min -> 1	manually set lower boundary of the base line (cycle number)
TmAnalysis.BaseLineRange.Max -> 5	manually set upper boundary of the base line (cycle number)
TmAnalysis.AutoThreshold -> 0	auto threshold for melting curve, Yes (-1), No (0)
TmAnalysis.FlipCurve -> 0	flip curve horizontally, On(-1), Off(0)
TmAnalysis.Scaling -> 1	All curves start at 100%(0), Maximum initial fluorescence = 100%(1)
Absolute Quantification	
AbsQuantAnalyzes.Smooth -> -1	smoothing On(-1), Off(0)
AbsQuantAnalyzes.SmoothMode -> 5	number of points used for smoothing
AbsQuantAnalyzes.Log -> 0	logarithmic chart On(-1), Off(0)
AbsQuantAnalyzes.BaseLineRange0.Min -> 3	manually set lower boundary of the base line (cycle number)

Audit-Trail print out	Description
AbsQuantAnalyzes.BaseLineRange0.Max -> 15	manually set upper boundary of the base line (cycle number)
AbsQuantAnalyzes.BaseLineRange1.Min -> 5	automatically set lower boundary of the base line (cycle number)
AbsQuantAnalyzes.BaseLineRange1.Max -> -1	calculate automatically upper boundary of the base line range Yes (-1), No (0)
AbsQuantAnalyzes.AutoBaseLine -> -1	automatic base line determination On (-1) or Off (0)
AbsQuantAnalyzes.AutoThreshold -> 0	auto threshold On(-1), Off(0)
AbsQuantAnalyzes.Filter -> -1	filter On(-1), Off(0)
AbsQuantAnalyzes.FilterOptions -> 1	filter strength slight(0), medium(1) or strong(2)
AbsQuantAnalyzes.FilterSmooth -> 0	smooth the filter data, Yes (-1), No (0)
AbsQuantAnalysis.Description -> Quantitation 1	title of this analysis
AbsQuantAnalysis.Group -> 0	valid for group
AbsQuantAnalysis.REF -> 0	passive reference used? Yes (-1), No (0)
AbsQuantAnalysis.GOI.Color -> FAM	dye of GOI
AbsQuantAnalysis.GOI.Gene ->	name of GOI
AbsQuantAnalysis.Threshold -> 1,13570592788773	threshold value the C_t values were calculated with
AbsQuantAnalysis.FitData.Count -> 4	number of standards
AbsQuantAnalysis.FitData.M -> -3,67716352286466	slope of the standard curve
AbsQuantAnalysis.FitData.N -> 28,0638862770259	intercept of the standard curve
AbsQuantAnalysis.FitData.R2 -> 0,999236478923372	correlation coefficient of the linear curve fit
AbsQuantAnalysis.FitData.Extern -> 0	Was the standard curve imported, Yes (-1), No (0)
MIQE.TypeInfo -> 1	MIQE documentation for DNA(0) or RNA(1)
LOCK -> 0	template or project locked? Yes (-1), No (0)
Relative Quantification	
RelQuantAnalyzes.GOISmooth -> -1	smoothing GOI, On(-1), Off(0)
RelQuantAnalyzes.GOISmoothMode -> 5	number of points used for smoothing, GOI
RelQuantAnalyzes.GOILog -> 0	logarithmic chart GOI On(-1), Off(0)
RelQuantAnalyzes.GOIBaseLineRange0.Min -> 3	manually set lower boundary of the base line (cycle number GOI)
RelQuantAnalyzes.GOIBaseLineRange0.Max -> 15	manually set upper boundary of the base line (cycle number GOI)
RelQuantAnalyzes.GOIBaseLineRange1.Min -> 5	automatically set lower boundary of the base line (cycle number GOI)
RelQuantAnalyzes.GOIBaseLineRange1.Max -> -1	calculate automatically upper boundary of the base line range Yes (-1), No (0)
RelQuantAnalyzes.GOIAutoBaseLine -> -1	automatic base line determination On (-1) or Off (0)
RelQuantAnalyzes.GORSmooth -> -1	smoothing reference gene, On(-1), Off(0)
RelQuantAnalyzes.GORSmoothMode -> 5	number of points used for smoothing of reference gene
RelQuantAnalyzes.GORLog -> 0	logarithmic chart GOI On(-1), Off(0), reference gene
RelQuantAnalyzes.GORBaseLineRange0.Min -> 3	manually set lower boundary of the base line (cycle number reference gene)
RelQuantAnalyzes.GORBaseLineRange0.Max -> 15	manually set upper boundary of the base line (cycle number reference gene)

Audit-Trail print out	Description
RelQuantAnalyzes.GORBaseLineRange1.Min -> 5	automatically set lower boundary of the base line (cycle number reference gene)
RelQuantAnalyzes.GORBaseLineRange1.Max -> -1	automatically set upper boundary of the base line (cycle number reference gene)
RelQuantAnalyzes.GORAutoBaseLine -> -1	automatic base line determination On (-1) or Off (0), reference gene
RelQuantAnalyzes.GOIAutoThreshold -> 0	auto threshold On(-1), Off(0), GOI
RelQuantAnalyzes.GORAutoThreshold -> 0	auto threshold On(-1), Off(0), Reference gene
RelQuantAnalyzes.GOIFilter -> -1	filter On(-1), Off(0), GOI
RelQuantAnalyzes.GOIFilterOptions -> 1	filter strength GOI, slight(0), medium(1) or strong(2)
RelQuantAnalyzes.GOIFilterSmooth -> 0	smooth the filter data GOI, Yes (-1), No (0)
RelQuantAnalyzes.GORFilter -> -1	filter On(-1), Off(0), reference gene
RelQuantAnalyzes.GORFilterOptions -> 1	filter strength reference gene, slight(0), medium(1) or strong(2)
RelQuantAnalyzes.GORFilterSmooth -> 0	smooth the filter data of reference gene, Yes (-1), No (0)
RelQuantAnalysis.Description -> RelQ	title of this analysis
RelQuantAnalysis.Group -> 0	valid for group
RelQuantAnalysis.REF -> 0	passive reference used? Yes (-1), No (0)
RelQuantAnalysis.GOI.Color -> FAM	dye of GOI
RelQuantAnalysis.GOI.Gene ->	name of GOI
RelQuantAnalysis.GOIThreshold -> 0,794712458619839	threshold value the C_t values of the GOI were calculated with
RelQuantAnalysis.GOR.Color ->	dye of reference gene
RelQuantAnalysis.GOR.Gene ->	name of the reference gene
RelQuantAnalysis.GORThreshold -> 50	threshold value the C_t values of the reference gene were calculated with
RelQuantAnalysis.GOIFitData.Count -> 4	number of standards, GOI
RelQuantAnalysis.GOIFitData.M -> -3,62354563433653	slope of the standard curve, GOI
RelQuantAnalysis.GOIFitData.N -> 27,5354442047509	intercept of the standard curve, GOI
RelQuantAnalysis.GOIFitData.R2 -> 0,999100282770293	correlation coefficient of the linear curve fit, GOI
RelQuantAnalysis.GOIFitData.Extern -> 0	Was the standard curve for GOI imported, Yes (-1), No (0)
RelQuantAnalysis.GORFitData.Count -> 0	number of standards, reference gene
RelQuantAnalysis.GORFitData.M -> 1	slope of the standard curve, reference gene
RelQuantAnalysis.GORFitData.N -> 0	intercept of the standard curve, reference gene
RelQuantAnalysis.GORFitData.R2 -> 0	correlation coefficient of the linear curve fit, reference gene
RelQuantAnalysis.GORFitData.Extern -> 0	Was the standard curve for reference gene imported, Yes (-1), No (0)
DeltaDeltaCt Analysis	
DeltaDeltaCtAnalyzes.GOISmooth -> -1	smoothing GOI, On(-1), Off(0)
DeltaDeltaCtAnalyzes.GOISmoothMode -> 5	number of points used for smoothing, GOI
DeltaDeltaCtAnalyzes.GOILog -> 0	logarithmic chart GOI On(-1), Off(0)
DeltaDeltaCtAnalyzes.GOIBaseLineRange0.Min -> 3	manually set lower boundary of the base line (cycle number GOI)

Audit-Trail print out	Description
DeltaDeltaCtAnalyzes.GOIBaseLineRange0.Max -> 15	manually set upper boundary of the base line (cycle number GOI)
DeltaDeltaCtAnalyzes.GOIBaseLineRange1.Min -> 5	automatically set lower boundary of the base line (cycle number GOI)
DeltaDeltaCtAnalyzes.GOIBaseLineRange1.Max -> -1	calculate automatically upper boundary of the base line range Yes (-1), No (0)
DeltaDeltaCtAnalyzes.GOIAutoBaseLine -> -1	automatic base line determination On (-1) or Off (0)
DeltaDeltaCtAnalyzes.GORSmooth -> -1	smoothing reference gene, On(-1), Off(0)
DeltaDeltaCtAnalyzes.GORSmoothMode -> 5	number of points used for smoothing of reference gene
DeltaDeltaCtAnalyzes.GORLog -> 0	logarithmic chart GOI On(-1), Off(0), reference gene
DeltaDeltaCtAnalyzes.GORBaseLineRange0.Min -> 3	manually set lower boundary of the base line (cycle number reference gene)
DeltaDeltaCtAnalyzes.GORBaseLineRange0.Max -> 15	manually set upper boundary of the base line (cycle number reference gene)
DeltaDeltaCtAnalyzes.GORBaseLineRange1.Min -> 5	automatically set lower boundary of the base line (cycle number reference gene)
DeltaDeltaCtAnalyzes.GORBaseLineRange1.Max -> -1	automatically set upper boundary of the base line (cycle number reference gene)
DeltaDeltaCtAnalyzes.GORAutoBaseLine -> -1	automatic base line determination On (-1) or Off (0), reference gene
DeltaDeltaCtAnalyzes.GOIAutoThreshold -> 0	auto threshold On(-1), Off(0), GOI
DeltaDeltaCtAnalyzes.GORAutoThreshold -> 0	auto threshold On(-1), Off(0), Reference gene
DeltaDeltaCtAnalyzes.GOIFilter -> -1	filter On(-1), Off(0), GOI
DeltaDeltaCtAnalyzes.GOIFilterOptions -> 1	filter strength GOI, slight(0), medium(-1) or strong(2)
DeltaDeltaCtAnalyzes.GOIFilterSmooth -> 0	smooth the filter data GOI, Yes (-1), No (0)
DeltaDeltaCtAnalyzes.GORFilter -> -1	filter On(-1), Off(0), reference gene
DeltaDeltaCtAnalyzes.GORFilterOptions -> 1	filter strength reference gene, slight(0), medium(1) or strong(2)
DeltaDeltaCtAnalyzes.GORFilterSmooth -> 0	smooth the filter data of reference gene, Yes (-1), No (0)
DeltaDeltaCtAnalysis.Description -> ddCt	title of this analysis
DeltaDeltaCtAnalysis.Group -> 0	valid for group
DeltaDeltaCtAnalysis.GOI.Color -> FAM	dye of GOI
DeltaDeltaCtAnalysis.GOI.Gene ->	name of GOI
DeltaDeltaCtAnalysis.GOIThreshold -> 0,794712458619839	threshold value the C_t values of the GOI were calculated with
DeltaDeltaCtAnalysis.GOR.Color ->	dye of reference gene
DeltaDeltaCtAnalysis.GOR.Gene ->	name of the reference gene
DeltaDeltaCtAnalysis.GORThreshold -> 50	threshold value the C_t values of the reference gene were calculated with
DeltaDeltaCtAnalysis.EfficiencyCalc -> 0	PCR efficiency calculation according to Livak (0) or Pfaffl (1)
DeltaDeltaCtAnalysis.EfficiencyType -> 0	if Pfaffl: calculate efficiency from standards (0) or entered values (1)
DeltaDeltaCtAnalysis.GOIEfficiency -> 1	PCR efficiency of the GOI
DeltaDeltaCtAnalysis.GOREfficiency -> 1	PCR efficiency of the reference gene
DeltaDeltaCtAnalysis.GOIFitData.Count -> 4	number of standards, GOI

Audit-Trail print out	Description
DeltaDeltaCtAnalysis.GOIFitData.M -> -3,62354563433653	slope of the standard curve, GOI
DeltaDeltaCtAnalysis.GOIFitData.N -> 27,5354442047509	intercept of the standard curve, GOI
DeltaDeltaCtAnalysis.GOIFitData.R2 -> 0,999100282770293	correlation coefficient of the linear curve fit, GOI
DeltaDeltaCtAnalysis.GORFitData.Count -> 0	number of standards, reference gene
DeltaDeltaCtAnalysis.GORFitData.M -> 1	slope of the standard curve, reference gene
DeltaDeltaCtAnalysis.GORFitData.N -> 0	intercept of the standard curve, reference gene
DeltaDeltaCtAnalysis.GORFitData.R2 -> 0	correlation coefficient of the linear curve fit, reference gene
DeltaDeltaCtAnalysis.ValFitData.Count -> 0	number of standards used for validation
DeltaDeltaCtAnalysis.ValFitData.M -> 1	slope of the validation curve
DeltaDeltaCtAnalysis.ValFitData.N -> 0	intercept of the validation curve
DeltaDeltaCtAnalysis.ValFitData.R2 -> 0	correlation coefficient of the linear curve fit, validation
Melt Curve Analysis	
MeltCurveAnalyzes.Smooth -> -1	smoothing GOI, On(-1), Off(0)
MeltCurveAnalyzes.SmoothMode -> 3	number of points used for smoothing
MeltCurveAnalyzes.Log -> 0	logarithmic chart On(-1), Off(0)
MeltCurveAnalyzes.BaseLineRange.Min -> 1	manually set lower boundary of the base line
MeltCurveAnalyzes.BaseLineRange.Max -> 5	manually set upper boundary of the base line
MeltCurveAnalyzes.AutoThreshold -> 0	auto threshold On(-1), Off(0), GOI
MeltCurveAnalyzes.FlipCurve -> -1	flip curve horizontally, On(-1), Off(0)
MeltCurveAnalyzes.Scaling -> 0	All curves start at 100%(0), Maximum initial fluorescence = 100%(1)
MeltCurveAnalysis.Description -> melt	title of this analysis
MeltCurveAnalysis.Group -> 0	valid for group
MeltCurveAnalysis.GOI.Color -> FAM	dye of GOI
MeltCurveAnalysis.GOI.Gene ->	name of GOI
MeltCurveAnalysis.Threshold -> 0	threshold value above which the melt temperatures are determined
Genotyping	
GenoTypingAnalyzes.GOISmooth -> -1	smoothing GOI, On(-1), Off(0)
GenoTypingAnalyzes.GOISmoothMode -> 5	number of points used for smoothing, GOI
GenoTypingAnalyzes.GOILog -> 0	logarithmic chart GOI On(-1), Off(0)
GenoTypingAnalyzes.GOIBaseLineRange0.Min -> 3	manually set lower boundary of the base line (cycle number GOI)
GenoTypingAnalyzes.GOIBaseLineRange0.Max -> 15	manually set upper boundary of the base line (cycle number GOI)
GenoTypingAnalyzes.GOIBaseLineRange1.Min -> 5	automatically set lower boundary of the base line (cycle number GOI)
GenoTypingAnalyzes.GOIBaseLineRange1.Max -> -1	automatically set upper boundary of the base line (cycle number GOI)
GenoTypingAnalyzes.GOIAutoBaseLine -> -1	automatic base line determination On (-1) or Off (0)
GenoTypingAnalyzes.GORSmooth -> -1	smoothing reference gene, On(-1), Off(0)

Audit-Trail print out	Description
GenoTypingAnalyzes.GORSmoothMode -> 5	number of points used for smoothing of reference gene
GenoTypingAnalyzes.GORLog -> 0	logarithmic chart GOI On(-1), Off(0), reference gene
GenoTypingAnalyzes.GORBaseLineRange0.Min -> 3	manually set lower boundary of the base line (cycle number reference gene)
GenoTypingAnalyzes.GORBaseLineRange0.Max -> 15	manually set upper boundary of the base line (cycle number reference gene)
GenoTypingAnalyzes.GORBaseLineRange1.Min -> 5	automatically set lower boundary of the base line (cycle number reference gene)
GenoTypingAnalyzes.GORBaseLineRange1.Max -> -1	calculate automatically upper boundary of the base line range Yes (-1), No (0)
GenoTypingAnalyzes.GORAutoBaseLine -> -1	automatic base line determination On (-1) or Off (0), reference gene
GenoTypingAnalyzes.GOIAutoThreshold -> 0	auto threshold On(-1), Off(0), GOI
GenoTypingAnalyzes.GORAutoThreshold -> 0	auto threshold On(-1), Off(0), Reference gene
GenoTypingAnalyzes.GOIFilter -> -1	filter On(-1), Off(0), GOI
GenoTypingAnalyzes.GOIFilterOptions -> 1	filter strength GOI, slight(0), medium(1) or strong(2)
GenoTypingAnalyzes.GOIFilterSmooth -> 0	smooth the filter data GOI, Yes (-1), No (0)
GenoTypingAnalyzes.GORFilter -> -1	filter On(-1), Off(0), reference gene
GenoTypingAnalyzes.GORFilterOptions -> 1	filter strength reference gene, slight(0), medium(1) or strong(2)
GenoTypingAnalyzes.GORFilterSmooth -> 0	smooth the filter data of reference gene, Yes (-1), No (0)
GenoTypingAnalyzes.SPType -> 1	genotyping based on C_t (1) or dRn (0)
GenoTypingAnalyzes.EPLast -> -1	end point is the last cycle (1) or enter cycle manually
GenoTypingAnalyzes.EPCycle -> -1	manually entered end point cycle
GenoTypingAnalyzes.InfoText -> wild type	text 1
GenoTypingAnalyzes.InfoText -> mutant	text 2
GenoTypingAnalyzes.InfoText -> heterozygote	text 3
GenoTypingAnalyzes.InfoText -> error	text 4
GenoTypingAnalysis.Description -> Geno	title of this analysis
GenoTypingAnalysis.Group -> 0	valid for group
GenoTypingAnalysis.REF -> 0	passive reference used? Yes (-1), No (0)
GenoTypingAnalysis.GOI.Color -> FAM	dye of GOI
GenoTypingAnalysis.GOI.Gene ->	name of GOI
GenoTypingAnalysis.GOIThreshold -> 0,794712458619839	threshold value the C_t values of the GOI were calculated with
GenoTypingAnalysis.GOR.Color ->	dye of reference gene
GenoTypingAnalysis.GOR.Gene ->	name of the reference gene
GenoTypingAnalysis.GORThreshold -> 50	threshold value the C_t values of the reference gene were calculated with
GenoTypingAnalysis.SPCutOff0 -> 0	cut off value 1, C_t
GenoTypingAnalysis.SPCutOff1 -> 0,794712458619839	cut off value 2, C_t
GenoTypingAnalysis.EPCutOff0 -> 49	cut off value 1, Cycle
GenoTypingAnalysis.EPCutOff1 -> 51	cut off value 2, cycle

Audit-Trail print out	Description
End Point Analysis	
EndPointAnalyzes.Smooth -> -1	smoothing GOI, On(-1), Off(0)
EndPointAnalyzes.SmoothMode -> 5	number of points used for smoothing, GOI
EndPointAnalyzes.Log -> 0	logarithmic chart GOI On(-1), Off(0)
EndPointAnalyzes.BaseLineRange0.Min -> 3	manually set lower boundary of the base line (cycle number GOI)
EndPointAnalyzes.BaseLineRange0.Max -> 15	manually set upper boundary of the base line (cycle number GOI)
EndPointAnalyzes.BaseLineRange1.Min -> 5	automatically set lower boundary of the base line (cycle number GOI)
EndPointAnalyzes.BaseLineRange1.Max -> 25	automatically set upper boundary of the base line (cycle number GOI)
EndPointAnalyzes.AutoBaseLine -> -1	automatic base line determination On (-1) or Off (0)
EndPointAnalysis.Description -> Endp	title of this analysis
EndPointAnalysis.Group -> 0	valid for group
EndPointAnalysis.GOI.Color -> FAM	dye of GOI
EndPointAnalysis.GOI.Gene ->	name of GOI
EndPointAnalysis.GOICutOff -> 2040,83542831301	cut off value of GOI
EndPointAnalysis.IPC.Color ->	Dye of the internal positive control (IPC)
EndPointAnalysis.IPC.Gene ->	gene name of the internal positive control
EndPointAnalysis.IPCCutOff -> 0	cut off value of the internal positive control
EndPointAnalysis.OptionCycles -> -1	end point analysis based on end point intensities, Yes (-1), No (0)
EndPointAnalysis.OptionLastCycles -> 2	number of last cycles used for calculation
EndPointAnalysis.OptionFromCycle -> 38	start cycle end point range
EndPointAnalysis.OptionToCycle -> 40	end cycle end point range
EndPointAnalysis.OptionCutOff -> -1	end point analysis based on NTC intensities, Yes (-1), No (0)
EndPointAnalysis.OptionCutOffNTC -> 10	factor applied to the NTC intensities for threshold calculation
EndPointAnalysis.OptionCutOffNTC_IPC -> 2	confidence intervals: (0)95%, (1)99%, (2)99.5%, (3)99.7%, (4)99.9%
EndPointAnalysis.OptionCutOffInput -> 0	use cut off values from table Yes(-1), No (0)
MIQE.TypeInfo -> 1	MIQE documentation for DNA(0) or RNA(1)
LOCK -> 0	template or project locked? Yes (-1), No (0)

Document Revision History

Date	Changes
07/2021	Minor updates of the user manual due to the changes in the Software and the QIAquant instrument Hardware
03/2020	Initial release

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