LightCycler® 96 System

For life science research only.
Not for use in diagnostic procedures.

How to use the LightCycler® 96 System Guides

Before reading, please review the section "Revision" for important information.

Quick Guide

Provides a short set of instructions for use in the laboratory, describing the basic handling steps. This shorter form of information is for routine use after you are familiar with the details of the LightCycler® 96 System described in the User Training Guide.

User Training Guide

Provides detailed step-by-step instructions for routine operation using the main applications of the LightCycler® 96 System, including instrument startup and shutdown.

Operator's Guide

Provides a detailed description of the LightCycler® 96 System, system components and all relevant software information not covered by the User Training Guide. For installation requirements, always refer to the Operator's Guide.

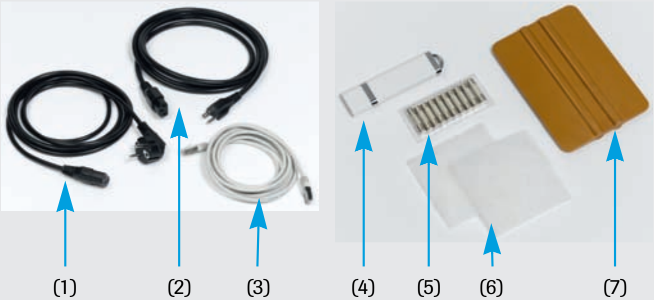
Revisions

Provides updates to the LightCycler® 96 System Guides, including new supplementary information and corrections to previous editions.

LightCycler® 96 System Quick Guide: System installation

Unpack the instrument

The LightCycler® 96 Instrument and the accessories are packaged in a shipping box.



Number	Quantity	Component
	1	LightCycler® 96 Instrument
(1)	1	Mains power cable (EU)
(2)	1	Mains power cable (US)
(3)	1	Ethernet cable (3 m)
(4)	1	LightCycler® 96 USB Drive
(5)	1	Package fuses FUSE 5x20 T8AH 250V ULR/IEC
(6)	2	Ventilation dust filters
(7)	1	Sealing foil applicator

Check for damage that may have occurred during transportation. Report any signs of damage to your local Roche Diagnostics representative.

Keep the shipping box and packaging in case of return.
If you have already disposed of the packaging, you can request it from Roche.

For detailed information on assembling the instrument and more detailed pictures, refer to the 'Operator's Guide' on the LightCycler® 96 USB Drive.

1. Remove the accessory box and the protective foam on the top.
2. Lift the LightCycler® 96 Instrument out of the box by holding it on the left and right sides, and place it on a solid level surface.
   To carry and lift the instrument, only use the recessed grips on the left and right sides of the instrument base plate.
   Caution: Due to the weight of the instrument, two persons may be needed to lift it.
3. Ensure that all components are present and intact.
   Report any missing items to your local Roche Diagnostics representative.

Assemble the instrument

1. Remove the protective foil from the touchscreen.
2. Connect the supplied mains power cable to the mains power socket of the instrument, and then to the wall outlet.
3. Optional, when connecting the instrument to an Ethernet network:
   - Connect one end of the Ethernet cable directly to the Ethernet port of your computer or the Ethernet port of your LAN.
   - Connect the other end of the Ethernet cable to the Ethernet port on the back of the LightCycler® 96 Instrument.

   For configuring the instrument using a direct connection or the local Ethernet, refer to the 'Operator's Guide' on the LightCycler® 96 USB Drive.
4. Optional: Connect the external handheld barcode scanner to the USB interface on the back of the instrument.

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LightCycler® 96 System Quick Guide: System installation

Remove the transport locking device

1. Switch on the instrument using the mains power switch on the back of the instrument. The initialization process begins.
2. When the instrument has successfully initialized, choose the *Eject* button on the touch-screen to release the loading module.

   

   The loading module is ejected.
3. Manually pull the loading module completely out of the instrument.
4. Remove the transport locking device from the mount.

   

   Keep the transport locking device in case the instrument has to be transported.
5. Push the loading module in until it starts moving automatically to its home position.

Install the LightCycler® 96 Application Software

1. Start the computer on which you want to install the software.

   For a detailed list of the system requirements, refer to the 'Operator's Guide' on the LightCycler® 96 USB Drive.
2. Insert the LightCycler® 96 USB Drive into a USB interface on your computer.
3. Log on to Microsoft Windows, and ensure that you have the administration rights to install the software.
4. Navigate to the USB drive, and install the software by double-clicking the *Setup_LightCycler96_<release>.exe* file.

   The installation process transfers files, extracts the files, and prepares the installation wizard.
5. For the optional connection of the application software and the instrument software (usable for online monitoring and data transfer):
   - Start the LightCycler® 96 Application Software.
   - Open the *Instrument Manager.*
   - Register the instrument with the application software.

   For registering the instrument and monitoring an instrument run via the network, refer to the 'Operator's Guide' on the LightCycler® 96 USB Drive.

New software releases and user guides for the LightCycler® 96 Instrument are available in the download area of the Roche Applied Sciences website.

Disclaimer

Before setting up operation of the LightCycler® 96 System, it is important to read the user documentation completely. Non-observance of the instructions provided or performing any operations not stated in the user documentation could produce safety hazards.

Version Information

Version 1.0, August 2012, Software Version 1.0.

Published by

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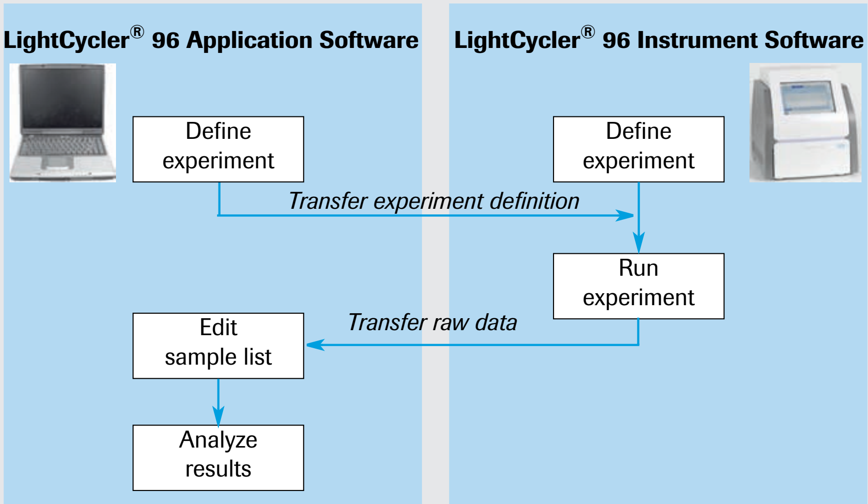
Sandhofer Straße 116
68305 Mannheim
Germany
www.roche-applied-science.com

LightCycler® 96 System Quick Guide: Programming and running an experiment

Workflow

To program and run an experiment, use:

- LightCycler® 96 Application Software for defining an experiment protocol and analyzing acquired data.
- LightCycler® 96 Instrument Software for defining an experiment protocol and performing experiments.



For detailed step-by-step information, refer to the 'LightCycler® 96 System User Training Guide' on the LightCycler® 96 USB Drive.

Set up the reaction mix

1. Identify the detection dye to be used in your experiment.
2. Prepare the PCR mix and set up the sample dilutions.
   When setting up the PCR mix, you should compensate for pipetting losses. It is recommended to prepare PCR mixes with 10% extra volume.
3. Pipette the PCR mix and the corresponding sample dilution into each well of the LightCycler® 480 Multiwell Plate.
4. Seal the multiwell plate with the LightCycler® 480 Sealing Foil using the sealing foil applicator (part of the system package).
5. Centrifuge the multiwell plate at 1,500 x *g* for 2 minutes in a standard swing-bucket centrifuge, using a rotor for multiwell plates and suitable adapters.

Define the experiment

1. Create a new experiment using the LightCycler® 96 Application Software or the LightCycler® 96 Instrument Software.
2. Open the *Run Editor* tab and define the temperature profile, including the heating and cooling cycles to be used.
3. Configure the detection format and the sample volume.
4. Save the experiment.

Run the experiment

1. If you have defined the experiment using the LightCycler® 96 Application Software, transfer the experiment file to the LightCycler® 96 Instrument.
   - If the instrument is connected to an Ethernet network, use the *Instrument Manager* in the application software to send the experiment file to the instrument.
   - If the instrument is not connected to an Ethernet network, use a USB drive to transfer the experiment file to the instrument.
2. Insert the LightCycler® 480 Multiwell Plate 96 with the samples into the LightCycler® 96 Instrument.
3. On the *Overview* tab on the touchscreen, select the experiment in the list.

LightCycler® 96 System Quick Guide: Programming and running an experiment

4. In the global action bar on the right, choose the *Start* button.
5. View the *Raw Data* tab to monitor the progress of the running experiment.

Run finished

The end of a run is indicated as follows:

- The status bar on the touchscreen displays the instrument status *Ready*.
- The LightCycler® 96 Instrument unlocks the loading module.
- The experiment progress bar shows the end time of the experiment run.
- The *Raw Data* tab provides the final raw data.

Transfer the experiment to the application software

1. - If the instrument is connected to an Ethernet network, use the *Instrument Manager* in the application software to retrieve the experiment file from the instrument.
   - If the instrument is not connected to an Ethernet network, use a USB drive to transfer the experiment file to your computer.
2. Edit the experiment according to your needs and save the file to your computer.

Edit the sample list

1. Open the *Plate View* tab of the *Sample Editor*.
2. Use the *Clear Wells* function to clear the empty wells. This eliminates the selected wells from further analyses.
3. Select a well or a range of wells.

4. In the *Reaction Properties* window area to the right of the multiwell plate image, edit the sample-specific properties.

   Ensure that the sample assignment on the 'Sample Editor' tab matches the pipetting scheme on the multiwell plate.
5. Save the experiment.

Analyze the data

1. On the *Analysis* tab, add the appropriate analysis type.
2. Open the *<analysis> Settings* dialog box and set up the analysis-specific parameters.
3. Exclude samples if necessary.
4. Select the results to be displayed.
5. Optional: Export the result data.

Disclaimer

Before setting up operation of the LightCycler® 96 System, it is important to read the user documentation completely. Non-observance of the instructions provided or performing any operations not stated in the user documentation could produce safety hazards.

Version Information

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LightCycler® 96 System
User Training Guide, Version 1.0

Software Version 1.0 **August 2012**

For life science research only.
Not for use in diagnostic procedures.

Prologue

I Revision history

User Training Guide Version	Software Version	Revision Date	Changes
V1.0	V1.0	August 2012	First edition

Questions or comments regarding the contents of this user training guide can be directed to your local Roche Diagnostics representative.

Every effort has been made to ensure that all the information contained in the LightCycler® 96 System User Training Guide is correct at the time of publishing.

However, Roche Diagnostics GmbH reserves the right to make any changes necessary without notice as part of ongoing product development.

II Contact addresses

	Manufacturer	Roche Diagnostics GmbH Sandhofer Straße 116 68305 Mannheim Germany
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SYBR is a registered trademark of Life Technologies Corporation.

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IV Intended use

The LightCycler® 96 Instrument is intended for performing rapid, accurate polymerase chain reaction (PCR) in combination with real-time, online detection of DNA-binding fluorescent dyes or labeled probes, enabling quantification or characterization of a target nucleic acid.

The LightCycler® 96 System is intended for life science research only. It must only be used by laboratory professionals trained in laboratory techniques, who have studied the Instructions for Use of this instrument. The LightCycler® 96 Instrument is not for use in diagnostic procedures.

The LightCycler® 96 System is intended for indoor use only.

V Preamble

Before setting up operation of the LightCycler® 96 System, it is important to read the user documentation completely. Non-observance of the instructions provided or performing any operations not stated in the user documentation could produce safety hazards.

VI Disclaimer of licenses

NOTICE: This product may be subject to certain use restrictions. Before using this product please refer to the Online Technical Support page (*http://technical-support.roche.com*) and search under the product number or the product name, whether this product is subject to a license disclaimer containing use restrictions.

VII Open Source licenses

Portions of the LightCycler® 96 Software might include one or more Open Source or commercial software programs. For copyright and other notices and licensing information regarding such software programs included with LightCycler® 96 Software, please refer to the USB drive provided with the product.

VIII Conventions used in this guide

Text conventions

To present information consistently and make it easy to read, the following text conventions are used in this guide:

Numbered list	Steps in a procedure that must be performed in the order listed.
Italic type	Used for operating instructions for the LightCycler® 96 Software. In addition, important notes and information notes are shown in italics.
Blue italic type	Refers to a different section in this User Training Guide, which should be consulted.
[ ]	Square brackets indicate keys on the keyboard.
< >	Angle brackets indicate variables to be replaced with appropriate values.

Abbreviations

The following abbreviations are used in this guide:

Abbreviation	Meaning
Cq	Quantification Cycle
Cy5	Cyanine 5
dsDNA	Double-stranded DNA
E	Efficiency
EPF	Endpoint Fluorescence
FAM	6-Carboxyl Fluorescein
GOI	Gene of Interest
HEX	Carboxyl-2',4,4',5',7,7'-Hexachlorofluorescein
NTC	No Template Control
NRTC	Non Reverse Transcription Control
PCR	Polymerase Chain Reaction
PE	Protection Earth
qPCR	Quantitative Real-Time PCR
RDML	Real Time Data Management Language
SNP	Single Nucleotide Polymorphism
SYBR	SYBR Green I (a common double-stranded binding dye)
T_m	Melting Temperature
USB	Universal Serial Bus
VIC	Reporter Dye for Hydrolysis Probes

Symbols used in this guide

Symbol	Meaning	Description
	WARNING	This symbol is used to alert you to the presence of important operating and maintenance instructions in the literature accompanying the instrument. There are no user-serviceable parts inside the instrument.
	HOT SURFACE	This symbol is used to label potentially hot instrument surfaces.
	BIO HAZARD	This symbol is used to indicate that certain precautions must be taken when working with potentially infectious material.
	DANGEROUS ELECTRICAL VOLTAGE	This symbol is used to indicate the danger of personal injury due to dangerous electrical voltage. Refers to an imminent danger that may result in death or serious personal injury.
	KEEP HANDS CLEAR	This symbol is used to indicate the risk of crushing hands in movable parts.
	IMPORTANT NOTE	Information critical to the success of the procedure or use of the product.
	INFORMATION NOTE	Additional information about the current topic or procedure.
▶ ▶ ▶		Procedure continued on next page.
■		End of procedure.

Symbols used on the instrument

Symbol	Meaning	Description
	MANUFACTURER OF DEVICE	Roche Diagnostics GmbH Sandhofer Strasse 116, D-68305 Mannheim Germany Made in Switzerland
	CE MARK	The CE mark on the instrument type plate indicates conformity with requirements of the directives relevant for this instrument.
	WARNING	On the instrument type plate.
	cUL MARK	On the instrument type plate.
	HOT SURFACE	On the loading module.
	BIO HAZARD	On the loading module.
	KEEP HANDS CLEAR	On the instrument housing (Only visible, when the loading unit is ejected).

In addition to these symbols, the following information is provided on the instrument typeplate:

- LightCycler® 96
- Instrument serial number in hexadecimal and in 1D barcode
- Power supply and mains power consumption: 100-125/200-240 Vac   50/60 Hz   600 VA

IX Warnings and precautions

In an emergency, immediately unplug the instrument.

The LightCycler® 96 Instrument must only be used by trained and skilled personnel.

It is essential that the following safety information required for installation and operation of the LightCycler® 96 Instrument is carefully read and observed. Please ensure that this safety information is accessible to all personnel working with the LightCycler® 96 Instrument.

Handling requirements

The LightCycler® 96 Instrument is an electromechanical instrument. There is a potential risk to the user from electric shock or physical injury if the instrument is not used according to the instructions given in this manual.

- Follow all safety instructions printed on or attached to the analytical instrument.
- Observe all general safety precautions which apply to electrical instruments.
- Do not access any electrical parts while the LightCycler® 96 Instrument is connected to the mains power supply.
- Never touch the power cable with wet hands.
- Never open the housing of the LightCycler® 96 Instrument .
- Never clean the instrument without disconnecting the power cable.
- Only authorized service personnel are allowed to perform service or repairs required for this unit.
- Do not use the network cable outdoors.

- Always wear safety goggles and gloves when dealing with toxic, caustic, or infectious materials.

- Although working with highly purified nucleic acids, for your own safety, please regard all biological material as potentially infectious. Handling and disposal of such material should be performed according to local safety guidelines. Spills should be immediately disinfected with an appropriate disinfectant solution to avoid contamination of laboratory personnel or equipment.
- For instructions on cleaning the LightCycler® 96 Instrument, refer to the *LightCycler® 96 System Operator's Guide*, chapter "Cleaning and care".

The multiwell plate mount may be hot after an experiment run.

Always keep your hands clear, when closing the loading unit.

General precautions

The LightCycler® 96 System contains software that allows it to be connected to a network. Please be aware that such a connection may have an adverse effect on the product's integrity, through, for example, infection with malicious code (viruses, Trojan horses, etc.) or access by unauthorized third parties, such as intrusion by hackers. Roche therefore highly recommends protecting the product against such risks by taking appropriate and state-of-the-art action.

As the product is not intended to be used within networks without an appropriate firewall and has not been designed for such use, Roche assumes no liability in this regard.

Incorrect positioning of the instrument can cause incorrect results and damage to the equipment. Follow the installation instructions carefully.

Danger of explosion through sparks. Keep all potentially inflammable or explosive material (for example, anesthetic gas) away from the instrument. Spraying liquid on electrical parts can cause a short circuit and result in fire. Keep the cover closed while the instrument is connected to the mains power supply and do not use sprays in the vicinity of the LightCycler® 96 Instrument. During fire fighting operations, disconnect the LightCycler® 96 Instrument from the mains power supply.

Do not disassemble the instrument.

Electrical safety

The LightCycler® 96 Instrument is designed in accordance with Protection Class I (IEC). The housing of the instrument is connected to protection earth (PE) by a cable. For protection against electric shock hazards, the instrument must be directly connected to an approved power source such as a three-wire grounded receptacle for the 115/230 V line. Where only an ungrounded receptacle is available, a qualified electrician must replace it with a properly (PE) grounded receptacle in accordance with the local electrical code. No extension must be used.

Any break in the electrical ground path, whether inside or outside the instrument, could create a hazardous condition. Under no circumstances should the operator attempt to modify or deliberately override the safety features of this instrument. If the power cable becomes cracked, frayed, broken, or otherwise damaged, it must be replaced immediately with the equivalent part from Roche Diagnostics.

Please observe the warnings regarding interactions and non-recommended functions. Also bear in mind the potential scope for misuse; it is advisable to draw attention to the possible consequences.

Starting the system

1 Overview

This section provides an overview of the following topics:

- The main components of the LightCycler® 96 System and your workflow for using them, see below.
- How to use this user training guide, see section *How to use this user training guide*, on page 14.

LightCycler® 96 System main components and workflow

The LightCycler® 96 System consists of two main components:

- The LightCycler® 96 Application Software on your computer, which provides all functions for defining an experiment protocol and for analyzing the data gathered during the experiment run.
- The LightCycler® 96 Instrument, which is controlled by the LightCycler® 96 Instrument Software. The LightCycler® 96 Instrument Software provides all functions for configuring and controlling the LightCycler® 96 Instrument. These include functions for managing, creating, and executing experiments, and for monitoring an experiment run. The instrument software is operated using the touchscreen of the instrument.

For starting a run, the experiment must be available on the LightCycler® 96 Instrument. After the experiment run, the raw data gathered by the software must be transferred to the application software for analysis.

The figure below shows the LightCycler® 96 System workflow and which software components are used to perform the individual workflow steps.

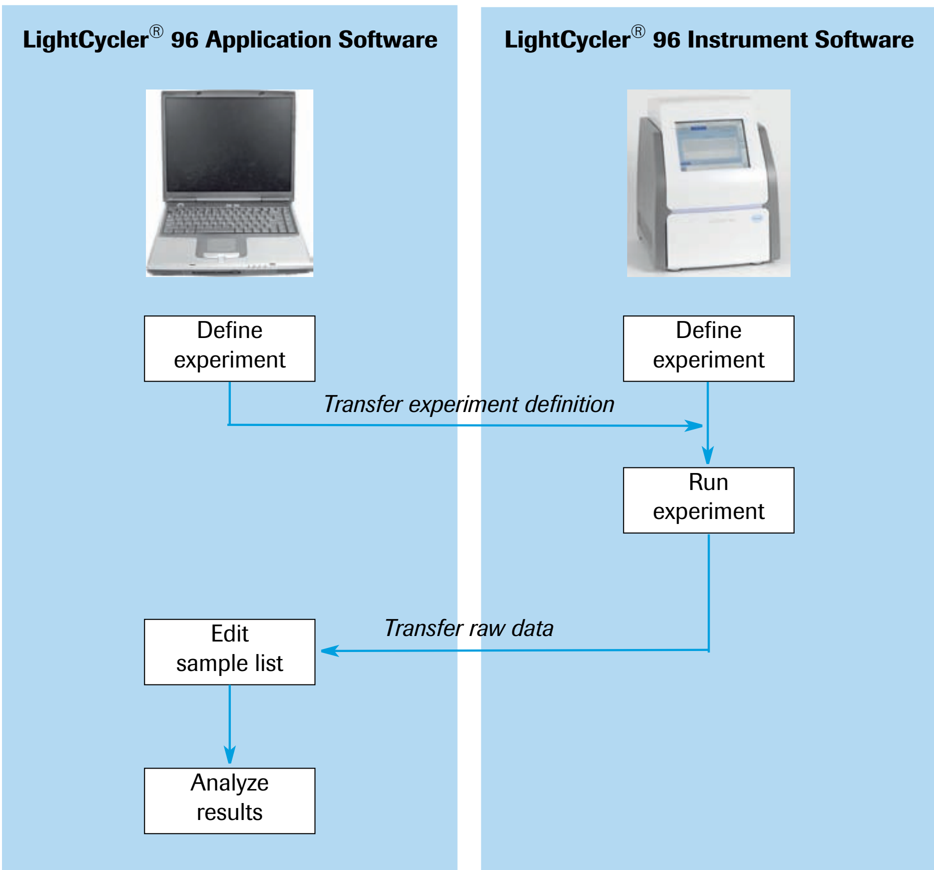


Figure 1: The LightCycler® 96 System workflow

A

How to use this user training guide

This user training guide is structured as follows:

Steps that are similar for all applications are described step-by-step in the chapter Programming and running an experiment. Read this chapter before starting an experiment.

Steps that are different for each application are described in the chapter Main applications, in a separate section for each application.

To perform experiments with the LightCycler® 96 System, follow the procedure below in the order given. This user training guide describes basic examples for each of the main applications.

1	Start the LightCycler® 96 Application Software. For step-by-step information, see section *Starting the LightCycler® 96 Application Software*, on page 15.
2	Start the LightCycler® 96 Instrument and the instrument software. For step-by-step information, see section *Starting the LightCycler® 96 Instrument*, on page 15.
3	Set up the samples. For detailed information for each application, see the corresponding section in the chapter *Main applications*, on page 55.
4	Define the experiment. ▶ For step-by-step information on how to program an experiment, see chapter *Programming and running an experiment*, on page 17. ▶ For details of the experiment run parameters for each described example, see the corresponding section in chapter *Main applications*, on page 55.
5	Run the experiment. For step-by-step information on how to run an experiment, see chapter *Programming and running an experiment*, on page 17.
6	Edit the sample list. ▶ For step-by-step information on how to edit a sample list, see chapter *Editing the sample list*, on page 48. ▶ For detailed information on analysis-specific parameters, see the corresponding section in the chapter *Main applications*, on page 55. *You can edit the sample list before running the experiment when using the LightCycler® 96 Application Software to define the experiment.*
7	Analyze the results. For detailed information, see the corresponding section in the chapter *Main applications*, on page 55.

2 Starting the LightCycler® 96 Application Software

Before starting the software, you must install it on your computer. For a detailed description of the installation, refer to the *LightCycler® 96 System Operator's Guide,* chapter A, section "Installation".

To start the LightCycler® 96 Application Software

1. Switch on the computer.
2. Double-click the *LightCycler® 96* icon on your desktop.

The LightCycler® 96 Application Software provides a splash screen with information on the initialization status. After initialization, the main window opens displaying the startup wizard.

3 Starting the LightCycler® 96 Instrument

Before starting, you must plug in the LightCycler® 96 Instrument. Refer to the *LightCycler® 96 System Operator's Guide,* chapter A "System Description" for a detailed description of the instrument parts, and chapter A, section "Installation" for a description of the installation.

The LightCycler® 96 Instrument Software is started together with the instrument.

To start the LightCycler® 96 Instrument

1. Use the mains power switch on the back of the instrument to switch it on.

The instrument and the instrument software are started.

A

Programming and running an experiment

For information on the order for performing the individual steps of a complete LightCycler® 96 System workflow, see section Overview, on page 13.

You can create an experiment and define the temperature profile and the dye-specific parameters either on the instrument using the LightCycler® 96 Instrument Software or on a computer using the LightCycler® 96 Application Software. For starting an experiment run, the experiment must be available on the instrument. Therefore, if you have programmed the experiment on a computer, it must be transferred to the instrument for the run.

B

This chapter describes both ways of programming an experiment:

- For detailed information on how to specify an experiment definition using the application software, see section *Programming the experiment with the LightCycler® 96 Application Software*, on page 18. For detailed information on how to transfer the experiment to the instrument, see section *Transferring the experiment to the instrument*, on page 28.
- For detailed information on how to specify an experiment definition using the instrument software, see section *Programming the experiment with the LightCycler® 96 Instrument Software*, on page 30.

After the experiment run, the raw data gathered by the software on the instrument must be transferred back to the application software for analysis. For detailed information on how to transfer the raw data to the application software, see section *Transferring the experiment from the instrument to the application software*, on page 45.

B

1 Programming the experiment with the LightCycler® 96 Application Software

The information provided in the experiment definition controls the LightCycler® 96 Instrument during an experiment run. The experiment definition specifies the target temperatures and hold times of the thermal block cycler, the number of cycles being executed, and other parameters.

For a comprehensive description of the LightCycler® 96 Application Software, refer to the "LightCycler® 96 System Operator's Guide", chapter "LightCycler® 96 Application Software".

To program an experiment:

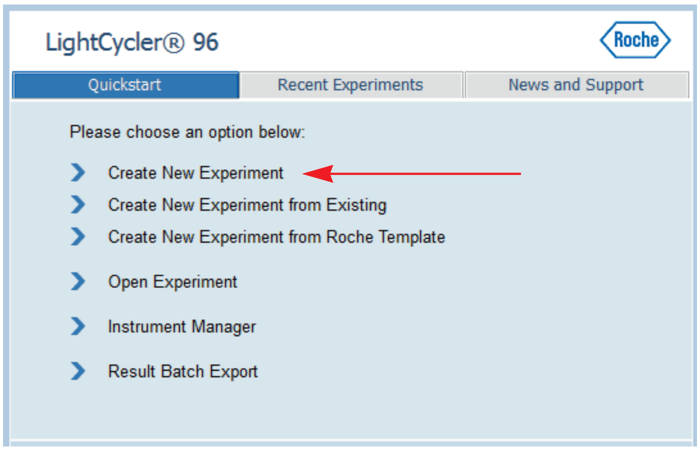
- Create a new experiment, see section *Creating the experiment*, below.
- Add one or more programs and define the temperature profile for each step of a program, see section *Creating the temperature profile*, on page 21.
- Specify the reaction volume and the detection format for the experiment , see section *Configuring the reaction volume and detection format*, on page 25.

1.1 Creating the experiment

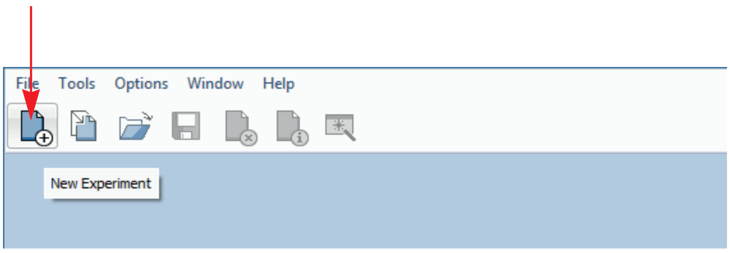
This user training guide describes how to generate a completely new experiment. For a detailed description of all options for creating an experiment with the LightCycler® 96 Application Software, refer to the *LightCycler® 96 System Operator's Guide*, chapter B, section "Experiments".

1 Perform one of the following steps:

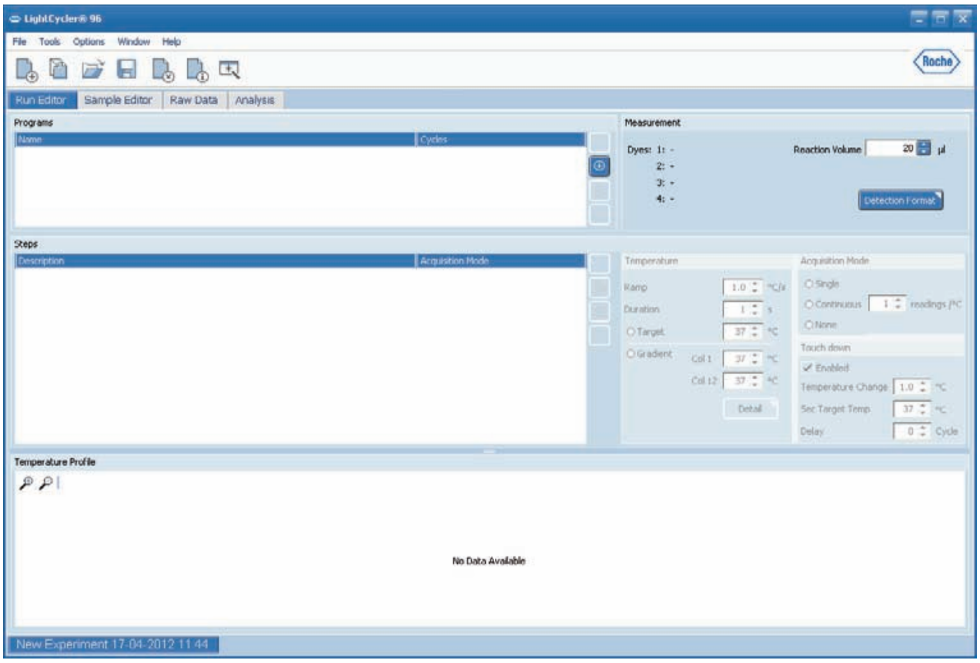
- In the startup wizard, choose *Create New Experiment*.



- In the tool bar, choose the *New Experiment* icon.



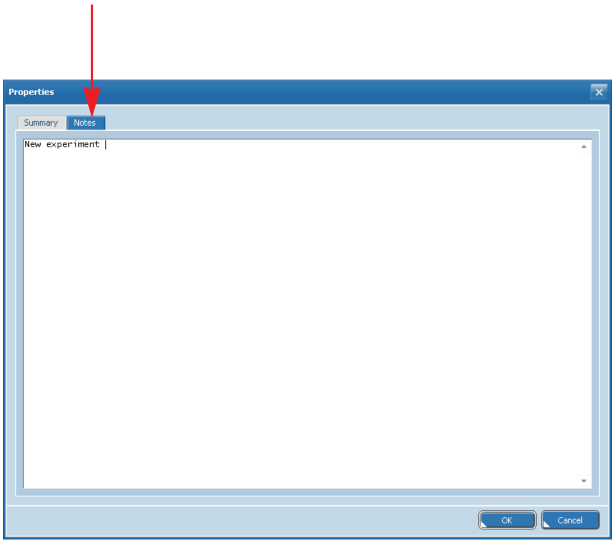
The LightCycler® 96 Application Software displays the new experiment in the main window. The new experiment has the default name *New Experiment <creation_date> <creation_time>*.



▶ ▶ ▶

2 Optional: Enter a description for the experiment.

- In the *File* menu, choose *Properties*.
- In the *Properties* dialog box, open the *Notes* tab.



- Enter a description.
- Choose *OK*.

1.2 Creating the temperature profile

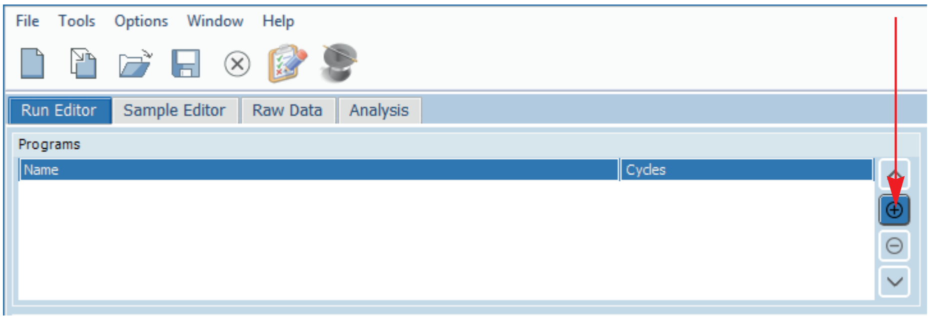
For detailed information on the applicable values for the experiment run parameters, see the corresponding section in the chapter Main applications, on page 55.

To create a temperature profile:

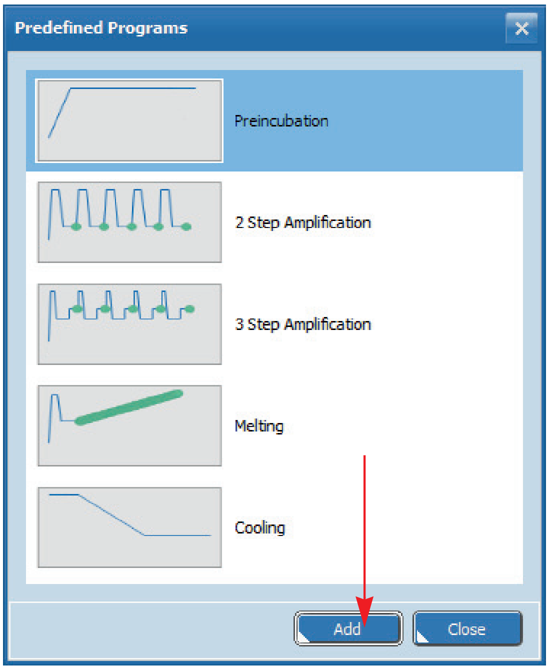
- Add one or more new programs to the temperature profile and create the cycling sequence, see section *To add a new program and specify the number of cycles*, below.
- Define the temperature profile for each step of a program, see section *To specify the temperature profile for each step of a program*, on page 23.

To add a new program and specify the number of cycles

1. Open the *Run Editor* tab.
2. In the *Programs* window area, choose the ⊕ button to open the *Predefined Programs* dialog box.



3. Select one of the available programs for the first program and choose *Add*.



The program is added to the *Programs* list and displayed in the *Temperature Profile* window area.

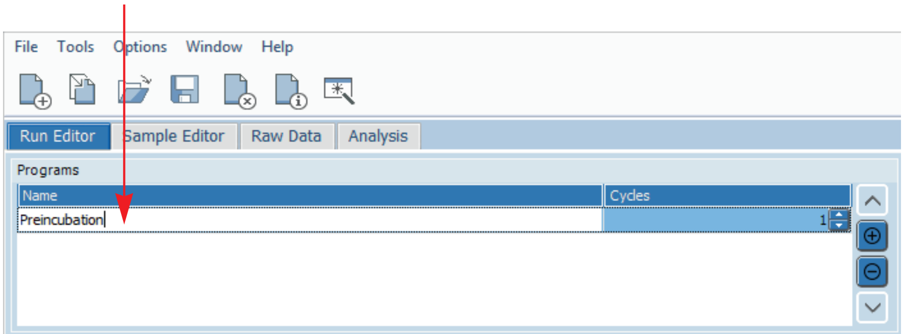
▶▶▶

B

B

4 Optional: To modify the name of the new program, proceed as follows:

- In the *Programs* list, select the new program.



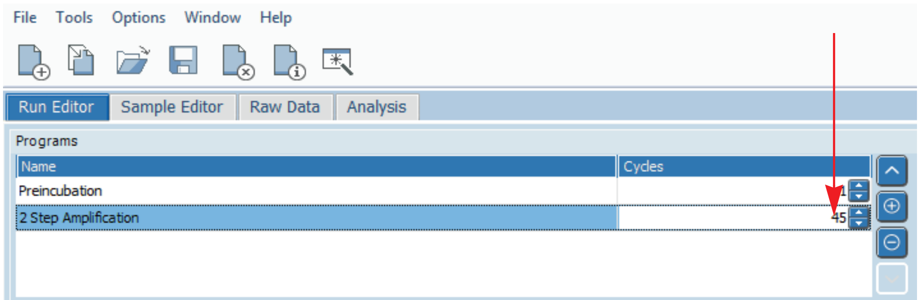
- Specify the name.

5 Repeat steps 1 to 4 to add further programs to your profile.

6 If necessary (for example, for an amplification program) proceed as follows to specify the number of repeats of a program (cycles):

- In the *Programs* list, select the new program.
- In the *Cycles* column, use the up and down arrows to specify how many times the cycle is to be repeated in this experiment, or type in a value (possible values: 1 to 99).

For detailed information on the applicable values for the number of cycles, see the corresponding section in the chapter Main applications, on page 55.



7 If necessary repeat step 6 to specify the corresponding number of cycles for further programs.

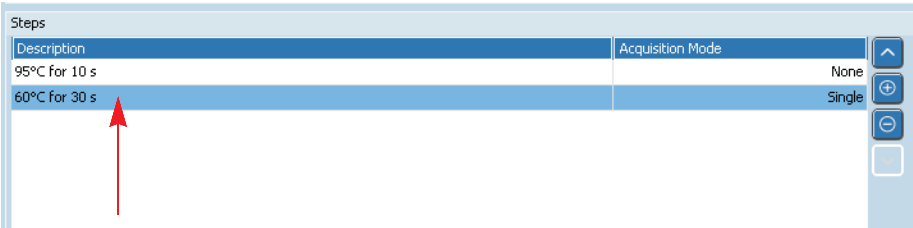
To specify the temperature profile for each step of a program

A step can only be edited as long as no run has been performed.

For a comprehensive description of all options of the LightCycler® 96 Application Software, refer to the "LightCycler® 96 System Operator's Guide", chapter "LightCycler® 96 Application Software".

---

1 ▶ In the *Steps* window area, select the step you want to edit.

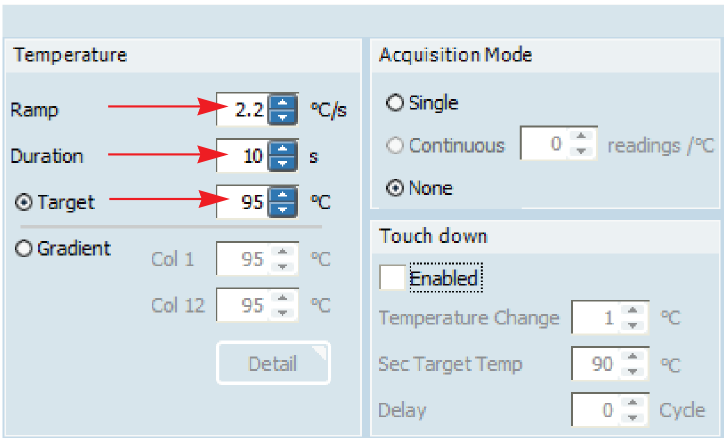


---

2 In the *Temperature* window area to the right of the *Steps* list, edit the default values of the following parameters for the selected step:

- *Ramp (°C/s)*:
  Maximum value for heating: 4.4°C/s
  Maximum value for cooling: 2.2°C/s
- *Duration* (*s*):
  Possible values: 1 to 7200 s (= 2 h)
- *Target (°C)*:
  Possible values: 37 to 98°C

For detailed information on the applicable values for the experiment run parameters, see the corresponding section in the chapter Main applications, on page 55.

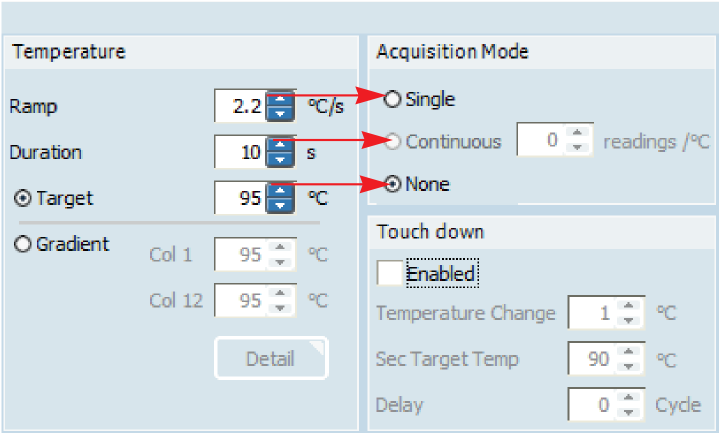


▶ ▶ ▶

3 In the *Acquisition Mode* window area to the right of the *Steps* list, choose one of the following options as the acquisition mode for the selected step.

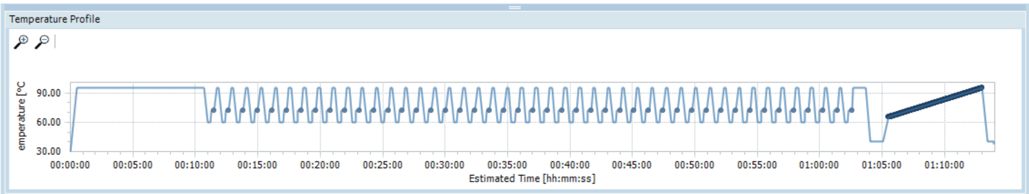
- *Single:* Applicable for amplification program steps (one measurement/cycle).
- *Continuous* (*readings/°C*): Applicable for melting program steps. You must also specify the number of optical acquisitions to be performed.
  Default value: 5 readings/°C
  Possible values: 1 to 25 readings/°C
- *None:* Applicable for steps that do not require fluorescence measurement.

For detailed information on the applicable values for the experiment run parameters, see the corresponding section in the chapter *Main applications, on page 55.*



4 Repeat steps 1 to 3 for each step of each program in your profile.

5 View the *Temperature Profile* window area for a graphical representation of the entire experimental protocol you have defined.



1.3 Configuring the reaction volume and detection format

To complete the run definition:

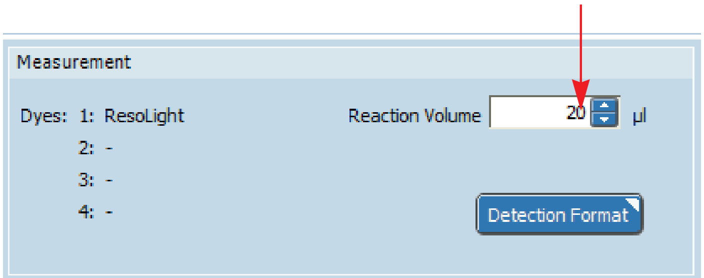
- Specify the reaction volume, see section *To specify the reaction volume for the experiment*, below.
- Specify the dye-specific parameters for the detection format, see section *To specify the detection format for the experiment*, below.
- Save the experiment, see section *To save the experiment*, on page 27.

To specify the reaction volume for the experiment

1. In the *Measurement* window area to the right of the *Programs* list, specify the reaction volume to be used in your experiment in the *Reaction Volume* field. The LightCycler® 96 Instrument supports reaction volumes from 10 to 50 µl.

   For detailed information on the applicable value for the reaction volume, see the corresponding section in the chapter Main applications, on page 55.

   As the LightCycler® 96 Instrument does not validate the reaction volume, ensure that the specified reaction volume matches the volume pipetted into the wells of the multiwell plate.



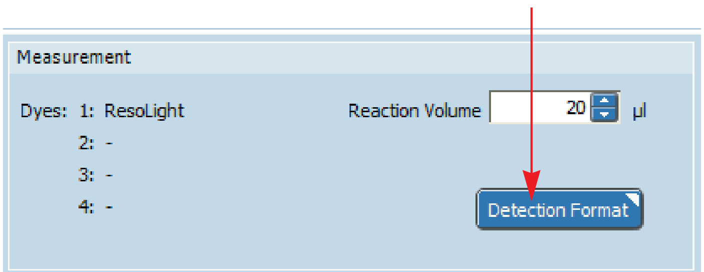
To specify the detection format for the experiment

The detection format specifies one or more excitation-emission filter combinations (detection channels) suitable for your experiment.

For detailed information on the applicable values for the dye-specific parameters for specifying the detection format, see the corresponding section in the chapter Main applications, on page 55.

For a comprehensive description of all options of the LightCycler® 96 Application Software, refer to the "LightCycler® 96 System Operator's Guide", chapter "LightCycler® 96 Application Software".

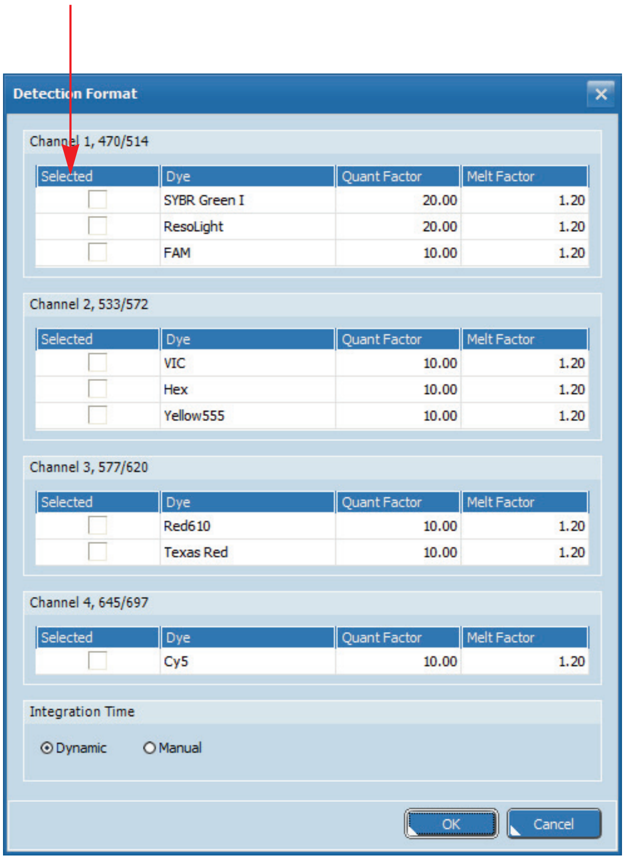
1. In the *Measurement* window area, choose *Detection Format*.



The *Detection Format* dialog box opens.

2 ▶ In the *Selected* column, select the check box of no more than one dye per detection channel, to specify that the corresponding channel is to be used.

Only one dye can be selected per channel. The software automatically deselects a check box if you try to select more than one dye in the same channel group.



▶ For *Integration Time*, leave the default value (*Dynamic*).

▶ For *Quant Factor* and *Melt Factor*, leave the default values.

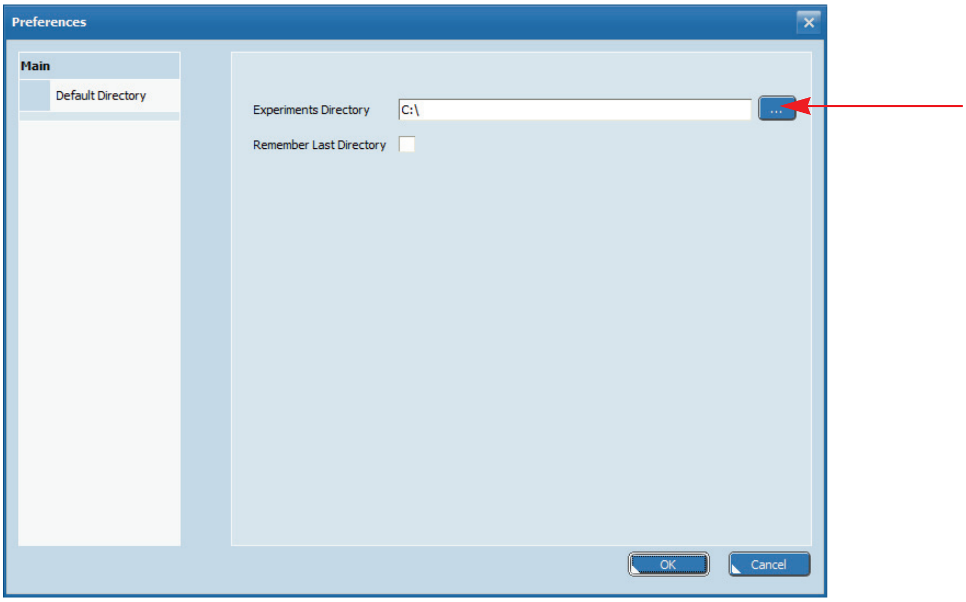
3 ▶ Optional: Repeat step 2 to specify another detection channel for your detection format.

You cannot combine SYBR Green I or the ResoLight dye with any dye of any other channel.

4 Choose *OK* to apply your settings to the corresponding parameters.

To save the experiment

1. Optional: Define a default directory for saving and loading experiment files.
   - In the *Options* menu, choose *Preferences*. The *Preferences* dialog box opens.



   - Choose the browse button next to the *Experiments Directory* field. The *Browse For Folder* dialog box opens.
   - Specify the default path.
   - Choose *OK*.

2. In the tool bar, choose the *Save Experiment* icon to save the new experiment. The *Save As* dialog box opens.
   For a detailed description of all saving options, refer to the *LightCycler® 96 System Operator's Guide*, chapter "LightCycler® 96 Application Software".

3. Navigate to the directory where you want to store the experiment file.

4. Enter a file name for the experiment.

5. Choose *Save*. The dialog box closes and the experiment is saved as a LightCycler® 96 file (.lc96).

B

2 Transferring the experiment to the instrument

If you have specified the experiment definition on a computer using the LightCycler® 96 Application Software, the experiment must be transferred to the instrument for the run.

To transfer the experiment to the instrument using a USB drive

1. Insert a USB drive into one of the USB interfaces of your computer.
2. Open Windows Explorer and navigate to the experiment file.
3. Copy the experiment file (.lc96) and paste it onto the USB drive.
4. Close Windows Explorer.
5. Remove the USB drive from your computer.
6. Switch on the LightCycler® 96 Instrument, see section *Starting the LightCycler® 96 Instrument*, on page 15.

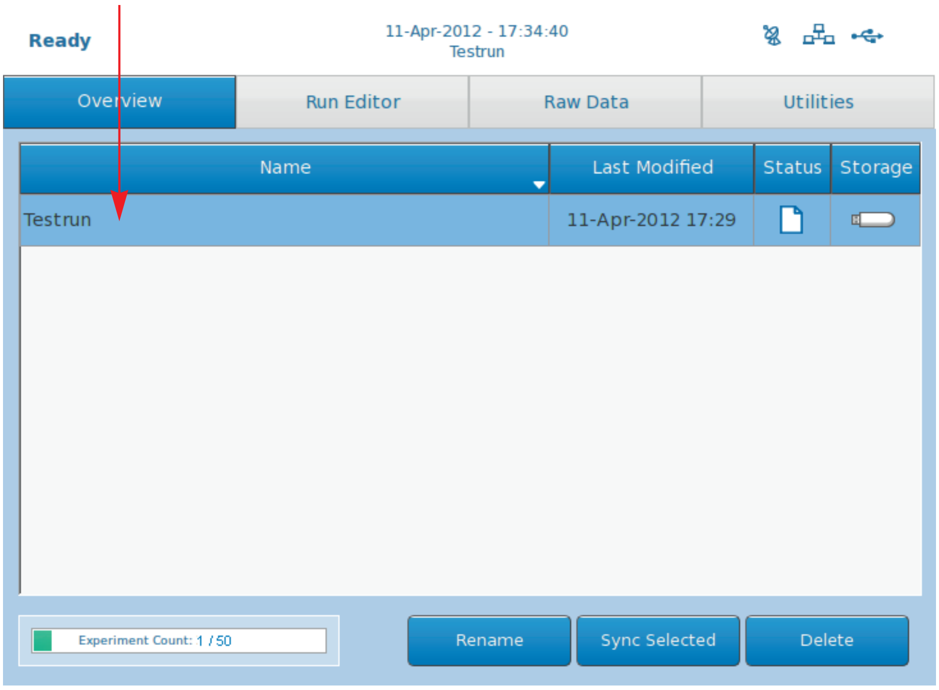
▶ ▶ ▶

B

7 Insert the USB drive into the USB interface on the right side of the instrument.

As soon as the USB icon is shown in the status bar of the LightCycler® 96 Instrument Software, the experiment file is added to the experiments table on the *Overview* tab.

In the corresponding *Storage* column, the USB icon is shown.



As long as you have not synchronized the storage locations, the experiment file is located only on the USB drive.

It is not necessary to synchronize the experiment to the instrument with the 'Sync Selected' button. Experiments can be run directly from the USB drive and when successfully finished are automatically saved back to the USB drive.

B

3 Programming the experiment with the LightCycler® 96 Instrument Software

The information provided in the experiment definition controls the LightCycler® 96 Instrument during an experiment run. The experiment definition specifies the target temperatures and hold times of the thermal block cycler, the number of cycles being executed, and other parameters.

For programming the experiment with the instrument software, the LightCycler® 96 Instrument must be started.

For a comprehensive description of the LightCycler® 96 Instrument Software, refer to the "LightCycler® 96 System Operator's Guide", chapter "LightCycler® 96 Instrument Software".

In addition, the help browser provides information on the currently open tab of the LightCycler® 96 Instrument Software.

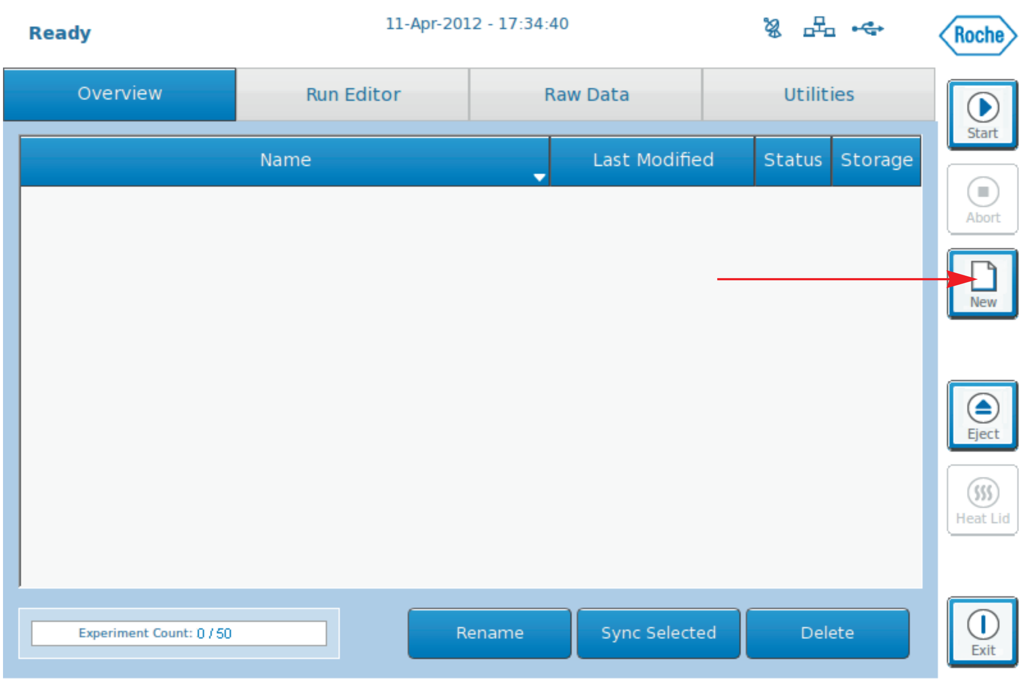
To program an experiment:

- Create a new experiment, see section *Creating the experiment*, below.
- Add one or more programs and define the temperature profile for each step of a program, see section *Creating the temperature profile*, on page 33.
- Specify the reaction volume and the detection format for the experiment, see section *Configuring the detection format and reaction volume*, on page 38.

3.1 Creating the experiment

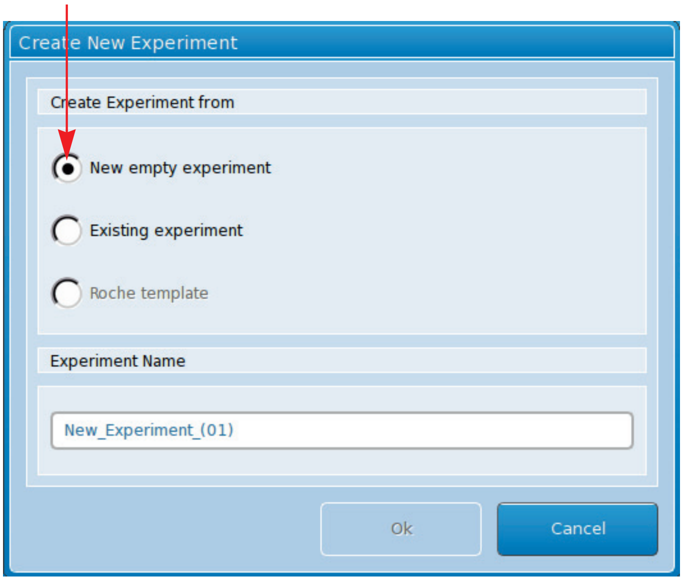
This user training guide describes how to generate a completely new experiment. For a detailed description of all options for creating an experiment with the LightCycler® 96 Instrument Software, refer to the *LightCycler® 96 System Operator's Guide*, chapter C, section "Experiments".

1 In the global action bar of the instrument software main window, choose *New*.

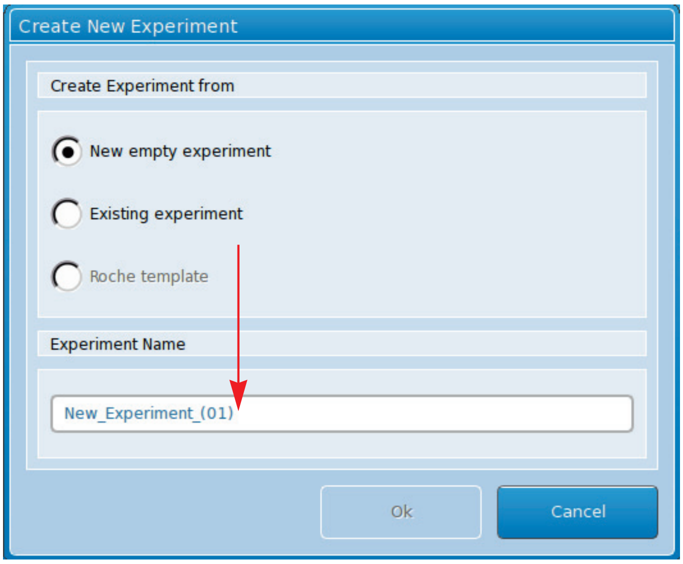


The *Create New Experiment* dialog box opens.

2 Choose *New empty experiment* to create a new, empty experiment.
The new experiment has the default name *New_Experiment_(<no>)*.

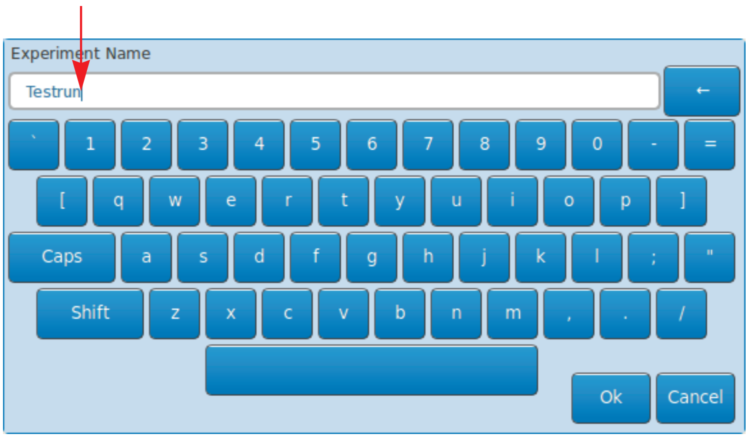


3 In the *Experiment Name* window area, choose the field.



A keyboard dialog box opens.

4 In the *Experiment Name* field, specify the name for the new experiment using the keys, and close the dialog box with *OK*.



5. In the *Create New Experiment* dialog box, choose *OK*.

   The LightCycler® 96 Instrument Software performs the following steps:

   - It adds the new experiment to the list in the *Overview* tab.
   - It opens the *Run Editor* tab for the new experiment.

3.2 Creating the temperature profile

For detailed information on the applicable values for the experiment run parameters, see the corresponding section in the chapter *Main applications*, on page 55.

You can only edit a program, and thus also a profile, as long as no run has been performed.

To create a temperature profile:

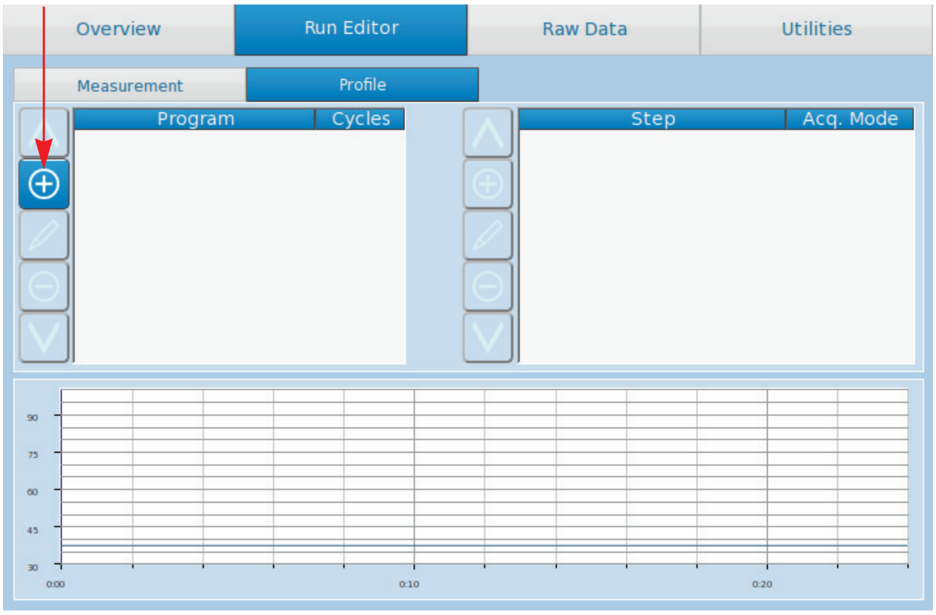
- Add one or more new programs to the temperature profile and specify the cycling sequence, see section *To add a new program and specify the number of cycles*, below.
- Define the temperature profile for each step of a program, see section *To specify the temperature profile for each step of a program*, on page 35.

B

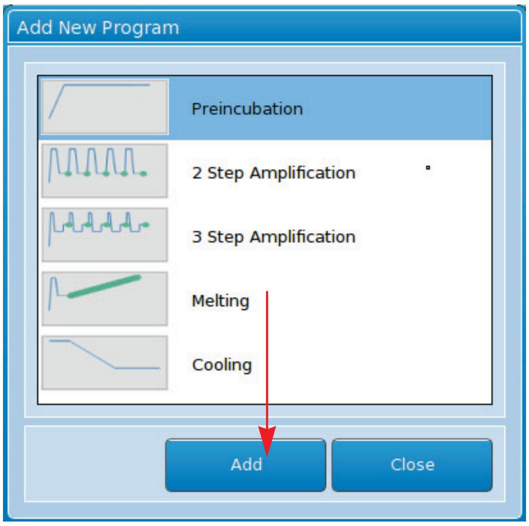
To add a new program and specify the number of cycles

1. Open the *Run Editor* tab.

2. On the *Profile* tab, choose the ⊕ button to open the *Add New Program* dialog box.



3. Select one of the available programs and choose *Add*.

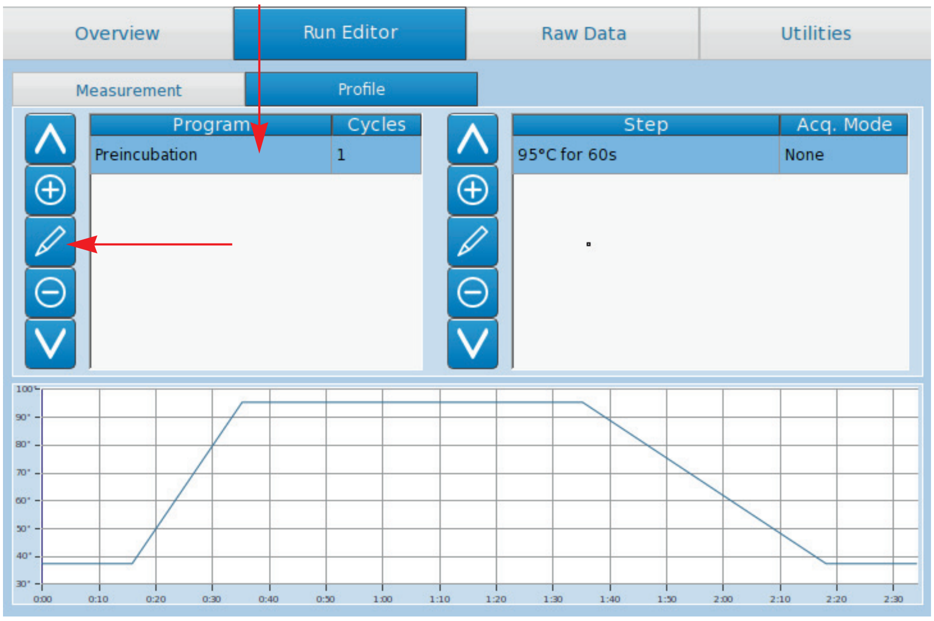


The program is added to the program list on the *Profile* tab.

▶▶▶

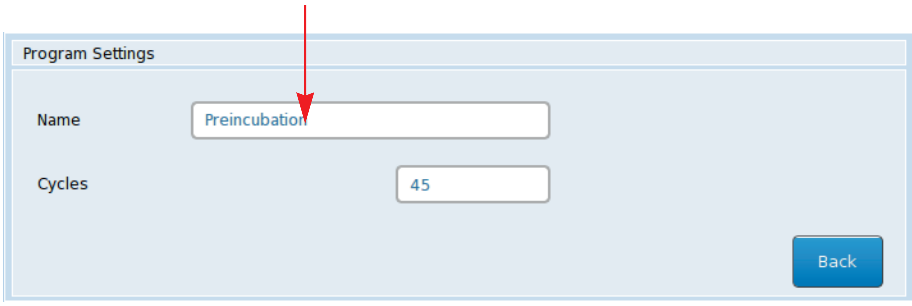
B

4 In the program list, choose the new program. Then choose the pencil button.



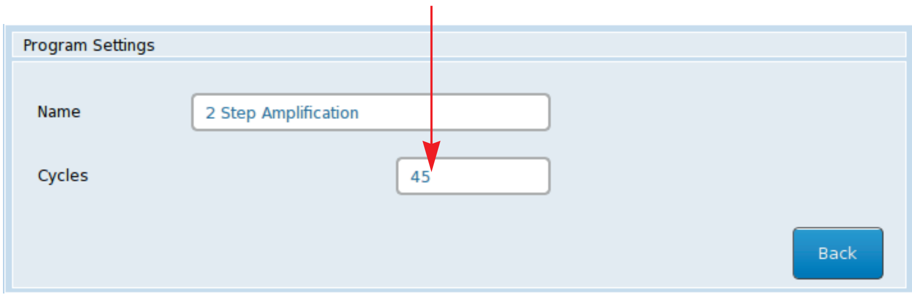
The *Program Settings* window area opens.

5 Optional: In the *Name* field, specify the name for the selected program.



6 If necessary (for example, for an amplification program) specify the number of repeats of the selected program (cycles).
Possible values: 1 to 99

For detailed information on the applicable values for the number of cycles, see the corresponding section in the chapter Main applications, on page 55.



7 Choose *Back* to apply your settings to the selected program.
The *Program Settings* window area is closed. The program list is displayed with the changed settings.

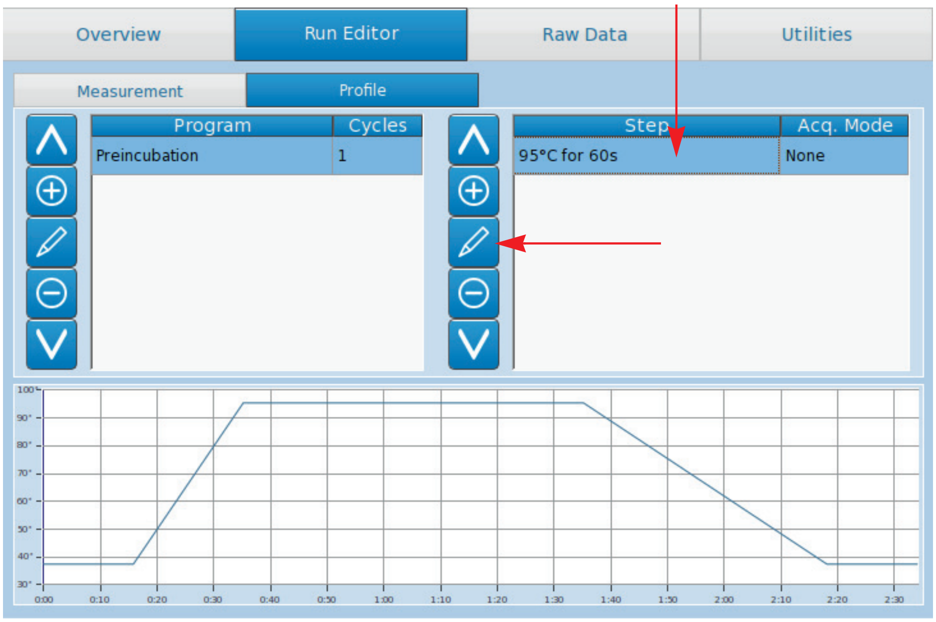
8 Optional: Repeat steps 1 to 7 to add further programs to your profile and specify the corresponding number of cycles if necessary.

To specify the temperature profile for each step of a program

A step can only be edited as long as no run has been performed.

For a comprehensive description of all options of the LightCycler® 96 Instrument Software, refer to the "LightCycler® 96 System Operator's Guide", chapter "LightCycler® 96 Instrument Software".

1 In the step list, select a step and choose the pencil button.

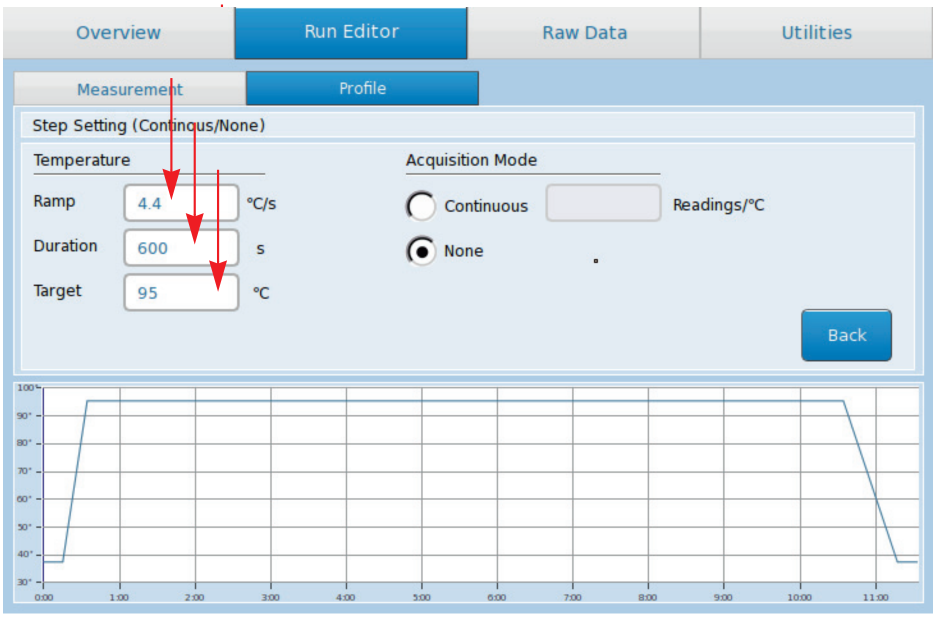


The *Step Setting* window area is displayed.

2 In the *Temperature* window area, choose the corresponding field one after the other to specify the default values of the following parameters for the selected step:

- *Ramp (°C/s)*:
  Maximum value for heating: 4.4°C/s
  Maximum value for cooling: 2.2°C/s
- *Duration* (*s*):
  Possible values: 1 to 7200 s (=2 h)
- *Target (°C)*:
  Possible values: 37 to 98°C
- For the steps of an amplification program: For *Mode*, leave the default option (*Standard*).

For detailed information on the applicable values for the experiment run parameters, see the corresponding section in the chapter Main applications, on page 55.



To specify the listed parameters, proceed as follows for each parameter of the selected step:

- Choose the field for each parameter. The corresponding dialog box opens.

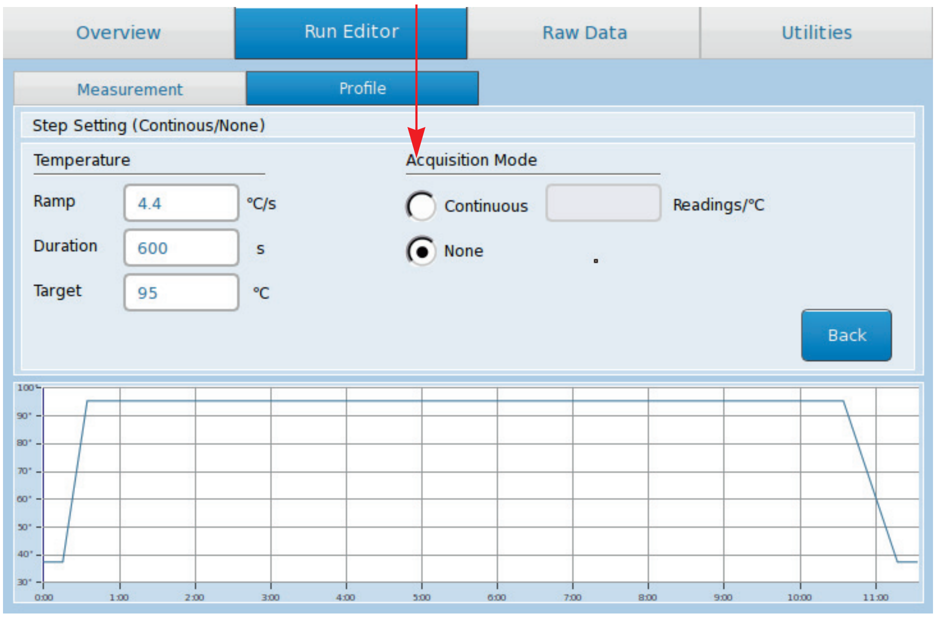
- In the dialog box, choose the relevant number buttons to specify the applicable value.
- Choose *OK* to apply your setting to the parameter.

▶ ▶ ▶

3 In the *Acquisition Mode* window area, choose one of the following options for the selected step.

- *Single:* Applicable for amplification program steps (one measurement/cycle).
- *Continuous (Readings/°C)*: Applicable for melting program steps. Also enter in the field the number of optical acquisitions to be performed in the corresponding list.
  Default value: 5 Readings/°C
  Possible values: 1 to 25 Readings/°C
- *None:* Applicable for steps that do not require fluorescence measurement.

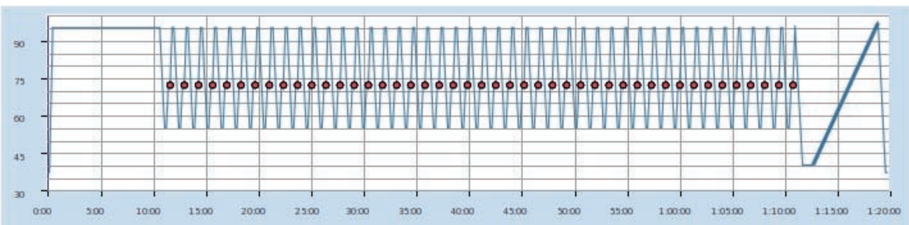
For detailed information on the applicable values for the experiment run parameters, see the corresponding section in the chapter Main applications, on page 55.



4 Choose *Back* to apply your settings to the corresponding parameters.

5 Optional: Repeat steps 1 to 4 for each step of each program in your profile.

6 View the temperature profile area for a graphical representation of the entire experimental protocol you have defined.



3.3 Configuring the detection format and reaction volume

You cannot change or customize the detection format definition after the run has started.

To complete the run definition:

- Specify the dye-specific parameters for the detection format, see section *To specify the detection format for the experiment*, below.
- Specify the reaction volume, see section *To specify the reaction volume for the experiment*, on page 40.

The LightCycler® 96 Instrument Software automatically saves all changes in the experiment file on the instrument.

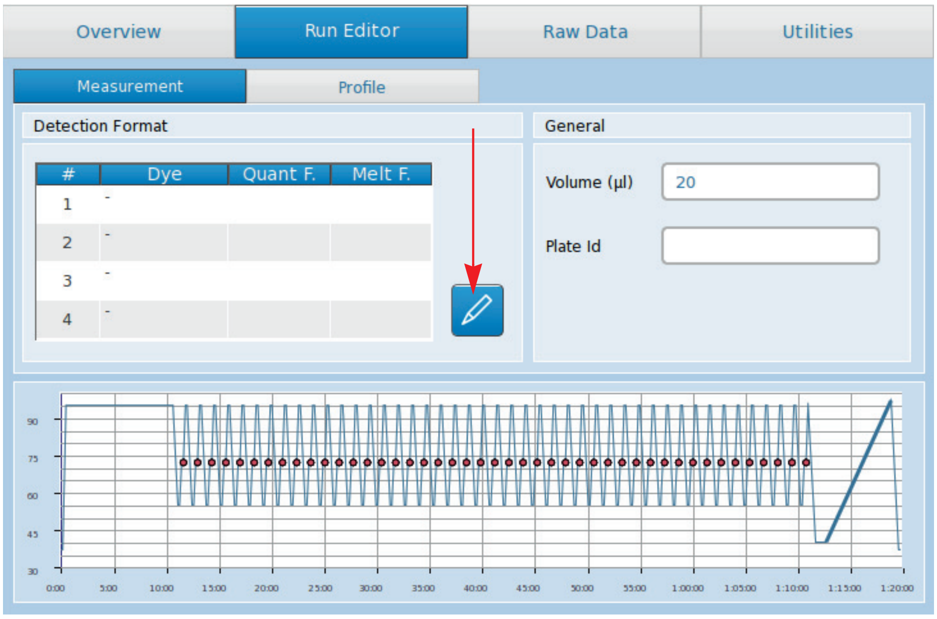
To specify the detection format for the experiment

The detection format specifies one or more excitation-emission filter combinations (detection channels) suitable for your experiment.

For detailed information on the applicable values for the detection format, see the corresponding section in the chapter Main applications, on page 55.

For a comprehensive description of all options of the LightCycler® 96 Instrument Software, refer to the "LightCycler® 96 System Operator's Guide", chapter "LightCycler® 96 Instrument Software".

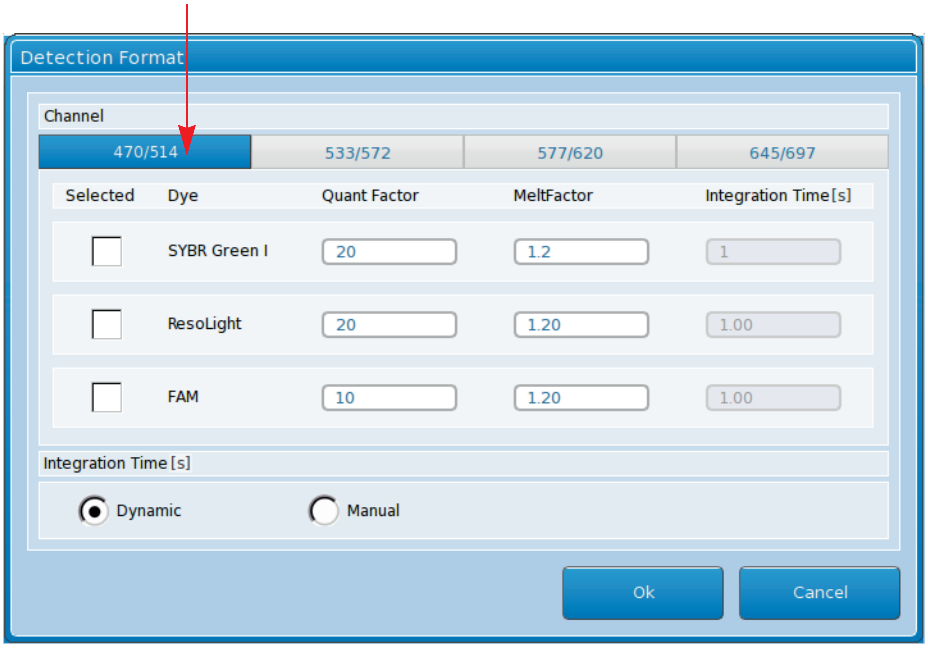
1. Open the *Measurement* tab.
2. In the *Detection format* window area, choose the pencil button.



The *Detection Format* dialog box opens.

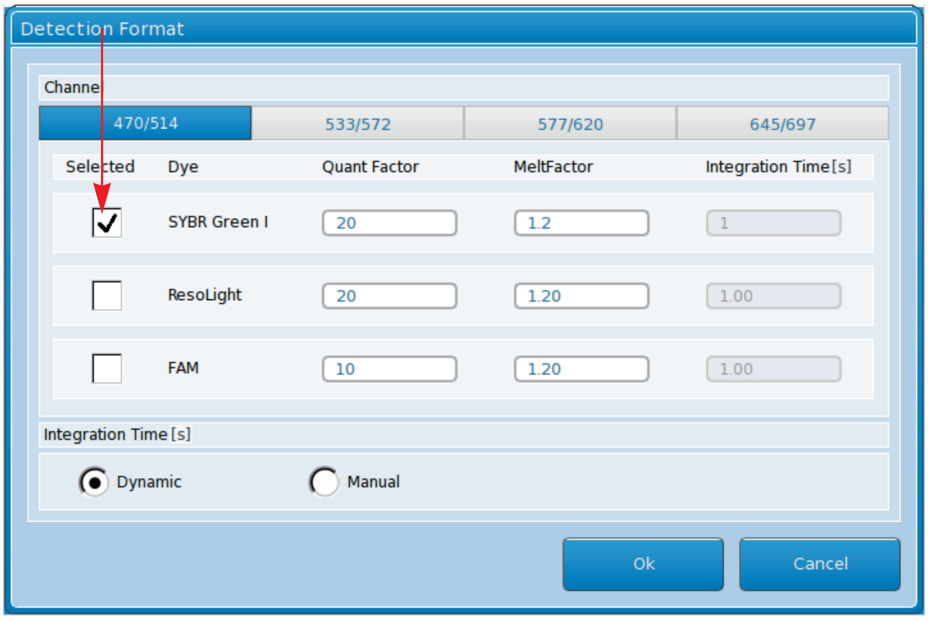
B

3 Choose the tab of the detection channel you want to use.



4 ▶ In the *Selected* column, select the check box of no more than one dye per detection channel, to specify that the corresponding channel is to be used.

Only one dye can be selected per channel. The software automatically deselects a check box if you try to select more than one dye in the same channel group.



▶ For *Quant Factor* and *Melt Factor*, leave the default values.

▶ For *Integration Time [s]*, leave the default value (*Dynamic*).

5 Repeat steps 3 and 4 to specify another detection channel for your detection format.

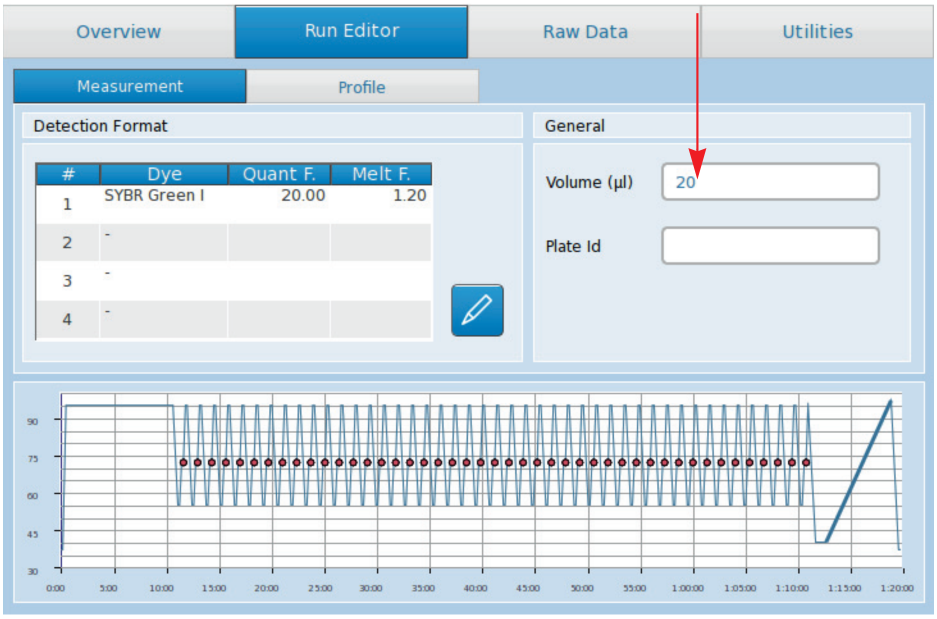
You cannot combine SYBR Green I or the ResoLight dye with any dye of any other channel.

6 Choose *OK* to apply your settings to the corresponding parameters.

B

To specify the reaction volume for the experiment

1. In the *General* window area, choose the *Volume (µl)* field. A dialog box for specifying the value opens.



2. Specify the applicable reaction volume to be used in your experiment.
The LightCycler® 96 Instrument supports reaction volumes from 10 to 50 µl.

For detailed information on the applicable value for the reaction volume, see the corresponding section in the chapter Main applications, on page 55.

As the LightCycler® 96 Instrument does not validate the reaction volume, ensure that the specified reaction volume matches the volume pipetted into the wells of the multiwell plate.

To save the experiment

The LightCycler® 96 Instrument Software automatically saves all changes in the experiment file on the instrument.

For detailed information on saving in the instrument software, refer to the "LightCycler® 96 System Operator's Guide", chapter "LightCycler® 96 Instrument Software".

4 Running the experiment

After defining the setup parameters (temperature profile, reaction volume, and detection format), and saving the definition, you are ready to run the LightCycler® 96 experiment.

For starting an experiment run, the experiment must be transferred to the LightCycler® 96 Instrument. An experiment run can only be started on the instrument using the LightCycler® 96 Instrument Software. For detailed information on how to transfer an experiment to the instrument, see section Transferring the experiment to the instrument, on page 28.

4.1 Starting the run

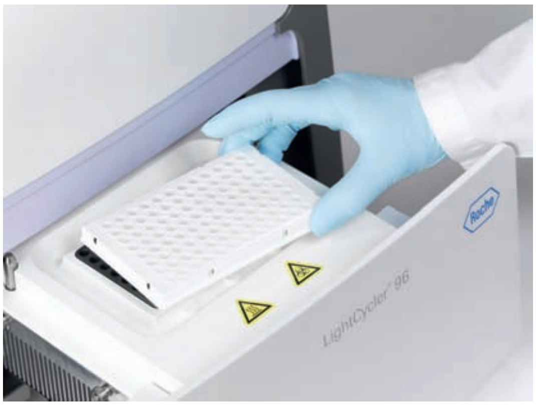
To start the experiment run

Before loading the multiwell plate into the LightCycler® 96 Instrument, it must be sealed with the self-adhesive sealing foil. Use the sealing foil applicator provided with the instrument for proper sealing.

Always centrifuge the filled and sealed plate before loading it into the instrument. For detailed information, see the corresponding section in the chapter Main applications, on page 55.

For detailed information on how to set up the samples, see the corresponding section in the chapter Main applications, on page 55. For comprehensive information, refer also to the "LightCycler® 96 System Operator's Guide", chapter "System description".

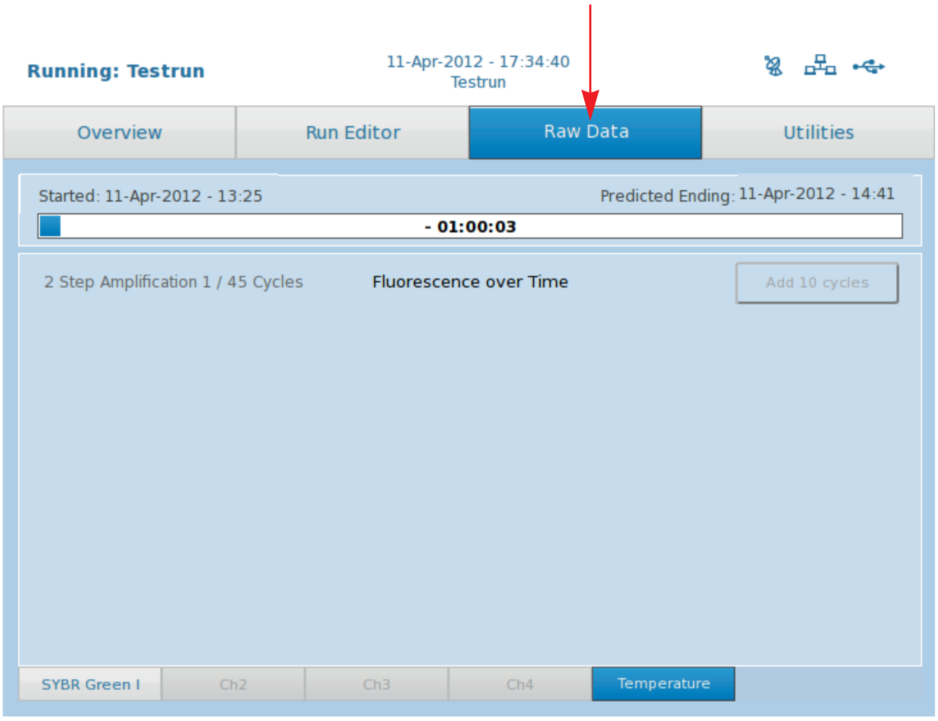
1 Load the LightCycler® 480 Multiwell Plate 96 with the samples into the LightCycler® 96 Instrument.



2 In the global action bar of the LightCycler® 96 Instrument Software main window, choose *Start*.

The 'Start' button is not enabled in the 'Standby' status of the instrument (block cycler cover switched off). To enable the 'Start' button, choose 'Heat Lid' in the global action bar. Reheating of the block cycler cover is initiated, which takes approximately 5 minutes. After heating the instrument changes to the 'Ready' status.

3 Choose the *Raw Data* tab to view the progress of the running experiment.



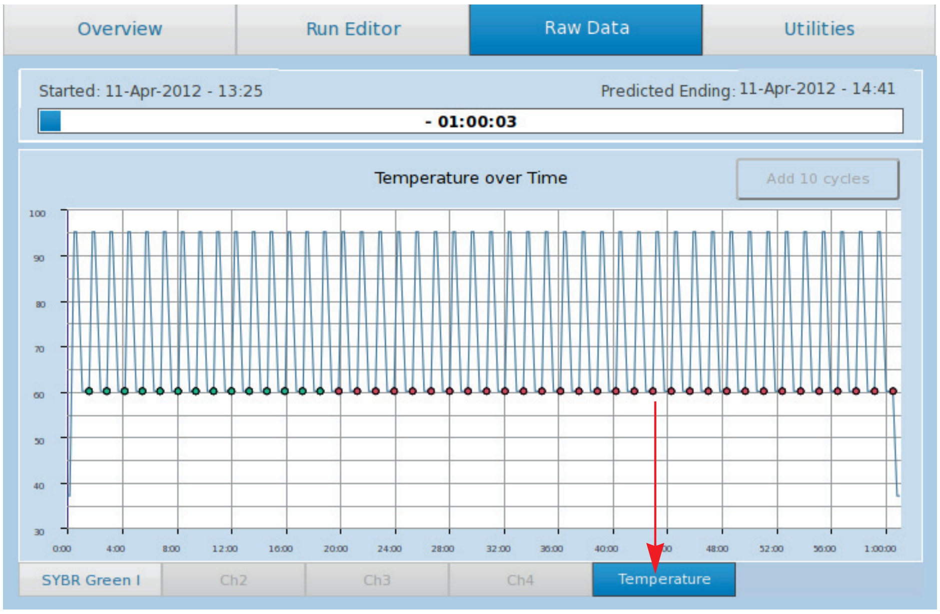
4.2 Monitoring the run

If the LightCycler® 96 Instrument and the computer running the LightCycler® 96 Application Software are not connected to a network, an experiment run can only be monitored on the instrument using the LightCycler® 96 Instrument Software.

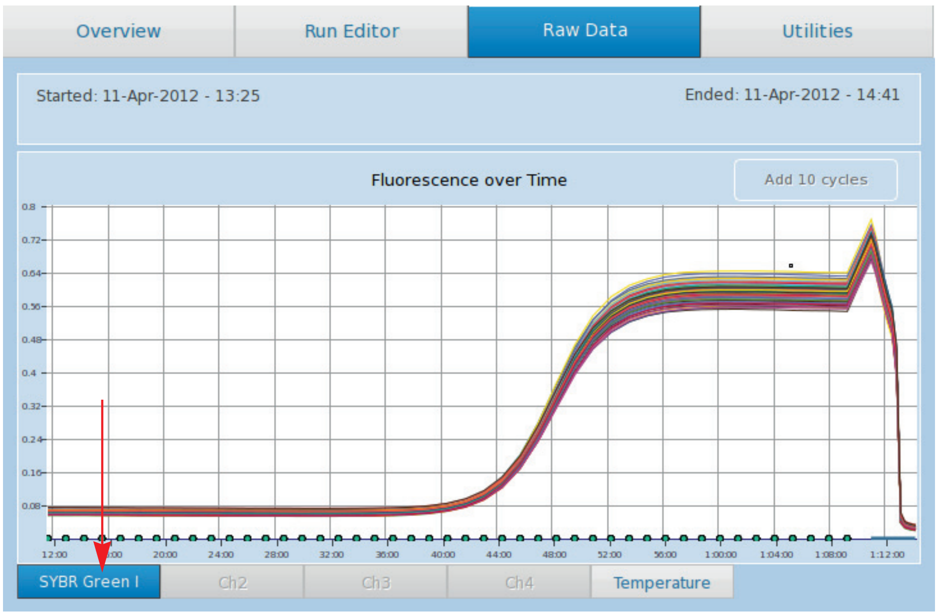
To monitor the experiment run

For a detailed description of the charts on the 'Raw Data' tab, refer to the "LightCycler® 96 System Operator's Guide", chapter "LightCycler® 96 Instrument Software".

1. On the *Raw Data* tab, choose the *Temperature* tab to monitor the summary of the programs selected for the experiment and their temperature and time settings in real time.



2. Choose one of the <*dye*> tabs to monitor the relevant fluorescence curves, that is, the fluorescence intensity against the time in hours, minutes, and seconds for the entire run in real time. There is one curve for each sample that has a gene labeled with the selected dye.



Run finished

The end of a run is indicated as follows:

- The status bar shows the instrument status *Ready*.
- On the *Overview* tab, the icon in the *Status* column changes to (Executed).
- The LightCycler® 96 Instrument unlocks the loading module.
- The experiment progress bar shows the end time.
- The *Raw Data* tab provides the final raw data.

5 Transferring the experiment from the instrument to the application software

After the experiment run, the raw data gathered by the instrument software must be transferred to the application software for analysis.

The LightCycler® 96 Instrument Software automatically saves all changes in an experiment file. The experiment file is saved according to its original location:

- On the LightCycler® 96 Instrument.
- On the USB drive.
- On both media if the operator has synchronized the storage locations.
  For detailed information on synchronizing, refer to the help browser of the LightCycler® 96 Instrument Software.

To transfer an experiment file including the raw data from the instrument to the application software:

- When the run is finished, save the experiment file including the raw data to a USB drive, see section *To save the experiment raw data to the USB drive*, below.
- Transfer the stored data to a computer on which the LightCycler® 96 Application Software is installed. Open the experiment in the application software for data analysis, see section *To transfer the stored data to the application software*, on page 47.

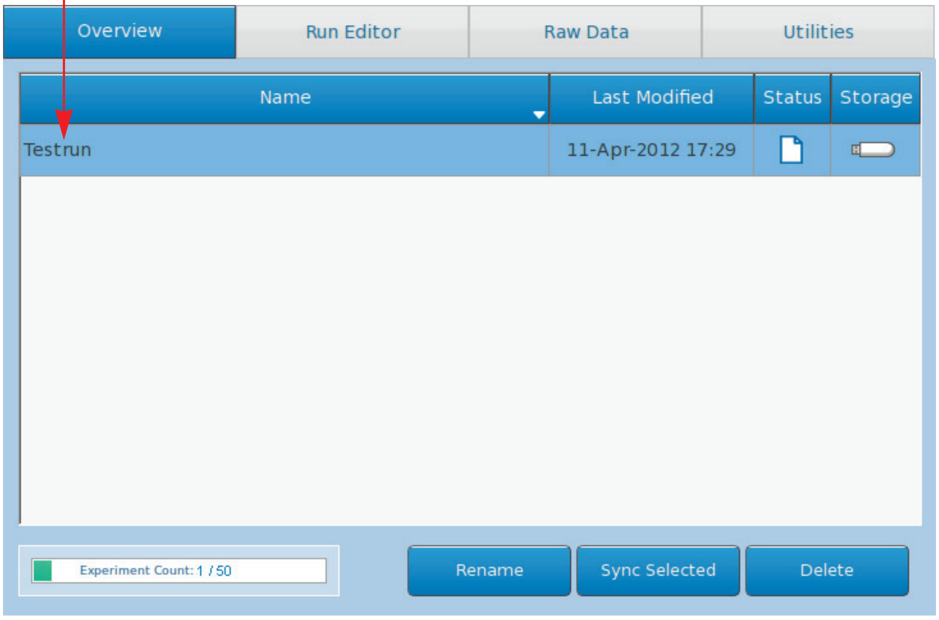
To save the experiment raw data to the USB drive

This procedure is optional. You only have to save the raw data to the USB drive if it is only saved on the instrument.

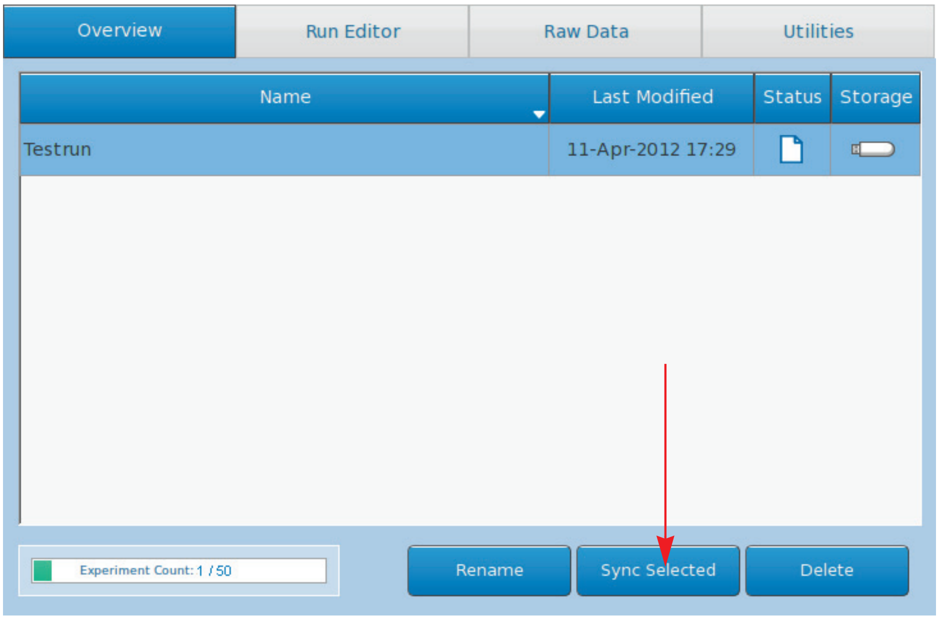
1. Insert a USB drive into the USB interface on the right side of the instrument.

2. Wait until the USB icon is displayed in the status bar of the instrument software.

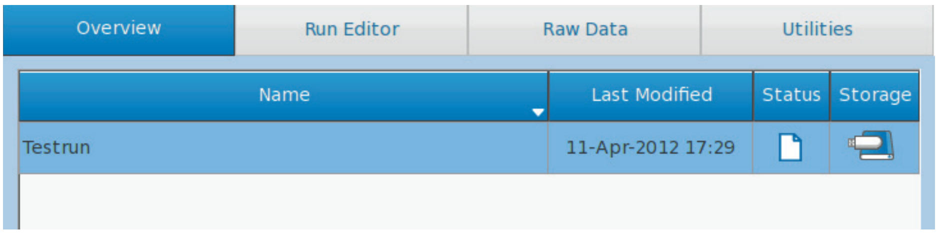
3 On the *Overview* tab, select the experiment you want to transfer to the application software.



4 Choose *Sync Selected* to store the experiment on the USB drive.



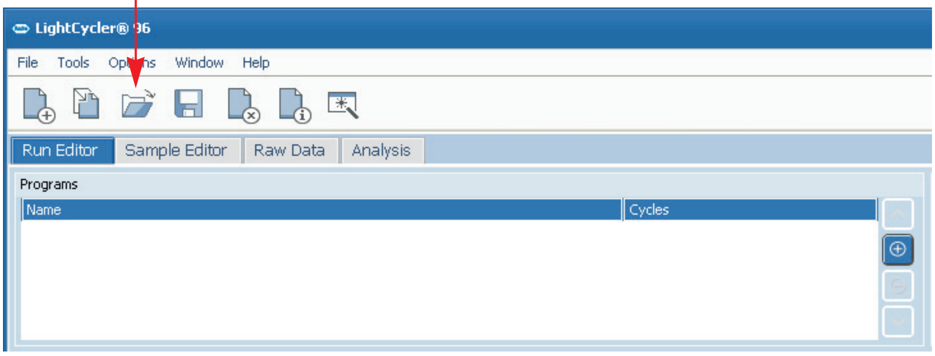
In the *Storage* column of the selected experiment, the *Synchronized* icon is shown.



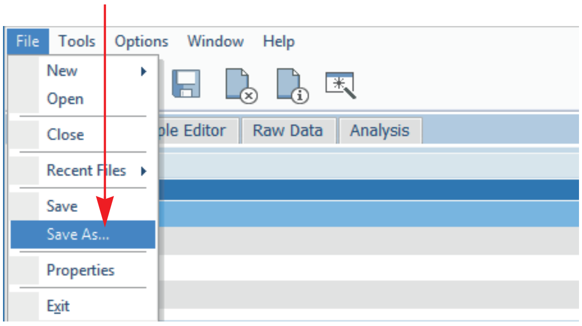
5 Remove the USB drive from the instrument.

To transfer the stored data to the application software

1. Insert the USB drive with the experiment file including the raw data into a USB interface on your computer running the LightCycler® 96 Application Software.
2. In the tool bar of the application software main window, choose the *Open Experiment* icon.



The *Open* dialog box for choosing an experiment opens.
3. Navigate to the USB drive and select the experiment file.
4. Choose *Open*. The experiment opens in the main window.
5. In the menu bar, choose *File > Save As* to store the data to a specified location on your computer.



The *Save As* dialog box opens.
6. Navigate to the directory where you want to store the experiment file.

   By default, the default experiment directory is displayed. For detailed information, refer to the "LightCycler® 96 System Operator's Guide", chapter "LightCycler® 96 Application Software".
7. Choose *Save*. The dialog box closes and the experiment is saved as a LightCycler® 96 file (.lc96).

6 Editing the sample list

For each experiment, you must edit the samples, that is, create, edit, delete, and rearrange samples and genes present in the wells, as well as the dyes used to label each gene. This sample data is then used to perform the analysis.

For editing the sample list, the experiment must be opened in the LightCycler® 96Application Software. You can edit the sample list before or after the experiment run, depending on your preferred routine.

For editing the sample list, the 'Sample Editor' requires information about the selected dye(s). When you edit the sample list before the experiment run (before transferring the experiment to the instrument for running), make sure that you have defined the run profile and selected the detection format in the 'Run Editor'.

This section provides step-by-step information on how to edit the sample list in general. For detailed information on further analysis-specific sample definitions for the different main applications, see the corresponding section in the chapter *Main applications*, on page 55.

For a comprehensive description of all options of the LightCycler® 96 Application Software, refer to the *LightCycler® 96 System Operator's Guide,* chapter "LightCycler® 96 Application Software".

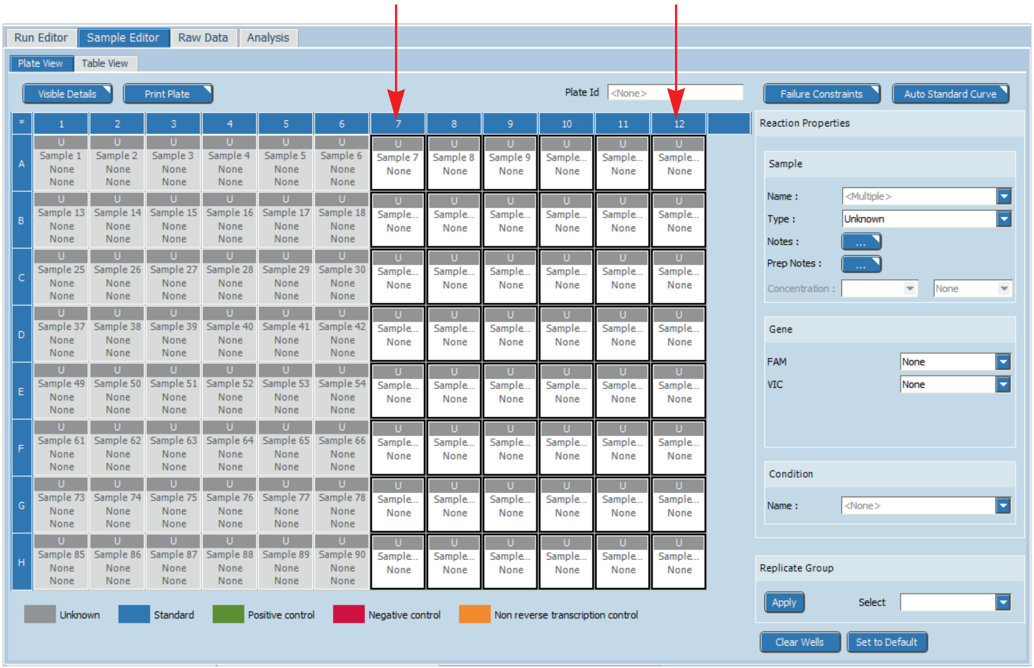
To edit the sample list:

- Clear the empty wells to display only the applicable data, see section *To clear empty wells*, on page 49.
- Specify the sample names and types, see section *To edit the samples*, on page 51.

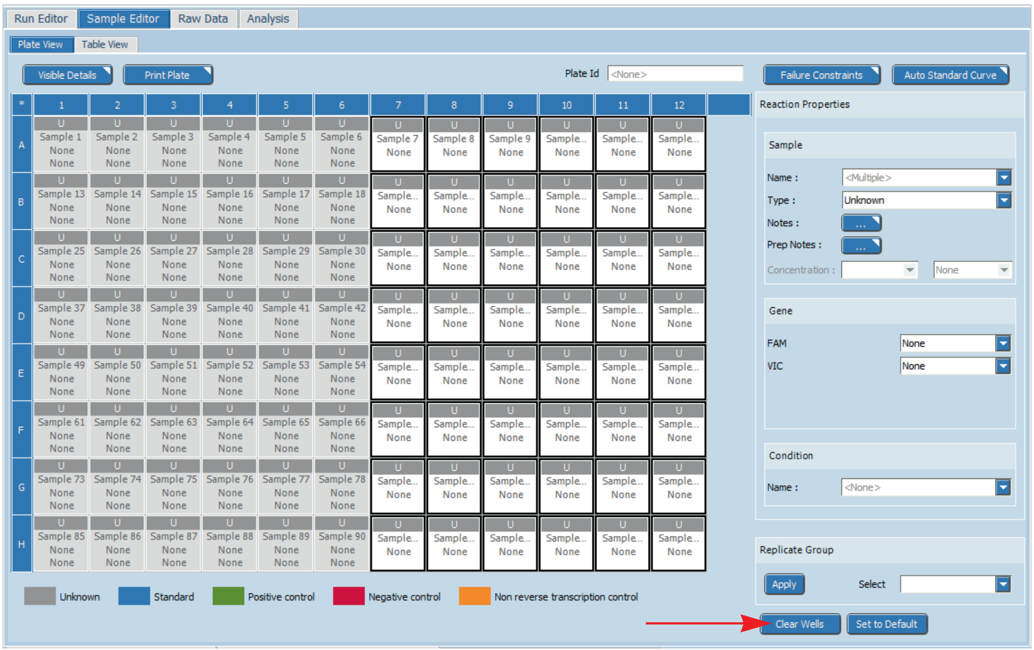
To clear empty wells

1. Open the *Sample Editor* tab.
2. Open the *Plate View*.
3. In the multiwell plate image, choose the wells you want to clear, for example, columns 7 to 12:
   - Choose table header *7* to start the selection.
   - Press and hold down the [Shift] key on your keyboard.
   - Choose table header *12* to finish the selection.

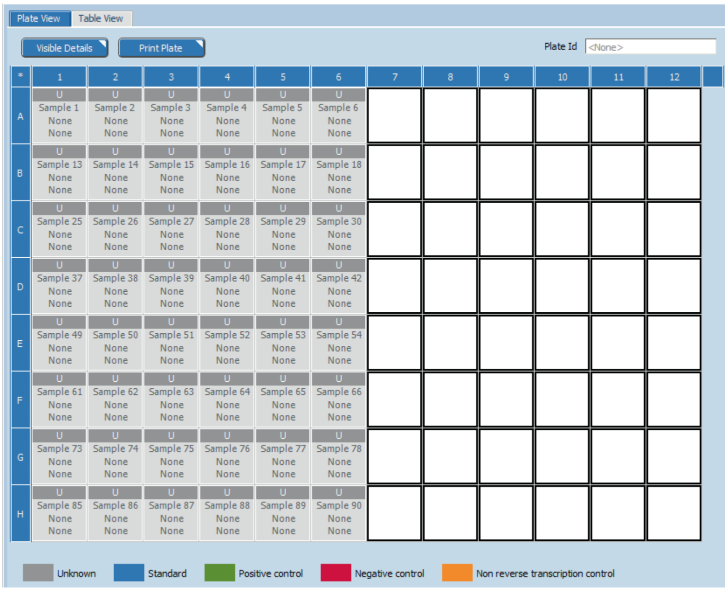
   All samples in the columns 7 to 12 are selected.



4 Choose *Clear Wells*.



All property values are removed from the selected wells and the wells are deactivated. This means they can no longer be edited and are not displayed in the table view or the analysis windows.



5 Repeat steps 3 and 4 for one or more rows of the multiwell plate image if necessary.

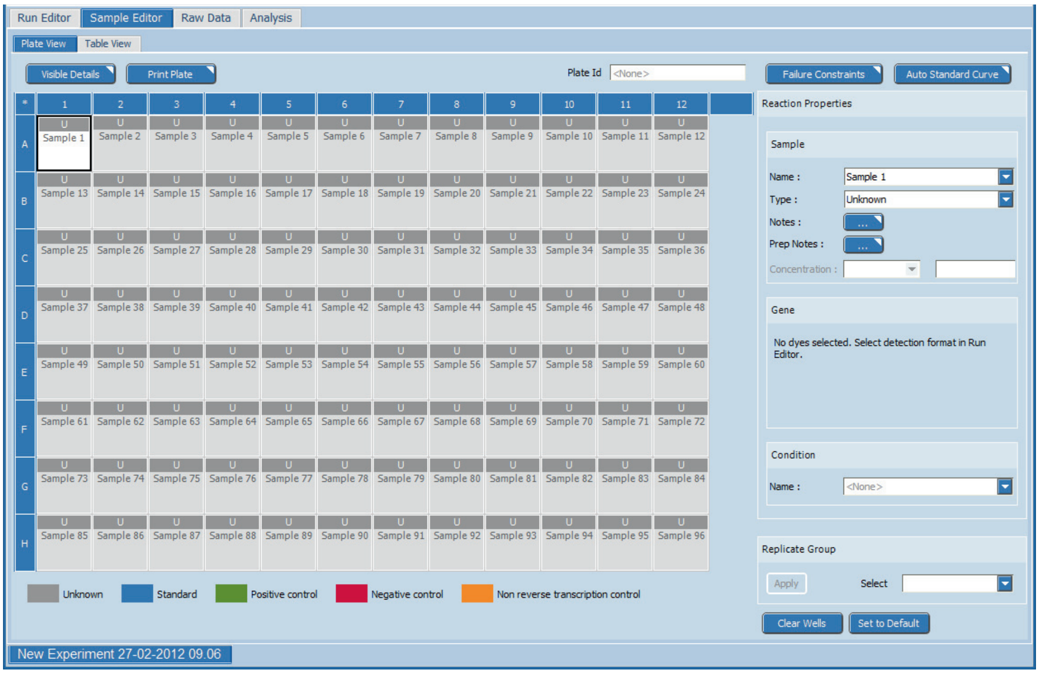
To edit the samples

The LightCycler® 96 Application Software offers two different views for editing the samples: the plate view and the table view. This user training guide describes how to edit the samples using the plate view, which shows the samples in 96 wells laid out to match the physical instrument. Changes in the plate view are immediately displayed in the table view and vice versa.

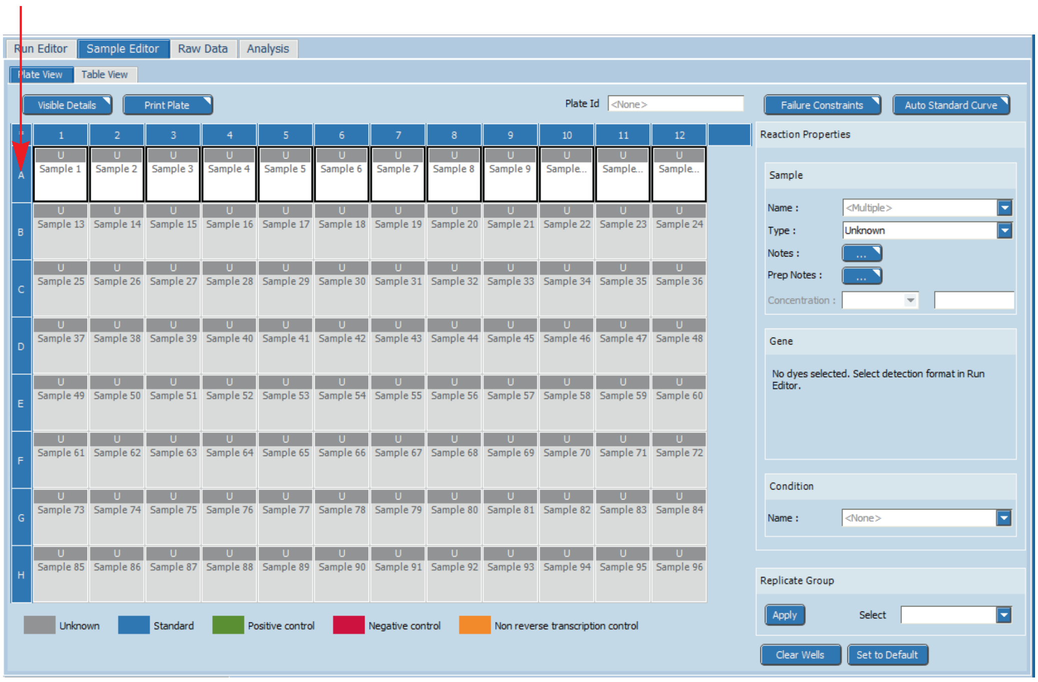
1. Open the *Sample Editor* tab.

   For a new experiment, the *Plate View* tab shows a schematic of the multiwell plate mount, that is, the multiwell plate image, with the following data for each well:

   - The default sample names *Sample 1* to *Sample 96*.
   - The sample type *Unknown* for all samples.



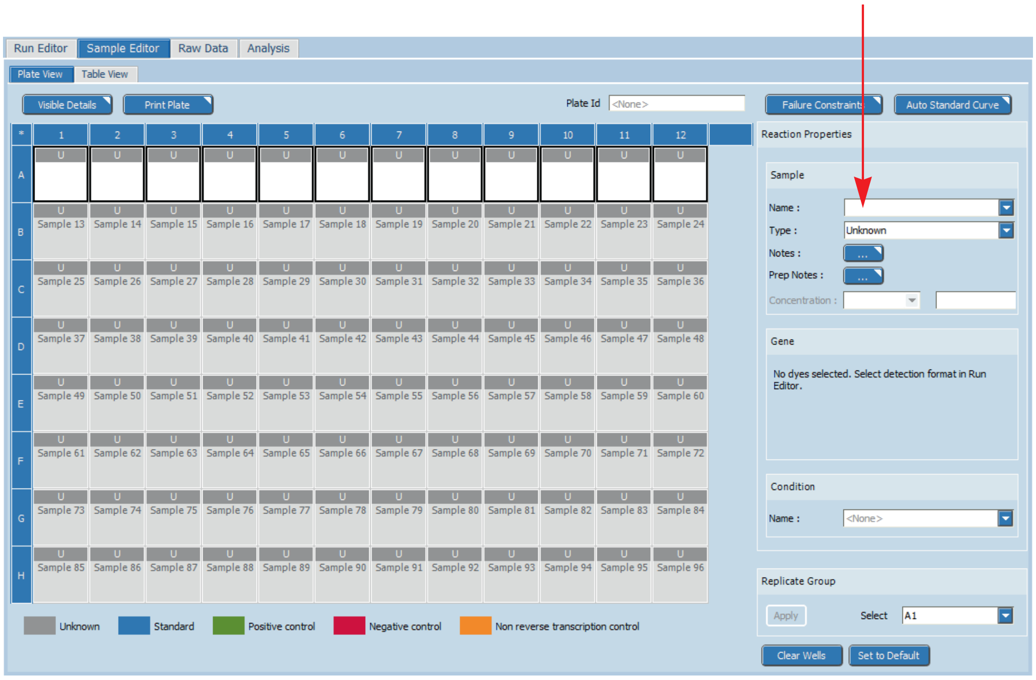
2. In the multiwell plate image, select a well or a range of wells to edit the corresponding sample-specific properties.



B

3 In the *Reaction Properties* window area, edit the sample name for the selected well(s):

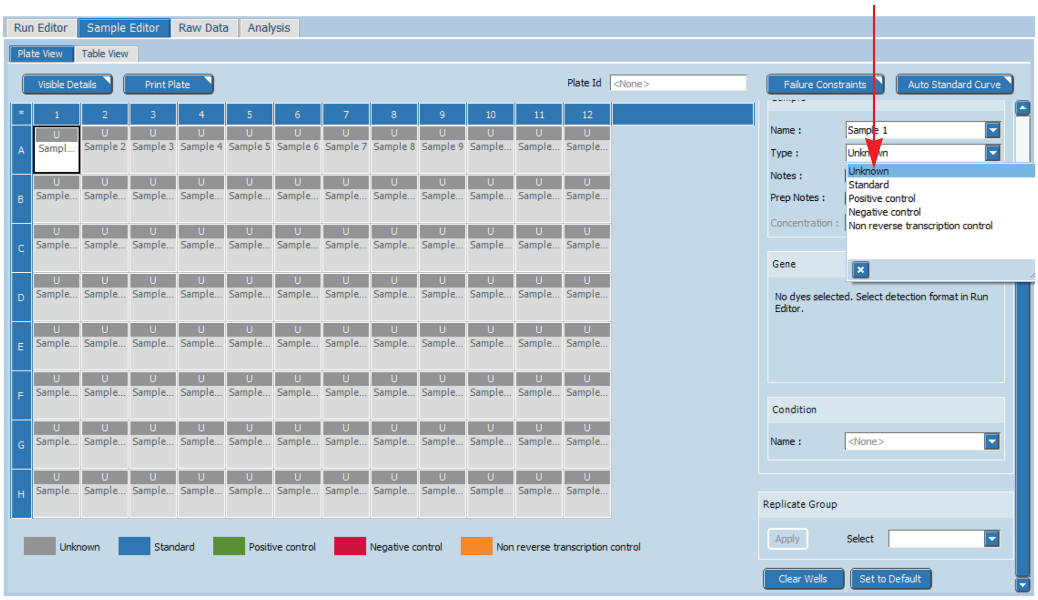
- Choose the *Name* list.
- Select the relevant name from the list or type in the name.



Ensure that the sample assignment on the 'Sample Editor' tab matches the pipetting scheme on the multiwell plate.

4 In the *Type* list, choose the applicable sample type for the selected well(s).

In the multiwell plate image, each well is colored to visualize the sample type.



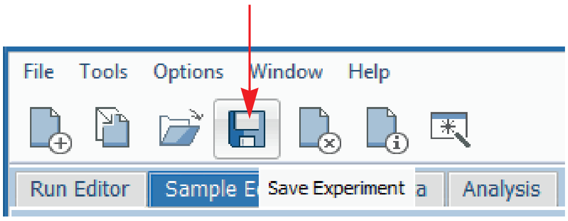
For detailed information on the applicable sample type for the samples in your specific experiment, see the corresponding section in the chapter Main applications, on page 55.
For detailed information on all available sample types, refer to the "LightCycler® 96 System Operator's Guide", chapter B, section "Sample Editor tab".

5 Repeat steps 2 to 4 to edit the corresponding sample-specific properties for another well or range of wells according to the relevant pipetting scheme. See the corresponding section in the chapter *Main applications*, on page 55.

The *Plate View* tab now displays the assigned samples.

To save the experiment

1. In the tool bar, choose the *Save Experiment* icon to save the experiment.



B

Main applications

For information on the order in which you must perform the individual work steps of a complete application workflow, see section Overview, on page 13.

This chapter shows how to perform experiments with the LightCycler® 96 System using the following examples:

- *Absolute quantification*, on page 56.
- *Endpoint SNP genotyping*, on page 74
- *Relative quantification*, on page 94.
- *Tm calling*, on page 116.

For all experiments a LightCycler® 480 Multiwell Plate 96, white, is used.

For each application, this chapter provides the following information:

- How to set up the nucleic acid samples.
- The applicable experiment run parameters relating to the temperature profile, detection format, and reaction volume.
- The applicable basic sample data parameters relating to the empty wells, the sample names, and the sample types.
- How to edit the special sample data settings.
- How to analyze the results.

Preconditions for all runs

A run can only be performed if:

- The instrument is started; see section *Starting the LightCycler® 96 Instrument*, on page 15.
- The experiment definition is transferred to the instrument if it was defined with the application software.

For a detailed description of the LightCycler® 96 Application Software and all elements of the graphical user interface, refer to the "LightCycler® 96 System Operator's Guide", chapter "LightCycler® 96 Application Software".

For a detailed description of the LightCycler® 96 Instrument Software and all elements of the graphical user interface, refer to the "LightCycler® 96 System Operator's Guide", chapter "LightCycler® 96 Instrument Software".

C

C

1 Absolute quantification

Absolute quantification is used to quantify a gene and express the final result as an absolute value (for example, copies/ml). Samples with an unknown quantity of gene are amplified alongside a dilution series of a gene-specific standard with known concentration. To obtain an absolute value for an unknown quantity of gene, the Cq of an unknown sample is compared to those of standards with known quantities.

In an absolute quantification analysis, the known concentration of each standard is automatically plotted against the measured Cq values. The resulting regression line is called the standard curve and shows the correlation between Cq and quantity. The concentration of an unknown sample is calculated by comparing its Cq with the standard curve.

For detailed information on absolute quantification analysis, refer to the LightCycler® 96 System Operator's Guide, chapter A, section "Analysis principles".

1.1 Experiment overview

The following example describes how to set up, run, and analyze an assay for gene quantification using a FAM-labeled hydrolysis probe. Quantification of samples is based on a 5-point standard curve derived from a plasmid standard dilution series covering a range of known quantities.

The assay is performed using a LightCycler® 480 Multiwell Plate 96, white. Each sample is set up in triplicate.

Samples	▶ Standard samples: Human Genomic DNA from human blood 0.1 ng to 1000 ng ▶ 5 DNA samples (unknown concentration)
Reagents	▶ FastStart Essential DNA Probes Master (2 x conc.) ▶ RealTime ready Catalog Assays Assay ID: 137341 (HSPA2) Configuration No.: 100 030 767

1.2 Setting up the samples

Sample dilution

The standard sample is diluted to a 10-fold dilution series covering a range of 0.1 to 1000 ng/5 µl.

Continuously cool the samples during setup by keeping the reaction tubes on ice.

Controls

To ensure the absence of contaminating nucleic acids in PCR reagents, it is highly recommended that you include a no template control (NTC) in your experiment.

PCR mix

When setting up the PCR mix, compensate for pipetting losses. We recommend preparing PCR mixes with 10% extra volume.

The table below shows the components included in the PCR mix for one 20 µl reaction. The PCR mix volume is 15 µl for a subsequent sample input of 5 µl/reaction.

Component	Concentration	Volume	Final conc.
Water, PCR grade		4 µl	
FastStart Essential DNA Probes Master	2 x conc.	10 µl	1 x conc.
RealTime ready Catalog Assays (HSPA2)	20 x conc.	1 µl	1 x conc.
Total volume (without sample DNA)		**15 µl**	

Pipetting scheme

1. Pipette 15 µl of the PCR mix into 33 wells of the multiwell plate according to the following scheme.
2. Pipette 5 µl of standard dilutions into the corresponding wells according to the following scheme (each in triplicate).
3. Pipette 5 µl of sample into the corresponding wells according to the following scheme (each in triplicate).
4. For the NTCs, pipette 5 µl of water (instead of DNA sample) into the corresponding wells according to the following scheme.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std1	Std2	Std3	Std4	Std5	NTC						
B	Std1	Std2	Std3	Std4	Std5	NTC						
C	Std1	Std2	Std3	Std4	Std5	NTC						
D	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5							
E	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5							
F	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5							
G												
H												

Centrifugation

1. Seal the multiwell plate with the LightCycler® 480 Sealing Foil using the sealing foil applicator (provided with the system package).
2. Centrifuge the multiwell plate at 1500 x *g* for 2 minutes in a standard swing-bucket centrifuge, using a rotor for multiwell plates.

   Make sure you balance the multiwell plate with a suitable counterweight (for example, another multiwell plate).

1.3 Experiment run parameters

For detailed information on how to program an experiment, see one of the following sections:
Programming the experiment with the LightCycler® 96 Application Software, on page 18.
Programming the experiment with the LightCycler® 96 Instrument Software, on page 30.

Use a standard PCR profile for hydrolysis probes.

The experiment includes the run parameters listed in the following tables.

Temperature profile

For detailed information on how to program a temperature profile, see one of the following sections:
For working with the LightCycler® 96 Application Software: Creating the temperature profile, on page 21.
For working with the LightCycler® 96 Instrument Software: Creating the temperature profile, on page 33.

For this example, use the following heating and cooling cycles:

Programs		Steps			
Name	**Number of cycles**	**Ramp (°C/s)**	**Duration (s)**	**Target (°C)**	**Acquisition Mode**
Preincubation	1	4.4	600	95	None
3-step amplification	45	4.4	10	95	None
		2.2	30	60	None
		4.4	1	72	Single
Cooling	1	2.2	30	37	None

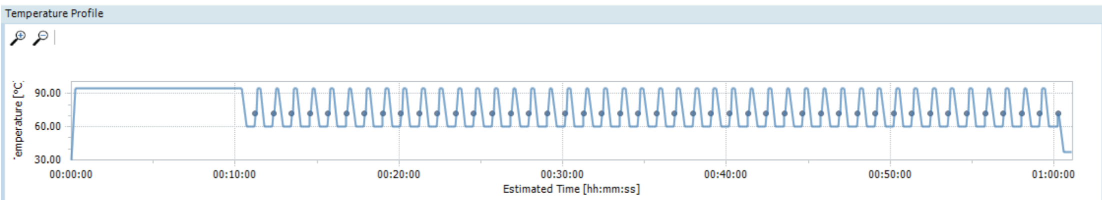
It is not necessary to add a separate cooling program at the end of the run. At the end of each run, the samples are automatically cooled to +37°C.

For the ramp rate for heating and cooling (*Ramp (°C/s)*), the default values are used in this example.

For the steps of the amplification program, the following default settings are used in this example:

- LightCycler® 96 Application Software: For *Gradient* and *Touch down*, the default settings are used.
- LightCycler® 96 Instrument Software: For *Mode*, the default setting *Standard* is used.

In the *Temperature Profile* window area, the following graphical summary of the programs selected for the experiment and their temperature and time settings is displayed.



Detection format

Selecting the dye for this mono-color experiment determines the channel combination for the measurement during the run. For all other parameters, the default values are used in this example.

For detailed information on how to specify the detection format, see the following sections:
For working with the LightCycler® 96 Application Software: To specify the detection format for the experiment, on page 25.
For working with the LightCycler® 96 Instrument Software: To specify the detection format for the experiment, on page 38.

For this example, use the following channel:

Dye	Channel
FAM	470/514

Reaction volume

As the LightCycler® 96 Instrument does not validate the reaction volume, ensure that the specified reaction volume matches the volume pipetted into the wells of the multiwell plate.

For detailed information on how to specify the reaction volume, see one of the following sections:
For working with the LightCycler® 96 Application Software: To specify the reaction volume for the experiment, on page 25.
For working with the LightCycler® 96 Instrument Software: To specify the reaction volume for the experiment, on page 40.

For this example, use the following reaction volume:

Reaction volume
20 µl

Experiment run

Once you have set up the samples and defined the experiment run parameters,you can start the run. For detailed information on how to run the experiment, see section *Running the experiment*, on page 41.

1.4 Editing the sample data

For editing the sample data, the experiment must be opened in the LightCycler® 96 Application Software. You can edit the sample list before or after the run, depending on your preferred routine.

The LightCycler® 96 Application Software offers two different views for editing the samples: the plate view and the table view. This user training guide describes how to edit the samples using the plate view, which shows the samples in 96 wells laid out to match the physical instrument. Changes in the plate view are immediately displayed in the table view and vice versa.

For detailed information on the 'Sample Editor' tab, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "Sample Editor tab".

To edit the sample list:

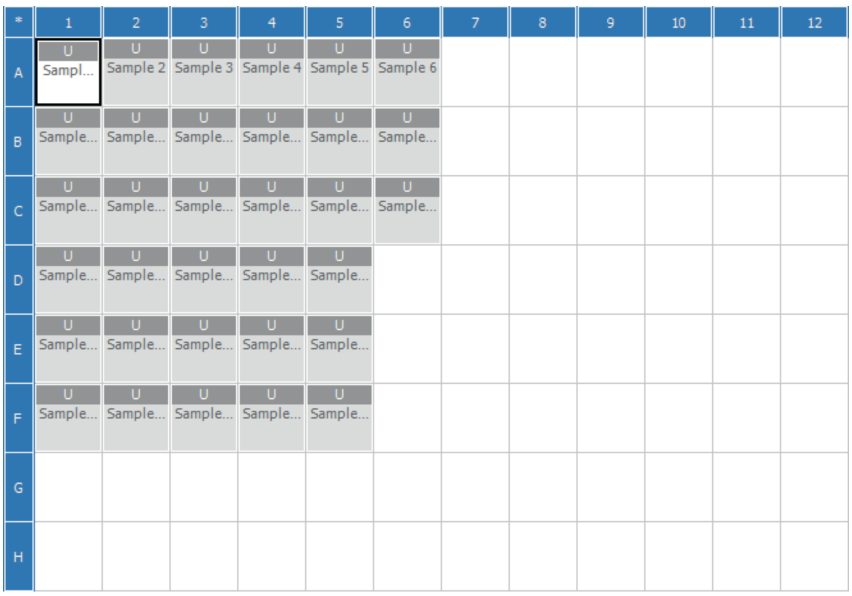
- Clear empty wells to eliminate them from the analysis, see section *Empty wells* below.
- Edit the sample names, see section *Sample names*, on page 61.
- Edit the sample types, see section *Sample types*, on page 62.
- Assign a gene for the dye, see section *To assign a gene to the dye*, on page 62.
- Specify the concentration values for the applicable standard quantity of the gene, see section *To define the concentration values*, on page 63.
- Check if the replicate groups are correctly assigned to all samples, see section *Replicate groups*, on page 65.

Empty wells

For detailed information on how to clear empty wells, see section To clear empty wells, on page 49.

For this example, clear the following wells (see also the multiwell plate image below):

- Columns *7* to *12*
- Rows *G* and *H*
- Wells *D6*, *E6*, *F6*

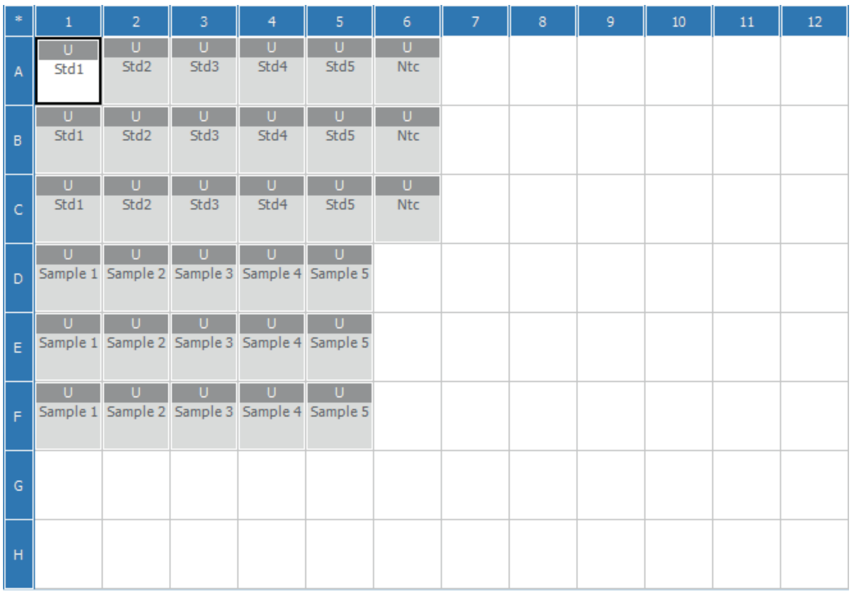


Sample names

For detailed information on how to edit the sample names, see section To edit the samples, on page 51.

For this example, the following sample names apply (see also the multiwell plate image below):

Name	Samples in the plate view
Std1	For the unknown samples in wells A1, B1, and C1
Std2	For the unknown samples in wells A2, B2, and C2
Std3	For the unknown samples in wells A3, B3, and C3
Std4	For the unknown samples in wells A4, B4, and C4
Std5	For the unknown samples in wells A5, B5, and C5
Sample1	For the unknown samples in wells D1, E1, and F1
Sample2	For the unknown samples in wells D2, E2, and F2
Sample3	For the unknown samples in wells D3, E3, and F3
Sample4	For the unknown samples in wells D4, E4, and F4
Sample5	For the unknown samples in wells D5, E5, and F5
Ntc	For the negative control in wells A6, B6, and C6



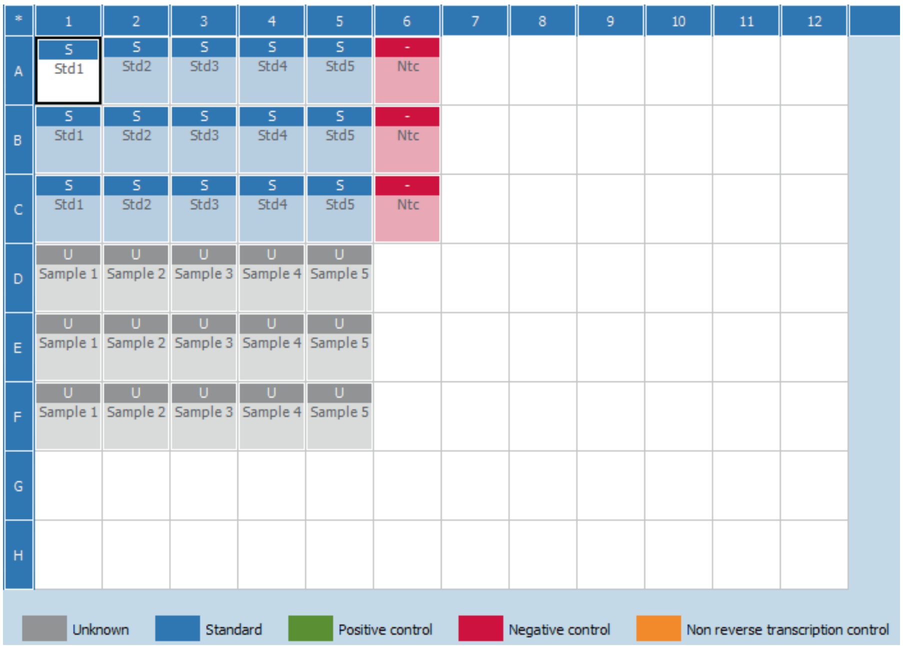
C

Sample types

For detailed information on how to edit the sample types, see section To edit the samples, on page 51.

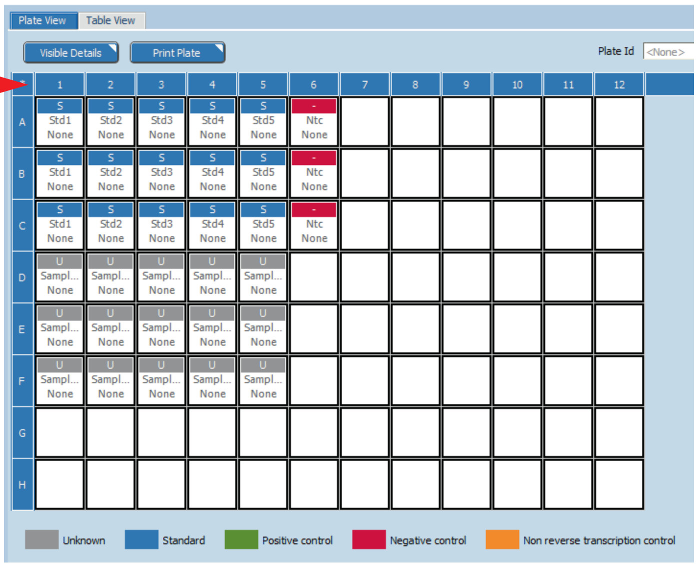
In this example the following sample types apply:

Type	Samples in the plate view
Standard	For the samples *Std1* to *Std5*
Unknown (default)	For the samples *Sample1* to *Sample5*
Negative control	For the samples *Ntc* (NTC)



To assign a gene to the dye

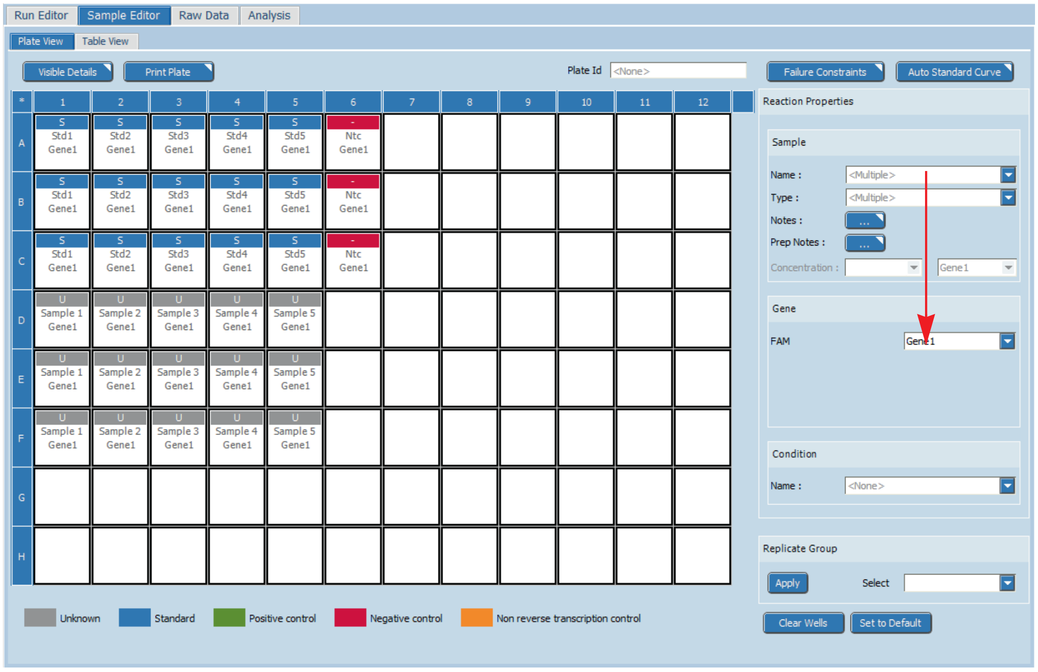
1. In the multiwell plate image, choose the asterisk (*) in the upper left corner to select all the wells.



2 In the *Reaction Properties* window area, choose the text field next to the *FAM* dye.

3 Type in *Gene 1*.

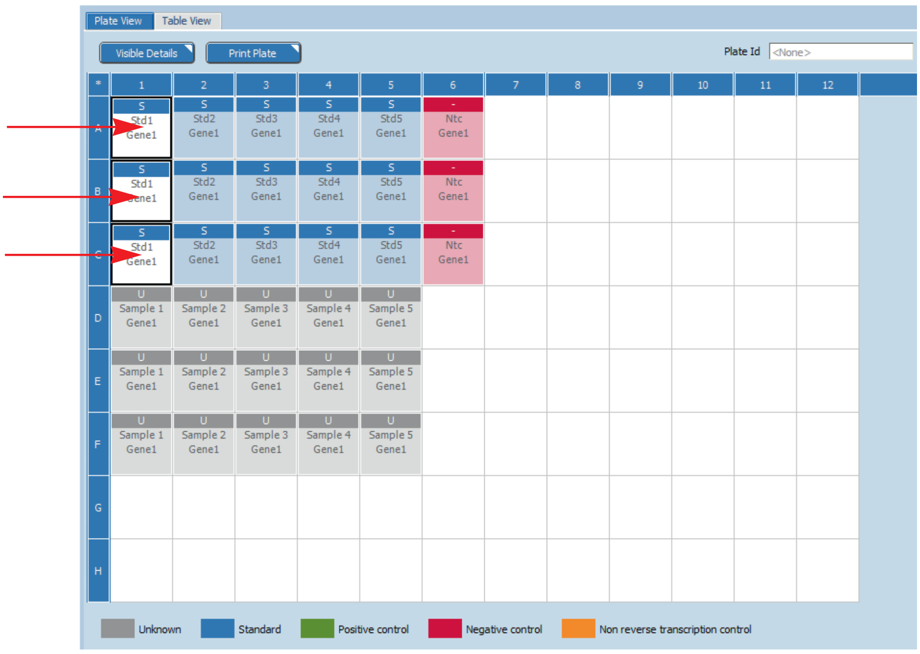
The gene *Gene 1* is assigned to all selected samples.



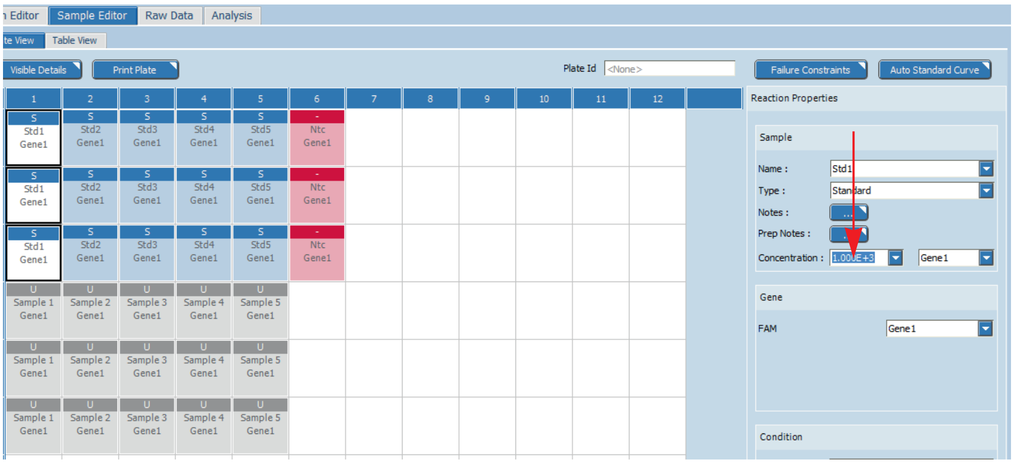
To define the concentration values

Samples of the sample type *Standard* have a known quantity of a specific gene. By comparing the Cq values of unknown samples of the same gene to the Cq values of these known standard quantities, the unknown quantities can be estimated. When specifying samples as standards, each gene in the reaction needs to be assigned a *Concentration* value.

1 In the multiwell plate image, select the samples *Std1* (positions A1, B1, C1).

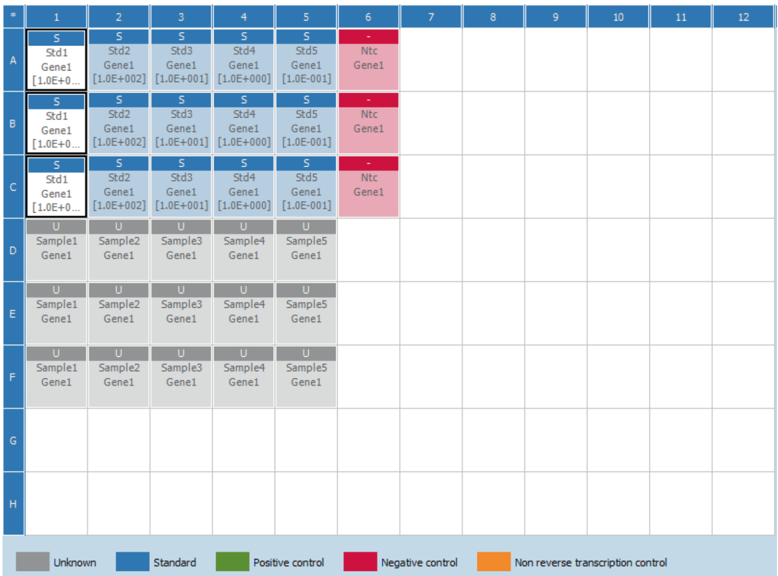


2 In the *Concentration* field, type in *1000*. The concentration 1000 is assigned to the selected samples. The multiwell plate image, the corresponding tooltips, and the *Concentration* field display *[1.000 E+3]*.



3 Repeat steps 1 and 2 for the following samples to define the corresponding concentrations listed below:

- Samples *Std2* (positions A2, B2, C2: concentration value *100* (*[1.000 E+2]* is displayed.)
- Samples *Std3* (positions A3, B3, C3: concentration value *10* (*[1.000 E+1]* is displayed.)
- Samples *Std4* (positions A4, B4, C4): concentration value *1* (*[1.000 E+0]* is displayed.)
- Samples *Std5* (positions A5, B5, C5): concentration value *0.1* (*[1.000 E-1]* is displayed.)



Replicate groups

The LightCycler® 96 Application Software automatically groups samples into replicate groups, provided they have identical values for the following properties:

- Sample name
- Sample type
- Concentration
- Gene name

Each replicate group is named according to the top leftmost of the grouped samples.

Changing one of these properties removes the corresponding sample from the replicate group.

1. Check if the multiwell plate image displays the same replicate groups for samples with identical values.

1.5 Analyzing the results

For detailed information on the 'Analysis' tab, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "Absolute quantification".

For detailed information on working with tables and graphs, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "General software conventions".

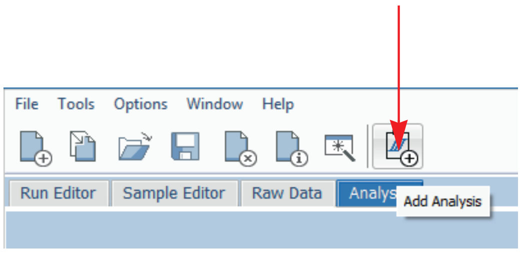
To analyze the calculated results of the absolute quantification application:

- Create the absolute quantification analysis, see section *Creating the analysis* below.
- Optional: Specify the settings for the absolute quantification analysis, see section *Analysis settings*, on page 68.
- On the different views of the *Abs Quant* tab, check the analysis results and customize the result data if necessary:
  - For the *Amplification Curves* view, see section *Amplification curves*, on page 69.
  - For the *Standard Curves* view, see section *Standard curve*, on page 69.
  - For the *Heat Map* view, see section *Heat map*, on page 70.
  - For the *Result Table* view, see section *Result table*, on page 70.

1.5.1 Creating the analysis

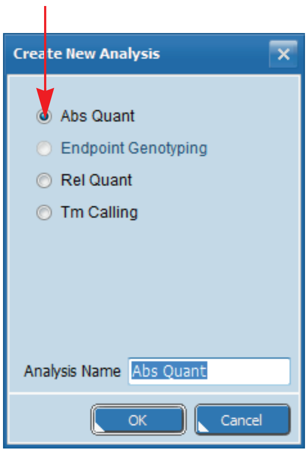
To create the Abs Quant analysis

1. Open the *Analysis* tab.
2. In the tool bar, choose the *Add Analysis* icon to add a new analysis.



The *Create New Analysis* dialog box opens.

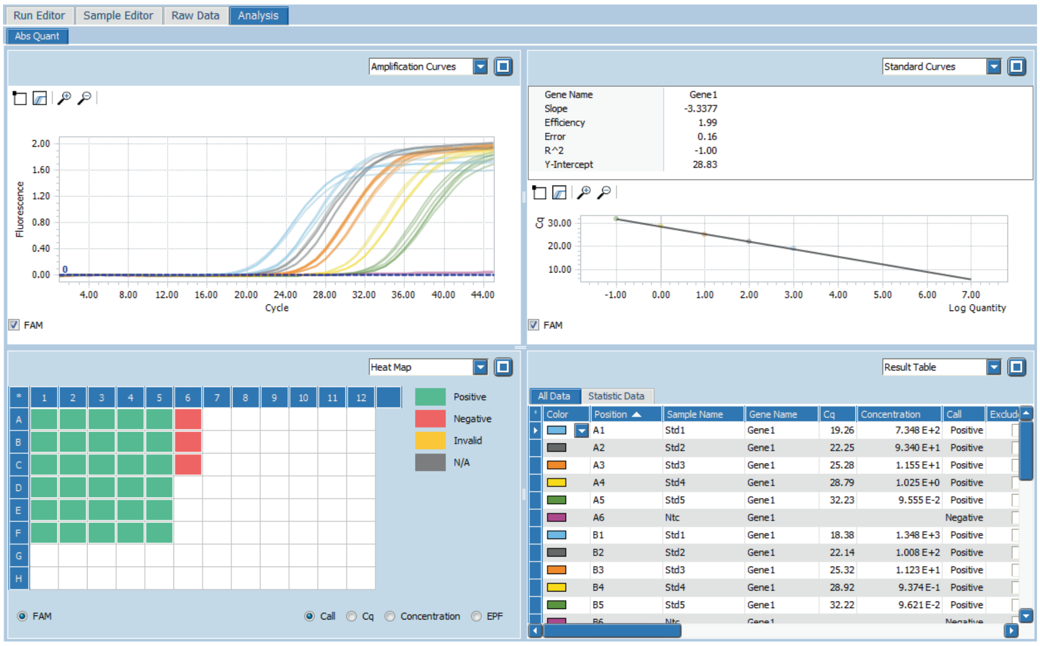
3. Choose *Abs Quant*.



4 Choose *OK*.

The *Analysis* tab displays four different views for the experiment using default values:

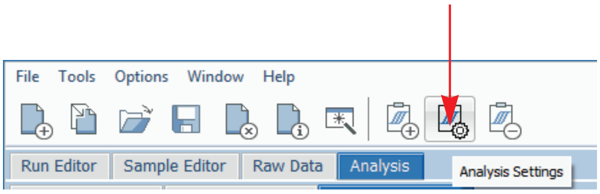
- *Amplification Curves*
- *Standard Curves*
- *Heat Map*
- *Result Table*



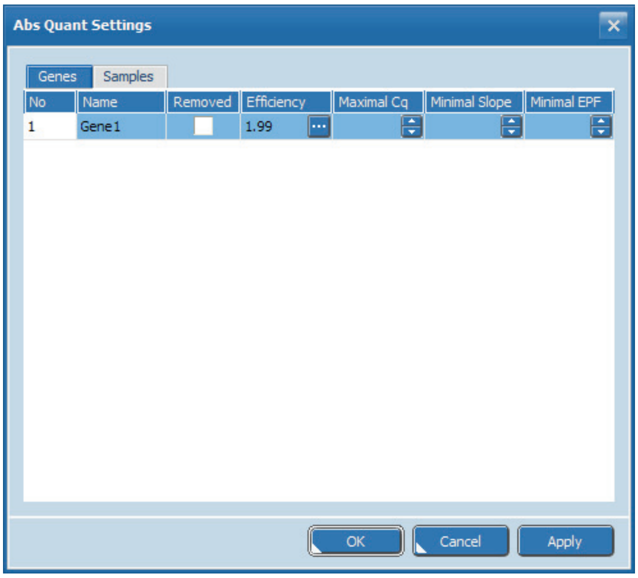
1.5.2 Analysis settings

Optional: To specify the analysis settings

1. In the tool bar, choose the *Analysis Settings* icon.



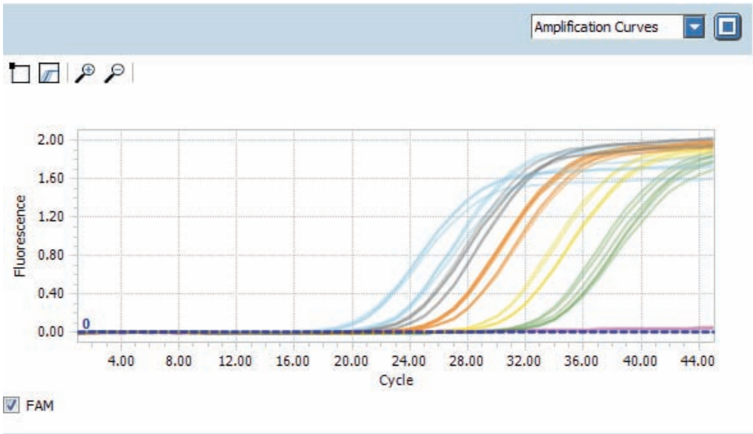
The *Abs Quant Settings* dialog box opens.



2. In the *Abs Quant Settings* dialog box, specify the analysis-specific settings, for example:
   - On the *Genes* tab, remove a gene from the analysis.
   - On the *Samples* tab, remove samples from the analysis.

1.5.3 Amplification curves

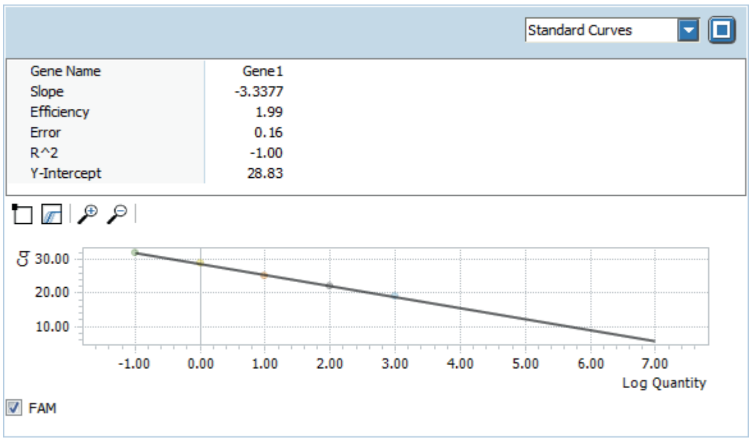
On the *Abs Quant* tab, amplification curves display the fluorescence intensity against the number of cycles in the amplification program. There is one curve for each sample that has a gene labeled with the selected dye.



1	Optional: For better distinction, color all curves for the standard samples in one color and the curves for the unknown samples in another. For detailed information on how to change the color of the samples, see section *To change the color of the samples*, on page 71.
2	Check the *Amplification Curves* chart for correct amplification.

1.5.4 Standard curve

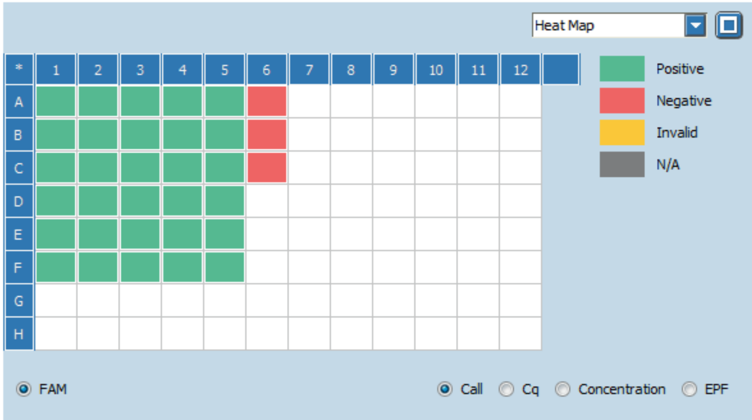
A standard curve displays a graph of Cq values against the base 10 logarithm of the quantity of each standard. For absolute quantification, the absolute values of the standard curve are used to assign quantities to unknown samples.



1.5.5 Heat map

The heat map shows an image of the multiwell plate used in the experiment for the specified channel (FAM).

A heat map only displays the samples contained in the sample list. Samples not contained in the sample list (that is, cleared wells and removed samples and genes) are displayed in white and samples excluded from calculation are displayed in gray.



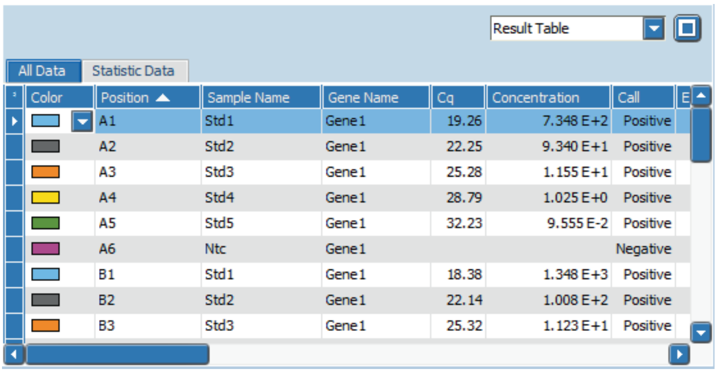
In this example the *Call* heat map is displayed. It shows the *Call* status of all samples contained in the sample list:

- The samples in columns 1 to 5 are green, that is, *Positive*.
- The NTCs in column 6 are red, that is, *Negative*.

1.5.6 Result table

The result table displays the calculated data results of the absolute quantification on two different tabs.

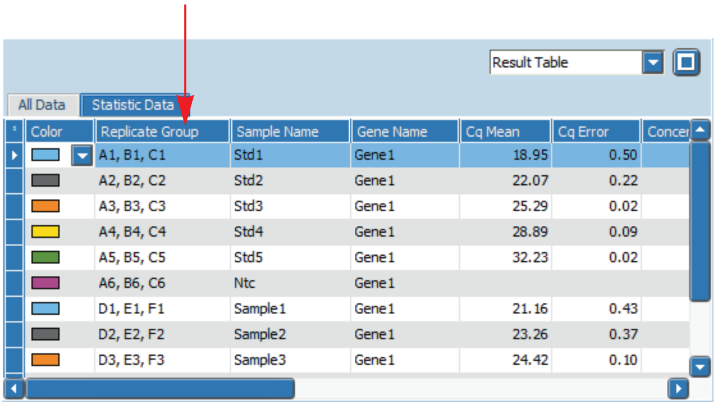
- On the *All Data* tab, all calculated data is displayed, for example, the calculated concentration of the gene present before amplification.


Result Table

All Data | Statistic Data

Color	Position	Sample Name	Gene Name	Cq	Concentration	Call
	A1	Std1	Gene1	19.26	7.348 E+2	Positive
	A2	Std2	Gene1	22.25	9.340 E+1	Positive
	A3	Std3	Gene1	25.28	1.155 E+1	Positive
	A4	Std4	Gene1	28.79	1.025 E+0	Positive
	A5	Std5	Gene1	32.23	9.555 E-2	Positive
	A6	Ntc	Gene1			Negative
	B1	Std1	Gene1	18.38	1.348 E+3	Positive
	B2	Std2	Gene1	22.14	1.008 E+2	Positive
	B3	Std3	Gene1	25.32	1.123 E+1	Positive

▶ The S*tatistic Data* tab summarizes all data for samples in replicate groups.


Result Table
All Data | Statistic Data

Color	Replicate Group	Sample Name	Gene Name	Cq Mean	Cq Error	Concer
	A1, B1, C1	Std1	Gene1	18.95	0.50	
	A2, B2, C2	Std2	Gene1	22.07	0.22	
	A3, B3, C3	Std3	Gene1	25.29	0.02	
	A4, B4, C4	Std4	Gene1	28.89	0.09	
	A5, B5, C5	Std5	Gene1	32.23	0.02	
	A6, B6, C6	Ntc	Gene1			
	D1, E1, F1	Sample1	Gene1	21.16	0.43	
	D2, E2, F2	Sample2	Gene1	23.26	0.37	
	D3, E3, F3	Sample3	Gene1	24.42	0.10	

For detailed information on all calculated results displayed in the 'Result Table' view, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "Absolute quantification".

1. In the *Result Table* view, check if the results show the expected dynamic range, for example, if all concentrations are positive over the complete range.

2. Optional: For better distinction, color all curves for the standard samples in one color and the curves for the unknown samples in another.
For detailed information on how to change the color of the samples, see section *To change the color of the samples*, below.

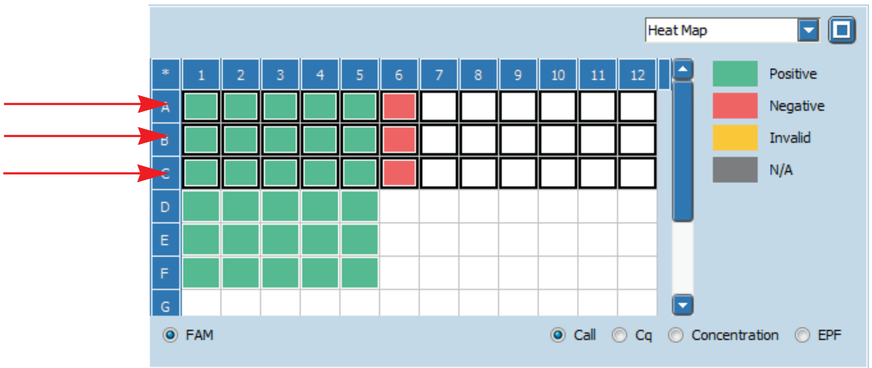
C

To change the color of the samples

For better distinction, color all curves for the standard samples in one color and the curves for the unknown samples in another.

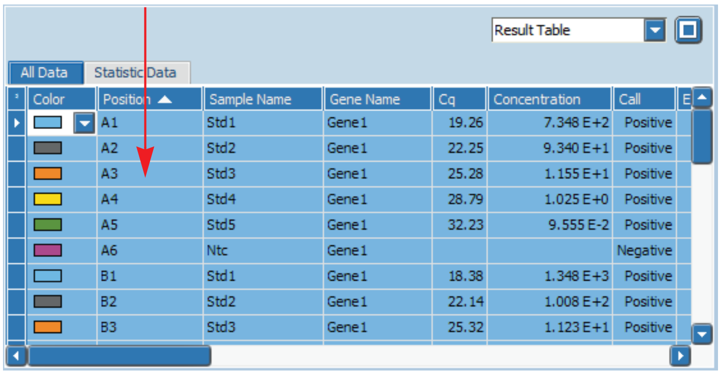
The color settings in the 'Result Table' view correspond to the color settings in the 'Amplification Curves' view.

1. In the *Heat Map* view, select rows A to C to select all standard samples (*Std* and *Ntc*).
The selected standard samples are highlighted in the heat map and in the result table (*All Data* tab).



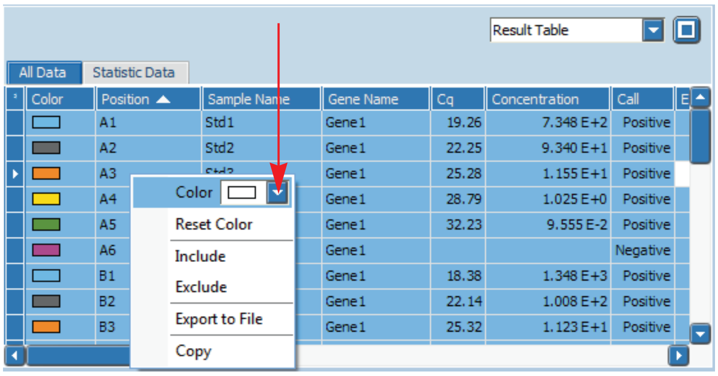
▶ ▶ ▶

2 In the *Result Table* view, right-click the selected samples.



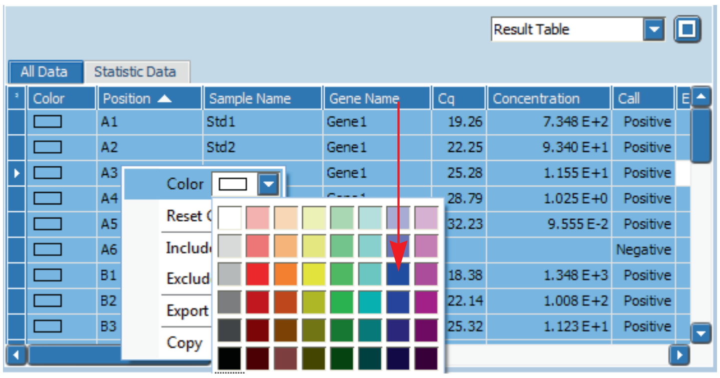
The corresponding shortcut menu opens.

3 On the shortcut menu, choose the down arrow next to *Color*.



The color selection dialog box opens.

4 Choose a color field to assign one color to all standard samples, for example, blue.

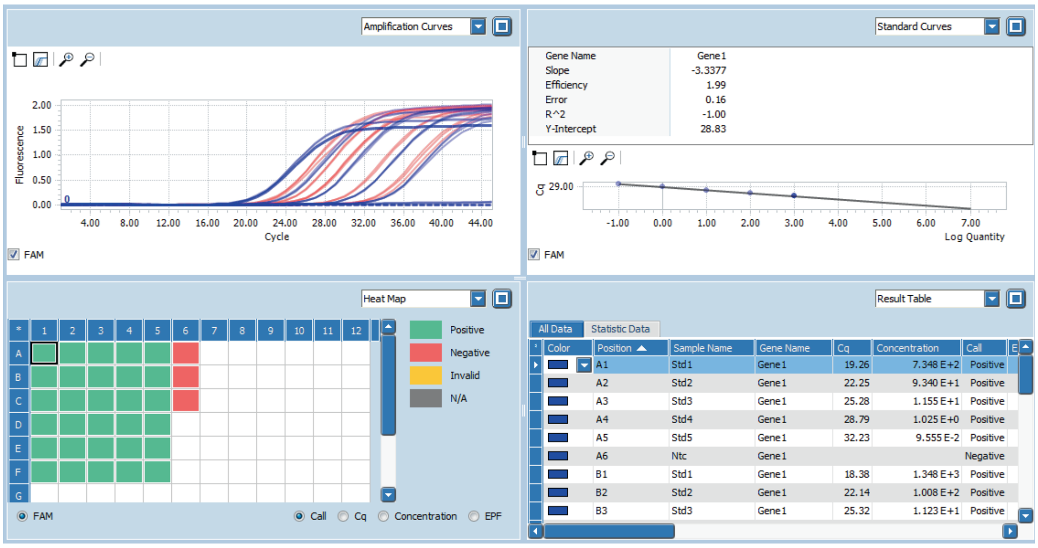


The color for the standard samples changes in the *Result Table* view and in the *Amplification Curves* view.

▶ ▶ ▶

5 Repeat steps 1 to 4 for the unknown samples in rows D to F. For example, assign red to all unknown samples.
The *Amplification Curves* chart and the *Result Table* view are customized accordingly:

- Standard samples are colored blue.
- Unknown samples are colored red.



1.6 Exporting result data

You can export the following result data to Microsoft Word or Excel:

- The result table as a text file
- The result graphs as a PNG file, GIF file, or text file.

For detailed information on how to export result data, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "Exporting analysis results".

C

2 Endpoint SNP genotyping

Endpoint SNP genotyping assays use hydrolysis probes for single-nucleotide polymorphism (SNP) genotyping. Two sequence-specific probes detect the wild type and mutant alleles. Each is labeled with different reporter dyes, most frequently with a FAM dye and a VIC dye. Fluorescence data are collected using PCR. To identify genotypes, only endpoint fluorescence intensities of the two reporter dyes are used. Relative dye intensities discriminate between homozygous for allele x, homozygous for allele y, and heterozygous alleles.

For detailed information, refer to the LightCycler® 96 System Operator's Guide, chapter A, sections "Detection formats" and "Endpoint genotyping analysis".

2.1 Experiment overview

The following example describes how to set up, run, and analyze a dual-color endpoint SNP genotyping assay.

The assay is performed using a LightCycler® 480 Multiwell Plate 96, white.

Samples	46 human genomic DNA samples, isolated with the MagNA Pure LC 2.0 Instrument (concentration approximately 90 ng/µl)
Reagents	▶ FastStart Essential DNA Probes Master (2 x conc.) ▶ Genotyping mix (40 x conc.), containing forward primer, reverse primer, and two hydrolysis probes labeled with FAM and VIC respectively

2.2 Setting up the samples

To set up the samples:

- Set up the sample dilutions, see section *Sample dilution* below.
- Include a no template control (NTC), see section *Controls* below.
- Prepare the PCR mix, see section *PCR mix*, on page 75.
- Pipette the sample dilution and the PCR mix, see section *Pipetting scheme*, on page 75.
- Centrifuge the multiwell plate, see section *Centrifugation*, on page 75

Continuously cool the samples and PCR mix during setup by keeping the reaction tubes on ice.

Sample dilution

The human genomic DNA samples (concentration approximately 90 ng/µl) are diluted to a consistent concentration of 5 ng/5 µl.

Controls

To ensure an accurate endpoint genotyping analysis, it is highly recommended that you include a no template control (NTC) in your experiment.

PCR mix

When setting up the PCR mix, compensate for pipetting losses. We recommend preparing PCR mixes with 10% extra volume.

The table below shows the components included in the PCR mix for one 20 µl reaction. The PCR mix volume is 15 µl for a subsequent sample input of 5 µl per reaction.

Component	Concentration	Volume	Final conc.
Water, PCR grade		4.5 µl	
FastStart Essential DNA Probes Master	2 x conc.	10 µl	1 x conc.
Genotyping mix	40 x conc.	0.5 µl	1 x conc:
Total volume (without sample DNA)		**15 µl**	

Pipetting scheme

1. Pipette 15 µl of the PCR mix into 48 wells of the multiwell plate according to the following scheme.
2. Pipette 5 µl of sample dilution in the PCR mix into each well.
3. For the two NTCs, pipette 5 µl of water (instead of DNA sample) into the corresponding wells,according to the following scheme.

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNA1	DNA2	DNA3	DNA4	DNA5	DNA6						
B	DNA7	DNA8	DNA9	DNA10	DNA11	DNA12						
C	DNA13	DNA14	DNA15	DNA16	DNA17	DNA18						
D	DNA19	DNA20	DNA21	DNA22	DNA23	DNA24						
E	DNA25	DNA26	DNA27	DNA28	DNA29	DNA30						
F	DNA31	DNA32	DNA33	DNA34	DNA35	DNA36						
G	DNA37	DNA38	DNA39	DNA40	DNA41	DNA42						
H	DNA43	DNA44	DNA45	DNA46	NTC	NTC						

Centrifugation

1. Seal the multiwell plate with the LightCycler® 480 Sealing Foil using the sealing foil applicator (provided with the system package).
2. Centrifuge the multiwell plate at 1500 x *g* for 2 minutes in a standard swing-bucket centrifuge, using a rotor for multiwell plates.

   Make sure you balance the multiwell plate with a suitable counterweight (for example, another multiwell plate).

2.3 Experiment run parameters

For detailed information on how to program an experiment, see one of the following sections:
Programming the experiment with the LightCycler® 96 Application Software, on page 18.
Programming the experiment with the LightCycler® 96 Instrument Software, on page 30.

For this experiment, a standard PCR profile for hydrolysis probes is used. The experiment includes the run parameters for the temperature profile, the detection format, and the reaction volume. These parameters are listed in the following tables.

Temperature profile

For detailed information on how to program a temperature profile, see one of the following sections:
For working with the LightCycler® 96 Application Software: Creating the temperature profile, on page 21.
For working with the LightCycler® 96 Instrument Software: Creating the temperature profile, on page 33.

For this example, use the following heating and cooling cycles:

Programs		Steps			
Name	**Number of cycles**	**Ramp (°C/s)**	**Duration (s)**	**Target (°C)**	**Acquisition Mode**
Preincubation	1	4.4	600	95	None
2-step amplification	45	4.4	10	95	None
		2.2	60	60	Single
Cooling	1	2.2	30	37	None

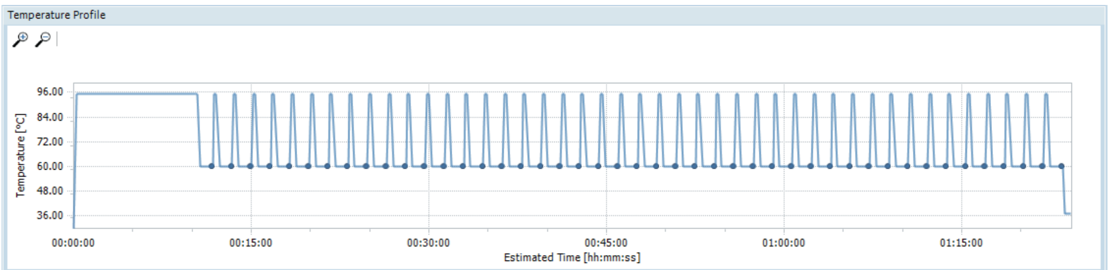
It is not necessary to add a separate cooling program at the end of the run. At the end of each run, the samples are automatically cooled to +37°C.

For the ramp rate for heating and cooling (*Ramp (°C/s)*), the default values are used in this example.

For the steps of the amplification program, the following default settings are used in this example:

- LightCycler® 96 Application Software: For *Gradient* and *Touch down*, the default settings are used.
- LightCycler® 96 Instrument Software: For *Mode*, the default setting *Standard* is used.

In the *Temperature Profile* window area, the following graphical summary of the programs selected for the experiment and their temperature and time settings is displayed.



Detection format

Selecting the dyes FAM and VIC for this dual-color experiment determines the channel combination for the measurement during the run. For all other parameters, the default values are used in this example.

For detailed information on how to specify the detection format, see the following sections:
For working with the LightCycler® 96 Application Software: To specify the detection format for the experiment, on page 25.
For working with the LightCycler® 96 Instrument Software: To specify the detection format for the experiment, on page 38.

For this example, use the following channel combination:

Dye	Channel
FAM	470/514
VIC	533/572

Reaction volume

As the LightCycler® 96 Instrument does not validate the reaction volume, ensure that the specified reaction volume matches the volume pipetted into the wells of the multiwell plate.

For detailed information on how to specify the reaction volume, see one of the following sections:
For working with the LightCycler® 96 Application Software: To specify the reaction volume for the experiment, on page 25.
For working with the LightCycler® 96 Instrument Software: To specify the reaction volume for the experiment, on page 40.

For this example, use the following reaction volume:

Reaction volume
20 µl

Experiment run

Once you have set up the samples and defined the experiment run parameters,you can start the run. For detailed information on how to run the experiment, see section *Running the experiment*, on page 41.

2.4 Editing the sample data

For editing the sample data, the experiment must be opened in the LightCycler® 96 Application Software. You can edit the sample list before or after the run, depending on your preferred routine.

The LightCycler® 96 Application Software offers two different views for editing the samples: the plate view and the table view. This user training guide describes how to edit the samples using the plate view, which shows the samples in 96 wells laid out to match the physical instrument. Changes in the plate view are immediately displayed in the table view and vice versa.

For detailed information on the 'Sample Editor' tab, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "Sample Editor tab".

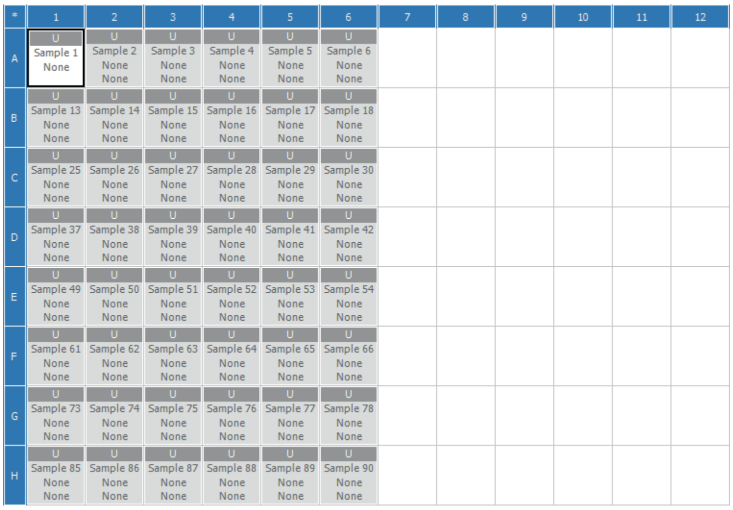
To edit the sample list:

- Clear empty wells to eliminate them from the analysis, see section *Empty wells* below.
- Edit the sample names, see section *Sample names*, on page 79.
- Edit the sample types, see section *Sample types*, on page 80.
- Assign a gene for each dye, see section *To assign a gene for each dye*, on page 81

Empty wells

For detailed information on how to clear empty wells, see section To clear empty wells, on page 49.

For this example, clear the wells in columns 7 to 12 (see also the multiwell plate image below).



Sample names

For detailed information on how to edit the sample names, see section To edit the samples, on page 51.

For this example, the following sample names apply (see also the multiwell plate image below):

Name	Samples in the plate view
DNA 1 to DNA 46	For the unknown samples in rows A to H
NTC	For the negative control in wells H5 and H6

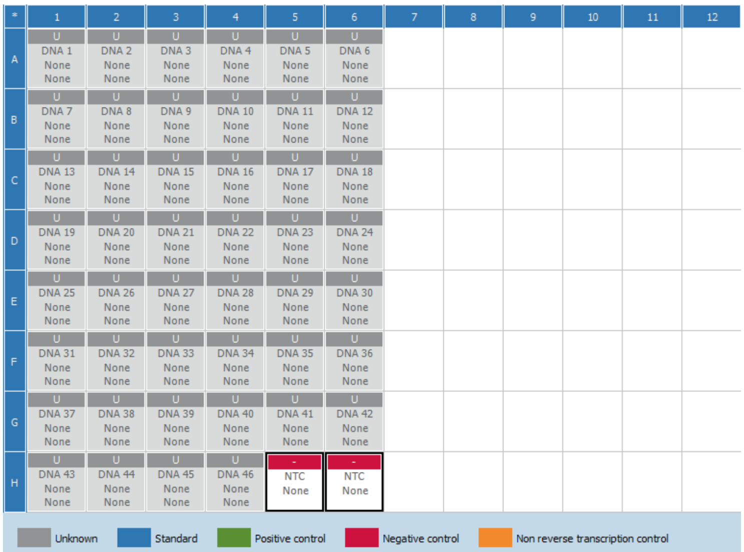
*	1	2	3	4	5	6	7	8	9	10	11	12
A	U DNA 1 None	U DNA 2 None None	U DNA 3 None None	U DNA 4 None None	U DNA 5 None None	U DNA 6 None None						
B	U DNA 7 None None	U DNA 8 None None	U DNA 9 None None	U DNA 10 None None	U DNA 11 None None	U DNA 12 None None						
C	U DNA 13 None None	U DNA 14 None None	U DNA 15 None None	U DNA 16 None None	U DNA 17 None None	U DNA 18 None None						
D	U DNA 19 None None	U DNA 20 None None	U DNA 21 None None	U DNA 22 None None	U DNA 23 None None	U DNA 24 None None						
E	U DNA 25 None None	U DNA 26 None None	U DNA 27 None None	U DNA 28 None None	U DNA 29 None None	U DNA 30 None None						
F	U DNA 31 None None	U DNA 32 None None	U DNA 33 None None	U DNA 34 None None	U DNA 35 None None	U DNA 36 None None						
G	U DNA 37 None None	U DNA 38 None None	U DNA 39 None None	U DNA 40 None None	U DNA 41 None None	U DNA 42 None None						
H	U DNA 43 None None	U DNA 44 None None	U DNA 45 None None	U DNA 46 None None	U NTC None None	U NTC None None						

Sample types

For detailed information on how to edit the sample types, see section To edit the samples, on page 51.

In this example the following sample types apply (see also the multiwell plate image below):

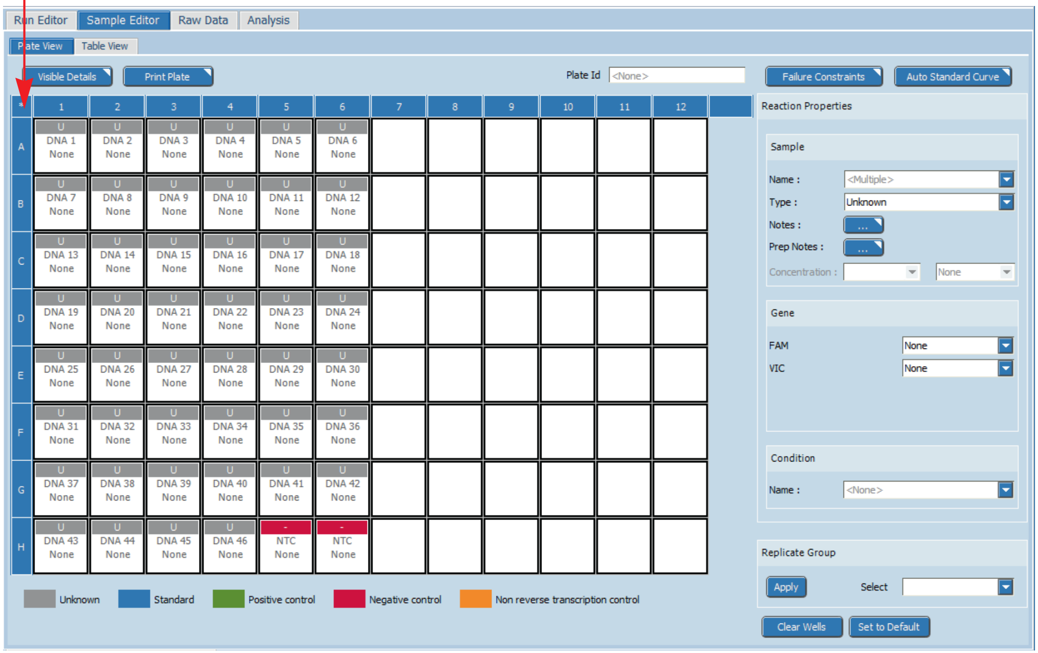
Type	Samples in the plate view
Unknown (default)	For the samples *DNA 1* to *DNA 46*
Negative control	For the samples *NTC*



To assign a gene for each dye

For endpoint genotyping analysis, it is essential to define identical gene names for both dyes. In case of different gene names, no endpoint genotyping analysis is possible.

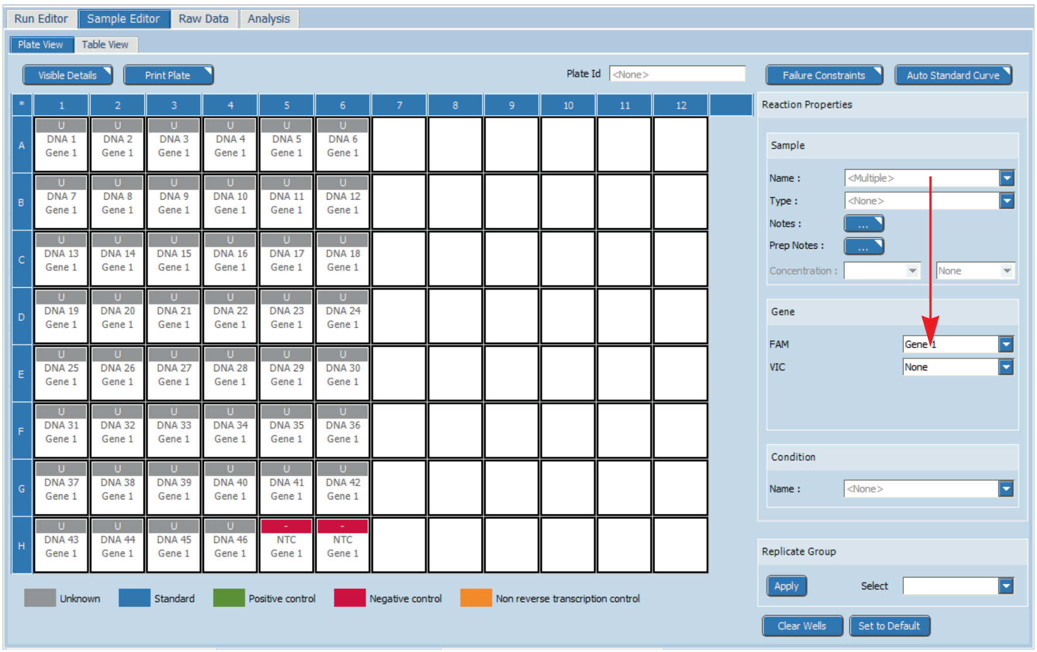
1. In the multiwell plate image, choose the asterisk (*) in the upper left corner to select all the wells.



2. In the *Reaction Properties* window area, choose the text field next to the *FAM* dye.

3. Type in *Gene 1*.

   The gene *Gene 1* is assigned to the *FAM* dye.

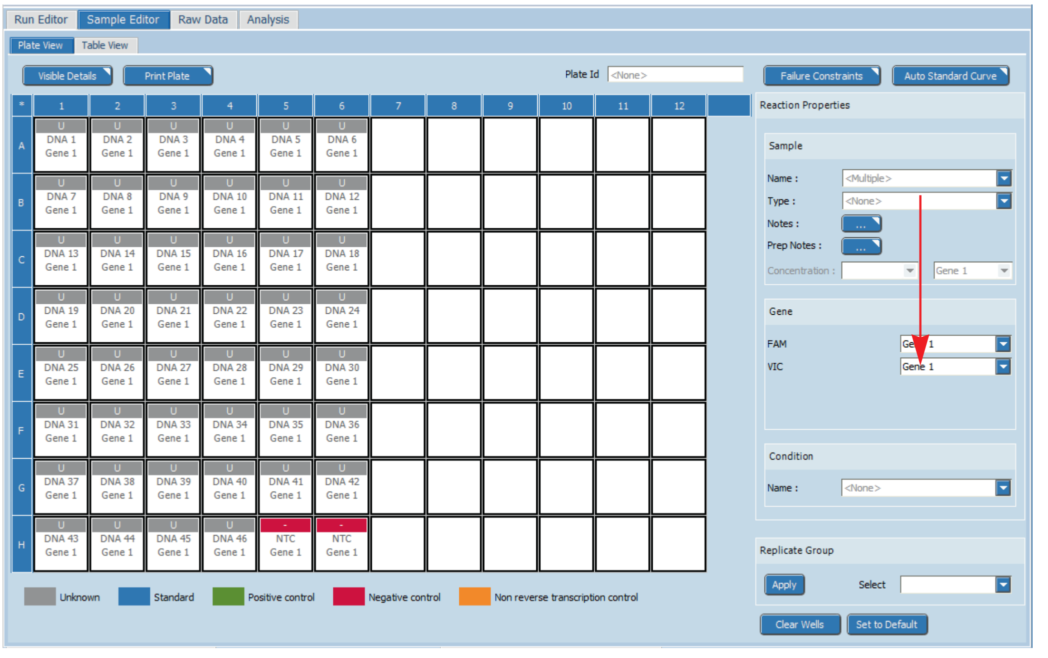


4. Choose the text field next to the *VIC* dye.

▶ ▶ ▶

5 Type in *Gene 1*.

The gene *Gene 1* is assigned to the *VIC* dye.



2.5 Analyzing the results

For detailed information on the 'Analysis' tab, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "Endpoint genotyping".

For detailed information on working with tables and graphs, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "General software conventions"

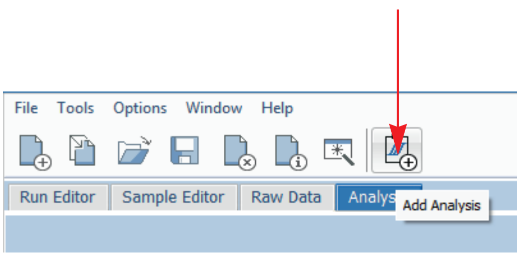
To analyze the calculated results of the endpoint genotyping application:

- Create the endpoint genotyping analysis, see section *Creating the analysis* below.
- Optional: Specify the genotyping settings, see section *Analysis settings*, on page 85.
- In the different views of the *Analysis* tab, check the analysis results and customize the result data if necessary:
  - In the *Amplification Curves* view, check the curves for plausibility, see section *To check the amplification curves*, on page 86.
  - In the *Scatter Plot* view, define the angle settings and thresholds for Gene 1, see section *To define the angle settings and thresholds for Gene 1*, on page 87.
  - For the *Heat Map* view, see section *Heat map*, on page 89
  - In the *Result Table* view:
    Rename the genotypes, see section *Optional: To rename a genotype*, on page 89.
    Filter the results, see section *Optional: To filter the results*, on page 91.
    Change the color of the samples, see section *Optional: To change the color of the samples*, on page 92.

2.5.1 Creating the analysis

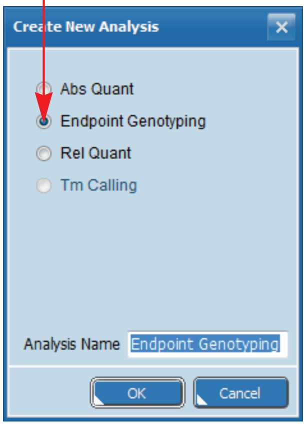
To create the endpoint genotyping analysis

1. Open the *Analysis* tab.
2. In the tool bar, choose the *Add Analysis* icon to add a new analysis.



The *Create New Analysis* dialog box opens.

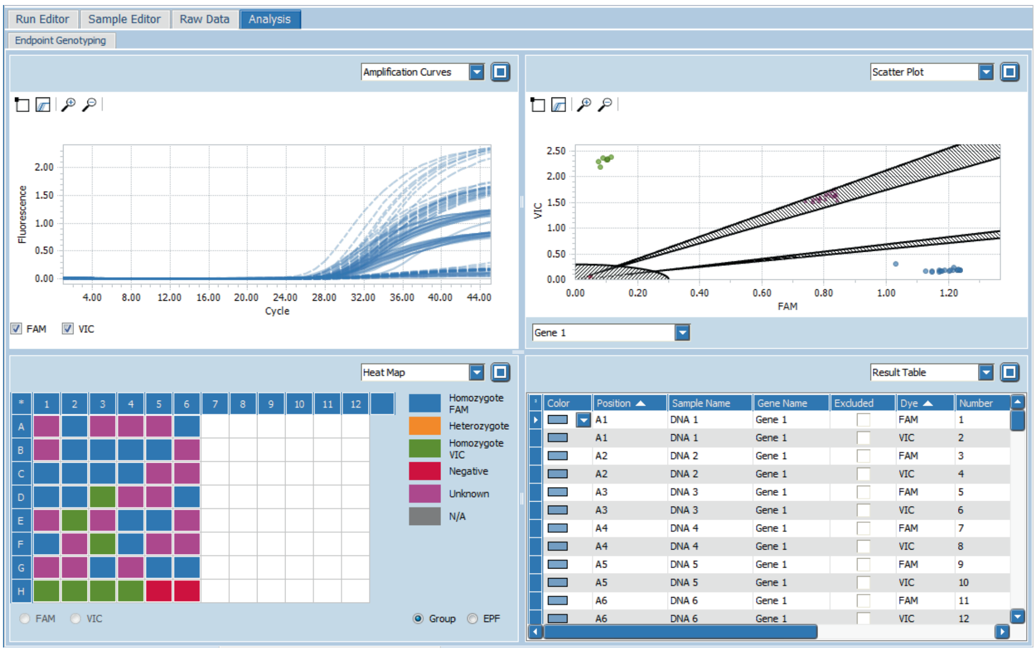
3 Choose *Endpoint Genotyping*.



4 Choose *OK*.

The *Analysis* tab displays four different views for the experiment using default values:

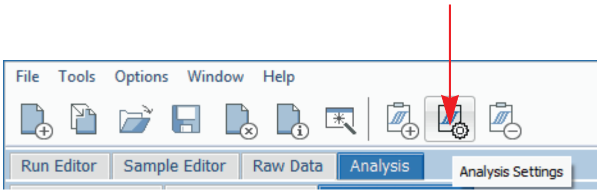
- *Amplification Curves*
- *Scatter Plot*
- *Heat Map*
- *Result Table*



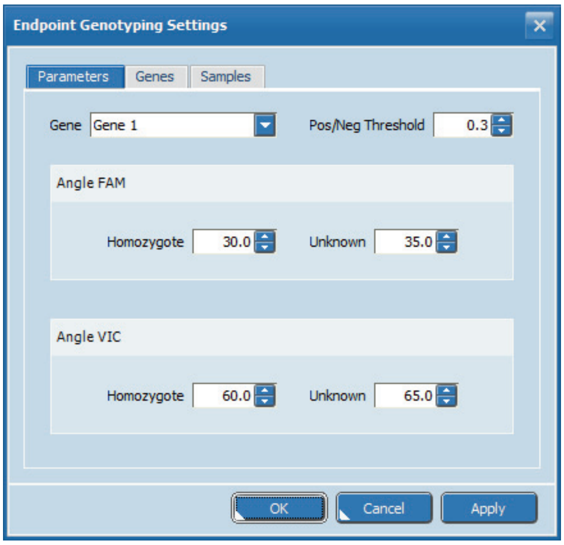
2.5.2 Analysis settings

Optional: To specify the analysis settings

1. In the tool bar, choose the *Analysis Settings* icon.



The *Endpoint Genotyping Settings* dialog box opens.



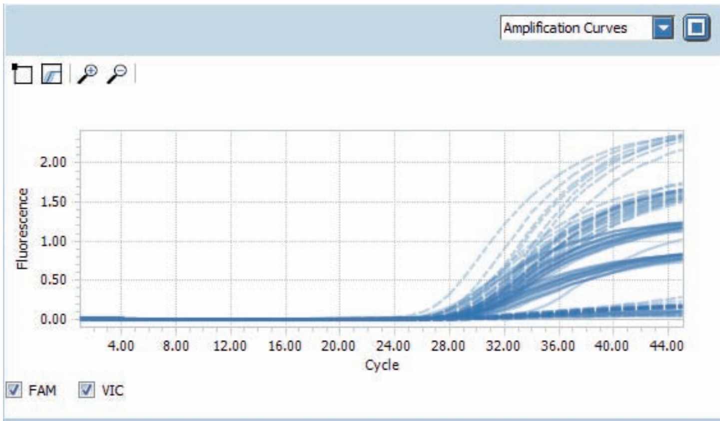
2. In the *Endpoint Genotyping Settings* dialog box, specify the analysis-specific settings, for example:
   - On the *Parameters* tab, specify the threshold and angle settings for Gene 1.

   The threshold and angle settings in the 'Endpoint Genotyping Settings' dialog box correspond to the slider settings in the scatter plot view. For detailed information, see section Scatter plot, on page 87.

   - On the *Genes* tab, exclude genes.
   - On the *Samples* tab, exclude samples.

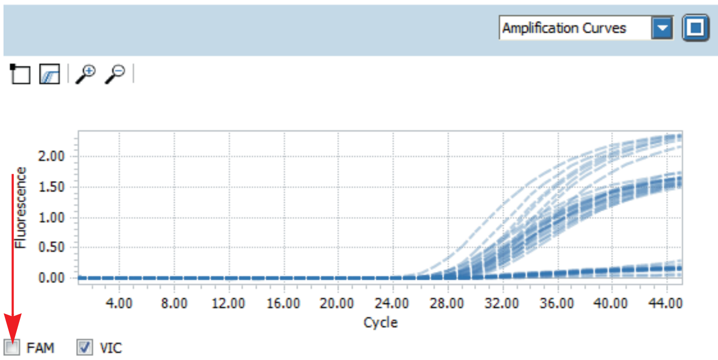
2.5.3 Amplification curves

The *Amplification Curves* chart displays the fluorescence intensity against the number of cycles in an amplification program. There is one curve for each sample that has a gene labeled with the selected dye.

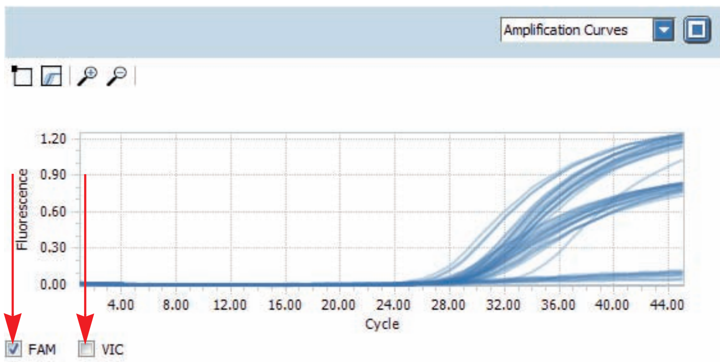


To check the amplification curves

1. Optional: Change the color of the samples to distinguish the corresponding amplification curves from each other.
For detailed information on how to change the color of the samples, see section *Optional: To change the color of the samples*, on page 92.
2. Check the *Amplification Curves* chart for correct amplification.
3. Check if you can identify the three diffent groups of genotypes: Homozygote: FAM, Homozygote: VIC, and Heterozygote for the respective channels FAM and VIC.
4. Clear the *FAM* check box to display only the curves for each sample that has a gene labeled with the VIC dye.



5. Select the *FAM* check box again and clear the *VIC* check box to display only the curves for each sample that has a gene labeld with the FAM dye.



2.5.4 Scatter plot

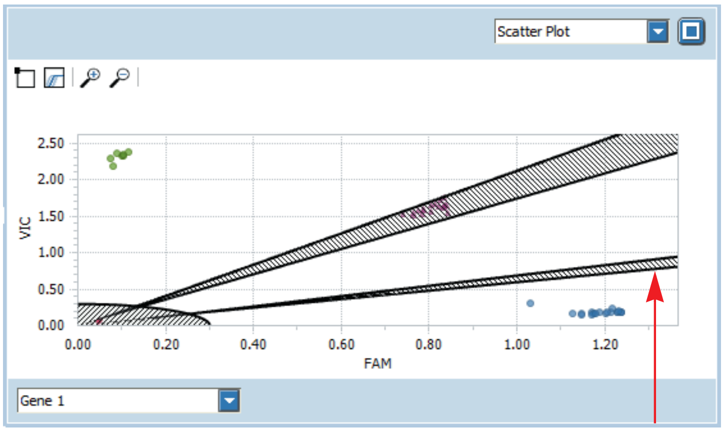
The *Scatter Plot* chart displays the endpoint fluorescence of the two selected dyes (representing the two alleles). Each point represents a sample, whose x-coordinate is the endpoint fluorescence level of FAM, and whose y-coordinate is the endpoint fluorescence level of VIC.

To define the angle settings and thresholds for Gene 1

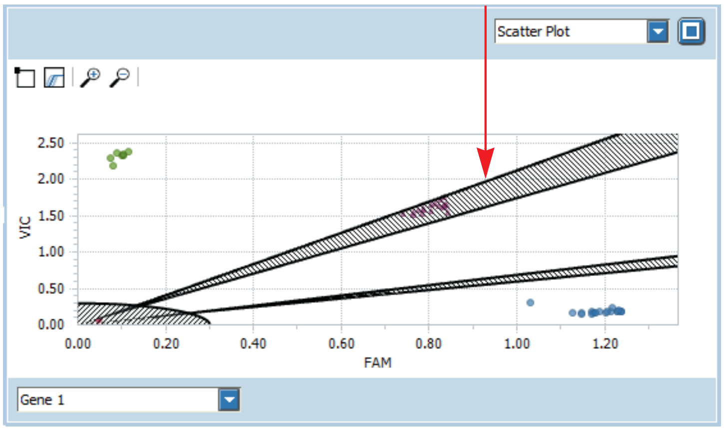
The slider settings on the scatter plot correspond to the threshold and angle settings in the 'Endpoint Genotyping Settings' dialog box.

1. In the *Scatter Plot* view, set the sliders manually to see the following three different groups of genotypes:
   - Homozygote: FAM
   - Homozygote: VIC
   - Heterozygote

2. To specify the area for *Homozygote: FAM,* drag the lowermost slider to the appropriate location: Any points below the lowermost slider are classified as *Homozygote: FAM.*

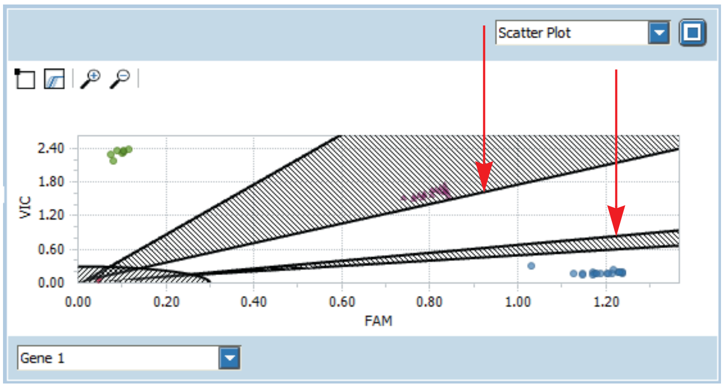


3. To specify the area for *Homozygote: VIC,* drag the uppermost slider accordingly: Any points above the uppermost slider are classified as *Homozygote: VIC.*

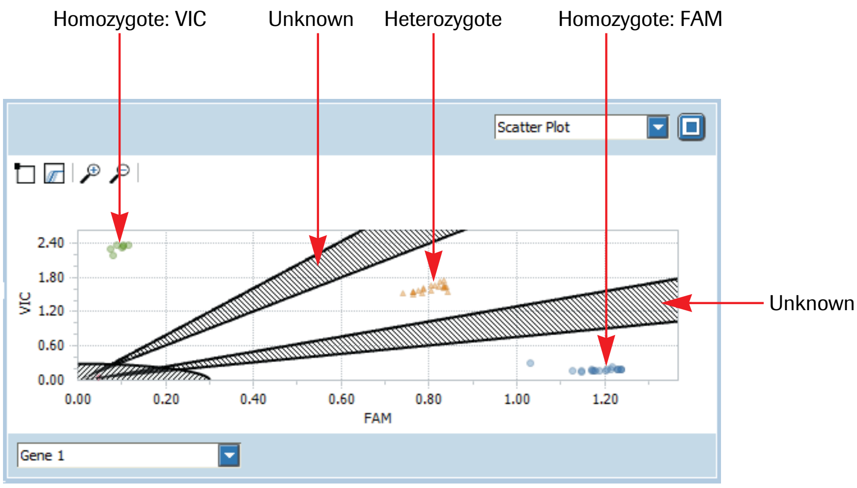


4 To specify the areas for unknown samples and to limit the area for heterozygote samples, drag the inner sliders accordingly:

- Any points in the shaded areas are classified as *Unknown*.
- Any points between the shaded areas are classified as *Heterozygote*.

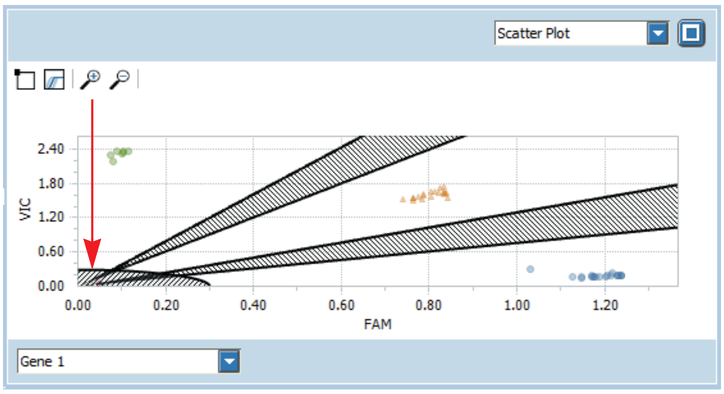


The *Scatter Plot* chart and the *Heat Map* are displayed according to the defined angles.



5 Check if the NTCs are in the *Pos/Neg Threshold* radius. In this example, keep the default value for the *Pos/Neg Threshold* radius.

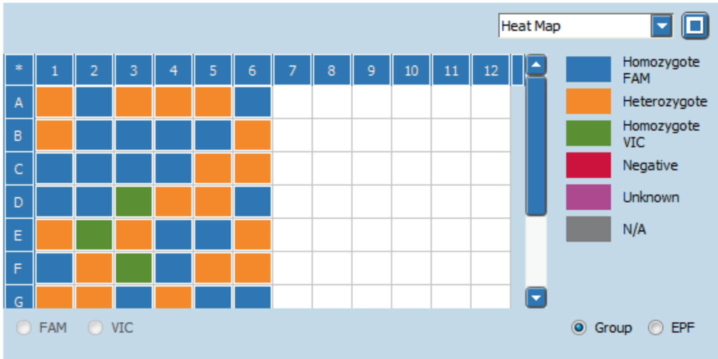
The *Scatter Plot* chart and the *Heat Map* are displayed according to the defined threshold and angles.



2.5.5 Heat map

The heat map shows an image of the multiwell plate used in the experiment for the specified gene.

The name settings of the genotypes in the 'Heat Map' view correspond to the name settings in the 'Result Table' view.



In this example the *Group* heat map is displayed. For all samples contained in the sample list, it shows the genotype the sample is assigned to, according to the threshold and angle settings.

- Blue: *Homozygote FAM*
- Orange: *Heterozygote*
- Green: *Homozygote VIC*

2.5.6 Result table

The result table displays the results of the endpoint genotyping analysis.

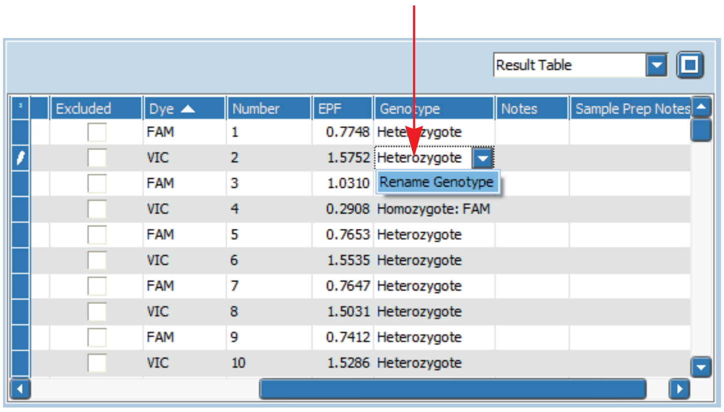
To customize the results:

- Rename the genotypes, see section *Optional: To rename a genotype* below.
- Filter the results, see section *Optional: To filter the results*, on page 91.
- Change the color of the samples, see section *Optional: To change the color of the samples*, on page 92.

Optional: To rename a genotype

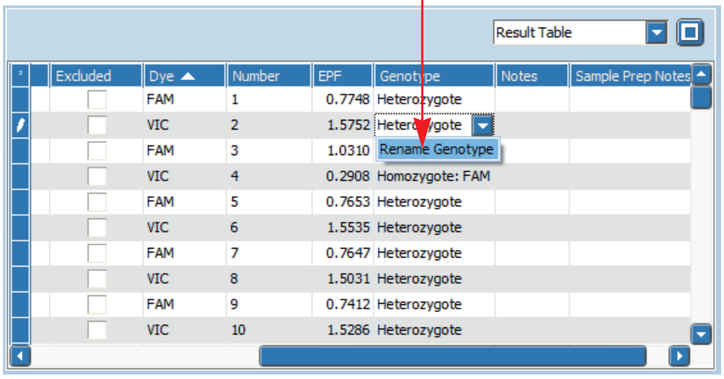
The name settings of the genotypes in the 'Result Table' view correspond to the name settings in the 'Heat Map' view.

1. In the *Result Table* view, select the genotype that you want to rename, for example, *Heterozygote*.



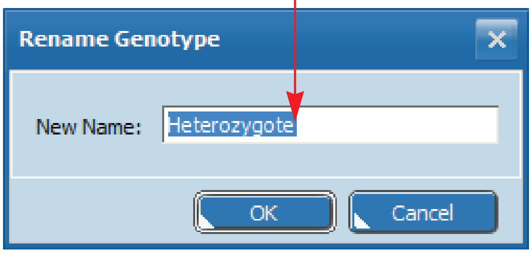
The shortcut menu opens.

2 On the shortcut menu, choose *Rename Genotype*.



The *Rename Genotype* dialog box opens.

3 In the *New Name:* field, type in the new name for the selected genotype, for example, *Heterozygote Gene 1*, and choose *OK*.

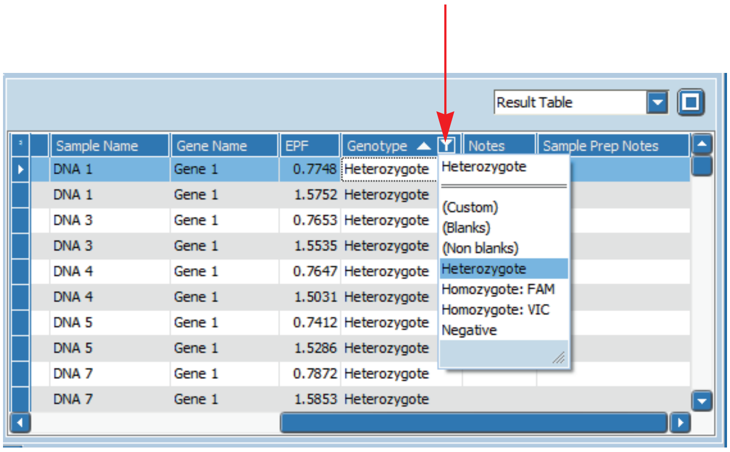


All genotypes of this group are renamed in the *Result Table* view.

4 Repeat steps 1 to 3 for another group of genotypes.

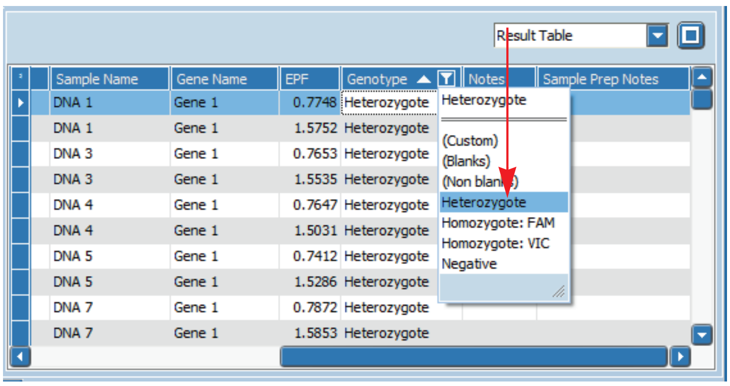
Optional: To filter the results

1. In the *Result Table* view, choose the filter icon in the header of the corresponding column, for example, *Genotype*.

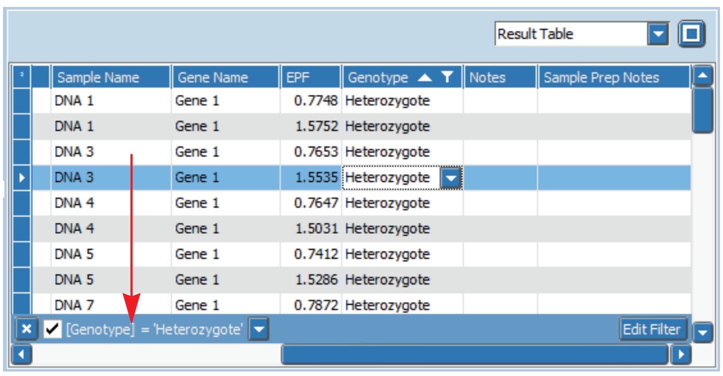


A list with all values found in this column and the category *(Custom)* is displayed.

2. Choose one of the values in this list, for example, *Heterozygote*.



The table is updated and the filter definition is displayed below.



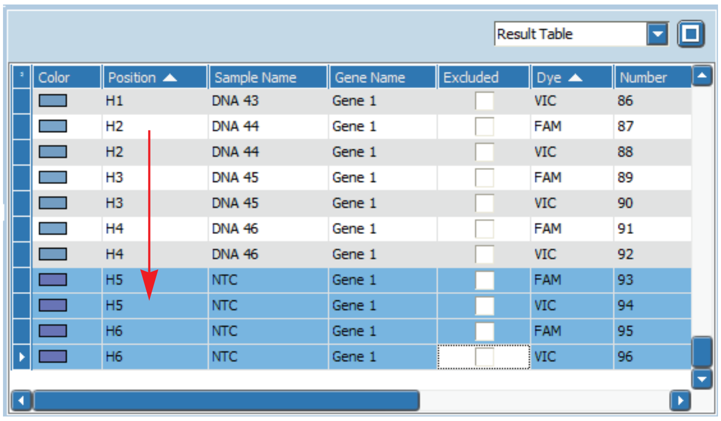
3. Repeat steps 1 and 2 to add additional values to the filter specification.
The items are filtered by these values. Only items matching all the filter conditions are displayed in the table.

Optional: To change the color of the samples

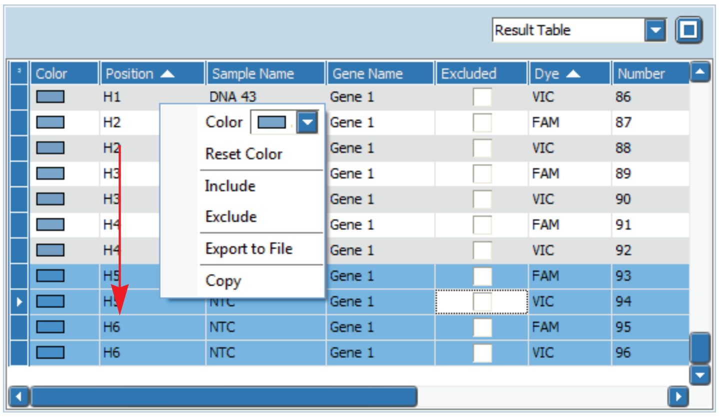
Change the color of the samples to distinguish the corresponding amplification curves from each other.

The color settings in the 'Result Table' view correspond to the color settings in the 'Amplification Curves' view.

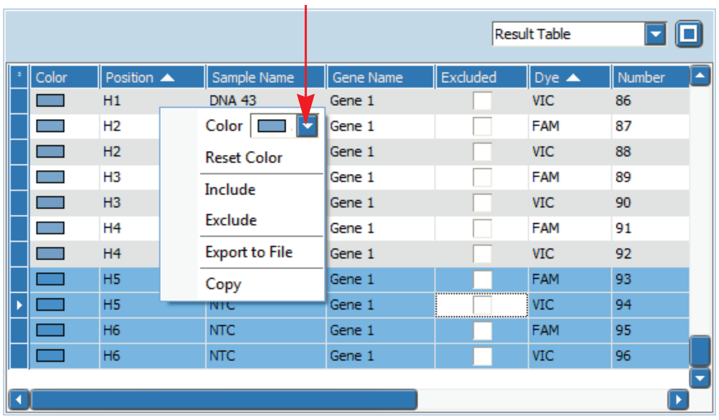
1. Choose the sample(s) for which you want to change the color, for example, the NTC samples.



2. Right-click the selected sample(s) to open the corresponding shortcut menu.

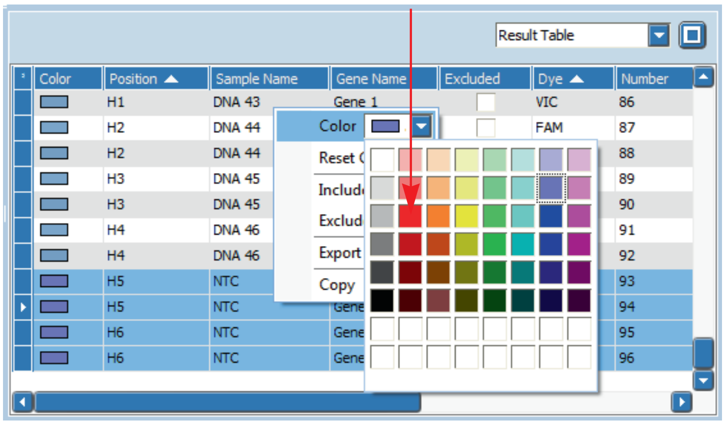


3. On the shortcut menu, choose the down arrow next to *Color*.



The color selection dialog box opens.

4 Choose a color field to assign a different color to the selected sample(s), for example, red.



The color for the seclected sample(s) changes in the *Result Table* view and in the *Amplification Curves* view.

5 Repeat steps 1 to 4 for another group of samples.

2.6 Exporting result data

You can export the following result data to Microsoft Word or Excel:

- The result table as a text file
- The result graphs as a PNG file, GIF file, or text file.

For detailed information on how to export result data, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "Exporting analysis results".

3 Relative quantification

Relative quantification compares the levels of two different gene sequences in a single sample, for example, target gene of interest and a reference gene, and expresses the result as a ratio. For comparison purposes, the reference gene is known to be present in constant numbers under all test conditions. This reference gene provides a basis for normalizing sample-to-sample differences.

The ratio of target gene to reference gene is a relative, dimensionless number, that is meaningful only when compared between samples. In addition to calculating sample-specific ratios, a normalized ratio is calculated by defining a "run calibrator" sample. The run calibrator is typically a positive sample with a stable ratio of target to reference. Its value is used to normalize all samples within one run, and also to provide a constant calibration point between several runs.

In addition to normalization, it is frequently required to measure the gene expression of each sample and each gene at different times or under different conditions. To generate a meaningful result, the sample-specific measurements are normalized to a common basis, that is, a certain experimental condition, to provide a scaled ratio. This condition is specified as the "study calibrator" condition.

For detailed information on relative quantification analysis, refer to the LightCycler® 96 System Operator's Guide, chapter A, section "Analysis principles".

3.1 Experiment overview

The following example describes how to set up, run, and analyze a gene expression assay. It comprises three samples tested at three different times (that is, at the three conditions *0 hours*, *1 hour*, and *2 hours*), one target gene and one reference gene.

The assay is performed using a LightCycler® 480 Multiwell Plate 96, white. Each sample is set up in duplicate.

Samples	cDNA samples. Three samples are tested at three different times.
Reagents	▶ FastStart Essential DNA Probes Master (2 x conc.) ▶ Target primer mix, 5 µM (10 x conc.) ▶ Target hydrolysis probe, 10 µM (50 x conc.) ▶ Reference primer mix, 5 µM (10 x conc.) ▶ Reference hydrolysis probe, 10 µM (50 x conc.)

3.2 Setting up the samples

To set up the samples:

- Set up the sample dilutions, see section *Sample dilution* below.
- Include a no template control (NTC), see section *Controls* below.
- Prepare the PCR mixes, see sections *PCR mix for the target gene* below, and *PCR mix for the reference gene*, on page 96.
- Pipette the sample dilution and the PCR mix, see section *Pipetting scheme*, on page 96.
- Centrifuge the multiwell plate, see section *Centrifugation*, on page 97.

Continously cool the samples during setup by keeping the tubes on ice.

Sample dilution

The 3 human cDNA samples from total RNA (concentration approximately 50 ng/µl) are diluted to a consistent concentration of 5 ng/µl.

Controls

To ensure an accurate relative quantification analysis, it is highly recommended that you include a no template control (NTC) in your experiment.

PCR mix for the target gene

When setting up the PCR mix, compensate for pipetting losses. We recommend preparing PCR mixes with 10% extra volume.

The table below shows the components included in the PCR mix for one 20 µl reaction. The PCR mix volume is 15 µl for a subsequent sample input of 5 µl per reaction.

Component	Concentration	Volume	Final conc.
Water, PCR grade		2.6 µl	
FastStart Essential DNA Probes Master	2 x conc.	10 µl	1 x conc.
Primer mix for target gene	10 x conc.	2.0 µl	500 nM each
UPL Probe for target gene (FAM)	50 x conc.	0.4 µl	200 nM
Total volume (without sample cDNA)		**15 µl**	

PCR mix for the reference gene

When setting up the PCR mix, compensate for pipetting losses. We recommend preparing PCR mixes with 10% extra volume.

The table below shows the components included in the PCR mix for one 20 µl reaction. The PCR mix volume is 15 µl for a subsequent sample input of 5 µl per reaction.

Component	Concentration	Volume	Final conc.
Water, PCR grade		2.6 µl	
FastStart Essential DNA Probes Master	2 x conc.	10 µl	1 x conc.
Primer mix for reference gene	10 x conc.	2.0 µl	500 nM each
UPL Probe for reference gene (FAM)	50 x conc.	0.4 µl	200 nM
Total volume (without sample cDNA)		**15 µl**	

Pipetting scheme

1. Pipette 15 µl of the PCR mix for the target gene into 20 wells, rows A and B, columns 1 to 10 of the multiwell plate according to the following scheme.
2. Pipette 15 µl of the PCR mix for the reference gene into 20 wells, rows C and D, columns 1 to 10 of the multiwell plate according to the following scheme.
3. Pipette 5 µl of cDNA sample into the corresponding wells according to the following scheme.
4. For the NTCs, pipette 5 µl of water (instead of cDNA sample) into the corresponding wells in column 10 according to the following scheme.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample1 0 h	Sample2 0 h	Sample3 0 h	Sample1 1 h	Sample2 1 h	Sample3 1 h	Sample1 2 h	Sample2 2 h	Sample3 2 h	NTC		
B	Sample1 0 h	Sample2 0 h	Sample3 0 h	Sample1 1 h	Sample2 1 h	Sample3 1 h	Sample1 2 h	Sample2 2 h	Sample3 2 h	NTC		
C	Sample1 0 h	Sample2 0 h	Sample3 0 h	Sample1 1 h	Sample2 1 h	Sample3 1 h	Sample1 2 h	Sample2 2 h	Sample3 2 h	NTC		
D	Sample1 0 h	Sample2 0 h	Sample3 0 h	Sample1 1 h	Sample2 1 h	Sample3 1 h	Sample1 2 h	Sample2 2 h	Sample3 2 h	NTC		
E												
F												
G												
H												

Centrifugation

1. Seal the multiwell plate with the LightCycler® 480 Sealing Foil using the sealing foil applicator (provided with the system package).
2. Centrifuge the multiwell plate at 1500 x *g* for 2 minutes in a standard swing-bucket centrifuge, using a rotor for multiwell plates.

   Make sure you balance the multiwell plate with a suitable counterweight (for example, another multiwell plate).

3.3 Experiment run parameters

For detailed information on how to program an experiment, see one of the following sections:
Programming the experiment with the LightCycler® 96 Application Software, on page 18.
Programming the experiment with the LightCycler® 96 Instrument Software, on page 30.

Run a standard PCR profile for hydrolysis probes including a 2-step amplification program. The experiment includes the run parameters for the temperature profile, the detection format, and the reaction volume. These parameters are listed in the following tables.

Temperature profile

For detailed information on how to program a temperature profile, see one of the following sections:
For working with the LightCycler® 96 Application Software: Creating the temperature profile, on page 21.
For working with the LightCycler® 96 Instrument Software: Creating the temperature profile, on page 33.

For this example, use the following heating and cooling cycles:

Programs		**Steps**			
Name	**Number of cycles**	**Ramp (°C/s)**	**Duration (s)**	**Target (°C)**	**Acquisition Mode**
Preincubation	1	4.4	600	95	None
2-step amplification	45	4.4	10	95	None
		2.2	30	60	Single
Cooling	1	2.2	30	40	None

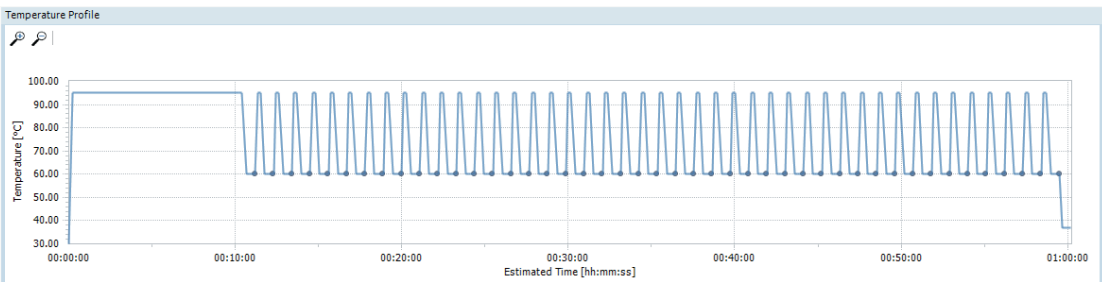
It is not necessary to add a separate cooling program at the end of the run. At the end of each run, the samples are automatically cooled to +37°C.

For the ramp rate for heating and cooling (*Ramp (°C/s)*), the default values are used in this example.

For the steps of the amplification program, the following default settings are used in this example:

- LightCycler® 96 Application Software: For *Gradient* and *Touch down*, the default settings are used.
- LightCycler® 96 Instrument Software: For *Mode*, the default setting *Standard* is used.

In the *Temperature Profile* window area, the following graphical summary of the programs selected for the experiment and their temperature and time settings is displayed.



Detection format

Selecting the dye (FAM) for this mono-color experiment determines the channel combination for the measurement during the run. For all other parameters, the default values are used in this example.

For detailed information on how to specify the detection format, see one of the following sections:
For working with the LightCycler® 96 Application Software: To specify the detection format for the experiment, on page 25.
For working with the LightCycler® 96 Instrument Software: To specify the detection format for the experiment, on page 38.

For this example, use the following channel:

Dye	Channel
FAM	470/514

Reaction volume

As the LightCycler® 96 Instrument does not validate the reaction volume, ensure that the specified reaction volume matches the volume pipetted into the wells of the multiwell plate.

For detailed information on how to specify the reaction volume, see one of the following sections:
For working with the LightCycler® 96 Application Software: To specify the reaction volume for the experiment, on page 25.
For working with the LightCycler® 96 Instrument Software: To specify the reaction volume for the experiment, on page 40.

For this example, use the following reaction volume:

Reaction volume
20 µl

Experiment run

Once you have set up the samples and defined the experiment run parameters,you can start the run. For detailed information on how to run the experiment, see section *Running the experiment*, on page 41.

3.4 Editing the sample data

For editing the sample data, the experiment must be opened in the LightCycler® 96 Application Software. You can edit the sample list before or after the run, depending on your preferred routine.

The LightCycler® 96 Application Software offers two different views for editing the samples: the plate view and the table view. This user training guide describes how to edit the samples using the plate view, which shows the samples in 96 wells laid out to match the physical instrument. Changes in the plate view are immediately displayed in the table view and vice versa.

For detailed information on the 'Sample Editor' tab, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "Sample Editor tab".

To edit the sample list:

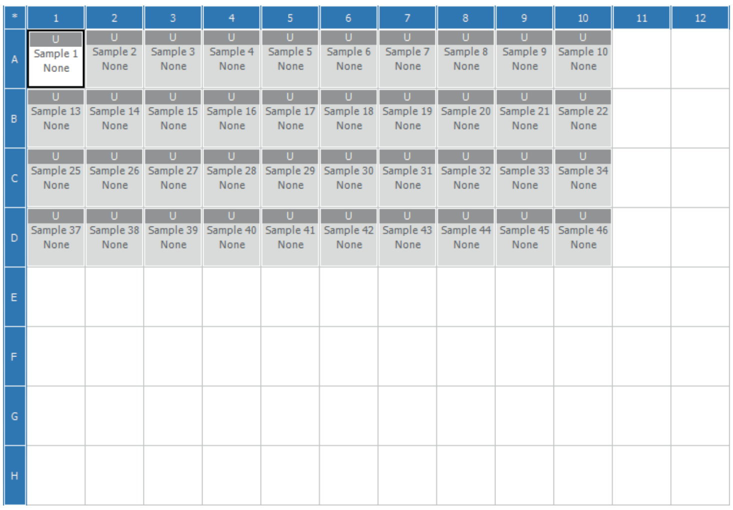
- Clear empty wells to eliminate them from the analysis, see section *Empty wells* below.
- Edit the sample names, see section *Sample names*, on page 101.
- Edit the sample types, see section *Sample types*, on page 102.
- Assign a gene for the dye, see section *To assign the target gene to the dye*, on page 103.
- Specify the three conditions for each sample, see section *To assign the reference gene to the dye*, on page 104.

Empty wells

For detailed information on how to clear empty wells, see section To clear empty wells, on page 49.

For this example, clear the following wells (see also the multiwell plate image below):

- Columns *11* to *12*
- Rows *E* to *H*



Sample names

For detailed information on how to edit the sample names, see section To edit the samples, on page 51.

For this example, the following sample names apply (see also the multiwell plate image below):

Name	Samples in the plate view
NTC	For the negative control in column 10
Sample1	For the unknown samples in columns 1, 4, and 7
Sample2	For the unknown samples in columns 2, 5, and 8
Sample3	For the unknown samples in columns 3, 6, and 9

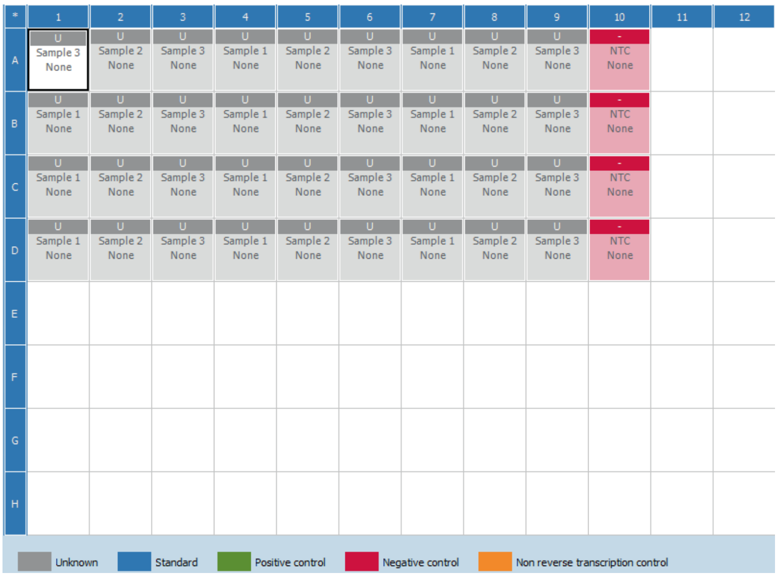
*	1	2	3	4	5	6	7	8	9	10	11	12
A	U Sample 1 None	U Sample 2 None	U Sample 3 None	U Sample 1 None	U Sample 2 None	U Sample 3 None	U Sample 1 None	U Sample 2 None	U Sample 3 None	U NTC None		
B	U Sample 1 None	U Sample 2 None	U Sample 3 None	U Sample 1 None	U Sample 2 None	U Sample 3 None	U Sample 1 None	U Sample 2 None	U Sample 3 None	U NTC None		
C	U Sample 1 None	U Sample 2 None	U Sample 3 None	U Sample 1 None	U Sample 2 None	U Sample 3 None	U Sample 1 None	U Sample 2 None	U Sample 3 None	U NTC None		
D	U Sample 1 None	U Sample 2 None	U Sample 3 None	U Sample 1 None	U Sample 2 None	U Sample 3 None	U Sample 1 None	U Sample 2 None	U Sample 3 None	U NTC None		
E												
F												
G												
H												

Sample types

For detailed information on how to edit the sample types, see section *To edit the samples, on page 51.*

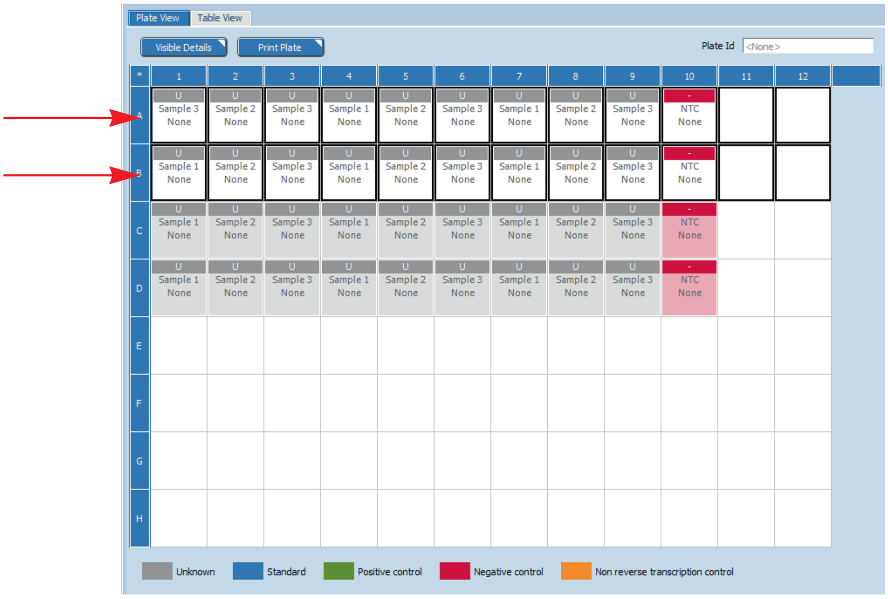
In this example the following sample types apply (see also the multiwell plate image below):

Type	Samples in the plate view
Negative control	For the samples *NTC*
Unknown (default)	For the samples *Sample1* to *Sample3*



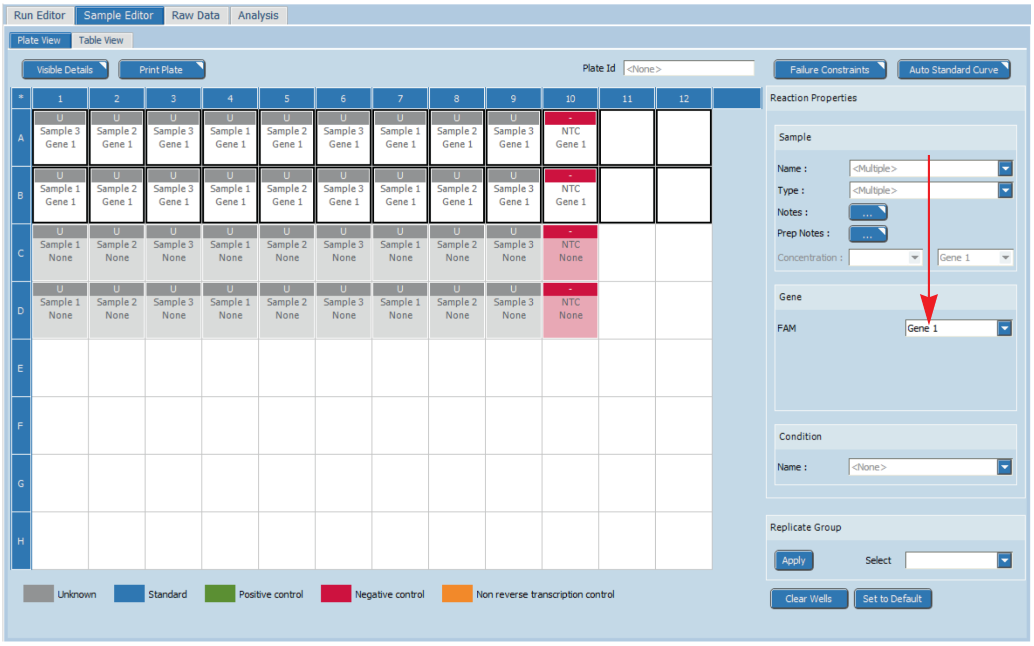
To assign the target gene to the dye

1. In the multiwell plate image, select the table rows A and B to select all wells in these rows.



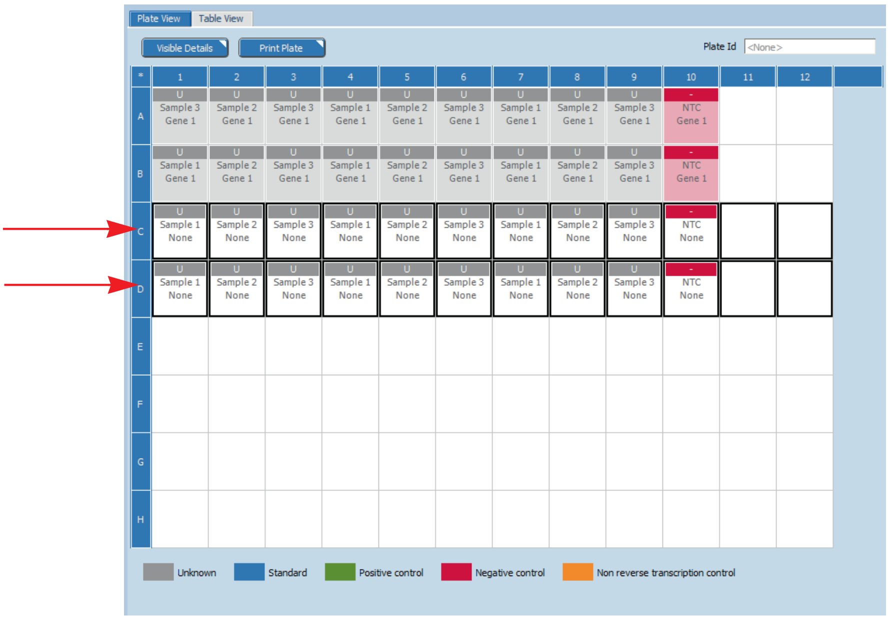
2. In the *Reaction Properties* window area, choose the text field next to the *FAM* dye.

3. Type in *Gene 1*.
   The gene *Gene 1* is assigned to all selected samples.



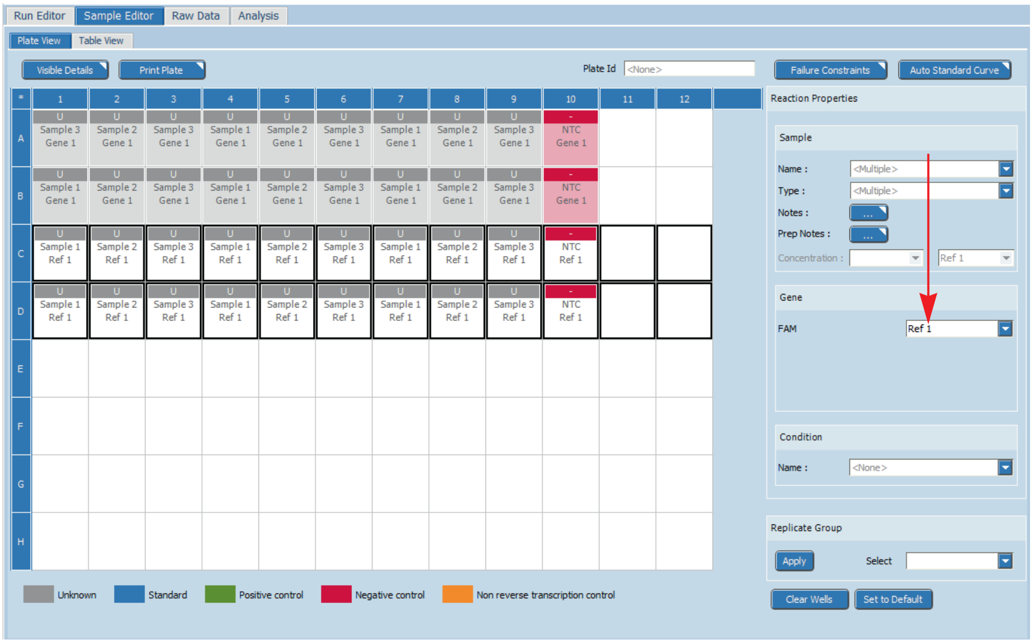
To assign the reference gene to the dye

1. In the multiwell plate image, select the table rows *C* and *D* to select all wells in these rows.



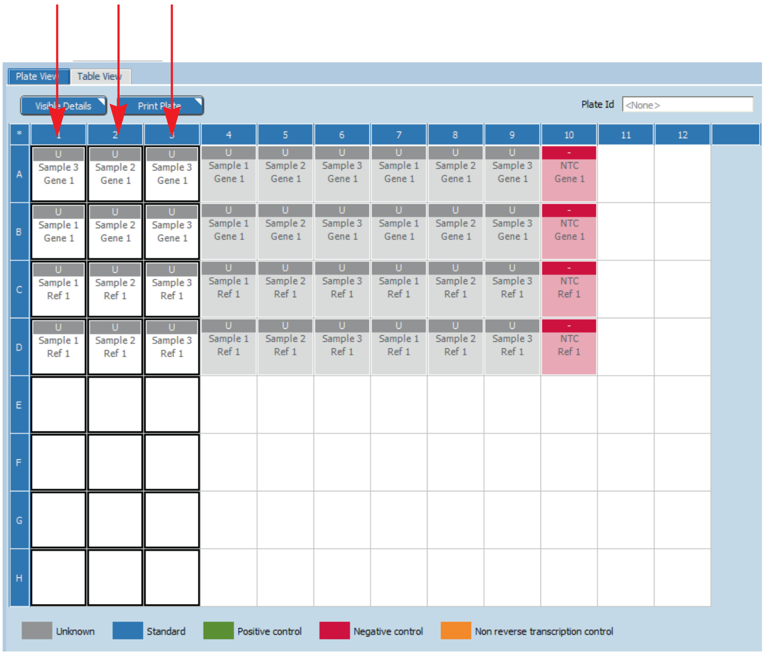
2. In the *Reaction Properties* window area, choose the text field next to the *FAM* dye.

3. Type in *Ref 1*.
The gene *Ref 1* is assigned to all selected samples.



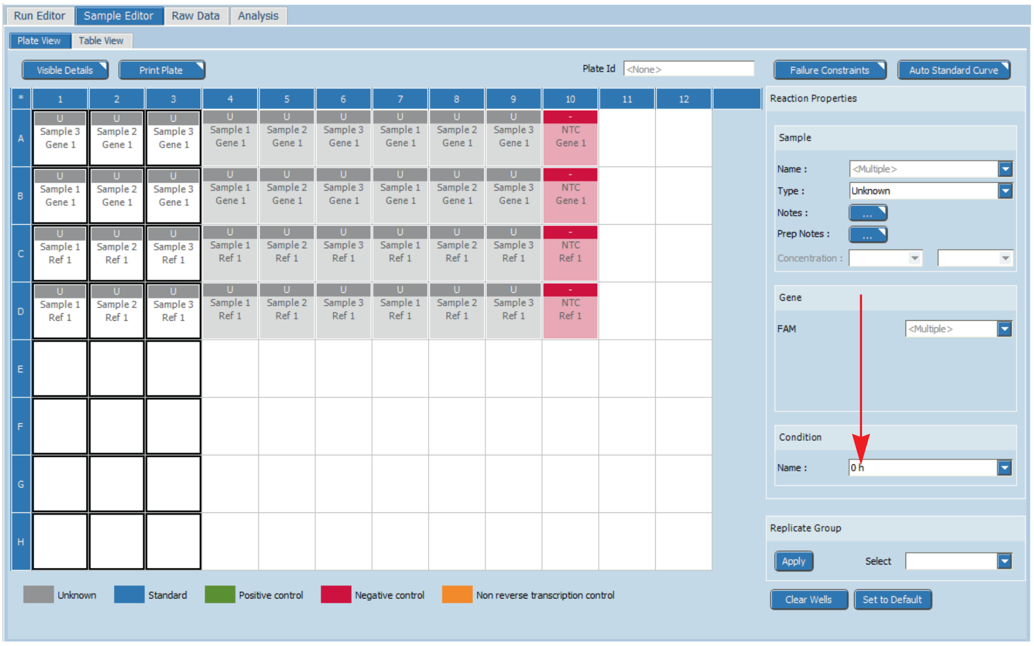
To specify the conditions

1. In the multiwell plate image, select columns *1*, *2*, and *3*.



2. In the *Reaction Properties* window area, choose the *Condition Name* text field.

3. Type in *0 h*.
The condition *0 h* is assigned to all selected samples.



4. As described in steps 1 to 3, assign the condition *1 h* to all wells in columns *4*, *5*, and *6*.

5 As described in steps 1 to 3, assign the condition *2 h* to all wells in columns *7*, *8*, and *9*.

*	1	2	3	4	5	6	7	8	9	10	11	12
A	U Sample 1 0 h Gene 1	U Sample 2 0 h Gene 1	U Sample 3 0 h Gene 1	U Sample 1 1 h Gene 1	U Sample 2 1 h Gene 1	U Sample 3 1 h Gene 1	U Sample 1 2 h Gene 1	U Sample 2 2 h Gene 1	U Sample 3 2 h Gene 1	- NTC Gene 1		
B	U Sample 1 0 h Gene 1	U Sample 2 0 h Gene 1	U Sample 3 0 h Gene 1	U Sample 1 1 h Gene 1	U Sample 2 1 h Gene 1	U Sample 3 1 h Gene 1	U Sample 1 2 h Gene 1	U Sample 2 2 h Gene 1	U Sample 3 2 h Gene 1	- NTC Gene 1		
C	U Sample 1 0 h Ref 1	U Sample 2 0 h Ref 1	U Sample 3 0 h Ref 1	U Sample 1 1 h Ref 1	U Sample 2 1 h Ref 1	U Sample 3 1 h Ref 1	U Sample 1 2 h Ref 1	U Sample 2 2 h Ref 1	U Sample 3 2 h Ref 1	- NTC Ref 1		
D	U Sample 1 0 h Ref 1	U Sample 2 0 h Ref 1	U Sample 3 0 h Ref 1	U Sample 1 1 h Ref 1	U Sample 2 1 h Ref 1	U Sample 3 1 h Ref 1	U Sample 1 2 h Ref 1	U Sample 2 2 h Ref 1	U Sample 3 2 h Ref 1	- NTC Ref 1		
E												
F												
G												
H												

3.5 Analyzing the results

For detailed information on the 'Analysis' tab, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "Relative quantification".

For detailed information on working with tables and graphs, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "General software conventions".

Relative quantification compares the levels of two different gene sequences, that is, the target gene of interest and a reference gene, in a single sample, and expresses the final result as a ratio of these genes.

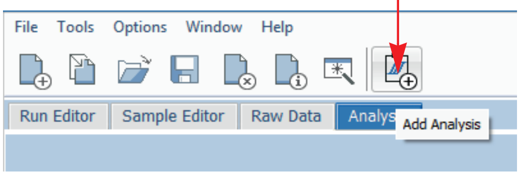
To analyze the calculated results of the relative quantification application:

- Create the relative quantification analysis, see section *Creating the analysis* below.
- Specify the settings for the relative quantification analysis, see section *Analysis settings*, on page 109.
- On the different views of the *Rel Quant* tab, check the analysis results and customize the result data if necessary:
  - For the *Amplification Curves* view, see section *Amplification curves*, on page 111.
  - For the *Ratio Bars* view, see section *Ratio bars*, on page 111.
  - For the *Heat Map* view, see section *Heat map*, on page 114.
  - For the *Result Table* view, see section *Result table*, on page 115.

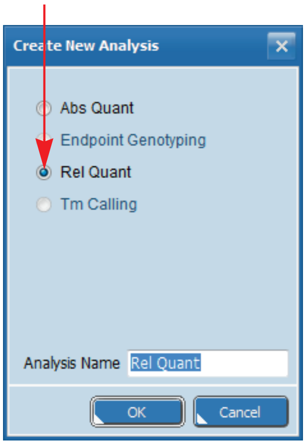
3.5.1 Creating the analysis

To create the relative quantification analysis

1. Open the *Analysis* tab.
2. In the tool bar, choose the *Add Analysis* icon to add a new analysis.



   The *Create New Analysis* dialog box opens.
3. Choose *Rel Quant*.



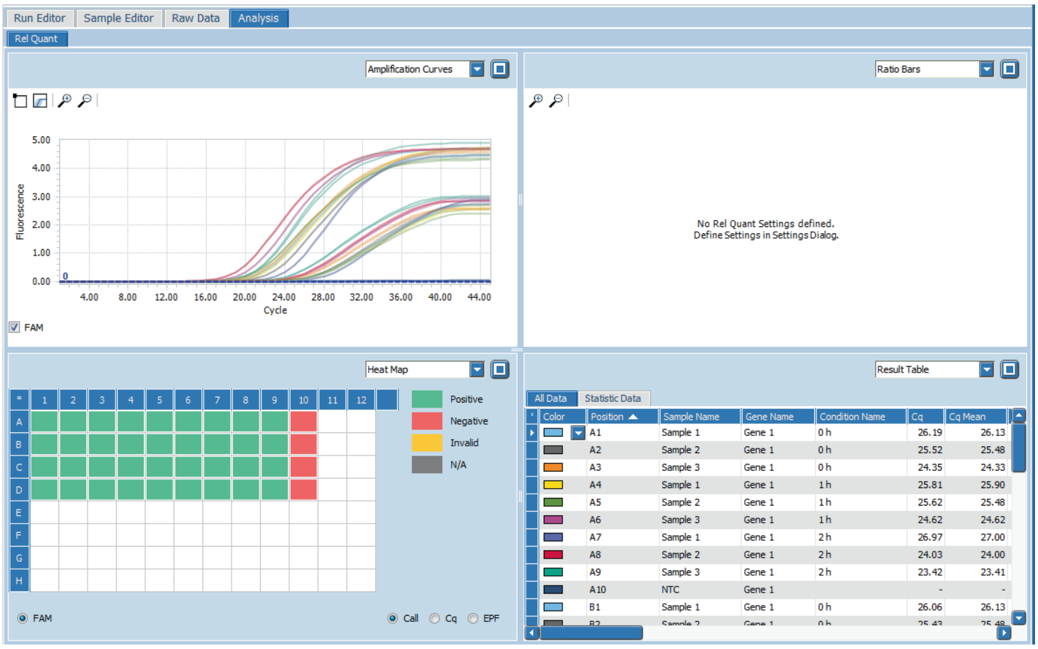
C

4 Choose *OK*.

The *Rel Quant* tab displays three different views for the experiment using default values:

- *Amplification Curves*
- *Heat Map*
- *Result Table*

The 'Ratio Bars' view is empty until you specify the analysis settings for the relative quantification analysis, see section Analysis settings*, on page 109.*



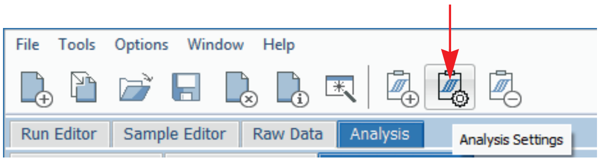
3.5.2 Analysis settings

In this example, specify the following settings:

- The reference gene.
- The study calibrator condition.
- For *Efficiency*, the default values are used, as no standard curve was calculated.

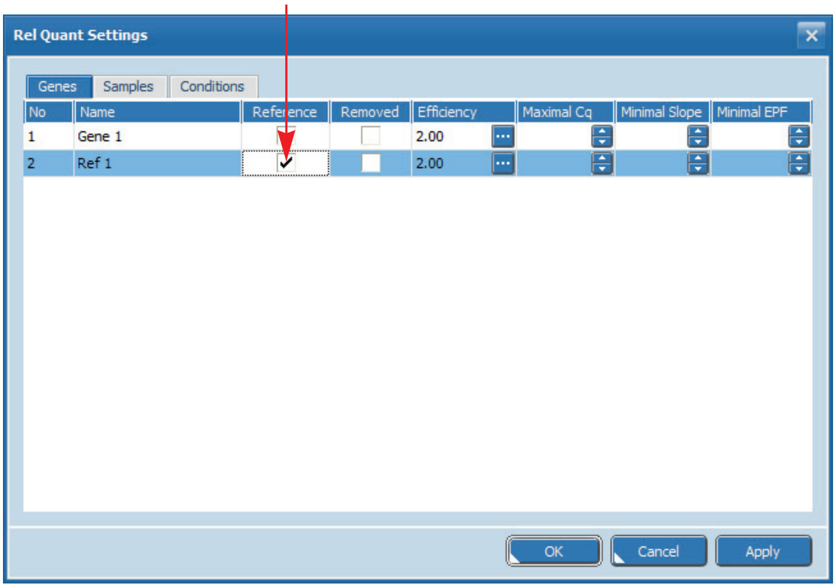
To specify the reference gene

1. In the tool bar, choose the *Analysis Settings* icon.



The *Rel Quant Settings* dialog box opens.

2. On the *Genes* tab, select the *Reference* check box for *Ref 1*.

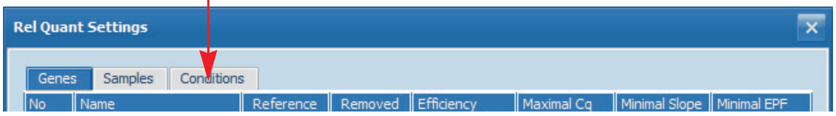


Choose *OK*.
The gene *Ref 1* is specified as the reference gene.

To specify the study calibrator condition

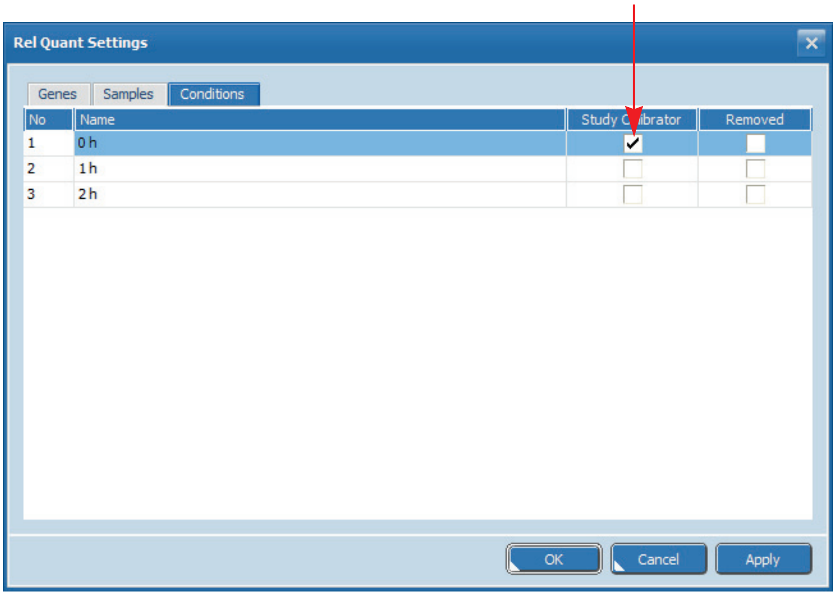
The study calibrator condition is used to normalize all sample-specific measurements to a common basis.

1. In the *Rel Quant Settings* dialog box, choose the *Conditions* tab.



The *Conditions* tab opens.

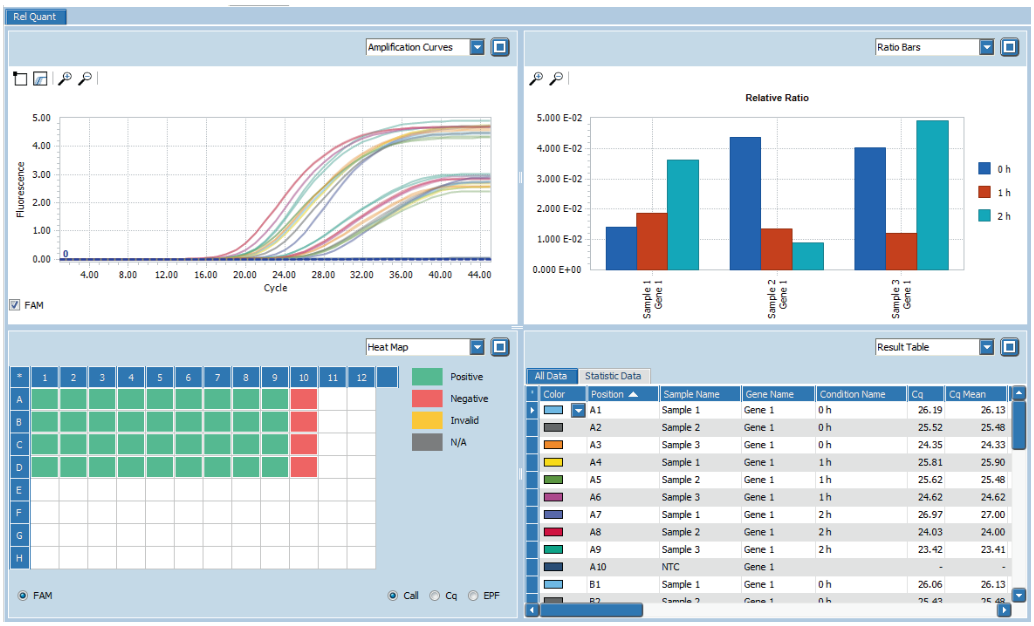
2. On the *Conditions* tab, select the *Study Calibrator* check box for the condition *0 h.*



Choose *OK*. The condition *0 h* (0 hours) is specified as the study calibrator condition.

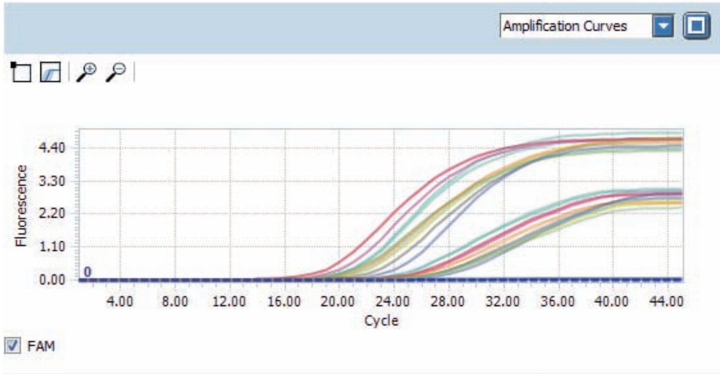
Results are recalculated based on the relative ratio measured for each sample at the study calibrator condition, that is, the start of the experiment.

The *Ratio Bars* chart is displayed.



3.5.3 Amplification curves

On the *Rel Quant* tab, amplification curves display the fluorescence intensity against the number of cycles in the amplification program. There is one curve for each sample that has a gene labeled with the selected dye.

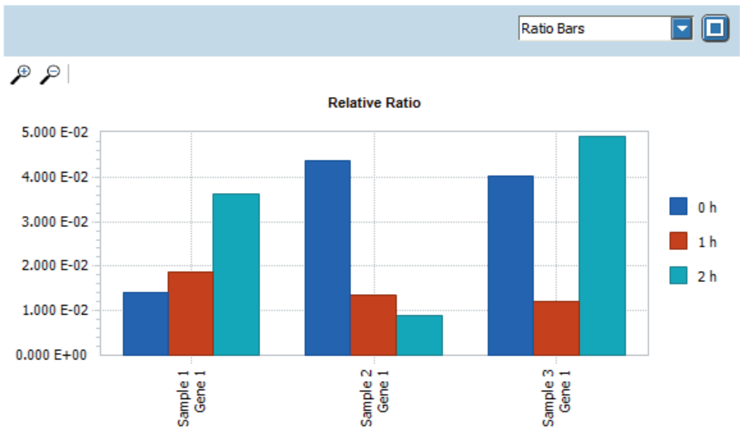


1 Check the amplification curves chart for correct amplification of target gene *Gene 1* and reference gene *Ref 1*.

3.5.4 Ratio bars

The *Ratio Bars* chart shows the corresponding ratio for each gene, sample, and condition. Each bar represents a ratio. The *Relative Ratio* chart is displayed by default.

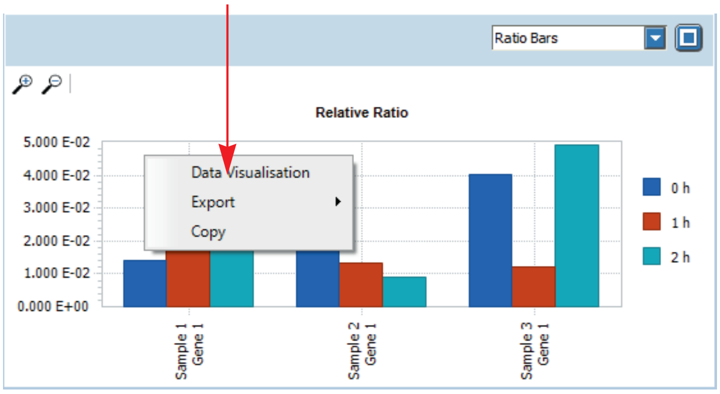
For detailed information on the possible ratios, refer to the LightCycler® 96 System Operator's Guide, chapter A, section "Relative quantification analysis".



C

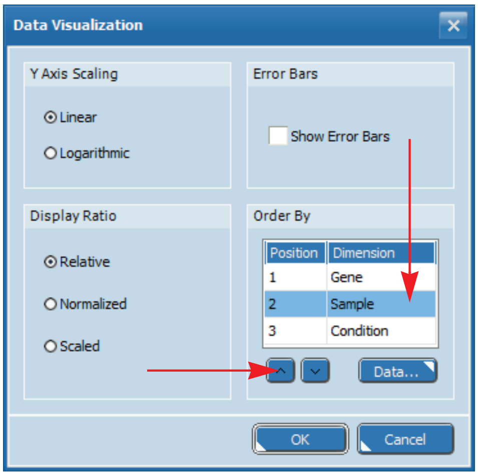
To customize the visualization in the Ratio Bars chart

1. Right-click the *Ratio Bars* chart and choose *Data Visualization* on the shortcut menu.



The *Data Visualization* dialog box opens.

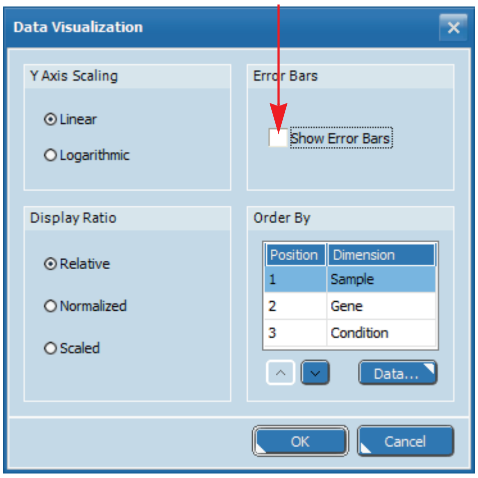
2. In the *Order By* section, choose *Sample* and then the up arrow to move the item to *Position 1*.



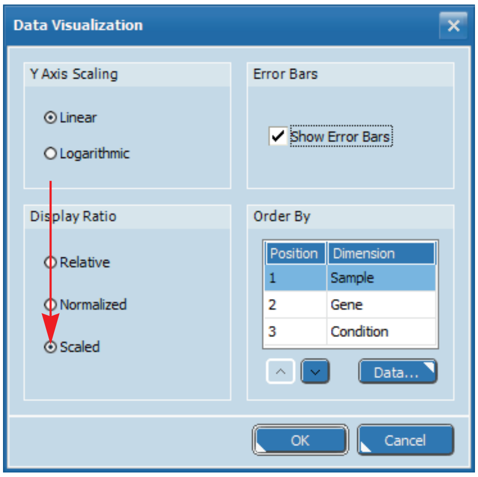
Sample is moved up to *Position 1*.
This means that the bars in the chart are ordered according to the following priority:

- 1: samples
- 2: genes
- 3: conditions

3. In the *Error Bars* section, select the *Show Error Bars* option to display error bars in the *Ratio Bars* chart.



4 In the *Display Ratio* section, select *Scaled,* as a study calibrator for calculating scaled ratios is used in this example (see section *To specify the study calibrator condition*, on page 110).

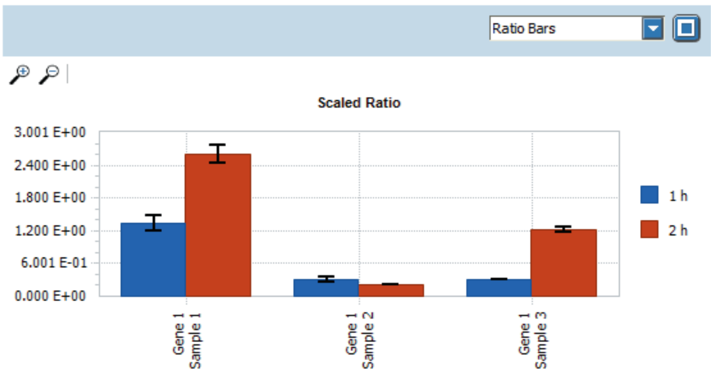


If no study calibrator condition is specified, keep the default option 'Relative' in the 'Display Ratio' section. Then the chart shows no bars for the reference genes.

5 Choose *OK.*

Results are recalculated based on the relative ratio measured for each sample at the study calibrator condition, that is, the start of the experiment.

The *Scaled Ratio* chart is displayed. This chart shows no bars for the study calibrator (condition *0 h*).

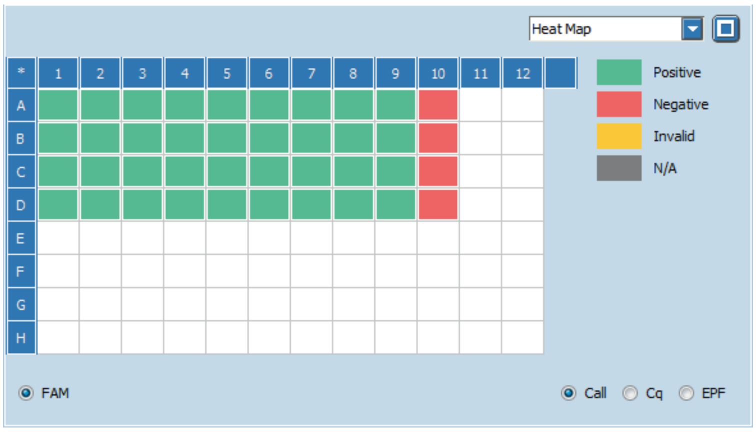


- *Sample 1*: The scaled ratio for gene 1 after two hours (condition *2 h*) is higher than the scaled ratio after one hour (condition *1 h*).
- *Sample 2*: The scaled ratio for gene 1 after two hours (condition *2 h*) is lower than the scaled ratio after one hour (condition *1 h*).
- *Sample 3*: The scaled ratio for gene 1 after two hours (condition *2 h*) is higher than the scaled ratio after one hour (condition *1 h*).

3.5.5 Heat map

The heat map shows an image of the multiwell plate used in the experiment for the specified channel (FAM).

A heat map only displays the samples contained in the sample list. Samples not contained in the sample list (that is, cleared wells and removed samples and genes) are displayed in white and samples excluded from calculation are displayed in gray.



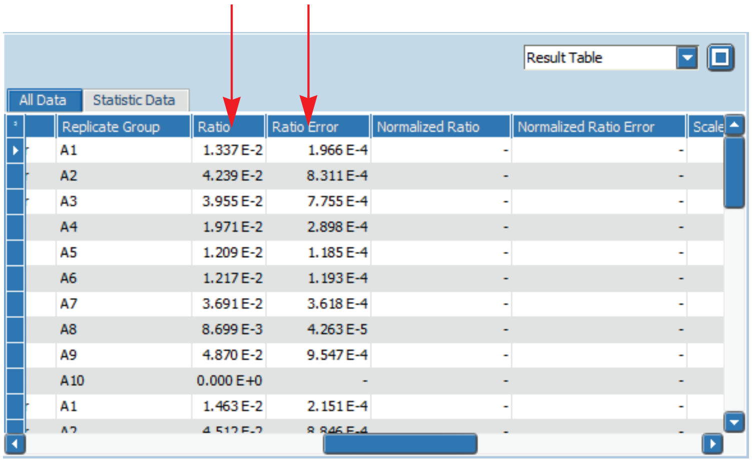
In this example the *Call* heat map is displayed. It shows the *Call* status of all samples contained in the sample list.

- The samples in columns 1 to 9 are green, that is, *Positive*.
- The NTCs in column 10 are red, that is, *Negative*.

3.5.6 Result table

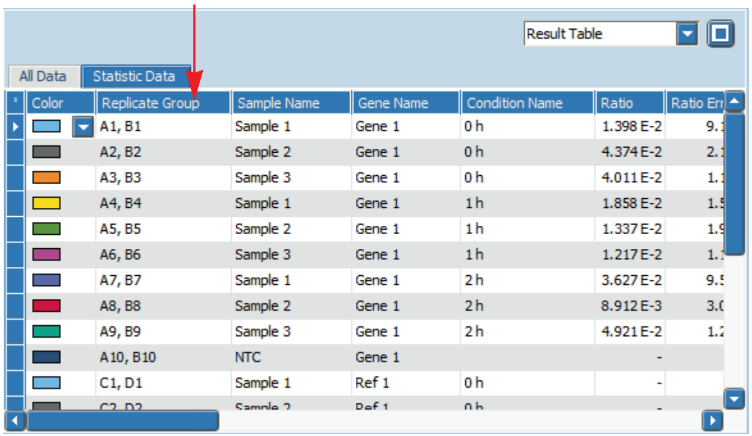
The result table displays the calculated data results of the relative quantification on two different tabs.

- The *All Data* tab shows the values for *Ratio* and *Scaled Ratio*, each with the corresponding errors.



Replicate Group	Ratio	Ratio Error	Normalized Ratio	Normalized Ratio Error	Scale
A1	1.337 E-2	1.966 E-4	-	-	
A2	4.239 E-2	8.311 E-4	-	-	
A3	3.955 E-2	7.755 E-4	-	-	
A4	1.971 E-2	2.898 E-4	-	-	
A5	1.209 E-2	1.185 E-4	-	-	
A6	1.217 E-2	1.193 E-4	-	-	
A7	3.691 E-2	3.618 E-4	-	-	
A8	8.699 E-3	4.263 E-5	-	-	
A9	4.870 E-2	9.547 E-4	-	-	
A10	0.000 E+0	-	-	-	
A1	1.463 E-2	2.151 E-4	-	-	

- The *Statistic Data* tab summarizes all data for samples in replicate groups.



Color	Replicate Group	Sample Name	Gene Name	Condition Name	Ratio	Ratio Err
	A1, B1	Sample 1	Gene 1	0 h	1.398 E-2	9.1
	A2, B2	Sample 2	Gene 1	0 h	4.374 E-2	2.1
	A3, B3	Sample 3	Gene 1	0 h	4.011 E-2	1.1
	A4, B4	Sample 1	Gene 1	1 h	1.858 E-2	1.5
	A5, B5	Sample 2	Gene 1	1 h	1.337 E-2	1.9
	A6, B6	Sample 3	Gene 1	1 h	1.217 E-2	1.1
	A7, B7	Sample 1	Gene 1	2 h	3.627 E-2	9.5
	A8, B8	Sample 2	Gene 1	2 h	8.912 E-3	3.0
	A9, B9	Sample 3	Gene 1	2 h	4.921 E-2	1.2
	A10, B10	NTC	Gene 1		-	
	C1, D1	Sample 1	Ref 1	0 h	-	

For detailed information on all calculated results displayed in the 'Result Table' view, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "Relative quantification".

1. In the *Result Table* view, check if the results show the expected dynamic range.

3.6 Exporting result data

You can export the following result data to Microsoft Word or Excel:

- The result table as a text file
- The *Amplification Curves* chart and the *Standard Curves* chart as a PNG file, GIF file, or text file.
- The *Ratio Bars* chart as a PNG file or GIF file.

For detailed information on how to export result data, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "Exporting analysis results".

4 T_m calling

When performing real-time PCR in the presence of an intercalating dye such as SYBR Green I, the increase in fluorescence is proportional to the amount of newly generated dsDNA. The SYBR Green I dye, however, binds all dsDNA, including specific and non-specific PCR products. This means that the presence of primer-dimers and other non-specific products can affect the quality of real-time PCR data produced using SYBR Green I. This also means that a melting curve analysis after the PCR is essential to verify product identification.

A melting curve analysis in the presence of SYBR Green I identifies PCR products by GC content and length. Both parameters determine the melting temperature (T_m) of a DNA fragment. Due to their small size, primer-dimers usually melt at a lower temperature than the specific PCR product.

For detailed information, refer to the LightCycler® 96 System Operator's Guide, chapter A, sections "Detection formats" and "Analysis principles".

4.1 Experiment overview

C

The following example describes how to set up, run, and analyze a real-time PCR experiment using the SYBR Green I detection format. A T_m calling analysis for product identification enables the verification of amplification results.

The assay is performed using a LightCycler® 480 Multiwell Plate 96, white. Each sample is set up in duplicate.

Samples	7 DNA samples (unknown concentration)
Reagents	▶ FastStart Essential DNA Green Master (2 x conc.) ▶ Primer mix (20 x conc.), containing forward and reverse primer, 5 µM each

4.2 Setting up the samples

To set up the samples:

- Include a no template control (NTC), see section *Controls* below.
- Prepare the PCR mix, see section *PCR mix*, on page 117.
- Pipette the sample dilution and the PCR mix, see section *Pipetting scheme*, on page 117.
- Centrifuge the multiwell plate, see section *Centrifugation*, on page 117.

Continously cool the samples during setup by keeping the tubes on ice.

Controls

To ensure the absence of contaminating nucleic acids in PCR reagents, it is highly recommended that you include a no template control (NTC) in your experiment.

PCR mix

When setting up the PCR mix, compensate for pipetting losses. We recommend preparing PCR mixes with 10% extra volume.

The table below shows the components included in the PCR mix for one 20 µl reaction. The PCR mix volume is 15 µl for a subsequent sample input of 5 µl per reaction.

Component	Concentration	Volume	Final conc.
Water, PCR grade		4 µl	
FastStart Essential DNA Green Master	2 x conc.	10 µl	1 x conc.
Primer mix	20 x conc.	1 µl	0.25 µM each
Total volume (without sample DNA)		**15 µl**	

Pipetting scheme

1. Pipette 15 µl of the PCR mix into 16 wells of the multiwell plate according to the following scheme.
2. Pipette 5 µl of sample into the corresponding wells according to the following scheme.
3. For the NTCs, pipette 5 µl of water (instead of DNA sample) into the corresponding wells according to the following scheme.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NTC										
B	Sample1	Sample1										
C	Sample2	Sample2										
D	Sample3	Sample3										
E	Sample4	Sample4										
F	Sample5	Sample5										
G	Sample6	Sample6										
H	Sample7	Sample7										

Centrifugation

1. Seal the multiwell plate with the LightCycler® 480 Sealing Foil using the sealing foil applicator (provided with the system package).
2. Centrifuge the multiwell plate at 1500 x *g* for 2 minutes in a standard swing-bucket centrifuge, using a rotor for multiwell plates.

   Make sure you balance the multiwell plate with a suitable counterweight (for example, another multiwell plate).

4.3 Experiment run parameters

For detailed information on how to program an experiment, see one of the following sections:
Programming the experiment with the LightCycler® 96 Application Software, on page 18.
Programming the experiment with the LightCycler® 96 Instrument Software, on page 30.

For an experiment using SYBR Green I, run a PCR profile including a 3-step amplification program and subsequent melting. The experiment includes the run parameters for the temperature profile, the detection format, and the reaction volume. These parameters are listed in the following tables.

Temperature profile

For detailed information on how to program a temperature profile, see the following sections:
For working with the LightCycler® 96 Application Software: *Creating the temperature profile, on page 21.*
For working with the LightCycler® 96 Instrument Software: *Creating the temperature profile, on page 33.*

For this example, use the following heating and cooling cycles:

Programs		Steps			
Name	**Number of cycles**	**Ramp (°C/s)**	**Duration (s)**	**Target (°C)**	**Acquisition Mode**
Preincubation	1	4.4	600	95	None
3-step amplification	45	4.4	10	95	None
		2.2	10	55	None
		4.4	10	72	Single
Melting	1	4.4	5	95	None
		2.2	60	65	None
		-		97	Continouous 5 readings /°C
Cooling	1	1.0	30	37	None

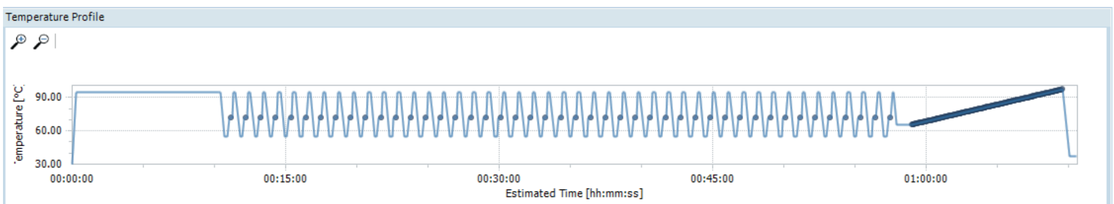
It is not necessary to add a separate cooling program at the end of the run. At the end of each run, the samples are automatically cooled to +37°C.

For the ramp rate for heating and cooling (*Ramp (°C/s)*), the default values are used in this example.

For the steps of the amplification program, the following default settings are used in this example:

- LightCycler® 96 Application Software: For *Gradient* and *Touch down*, the default settings are used.
- LightCycler® 96 Instrument Software: For *Mode*, the default setting *Standard* is used.

In the *Temperature Profile* window area, the following graphical summary of the programs selected for the experiment and their temperature and time settings is displayed.



Detection format

Selecting the dye (SYBR Green I) for this mono-color experiment determines the channel combination for the measurement during the run. For all other parameters, the default values are used in this example.

For detailed information on how to specify the detection format, see the following sections:
For working with the LightCycler® 96 Application Software: To specify the detection format for the experiment, on page 25.
For working with the LightCycler® 96 Instrument Software: To specify the detection format for the experiment, on page 38.

For this example, use the following channel:

Dye	Channel
SYBR Green I	470/514

Reaction volume

As the LightCycler® 96 Instrument does not validate the reaction volume, ensure that the specified reaction volume matches the volume pipetted into the wells of the multiwell plate.

For detailed information on how to specify the reaction volume, see the following sections:
For working with the LightCycler® 96 Application Software: To specify the reaction volume for the experiment, on page 25.
For working with the LightCycler® 96 Instrument Software: To specify the reaction volume for the experiment, on page 40.

For this example, use the following reaction volume:

Reaction volume
20 µl

Experiment run

Once you have set up the samples and defined the experiment run parameters,you can start the run. For detailed information on how to run the experiment, see section *Running the experiment*, on page 41.

4.4 Editing the sample data

For editing the sample data, the experiment must be opened in the LightCycler® 96 Application Software. You can edit the sample list before or after the run, depending on your preferred routine.

The LightCycler® 96 Application Software offers two different views for editing the samples: the plate view and the table view. This user training guide describes how to edit the samples using the plate view, which shows the samples in 96 wells laid out to match the physical instrument. Changes in the plate view are immediately displayed in the table view and vice versa.

For detailed information on the ‘Sample Editor’ tab, refer to the LightCycler® 96 System Operator’s Guide, chapter B, section "Sample Editor tab".

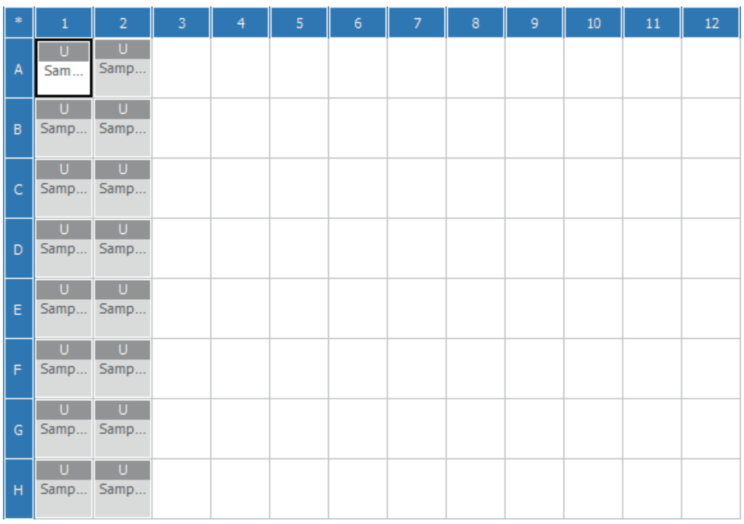
To edit the sample list:

- Clear empty wells to eliminate them from the analysis, see section *Empty wells* below.
- Edit the sample names, see section *Sample names*, on page 121.
- Edit the sample types, see section *Sample types*, on page 121.
- Assign a gene for the dye, see section *To assign a gene for the dye*, on page 122.

Empty wells

For detailed information on how to clear empty wells, see section To clear empty wells, on page 49.

For this example, clear the wells in columns 3 to 12 (see also the multiwell plate image below).

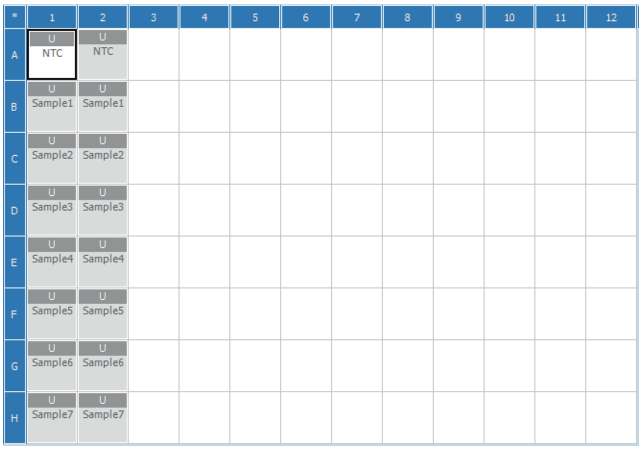


Sample names

For detailed information on how to edit the sample names, see section [*To edit the samples*]*, on page 51.*

For this example, the following sample names apply (see also the multiwell plate image below):

Name	Samples in the plate view
NTC	For the negative control in wells A1 and A2.
Sample1 to Sample7	For the unknown samples in rows B to H. Each sample is set up in duplicate.

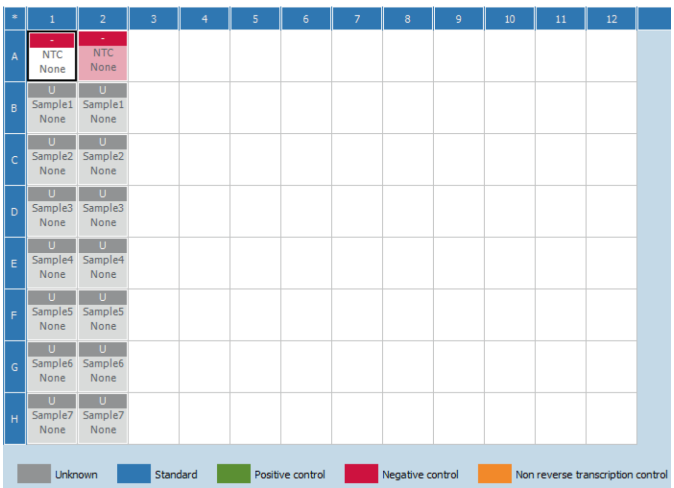


Sample types

For detailed information on how to edit the sample types, see section [*To edit the samples*]*, on page 51.*

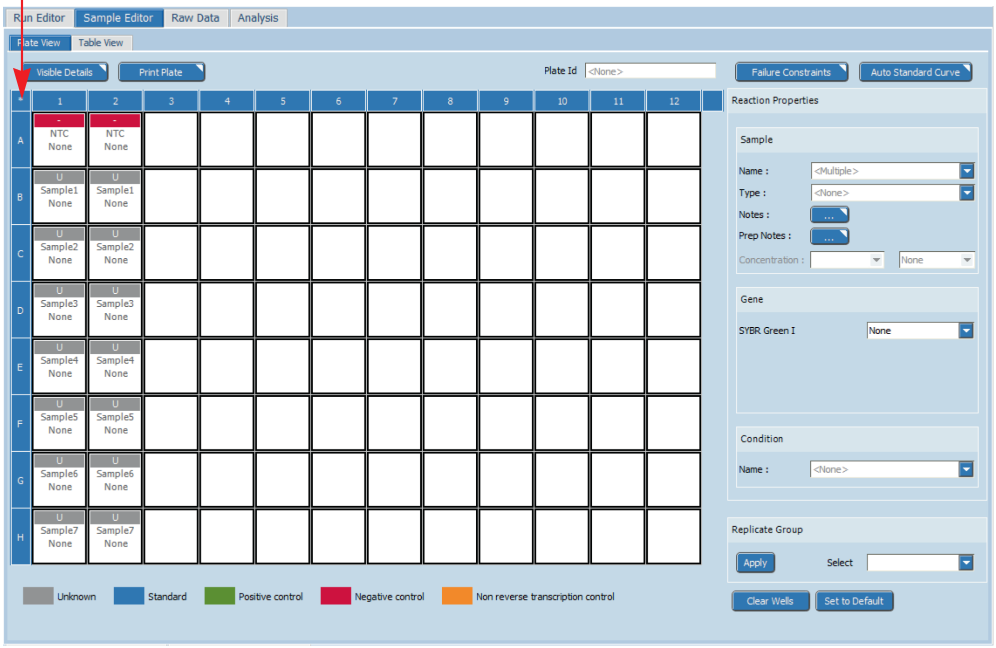
In this example the following sample types apply:

Type	Samples in the plate view
Negative control	For the samples *NTC*
Unknown (default)	For the samples *Sample1* to *Sample7*



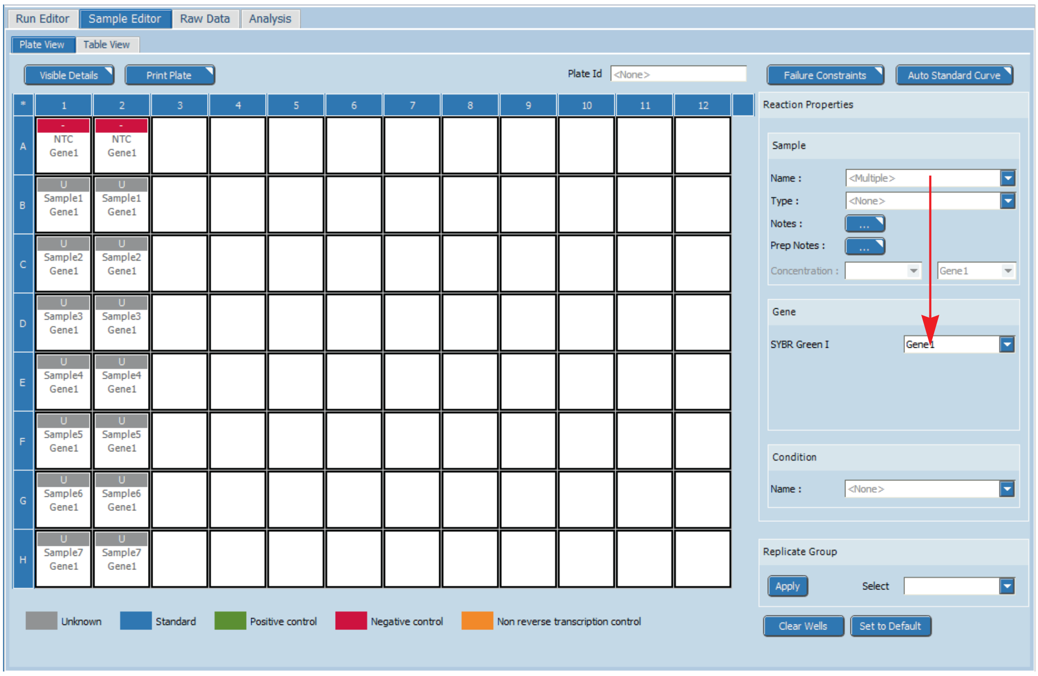
To assign a gene for the dye

1. In the multiwell plate image, choose the asterisk (*) in the upper left corner to select all wells.



2. In the *Reaction Properties* window area, choose the text field next to the *SYBR Green I* dye.

3. Type in *Gene 1*.



4.5 Analyzing the results

For detailed information on the 'Analysis' tab, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "T_m Calling".

For detailed information on working with tables and graphs, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "General software conventions".

To analyze the calculated results of the T_m Calling application:

- Create the Abs Quant analysis and the T_m Calling analysis, see section *Creating the analysis* below.
- Optional: Specify the melting analysis parameters, see section *Analysis settings*, on page 125.
- In the different views of the *Abs Quant* and *Tm Calling* tabs, check the analysis results and customize the result data if necessary:
  - On the *Abs Quant* tab, check the amplification curves for plausibility, see section *Amplification curves*, on page 125.
  - For the *Melting Curves* view, see section *Melting curves*, on page 126.
  - In the *Melting Peaks* view, specify a melting peak area, see section *To specify the melting peak area Area 1*, on page 126.
  - For the *Heat Map* view, see section *Heat map*, on page 127.
  - In the *Result Table* view, check the melting temperature values, see section *Result table*, on page 128.

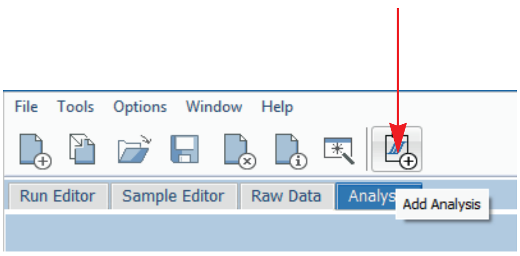
4.5.1 Creating the analysis

To create the Abs Quant analysis

For detailed information on how to create the Abs Quant analysis, see section To create the Abs Quant analysis, on page 66.

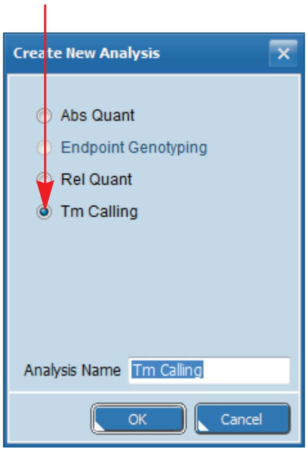
To create the T_m Calling analysis

1. Open the *Analysis* tab.
2. In the tool bar, choose the *Add Analysis* icon to add a new analysis.



The *Create New Analysis* dialog box opens.

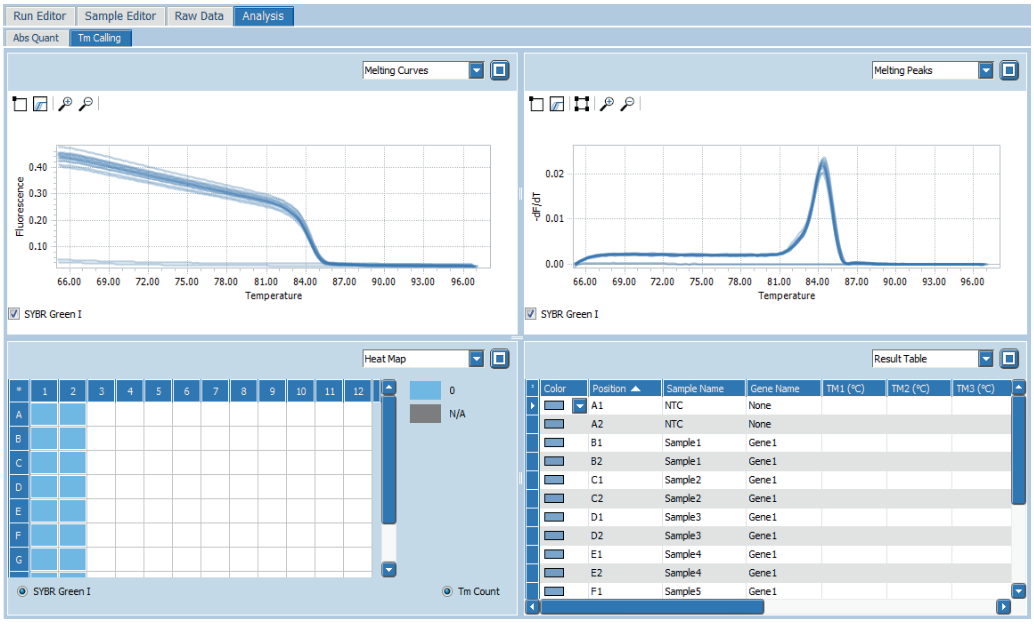
3 Choose *Tm Calling*.



4 Choose *OK*.

The *Tm Calling* tab displays four different views for the experiment using default values:

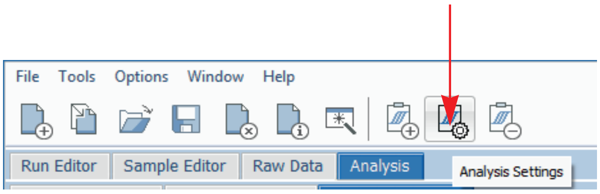
- *Melting Curves*
- *Melting Peaks*
- *Heat Map*
- *Result Table*



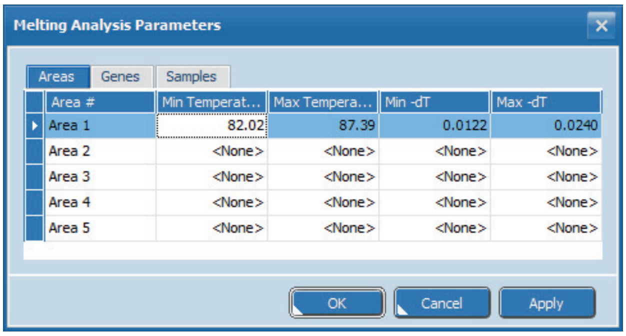
4.5.2 Analysis settings

Optional: To specify the analysis settings

1. In the tool bar, choose the *Analysis Settings* icon.



The *Melting Analysis Parameters* dialog box opens.

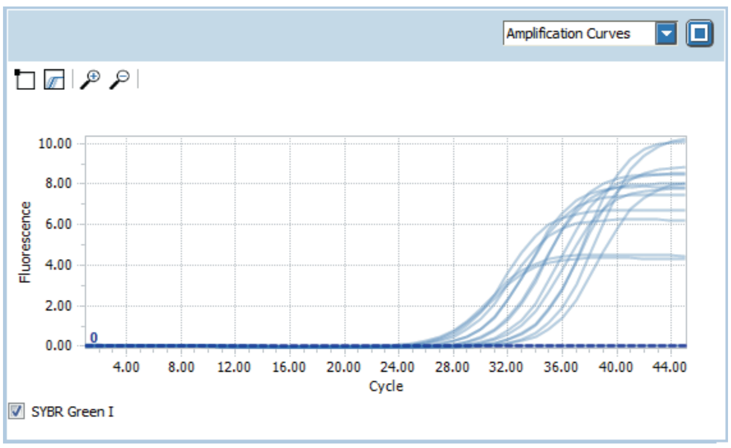


2. In the *Melting Analysis Parameters* dialog box, specify the analysis-specific settings, for example:
   - On the *Areas* tab, specify the areas where melting peaks are to be called. An area is displayed as a rectangle which represents a temperature range and a fluorescence threshold.

     The temperature and fluorescence settings in the Melting Analysis Parameters dialog box correspond to the area marking in the Melting Peaks graph. For detailed information, see section Melting peaks, on page 126.
   - On the *Genes* tab, remove a gene from the analysis.
   - On the *Samples* tab, remove samples from the analysis.

4.5.3 Amplification curves

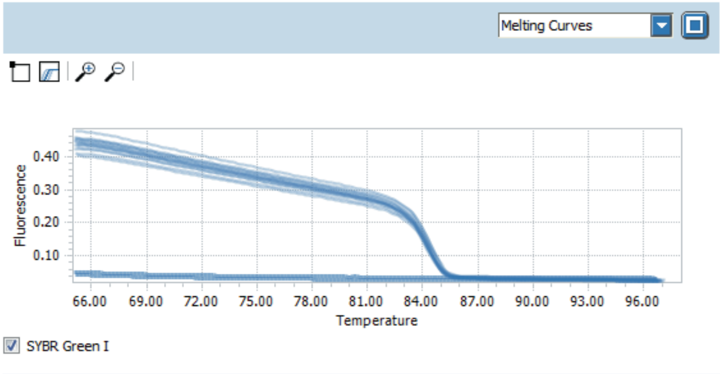
On the *Abs Quant* tab, amplification curves display the fluorescence intensity against the number of cycles in the amplification program. An amplification curves graph is only available when an amplification program has been performed.



1. Check the *Amplification Curves* chart for correct amplification.

4.5.4 Melting curves

On the *Tm Calling* tab, melting curves show the raw fluorescence intensity against the temperature in °C.



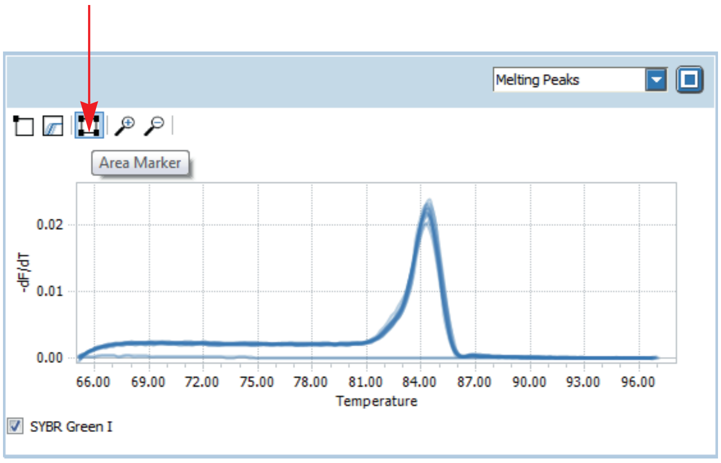
4.5.5 Melting peaks

The melting peaks graph displays the first negative derivative of the fluorescence with respect to the temperature in the melting program (-dF/dT). The graph shows a single melting peak, meaning that no additional by-products are detected in the experiment.

To specify the melting peak area Area 1

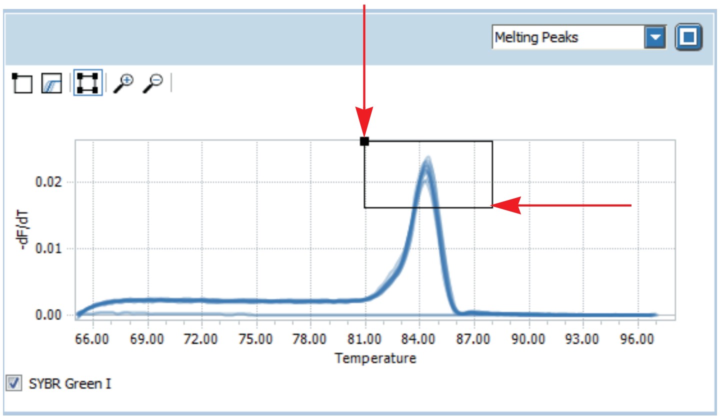
The area marking in the melting peaks graph corresponds to the settings on the 'Areas' tab in the 'Melting Analysis Parameters' dialog box, see section Analysis settings, on page 125.

1. In the *Melting Peaks* view, choose *Area Marker* to indicate that a marking action follows.



2 To specify the melting peak area *Area 1*:

- Move the cursor to the point for the top left corner of the area.
- Hold down the left mouse button and drag the cursor to the point for the bottom right corner of the area.
- Release the mouse button.



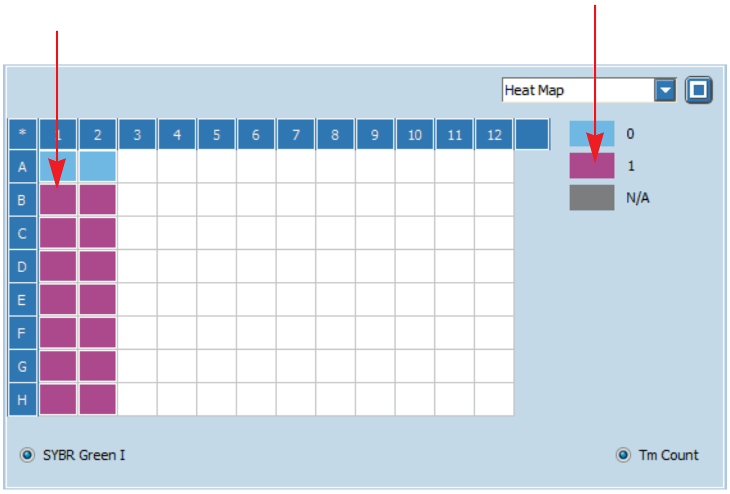
- To change the size of the specified area, grab the relevant side or corner of the rectangle and drag it accordingly.

The LightCycler® 96 Application Software allows five areas to be defined in one graph. To specify an additional area, one of the existing areas must be deleted.

4.5.6 Heat map

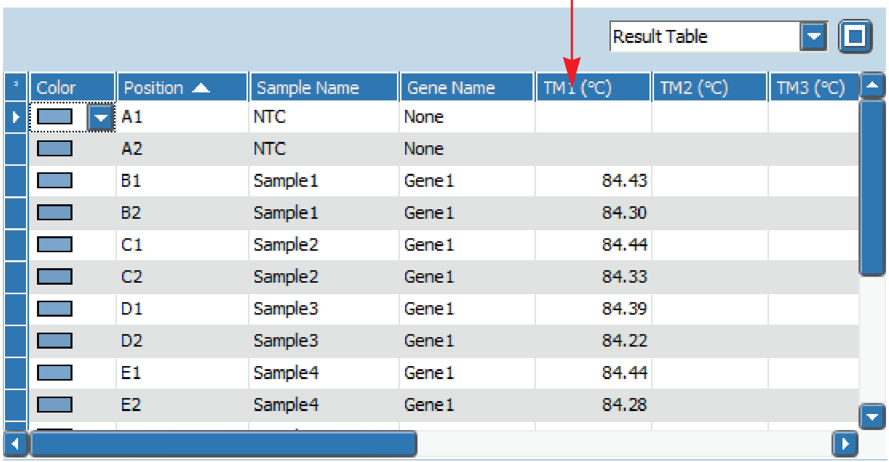
The heat map shows the melting temperatures for the melting peak area *Area 1*.

All samples for which the melting peak lies in the melting peak area *Area 1* are assigned the corresponding area number *1* and the color magenta, in this example the samples B1 to H2.



4.5.7 Result table

The result table displays the results of the T_m calling analysis. The *TM1(°C)* column shows the melting temperature (T_m) of any peak identified in *Area 1* in the melting peaks graph. Where no peak is present, the table cells are blank.



Result Table

Color	Position	Sample Name	Gene Name	TM1 (°C)	TM2 (°C)	TM3 (°C)
	A1	NTC	None			
	A2	NTC	None			
	B1	Sample1	Gene1	84.43		
	B2	Sample1	Gene1	84.30		
	C1	Sample2	Gene1	84.44		
	C2	Sample2	Gene1	84.33		
	D1	Sample3	Gene1	84.39		
	D2	Sample3	Gene1	84.22		
	E1	Sample4	Gene1	84.44		
	E2	Sample4	Gene1	84.28		

4.6 Exporting result data

You can export the following result data to Microsoft Word or Excel:

- The result table as a text file
- The result graphs as a PNG file, GIF file, or text file.

For detailed information on how to export result data, refer to the LightCycler® 96 System Operator's Guide, chapter B, section "Exporting analysis results".

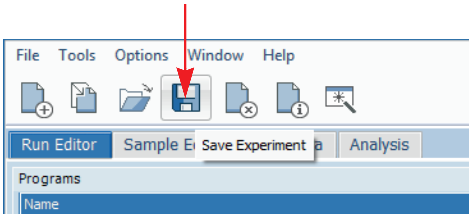
Shutting down the system

You shut down the system as follows:

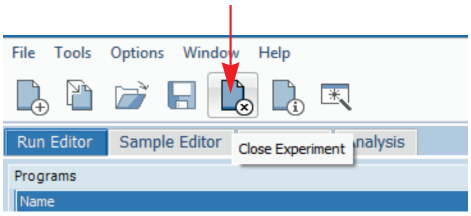
- Exit the LightCycler® 96 Application Software, see section *To exit the LightCycler® 96 Application Software*, below.
- Exit the LightCycler® 96 Instrument Software and switch off the instrument, see section *To exit the LightCycler® 96 Instrument Software and switch off the instrument*, on page 130.

To exit the LightCycler® 96 Application Software

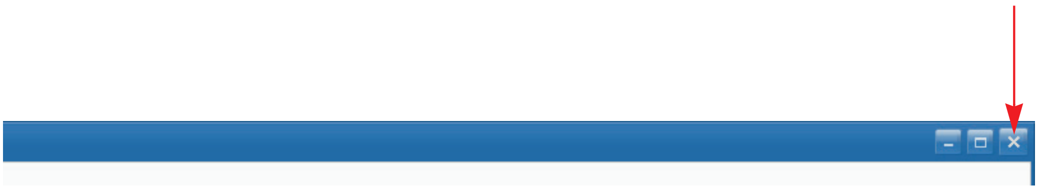
1. Ensure that all necessary data is saved.
   In the tool bar, choose the *Save Experiment* icon.



2. In the tool bar, choose the *Close Experiment* icon to close any opened experiments.

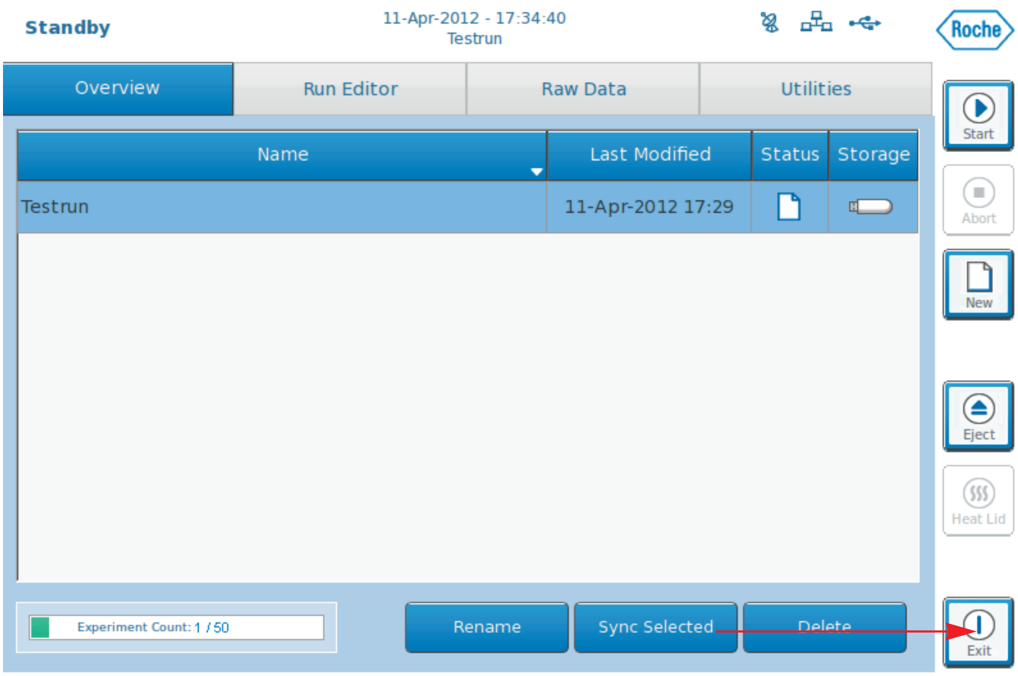


3. In the title bar of the main window, choose the (Close) button to exit the software.



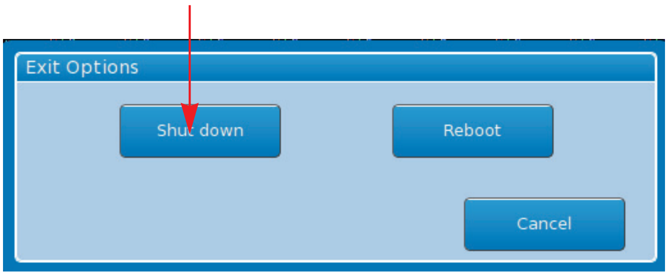
To exit the LightCycler® 96 Instrument Software and switch off the instrument

1. In the global action bar of the LightCycler® 96 Instrument Software main window, choose *Exit*.



The *Exit Options* dialog box opens.

2. Choose *Shut down*.



The instrument software completes all currently running actions and shuts down.

D

3. Use the mains power switch on the back of the instrument to switch off the instrument.

   Do not switch off the instrument before shutting down the software, otherwise data could be lost.

D

Appendix

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For life science research only. Not for use in diagnostic procedures.

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68305 Mannheim
Germany

www.roche-applied-science.com

① 0812

LightCycler® 96 System Operator's Guide, Version 1.0

Software Version 1.0

August 2012

For life science research only.
Not for use in diagnostic procedures.

Prologue

I Revision history

Operator's Guide Version	Software Version	Revision Date	Changes
V1.0	V1.0	August 2012	First edition

Questions or comments regarding the contents of this Operator's Guide can be directed to your local Roche Diagnostics representative.

Every effort has been made to ensure that all the information contained in the LightCycler® 96 System Operator's Guide is correct at the time of publishing.

However, Roche Diagnostics GmbH reserves the right to make any changes necessary without notice as part of ongoing product development.

II Contact addresses

Manufacturer	Roche Diagnostics GmbH Sandhofer Straße 116 68305 Mannheim Germany
Distribution	Roche Diagnostics GmbH Sandhofer Straße 116 68305 Mannheim Germany
Distribution in USA	Roche Diagnostics 9115 Hague Road PO Box 50457 Indianapolis, IN 46250 USA

III Declaration of conformity

The instrument meets the requirements laid down in Council Directive 2004/108/EC relating to "Electromagnetic Compatibility" and Council Directive 2006/95/EC relating to "Low Voltage Equipment".

The following standards were applied: IEC/EN 61326-1 (EMC), IEC/EN 61010-1 (Safety), and IEC/EN 61010-2-081.

IV Warranty

The warranty conditions are specified in the sales contract. Contact your local Roche Diagnostics representative for further information.

Any unauthorized modification of the LightCycler® 96 Instrument and/or the LightCycler® 96 Software will result in the invalidation of the guarantee and service contract.

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VI Intended use

The LightCycler® 96 Instrument is intended for performing rapid, accurate polymerase chain reaction (PCR) in combination with real-time, online detection of DNA-binding fluorescent dyes or labeled probes, enabling quantification or characterization of a target nucleic acid.

The LightCycler® 96 System is intended for life science research only. It must only be used by laboratory professionals trained in laboratory techniques, who have studied the Instructions for Use of this instrument. The LightCycler® 96 Instrument is not for use in diagnostic procedures.

The LightCycler® 96 System is intended for indoor use only.

VII License statements for the LightCycler® 96 Instrument

NOTICE: This product may be subject to certain use restrictions. Before using this product please refer to the Online Technical Support page (*http://technical-support.roche.com*) and search under the product number or the product name, whether this product is subject to a license disclaimer containing use restrictions.

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IX Open Source licenses

Portions of the LightCycler® 96 Software might include one or more Open Source or commercial software programs. For copyright and other notices and licensing information regarding such software programs included with LightCycler® 96 Software, please refer to the USB drive provided with the product.

X Preamble

Before setting up operation of the LightCycler® 96 System, it is important to read the user documentation completely. Non-observance of the instructions provided or performing any operations not stated in the user documentation could produce safety hazards.

XI Contents of this operator's guide

This operator's guide describes the operation of the LightCycler® 96 Instrument. It contains the following chapters:

Chapter A. System description contains the installation requirements of the LightCycler® 96 System and a description of the system's components and disposables. This chapter contains the installation and configuration procedure and also gives a short overview of the basic PCR analysis workflow.

Chapter B. LightCycler® 96 Application Software explains the functions of the LightCycler® 96 Application Software, running on the customer's computer, in detail.

Chapter C. LightCycler® 96 Instrument Software explains the functions of the LightCycler® 96 Instrument Software, running on the LightCycler® 96 Instrument, in detail.

Chapter D. Cleaning and care describes the cleaning and care procedures required for the LightCycler® 96 Instrument.

Chapter E. Troubleshooting provides troubleshooting and error code information for the LightCycler® 96 Instrument.

Chapter F. Appendix contains ordering information and the index.

XII Conventions used in this guide

Text Conventions

To present information consistently and make it easy to read, the following text conventions are used in this guide:

Numbered list	Steps in a procedure that must be performed in the order listed.
Italic type	Used for operating instructions for the LightCycler® 96 Software. In addition, important notes and information notes are shown in italics.
Blue italic type	Refers to a different section in this Operator's Guide, which should be consulted.
[ ]	Square brackets indicate keys on the keyboard.
< >	Angle brackets indicate variables to be replaced with appropriate values.

Abbreviations

The following abbreviations are used in this guide:

Abbreviation	Meaning
AC	Alternating Current
AT/GC ratio	Adenine-Thymine/Guanine-Cytosine ratio
Cq	Quantification Cycle
CCD	Charge-Coupled Device
CSV	Comma-Separated Value
Cy5	Cyanine 5
DNS	Domain Name Service
DHCP	Dynamic Host Configuration Protocol
dsDNA	Double-stranded DNA
E	Efficiency
EPF	Endpoint Fluorescence
FAM	6-Carboxyl Fluorescein
FRET	Fluorescence Resonance Energy Transfer
GOI	Gene of Interest
HEX	Carboxyl-2',4,4',5',7,7'-Hexachlorofluorescein
HRM	High Resolution Melting
LED	Light Emitting Diode
LAN	Local Area Network
NTC	No Template Control
NRTC	Non Reverse Transcription Control
PCR	Polymerase Chain Reaction
PE	Protection Earth
PNG	Portable Network Graphics
qPCR	Quantitative Real-Time PCR

Abbreviation	Meaning
RDML	Real Time Data Management Language
SNP	Single Nucleotide Polymorphism
SD	Standard Deviation
SVG	Scalable Vector Graphics
SYBR	SYBR Green I (a common double-stranded binding dye)
TCP/IP	Transmission Control Protocol/Internet Protocol
T_m	Melting Temperature
UPS	Uninterruptible Power Supply
USB	Universal Serial Bus
VIC	Reporter Dye for Hydrolysis Probes

Symbols used in this guide

Symbol	Meaning	Description
	WARNING	This symbol is used to alert you to the presence of important operating and maintenance instructions in the literature accompanying the instrument. There are no user-serviceable parts inside the instrument.
	HOT SURFACE	This symbol is used to label potentially hot instrument surfaces.
	BIO HAZARD	This symbol is used to indicate that certain precautions must be taken when working with potentially infectious material.
	DANGEROUS ELECTRICAL VOLTAGE	This symbol is used to indicate the danger of personal injury due to dangerous electrical voltage. Refers to an imminent danger that may result in death or serious personal injury.
	KEEP HANDS CLEAR	This symbol is used to indicate the risk of crushing hands in movable parts.
	IMPORTANT NOTE	Information critical to the success of the procedure or use of the product.
	INFORMATION NOTE	Additional information about the current topic or procedure.
▶ ▶ ▶		Procedure continued on next page.
■		End of procedure.

Symbols used on the instrument

Symbol	Meaning	Description
	MANUFACTURER OF DEVICE	Roche Diagnostics GmbH Sandhofer Strasse 116, D-68305 Mannheim Germany Made in Switzerland
	CE MARK	The CE mark on the instrument type plate indicates conformity with requirements of the directives relevant for this instrument.
	WARNING	On the instrument type plate.
	cUL MARK	On the instrument type plate.
	HOT SURFACE	On the loading module.
	BIO HAZARD	On the loading module.
	KEEP HANDS CLEAR	On the instrument housing (Only visible, when the loading unit is ejected).

In addition to these symbols, the following information is provided on the instrument type plate:

- LightCycler® 96
- Instrument serial number in hexadecimal and in 1D barcode
- Power supply and mains power consumption: 100-125/200-240 Vac 50/60 Hz 600 VA

XIII Warnings and precautions

In an emergency, immediately unplug the instrument.

The LightCycler® 96 Instrument must only be used by trained and skilled personnel.

It is essential that the following safety information required for installation and operation of the LightCycler® 96 Instrument is carefully read and observed. Please ensure that this safety information is accessible to all personnel working with the LightCycler® 96 Instrument.

Handling requirements

The LightCycler® 96 Instrument is an electromechanical instrument. There is a potential risk to the user from electric shock or physical injury if the instrument is not used according to the instructions given in this manual.

- Follow all safety instructions printed on or attached to the analytical instrument.
- Observe all general safety precautions which apply to electrical instruments.
- Do not access any electrical parts while the LightCycler® 96 Instrument is connected to the mains power supply.
- Never touch the power cable with wet hands.
- Never open the housing of the LightCycler® 96 Instrument.
- Never clean the instrument without disconnecting the power cable.
- Only authorized service personnel are allowed to perform service or repairs required for this unit.
- Do not use the network cable outdoors.

- Always wear safety goggles and gloves when dealing with toxic, caustic, or infectious materials.

- Although working with highly purified nucleic acids, for your own safety, please regard all biological material as potentially infectious. Handling and disposal of such material should be performed according to local safety guidelines. Spills should be immediately disinfected with an appropriate disinfectant solution to avoid contamination of laboratory personnel or equipment.
- For instructions on cleaning the LightCycler® 96 Instrument, see chapter *Cleaning and care*, on page 231.

The multiwell plate mount may be hot after an experiment run.

Always keep your hands clear, when closing the loading unit.

General Precautions

The LightCycler® 96 System contains software that allows it to be connected to a network. Please be aware that such a connection may have an adverse effect on the product's integrity, through, for example, infection with malicious code (viruses, Trojan horses, etc.) or access by unauthorized third parties, such as intrusion by hackers. Roche therefore highly recommends protecting the product against such risks by taking appropriate and state-of-the-art action.

As the product is not intended to be used within networks without an appropriate firewall and has not been designed for such use, Roche assumes no liability in this regard.

Incorrect positioning of the instrument can cause incorrect results and damage to the equipment. Follow the installation instructions carefully.

Danger of explosion through sparks. Keep all potentially inflammable or explosive material (for example, anesthetic gas) away from the instrument. Spraying liquid on electrical parts can cause a short circuit and result in fire. Keep the cover closed while the instrument is connected to the mains power supply and do not use sprays in the vicinity of the LightCycler® 96 Instrument. During fire fighting operations, disconnect the LightCycler® 96 Instrument from the mains power supply.

Do not disassemble the instrument.

Electrical safety

The LightCycler® 96 Instrument is designed in accordance with Protection Class I (IEC). The housing of the instrument is connected to protection earth (PE) by a cable. For protection against electric shock hazards, the instrument must be directly connected to an approved power source such as a three-wire grounded receptacle for the 115/230 V line. Where only an ungrounded receptacle is available, a qualified electrician must replace it with a properly (PE) grounded receptacle in accordance with the local electrical code. No extension must be used.

Any break in the electrical ground path, whether inside or outside the instrument, could create a hazardous condition. Under no circumstances should the operator attempt to modify or deliberately override the safety features of this instrument. If the power cable becomes cracked, frayed, broken, or otherwise damaged, it must be replaced immediately with the equivalent part from Roche Diagnostics.

Please observe the warnings regarding interactions and non-recommended functions. Also bear in mind the potential scope for misuse; it is advisable to draw attention to the possible consequences.

XIV Disposal of the instrument

Disposal recommendations

All electrical and electronic products should be disposed of separately from the municipal waste system. Proper disposal of your old appliance prevents potential negative consequences for the environment and human health.

	The LightCycler® 96 Instrument must be treated as biologically contaminated hazardous waste. Decontamination (that is, a combination of processes, including cleaning, disinfection, and/or sterilization) is required before reuse, recycling, or disposal. Dispose of the instrument according to local and/or laboratory regulations. For more information, contact your local Roche Diagnostics representative.
	The LightCycler® 96 Instrument USB Drive and the external handheld barcode scanner are covered by the European Directive 2002/96/EC on waste electrical and electronic equipment (WEEE) of the European Parliament and the Council of January 27, 2003. The USB drive and the barcode scanner must be disposed of via designated collection facilities appointed by government or local authorities. For more information on disposing of your product, please contact your city authorities, waste disposal service, or your local Roche Diagnostics representative.

Chapter A
System description

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System description

1 Introduction

The LightCycler® 96 System enables you to perform real-time, online PCR combined with rapid cycling of up to 96 samples.

After monitoring fluorescence during nucleic acid amplification, results can be analyzed, for example, by quantification. The outstanding thermal homogeneity and cycling speed of the LightCycler® 96 System provide exact results in a short time.

The optical detection system offers the flexibility to detect a broad range of sequence-dependent probes (for example, hydrolysis probes) and sequence-independent dyes (for example, SYBR Green).

The LightCycler® 96 Application Software and the LightCycler® 96 Instrument Software provide excellent tools to generate high quality data. Advanced software tools facilitate fast, intuitive navigation, allowing easy programming, data capture and analysis. The new software offers a broad range of supported applications combined with a versatile analysis workflow for each application.

- Acquire relative quantification results in an easy setup of data you provide to the instrument. A correlation matrix will be provided in an attractive, publishable format that takes all relevant parameters into account.
- Easy import and export functions, email notifications after the run, online monitoring features and server-based network capabilities enable you to communicate your data in whichever way serves your needs.

For detailed information on the broad range of software capabilities and their usage, see chapters *LightCycler® 96 Application Software*, on page 77 and *LightCycler® 96 Instrument Software*, on page 183.

Based on the latest improvements, the LightCycler® 96 Instrument employs advanced state-of-the-art instrument parts (for example, novel optical system and thermal block cycler).

For more detailed information on the LightCycler® 96 System, visit the LightCycler® 96 System Special Interest Site at *www.lightcycler96.com*.

2 Specifications of the LightCycler® 96 Instrument

LightCycler® 96 Instrument	Cat. No. 05 815 916 001

2.1 General specifications

Dimensions	40 × 40 × 53 cm (W × D × H)
Weight	Approximately 25 kg
Power supply	100 to 125 V / 200 to 240 V (+/-10%) 50/60 Hz (+/-5%)
Mains power consumption	600 W
Noise level ▶ During run ▶ In standby (block cycler cover switched off)	 < 43 dB (A9) 27 dB (A)
Protection class AC adapter	I
Protection class instrument	I
Electromagnetic emission	Class B according to ▶ EN 61326-1 ▶ 47 CFR, Part 15
Electromagnetic immunity	Compliant with EN 61326-1
Heat output ▶ During run (mean value) ▶ In standby (block cycler cover switched off)	 350 W 100 W

2.1.1 Environmental parameters

Temperatures allowed during transportation/ storage/packaging	-20 to +60°C
Relative humidity allowed during transportation/ storage/packaging	10% to 95%, no condensation
Altitude/pressure allowed during transportation/ storage/packaging	0 to 3000 m above sea level 106 to 70 kPa
Temperatures allowed during operation	+15 to +32°C
Relative humidity allowed during operation	Max. 80% at +32°C, no condensation Min. 30% at +15 to +32°C
Altitude/pressure allowed during operation	0 to 2000 m above sea level 106 to 80 kPa
Atmospheric conditions during operation	Pollution Degree II

2.1.2 Interfaces

The LightCycler® 96 Instrument provides the following external interfaces:

Interface	Device
Ethernet 100 Base T	Connection to a computer for instrument control and data transfer
USB 2.0 (on the right side of the instrument)	Connection to USB drive
USB 2.0 (on the instrument back)	Connection to external handheld barcode scanner

2.2 Technical specifications

All values are determined under standard laboratory conditions.

Number of samples per run	Maximum 96
PCR volume	10 to 50 µl
Processing time	Approximately 40 to 50 minutes for standard PCR protocols

2.3 Specifications of the detection unit

2.3.1 Excitation

Type	White LED
Average lifetime	Approximately 10 000 h

2.3.2 Detection

Type	Charge-Coupled Device (CCD) camera
Integration time ▶ Dynamic mode ▶ Manual mode	 10 ms to 1 s up to 4 s
Integration time selection	Dynamic or manual
Reproducibility	≤ 1% CV
Well-to-well crosstalk	< 0.5%
Spectral crosstalk	< 4%

2.4 Filter set

Excitation wavelength [nm]		Emission wavelength [nm]	
Bandpass	**Bandwidth**	**Bandpass**	**Bandwidth**
470	30	514	20
533	15	572	20
577	20	620	25
645	20	697.5	45

2.5 Specifications of the thermal block cycler

Temperature control	Peltier-based heating and cooling
Temperature range	+37 to +98°C
Heating rate	4.4°C/s
Cooling rate	2.2°C/s
Thermal homogeneity	±0.3°C
Thermal accuracy	±0.2°C
Block cycler cover during cycling	105°C +/-3°C

2.6 Specifications of the external handheld barcode scanner

Customers can purchase the external handheld barcode scanner for the LightCycler® 96 Instrument as an optional accessory:

LightCycler® USB Handheld Scanner	Cat. No. 05 825 601 001

The external handheld barcode scanner is used to scan the ID of a multiwell plate labeled with a barcode into the *Plate Id* field of the LightCycler® 96 Software. It is connected to the LightCycler® 96 Instrument via the USB interface on the back of the instrument.

The LightCycler® 96 System supports the following barcode types by default:

Barcode type	Resolution	Checkdigit	Min. data characters
Code 39	250 to 500	x start/stop character not transmitted	1
Code 2 of 5	250 to 500	x	1
Code 128	250 to 500	–	1

For detailed information on specifying customer-specific barcode types, refer to the documentation provided with the external handheld barcode scanner. It is possible to restore the default settings if required.

Note that the type of the external handheld barcode scanner is subject to change without notice. The specifications listed here apply to the type provided at the time of publishing of this Operator's Guide.

3 The LightCycler® 96 System Package

The table below lists the contents of the LightCycler® 96 System Package. Use this list to verify that nothing is missing.

After opening, check for any damage that may have occurred in transit. Report any signs of damage to your local Roche Diagnostics representative.

Quantity	Component
1	LightCycler® 96 Instrument
2	LightCycler® 96 Quick Guides: ▶ System installation ▶ Programming and running an experiment
1	LightCycler® 96 USB Drive containing: ▶ Executables for the software ▶ Release notes ▶ License texts ▶ LightCycler® 96 System Guides ▶ Decontamination and shipment preparation ▶ Demo data and experiment templates provided by Roche
1	Mains power cable (EU)
1	Mains power cable (US)
1	LAN cable (3 m)
2	Ventilation dust filters
1	Package fuses FUSE 5x20 T8AH 250V ULR/IEC
1	Sealing foil applicator

4 System description

The LightCycler® 96 System comprises the following main components:

- The LightCycler® 96 Instrument; see below.
- The LightCycler® 96 Instrument Software, which is installed on the instrument; see chapter *LightCycler® 96 Instrument Software*, on page 183.
- The LightCycler® 96 Application Software, which is installed on a customer's computer; see chapter *LightCycler® 96 Application Software*, on page 77.
- The disposables to be used with the LightCycler® 96 Instrument; see section *Disposables*, on page 39.
- The reagents to be used in experiments on the LightCycler® 96 Instrument; see section *Reagents*, on page 41.

4.1 The LightCycler® 96 Instrument

The LightCycler® 96 Instrument is a rapid thermal block cycler with integrated real-time, online detection capabilities. This setup enables homogeneous PCR (simultaneous amplification and detection of target nucleic acids). Detection of target nucleic acids is performed by adding either a fluorescent double-stranded-DNA-specific dye or sequence-specific oligonucleotide probes labeled with fluorophores.

Both approaches allow operators to measure the generation of PCR products during amplification, the basis of quantitative real-time PCR (qPCR). Post-PCR analysis of previously generated PCR products using a melting program is used for PCR product characterization. For detailed information on the available detection formats, see section *Detection formats*, on page 60.

The LightCycler® 96 Instrument comprises two main components:

- The block cycler unit, including the thermal block cycler with the multiwell plate mount, the cooling elements, the Peltier elements, and the electronics interface; for detailed information, see section *Block cycler unit*, on page 33.
- The detection unit, including the optic module, the filter module, and the CCD camera; for detailed information, see section *Detection unit*, on page 35.

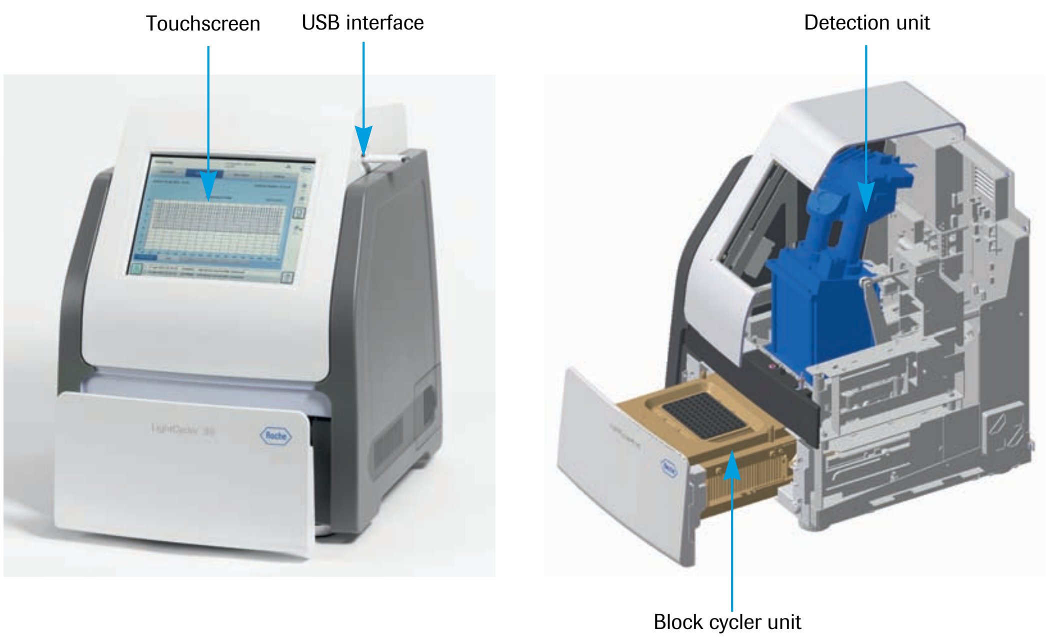

Figure 1: Main components of the LightCycler® 96 Instrument

4.1.1 Loading module

The loading module of the LightCycler® 96 Instrument houses the complete thermal block cycler, the electronic interface and the block cycler ventilation. For a detailed description of these components, see the following sections.

The loading module can have the following states:

Status	Description
Open	The loading module is completely opened and ready for loading or unloading the multiwell plate.
Locked	The loading module is locked when closed manually after loading the multiwell plate and during the following experiment run.
Closed	When the experiment run is finished, the LightCycler® 96 Instrument unlocks the loading module. When using the *Eject* button on the touchscreen, the loading module is pushed forward. The operator can open the loading module completely using the recessed grip. *The loading module may only be opened using the 'Eject' button on the touchscreen. Otherwise the instrument changes to the 'Error' state and has to be rebooted. For detailed information, see section Global action bar, on page 188.*

For detailed information on cleaning the loading module, see section *Cleaning instructions*, on page 233.

4.1.2 Touchscreen

The touchscreen on the LightCycler® 96 Instrument provides the instrument software. For detailed information, see chapter *LightCycler® 96 Instrument Software*, on page 183.

The touchscreen provides a screen resolution of 800 x 600 pixels. It is operated by pressing with a finger.

4.1.3 USB interfaces

The LightCycler® 96 Instrument provides two USB interfaces:

- The USB interface on the right side of the instrument only accepts the following USB drives:
  - USB drives supporting USB 2.0.
  - USB drives containing only one partition. The instrument cannot identify USB drives with several partitions.

  With a USB drive, an experiment can be transferred to the instrument and performed without a connected computer running the LightCycler® 96 Application Software.
- The USB interface on the back of the instrument exclusively allows connection of an external handheld barcode scanner. It does not allow connection of a USB drive. For detailed information, see section *Specifications of the external handheld barcode scanner*, on page 27.

4.1.4 Instrument back

The back of the instrument houses the power box with the instrument's mains power socket and mains power switch, the USB interface for connecting an external handheld barcode scanner, as well as the Ethernet interface required for connecting the LightCycler® 96 Instrument to a network or directly to a computer. For details of the instrument's power supply and Ethernet connection, see section *Installation*, on page 42.

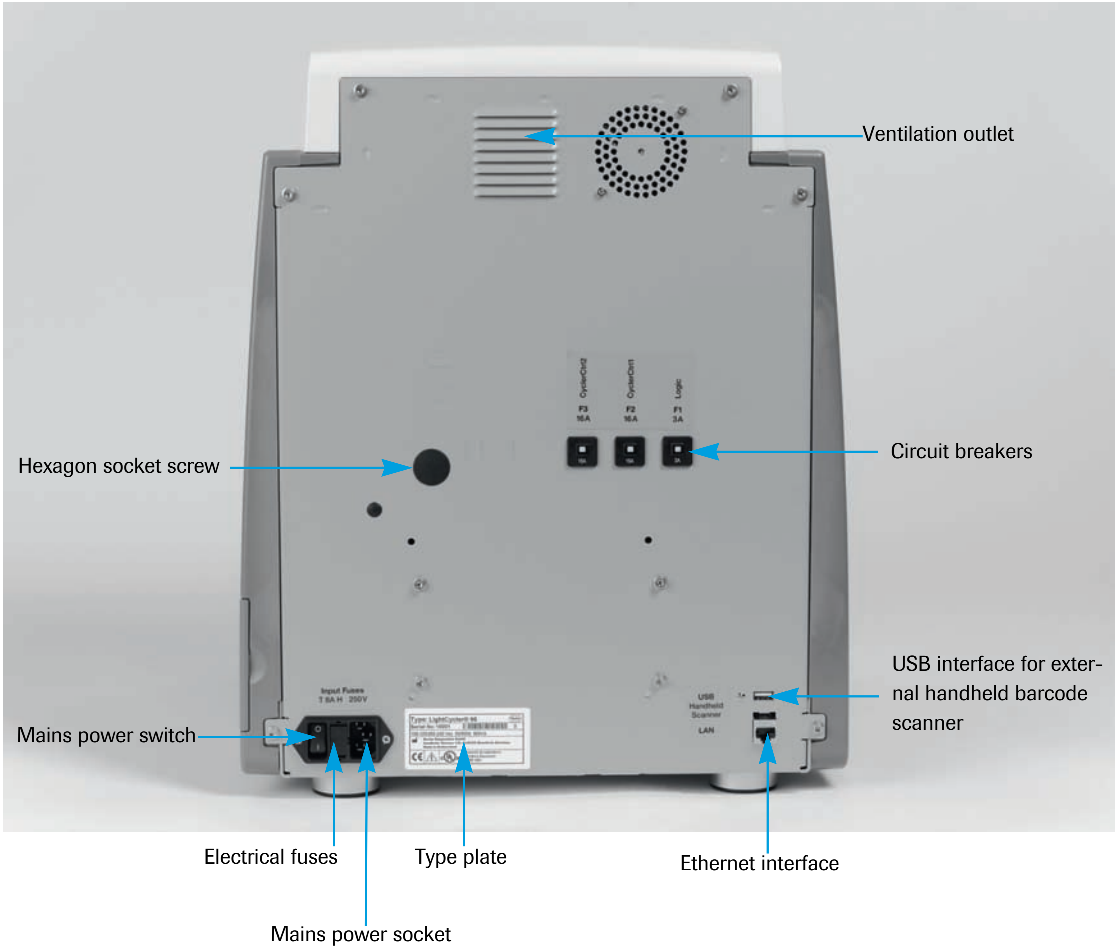

Figure 2: LightCycler® 96 Instrument back view

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4.1.5 Ventilation

Air supply and air evacuation are arranged as follows:

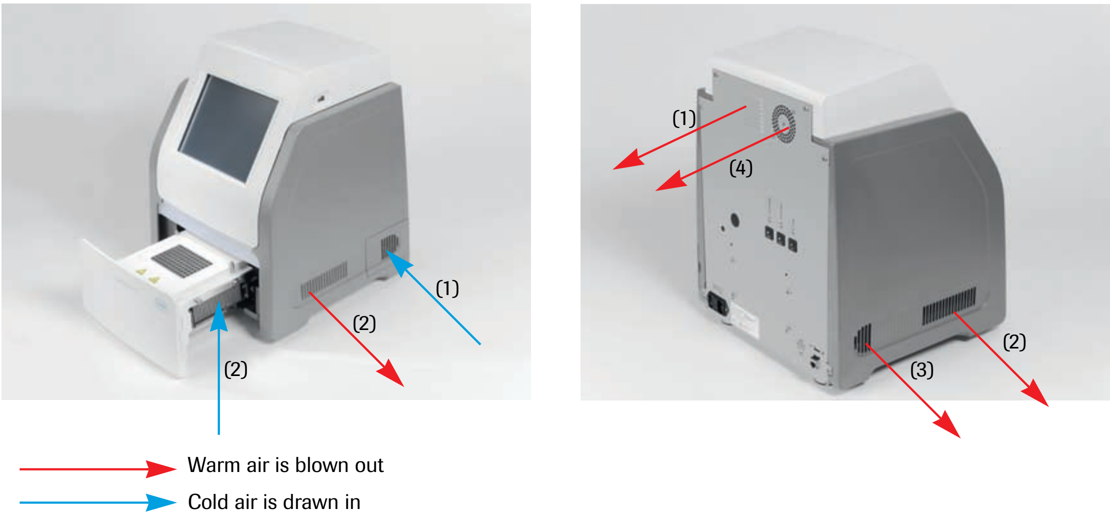

Figure 3: Schematic ventilation overview

(1)	System and LED ventilation. The ventilation inlet on the right side of the instrument is equipped with a ventilation dust filter. For detailed information on changing the ventilation dust filter, see section *Exchanging the ventilation dust filters*, on page 237.
(2)	Block cycler ventilation. The fan in the block cycler unit is only operated if the cooling elements need to be cooled down.
(3)	Power box ventilation.
(4)	Electronics ventilation.

To facilitate adequate ventilation, the ventilation inlets and outlets must not be obstructed. For detailed information, see section Installation requirements, on page 42.

4.2 Block cycler unit

The block cycler unit consists of the following main components:

- Thermal block cycler, which includes the Peltier elements, thermal interface, cooling elements, and electronics interface; for detailed information, see section *Thermal block cycler* below.
- Block cycler cover.
- Multiwell plate mount; for detailed information, see section *Multiwell plate mount*, on page 34.

4.2.1 Thermal block cycler

The thermal block cycler provides rapid, precise, and accurate temperature control. A heated lid prevents changes in reaction volume and optical artifacts due to condensation. The silver mount, which has a non-stick coating, has a high thermal conductivity and low thermal mass, allowing speed and precision. The thermal block cycler is driven by six Peltier elements.

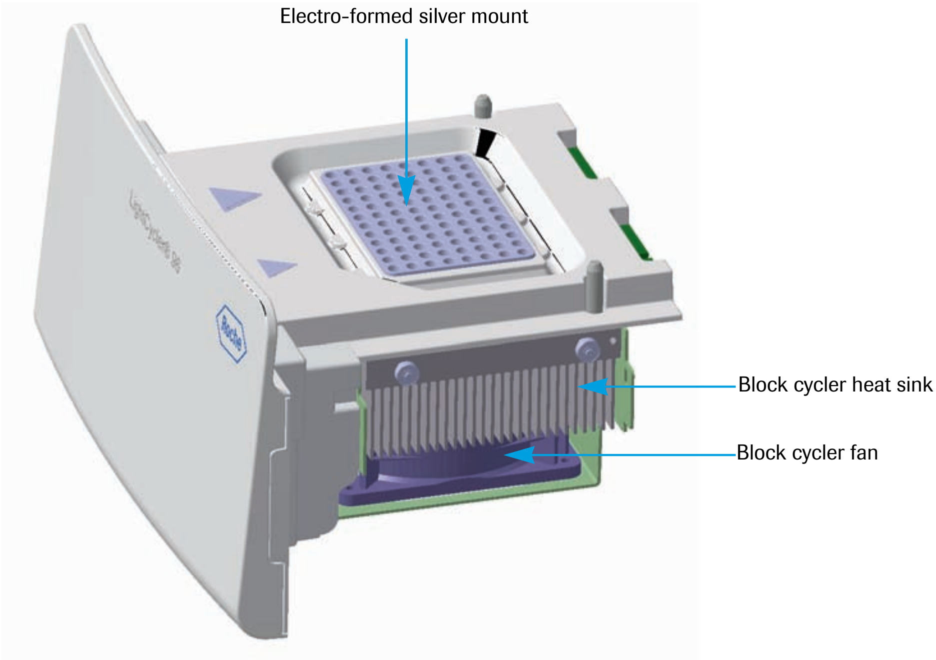

Figure 4: Thermal block cycler

Ventilation

To cool the thermal block cycler during operation, the block cycler is fitted with a high-efficiency fan. The fan in the block cycler unit is only operated if the eletronic power amplifier of the cooling elements exeeds a certain temperature. The air flow is guided through the instrument and expelled on the right and left sides. For detailed information, see section *Ventilation*, on page 32.

Block cycler cover

The thermal block cycler has a matching block cycler cover. The lid has 96 pinholes allowing fluorescence detection by the detection unit through the closed lid. During cycling the block cycler cover is pressed onto the multiwell plate and heated. This heating prevents changes in reaction volume and optical artifacts due to condensation.

If the instrument is not used for some time, it changes to standby mode and the block cycler cover is switched off. The cover must be heated again when the operator wants to start an experiment run. The experiment run can be started when the instrument changes to *Ready*.

4.2.2 Multiwell plate mount

The mount is made of silver, has a nonstick coating, and accepts the following disposables:

- A LightCycler® 480 Multiwell Plate 96. A mechanical coding prevents incorrect loading orientation of the multiwell plate.

  LightCycler® 480 Multiwell Plates are labeled with a barcode that can be identified using an external handheld barcode scanner. The LightCycler® 96 Software saves this ID to the corresponding experiment file. For detailed information, see section *Specifications of the external handheld barcode scanner*, on page 27.

- Up to 12 LightCycler® 8-Tube Strips.

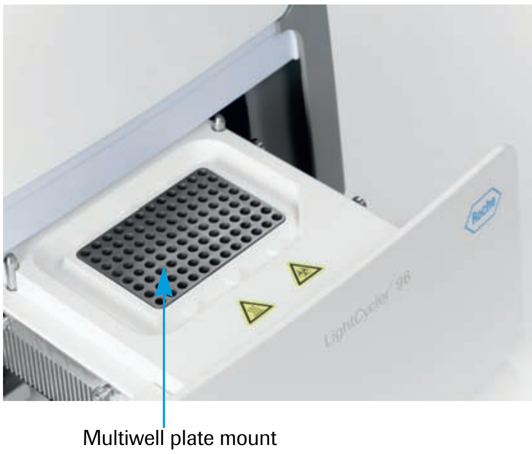

Figure 5: Multiwell plate mount

For the LightCycler® 8-Tube Strips the operator must ensure, that the load pattern on the mount is symmetrical. Do not use only one strip. Start with the outer columns, for example, two strips in the outer columns 1 and 12 or 2 and 11.

If the mount is loaded asymmetrically, the block cycler cover is charged asymmetrically and the instrument could be damaged.

Additionally the operator must ensure, that the orientation of the strips on the mount matches the sample asignment in the 'Sample Editor' tab. Otherwise the analysis results are incorrectly assigned to the samples. For detailed information on assigning the samples, see section Sample Editor tab, *on page 128.*

4.3 Detection unit

The detection unit consists of the following main components:

- The optic module containing 2 x 96 glass fibres for providing the excitation light and collecting the emitted light to and from each well, and one fibre for the reference channel.
- The LED light source; the LightCycler® 96 Instrument uses a white high power LED as the excitation light source. The actual wavelength used for excitation of fluorophores in the reaction is determined by the chosen excitation filter.
- The filter module containing the filter wheel with four excitation and four emission filters.
- The CCD camera for measuring the intensity of the emitted light.

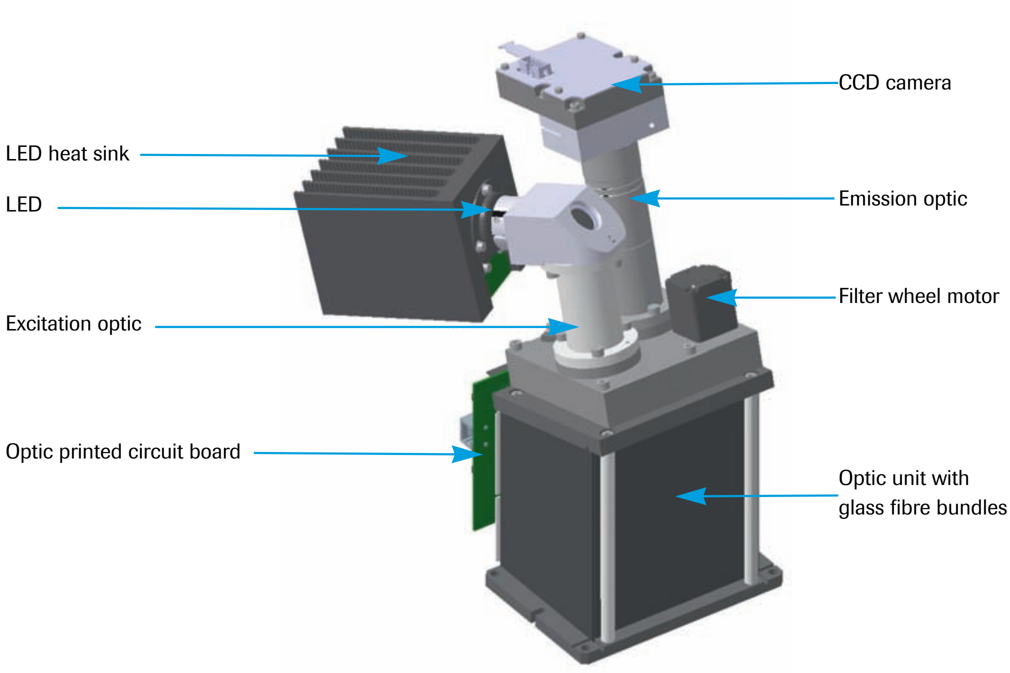

Figure 6: Schematic detection unit overview

4.3.1 Optic module

Fiber optics provide efficient optical coupling between the excitation source, PCR, emission source, and CCD camera, enabling high sensitivity. The glass fibres in the optic module distribute the excitation light to the 96 wells of the multiwell plate and collect the emitted light.

After passing through the excitation filter, the light is projected via the glass fibres in the optic module onto the wells in the multiwell plate. In the same way, light emitted by the fluorophores is passed vertically into the optic module. This ensures that there are no shading effects within the plate wells and no distortions or variations in the signals coming from wells located at the edges of the PCR multiwell plate compared to center wells, enabling homogeneous sensitivity over the complete plate.

The fluorescent signals are then guided to the emission filter contained in the filter module and detected using the CCD camera. For detailed information, see sections *Filter module*, on page 36 and *CCD camera*, on page 37.

In addition an extra glass fibre measures the intensity of the LED during a run. These values are used to compensate for possible intensity fluctuation.

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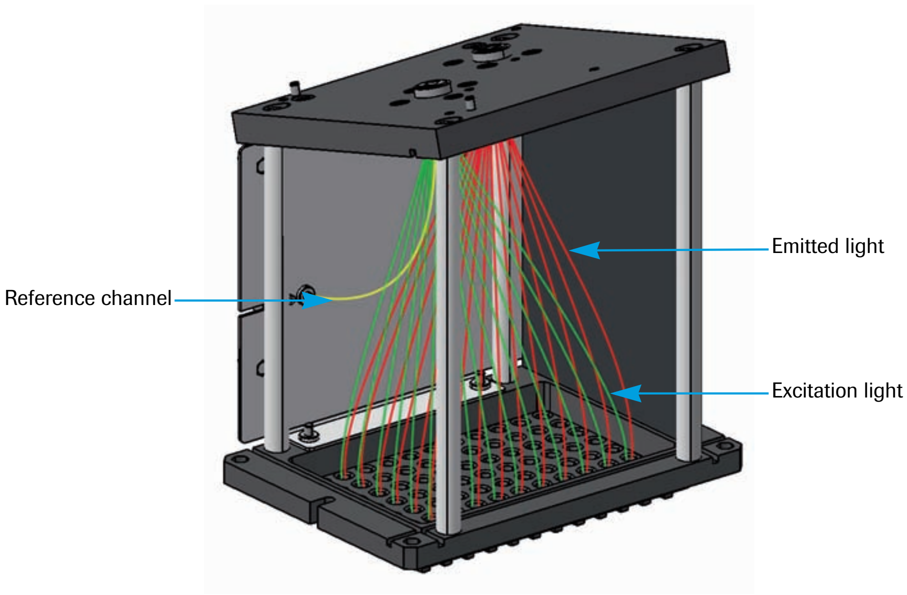

Figure 7: Optic unit with glass fibre bundles

4.3.2 Filter module

The filter module contains a filter wheel with four excitation and four emission filters. The filters are hard-coated and do not need to be customized. The LightCycler® 96 Instrument provides four different filter combinations. The corresponding excitation and emission filters are positioned opposite on the filter wheel.

The filter wheel is driven by a stepper motor with four positions according to the filter combinations. The sensor of the filter wheel ensures that the correct filter combination is always set. For detailed information on the filter set, see section *Filter set*, on page 27.

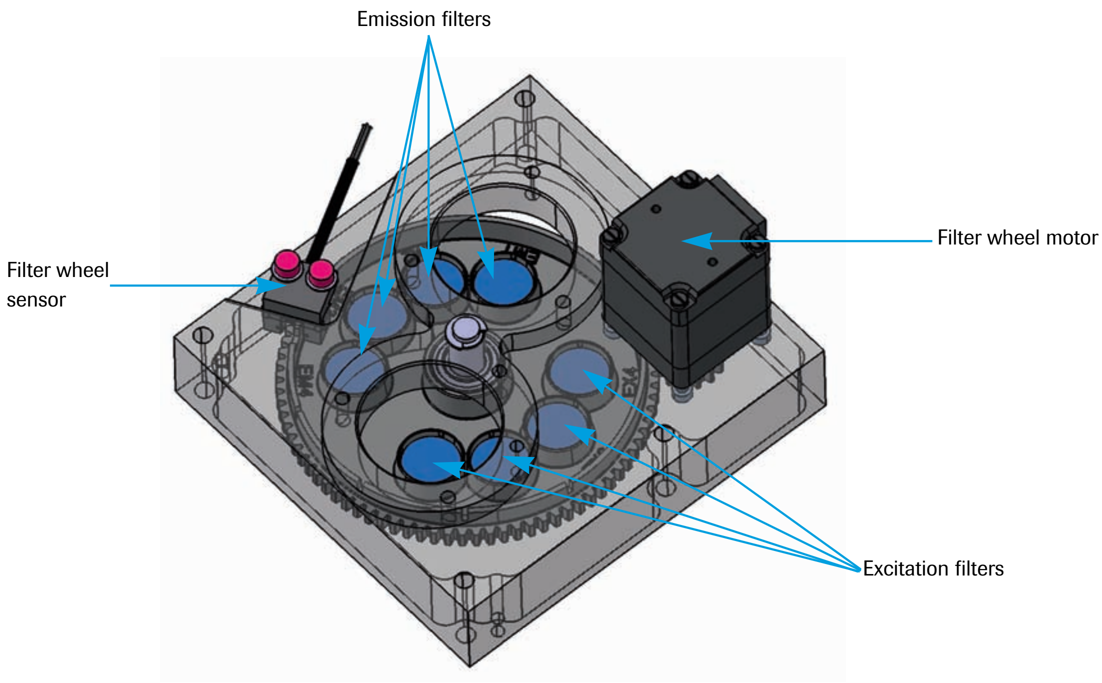

Figure 8: Filter module with filter wheel

4.3.3 CCD camera

The light-sensitive CCD camera contains a CCD chip. The acquisition time of the CCD camera is adjusted either manually or dynamically using the LightCycler® 96 Software. After measurements are transferred to the software, it performs further corrections and noise reduction.

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4.4 Detection channels

As described in section *Filter module*, on page 36, the LightCycler® 96 Instrument provides four different combinations of excitation and emission filters to enable optimal excitation of fluorophores and exact measurement of emitted fluorescence signals. These filter combinations represent the four detection channels.

The excitation-emission filter pairs can either be used singly in monocolor applications or in successive combination for multicolor applications. For detailed information, see section *Detection formats*, on page 60.

The table below shows the excitation-emission filter combinations of the LightCycler® 96 Instrument used in the different detection formats:

Fluorophore	Excitation filter	Emission filter	Detection format
SYBR Green I ResoLight Dye FAM	470	514	Intercalating dye
VIC HEX Yellow555	533	572	Hydrolysis probes/ Universal ProbeLibrary probes
Red610 Texas Red	577	620	Hydrolysis probes
Cy5	645	697.5	Hydrolysis probes

The LightCycler® 96 Instrument can detect signals from up to four dyes, making it possible to obtain more information from a single reaction. The channels chosen for analysis depend on the fluorescent dyes used in the experiment.

Besides the dyes listed in the table above, all other fluorescent dyes that are compatible with the excitation-emission filter wavelengths can be measured using the LightCycler® 96 Instrument.

4.5 Disposables

The LightCycler® 96 Instrument requires specific disposables for operation. The LightCycler® 96 Disposables meet the demands of real-time PCR applications supported by the instrument. The following disposables are available for the LightCycler® 96 Instrument:

- LightCycler® 480 Multiwell Plate 96, white
- LightCycler® 480 Multiwell Plate 96, clear
- LightCycler® 480 Sealing Foil
- LightCycler® 8-Tube Strips (white)
- LightCycler® 8-Tube Strips (clear)

For detailed information on the LightCycler® 96 Disposables, please visit our Special Interest Site for Real-Time PCR Systems at *www.lightcycler96.com*.

The LightCycler® 480 Multiwell Plates and the LightCycler® 8-Tube Strips are intended for single use only. Never use a multiwell plate or tube strip more than once (even after cleaning it), as this can lead to unreliable results or incorrect integration time.

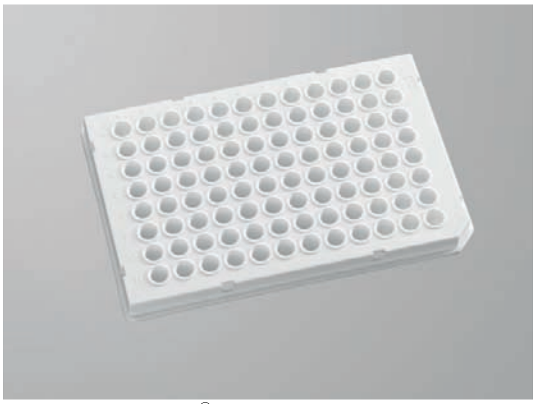
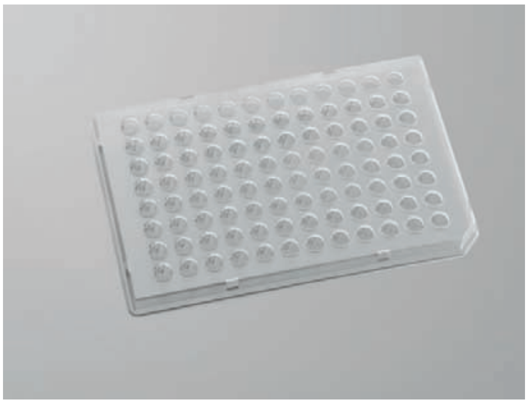

Figure 9: LightCycler® 480 Multiwell Plate 96, white and clear

The multiwell plates carry a barcode label on the long side at row A. This barcode label represents a running plate ID that can be read by the external handheld barcode scanner.

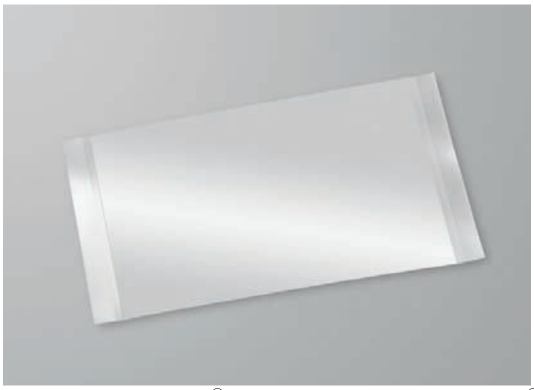


Figure 10: LightCycler® 480 Sealing Foil and LightCycler® 480 Sealing Foil Applicator

Before the multiwell plate is loaded into the LightCycler® 96 Instrument, it must be sealed with the self-adhesive sealing foil. Use the sealing foil applicator provided with the instrument for proper sealing. You can order further sealing foil applicators directly from your local Roche Diagnostics representative. Sealing the plate is crucial to eliminate evaporation at high temperatures. Use only the recommended foil.

Always centrifuge the filled and sealed plate before loading it into the instrument.

Always wear gloves and only handle the sealed plate by its edges. Fingerprints and other staining on the plate can affect the results.

Figure 11: LightCycler® 8-Tube Strips (white and clear)

Close the tubes by firmly pressing a strip of caps into place. Make sure the tubes are closed properly, otherwise the contents could evaporate during the run.

Place the strips symmetrically on the multiwell plate mount to ensure consistent temperature distribution in the samples. For detailed information, see section Multiwell plate mount, on page 34.

Always centrifuge the filled and capped tube strips before loading them into the instrument.

Always wear gloves and take care not to contaminate the caps. Fingerprints and other staining on the caps can affect the results.

4.6 Reagents

Optimal performance of the system is achieved using the LightCycler® 96 Instrument in combination with dedicated reagents. For detailed information on the LightCycler® 96 Reagents, please visit our Special Interest Site for Real-Time PCR Systems at *www.lightcycler96.com*.

4.7 Additional equipment

The following additional equipment is required to perform real-time PCR assays with the LightCycler® 96 System:

- Standard swing-bucket centrifuge containing a rotor for multiwell plates with suitable adapters
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing master mixes and dilutions

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5 Installation

5.1 Installing the LightCycler® 96 Instrument

The LightCycler® 96 Instrument is packed in a shipping box. The box includes the LightCycler® 96 Instrument and the accessories.

- For the scope of delivery, see section *The LightCycler® 96 System Package*.
- For details of how to assemble the LightCycler® 96 Instrument, see section *Assembling the instrument*.

The original shipping container must be transferred unopened to the installation site.
On delivery, carefully inspect the shipping box for damage. Report any damage to your local Roche Diagnostics representative before accepting the unit.

Keep the shipping box and packaging in case of return.
If you have already diposed of the packaging, you can request it from Roche.

Lift the LightCycler® 96 Instrument only by the sides using the recessed grips on the left and right sides of the instrument base plate.

Caution!
Due to the weight of the instrument, two persons may be needed to lift it.

5.1.1 Installation requirements

- The LightCycler® 96 System is for indoor use only.
- When installing a LightCycler® 96 Instrument that has been stored in a cold room or transported at low temperatures, condensation may occur, which can cause malfunction of the instrument. The instrument must be acclimated to room temperature for at least one hour prior to installation.
- Place the LightCycler® 96 Instrument on a solid, level surface in the upright position.
- Do not place the instrument in direct sunlight or close to radiators or heating devices.
- Do not place the LightCycler® 96 Instrument next to instruments that cause vibration, electromagnetic interference, or have high inductance (for example, refrigerators, centrifuges, or mixers).
- Peripheral instruments connected to the LightCycler® 96 Instrument must meet the IEC 60950 (UL 60950) standard.
- Do not place anything on top of the instrument.
- Use only the power cables and Ethernet cable supplied with the system package.
- Do not use the Ethernet cable outdoors.

5.1.2 Space and power requirements

Place the LightCycler® 96 Instrument in a location that can support the following instrument requirements:

Dimensions	The LightCycler® 96 Instrument is 40 cm wide, 40 cm long, and 53 cm high.
Weight	The LightCycler® 96 Instrument weighs approximately 25 kg.
Power	▶ The LightCycler® 96 Instrument operates at 100 to 125 V / 200 to 240 V (50/60 Hz). ▶ The instrument can be connected to a single-phase or dual-phase supply only. The current consumption capacity of the mains power supply must not be exceeded. ▶ There are no special provisions for protective grounding. ***Caution!*** *The instrument requires proper grounding. Any break in the electrical ground wire, whether inside or outside the instrument, or disconnection of the electrical ground connection, could create a hazardous condition.* ***Caution!*** *Do not under any circumstances attempt to modify or deliberately override the safety features of this system.* ▶ The LightCycler® 96 Instrument uses 600 VA. *Depending on the quality of electrical grounding, an uninterruptible power supply (UPS) with line conditioner and support for "Online/Direct Mode" may be required. A UPS is not provided with the LightCycler® 96 Instrument. Roche recommends contacting a local supplier who can provide a UPS in accordance with the electrical requirements.*
Ventilation	▶ Do not place anything under the instrument, such as sheets of paper, since these could block the air inlet. ▶ Do not cover the instrument – in particular do not block the ventilation holes.
Access	▶ A gap of 10 cm is recommended between the back of the instrument and the wall to allow access to the mains power switch and the Ethernet interface. ▶ A gap of 20 cm is required on both sides to allow adequate air flow. ▶ A gap of 5 cm is required above the instrument cover to access the USB interface.

5.1.3 Assembling the instrument

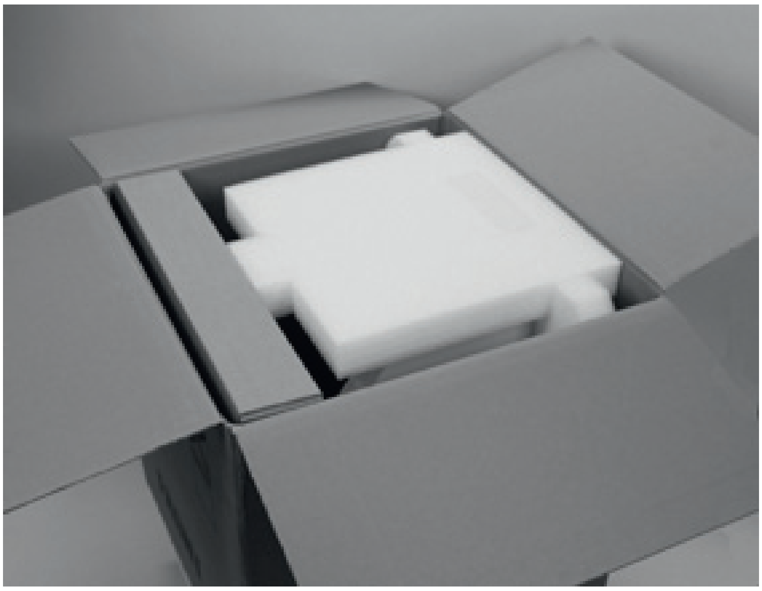
The LightCycler® 96 Instrument and accessories are protected in a shipping box.

To assemble the instrument:

- Unpack and install the LightCycler® 96 Instrument (see section *To unpack and install the LightCycler® 96 Instrument*, below).
- Remove the transport locking device (see section *To remove the transport locking device*, on page 48).

To unpack and install the LightCycler® 96 Instrument

1. Position the shipping box on a solid, level surface in the upright position.
2. Open the carton on the top.
3. Remove the accessory box and the protective foam on the top.



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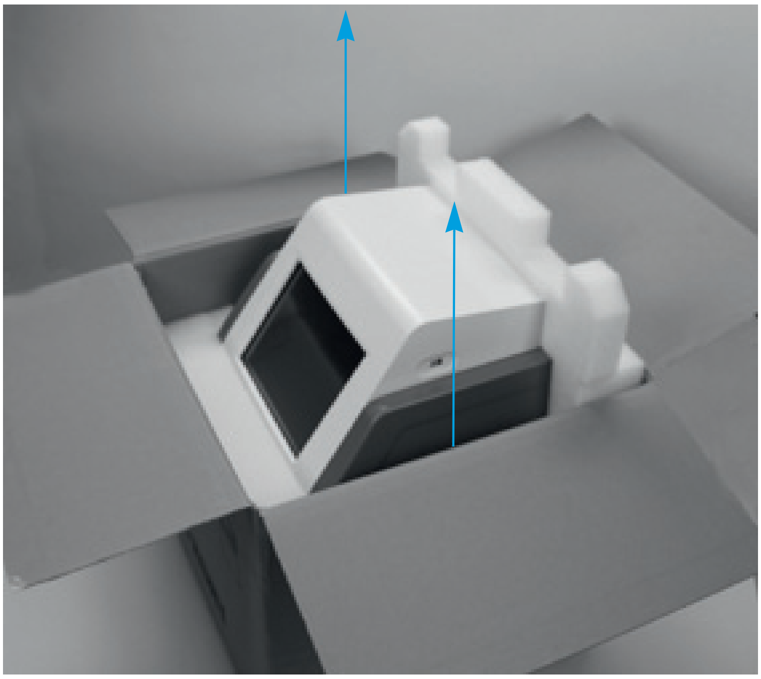
4 Lift the LightCycler® 96 Instrument out of the box by holding it on the left and right sides and place it on a solid level surface.

To carry and lift the instrument, only use the recessed grips on the left and right sides of the instrument base plate.

Caution:
Due to the weight of the instrument, two persons may be needed to lift it.



Check for damage that may have occurred in transit. Report any signs of damage to your local Roche Diagnostics representative.

5 Position the instrument on the workbench in the upright position.

6 Ensure that all components are present and intact.

For a detailed list, see section *The LightCycler® 96 System Package*, on page 28.

Report any missing items to your local Roche Diagnostics representative.

7 Remove the protective foil from the touchscreen.

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8 Connect the supplied mains power cable to the mains power socket of the instrument, and then to the wall outlet.

Mains power socket

Do not touch mains power cables when your hands are wet. Do not attempt to connect or disconnect either of the mains power cables when the instrument is switched on. If any power connector becomes worn or frayed, it must be replaced immediately with an approved cable. Always connect the equipment to a grounded wall outlet.

9 Optional: Connect one end of the Ethernet cable provided with the instrument to one of the following Ethernet ports:

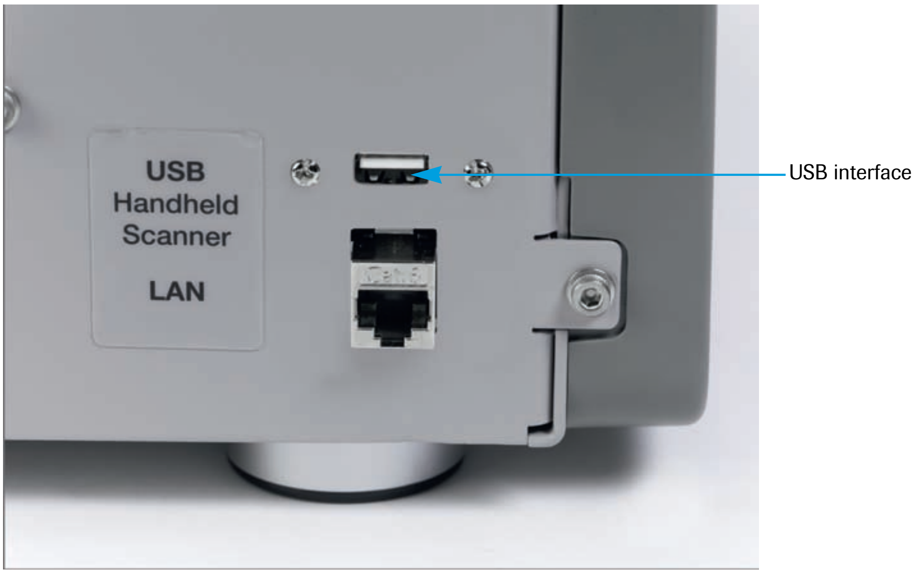
- The Ethernet port of your computer.
- The Ethernet port of your LAN.

10 Optional: Connect the other end of the Ethernet cable to the Ethernet port on the back of the LightCycler® 96 Instrument. For network settings on the instrument see *Configuring the LightCycler® 96 Instrument in the network*, on page 49.

Ethernet port

▶ ▶ ▶

11 Optional: Connect the external handheld barcode scanner to the USB interface on the back of the instrument.



12 Switch on the instrument using the mains power switch on the back of the instrument.

To remove the transport locking device

1. Once the instrument has been successfully initialized, choose the *Eject* button on the touchscreen to release the loading module.

The loading module is ejected.

2. Manually pull the loading module completely out of the instrument.

3. Remove the transport locking device from the mount.

Keep the transport locking device in case the instrument has to be transported.

4. Push the loading module back until it starts moving automatically to its home position.

5.2 Installing and configuring the LightCycler® 96 Instrument Software

The LightCycler® 96 Instrument is controlled by the LightCycler® 96 Instrument Software. Each configuration (instrument and instrument software) works as an independent system. The LightCycler® 96 Instrument Software operates the LightCycler® 96 Instrument using the information provided by the user who defines the experiment run conditions.

- For detailed information on monitoring the instrument and defining an experiment run, see chapter *LightCycler® 96 Instrument Software*, on page 183.
- For detailed information on handling updates of the LightCycler® 96 Instrument Software, see section *Managing updates*, on page 58.

5.2.1 Installing the LightCycler® 96 Instrument Software

LightCycler® 96 Instrument Software is pre-installed on the instrument. For updating the software on the instrument, see section *Managing updates*, on page 58.

5.2.2 Configuring the LightCycler® 96 Instrument in the network

The LightCycler® 96 Instrument is compatible for use in a local point-to-point connection over Ethernet. The LightCycler® 96 Instrument Software allows for configuring the instrument in the network. There are two ways of connecting the instrument:

- A direct connection between the instrument and the computer, using a single cable; see section *Using a direct connection* below.
- A connection between the instrument and the local Ethernet; see section *Using the local point-to-point connection*, on page 54.

For sending and retrieving experiment files to/from the LightCycler® 96 Application Software via the network, the instrument must be registered with the application software. For detailed information, see section Instrument Manager, on page 110.

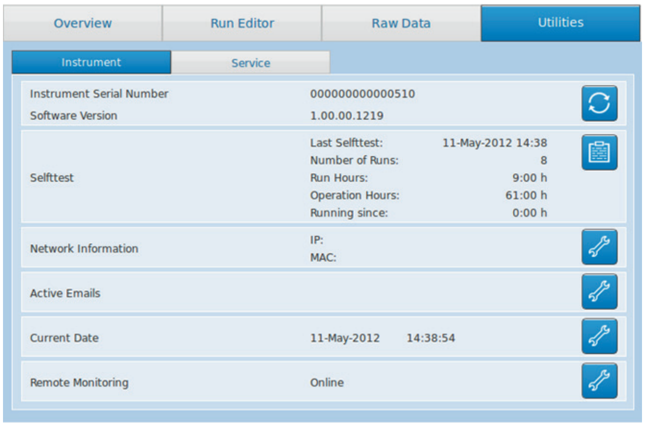
Using a direct connection

For using a direct connection between the LightCycler® 96 Instrument and the computer running the LightCycler® 96 Application Software, both devices must be configured.

To configure the direct connection on the instrument

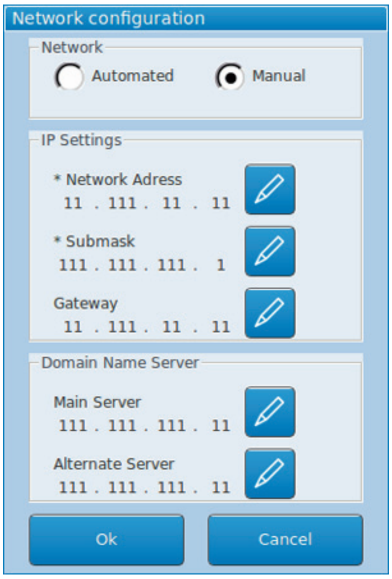
1	Connect the LightCycler® 96 Instrument to the computer, using the supplied network cable. For detailed information, see section *Assembling the instrument*, on page 44.
2	Switch on the instrument.

3 On the touchscreen, open the *Utilities* tab and then the *Instrument* tab.



4 Choose the tools icon next to *Network Information*. The *Network configuration* dialog box opens.

5 In the *Network* window area, choose *Manual*. The values displayed in the dialog box are retrieved from the last *Automated* settings.



6 Use the pencil button next to the *Network Address* field to open the *Edit IP Address: Network Address* dialog box.

7 Enter the IP address.

8 Choose *OK*.

9 Repeat steps 6 to 8 to specify the subnet mask accordingly.

Contact your local IT administrator for the correct IP addresses.

10 Choose *OK* to apply the network settings and close the dialog box.

If the configuration was completed successfully, the network icon is displayed in the status bar of the LightCycler® 96 Instrument Software. Otherwise the icon is crossed out red. For detailed information, see section *Status bar*, on page 187.

It can take a few minutes, until the changes to take effect.

The LightCycler® 96 Instrument is now configured for a direct connection. The IP address is displayed in the *Network Information* field of the *Utilities* tab.

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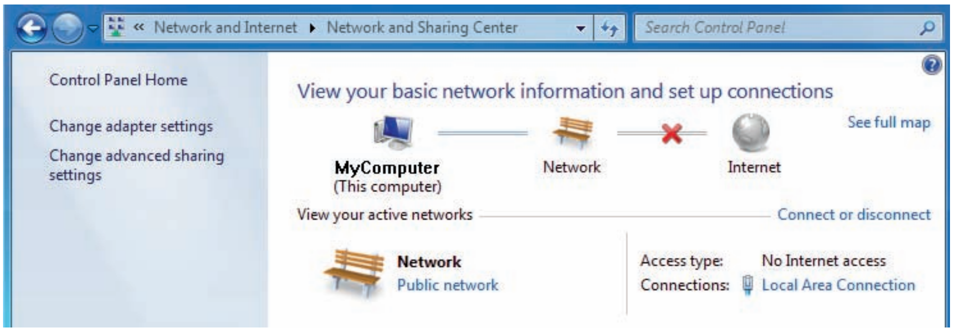
To configure the direct connection on the computer

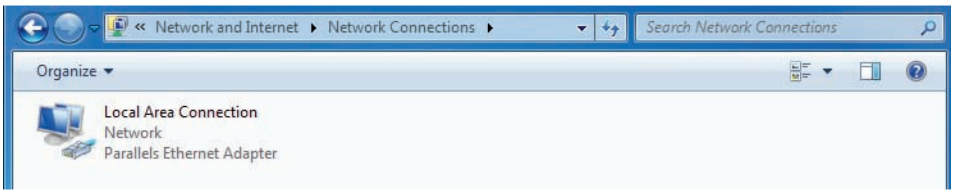
1. Log in to Microsoft Windows and ensure that you have the administration rights to edit the network configuration.

2. Click the *Start* menu and select *Control Panel*.

3. In the *Network and Internet* section, click *View network status and tasks*.



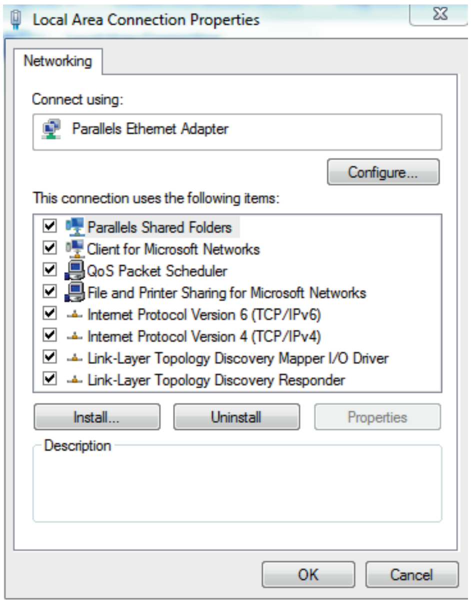
4. In the top left, click *Change adapter settings*. The available adapters are displayed:



5. Select the adapter you plan to connect the network cable to. Usually the adapter will be labeled *Local Area Connection*, as displayed above.

▶ ▶ ▶

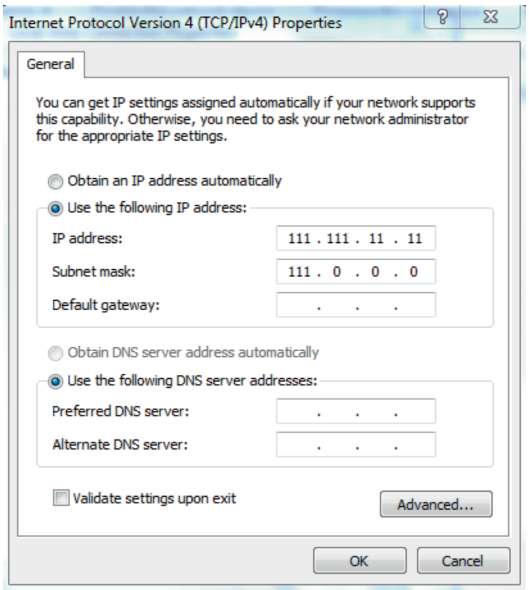
6 Right-click the adapter icon and select *Properties* on the shortcut menu. The *Local Area Connection Properties* dialog box opens.



7 Select the item *Internet Protocol Version 4 (TCP/IPv4)*.

Ensure that you have selected 'Version 4' (not 'Version 6').

8 Click *Properties*. The *Internet Protocol Version 4 (TCP/IPv4) Properties* dialog box opens.



9
- Select the *Use the following IP address* option.
- Enter a valid IP address for local point-to-point connections and the corresponding subnet mask.

Contact your local IT administrator for the correct IP addresses.
Ensure that you type the correct numbers in each section, separated by the dots.

10 Click *OK* and then *Close*.

The computer is now configured for a direct connection.

Using the local point-to-point connection

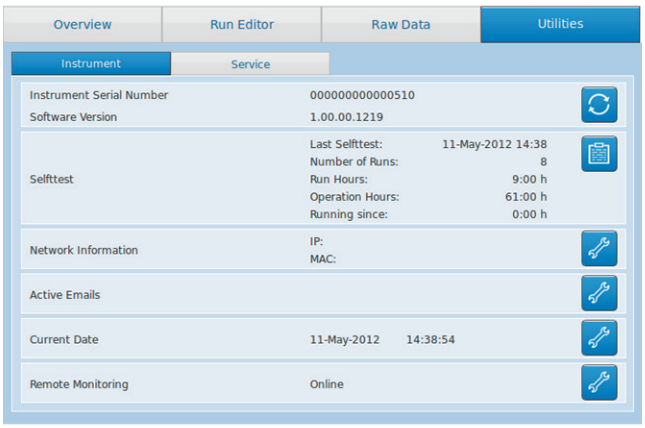
For connecting the LightCycler® 96 Instrument to the local point-to-point connection Ethernet, the operator has the following options to configure the network connection:

- Using a standard network, where the TCP/IP addresses are assigned automatically by a Dynamic Host Configuration Protocol (DHCP) server.
- Assigning the IP addresses manually.

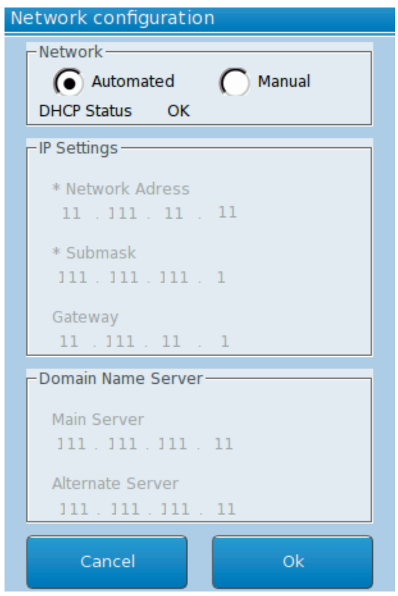
A standard network is the type of network you are likely to have if you do not need to configure the IP address of your computer manually.

To configure the local Ethernet on the instrument

1. Connect the LightCycler® 96 Instrument to the network in the same way as a computer, using the supplied network cable to connect to a wall socket or a network switch/hub. For detailed information, see section *Assembling the instrument*, on page 44.
2. Switch on the instrument.
3. On the touchscreen, open the *Utilities* tab.



4. Choose the tools icon next to *Network Information*. The *Network configuration* dialog box opens.



5 In the *Network* window area, choose whether you want to obtain the IP address automatically or manually.

- *Automated*:
  The IP address is searched for automatically. The IP address, subnet mask, default gateway address, and the DNS addresses are displayed in the corresponding fields. Proceed with step 10.

  Write down the IP address for later use when connecting the instrument to a computer.

- *Manual*:
  Proceed with step 6 below.

6 Use the pencil button next to the *Network Address* field to open the *Edit IP Address: Network Address* dialog box.

7 Enter the IP address.

8 Choose *OK*.

9 Edit the *Submask* field accordingly.

10 Optional:

- If you want to use the Domain Name Service (DNS) to assign a name to the instrument.
- If you want to send email notifications containing the status of the experiment to specified recipients. In this case you need the DNS to resolve the server addresses for the email configuration. For detailed information, see section *Active Emails*, on page 215.

In the *Domain Name Server* window area, use the pencil button next to the *Main Server* field to open the *Edit IP Address: Main Server* dialog box.

11 Enter the IP address of the DNS main server.

12 Choose *OK*.

13 Edit the *Alternate Server* field accordingly.

14 Choose *OK* to apply the network settings and close the dialog box.

The LightCycler® 96 Instrument is now configured in the network. The IP address is displayed in the *Network Information* field of the *Utilities* tab.

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5.3 Installing the LightCycler® 96 Application Software

The LightCycler® 96 Application Software is installed on a computer which can be connected to the LightCycler® 96 Instrument either via a standard network or directly, as well as on a computer that is not connected to a LightCycler® 96 Instrument.

- For detailed information on connecting to a LightCycler® 96 Instrument, see section *To register an instrument*, on page 112.
- For detailed information on defining an experiment run and on analyzing the results, see chapter *LightCycler® 96 Application Software*, on page 77.
- For detailed information on handling updates of the LightCycler® 96 Application Software, see section *Managing updates*, on page 58.

5.3.1 System requirements

To install and run the LightCycler® 96 Application Software, the computer must satisfy the following minimum requirements:

- Processor: Intel Core 2 duo 2.4 GHz
- Memory: 2 GB
- Hard disk: 250 GB
- LAN: RJ45 Ethernet (100 MBit)
- USB: USB 2.0
- Display resolution: 1280 * 1024
- Operating system: Microsoft Windows 7
- Microsoft .NET Framework 4.0 installed
- Setting for regional and language options: English (USA)

5.3.2 Installing the LightCycler® 96 Application Software

The LightCycler® 96 Application Software is provided on the separate USB drive which is part of the system package. The software is installed via a standard installation program. Software updates are provided via the download area of the *Roche Applied Science* website. For detailed information on handling updates, see section *Managing updates*, on page 58.

To install the LightCycler® 96 Application Software

1. Start the computer on which you want to install the software.
2. Insert the USB drive.
3. Log in to Microsoft Windows, and ensure that you have the administration rights to install the software.
4. Open Windows Explorer and navigate to the USB drive.
5. Double-click the *Setup_LightCycler96_<release>.exe* file.
   The installation process transfers files, extracts the files, and prepares the installation wizard. The *Welcome to the LightCycler® 96 Setup Wizard* dialog box opens.
   Click *Next*.
6. You are prompted to agree to the license conditions:
   - Read the license agreement.
   - Check the *I agree* option.
   - Click *Next* to proceed.

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7	In the *Select Installation Folder* dialog box: ▶ Select the location for the LightCycler® 96 Application Software: Either keep the default settings or browse to select a location for the installation. *By default, the software is installed in the 'C:\Program Files\Roche Diagnostics\LightCycler® 96' directory.* ▶ Choose whether the software is to be accessed only by yourself or by anyone on the computer.
8	In the *Confirm Installation* dialog box, click *Next*.
9	The selected components are installed. When the installation process has finished, the *Installation Complete* dialog box opens. Click *Close*.

5.3.3 The LightCycler® 96 Application Software home directory

The LightCycler® 96 Application Software is installed in the home directory you specified during the installation process. By default, the home directory is created under:
C:\Program Files\Roche Diagnostics\LightCycler® 96.

When the installation is complete, the home directory contains the following directories:

Directory	Description
bin	Program libraries, configuration files, and executable files for the LightCycler® 96 Software application
Manuals	LightCycler® 96 System Guides
Templates	Non-executed experiments with run settings to be used as templates

5.3.4 Uninstalling the LightCycler® 96 Application Software

1	Start the computer with the LightCycler® 96 Application Software.
2	Log in to Windows and ensure that you have the administration rights to uninstall the software.
3	In the *Start* menu, navigate to the *LightCycler® 96* folder and choose *Uninstall LightCycler® 96.*
4	You are prompted to confirm the uninstallation. Choose *Yes*.
5	During the installation, the directories *bin*, *Manuals*, and *Templates* are deleted. When the uninstallation is finished, a message states that the software has been completely removed from your computer. Choose *OK*.

Alternatively, you can use the Microsoft Windows Control Panel to uninstall the LightCycler® 96 Application Software.

5.4 Managing updates

New software releases and user guides for the LightCycler® 96 Instrument are available in the download area of the *Roche Applied Science* website. New user guides are also available on the *Roche Technical Support* website.

5.4.1 Installing a LightCycler® 96 Application Software update

When installing an update of the LightCycler® 96 Application Software, there is no need to uninstall the existing version. Administrators can install the software update on the computer, as described in section *Installing the LightCycler® 96 Application Software*.

During an upgrade, all settings of the prior software version are adopted so no configuration is required.

5.4.2 Installing a LightCycler® 96 Instrument Software update

When installing an update of the LightCycler® 96 Instrument Software, there is no need to uninstall the existing version. During an upgrade, all settings of the prior software version are adopted so no configuration is required.

To install a LightCycler® 96 Instrument Software update

1. Download the new software release from the download area of the *Roche Applied Science* website and save it to a USB drive.

   The installation file must be located on the top level of the USB drive.

2. Start the LightCycler® 96 Instrument and wait for the *Ready* status to be displayed.

3. Insert the USB drive into the USB interface on the right side of the instrument.

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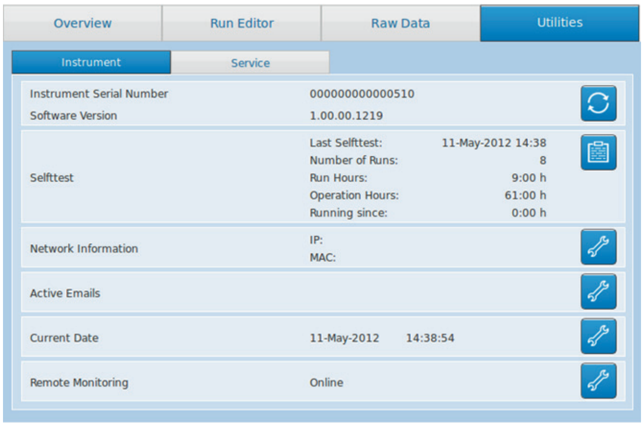
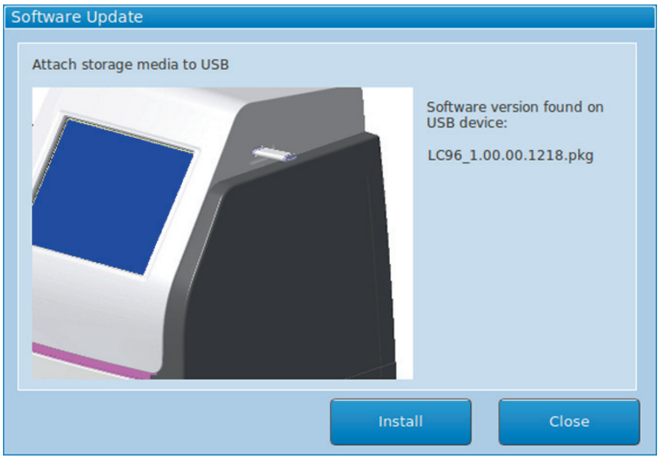
4 On the touchscreen, open the *Utilities* tab.

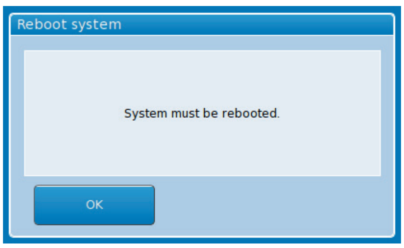


5 Choose the update button next to the *Software Version* field. The *Software Update* dialog box opens.
The dialog box displays the software version available on the USB drive.



6 Select the corresopnding software version.

7 Choose *Install*.
The file is copied from the USB drive to the instrument.
After installation, the system must be rebooted. The software displays the following dialog box:



8 Choose *OK*.
The instrument automatically shuts down and restarts. The instrument is then ready for further use.

5.4.3 Installing a firmware update

In some cases, the software will include improvements for the LightCycler® 96 Instrument, which will require the instrument to be updated. New software releases for the firmware will be delivered together with the LightCycler® 96 Instrument Software and are automatically installed when the instrument software is updated. For detailed information, see section *Installing a LightCycler® 96 Instrument Software update*, on page 58.

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6 Detection formats

The LightCycler® 96 Instrument makes use of fluorescent dyes for online, real-time monitoring of both the generation of PCR products during cycling and the melting of PCR products. Fluorescence signals measured during cycling are correlated with the amount of PCR product in the reaction, allowing calculation of the input copy number of the target nucleic acid (possible both with sequence-specific and sequence-independent detection formats). With sequence-independent detection formats, fluorescence measurements are also used for PCR product characterization by melting curve analysis. For maximum flexibility, the LightCycler® 96 Instrument supports several fluorescent detection formats and can be used with a broad range of probes and dyes:

- Sequence-Specific Probe Binding Assays

  Rely on fluorophores coupled to sequence-specific oligonucleotide probes that hybridize to their complementary sequence in target PCR products, for example:

  - Hydrolysis probes (5´-nuclease assay)
  - Universal ProbeLibrary probes (5´-nuclease assay)
- Sequence-Independent Detection Assays using double-stranded DNA-binding dyes.

Optimal performance of the system is achieved using the LightCycler® 96 Instrument in combination with dedicated reagents. For detailed information on the LightCycler® 96 Reagents, please visit our Special Interest Site for Real-Time PCR Systems at www.lightcycler96.com.

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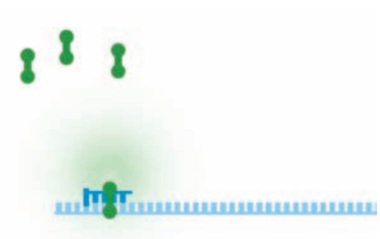
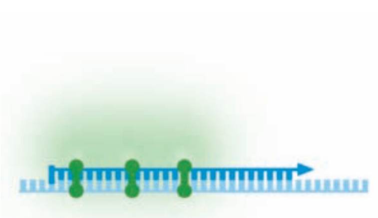
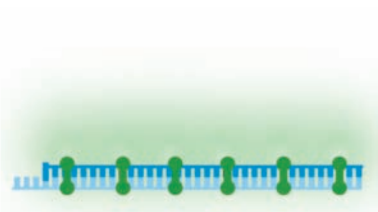
6.1 Monitoring PCR with an intercalating fluorescent dye

Generation of PCR products is detected by measuring the increase in the dye fluorescence (measured at 510 nm). In solution, unbound dye exhibits very little fluorescence. However, fluorescence is greatly enhanced after dye intercalation into the helix of dsDNA. During PCR, the increase in fluorescence is proportional to the amount of newly generated dsDNA.

The following are the basic steps of DNA detection using intercalating dyes such as SYBR Green I during real-time PCR on the LightCycler® 96 System:

1		During annealing, PCR primers hybridize to the target DNA strand and form small regions of dsDNA where the dye intercalates: the amount of fluorescence signal increases.
2		In the elongation phase, more dsDNA is formed and more dye can intercalate: the fluorescence signal increases exponentially.
3		At the end of the elongation phase, each strand of dsDNA contains the maximum amount of intercalated dye. For this reason, the fluorescence is measured (510 nm) at the end of each elongation phase when using intercalating dyes in real-time PCR.

Since the dye binds all dsDNA, regardless of the DNA sequence, it cannot discriminate between specific PCR products, primer-dimers and other nonspecific products. Any double-stranded PCR artifact will contribute to signal increase, which could result in overestimation of the concentration of the target sequence.

To determine whether only the desired PCR product has been amplified, a melting curve analysis should be performed directly after PCR. PCR product characterization by melting curve analysis is based on the fact that each particular dsDNA sequence has its characteristic melting point (the temperature at which 50% of the DNA is double-stranded and 50% is melted, and becomes single-stranded). The most important factors that determine the thermal stability of dsDNA are length in base pairs and GC content of the sequence.

During a melting experiment, the reaction mixture is slowly heated up, for example, from +60°C to +95°C. When the temperature reaches the melting point of a PCR product present in the reaction, the DNA strands separate and the fluorescence of the released dye decreases sharply.

The LightCycler® 96 Instrument continuously monitors the fluorescence over the temperature transition. In the LightCycler® 96 Software, these data are displayed as a melting peaks chart (first negative derivative of fluorescence [F] vs. temperature [T]), where the temperature of the melting peak maximum corresponds to the point of inflection in the melting curve that defines the melting point of the specific dsDNA fragment.

If the real-time PCR produces only one specific DNA amplicon, the melting curve analysis will show only one melting peak. If primer-dimers or other nonspecific products are present, they will be shown as additional melting peaks. Checking the melting temperature of a PCR product is thus used to confirm the specificity of the PCR product.

6.2 Monitoring PCR with hydrolysis probes

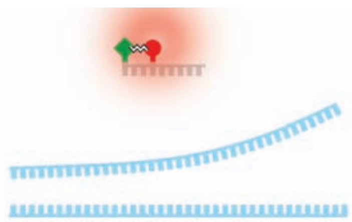
Hydrolysis probe assays can technically be described as homogeneous 5´-nuclease assays, since a single 3´-nonextendable probe, which is cleaved during PCR amplification, is used to detect the accumulation of a specific target DNA sequence. This single probe contains two labels, a fluorescent reporter and a quencher, in close proximity to each other. When the probe is intact, the quencher dye is close enough to the reporter dye to suppress the reporter fluorescent signal. Fluorescence quenching takes place via Fluorescence Resonance Energy Transfer (FRET).

During PCR, the 5´-nuclease activity of the polymerase cleaves the hydrolysis probe, separating reporter and quencher. After cleavage, the reporter is no longer quenched and emits a fluorescence signal when excited. The LightCycler® 96 Instrument can detect hydrolysis probes that are labeled with 4 classes of reporter dyes:

- Class 1: FAM
- Class 2: VIC, Hex or Yellow555
- Class 3: Red610 or Texas Red
- Class 4: Cy5

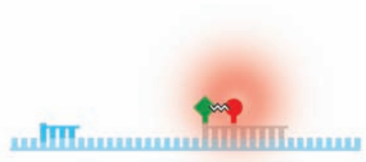
Hydrolysis probes can be used separately or in combination, which permits either single-color or multi-color detection.

1



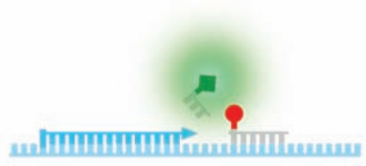
A hydrolysis probe carries two fluorescent dyes in close proximity, with the quencher dye suppressing the reporter fluorescence signal. The 3´-end of the probe is phosphorylated, so it cannot be extended during PCR.

2



In the annealing phase of PCR, primers and probes specifically anneal to the target sequence.

3



As the DNA polymerase extends the primer, it encounters the probe. The polymerase then cleaves the probe with its inherent 5´-nuclease activity, displacing the probe fragments from the target, and continues to polymerize the new amplicon.

4		In the cleaved probe, the reporter dye is no longer quenched and therefore can emit fluorescence that can be measured in the appropriate detector channel of the LightCycler® 96 Instrument. The increase in fluorescence from the reporter dye correlates to the accumulation of released reporter dye molecules (and thus indirectly to the amount of PCR products). The fluorescence signal of the reporter dye is measured at the end of the elongation phase.

In the hydrolysis probe format, PCR products cannot be characterized by melting curve analysis, because the generation of fluorescence signals does not depend on the hybridization status but on irreversible digestion of the probe.

For a 5'-nuclease digestible hybridization complex to form correctly, the melting temperature of the probe should be higher than the melting temperature of the PCR primer.

7 Analysis principles

The LightCycler® 96 Application Software provides different analyses modules for a PCR experiment:

- Absolute quantification; see section *Absolute quantification analysis*, on page 68.
- Relative quantification; see section *Relative quantification analysis*, on page 68.
- Endpoint genotyping; see section *Endpoint genotyping analysis*, on page 73.
- T_m calling; see section *Tm calling*, on page 74.

Color compensation

To correct the fluorescence crosstalk, color compensation is applied automatically before data analysis. The LightCycler® 96 Application Software automatically reassigns the fluorescence in each channel to the appropriate dye. This results in the detection of only one dye in each channel.

For multicolor experiments, color compensation is performed automatically for all analysis modules. When measuring a typical multicolor amplification data set, all amplification curves detected in heterologous channels are called *Negative* by the automatic positive/negative algorithm. For detailed information on the algorithm, see section *Positive/negative filter*, on page 65.

7.1 Quantification analysis

A quantification analysis can be performed on any experiment in which a nucleic acid is:

- Amplified using a cycling program and
- Detected via fluorescent signals that originate from DNA-binding generic dyes or sequence-specific probes

Taking advantage of real-time, online monitoring of PCR, the software considers fluorescence values measured in the exponentially growing log-linear phase of the PCR amplification process for analysis of the quantification data.

A typical quantification experiment performed on the LightCycler® 96 Instrument is shown in the figure below. The reaction profile contains three phases: the initial background phase, an exponential (log-linear) growth phase, and a final plateau phase. The initial phase lasts until the fluorescence signal from the PCR product is greater than the background fluorescence. The exponential log phase begins when sufficient product has accumulated to be detected above the background and ends when the reaction efficiency falls as the reaction enters the plateau.

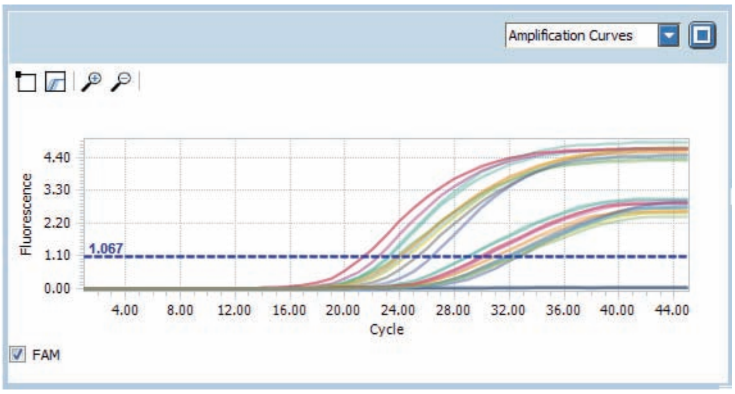


Figure 12: Typical quantification experiment

A perfect amplification reaction with an efficiency of 2 (that is, every PCR product is replicated once in every cycle) can be described during the log-linear phase by $T_n = T_0 \times 2^n$.

Real-time PCR experiments, however, are influenced by many factors, and therefore efficiency may not be perfect. Thus PCR amplification is more accurately described as: $T_n = T_0 \times E^n$,
where T_n is the amount of gene molecules at cycle n, T_0 is the initial number of gene molecules, n is the number of amplification cycles, and E is the efficiency of amplification.

The figure above shows that the cycle where each reaction first rises above the background depends on the amount of the gene that is present at the beginning of the reaction. The cycle at which the fluorescence of a sample rises above the background fluorescence is called the quantification cycle (Cq) of the sample, also known as "crossing point" (Cp) or "threshold cycle" (Ct).

The estimated quantity may be:

- Absolute in terms of an actual copy number or quantity, or
- Relative by comparing a target gene to a reference gene

Absolute quantification and relative quantification share many common features, including calling of Cq values from amplification curves, use of standards, positive/negative calling, etc. However, since both methods are based on different calculation workflows, they are separated in different analysis modules.

Positive/negative filter

The LightCycler® 96 Application Software automatically applies a positive/negative filter to produce a qualitative call. The positive/negative algorithm calls three different result types:

- Positive:
  The fluorescence curve shows an adequate signal/noise ratio and an appropriate sigmoid curve shape.
- Negative:
  The fluorescence curve does not show an adequate signal/noise ratio and shows an inappropriate sigmoid curve shape.
- Invalid:
  Artifacts (very rare cases)

The LightCycler® 96 Application Software provides a function to manually define gene-specific thresholds for the Cq (maximum value), slope (minimum value) and endpoint fluorescence (minimum value) to change the positive/negative call. For detailed information on these settings, see sections *Abs Quant Settings*, on page 150 and *Rel Quant Settings*, on page 159.

Cycle of Quantification (Cq)

In an amplification reaction, the cycle at which the fluorescence of a sample rises above the background fluorescence is called the cycle of quantification (Cq) of the sample. The Cq is thus a single value reflecting the cycle number used for quantification.

The larger the quantity (or concentration) of the gene present before amplification, the fewer cycles of amplification will be required to amplify that gene to a detectable level. As the gene reaches a detectable level, the exponential increase of the gene becomes visible as the fluorescence signal from the PCR product is greater than the background fluorescence.

This correlation between amount of template and value of Cq facilitates all types of real-time PCR-based quantitative analysis. Because of this relationship between Cq and starting quantity, real-time PCR makes data acquisition and analysis during the exponential phase easy and therefore allows sensitive quantification of a given target.

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The LightCycler® 96 Application Software uses predefined fluorescence threshold values to calculate the Cq value of a sample. The predefined fluorescence threshold values depend on the detection format (dye) used for the experiment. For detailed information on how to specify the detection format, see section *Detection Format*, on page 126.

The Cq value for a sample is only displayed in the results if the sample is determined 'Positive' by the positive/negative filter algorithm or by the gene-specific threshold set manually by the operator.

*The Cq threshold and the positive/negative threshold are two independent thresholds that are not correlated. Cq values are calculated **once** for each sample and do **not** change when the positive/negative threshold is changed manually by the operator. The Cq threshold cannot be changed by the operator.*

Endpoint fluorescence

The endpoint fluorescence (EPF) of an amplification curve indicates the last fluorescence value of the background-corrected curve.

Background correction in all quantification analysis modules is performed by dividing each data point by the background value of the relevant curve and subsequently subtracting a value of "1" (proportional background correction).

Standard curves

In a quantification analysis, a standard curve is used to determine the quantity of unknown samples and the amplification efficiency of a certain gene. In a standard curve, the quantities of standard samples are plotted against the Cqs of the samples. The x-axis represents the log of the initial target quantity, and the y-axis represents Cq in cycles. The standard curve is a linear regression line through these plotted data points.

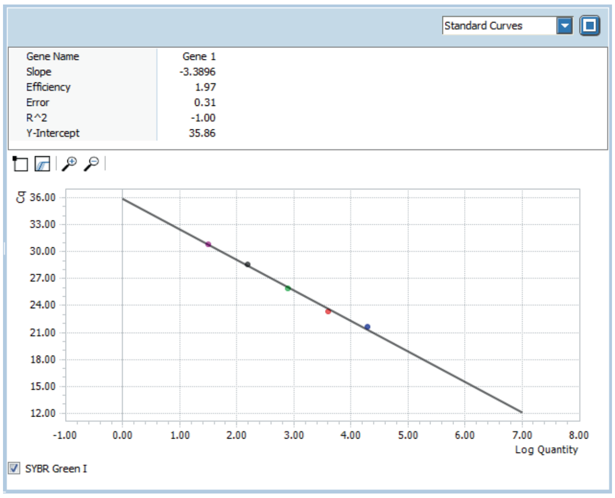

Figure 13: Standard curve in a quantification experiment

A standard curve is set up with at least three dilution steps. The quantities chosen for the standard curve should fall between the expected quantity range of the gene of interest.

In the LightCycler® 96 Application Software, at least three standard concentrations are required to calculate the standard curve. Otherwise, no standard curve is calculated.

- The PCR *Efficiency* can be calculated using the formula: $E = 10^{-1/\text{slope}}$
(for example, slope = -3.3 → E = 2).
The software automatically calculates the efficiency and displays it in the standard curve chart.

For the valid use of the standard curve, PCR amplification must be highly reproducible and reaction conditions must be constant for all experiments. It is recommended to run tests to ensure stable PCR efficiency and to use replicate samples (especially for low quantities) to create the standard curve. Also, you should include a previously quantified sample in each analyzed run, to verify that the calculated values are reproducible.

- The *Slope* of the standard curve describes the kinetics of the PCR amplification. It indicates how quickly the amount of target nucleic acid (NA) can be expected to increase with the amplification cycles. The slope of the standard curve can be calculated from the *Efficiency* of the amplification reaction using the formula $s = -\log^{E}$. A perfect amplification reaction would produce a standard curve with an efficiency of 2.00.
- The display of a standard curve shows a *Y-Intercept*. The x-axis is adjusted to display the calculated values properly.
- The *Standard Error of Estimate* value (mean squared error of the single data points fitted to the regression line) is a measure of the accuracy of the quantification result based on the standard curve (an acceptable value should be < 0.2).
- The *Correlation Coefficient* of a standard curve shows the r squared value of the correlation.

7.1.1 Absolute quantification analysis

Absolute quantification enables you to quantify a single or multiple genes and express the final result as an absolute value (for example, copies/ml). The absolute values for quantity or copy number are derived from dilutions of gene-specific standards with known quantity/copy number.

The easiest way to obtain an absolute value for an unknown quantity of gene is to compare the Cq of an unknown sample against those of standards with known quantities. Standard material can be selected from various sources (for example, linearized plasmid DNA carrying the cloned target sequence, purified PCR products). The gene quantity in the standard must be known. PCR is then performed with a series of dilutions of the standard, which represent different gene quantities in a reaction.

The known template amount of each standard dilution is automatically plotted against the measured Cq values. The resulting regression line is called the standard curve and shows the correlation between Cq and quantity. By comparing the Cq values from samples with unknown amounts of template to this standard curve, one can determine the starting amount of template in each sample.

7.1.2 Relative quantification analysis

Ratio

Relative quantification compares the levels of two different gene sequences in a single sample (for example, target gene of interest (GOI) and a reference gene), and expresses the final result as a ratio of these genes.

$$\text{ratio} = \frac{\text{target concentration}}{\text{reference concentration}}$$

For comparison purposes, the reference gene is assumed to be present in constant numbers under all test conditions. This reference gene provides a basis for normalizing sample-to-sample differences. To improve this normalization step, multiple reference genes can be chosen. Each target gene is then normalized to the geometric mean quantity of all reference genes. If no single reference gene is suitable for all samples, consider using more than one reference gene and averaging their assay levels to form a single reference value.

Normalized ratio

In addition to calculating sample-specific ratios, a normalized ratio can be calculated by defining a special run-specific sample type, the "run calibrator" sample. This run calibrator is a positive sample with a stable ratio of target-to-reference and is used to normalize all samples within one run. It also provides a constant calibration point between several LightCycler® 96 System runs.

The result is expressed as a normalized ratio, that is, target/reference ratio (unknown sample) divided by target/reference ratio (calibrator):

$$\text{normalized ratio} = \frac{\text{target/reference}_{\text{(unknown sample)}}}{\text{target/reference}_{\text{(run calibrator)}}}$$

While a calibrator corrects for differences in detection sensitivity between target and reference caused by differences in probe annealing, probe labeling, or dye extinction coefficients, it does not correct for differences in PCR efficiency between the target and reference gene.

Scaled ratio

In addition to normalization, it is frequently required to measure the gene expression of each sample and each gene at different times or under different conditions. To generate a meaningful result, the sample-specific measurements are normalized to a common basis, that is, a certain experimental condition, to provide a scaled ratio. This condition is specified as the "study calibrator" condition.

The scaled ratio for a specific sample is calculated by dividing all ratios by the ratio of the selected base value (that is, the value in the study calibrator condition):

$$\text{scaled ratio} = \frac{\text{target/reference}_{\text{(unknown sample at condition x)}}}{\text{target/reference}_{\text{(unknown sample at study calibrator condition)}}}$$

The following example shows the multiwell plate scheme for a simple relative quantification experiment with multiple samples measured under multiple conditions. In this example, "T_{S1}" represents the target gene in Sample 1, "R_{S1}" the reference gene in Sample 1, "T_{S2}" the target gene in Sample 2, and so on.

The study calibrator condition is specified as "0 hours". Then the base value for the calculation is the relative gene expression ratio measured for each sample at the study calibrator condition, that is, the start of the experiment.

	0 h		1 h		2 h		3 h		2 d		4 d		
	1	2	3	4	5	6	7	8	9	10	11	12	
A	T_{S1}	R_{S1}	T_{S1}	R_{S1}	T_{S1}	R_{S1}	T_{S1}	R_{S1}	T_{S1}	R_{S1}	T_{S1}	R_{S1}	Sample 1
B	T_{S2}	R_{S2}	T_{S2}	R_{S2}	T_{S2}	R_{S2}	T_{S2}	R_{S2}	T_{S2}	R_{S2}	T_{S2}	R_{S2}	Sample 2
C	T_{S3}	R_{S3}	T_{S3}	R_{S3}	T_{S3}	R_{S3}	T_{S3}	R_{S3}	T_{S3}	R_{S3}	T_{S3}	R_{S3}	Sample 3
D	T_{S4}	R_{S4}	T_{S4}	R_{S4}	T_{S4}	R_{S4}	T_{S4}	R_{S4}	T_{S4}	R_{S4}	T_{S4}	R_{S4}	Sample 4
E	T_{S5}	R_{S5}	T_{S5}	R_{S5}	T_{S5}	R_{S5}	T_{S5}	R_{S5}	T_{S5}	R_{S5}	T_{S5}	R_{S5}	Sample 5
F	T_{S6}	R_{S6}	T_{S6}	R_{S6}	T_{S6}	R_{S6}	T_{S6}	R_{S6}	T_{S6}	R_{S6}	T_{S6}	R_{S6}	Sample 6
G	T_{S7}	R_{S7}	T_{S7}	R_{S7}	T_{S7}	R_{S7}	T_{S7}	R_{S7}	T_{S7}	R_{S7}	T_{S7}	R_{S7}	Sample 7
H	T_{S8}	R_{S8}	T_{S8}	R_{S8}	T_{S8}	R_{S8}	T_{S8}	R_{S8}	T_{S8}	R_{S8}	T_{S8}	R_{S8}	Sample 8

The scaled ratio for Sample 1, for example after two hours, is calculated as follows:

$$\text{scaled ratio}_{S1,\,2h} = \frac{\text{target/reference}_{(S1,\,2h)}}{\text{target/reference}_{(S1,\,0h)}}$$

The scaled ratio for Sample 2, for example after two hours, is calculated as follows:

$$\text{scaled ratio}_{S2,\,2h} = \frac{\text{target/reference}_{(S2,\,2h)}}{\text{target/reference}_{(S2,\,0h)}}$$

Scaled ratio including run calibrator

The LightCycler® 96 Application Software provides a third ratio calculation to obtain scaled and normalized ratios. If, in addition to the study calibrator, a run calibrator sample is specified, the scaled normalized ratio for each sample is calculated by dividing the normalized ratios of all conditions by the normalized ratio of the study calibrator condition:

$$\text{scaled normalized ratio} = \frac{\dfrac{\text{target/reference}_{\text{(unknown sample at condition x)}}}{\text{target/reference}_{\text{(run calibrator)}}}}{\dfrac{\text{target/reference}_{\text{(unknown sample at study calibrator condition)}}}{\text{target/reference}_{\text{(run calibrator)}}}}$$

Relative quantification usually requires the use of either a run calibrator or a study calibrator per single multiwell plate to generate detection-sensitivity-corrected results for all measured genes. Only when multiple plates are combined in a relative quantification study, might special plate setups require the use of both, a run calibrator and a study calibrator.

PCR efficiency correction

The reliability of all quantitative real-time PCR applications and, consequently, of all relative quantification calculations depends on the quality of the PCR. The final ratio resulting from the relative quantification is a function of PCR efficiency and of the quantification cycles. It does not require the knowledge of absolute copy numbers at the detection threshold. Thus, the analysis does not determine the actual quantity of DNA in the samples.

A target-specific efficiency can be either:

- Derived from in-run target-specific standard curves, or
- Set manually if no standards are defined or if standards are excluded from the calculation.

This efficiency-corrected ratio calculation is automatically performed by the LightCycler® 96 Application Software.

The basic prerequisites for accurate relative quantification are:

- *When standards are used, the efficiencies of the relative standards and the unknown samples are identical.*
- *The efficiencies of both target and reference PCR do not vary from sample to sample.*
- *The reference gene is not regulated in the system being examined.*

Ratio calculation for a single reference gene

If only one reference gene is used in experiments, the following basic calculations for ratio, normalized ratio and scaled ratio are performed:

$$\text{ratio} = \frac{E_R^{Cq_R}}{E_T^{Cq_T}}$$

$$\text{normalized or scaled ratio} = \frac{E_T^{(Cq_{T,\,cal} - Cq_T)}}{E_R^{(Cq_{R,\,cal} - Cq_R)}}$$

E_T	Amplification efficiency of the target gene
E_R	Amplification efficiency of the reference gene
Cq_T	Quantification cycle of the target gene
Cq_R	Quantification cycle of the reference gene
cal	Run or study calibrator

Ratio calculation for multiple reference genes

If multiple reference genes are used in an experiment, the geometric mean of the concentration ratios is calculated. In a case with two references and use of a run calibrator or study calibrator, the following formula applies:

$$\text{ratio} = \frac{\sqrt{E_{R1}^{Cq_{R1}} \times E_{R2}^{Cq_{R2}}}}{E_T^{Cq_T}}$$

$$\text{normalized or scaled ratio} = \frac{E_T^{(Cq_{T,\,cal} - Cq_T)}}{\sqrt{E_{R1}^{(Cq_{R1,\,cal} - Cq_{R1})} \times E_{R2}^{(Cq_{R2,\,cal} - Cq_{R2})}}}$$

Calculations with more than two reference genes are performed, taking higher orders of the root by calculating the product of all terms.

$$\text{ratio} = \frac{\sqrt[n]{\Pi_{i=1}^{n} E_{Ri}^{Cq_{Ri}}}}{E_T^{Cq_T}}$$

$$\text{normalized or scaled ratio} = \frac{E_T^{(Cq_{T,\,cal} - Cq_T)}}{\sqrt[n]{\Pi_{i=1}^{n} E_{Ri}^{(Cq_{Ri,\,cal} - Cq_{Ri})}}}$$

E_T	Amplification efficiency of the target gene
E_{Ri}	Amplification efficiency of the reference gene i
Cq_T	Quantification cycle of the target gene
Cq_{Ri}	Quantification cycle of the reference gene i
cal	Run or study calibrator

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Error calculation

The calculations in the LightCycler® 96 Application Software are based on measured values which have uncertainties (represented by the standard deviations of technical replicates). The functions employed in relative quantification analysis calculations combine these values and lead to a complex error propagation.

The LightCycler® 96 Application Software follows the principle of linearization and standard distribution when statistical variables and error propagation occur. The employed method is also known as the "Delta Method" in statistics and is based on taking the first derivative of the functions calculating ratio, normalized ratio or scaled ratio. For variable calculation a first order linear approximation is performed (known as "first order Taylor series expansion").

The calculations in relative quantification analysis are based on the quantification cycle (Cq) and the PCR efficiency (E). However, for error calculation only the Cq values are considered uncertain. The gene-specific PCR efficiencies are regarded as exact values.

In the case of uncorrelated uncertainties and assumption of a normal distribution, the standard deviation of a function can be written as the sum of weighted variances of the components.

$$\mathrm{sd(ratio)} \approx \sqrt{\Sigma_{k=1}^{n}\left(\left(\frac{\delta \mathrm{ratio}}{\delta Cq_k}\right)^2 \mathrm{var}(Cq_k)\right)}$$

The weighted terms for each individual gene (each technical replicate group) are calculated by the following formula and hold true for any number of genes and combinations thereof:

$$\left(\frac{\delta r_j}{\delta Cq_g}\right)^2 = \left(\frac{r_j}{n_g} \times \ln(E_g)\right)^2$$

$\frac{\delta r_j}{\delta Cq_g}$	Partial first derivative of r_j with respect to Cq_g
r_j	Ratio j
Cq_g	Quantification cycle of gene g
n_g	Number of genes of a certain type (target or reference)
E_g	Efficiency of gene g

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7.2 Endpoint genotyping analysis

Endpoint genotyping analysis is used for SNP (single nucleotide polymorphism) genotyping (also known as allelic discrimination). Endpoint genotyping assays use hydrolysis probes. For detailed information, see section *Monitoring PCR with hydrolysis probes*, on page 62.

An endpoint genotyping assay includes two sequence-specific probes that are designed to detect allele x and allele y and are labeled with different reporter dyes.

In a standard setup

- The FAM dye detects samples that are homozygous for allele x.
- The VIC dye detects samples that are homozygous for allele y.

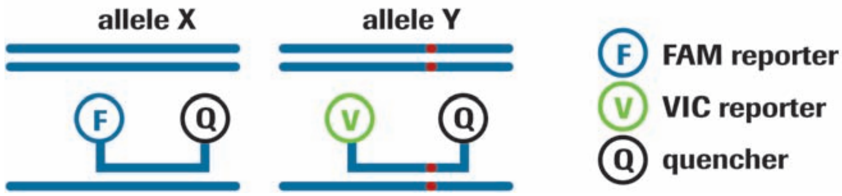

Figure 14: Dual-color principle of endpoint genotyping

Fluorescence data are collected throughout the PCR amplification. However, only the endpoint signal intensities of the two reporter dyes are used to identify the genotypes. The relative dye intensities can be visualized on a scatter plot, simplifying discrimination into homozygous x, homozygous y, and heterozygous samples. After manually setting up the thresholds, the LightCycler® 96 Application Software groups the samples based on the intensity distribution of the two dyes.

LightCycler® 96 Application Software allows manual grouping of samples with similar fluorescence distribution in the scatter plot. The following groups can be defined by the operator:

- Negatives
- Homozygous for allele x
- Homozygous for allele y
- Heterozygous
- Unknowns

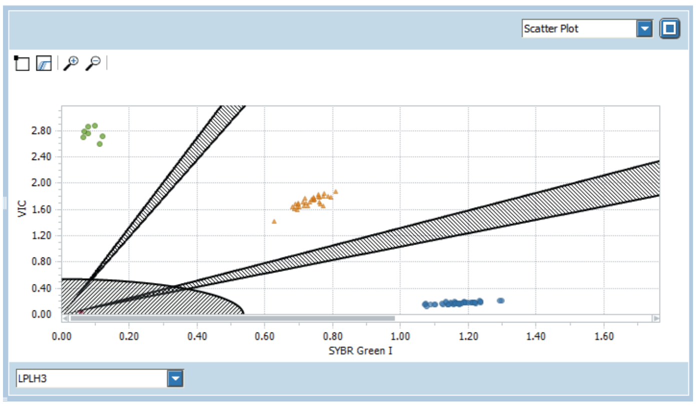

Figure 15: Scatterplot for an endpoint genotyping experiment

Each point represents a sample, whose x-coordinate is the endpoint level of allele x, and whose y-coordinate is the endpoint level of allele y.

7.3 T_m calling

The temperature at which DNA strands separate or melt when heated can vary over a wide range, depending on the sequence, the length of the strand, DNA quantity, and buffer composition. For example, melting temperatures can vary for products of the same length but different Adenine-Thymine/Guanine-Cytosine ratio (AT/GC ratio), or for products with the same length and GC content but with a different GC distribution. Also, base pair mismatches between two DNA molecules lead to a decrease in melting temperature. This effect is more pronounced for short DNA amplicons.

The purpose of T_m calling is to determine the characteristic melting temperature of the target DNA.

To analyze sample melting temperature profiles, the fluorescence of the samples must be monitored while the temperature of the LightCycler® 96 Instrument thermal block cycler is steadily increased. As the temperature increases, sample fluorescence decreases. In the case of the double-stranded DNA-specific dye SYBR Green I or the ResoLight dye, this is due to the separation of the DNA strands and consequently the release of dye molecules.

A T_m calling analysis can be performed on any experiment that includes a melting program. A melting program is usually performed after amplification of the target DNA. A typical melting program includes three segments:

- The samples are rapidly heated to a temperature high enough to denature all DNA molecules.
- The samples are cooled below the annealing temperature of the target DNA.
- The samples are slowly heated while measuring sample fluorescence as the target DNA melts.

You can use melting temperatures from +37 to +98°C.

Melting curves

The analysis displays a melting curves chart of sample fluorescence against temperature. The chart shows the downward curve in fluorescence for the samples as they melt.

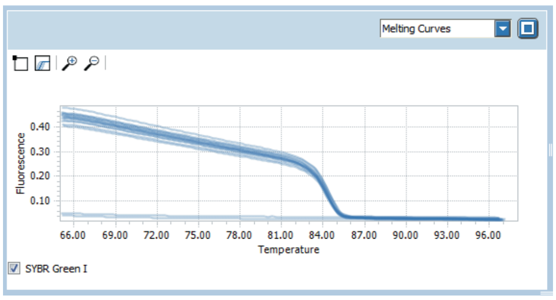

Figure 16: Melting curves chart

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Melting peaks

The analysis also displays a melting peaks chart that plots the first negative derivative of the sample fluorescent curves. In this chart, the melting temperature of each sample appears as a peak. Displaying the melting temperatures as peaks makes it easier to distinguish each sample's characteristic melting profile and to discern differences between samples.

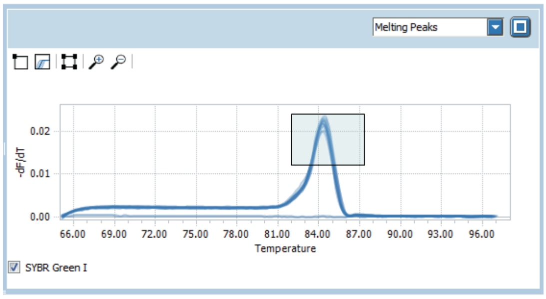

Figure 17: Melting peaks chart

The decrease in fluorescence during melting is, in the case of DNA-binding dyes (for example, SYBR Green I, ResoLight dye), due to the separation of DNA strands and consequently the release of dye molecules. As these dyes only fluoresce at 530 nm if bound to double-stranded DNA, melting drastically decreases fluorescence at this wavelength. The melting temperature, or T_m, is defined as the point at which half of the DNA is double-stranded and half is single-stranded.

After amplification in a hydrolysis probe PCR assay, all probes are hydrolyzed. Thus, T_m calling cannot be performed.

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Chapter B

LightCycler® 96 Application Software

B

B

B

LightCycler® 96 Application Software

1 Overview

The LightCycler® 96 Application Software provides all functions for defining an experiment protocol and analyzing the data gathered during the experiment run.

- Specifying the temperature profile and the dye-specific parameters for an experiment run; see section *Run Editor tab*, on page 120.
- Creating, editing, deleting, and rearranging samples and genes present in the wells, as well as the dyes used to label each gene; see section *Sample Editor tab*, on page 128.
- Creating analysis, defining analysis settings, and viewing the calculated results of an experiment run; see section *Analysis tab*, on page 145.

For starting a run, the experiment must be transferred to the LightCycler® 96 Instrument. For analysis, the raw data gathered by the LightCycler® 96 Instrument Software must be transferred back to the application software. For detailed information on the LightCycler® 96 Instrument Software, see chapter *LightCycler® 96 Instrument Software*, on page 183.

The computer running the LightCycler® 96 Application Software can be connected to the LightCycler® 96 Instrument either via a local Ethernet network or directly (point-to-point connection). In both cases, the operator can register the instrument with the software for monitoring the instrument and for sending and retrieving experiments. For detailed information, see section *Instrument Manager*, on page 110.

The LightCycler® 96 Application Software may be installed on a computer which is connected to a network. Please be aware that such connection may have an adverse effect on the product's integrity, for example, through infection with malicious code (viruses, Trojan horses, etc.) or access by unauthorized third parties (suc h as intrusion by hackers). Roche therefore highly recommends protecting the product against such risks by taking appropriate and state-of-the-art action.

As the product is not intended to be used within networks without an appropriate firewall, and has not been designed for such use, Roche assumes no liability in this regard.

1.1 Experiment file types

The LightCycler® 96 Application Software supports the following experiment file types:

- *.lc96 (LightCycler® 96 experiment files)
- *.rdml (Real-time PCR Data Markup Language files)

By default, all newly generated experiment files have the file type *.lc96. Operators can save experiment files as LightCycler® 96 files or as RDML files without changing their content.

Both file types can be opened in the LightCycler® 96 Application Software. To enable opening of LightCycler® 96 experiment files with a third-party RDML-compatible software, the files must be saved as *.rdml.

1.2 Starting the software

Before starting the software, it must be installed on your computer. For detailed information on the installation, see section *Installing the LightCycler® 96 Application Software*, on page 56.

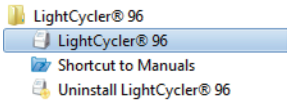
To start the LightCycler® 96 Application Software

1. Switch on the computer.

2. Perform one of the following steps:
   - Double-click the *LightCycler® 96* icon on your desktop.

   - In the *Start* menu, navigate to the *LightCycler® 96* folder and choose *LightCycler® 96*.

   The LightCycler® 96 Application Software provides a splash screen with information on the initialization status. After initialization, the main window opens displaying the startup wizard (see below).

1.3 Startup wizard

The startup wizard opens after the LightCycler® 96 Application Software is launched. It provides shortcuts to high-level tasks like creating or opening an experiment, and links to additional information.

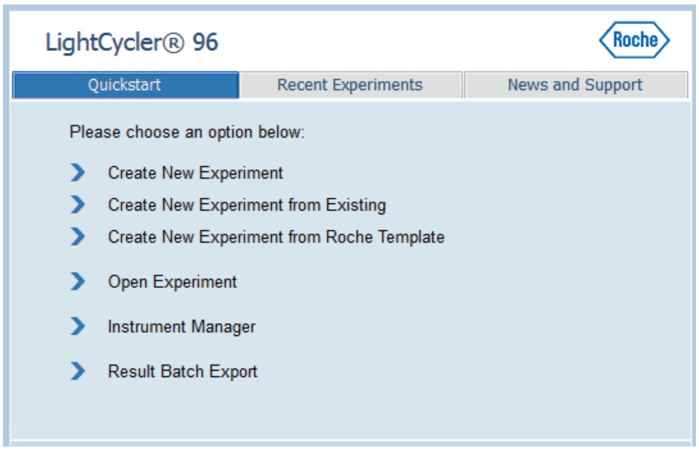


Figure 18: Startup wizard, Quickstart tab

B

Quickstart tab

The *Quickstart* tab (see *Figure 18* above) provides the following shortcuts:

Shortcut	Description
Create New Experiment	Creates a new, empty experiment. For detailed information, see section *To generate a new experiment*, on page 102.
Create New Experiment from Existing	Opens the *Open* dialog box for choosing an existing experiment to be used as a template. For detailed information, see section *To use an existing experiment as a template*, on page 102.
Create New Experiment from Roche Template	Opens the *Open* dialog box for choosing an experiment template provided by Roche. For detailed information, see section *To use a Roche template*, on page 103.
Open Experiment	Opens the *Open* dialog box for choosing an experiment. For detailed information, see section *To open an experiment*, on page 104.
Instrument Manager	Opens the *Instrument Manager* dialog box for managing the LightCycler® 96 Instruments registered with the application software. For detailed information, see section *Instrument Manager*, on page 110.
Result Batch Export	Opens the *Result Batch Export* wizard for creating a file with result table data collected from different experiments of the same type. For detailed information, see section *Result Batch Export*, on page 116.

Recent Experiments tab

Figure 19: Startup wizard, Recent Experiments tab

The *Recent Experiments* tab lists the 10 last-opened experiments. If one of the recent experiments is no longer available, the according entry is deleted.

Choosing one of the list entries opens the corresponding experiment.

News and Support tab

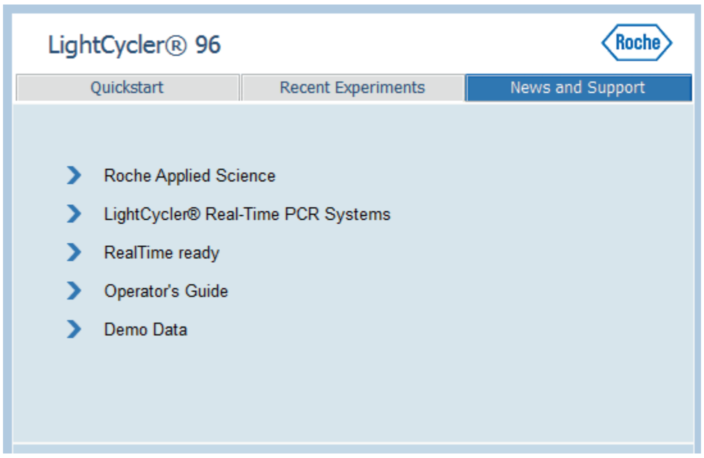


Figure 20: Startup wizard, News and Support tab

The *News and Support* tab provides links to the following websites and locations:

Shortcut	Link/Description
Roche Applied Science	*http://www.roche-applied-science.com*
LightCycler® Real-Time PCR Systems	*http:www.lightcycler.com*
RealTime ready:	*http:realtimeready.roche.com*
Operator's Guide:	Displays the LightCycler® System Guides.
Demo Data:	Displays the demo experiment files delivered with the software. The files are located under: *C:/Users/<user>/AppData/Roaming/Roche Diagnostics/ LightCycler® 96/DemoData*

1.4 The main window

The figure below shows the main window of the LightCycler® 96 Application Software (in this example, the *Run Editor* tab of an experiment is shown). The main window contains the following areas, described below:

- Menu bar
- Tool bar
- Working window area with working window area tabs representing the main software functions
- Experiment bar

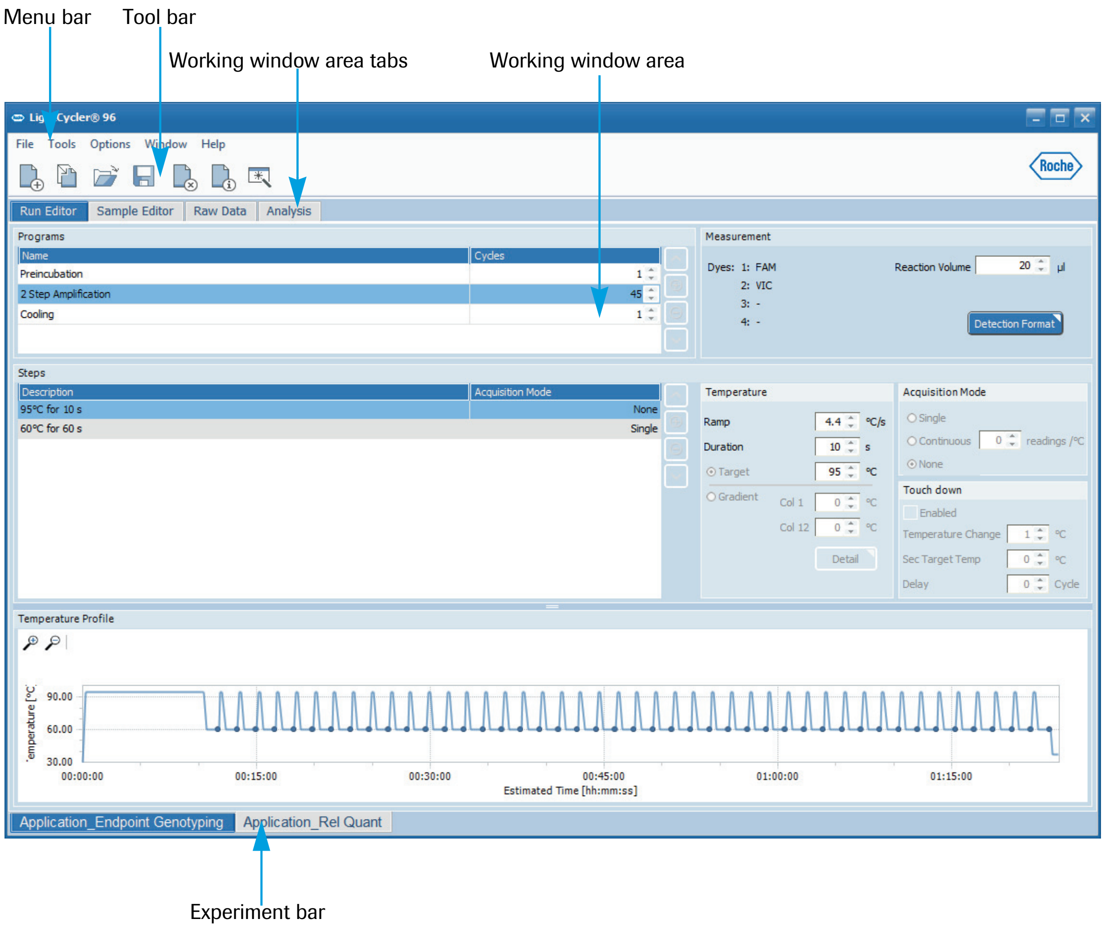


Figure 21: LightCycler® 96 Application Software main window

1.4.1 Menu bar

The menu bar provides access to the software functions.

File menu

The commands in the *File* menu provide functions for managing experiments and for exiting the LightCycler® 96 Application Software.

Command	Description
New > Experiment	Creates a new, empty experiment. For detailed information, see section *To generate a new experiment*, on page 102.
New > Experiment from Existing	Opens a file selection dialog box for choosing an existing experiment to be used as a template. For detailed information, see section *To use an existing experiment as a template*, on page 102.
New > Experiment from Roche Template	Opens a file selection dialog box for choosing an experiment template provided by Roche. For detailed information, see section *To use a Roche template*, on page 103.
Open	Opens a file selection dialog box for choosing an experiment. For detailed information, see section *To open an experiment*, on page 104.
Close	Closes the current experiment file. The operator is prompted to save unsaved data before the experiment is closed.
Recent Files	Lists the recently opened experiments. Choosing one of the list entries opens the corresponding experiment.
Save	Saves the current experiment to its associated file. For detailed information, see section *To save an experiment*, on page 105.
Save As	Saves the current experiment to a new file. For more information, see section *To save an experiment*, on page 105.
Properties	Opens the *Properties* dialog box displaying the experiment summary and the experiment notes. For detailed information, see section *Experiment properties*, on page 106.
Exit	Exits the LightCycler® 96 Application Software. The operator is prompted to save unsaved data before the application shuts down.

Tools menu

The *Tools* menu provides access to the following functions:

Command	Description
Instrument Manager	Opens the *Instrument Manager* wizard for managing and monitoring the LightCycler® 96 Instruments registered with the application software. For detailed information, see section *Instrument Manager*, on page 110.
Result Batch Export	Opens the *Result Batch Export* wizard, which allows for exporting the result table data collected from multiple experiment files. For detailed information, see section *Result Batch Export*, on page 116.

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Options menu

The *Options* menu provides general configuration functions. The *Preferences* command opens the *Preferences* dialog box, where the default directory for searching for and saving experiment files is to be defined. For detailed information, see section *Preferences*, on page 119.

Window menu

The *Show Startup Wizard* command in the *Window* menu provides access to the LightCycler® 96 Application Software startup wizard. For detailed information, see section *Startup wizard*, on page 81.

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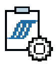
Help menu

The *Help* menu provides access to the following information:

Command	Description
Operator's Guide	Shortcut to the LightCycler® 96 System Guides in the installation folder.
About	Opens the *About* dialog box, which displays the software version and copyright information about the software.

1.4.2 Tool bar

The icons in the tool bar provide quick access to the following functions:

Icon	Function	Description
	New Experiment	Creates a new, empty experiment. For detailed information, see section *To generate a new experiment*, on page 102.
	New Experiment from Existing	Opens the *Open* dialog box for choosing an existing experiment to be used as a template. For detailed information, see section *To use an existing experiment as a template*, on page 102.
	Open Experiment	Opens the *Open* dialog box for choosing an experiment. For detailed information, see section *To use a Roche template*, on page 103.
	Save Experiment	Only available if an experiment is opened: Saves the current experiment to its associated file. For detailed information, see section *To save an experiment*, on page 105.
	Close Experiment	Only available if an experiment is opened: Closes the current experiment file. The operator is prompted to save unsaved data before the experiment is closed.
	Properties	Only available if an experiment is opened: Opens the *Properties* dialog box displaying the experiment summary. For detailed information, see section *Experiment properties*, on page 106.
	Show Startup Wizard	Opens the LightCycler® 96 Application Software startup wizard. For detailed information, see section *Startup wizard*, on page 81.
	Add Analysis	Only displayed if the *Analysis* tab is opened: Opens the *Create New Analysis* dialog box to select a new analysis and edit the name of the corresponding tab. For detailed information, see section *Adding a new analysis*, on page 147.
	Analysis Settings	Only displayed if the *Analysis* tab is opened and at least one analysis is defined: Opens the *<analysis> Settings* dialog box for specifiying the analysis-specific settings. For detailed information, see the descriptions of the corresponding analysis tabs.  *Changing the analysis settings invalidates the results and causes an automatic recalculation to be performed.*
	Delete Analysis	Only displayed if the *Analysis* tab is opened and at least one analysis is defined: Removes the analysis from the experiment.

1.4.3 Working window area tabs

The LightCycler® 96 Application Software provides the complete workflow via the tabs in the working window area of the experiment:

Tab	Description
Run Editor	Provides the following functions: ▶ Defining the detection format and dye settings for the experiment ▶ Defining the temperature and cycling sequence for the experiment For detailed information, see section *Run Editor tab*, on page 120.
Sample Editor	Provides the following functions: ▶ Defining sample and gene names ▶ Defining the sample types For detailed information, see section *Sample Editor tab*, on page 128.
Raw Data	Provides the raw data collected during an experiment run. For detailed information, see section *Raw Data tab*, on page 142.
Analysis	Provides the functions and methods for analyzing the results of an experiment run. For detailed information, see section *Analysis tab*, on page 145.

1.4.4 Experiment bar

The experiment bar provides an entry for each open experiment. Choosing one of these entries displays the last opened tab for the corresponding experiment.

Each entry in the experiment bar provides a tooltip showing the location where the experiment file is stored. The tooltip for a new experiment shows the default name for new experiments.

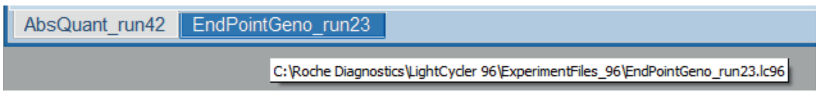


Figure 22: Experiment bar

1.5 General software conventions

1.5.1 Regional settings

The LightCycler® 96 Application Software user interface displays date and floating-point numbers in English (USA) specified by the Microsoft Windows Regional and Language Options.

1.5.2 Buttons

Button design

In the LightCycler® 96 Application Software user interface, general button design conventions illustrate the function of each button using specific button indicators:

Button	Marking	Description
OK	White triangle in bottom left corner	Completes the action and closes the dialog.
Detection Format	White triangle in top right corner	Opens a secondary dialog.
Apply	No triangle	Performs a specified action in the current dialog.
Apply	White border, label gray, button transparent	Button is disabled.

Standard buttons

The LightCycler® 96 Application Software user interface uses the following standard buttons in dialog boxes:

Button	Description
OK	Closes the dialog box and applies the settings to the corresponding parameters.
Apply	Applies the settings to the corresponding parameters without closing the dialog box.
Cancel	Closes the dialog box and discards the settings.
...	Browse button: ▶ Opens a *Browse For Folder* dialog box, where the operator can navigate to a specific location. ▶ Opens a text input dialog box, where the operator can enter text, for example, to describe a sample.

1.5.3 Input fields

The LightCycler® 96 Application Software user interface provides several options for entering data into input fields:

Input Field	Description
Text field	Click the field and type the text according to the rules specified for the parameter. You can enter up to 100 characters in an input field.
Text field with a list	Click the field and select a value from the pull-down list.
Numeric values field with up and down arrows	Click the field and specify a value by clicking the up and down arrows or enter the value directly.

B

1.5.4 Working with tables

The LightCycler® 96 Application Software provides functions to be used in all tables displayed in the user interface. This section describes functions that are shared between multiple different tables.

Several functions allow for changing the display of a table, for example, hiding or showing columns. This customized view of a table is saved to the experiment file. When loading an experiment file, the customized window configuration is displayed automatically.

Table header shortcut menu

The table header shortcut menu provides access to most of the table functions. The shortcut menu opens with a right-click on the table header.

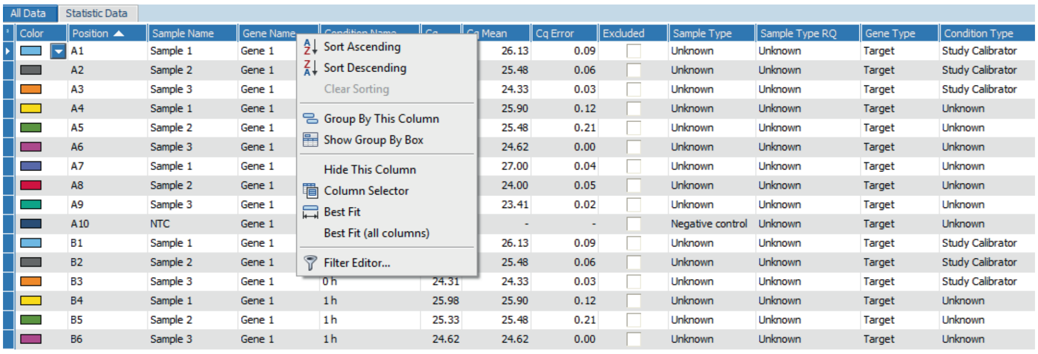


Figure 23: Table header shortcut menu

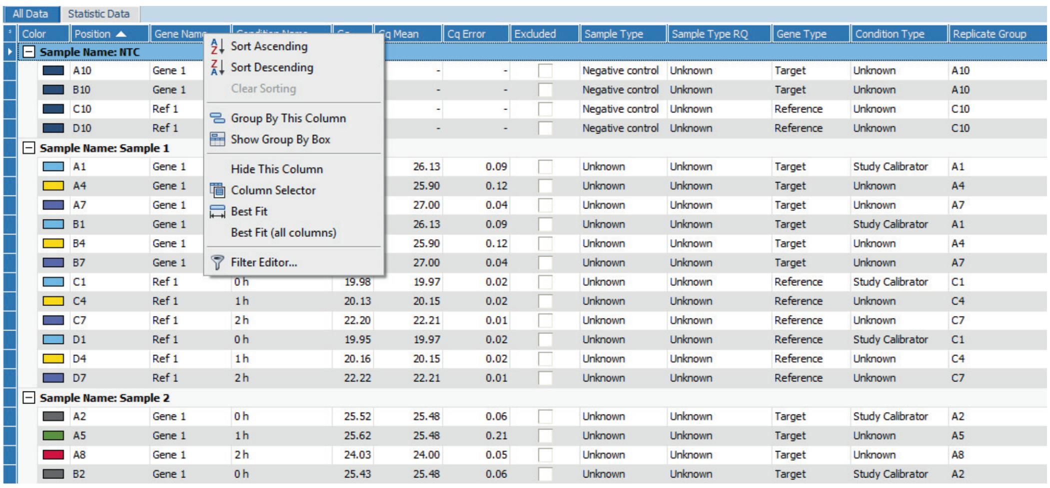


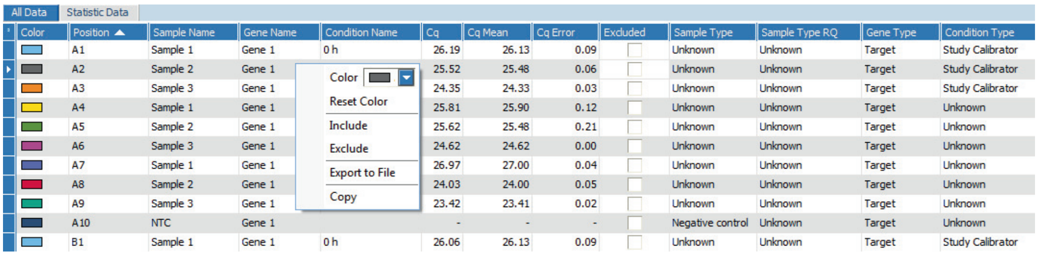
Figure 24: Table with grouped rows

Command	Description
Sort Ascending/ Sort Descending	Sorts the corresponding column.
Clear Sorting	Resets the row order to the default value.
Group By This Column	Allows for grouping the values in a table by one or more selected columns; see *Figure 24* above.
Show Group By Box	Shows the header of the column(s) selected with *Group By This Column* in a box above the table header. Using the shortcut menu in this <*column*> box, the rows in the grouped table can be rearranged as follows: ▶ *Full Expand* Expands all rows in the table to show the values in the other columns. ▶ *Full Collapse* Collapses all rows in the table to hide the values. ▶ *Ungroup* Undoes the grouping of the columns. ▶ *Hide Group By Box* Hide the <*column*> box.
Remove This Column/ Show This Column	Hides/shows the selected column.
Column Selector	Opens the *Column Selector* dialog box which lists the removed columns. For detailed information on the column selector, see section *Hiding or showing columns*, on page 94.
Best Fit	Changes the column width to the best fit for the selected column.
Best Fit (all columns)	Changes the column width to the best fit for all columns.
Clear Filter	Resets the filter specified for the column to the default value.
Filter Editor	Displays the *Filter Builder* dialog box. For detailed information on specifying filters, see section *Filtering table items*, on page 95.

The commands are disabled if the function is not allowed in the current context.

Table shortcut menu

The tables on the *Raw Data* tab and the *Analysis* tab additionally provide the table shortcut menu, which offers functions affecting one or more selected table items. The table shortcut menu opens with a right-click on a selected item or a range of selected items. For detailed information on selecting table items, see section *To select and deselect items*, on page 92.



Color	Position	Sample Name	Gene Name	Condition Name	Cq	Cq Mean	Cq Error	Excluded	Sample Type	Sample Type RQ	Gene Type	Condition Type
	A1	Sample 1	Gene 1	0 h	26.19	26.13	0.09		Unknown	Unknown	Target	Study Calibrator
	A2	Sample 2	Gene 1		25.52	25.48	0.06		Unknown	Unknown	Target	Study Calibrator
	A3	Sample 3	Gene 1		24.35	24.33	0.03		Unknown	Unknown	Target	Study Calibrator
	A4	Sample 1	Gene 1		25.81	25.90	0.12		Unknown	Unknown	Target	Unknown
	A5	Sample 2	Gene 1		25.62	25.48	0.21		Unknown	Unknown	Target	Unknown
	A6	Sample 3	Gene 1		24.62	24.62	0.00		Unknown	Unknown	Target	Unknown
	A7	Sample 1	Gene 1		26.97	27.00	0.04		Unknown	Unknown	Target	Unknown
	A8	Sample 2	Gene 1		24.03	24.00	0.05		Unknown	Unknown	Target	Unknown
	A9	Sample 3	Gene 1		23.42	23.41	0.02		Unknown	Unknown	Target	Unknown
	A10	NTC	Gene 1		-	-	-		Negative control	Unknown	Target	Unknown
	B1	Sample 1	Gene 1	0 h	26.06	26.13	0.09		Unknown	Unknown	Target	Study Calibrator

Figure 25: Table shortcut menu

Command	Description
Color	Opens the color selection dialog box. For detailed information, see section *Editing cells*, on page 93.
Reset Color	Resets the color to the default value.
Include	Includes the corresponding sample in the analysis.
Exclude	Excludes the corresponding sample from the analysis. By default, all samples are included, so all samples are deselected in the *Excluded* column. Excluding samples can be useful when a sample is clearly an outlier, or if an error has occurred in pipetting or amplification.
Export to File	Opens the *Save As* dialog and saves the corresponding table data including the header line to the specified location. for detailed information, see section *Exporting the result table*, on page 182. The data are saved as a text file (.txt) in table format.
Copy	Copies the current selection including the header line to the clipboard for pasting into other applications, for example, Microsoft Excel.

The commands are disabled if the function is not allowed in the current context.

To select and deselect items

1. Click a row in a table to select the corresponding item. The selected items are highlighted.
2. To add or remove rows from the selection, use the [Shift] key and [Ctrl] key on your keyboard.
3. To select all rows in a table, click the table and press [Ctrl] + [a] on your keyboard.

B

Editing cells

Tables often allow direct editing of the contents of individual cells. Different types of cells respond slightly differently to editing:

Cell Type	Editing
Text or numbers	To enable editing: ▶ Double-click in the cell. Edit the contents in the displayed input field. ▶ Select the cell (by clicking or using arrow keys) and start typing. To stop editing: ▶ Press the [Enter] key to complete the change and start editing the next cell below. ▶ Press the [Tab] key to complete the change and start editing the next cell to the right. ▶ Click outside the cell to complete the change and stop editing cells.
Boolean value	▶ Click the cell to toggle the value. ▶ If the cell is selected, press any key to toggle the value.
Color	▶ Select the cell. The color selection dialog box opens. ▶ Click a color in the color palette.
Choice	▶ Click the cell and select an entry from the list displayed.

To sort tables

1. Click the header of a column to sort the table by the column values.
   Clicking the header several times toggles the sort order between descending and ascending.
   In a 'Position' column, showing the position of a sample on the multiwell plate, clicking the header toggles between sorting by row (A1, A2, A3, ...) or sorting by column (A1, B1, C1, ...).
   Clicking the header of the 'Number' column allows for sorting a table upwards or downwards.
2. To sort the table by multiple columns, press the [Ctrl] key and click the headers of the columns to be included in the sort. Start from the least significant column and proceed through to the most significant column.

To change the column width

1. Move the cursor to the border of the table header you want to change.
2. When the cursor changes to a left and right arrow, hold down the left mouse button and drag the border until the column has the appropriate width.

Hiding or showing columns

Operators can hide or show columns of a table using the *Column Selector* dialog box.

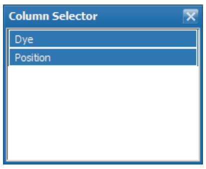


Figure 26: Column Selector dialog box

The dialog box lists all hidden columns (that is, all columns removed from the table).

To hide a column

1	Right-click the header of the column you want to hide.
2	On the table header shortcut menu, choose *Hide This Column*.

Alternatively, you can drag and drop a column to the *Column Selector* dialog box.

1	On the table header shortcut menu, choose *Column Selector.* The *Column Selector* dialog box opens in the bottom right corner of the table.
2	Click the header of the column you want to hide.
3	Drag the column into the *Column Selector* dialog box.

To show a column

1	On the table header shortcut menu, choose *Column Selector.* The *Column Selector* dialog box opens in the bottom right corner of the table.
2	Perform one of the following steps: ▶ Double-click the column you want to show. ▶ Right-click the column you want to show and choose S*how This Column* on the shortcut menu. ▶ Click the column you want to show and drag it back to the table header.

Filtering table items

Operators can filter items in a table to reduce the number of displayed rows by the values in specified columns.

When a filter is active, the filter control bar is displayed below the table. This bar allows for quick switching between filter conditions and enabling/disabling filters.

Figure 27: Filter control bar

To filter table items with the filter icon

1. Click the filter icon in the header of the corresponding column.

   The filter icon is only displayed if filtering is supported for the table.

   A list with all values found in this column and the category (custom) is displayed.

2. Perform one of the following steps:
   - Choose one of the values in this list.
   - Choose the category (custom).
     The *Custom Autofilter* dialog box is displayed. The *Custom Autofilter* function allows you to combine two values for filtering.

   The table is updated and the filter definition is displayed below.

3. Repeat these steps to add additional values to the filter definition.

   The items are filtered by these values. Only items matching all the filter conditions are displayed and selected in the table.

Filtering table items with the Filter Editor

Alternatively, you can filter the items using the appropriate expressions which you create with the *Filter Editor* function:

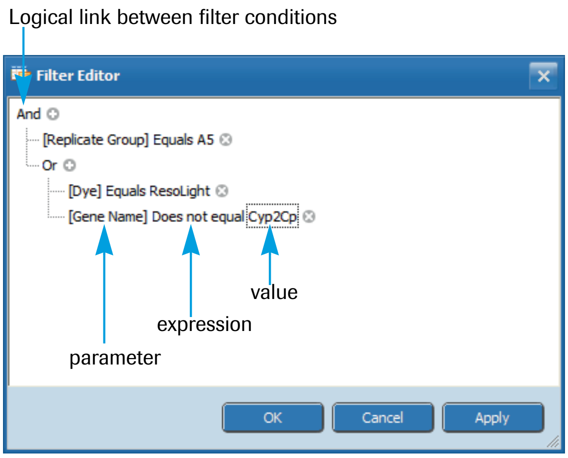


Figure 28: Filter Editor dialog box

B

To filter table items with the Filter Editor

1	Perform one of the following steps: ▶ Filter the items as described above and click *Edit Filter*. *The 'Edit Filter' button is only displayed after you have specified a column filter setting.* ▶ Right-click the column header and choose *Filter Editor* on the shortcut menu. The *Filter Editor* dialog box is displayed, see above.
2	Click the *<logical link>* field and choose the appropriate expression from the pull-down list. *The 'Add Group' function in this list allows a different logical link between conditions within a group and between groups of conditions.*
3	Click the + symbol to add a filter expression.
4	Click the *<parameter>* field and choose the corresponding parameter from the pull-down list.
5	Click *<expression>* and choose the appropriate expression from the pull-down list.
6	Click the *<value>* field and type the necessary value.
7	Perform one of the following steps: ▶ Click *Apply* to filter the samples via the specified expression. The *Filter Builder* dialog box remains open. ▶ Click *OK* to filter the samples via the specified expression and close the *Filter Builder* dialog box. The table is updated and the filter definition is displayed below.

■

1.5.5 Working with graphs

This section describes functions that are shared between multiple different graphs (in this example, an amplification curves graph is shown).

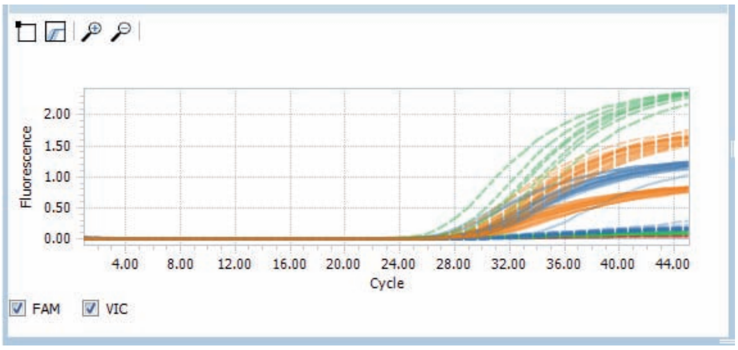
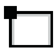
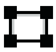


Figure 29: Graphs window area

As selection, zooming, and marking share the same mouse actions, the operator must indicate which function will follow. Graphs in the software have several common tools to manage the display:

Icon	Function	Usage
	Selection	▶ Choose the icon to select it. ▶ Move the cursor over the graph window area, to the left of the curves you wish to select. ▶ Hold down the left mouse button and drag the cursor to a point in the middle of the curves. As you drag the cursor, a box will show the window area where curves will be selected. ▶ Release the mouse button to make the selection. All curves which pass through the box will be selected, and other curves will be deselected. The selected curves are displayed bold.
	Show Selection	Choose this icon to hide or show the selected curves.
	Area Marker	Only displayed in melting peaks graphs: ▶ Choose the icon to indicate that a marking action will follow. ▶ Move the cursor over the graph window area to the point for the top left corner of the area. ▶ Hold down the left mouse button and drag the cursor to the point for the bottom right corner of the area. ▶ Release the mouse button. ▶ To change the size of the specified area, select the corresponding side or corner of the rectangle, and drag it accordingly.  *The LightCycler® 96 Application Software allows five areas to be defined in one graph. To specify an additional area, one of the existing areas must be deleted.*
	Zoom	▶ Choose the icon to zoom into the graph. ▶ Move the cursor to the top left of the area you wish to zoom to. ▶ Hold down the left mouse button and move the cursor to the bottom right of the graph you wish to zoom into. As you drag the cursor, a box will show the graph that will be zoomed into. ▶ Release the mouse button to make the selection. The graph will now have axes set to display the selected window area.
	Cancel Zoom	Choose this icon to zoom out back to normal size.

Dye selection

Each graph provides options to show only the curves according to the selected dye(s). An option is displayed for each dye assigned in the corresponding detection format. For detailed information on how to define a detection format, see section *Detection Format*, on page 126.

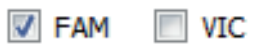


Figure 30: Dye selection in a graph

Tooltips

Many graphs will display tooltips that provide extra information associated with a curve, such as the position of the sample, etc.

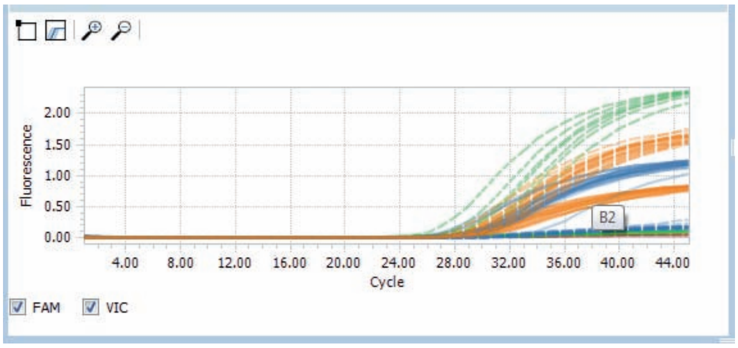


Figure 31: Tooltips in a graph

To display a tooltip

1. Hover the cursor over a curve. A gray box (tooltip) will appear showing the additional information.
   When a tooltip has appeared, moving the cursor around will continue to display the information associated with the curve nearest to the cursor.
2. To stop displaying tooltips, move the cursor away from the curves.

Graphs shortcut menu

The shortcut menu provides functions for copying and exporting the corresponding graphs.

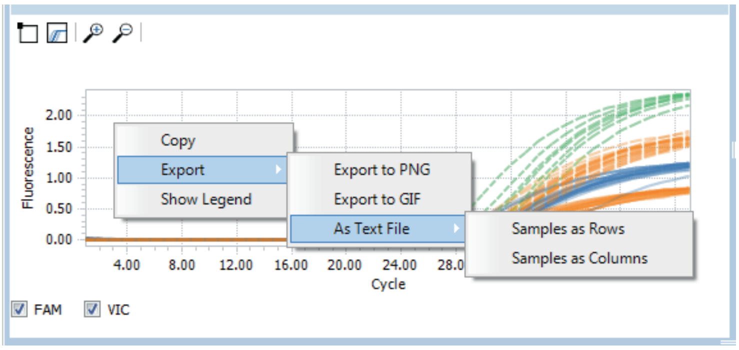


Figure 32: Graphs shortcut menu

Command	Description
Copy	Copies the graph as a picture to the clipboard. You can paste the copied picture into another software program.
Export	Opens the *Save As* dialog and saves the data to the specified location. The following commands are available: ▶ *Export to PNG* The chart is saved as a PNG file (.png). ▶ *Export to GIF* The chart is saved as a GIF file (.gif) ▶ *Export As Text File > Samples as Rows* The chart data are saved as a text file (.txt) in table format with the sample name as the row header. ▶ *Export As Text File > Samples as Columns* The chart data are saved as a text file (.txt) in table format with the sample name as the column header.
Show Legend/ Hide Legend	Show a Legend to display the line style which is used for the according dye or hides it.
Set Slider Here	Only displayed in amplification curves for the EPF value: Specifies the minimum EPF threshold. By default the slider is set to 0. In this position no EPF threshold is applied. For detailed information on setting thresholds, see section *Minimum EPF threshold*, on page 152.

1.5.6 Working with plate views

LightCycler® 96 Application Software contains plate views, which reflect the arrangement of samples in the multiwell plate mount of the LightCycler® 96 Instrument (in this example, the plate view on the *Sample Editor* tab is shown):

Figure 33: Plate view

The plate view shows the arrangement of 96 wells in an array of 12 columns and 8 rows. The plate view is marked with the same numbers and letters as found on the LightCycler® 480 Multiwell Plate 96. Each well is displayed as a rectangle, colored according to the sample type of the well.

The LightCycler® 96 Application Software provides functions to be used in all plate views displayed in the user interface.

To select and deselect wells

1. Click a well in a plate view to select the corresponding item.
   The selected well is highlighted.
2. To add or remove wells from the selection, use the [Shift] key and [Ctrl] key on your keyboard.
3. To select all wells in a plate view, perform one of the following steps:
   - Click one well and press [Crl] + [a] on your keyboard.
   - Click the asterisk (*) in the upper left corner of the plate view.

Tooltips

The plate views also display tooltips that provide additional information associated with a well, such as the well position, the sample name, etc.

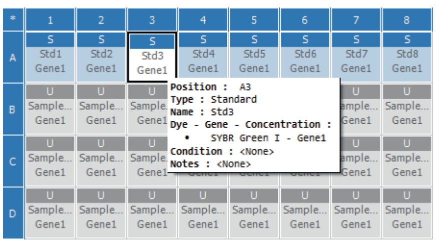


Figure 34: Tooltips in the plate view

To display a tooltip

1	Hover the cursor over a well. A gray box (tooltip) will appear showing the additional information. When a tooltip has appeared, moving the cursor around will continue to display the information associated with the well nearest to the cursor.
2	To stop displaying tooltips, move the cursor away from the wells.

1.5.7 Working with sections

The *Raw Data* tab and the *Analysis* tab in the LightCycler® 96 Application Software provide several sections in the working window area. Sections allow the operator to combine different views of the data.

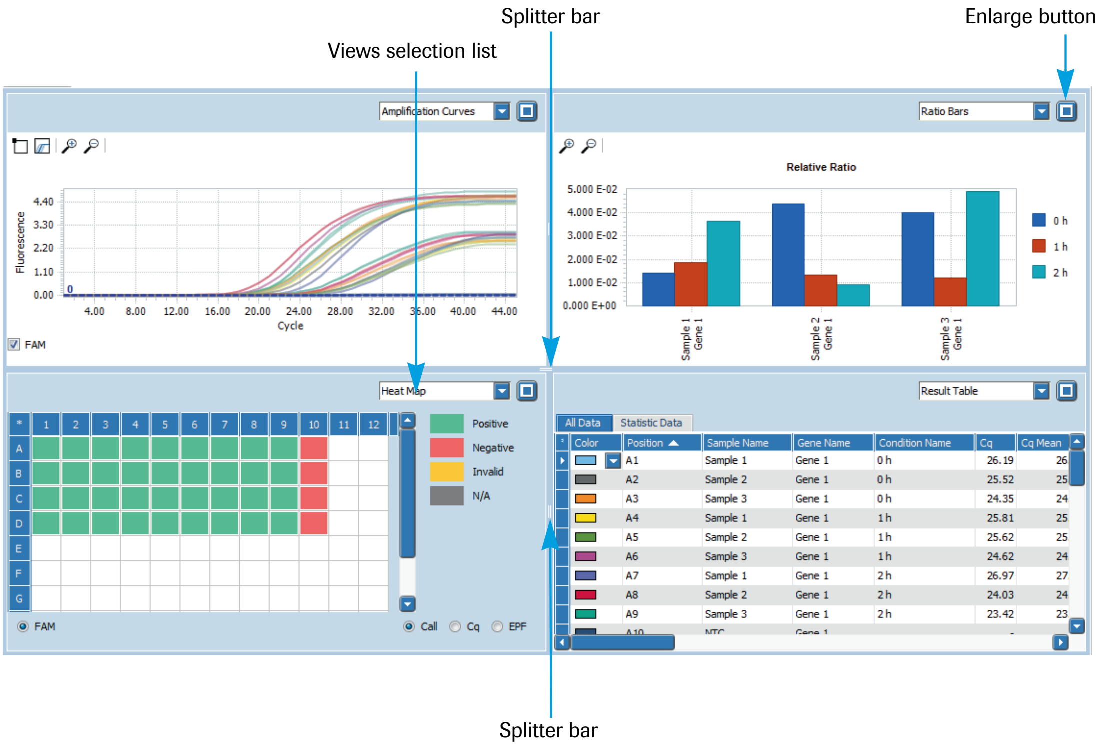


Figure 35: Sections in the working window area

By default the tabs provide four sections. Each section contains a list for choosing the data to be displayed and a button to enlarge the section or restore the previous view. The customized view is saved to the experiment file. When loading an existing experiment file, the customized window configuration is displayed automatically.

To change the size of a section

1	Click the splitter bar between the corresponding sections and drag it accordingly.

To change the size of a section

1	To enlarge a special section, click the ▣ button. The section is enlarged to the complete working area. To restore the previous view, click the icon again.

1.6 Experiments

The information provided in the experiment definition controls the LightCycler® 96 Instrument during an experiment run. The experiment definition specifies the target temperatures and hold times of the thermal block cycler, the number of cycles being executed, and other parameters.

For starting an experiment run, the experiment must be transferred to the instrument. An experiment run can only be started on the instrument using the LightCycler® 96 Instrument Software. For detailed information on how to send an experiment to the instrument, see section *Instrument Manager, on page 110.*

B

1.6.1 Creating an experiment

Before a LightCycler® 96 Instrument run can be started, a new experiment has to be created. The operator has the following options for creating a new experiment:

- Generating a completely new experiment
- Using an existing experiment as a template
- Using a predefined Roche template

To generate a new experiment

1. Perform one of the following steps:
   - On the start screen, choose the *Quickstart* tab and then *Create New Experiment*.
   - In the *File* menu, choose *New > Experiment*.
   - In the tool bar, choose the *New Experiment* icon.

   The LightCycler® 96 Application Software displays the new experiment in the main window. The new experiment has the default name *New Experiment <creation_date> <creation_time>*.
2. Optional: Enter a description of the experiment.
   - In the *File* menu, choose *Properties*.
   - In the *Properties* dialog box, choose the *Notes* tab.
   - Enter a description.
   - Choose *OK*.

   For detailed information on the *Properties* dialog box, see section *Experiment properties*, on page 106.

To use an existing experiment as a template

To create a new experiment from an existing one (that is, to copy all settings of an experiment), the operator must open the experiment file as a template. In this case, the raw data of the experiment is deleted. The experiment run settings, the sample editor settings, and the analysis settings of the experiment are provided for editing.

When the option 'Create New Experiment from existing' is used in the LightCycler® 96 Application Software, the analyses and all their settings are retained. After the experiment run, the analyses are created automatically.

When the option 'Create New Experiment from existing' is used in the LightCycler® 96 Instrument Software, all analyses and their settings are lost.

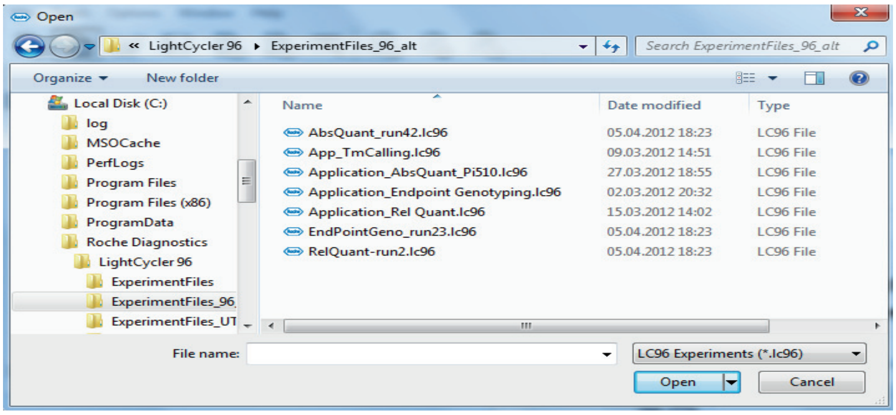
1. Perform one of the following steps:
   - On the start screen, choose the *Quickstart* tab and then *Create New Experiment from Existing*.
   - In the *File* menu, choose *New* > *Experiment from Existing*.
   - In the tool bar, choose the *New Experiment from Existing* icon.

   The *Open* dialog box opens.

2. Navigate to the corresponding directory.

   By default, the default experiment directory is displayed. This directory can be specified in the 'Default Directory' dialog box. For detailed information, see section *Preferences, on page 119.*

3. In the list next to the *File name* field, select the file type to be displayed: **.lc96*



4. Select the experiment and choose *Open*. The experiment opens in the main window.

5. Change all settings according to your needs.

   When detection format settings are changed (for example, the dye type in the same channel), all gene-specific settings (that is, gene name and concentration) are set to their default values.

   Changing sample names or gene names might result in non-matching analysis-specific settings (for example, changing the gene name of a reference gene in relative quantification).

■

To use a Roche template

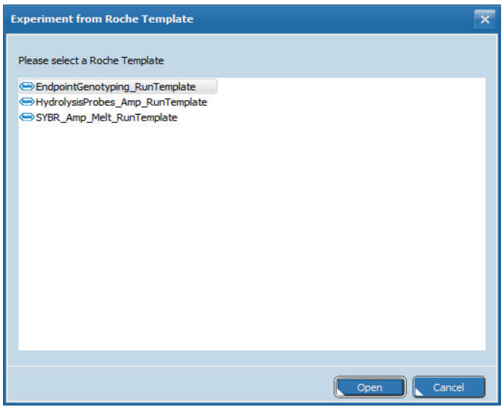
Roche provides a number of predefined experiments as templates. An experiment template contains the temperature profile and the dye-specific parameters for an experiment. The templates are located in the LightCycler® 96 Application Software home directory.

1. Perform one of the following steps:
   - On the start screen, choose the *Quickstart* tab and then *Create New Experiment from Roche Template*.
   - In the *File* menu, choose *New* > *Experiment from Roche Template*.

   The *Experiment from Roche Template* dialog box opens.

▶ ▶ ▶

B

2	Select the corresponding template. 
3	Choose *Open*. The experiment opens in the main window.
4	Change all settings according to your needs.

1.6.2 Opening an experiment

Experiments can be saved and reopened at any time during the experiment definition, after the experiment run, or after defining the analysis parameters.

To open an experiment

1	Perform one of the following steps: ▶ In the startup wizard, choose the *Recent experiments* tab and then the experiment to be displayed. The experiment opens in the main window. ▶ In the *File* menu, choose *Recent Experiments* and then the experiment to be displayed. The experiment opens in the main window. ▶ In the *File* menu, choose *Open*. The *Open* dialog box opens. Proceed with step 2 ▶ In the tool bar, choose the *Open Experiment* icon. The *Open* dialog box opens. Proceed with step 2.
2	Navigate to the corresponding directory and select the relevant experiment file. *By default, the default experiment directory is displayed. This directory can be specified in the 'Default Directory' dialog box. For detailed information, see section Preferences, on page 119.*
3	Choose *Open*. The experiment opens in the main window.

1.6.3 Saving an experiment

Experiments can be saved at any time during the experiment definition, after the experiment run, or after defining the analysis parameters.

After the run is finished, you can no longer change any of the run parameters. For detailed information on copying and reusing an existing experiment, see section *To use an existing experiment as a template, on page 102.*

To save an experiment

1. Perform one of the following steps:
   - In the *File* menu, choose *Save* to save the currently opened experiment. If no file exists, the *Save As* dialog box opens.
   - In the *File* menu, choose *Save As* to save the experiment to a specified location. The *Save as* dialog box opens.
   - In the tool bar, choose the *Save Experiment* icon to save the currently opened experiment. If no file exists, the *Save As* dialog box opens.
2. Navigate to the directory where you want to store the experiment file.

   By default, the default experiment directory is displayed. This directory can be specified in the 'Default Directory' dialog box. For detailed information, see section Preferences, on page 119.
3. Enter a file name for the experiment.
4. Click *Save*. The dialog box closes and the experiment is saved as a LightCycler® 96 file (.lc96).

   By default, experiments are saved as LightCycler® 96 files. (.lc96). The dialog additionally provides an option for saving experiments as RDML files (.rdml). Both file types are compatible with the LightCycler® 96 Application Software. To enable opening LightCycler® 96 experiment files with a third-party RDML-compatible software, save the files as .rdml.

B

1.6.4 Experiment properties

When an operator creates a new experiment, the LightCycler® 96 Application Software generates the experiment summary. The summary is updated each time the experiment file is saved. The summary is provided in the *Properties* dialog box, which operators access via the *File* menu or the *Properties* icon in the tool bar:

File > Properties

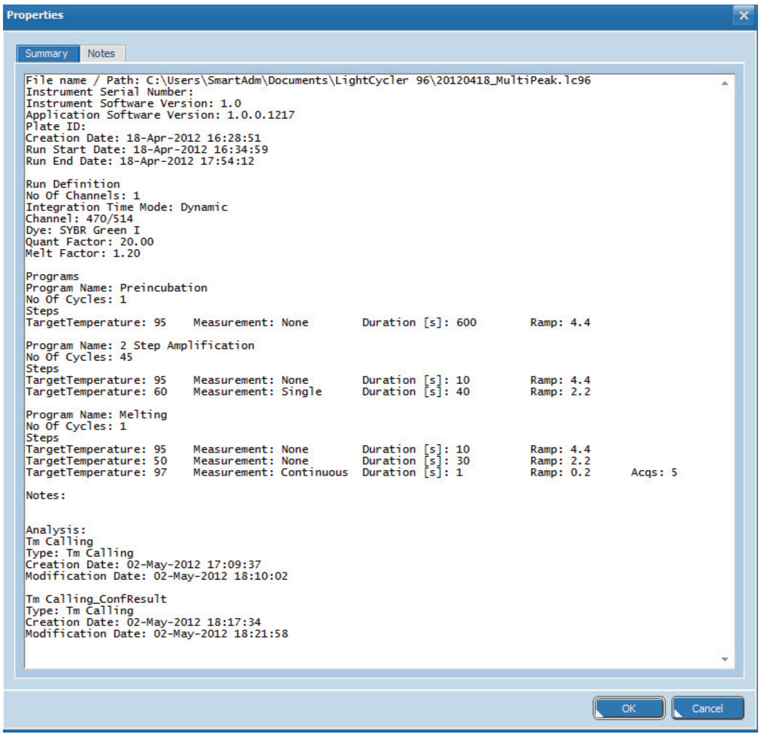


Figure 36: Properties dialog box

Summary tab

The experiment summary includes the following information:

- The file name and path of the experiment. The file name is also displayed in the experiment bar.
- The plate ID of the LightCycler® 480 Multiwell Plate 96
- The date and time the experiment was created
- The LightCycler® 96 Application Software version
- The start and end date of the experiment run
- The LightCycler® 96 Instrument serial number
- The LightCycler® 96 Instrument Software version
- The measurement settings specified in the run definition
- The programs contained in the run definition
- The experiment notes
- The applied analysis
- The date and time the analysis was created or modified

Notes tab

The *Notes* tab is a text input field for up to 10 000 characters in which you can add notes to the experiment if necessary. The notes are saved to the experiment file.

1.7 Import, export, and file transfer options

The following figure shows the input and output data flow offered by the LightCycler® 96 Application Software architecture. The data to be imported and exported is described below.

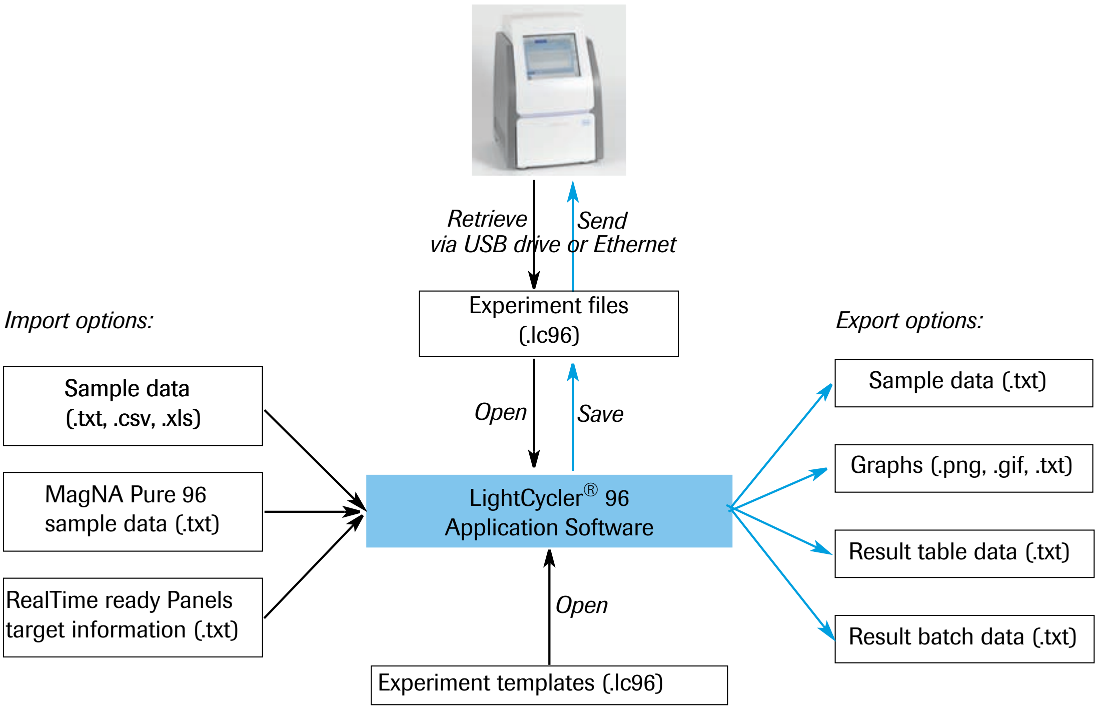


Figure 37: LightCycler® 96 Application Software input and output data flow

1.7.1 Import data

To define a new experiment, the following data can be imported into the LightCycler® 96 Application Software:

Data	File Format	Description
Experiment template	.lc96	Predefined operator settings to be specified in the experiment definition: ▶ Temperature profile ▶ Dye-specific parameters ▶ Sample and gene information For detailed information, see section *To use an existing experiment as a template*, on page 102.
Sample data	.txt .csv .xls	Sample information for an experiment, including sample names, gene names, and dye assignment. For detailed information, see section *To import sample data into the sample list*, on page 141.

B

Data	File Format	Description
MagNA Pure 96 sample data	.txt	Samples file exported from the MagNA Pure 96 Software in LightCycler® System readable format (.txt). The file contains the following settings: ▶ *Position* ▶ *Sample Name* ▶ *Sample Note* ▶ *Sample ID* ▶ *Sample Prep Notes* The *Sample ID* information is ignored during import. For detailed information, see section *To import sample data into the sample list*, on page 141.
RealTime ready Panels target information	.txt	Text files generated for import of target information for RealTime ready Panels 96. The file contains the following settings: ▶ *Pos* ▶ *Target Name* ▶ *Combined sample/target type* The combined sample/target type information is ignored during import.

1.7.2 Export data

To store the results of an experiment or transfer the results to other software programs, the corresponding files must be exported. The LightCycler® 96 Application Software provides export functions for the following data:

Data	File Format	Description
Sample data	.txt	Sample and gene data specified on the *Sample Editor* tab of the experiment definition. For detailed information, see section *Exporting the sample list*, on page 139.
Graph	.png .gif .txt	Result charts data saved as a bitmap format file (.png), a Graphics Interchange Format file (.gif), or a text file with the samples as columns or rows. For detailed information, see section *Working with graphs*, on page 97.
Result table data	.txt	Tab-delimited text file (.txt) containing the currently displayed result table including the header line. For detailed information, see section *Exporting the result table*, on page 182.
Result batch data	.txt	Tab-delimited text file (.txt) containing the result table data collected from multiple experiment files, including the header line and the original file name and plate ID for each sample. For detailed information, see section *Exporting multiple result data*, on page 182.

1.8 Disregarding positions from an analysis

The LightCycler® 96 Application Software provides the following options for eliminating positions from the analysis:

- The *Clear Wells* function, provided on the *Plate View* tab of the *Sample Editor*. *Clear Wells* eliminates the selected wells from all analyses.

It is strongly recommended to use the 'Clear Wells' function for all empty wells of LightCycler® 480 Multiwell Plates 96 and for positions not occupied by LightCycler® 8-Tube Strips.

- The *Remove* function, provided in the *<analysis> Settings* dialog box of an analysis. *Remove* eliminates samples, genes, and/or conditions (only for relative quantification) from the corresponding analysis.
- The *Exclude* function, provided in the analysis result tables. *Exclude* eliminates the selected samples from the result calculation of the corresponding anylysis.

For detailed information, see section *Eliminating positions from the analysis*, on page 148.

1.9 Exiting the software

To exit the LightCycler® 96 Application Software

1. Perform one of the following steps:
   - In the *File* menu, choose *Exit*.
   - In the title bar of the main window, choose the ✕ button.

   You are prompted to save unsaved data before the application shuts down.

B

2 Tools

In the *Tools* menu, the LightCycler® 96 Application Software provides several tools for managing and monitoring LightCycler® 96 Instruments and for exporting result data from multiple experiment files.

2.1 Instrument Manager

The *Instrument Manager* provides access to the following functions:

- Registering a LightCycler® 96 Instrument with the application software, see section *Instruments window area*, on page 111.
- Transferring experiments to and from the selected instrument, see section *Send/Receive Experiments tab*, on page 113.
- Opening an experiment in the application software or deleting an experiment from the instrument, see section *Send/Receive Experiments tab*, on page 113.
- Monitoring an experiment run on an instrument, see section *Online Monitoring tab*, on page 115.

Preconditions

For using the functions of the *Instrument Manager* the following preconditions apply:

- The LightCycler® 96 Instrument is switched on.
- The instrument is configured for use in the network; see section *Configuring the LightCycler® 96 Instrument in the network*, on page 49.
- The instrument is connected to the network; see section *USB interfaces*, on page 30.
- The computer running the LightCycler® 96 Application Software is connected to the network.
- The *Remote Monitoring* function is activated and configured accordingly in the LightCycler® 96 Instrument Software; see section *Remote Monitoring*, on page 219.

The *Instrument Manager* wizard is accessed via the *Tools* menu (in this example, the *Information* tab is shown):

Tools > Instrument Manager

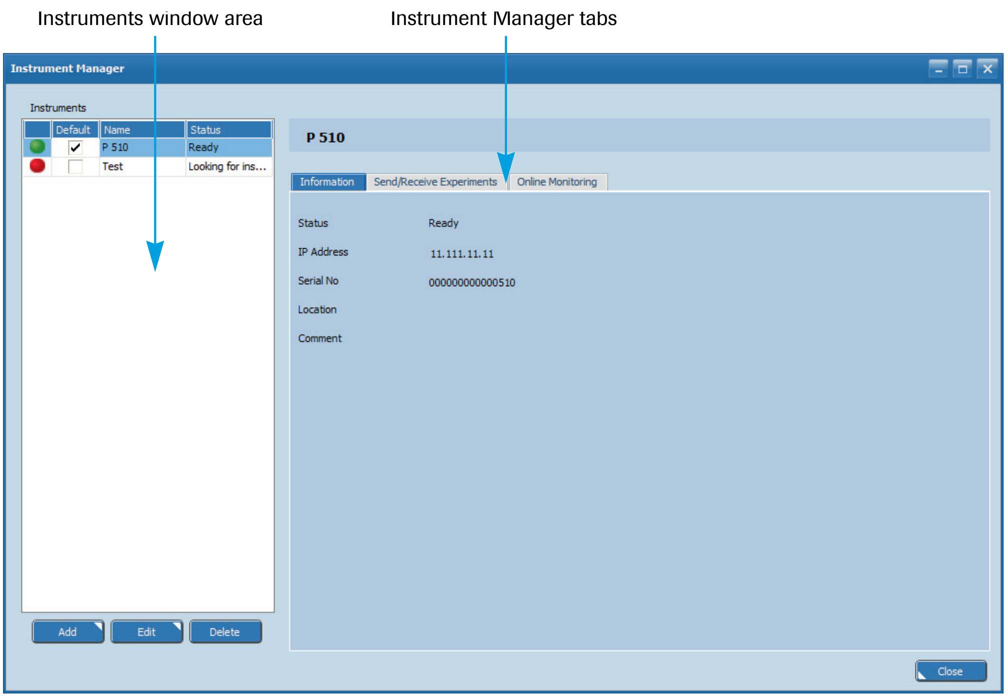


Figure 38: Instrument Manager

2.1.1 Instruments window area

The columns in the *Instruments* table show the properties for each registered instrument. For detailed information on registering instruments, see section *To register an instrument*, on page 112.

Column	Description
	Connection status of the instrument: ▶ Online: Green dot ▶ Offline: red dot
Default	Specifies whether the instrument is the default instrument; see section *To register an instrument*, on page 112.
Name	Name of the instrument, defined in the *Add/Edit Instrument* dialog box.
Status	Processing status of the instrument.

The buttons below the *Instruments* table perform the following functions:

Button	Description
Add	Opens the *Add/Edit Instrument* dialog box for editing the properties and registering an instrument with the LightCycler® 96 Application Software.
Edit	Opens the *Add/Edit Instrument* dialog box for editing the properties of an instrument already registered with the application software.
Delete	Deletes the selected instrument from the table. The instrument is no longer registered with the application software.

Add/Edit Instrument dialog box

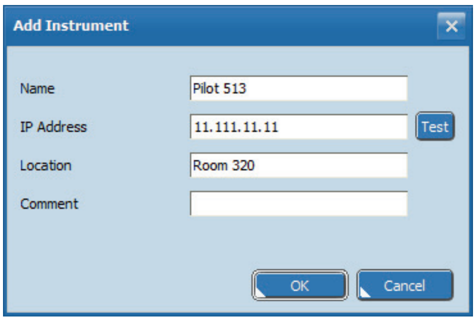


Figure 39: Add/Edit Instrument dialog box

In the *Add/Edit Instrument* dialog box, operators specify the properties of an instrument to be registered with the LightCycler® 96 Application Software:

Setting	Description
Name	Optional: Name of the instrument in the application software.
IP Address	IP address of the instrument, specified in the LightCycler® 96 Instrument Software.
Location	Optional: Location of the instrument.
Comment	Optional: Description of the instrument

B

To register an instrument

The instrument must be registered with the application software for sending and retrieving experiment files to/from the LightCycler® 96 Instrument Software or for monitoring a LightCycler® 96 Instrument. The application software allows for registering up to 10 instruments.

1. In the Tools menu, choose *Instrument Manager*.
   The *Instrument Manager* window opens.
2. Below the *Instruments* table, choose *Add*.
   The *Add/Edit Instrument* dialog box opens.
3. Optional: In the *Name* field, enter the name of the instrument to be used in the application software.
4. In the *IP Address* field, enter the IP address of the instrument in the network.
5. Optional: Choose the *Test* button to test the connection to the IP address.
   If the IP address is not available, the software displays an error message:
   - *Warning: Test Connection*
     Communication failed: Could not get instrument data.
   - *Error: Test Connection*
     Communication failed: Could not reach instrument.
6. Optional: In the *Location* field, enter the location of the instrument.
7. Optional: In the *Comment* field, enter a description of the instrument.
8. Choose *OK*. The dialog box closes.
   - The instrument is registered with the application software and displayed in the *Instruments* list.
   - The *Information* tab shows the properties of the instrument; see section *Information tab* below.
9. Optional: To specify the instrument as the default, select the check box in the *Default* column.

2.1.2 Information tab

The Information tab displays the properties of a LightCycler® 96 Instrument selected in the *Instruments* table.

Property	Description
Status	Processing status of the instrument.
IP Address	IP address of the instrument, specified in the LightCycler® 96 Instrument Software.
Serial No	Serial number of the instrument.
Location	Optional: Location of the instrument.
Comment	Optional: Description of the instrument.

2.1.3 Send/Receive Experiments tab

The *Send/Receive Experiments* tab provides a transfer function for experiments to and from a registered LightCycler® 96 Instrument. If more than one experiment exists in the network, the operator must select the corresponding instrument.

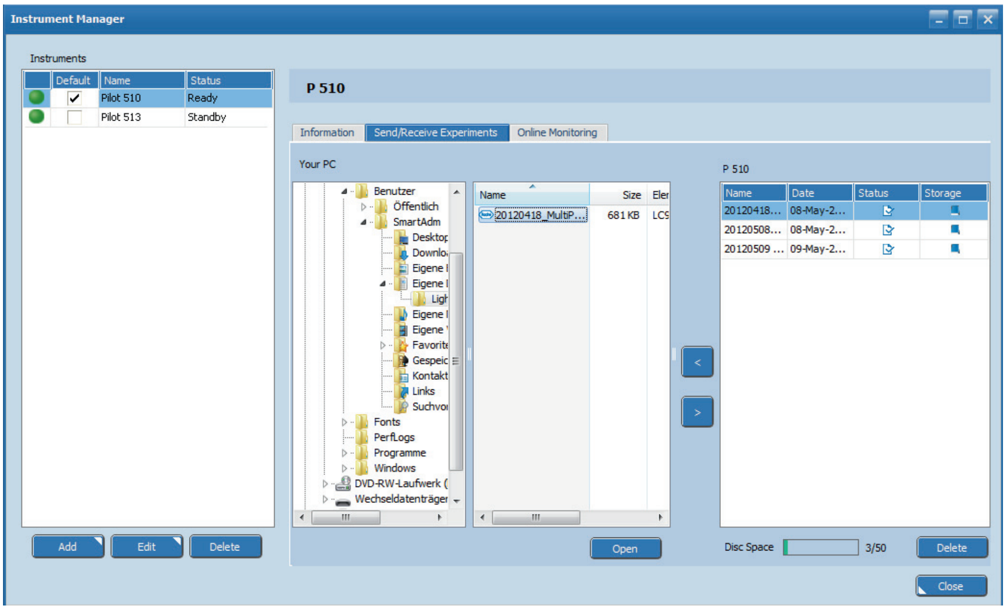


Figure 40: Instrument Manager, Send/Receive tab

Your PC window area

The *Your PC* window area provides a file explorer for navigating to the location of the experiment file.

<instrument> window area

The *<instrument>* window area provides the following data:

- The *Disc Space* bar showing the number of experiments saved on the instrument in relation to the possible maximum number of experiments.
- The experiments table displaying all experiments available on the instrument.

Column	Description
Name	Experiment name.
Date	Date and time of the last modification.
Status	Status of the experiment: *Running*, *Processed*, *Unprocessed*, or *Aborted*.
Storage	Location of the experiment file: On the instrument, on the connected USB drive, or on both locations.

The buttons in the *Send/Receive Experiments* tab perform the following functions:

Button	Description
Open	Opens the selected experiment in the application software.
Delete	Deletes the selected experiment from the instrument.

To send an experiment to an instrument

1. In the Tools menu, choose *Instrument Manager*.
   The *Instrument Manager* window opens.
2. Open the *Send/Receive Experiments* tab.
3. In the *Instruments* table, select the instrument the experiment is to be sent to.
4. In the *Your PC* window area, navigate to the directory containing the experiment. All experiments in this directory are displayed.
5. Select the experiment to be sent to the instrument.
6. Choose the > button.
   The experiment is sent to the selected instrument.

   You are notified if the instrument does not have enough space for the experiment.

   If an experiment with the same name already exists on the instrument, you are prompted for a new name.

To retrieve an experiment from an instrument

1. In the Tools menu, choose *Instrument Manager*.
   The *Instrument Manager* window opens.
2. Open the *Send/Receive Experiments* tab.
3. In the *Instruments* table, select the instrument the experiment is to be retrieved from.
4. In the *Your PC* window area, navigate to the directory the experiment is to be transferred to.
5. In the *<instrument>* area, select the experiment to be retrieved.
6. Choose the < button.
   The selected experiment is retrieved from the instrument and saved to the specified directory on your computer.

   If an experiment with the same name already exists in the selected directory, you are prompted for a new name.

2.1.4 Online Monitoring tab

The *Online Monitoring* tab provides the raw fluorescence data collected during an experiment run in real time.

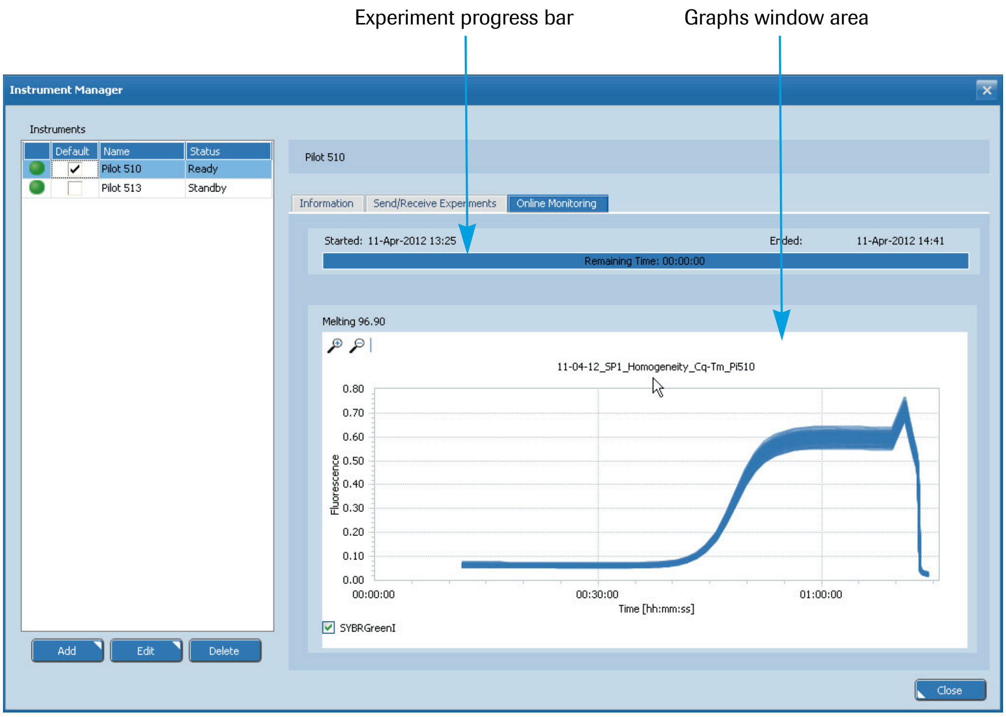


Figure 41: Instrument Manager, Online Monitoring tab

Experiment progress bar

The experiment progress bar at the top of the tab provides the following information:

- The start time of the experiment
- The predicted end time of the experiment
- The predicted time the experiment run will take

Graphs window area

The graphs window area provides the fluorescence curves, that is, the fluorescence intensity against the time in hours, minutes, and seconds for the entire run. There is one curve for each sample that has a gene labeled with the selected dye.

2.2 Result Batch Export

The *Result Batch Export* wizard provides a batch export tool that allows for creating a file with result table data collected from different experiments of the same type. The batch export function exports the collected result table data to a tab-delimited text file (.txt). You can open this file using Microsoft Excel.

The resulting text file contains all result data, including the header rows, the experiment name and plate ID for each sample. The result batch export includes the following items:

- Hidden columns
- Excluded positions
- Deselected positions

Filter settings are not applied during result batch export.

The *Result Batch Export* wizard is accessed via the *Tools* menu:

Tools > Result Batch Export

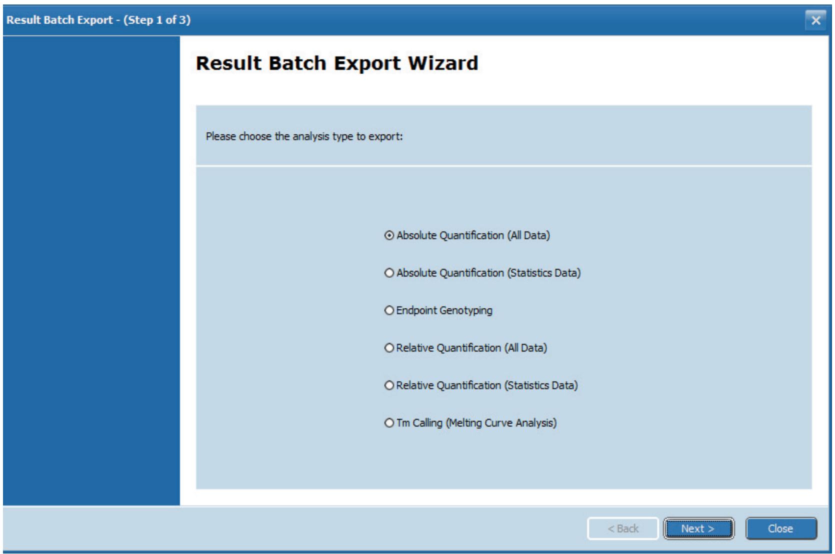


Figure 42: Result Batch Export wizard

The buttons in the *Result Batch Export* wizard perform the following functions:

Button	Description
Back	Returns to the previous page of the wizard.
Next	Opens the next page.
Export	Only displayed on the *Export* page: Exports the selected data to a tab-delimited text file (.txt).
Close	Closes the wizard and discards all settings.

2.2.1 Analysis type

The first page of the *Result Batch Export* wizard allows for selecting one of the following analysis types:

- Absolute quantification, all data
- Absolute quantification, statistical data
- Relative quantification, all data
- Relative quantification, statistical data
- Endpoint genotyping
- T_m calling

2.2.2 File selection

Choosing *Next* opens the *Experiment Files Selection* page:

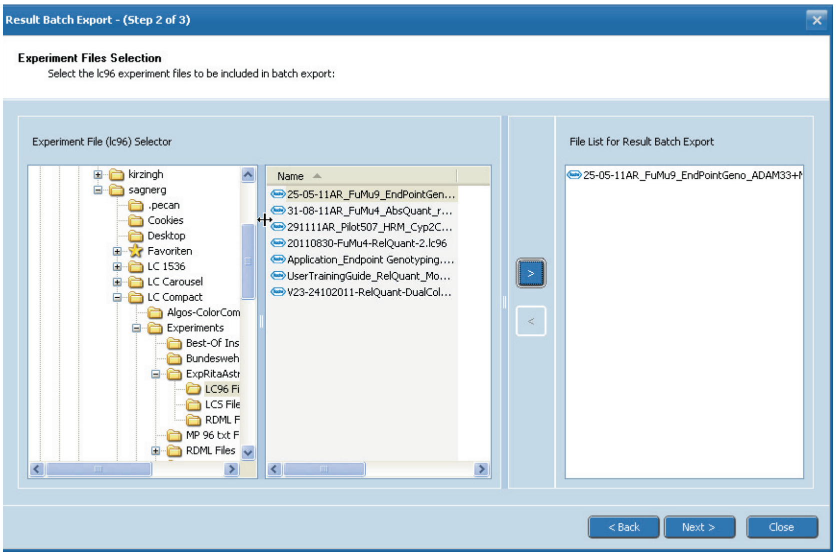


Figure 43: Result Batch Export wizard, Experiment Files Selection

Window area	Description
Experiment File (lc96) Selector	File explorer for navigating to the location of the experiment files.
File List for Result Batch Export	Experiment files the operator has selected for export.

2.2.3 Export

Choosing *Next* opens the *Export* page:

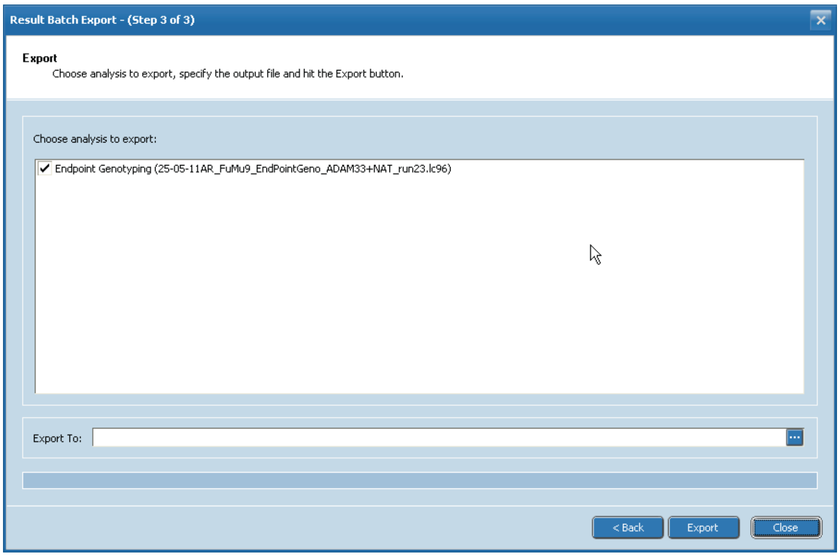


Figure 44: Result Batch Export wizard, Export

Window area/field	Description
Choose analysis to export	Analyses contained in the selected experiment files.
Export To	Path and name for the batch export result file.

To export multiple result data

1. In the *Tools* menu, choose *Result Batch Export*.
   The *Result Batch Export* wizard opens.
2. Choose the analysis type to be exported.
3. Choose *Next*.
   The *Experiment Files Selection* page opens.
4. In the *Experiment File (lc96) Selector* window area, navigate to the directory containing the corresponding experiments. All experiments in this directory are displayed.
5. Select the experiments to be exported.
6. Choose the > button to the right of the experiments list.
   The selected experiments are added to the *File List for Result Batch Export.*
7. Optional:
   - Repeat steps 4 to 6 to add experiments stored in other directories.
   - If necessary, use the < button to remove experiments from the list.
8. Choose *Next*.
   The *Export* page opens.
9. In the *Choose analysis to export* list, choose the analyses to be exported.
10. In the *Export To* field, specify the path and the name for the batch export result file.
11. Choose *Export*.
    - The progress bar at the bottom of the page shows the progress of the export process.
    - You are notified when the export process is finished.

3 Preferences

The LightCycler® 96 Application Software provides the *Preferences* dialog box, where general settings are specified, for example, the default directories to be used. The dialog box is accessed via the *Options* menu:

Options > Preferences

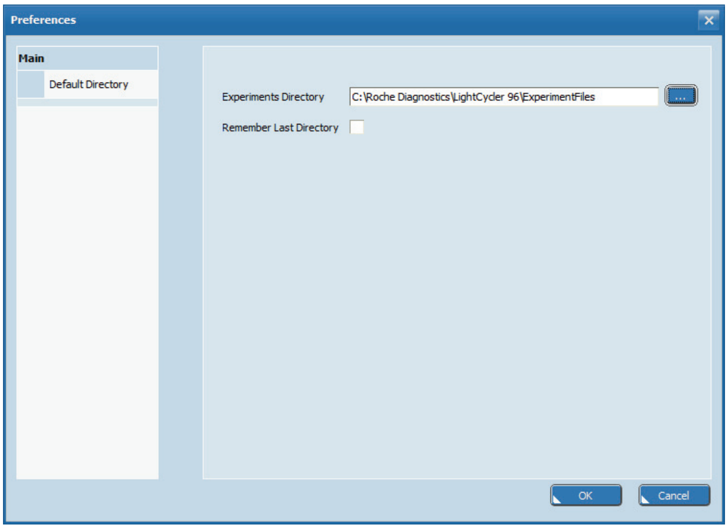


Figure 45: Preferences dialog box

Setting	Description
Experiments Directory	Default path for saving and loading experiment files
Remember Last Directory	The last opened directory is to be remembered as long as the application is running. *After a restart, the software opens the specified default directory.*

B

4 Run Editor tab

On the *Run Editor* tab, the temperature profile and the dye-specific parameters for an experiment run are specified.

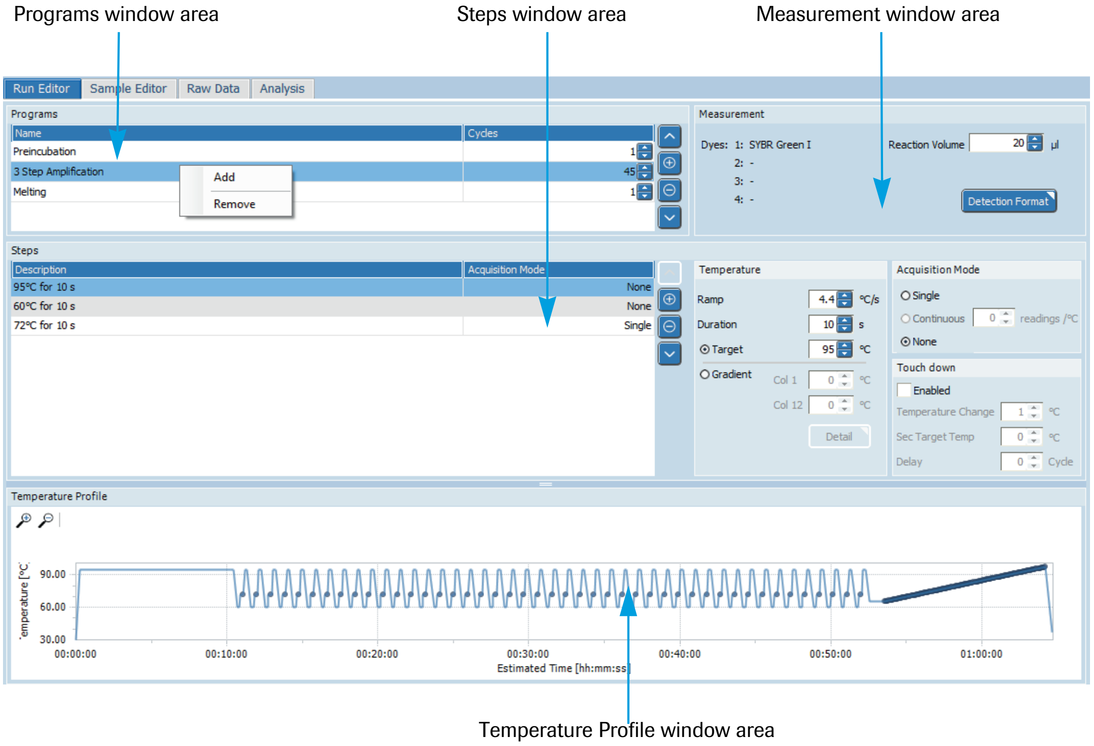


Figure 46: Run Editor tab

For a new experiment, this tab shows no data. For detailed information on how to create a temperature profile and set the dye-specific parameters, refer to the *LightCycler® 96 System User Training Guide*.

4.1 Programs window area

All profiles are comprised of programs, which are run by the instrument in the order they are displayed in the *Programs* window area. Each program performs a role within the experiment and can be specified separately.

The programs are displayed in a list and have the following properties:

Parameter	Description
Name	Name of the program.
Cycles	Specifies how many times the cycle is to be repeated, for example 45 times.

A program, and thus also a profile, can only be edited as long as no run has been performed. Every change is displayed immediately in the *Temperature Profile* window area.

4.1.1 Programs list buttons

The *Programs* list can be edited with the following buttons:

Button	Function	Description
	Up	Moves the selected program up one place. If there is no selected program, or the selected item is first in the list, this button is disabled.
	Add	Opens the *Add New Program* dialog box which allows for adding a new program to the list. The new program is added to the end of the list. For detailed information, see *Adding a new program* below.
	Remove	Deletes the selected program from the list. If no program is selected, this button is disabled.
	Down	Moves the selected program down one place. If there is no selected program, or the selected item is last in the list, this button is disabled.

4.1.2 Programs list shortcut menu

The shortcut menu in the *Programs* list provides functions for adding and removing programs.

Command	Description
Add	Opens the *Add New Program* dialog box which allows for adding a new program to the list. The new program is added to the end of the list. For detailed information, see *Adding a new program* below.
Remove	Deletes the selected program from the list. If no program is selected, this button is disabled.

4.1.3 Adding a new program

The  button in the *Programs* list or the *Add* command on the shortcut menu opens the *Predefined Programs* dialog box, which allows for selecting a new program and adding it to the *Programs* list.

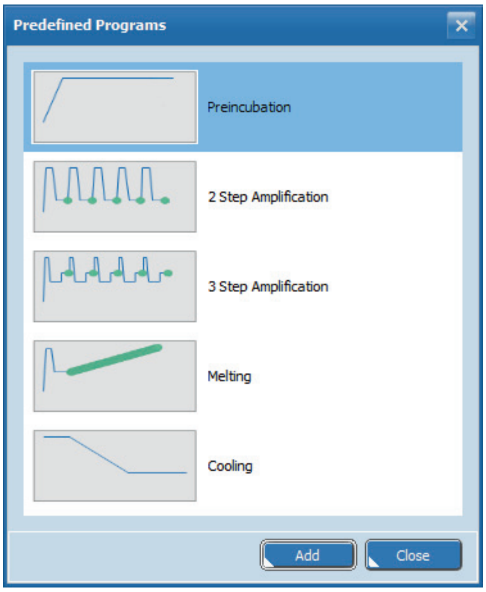


Figure 47: Predefined Programs dialog box

B

The following programs are available:

Program	Description
Preincubation	Holds a specified temperature for a defined time.
2 Step/3 Step Amplification	Cycling program; defines a program of the experiment where the instrument will repeatedly heat and cool to a defined series of temperatures. Each repeat is called a cycle. The touchdown function for amplification programs allows the operator to specify that one of the stages of each cycle will have its target temperature modified as the cycling proceeds. This allows for the early cycles of a PCR to have a higher annealing temperature specified, leading to more specific amplification. For detailed information, see section *Touchdown*, on page 125.
Melting	Defines a program where the instrument will ramp to an initial temperature, then ramp to a final temperature. While ramping to the final temperature, optical acquisitions will be made continuously. These can then be analyzed to yield melting peaks.
Cooling	Defines a program where the instrument will cool down to a final temperature and then hold the specified temperature for a defined time.

It is not necessary to add a separate cooling program at the end of the run. At the end of each run, the samples are automatically cooled to +37°C.

4.2 Steps window area

A program consists of one or more steps, which are run by the instrument in the order they are displayed in the *Steps* window area. A step specifies the following data:

- The temperatures used by the instrument
- The time for which these temperatures are to be held
- The rate at which to heat and cool
- The points at which the instrument will acquire optical data

The minimum experiment definition has one program with one cycle and one valid step.

If multiple amplification programs with acquisitions are defined, only the first amplification program is considered for result calculation and chart display.

If multiple melting programs with acquisitions are defined, the 'Melting Selection' dialog box opens for selecting the melting program for result calculation. For detailed information, see section Adding a new analysis, on page 147.

The LightCycler® 96 Instrument adjusts the temperature between the steps automatically, cooling or heating up to meet the temperature specified for the next step.

The steps are displayed in a list and have the following properties. The LightCycler® 96 Application Software derives these properties from the settings in the *Temperature* and *Measurement* areas to the right of the *Steps* list (see section *Step settings*, on page 124).

Parameter	Description
Description	Target temperature and duration in seconds for which the temperature is to be held.
Acquisition Mode	Acquisition mode: *None*, *Single*, or *Continuous*.

4.2.1 Steps list buttons

The *Steps* list can be edited with the following buttons:

Button	Function	Description
	Up	Moves the selected step up one place. If there is no selected step, or the selected item is first in the list, this button is disabled.
	Add	Adds a new step to the list. The new step is added to the end of the list.
	Remove	Deletes the selected step from the list. If no step is selected, this button is disabled.
	Down	Moves the selected step down one place. If there is no selected program, or the selected item is last in the list, this button is disabled.

4.2.2 Steps list shortcut menu

The shortcut menu in the *Steps* list provides functions for adding and removing steps.

Command	Description
Add	Adds a new step to the list. The new step is added to the end of the list.
Remove	Deletes the selected step from the list. If no step is selected, this button is disabled.

4.2.3 Step settings

A step can only be edited as long as no run has been performed. The settings of a selected step are displayed in the *Temperature*, *Measurement*, and *Touchdown* areas to the right of the *Steps* list. Every change is displayed immediately in the *Steps* list and the *Temperature Profile* window area.

The following settings can be specified for each step in a program:

Temperature

Setting	Parameter	Description
Temperature	*Ramp (°C/s)*	Rate of temperature change in °C per second, which the LightCycler® 96 Instrument uses for heating or cooling until the defined temperature is reached.
	Duration (s)	Duration in seconds for which the temperature is to be held.
	Target (°C)	Temperature in °C, which is to be held for a defined time.
Gradient	Only available for amplification programs and when no touchdown is specified: Temperature grading used by the LightCycler® 96 Instrument for heating the different Peltier elements in the thermal block cycler. Operators can specify temperature gradients from 37 to 98°C. *The 'Detail' button opens the 'Gradient' dialog box, which displays a detailed view of the specified temperature gradient. For detailed information, see below.*	
	Col 1 (°C)	Minimum temperature for the gradient. This temperature is applied to the leftmost column of the multiwell plate.
	Col 12 (°C)	Maximum temperature for the gradient. This temperature is applied to the rightmost column of the multiwell plate.

The *Detail* button opens the *Gradient* dialog box, which displays a detailed view of the specified temperature gradient.

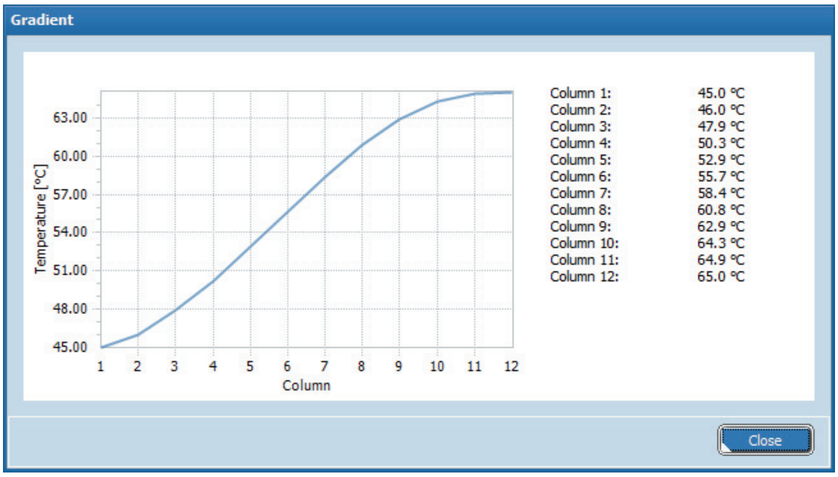


Figure 48: Gradient dialog box

The curve in the dialog box shows the applied temperature gradient according to the columns on the multiwell plate.

Acquisition Mode

Setting	Parameter	Description
Acquisition Mode	*Single*	Only available for amplification programs: Acquires fluorescence data once only, when the temperature target is reached and the hold time completed.
	Continuous (readings/°C)	Not available for amplification programs: Number of optical acquisitions to be performed. The fluorescence data are acquired continuously until the temperature target is reached.
	None	No fluorescence data are acquired.

Touchdown

Setting	Parameter	Description
Touch down	*Enabled*	Only available for amplification programs and when no gradient is specified: Enables/disables the touchdown function. *Touchdown is only available for amplification programs and if no gradient is specified and vice versa.*
	Temperature Change [°C]	Rate of temperature change in °C per cycle, at which the touchdown phase proceeds to the final phase temperature.
	Sec Target Temp [°C]	Second target temperature to be reached by the last cycle of the program. This temperature is used to change the target temperature of a segment during the amplification reaction.
	Delay [Cycles]	Number of cycles after which the temperature change is first applied.

4.3 Measurement window area

In the *Measurement* window area the operator specifies the dye-specific settings for an experiment run. The *Measurement* window area provides the following settings:

Setting	Description
Dyes	Dye to be used in this experiment. You choose the dye in the *Detection Format* dialog box. For detailed information, see section *Detection Format*, on page 126.
Reaction Volume [µl]	Reaction volume to be used in the experiment. The LightCycler® 96 Instrument supports reaction volumes from 10 to 50 µl. *As the LightCycler® 96 Instrument does not validate the reaction volume, the operator must ensure that the specified reaction volume matches the volume pipetted into the wells of the multiwell plate.*

Detection Format

The *Detection Format* button opens the *Detection Format* dialog box. By setting the detection formats, the operator chooses the filter combinations suitable for the experiment. A detection format specifies one or more excitation-emission filter combinations. For detailed information, see section *Detection channels*, on page 38.

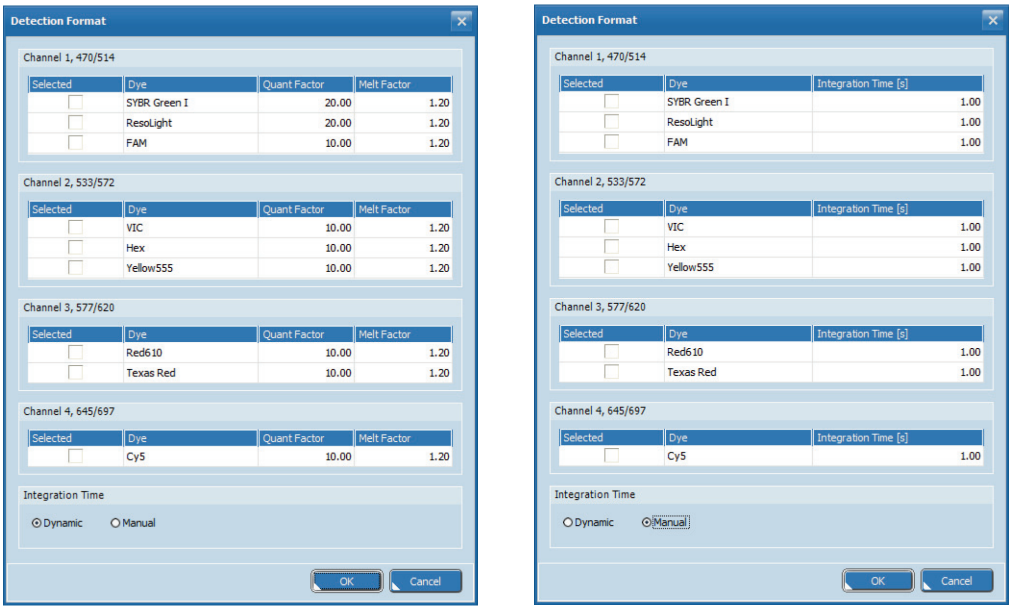


Figure 49: Detection Format dialog box, Dynamic versus Manual mode

The dialog box shows the following settings for each channel (that is, for each filter combination):

Column	Description	Possible values
Selected	Specifies whether the channel is to be used by selecting the corresponding dye. *Only one dye can be selected per channel. The software automatically deselects a check box when you try to select more than one dye in the same channel group.* *SYBR Green I and ResoLight Dye cannot be combined with any dye of another channel.*	
Dye	Name of the dye.	

Column	Description	Possible values
Quant Factor	Only displayed if the integration time mode is set to *Dynamic*: Multiplication factor to be applied to the filter combination for a quantification program. The *Quant Factor* represents the fold signal stroke from the initial background fluorescence to the plateau phase.	1.0 to 500
Melt Factor	Only displayed if the integration time mode is set to *Dynamic*: Multiplication factor to be applied to the filter combination for a melting program.	1.0 to 500
Integration Time [s]	Only displayed if the integration time mode is set to *Manual*: Amount of time for which the LED will be used to excite the wells during a single optical reading. The higher this value is set, the more light will be emitted from the fluorophores in the well, and the larger the resulting spectral values are.	0.1 to 10 sec

The integration time is defined as the acquisition time of the CCD camera. Depending on the integration time mode chosen for the detection format, the dialog box shows the following settings:

Mode	Description	Setting
Dynamic	The integration time is set automatically based on the fluorescence of the individual plate. *For quantification and melting programs an independent value can be set.*	Melt Factor Quant Factor
Manual	The integration time is set manually.	Integration Time

4.4 Temperature Profile window area

The *Temperature Profile* window area provides a summary of the programs selected for the experiment and their temperature and time settings.

When starting an experiment run, the operator must ensure that the correct temperature profile is used.

5 Sample Editor tab

The *Sample Editor* tab allows operators to create, edit, delete, and rearrange samples and genes present in the wells, as well as the dyes used to label each gene. A single sample can be present in one or more different wells and can have one or more genes of interest. This sample and gene data will then be used to perform different analysis methods. In addition to sample names and gene names, operators can also edit condition names. This property is only relevant for relative quantification analysis. For detailed information, see see section *Relative quantification*, on page 158).

The operator can perform the corresponding tasks in the *Plate View* or *Table View* tabs. Changes on the *Plate View* tab are immediately displayed in the *Table View* tab and vice versa.

The operator must ensure that the sample assignment in the 'Sample Editor' tab matches the pipetting scheme on the multiwell plate.

5.1 Plate View tab

The *Plate View* tab of the *Sample Editor* shows the assigned samples and genes in wells laid out as a LightCycler® 480 Multiwell Plate 96.

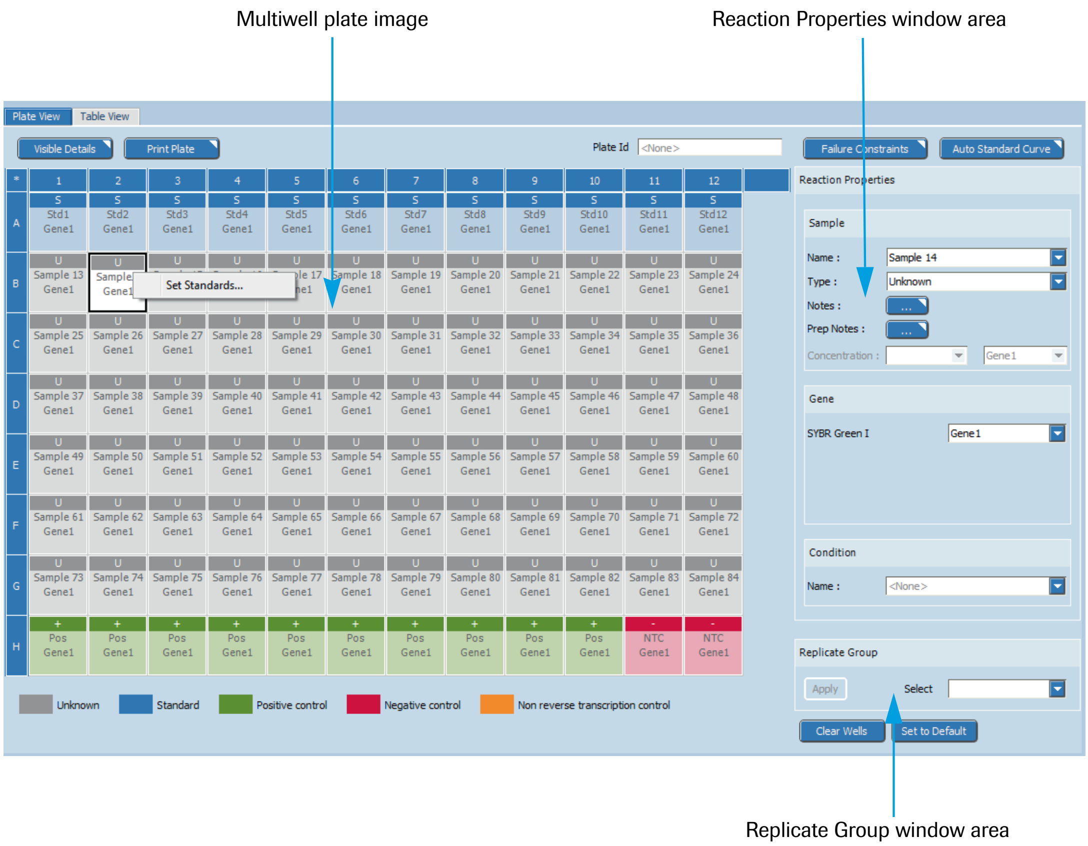


Figure 50: Plate View tab

For a new experiment, this tab shows the following data:

- The default sample names *Sample 1* to *Sample 96*
- The sample type *Unknown* for all samples

For detailed information on how to define the reaction properties according to different applications, refer to the *LightCycler® 96 System User Training Guide*.

5.1.1 Multiwell plate image

The multiwell plate image provides a schematic of the multiwell plate mount for editing the range of samples. Each row is allocated a letter from A to H. The wells of a row are displayed as columns and numbered from left to right.

- Each well displays the sample properties according to the operator's selection. For detailed information on how to customize the plate view, see section *Visible Details*, on page 132.
- Each well is colored to match the sample type in the corresponding well.
- Hovering the cursor over a well displays a tooltip with the properties of the sample.
- Selecting a well or a range of wells allow editing of the corresponding properties using the fields in the window areas displayed to the right of the multiwell plate image.

Plate image shortcut menu

The multiwell plate image provides a shortcut menu containing the following command:

Command	Description
Set Standards	Opens the *Auto Standard Curve* dialog box. For detailed information, see section *Auto Standard Curve*, on page 135

5.1.2 Reaction Properties window area

The *Reaction Properties* window area allows operators to create or edit the following properties of samples and genes in a selected well or a range of wells. Property changes are immediately displayed in the multiwell plate image.

The Sample Editor allows operators to edit the sample- and/or gene-specific properties, and the condition names (only relevant for relative quantification). Settings affecting the analysis of the data are to be specified in the corresponding 'Analysis' tab. For detailed information, see section Analysis tab, on page 145.

Setting	Property	Description
Sample	*Name*	Sample name. The name is used to identify the sample in tables, wells, graphs, etc.
	Type	Sample type. The following sample types are possible: ▶ *Unknown* Sample with unknown quantity of a specific gene. *For relative quantification analysis, only the type 'Unknown' is used for calculating the corresponding ratios.* ▶ *Standard* Sample with known quantity of a specific gene. By comparing the Cq values of unknown samples of the same gene to the Cq values of these known standard quantities, the unknown quantities can be estimated. You may provide as many standards as required to cover the expected range of quantities of unknown genes. When specifying samples as standards, each gene in the reaction needs to be assigned a *Concentration* value. ▶ *Positive control* Sample containing a specific gene. ▶ *Negative control* Sample without a specific gene. ▶ *Non reverse transcription control* Reaction without reverse transcriptase enzyme to check for genomic DNA contamination.
	Notes	Description of the sample. Clicking the button opens the *Edit Sample Note* dialog box, which allows for a longer, multi-line description.
	Prep Notes	Notes as specified in imported MagNA Pure 96 sample data files. Operators can edit the displayed text.
	Concentration	Only available for sample type *Standard:* Concentration value for the standard quantity of the gene selected in the list.
Gene	*<dye>*	Associates the gene with the displayed dye. Where multiple dyes are used (for example, hydrolysis probes with different wavelengths), the instrument allows multiple genes in the same well. ▶ The *<dye>* list provides all dyes assigned in the corresponding detection formats. For detailed information on how to define a detection format, see section *Detection Format*, on page 126. ▶ For each dye, a gene can be assigned by editing the text field or choosing a gene from the list. The list provides the gene names which are already defined.
Condition	*Name*	Only relevant for relative quantification analysis: Condition name for grouping the samples according to different conditions during the experiment run. One of these conditions is to be specified in the analysis as a study calibrator for calculating the scaled ratio. For detailed information, see section *Scaled ratio*, on page 69.

B

5.1.3 Replicate Group window area

The *Replicate Group* window area allows operators to create new replicate groups and arrange samples in them. The replicate group is always named according to the top leftmost of the corresponding wells. If this "master replicate" is removed, the next top leftmost position takes on the role.

- If properties of the master replicate are changed, all changes are assigned to all replicate group members.
- If properties of one of the other replicate group members are changed, the corresponding sample is removed from the replicate group.

Property	Description
Select	Name of the replicate group. The list provides the already defined replicate groups.

The LightCycler® 96 Application Software automatically groups samples in replicate groups, provided they have identical values for the following properties:

- Sample name
- Sample type
- Concentration
- Gene name
- Condition

To create a replicate group

1. In the multiwell plate image, select the corresponding wells.
2. In the *Replicate Group* window area, choose *Apply*.
   The replicate group is created and named according to the top leftmost of the selected wells. The replicate group is now displayed in the *Select* list.

To select wells by replicate group

1. In the *Replicate Group* window area, choose a replicate group from the *Select* list.

To add samples to a replicate group

1. In the *Replicate Group* window area, choose a replicate group from the *Select* list.
2. In the multiwell plate image, select the additional wells.
3. In the *Replicate Group* window area, choose *Apply*.
   The properties of the samples in the replicate group are assigned to the additional samples.

5.1.4 Visible Details

The *Visible Details* button opens the *Visible Details* dialog box, which allows for customizing the sample editor display.

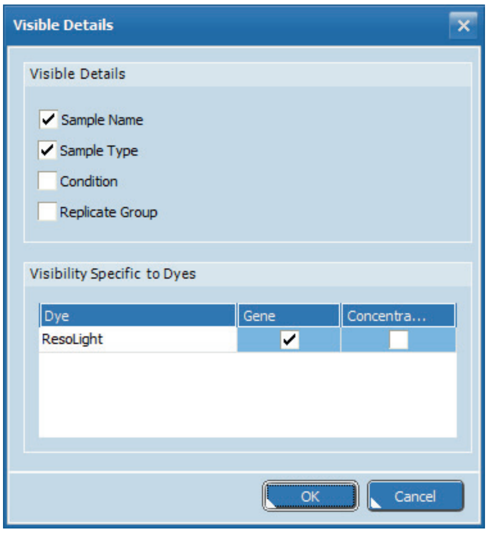


Figure 51: Visible Details dialog box

Setting	Description
Visible Details	Properties to be displayed for the wells in the plate window area. Selected properties are displayed, unselected properties are hidden.
Visibility Specific to Dyes	Dye-specific properties to be displayed for the wells in the plate window area. The table shows a row for each dye assigned to the sample.

5.1.5 Print Plate

The *Print Plate* button opens the *Print Plate Preview*. Operators can print the plate view of an experiment as pipetting information. The printout matches the view on the screen, meaning the visibility of the properties are identical. The printout also shows the following data:

- The experiment name
- The plate ID
- The LightCycler® 96 Application Software version
- The print date

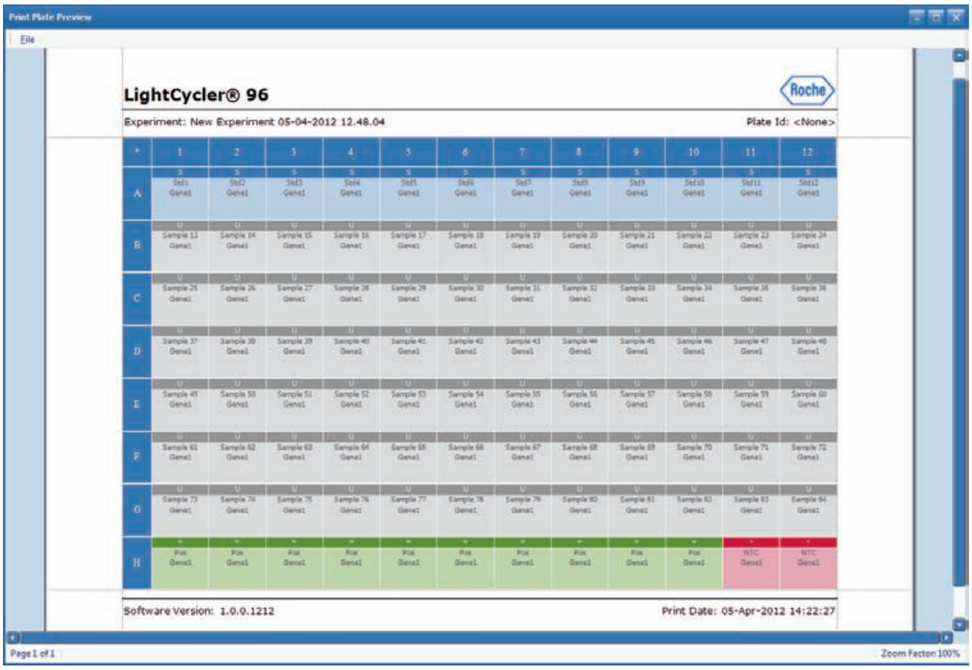


Figure 52: Print Plate Preview

Printing and saving the plate view is done via the commands in the *File* menu:

Command	Description
Page Setup ...	Opens the *Page Setup* dialog box to specify the settings for the print page.
Print ...	Opens the *Print* dialog box to choose the printer settings and start printing the current plate view.
Print	Prints the current plate view. The information is printed on the operator's system default printer. If the printer is not ready or an alarm occurs during the print process, an error message is displayed.
Save as Image	Opens the *Save As* dialog and saves the plate view as an image to the specified location. The following file types are available: ▶ *JPEG image (.jpeg)* ▶ *PNG image (.png)* ▶ *BMP image (.bmp)*
Exit	Closes the print preview.

5.1.6 Plate ID

The plate ID is saved to the experiment file for identification of the experiment.

When using the external handheld barcode scanner, the *Plate Id* field displays the barcode of the loaded multiwell plate. *<None>* is displayed if no barcode is available or no multiwell plate is loaded.

5.1.7 Failure Constraints

The *Failure Constraints* button opens the *Failure Conditions* dialog box, in which operators can define additional failure constraints, for example, for Cq and efficiency values in quantification analyses. A failure is raised if one or more of these constraints are not met. The failures are displayed in the result table of the corresponding analysis. For detailed information, see the description of the corresponding result tables in section *Analysis tab*, on page 145.

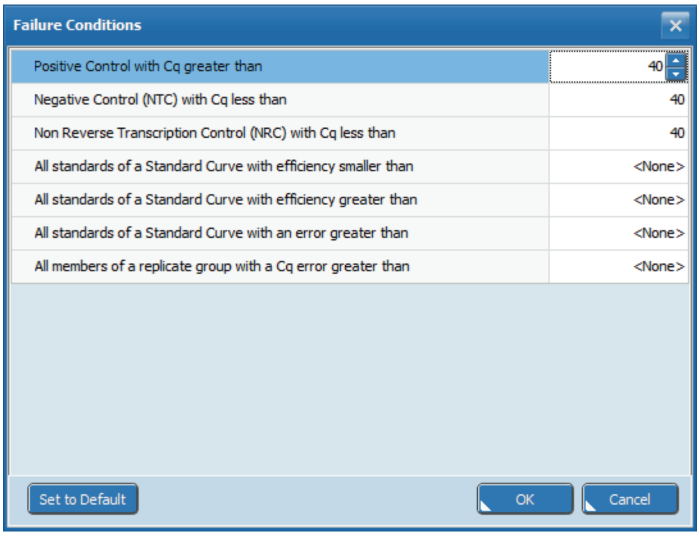


Figure 53: Failure Conditions dialog box

The dialog box provides the following failure constraints:

- *Positive Control with Cq greater than*
- *Negative Control (NTC) with Cq less than*
- *Non Reverse Transcription Control (NRC) with Cq less than*
- *All standards of a Standard Curve with efficiency smaller than*
- *All standards of a Standard Curve with efficiency greater than*
- *All standards of a Standard Curve with an error greater than*
- *All members of a replicate group with a Cq error greater than*

Each row in the list provides an input field for specifying the value for the corresponding condition.

A failure only means a notification for the operator. Positive/negative calls and Cq values are not changed and the corresponding samples are not automatically excluded from the calculation.

5.1.8 Auto Standard Curve

The *Auto Standard Curves* button opens the *Auto Standard Curve* dialog box. This dialog box allows operators to set up a dilution series without having to define each well.

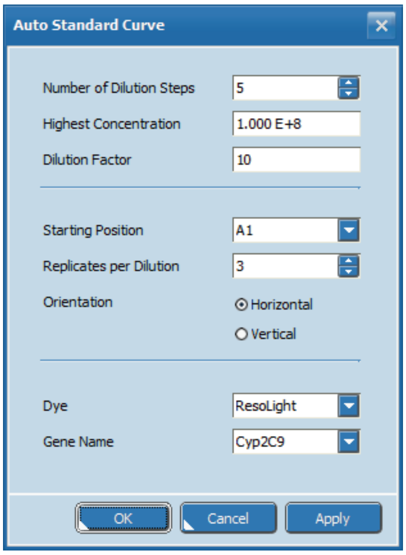


Figure 54: Auto Standard Curve dialog box

Setting	Description
Number of Dilution Steps	Number of different concentrations per series.
Highest Concentration	Highest concentration (that is, the starting concentration).
Dilution Factor	Dilution factor between adjacent dilution steps.
Starting Position	Position of the well to start the series. Default: *A1* If the dialog box is opened with the *Set standards* command on the multiwell plate image shortcut menu, the position of the selected well is displayed here.
Replicates per Dilution	Number of replicates per dilution.
Orientation	Alignment of the series on the multiwell plate: ▶ *Horizontal* from left to right ▶ *Vertical* from top to bottom
Dye	Dye to be used for the corresponding gene of interest. The list provides the dyes assigned in the corresponding detection formats. For detailed information on how to define a detection format, see section *Detection Format*, on page 126.
Gene Name	Gene for which the dilution series is defined. The list provides the gene names which are already defined in the experiment. ▶ If the operator has previously assigned the same gene name to all affected wells, this name is displayed as the default. ▶ If the operator has assigned different gene names to the affected wells, *<Multiple>* is displayed.

5.1.9 Clear Wells/Set to Default

The buttons below the *Replicate Group* area perform the following functions:

Button	Description
Clear Wells	Removes all property values from the selected wells. *Cleared wells are deactivated. This means they can no longer be edited and are not displayed in the table view and the analysis windows.* *To reactivate the wells, use the 'Set To Default' button.* *It is strongly recommended to use the 'Clear Wells' function for all empty wells of LightCycler® 480 Multiwell Plates 96 and for positions not occupied by LightCycler® 8-Tube Strips.*
Set to Default	Sets all property values to the corresponding default values.

5.2 Table View tab

The *Table View* tab of the *Sample Editor* shows the assigned samples and genes in table format. For detailed information on editing cells and sorting and filtering the table, see section *Working with tables*, on page 90.

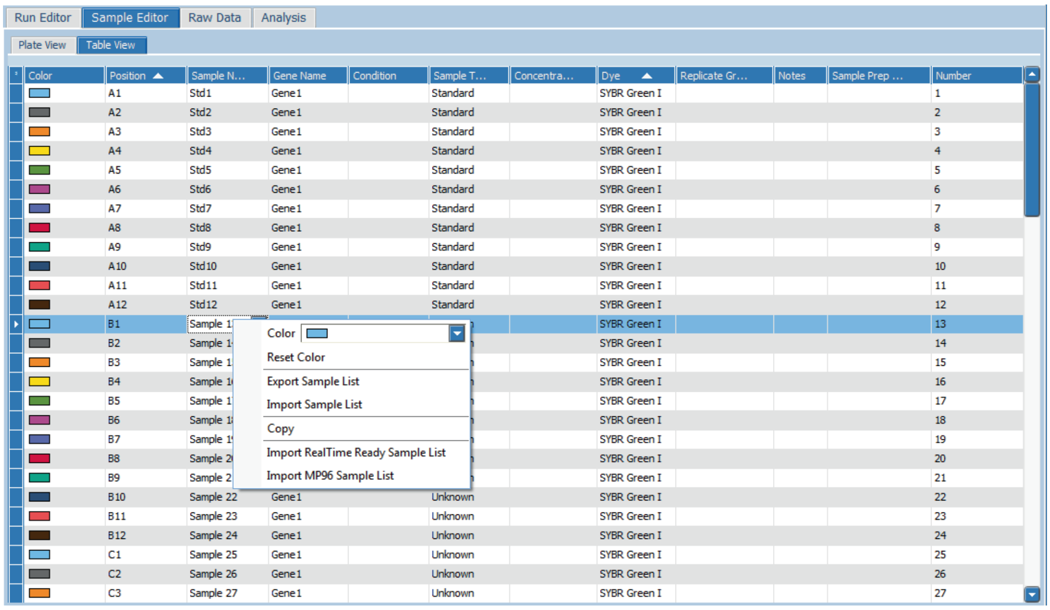


Figure 55: Table View tab

For a new experiment, this tab shows the following data:

- Number and position of a sample
- The default sample names *Sample 1* to *Sample 96*
- The sample type *Unknown* for all samples

For detailed information on how to define the reaction properties according to different applications, refer to the *LightCycler® 96 System User Training Guide*.

5.2.1 Samples table

The columns in the samples table show the following sample properties. For detailed information on the properties, see sections *Reaction Properties window area*, on page 129 and *Replicate Group window area*, on page 131.

Property	Description
Color	Color of the corresponding sample in table cells and graph lines.
Position	Position of the well in the multiwell plate. Each row is allocated a letter from A to H. The wells of a row are numbered from left to right.
Sample Name	Sample name.
Gene Name	Name of the gene of interest.
Condition Name	Only relevant for relative quantification analysis: Condition for grouping the samples according to different conditions during the experiment run.
Sample Type	Sample type; the following sample types are possible: ▶ *Unknown* ▶ *Standard* ▶ *Positive control* ▶ *Negative control* ▶ *Non reverse transcription control* For a detailed description of the sample types, see section *Reaction Properties window area*, on page 129.
Concentration	Only available for sample type *Standard*: Concentration value for the standard quantity of the gene.
Dye	Name of the associated dye; if multiple dyes are used in one well, the table provides a row with the same number and position for each dye.
Replicate Group	Replicate group the sample belongs to.
Notes	Description of the sample.
Sample Prep Notes	Notes as specified in imported MagNA Pure 96 sample data files. Operators can edit the displayed text.
Number	Index number of a well per channel. Index numbers are counted sequentially from left to right and from top to bottom.

Samples table shortcut menu

The samples table provides a shortcut menu containing the following commands:

Command	Description
Color	Opens the color selection dialog box. For detailed information, see section *Editing cells*, on page 93.
Reset Color	Resets the color to the default value.
Export Sample List	Exports the sample data to a tab-delimited text file (.txt). For detailed information, see section *Exporting the sample list*, on page 139.
Import Sample List	Imports the sample data available on the clipboard to the samples table. For detailed information, see section *Importing sample data*, on page 140.
Copy	Copies the selected rows, including the header line, to the clipboard.
Import RealTime Ready Sample List	Imports a RealTime ready sample list. The command opens a file selection dialog box, in which operators can choose a corresponding text file generated for import of target information for RealTime ready Panels.
Import MP96 Sample List	Imports a MagNA Pure 96 sample data file.

5.2.2 Exporting the sample list

The sample list of an experiment contains the complete sample data provided on the *Sample Editor* tab. Operators can export these data to a text file that can be opened with a text file editor or imported to a spreadsheet application, for example, Microsoft Excel.

To export the sample list

1. In the *Sample Editor*, open the *Table View* tab.
2. On the table shortcut menu, choose *Export Sample List*.
   The *Save As* dialog box opens.
3. Navigate to the directory where you want to store the sample list file.
   By default, the default experiment directory is displayed. This directory can be specified in the 'Default Directory' dialog box. For detailed information, see section Preferences, on page 119.
4. Enter a name for the sample list file.
5. Click *Save*. The dialog box closes and the sample data are saved as a text file (.txt).

5.2.3 Importing sample data

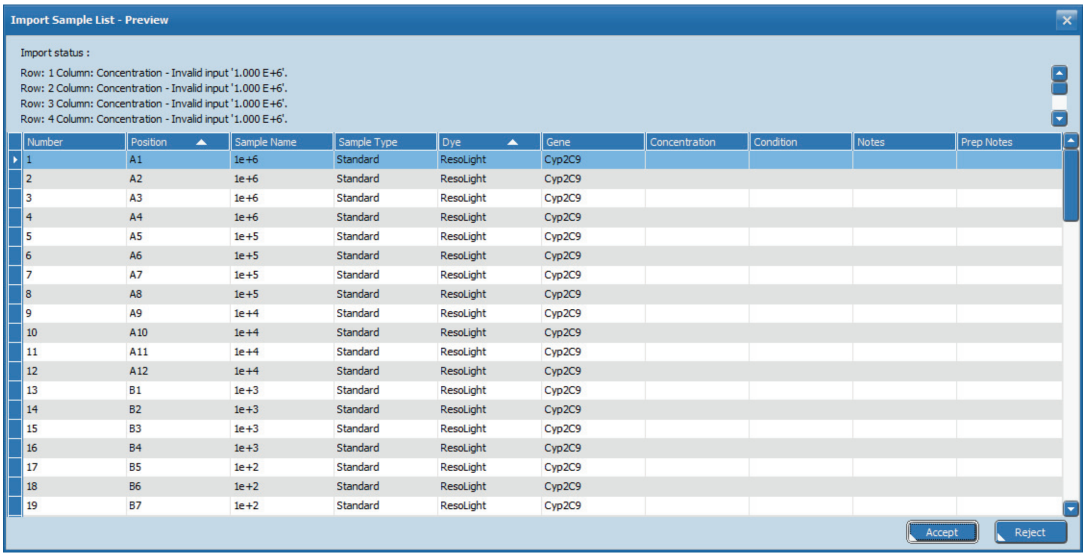
The import function allows the operator to import sample data, that is, to paste data into the samples table.

Importable files must contain position, dye and header line information matching the position, dye and header line text on the Sample Editor tab.

The data can be imported from one of the following sources:

- A sample list exported from another experiment; for detailed information on exporting the sample list, see section *Raw Data tab*, on page 142.
- A Microsoft Excel file (.xls, .xlsx) containing a sample list.
- A CSV file (.csv) containing a sample list.
- A results file exported from the MagNA Pure 96 Software in LightCycler® System readable format (.txt or .xml).
- A text file generated for RealTime ready Panels and Custom Panels.

The *Import Sample List* command on the samples table shortcut menu opens the *Import Sample List - Preview* window:


Import Sample List - Preview

Import status :
Row: 1 Column: Concentration - Invalid input '1.000 E+6'.
Row: 2 Column: Concentration - Invalid input '1.000 E+6'.
Row: 3 Column: Concentration - Invalid input '1.000 E+6'.
Row: 4 Column: Concentration - Invalid input '1.000 E+6'.

Number	Position	Sample Name	Sample Type	Dye	Gene	Concentration	Condition	Notes	Prep Notes
1	A1	1e+6	Standard	ResoLight	Cyp2C9				
2	A2	1e+6	Standard	ResoLight	Cyp2C9				
3	A3	1e+6	Standard	ResoLight	Cyp2C9				
4	A4	1e+6	Standard	ResoLight	Cyp2C9				
5	A5	1e+5	Standard	ResoLight	Cyp2C9				
6	A6	1e+5	Standard	ResoLight	Cyp2C9				
7	A7	1e+5	Standard	ResoLight	Cyp2C9				
8	A8	1e+5	Standard	ResoLight	Cyp2C9				
9	A9	1e+4	Standard	ResoLight	Cyp2C9				
10	A10	1e+4	Standard	ResoLight	Cyp2C9				
11	A11	1e+4	Standard	ResoLight	Cyp2C9				
12	A12	1e+4	Standard	ResoLight	Cyp2C9				
13	B1	1e+3	Standard	ResoLight	Cyp2C9				
14	B2	1e+3	Standard	ResoLight	Cyp2C9				
15	B3	1e+3	Standard	ResoLight	Cyp2C9				
16	B4	1e+3	Standard	ResoLight	Cyp2C9				
17	B5	1e+2	Standard	ResoLight	Cyp2C9				
18	B6	1e+2	Standard	ResoLight	Cyp2C9				
19	B7	1e+2	Standard	ResoLight	Cyp2C9				

Accept Reject

Figure 56: Import Sample List - Preview

The preview shows the sample table with the import status and the expected result. Overwritten cells are highlighted.

The buttons below the table perform the following functions:

Button	Description
Accept	Closes the preview and applies the changes to the corresponding rows.
Reject	Closes the preview and discards the changes.

To import sample data into the sample list

1	Open the file containing the data to be imported.
2	Select the corresponding data and copy them to the clipboard.
3	In the *Sample Editor*, open the *Table View* tab.
4	On the shortcut menu, choose *Import Sample List*. The *Import Sample List - Preview* window opens, showing the sample table with the expected results.
5	Choose *Accept*. The *Import Sample List - Preview* window closes and the data are imported into the sample list.

6 Raw Data tab

The *Raw Data* tab shows the temperature and optical data collected during an experiment run. The raw data of an experiment contain all instrument corrections but no color compensation, drift or background correction.

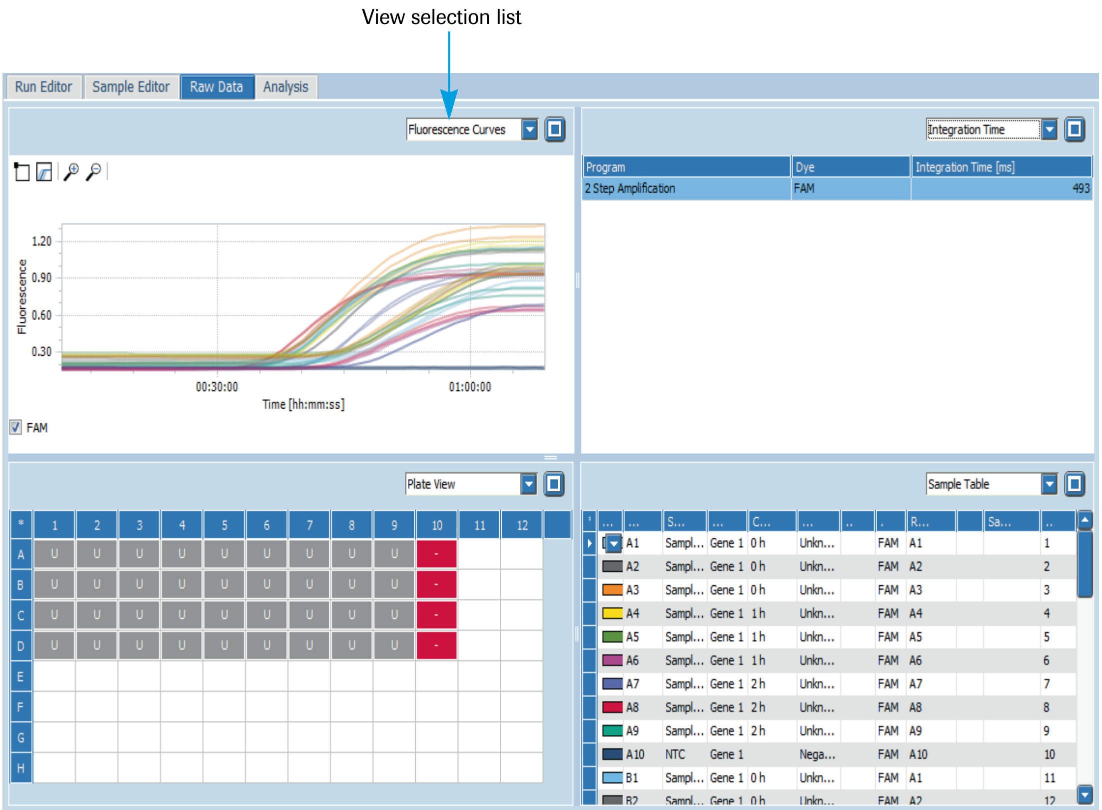


Figure 57: Raw Data tab

By default the *Raw Data* tab shows four sections. For detailed information on changing the number of displayed sections and on resizing the sections, see section *Working with sections*, on page 101.

All sections on the *Raw Data* tab use the same selection: When the operator selects a curve in one of the graphs or a well in the *Plate View* or *Sample Table*, the same wells are selected in each section.

The view selection list in the sections of the *Raw Data* tab provides the following data:

- *Plate View*; see section *Plate View*, on page 143.
- *Fluorescence Curves*; see section *Fluorescence Curves*, on page 143.
- *Melting Curves*; see section *Melting Curves*, on page 143.
  This option is only available for melting programs.
- *Amplification Curves*; see section *Amplification Curves*, on page 144.
- *Sample Table*; see section *Sample Table*, on page 144.
- *Integration Time*; see section *Integration Time*, on page 144.

Plate View

The *Plate View* shows the wells as they are laid out in the Sample Editor, allowing operators to select the wells for display. Each well is colored to match the sample type.

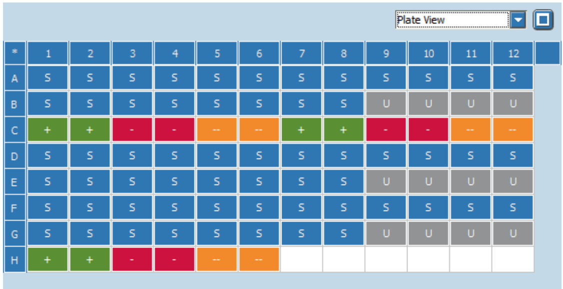


Figure 58: Raw Data tab, Plate View

Fluorescence Curves

Fluorescence Curves display the fluorescence intensity against the time in hours, minutes, and seconds for the entire run. There is one curve for each sample that has a gene labeled with the selected dye.

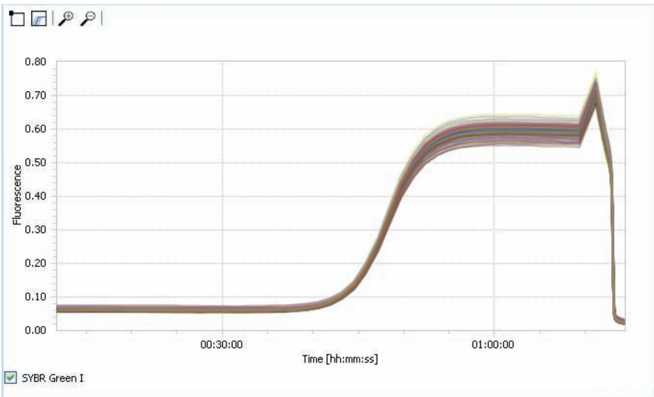


Figure 59: Raw Data tab, Fluorescence Curves

Melting Curves

Melting Curves display the fluorescence intensity against the temperature in °C for a melting program.

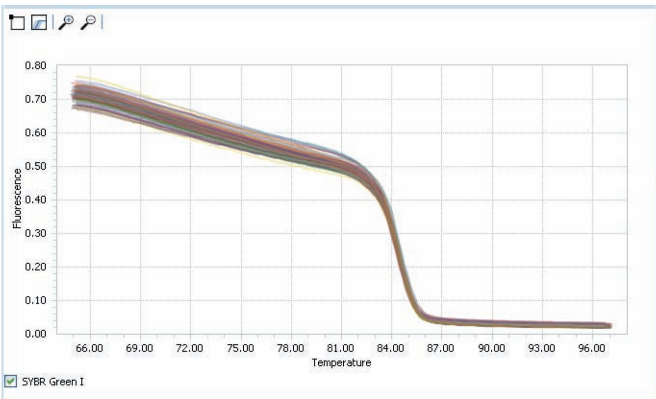


Figure 60: Raw Data tab, Melting Curves

B

Amplification Curves

Amplification Curves display the fluorescence intensity against the number of cycles in an amplification program. There is one curve for each sample that has a gene labeled with the selected dye.

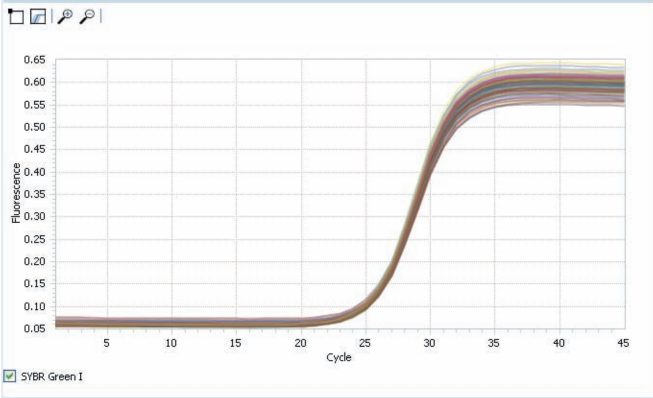
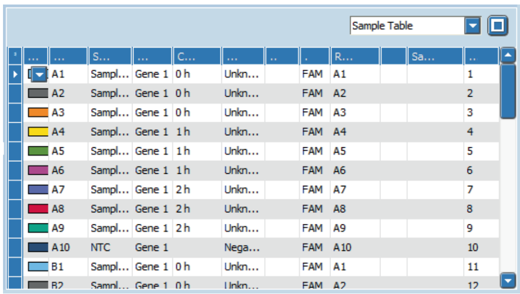


Figure 61: Raw Data tab, Amplification Curves

Sample Table

The *Sample Table* shows all information of the sample editor *Table View* tab and allows selection of one or more wells to be highlighted in the graphs.



Sample Table

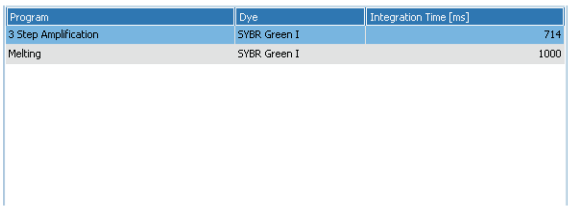
...	...	S...	...	C...	...	..	.	R...		Sa...	..
	A1	Sampl...	Gene 1	0 h	Unkn...		FAM	A1			1
	A2	Sampl...	Gene 1	0 h	Unkn...		FAM	A2			2
	A3	Sampl...	Gene 1	0 h	Unkn...		FAM	A3			3
	A4	Sampl...	Gene 1	1 h	Unkn...		FAM	A4			4
	A5	Sampl...	Gene 1	1 h	Unkn...		FAM	A5			5
	A6	Sampl...	Gene 1	1 h	Unkn...		FAM	A6			6
	A7	Sampl...	Gene 1	2 h	Unkn...		FAM	A7			7
	A8	Sampl...	Gene 1	2 h	Unkn...		FAM	A8			8
	A9	Sampl...	Gene 1	2 h	Unkn...		FAM	A9			9
	A10	NTC	Gene 1		Nega...		FAM	A10			10
	B1	Sampl...	Gene 1	0 h	Unkn...		FAM	A1			11
	B2	Sampl...	Gene 1	0 h	Unkn...		FAM	A2			12

Figure 62: Raw Data tab, Sample Table

For a description of the properties displayed in the table, see section *Samples table*, on page 138.

Integration Time

The *Integration Time* table shows the dye-specific integration time for each measuring program.



Program	Dye	Integration Time [ms]
3 Step Amplification	SYBR Green I	714
Melting	SYBR Green I	1000

Figure 63: Raw Data tab, Integration Time table

7 Analysis tab

On the *Analysis* tab, operators create an analysis, define analysis settings, and view the calculated results of an experiment run (in this example, the *Analysis* tab for a relative quantification analysis is shown).

Figure 64: Analysis tab

<analysis> tabs

The LightCycler® 96 Application Software provides a tab for each analysis added to the experiment. The selected analysis determines the type and contents of the sections on the *Analysis* tab. By default each <*analysis*> tab shows four sections. For detailed information on changing the number of displayed sections and on resizing the sections, see section *Working with sections*, on page 101.

The name of an <*analysis*> tab can be edited using the *Rename Analysis* command on the analysis shortcut menu. For detailed information, see *Analysis shortcut menu*, on page 146.

All sections on the *Analysis* tab use the same selection: When the operator selects a curve in one of the graphs or a well in a *Heat Map* or in the *Sample Table*, the same wells are selected in each section.

Analysis shortcut menu

Each <*analysis*> tab provides a shortcut menu. The shortcut menu opens with a right-click on the header of the tab:

Command	Description
Analysis Settings	Opens the <*analysis*> *Settings* dialog box for specifiying the analysis-specific settings. For detailed information, see the descriptions of the corresponding analysis tabs. *Changing the analysis settings invalidates the results and, after confirmation, causes an automatic recalculation.*
Remove Analysis	Removes the analysis from the *Analysis* tab. The operator is prompted to confirm the action.
Rename Analysis	Opens the *Rename Analysis* dialog box to specify a new name for the analysis.

View selection list

The view selection list in each section of the *Analysis* tab provides the data to be displayed. The available options depend on the selected analysis. For detailed information, see the description of the corresponding analysis.

Analysis tool bar icons

The following icons in the tool bar are only displayed when the *Analysis* tab is opened:

Icon	Function	Description
	Add Analysis	Opens the *Create New Analysis* dialog box to select a new analysis and edit the name of the corresponding tab. For detailed information, see below.
	Analysis Settings	Only displayed if at least one analysis is defined: Opens the <*analysis*> *Settings* dialog box for specifiying the analysis-specific settings. For detailed information, see the descriptions of the corresponding analysis tabs. *Changing the analysis settings invalidates the results and causes an automatic recalculation.*
	Delete Analysis	Only displayed if at least one analysis is defined: Removes the selected analysis from the experiment.

Adding a new analysis

The *Add Analysis* icon in the tool bar opens the *Create New Analysis* dialog box:

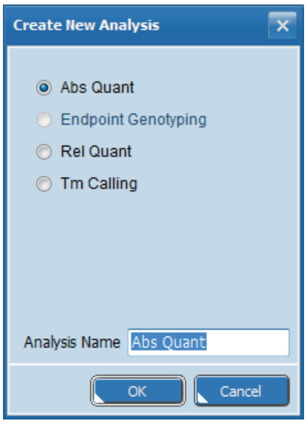


Figure 65: Create New Analysis dialog box

The dialog box allows for selecting a new analysis and specifying the name of the corresponding tab:

Setting	Description
<analysis>	Type of analysis to be added.
Analysis Name	Name of the correspondig tab. By default the name of the analysis type is provided.

If multiple melting programs with acquisitions are defined, the *Melting Selection* dialog box opens for selecting the melting program for result calculation.

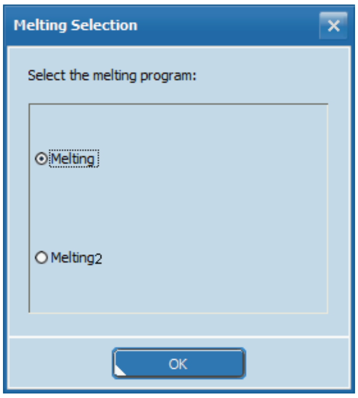


Figure 66: Melting Selection dialog box

B

Eliminating positions from the analysis

The LightCycler® 96 Application Software provides functions in three levels for excluding positions from the analysis:

- Clearing wells:
  The *Clear Wells* function is provided on the *Plate View* tab of the *Sample Editor*. *Clear Wells* eliminates the selected wells from all analyses. Cleared wells are deactivated. This means they can no longer be edited and are not displayed in the table view or the analysis windows.
  For detailed information, see section *Clear Wells/Set to Default*, on page 136.

It is strongly recommended to use the 'Clear Wells' function for all empty wells of LightCycler® 480 Multiwell Plates and for positions not occupied by LightCycler® 8-Tube Strips.

- Removing samples, genes, and/or conditions (for relative quantification only):
  The *Remove* function is provided in the *<analysis> Settings* dialog box of the corresponding analyses. Removed samples, genes, or conditions are no longer displayed in tables and charts of the analysis. For detailed information, see the descriptions of the corresponding analyses.

- Excluding selected samples from the analysis:
  The *Exclude* function for specified samples is provided in the result tables of the corresponding analyses. Excluding samples can be useful when a sample is clearly an outlier, or if an error has occurred in pipetting or amplification. Excluded samples are displayed in tables and charts, but do not show any result values (for example, Cq values, T_m values, or ratios).
  For detailed information, see the descriptions of the corresponding analysis result tables.

7.1 Absolute quantification

Absolute quantification uses a Cq calling algorithm and an algorithm for positive/negative determination. Absolute quantification calculates the concentration based on gene-specific standard curves. By default the tab for viewing an absolute quantification analysis is called *Abs Quant*.

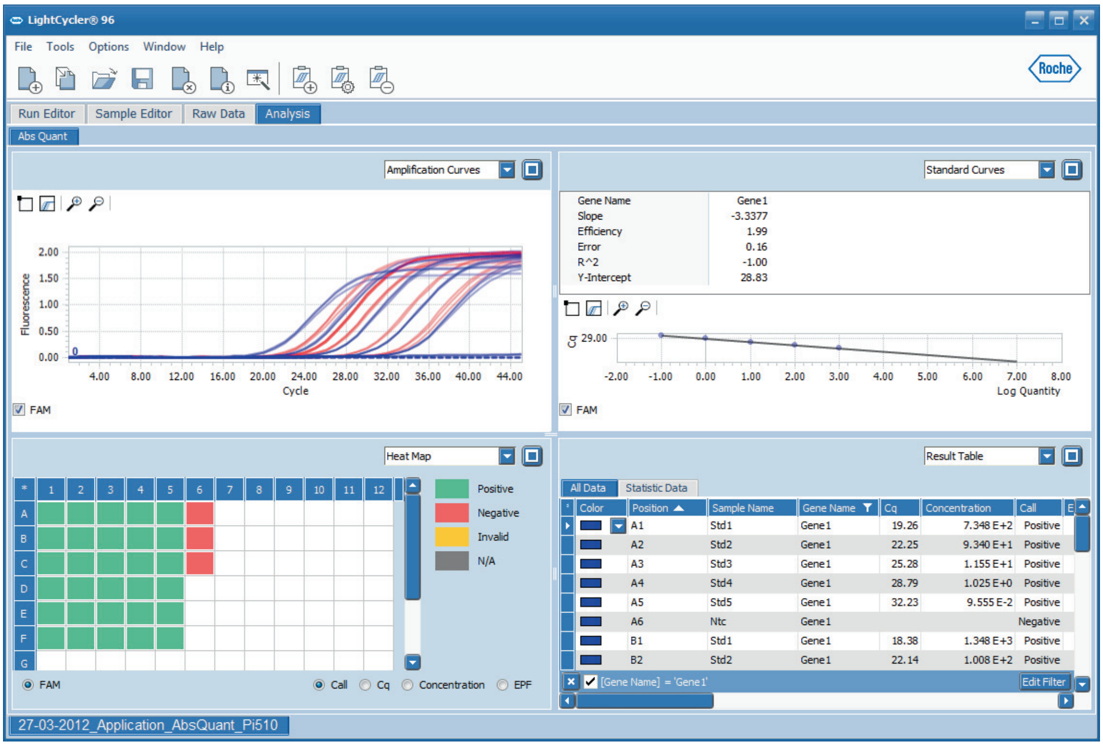


Figure 67: Abs Quant tab

The views selection list in the sections of the *Analysis* tab provides the following data:

- *Amplification Curves*; see section *Amplification Curves*, on page 152.
- *Heat Map*; see section *Heat Map*, on page 153.
- *Result Table*; see section *Result Table*, on page 154.
- *Standard Curves*; see section *Standard Curves*, on page 157.
- *Melting Peaks*; see section *Melting Peaks*, on page 178.
  This option is only available if a melting program has been performed.

7.1.1 Abs Quant Settings

The *Abs Quant Settings* dialog box allows operators to specify the analysis-specific settings. The dialog box is accessed via the *Analysis Settings* command on the analysis shortcut menu or by clicking the *Analysis Settings* icon in the tool bar.

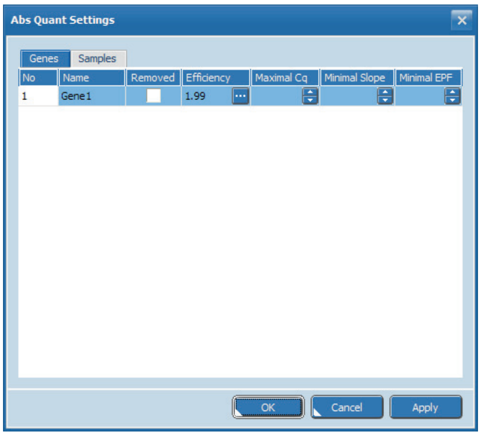


Figure 68: Abs Quant Settings dialog box

Each tab shows a table for editing the gene- or sample-specific settings.

Genes tab

Column	Setting
No	Numbering of the defined genes.
Name	Gene name; the genes are listed in the same order as specified in the *Sample Editor* tab.
Removed	Removes the gene from the analysis. Removed genes are no longer displayed in tables and charts of the corresponding analysis.
Efficiency	Efficiency used for calculating efficiency-corrected concentrations. Clicking the browse button in this column opens the *Efficiency Editor* dialog box for specifiying the efficiency to be used: ▶ *E=* Only available if no in-run standard curve for the relevant gene is present and if a single standard concentration is defined: The efficiency can be specified manually in the displayed field. Default value: 2.0 ▶ *E from standard curve* The efficiency has been derived from the standard curve.
Maximal Cq	Specifies the maximum Cq value, that is, the threshold for a positive call. ▶ For all samples with a Cq value greater than the specified maximum Cq, the Cq values are removed from the result table. ▶ The corresponding call status is set to *Negative*. ▶ The *Edited Call* column in the result table is checked.
Minimal Slope	Specifies the minimum slope of the amplification curve, that is, the threshold for a positive call. ▶ For all samples with a slope value less than the specified minimum, the corresponding call status is set to *Negative.* ▶ The resulting Cq value is removed from the result table. ▶ The *Edited Call* column in the result table is checked.

Column	Setting
Minimal EPF	Specifies the minimum EPF value, that is, the threshold for a positive call. The minimum value to apply as an EPF threshold corresponds to the predefined fluorescence thresholds, see section Cycle of Quantification (Cq), on page 65. ▶ For all samples with an EPF value less than the specified minimum EPF value, the corresponding call status is set to *Negative*. ▶ The resulting Cq value is removed from the result table. ▶ The *Edited Call* column in the result table is checked. *It is also possible to specify the minimum EPF threshold using the slider in the 'Amplification Curves' chart. For detailed information, see section To specify the EPF threshold using the slider, on page 152.* *The 'Minimal EPF' cannot be lower the the threshold value which is used by the Cq algorithm. For detailed information, see section Cycle of Quantification (Cq), on page 65.*

B

Samples tab

The *Samples* tab allows for removing samples from the analysis. Removed samples are no longer displayed in tables and charts of the corresponding analysis.

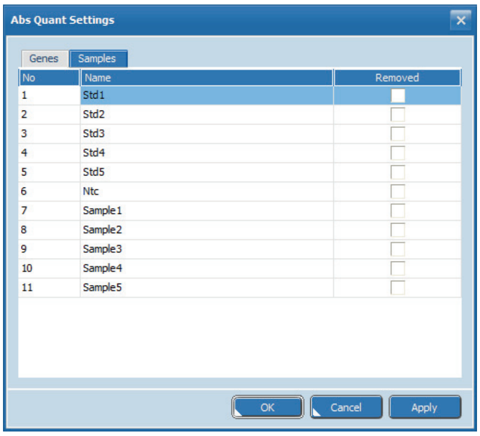


Figure 69: Abs Quant Settings dialog box, Samples tab

B

7.1.2 Amplification Curves

Amplification curves display the fluorescence intensity against the number of cycles in an amplification program. There is one curve for each sample that has a gene labeled with the selected dye. For detailed information on selecting and deselecting curves, zooming, and using the graphs shortcut menu, see section *Working with graphs*, on page 97.

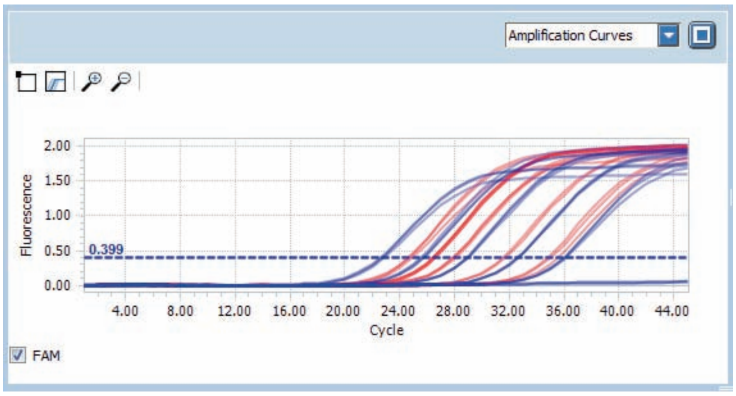


Figure 70: Amplification Curves graph

The selected curves are colored according to the sample type. Selected wells are highlighted. The graph can be filtered by selecting the dye for which the graph is displayed. Since one dye can be used for multiple genes, all genes associated with the dye will be displayed.

Minimum EPF threshold

The slider in the amplification curve specifies the minimum EPF threshold for a positive call.

By default the slider is set to zero and does not determine the positive/negative call. In the default state the positive/negative call is determined by the automated positive/negative filter algorithm. Changing the EPF threshold value overrules the automated positive/negative call. For detailed information on the filter algorithm, see section *Positive/negative filter*, on page 65.

To specify the EPF threshold using the slider

1 Perform one of the following steps:
- Click the slider in the amplification chart and move it to the appropriate position.
- Right-click the amplification chart and choose *Set slider here*.

Moving the slider displays the current fluorescence value.

The minimum value to apply as an EPF threshold corresponds to the predefined fluorescence thresholds, see section Cycle of Quantification (Cq), on page 65.
You can move the slider to any position in the amplification chart, but you cannot apply a threshold below the florescence threshold of the Cq algorithm.

2 Perform one of the following steps:
- Right-click the slider and choose *All* on the shortcut menu to apply the threshold to all genes.
- Right-click the slider, choose the corresponding dye on the shortcut menu, and then the gene the threshold is to be applied to.

The threshold is applied to the corresponding genes:
- For all samples with an EPF value greater than the specified minimum, the corresponding call status is set to *Positive*.
- For all samples with an EPF value less than the specified minimum, the corresponding call status is set to *Negative* and the resulting Cq value is removed from the result table.

7.1.3 Heat Map

A heat map shows an image of the multiwell plate used in the experiment for the specified channel. Hovering the cursor over a well will display a tooltip with the properties of the sample (in this example, the *Cq* heatmap is shown).

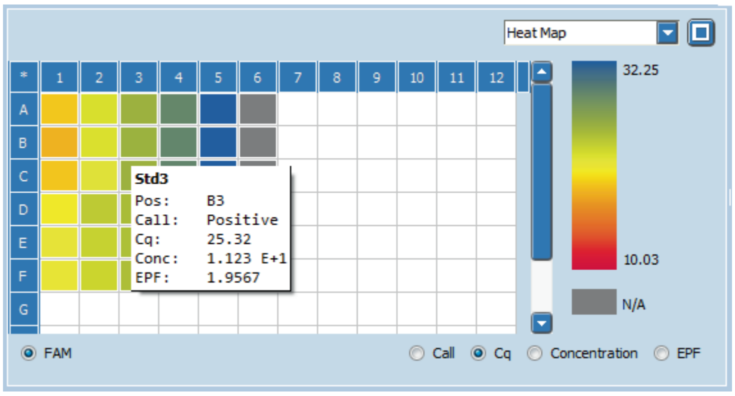


Figure 71: Absolute quantification, Heat Map

A heat map has the following options for displaying values:

Option	Description
<dye>	Dye assigned in the corresponding detection format. If you have specified a dual color experiment, the LightCycler® 96 Application Software provides a heat map for each filter combination. For detailed information on how to define a detection format, see section *Detection Format*, on page 126.
Call	Call status of all samples contained in the sample list. The following values are possible: ▶ Green: Positive ▶ Red: Negative ▶ Yellow: Invalid ▶ Gray: N/A (not available)
Cq	Quantification cycle values of the samples as a continuous spectrum from red (lowest Cq) to blue (highest Cq); each well is colored according to the Cq value called for a particular dye in that well.
Concentration	Concentration values of the samples as a continuous spectrum from red (highest concentration value) to blue (lowest concentration value); each well is colored according to the concentration value calculated for a particular dye in that well.
EPF	EPF values of the samples as a continuous spectrum from red (largest EPF value) to blue (smallest EPF value); each well is colored according to the EPF value called for a particular dye in that well.

A heat map only displays the samples contained in the sample list. Samples not contained in the sample list (that is, cleared wells, and removed samples and genes) are displayed in white and samples excluded from calculation are displayed in gray.

7.1.4 Result Table

The result table displays the results of the absolute quantification on two tabs:

- *All Data* tab, see below
- *Statistic Data* tab, see section *Statistic Data tab*, on page 156

For detailed information on editing cells and sorting and filtering the table, see section *Working with tables*, on page 90.

All Data tab

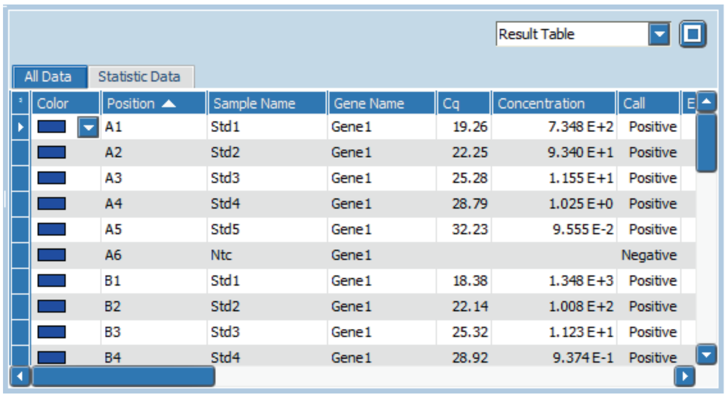


Figure 72: Absolute quantification, All Data tab

The columns of the table have the following meanings:

Column	Description
Color	Color coding of the sample. For detailed information on how to change the colors, see section *Editing cells*, on page 93.
Position	Position of the well in the multiwell plate. Each row is allocated a letter from A to H. The wells of a row are numbered from 1 to 12 from left to right.
Sample Name	Name of the sample present in the well.
Gene Name	Name of the gene of interest.
Cq	Calculated Cq value. *The Cq is only displayed for samples with a positive 'Call'.*
Concentration	Calculated concentration of the gene present before amplification.
Call	Calculated positive/negative status. ▶ *Positive:* The fluorescence curve shows a satisfactory signal/noise ratio and sigmoid curve shape. ▶ *Negative:* The fluorescence curve does not show a satisfactory signal/noise ratio or sigmoid curve shape. ▶ *Invalid: Artifacts (very rare cases).*
Excluded	Specifies whether the sample is excluded from the analysis. By default, all samples are included, so all samples are deselected in the exclusion column. Excluding samples can be useful when a sample is clearly an outlier, or if an error has occurred in pipetting or amplification.
Sample Type	Sample type, as defined in the *Sample Editor* tab; for a detailed description of the sample types, see section *Reaction Properties window area*, on page 129.
Standard	Concentration value for sample of the type *Standard.*
Cq Mean	Calculated mean Cq value for the samples in the corresponding replicate group.

Column	Description
Cq Error	Calculated error (standard deviation) for the samples in the corresponding replicate group.
Concentration Mean	Calculated mean concentration for the samples in the corresponding replicate group.
Concentration Error	Calculated mean deviation for the samples in the corresponding replicate group.
Replicate Group	Master position of the replicate group the sample belongs to.
Dye	Name of the associated dye.
Edited Call	Modification status of the sample. This check box is selected when an applied threshold has changed the call status of a sample.
Slope	Calculated slope value; the slope indicates the maximum fluorescence increase between two acquisitions.
EPF	Calculated endpoint fluorescense (EPF value).
Failure	Specifies whether a failure occurred during the experiment run. A failure is automatically raised if one or more of the following criteria are met: ▶ A positive control is negative. ▶ A negative control or a non reverse transcription control is positive. ▶ A standard is negative. ▶ A replicate group contains positive and negative calls. The operator can define additional failure constraints using the *Failure Conditions* dialog box in the sample editor. A failure is raised if one or more of these constraints are met. For detailed information, see section *Failure Constraints*, on page 134. *A failure only means a notification for the operator. The corresponding samples are not automatically excluded from the calculation.*
Notes	Description of the sample.
Sample Prep Notes	Notes as specified in imported MagNA Pure 96 sample data files.
Number	Index number of a well per channel. Index numbers are counted sequentially from left to right and from top to bottom.

B

B

Statistic Data tab

The *Statistic Data* table summarizes all data for samples in replicate groups. The table displays the master positions of replicate groups and all positions not contained in replicate groups:

Column	Description
Color	Color coding of the sample. For detailed information on how to change the colors, see section *Editing cells*, on page 93.
Replicate Group Member	Positions of the replicate group members the sample belongs to.
Replicate Group	Master position of the replicate group the sample belongs to.
Sample Name	Name of the sample present in the well.
Gene Name	Name of the gene of interest.
Cq Mean	Calculated mean Cq value for the samples in the corresponding replicate group.
Cq Error	Calculated error (standard deviation) for the samples in the corresponding replicate group.
Concentration Mean	Calculated mean concentration for the samples in the corresponding replicate group.
Concentration Error	Calculated mean deviation for the samples in the corresponding replicate group.
Sample Type	Sample type, as defined in the *Sample Editor* tab; for a detailed description of the sample types, see section *Reaction Properties window area*, on page 129.
Standard	Concentration value for sample of the type *Standard.*
Excluded	Specifies whether the sample is excluded from the analysis. By default, all samples are included, so all samples are deselected in the exclusion column. Excluding samples can be useful when a sample is clearly an outlier, or if an error has occurred in pipetting or amplification.
Dye	Name of the associated dye.
Number	Index number of a well per channel. Index numbers are counted sequentially from left to right and from top to bottom.
Position	Position of the well in the multiwell plate. Each row is allocated a letter from A to H. The wells of a row are numbered from 1 to 12 from left to right.
Failure	Provides an option showing whether a failure has occurred.

B

7.1.5 Standard Curves

A standard curve displays a graph of Cq values against the base 10 logarithm of the quantity of each standard. For absolute quantification, the absolute values of the standard curve are used to assign quantities to unknown samples.

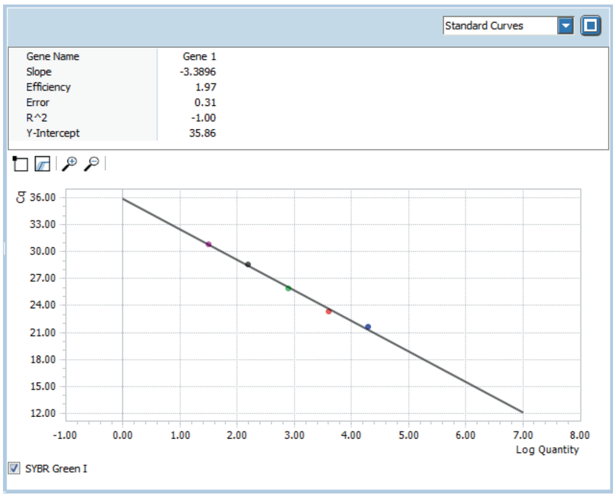


Figure 73: Standard curves graph

Standard curves additionally display the following gene-specific values:

- *Gene Name*
- *Slope*
- *Efficiency*
- *Error*
- *R^2 (correlation coefficient)*
- *Y-Intercept*

For detailed information on standard curves and the displayed values, see section *Standard curves*, on page 66.

7.1.6 Melting Peaks

A melting peaks chart plots the first negative derivative of the fluorescence decrease (-dF/dT) and displays the melting temperatures of the samples as peaks. A melting peaks graph is only available if a melting program has been performed. For detailed information, see section *Tm calling*, on page 74.

7.2 Relative quantification

Relative quantification compares the levels of two different gene sequences in a single sample (for example, target gene of interest and a reference gene), and expresses the final result as a ratio of these genes. By default the tab for viewing a relative quantification analysis is called *Rel Quant*.

Figure 74: Rel Quant tab

The views selection list in the sections of the *Analysis* tab provides the following data:

- *Amplification Curves*; see section *Amplification Curves*, on page 152.
- *Ratio Bars*; see section *Ratio Bars*, on page 162.
- *Result Table*; see section *Result Table*, on page 163.
- *Standard Curves*; see section *Standard Curves*, on page 157.
- *Melting Peaks*; see section *Melting Peaks*, on page 178.
  This option is only available if a melting program has been performed.
- *Heat Map*; see section *Heat Map*, on page 153.

7.2.1 Rel Quant Settings

The *Rel Quant Settings* dialog box allows operators to specify the analysis-specific settings, particularly the reference gene, run calibrator and study calibrator. The dialog box is accessed via the *Analysis Settings* command on the analysis shortcut menu or by clicking the *Analysis Settings* icon in the tool bar.

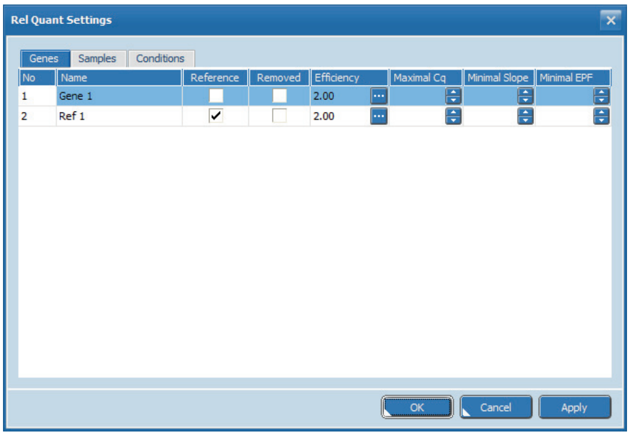


Figure 75: Rel Quant Settings dialog box

Each tab shows a table for editing the gene- or sample-specific settings.

Genes tab

Column	Setting
No	Numbering of the defined genes.
Name	Gene name; the genes are listed in the same order as specified in the *Sample Editor* tab.
Reference	Marks the selected gene as a reference. This gene will be used as a reference, with all other quantities calculated relative to it. For detailed information, see section *Normalized ratio*, on page 68.
Removed	Removes the gene from the analysis. Removed genes are no longer displayed in tables and charts of the corresponding analysis.
Efficiency	Efficiency used for calculating efficiency-corrected concentrations. Clicking the browse button in this column opens the *Efficiency Editor* dialog box for specifiying the efficiency to be used: ▶ *E=* Only available if no in-run standard curve for the relevant gene is present and if a single standard concentration is defined: The efficiency can be specified manually in the displayed field. Default value: 2.0 ▶ *E from standard curve* The efficiency has been derived from the standard curve.
Maximal Cq	Specifies the maximum Cq value, that is, the threshold for a positive call. ▶ The corresponding call status is set to *Negative*. ▶ All result Cq values greater than the specified maximum Cq value are removed from the result table. ▶ The *Edited Call* column in the result table is checked.
Minimal Slope	Specifies the minimum slope of the amplification curve, that is, the threshold for a positive call. ▶ For all samples with a slope value less than the specified minimum, the corresponding call status is set to *Negative.* ▶ The resulting Cq value is removed from the result table. ▶ The *Edited Call* column in the result table is checked.

Column	Setting
Minimal EPF	Specifies the minimum EPF value, that is, the threshold for a positive call. The minimum value to apply as an EPF threshold corresponds to the predefined fluorescence thresholds, see section *Cycle of Quantification (Cq)*, on page 65. ▶ For all samples with an EPF value greater than the specified minimum, the corresponding call status is set to *Positive*. ▶ For all samples with an EPF value less than the specified minimum, the corresponding call status is set to *Negative* and the resulting Cq value is removed from the result table. ▶ The *Edited Call* column in the result table is checked. *It is also possible to specify the minimum EPF threshold using the slider in the 'Amplification Curves' chart. For detailed information, see section To specify the EPF threshold using the slider, on page 152.*

Samples tab

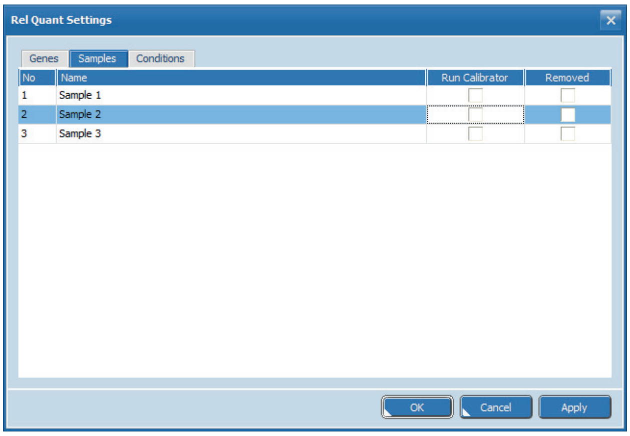


Figure 76: Rel Quant Settings dialog box, Samples tab

Column	Setting
No	Numbering of the defined samples.
Name	Sample name.
Run Calibrator	Marks the selected sample as the run calibrator. The run calibrator sample is used to normalize all samples within one run. For detailed information, see section *Normalized ratio*, on page 68. *Only samples with the sample types 'Unknown' or 'Positive Control' can be selected as a run calibrator.*
Removed	Removes the sample from the analysis. Removed samples are no longer displayed in tables and charts of the corresponding analysis.

Conditions tab

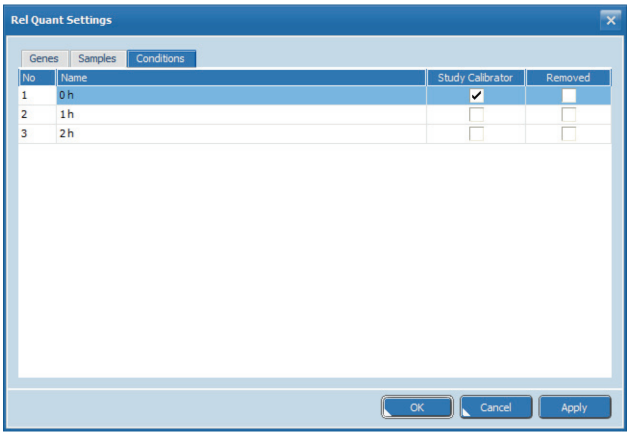


Figure 77: Rel Quant Settings dialog box, Conditions tab

Column	Setting
No	Numbering of the defined conditions.
Name	Condition name; the conditions are listed in the same order as specified in the *Sample Editor* tab. For detailed information, see section *Reaction Properties window area*, on page 129.
Study Calibrator	Marks the selected condition as the study calibrator. The study calibrator sample is used to normalize all sample-specific measurements to a common basis. For detailed information, see section *Scaled ratio*, on page 69.
Removed	Removes the condition from the analysis. Removed conditions are no longer displayed in tables and charts of the analysis.

7.2.2 Amplification Curves

The display of the amplification curves in relative quantification analysis corresponds to their display in absolute quantification analysis. For detailed information, see section *Amplification Curves*, on page 152.

B

7.2.3 Ratio Bars

The *Ratio Bars* chart shows the same ratio data as the result table, but in a bar chart format. Each bar represents a ratio, a normalized ratio, or a scaled ratio.

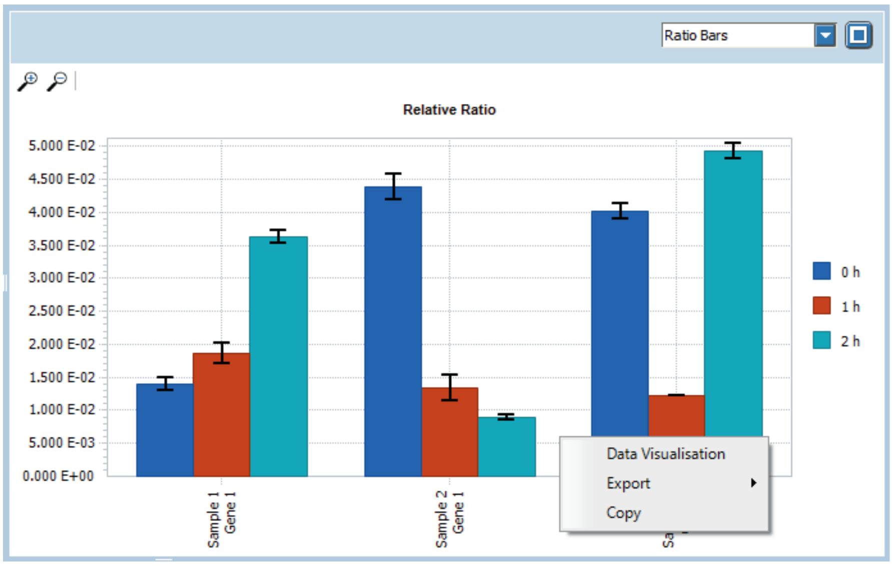


Figure 78: Ratio Bars chart

The *Ratio Bars* chart shows the corresponding ratio, normalized ratio, or scaled ratio for each gene, sample, and condition.

Data Visualization

The ratios displayed in the *Ratio Bars* chart, as well as how they are scaled and ordered, depend on the settings the operator specifies in the *Data Visualization* dialog box. The dialog box is accessed via the *Data Visualization* command on the chart's shortcut menu.

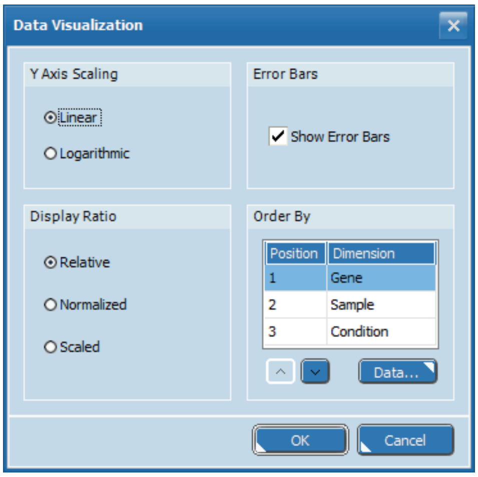


Figure 79: Data Visualization dialog box

Setting	Description
Y Axis Scaling	Specifies whether the y-axis is to be scaled linear or logarithmic. By default, the y-axis is scaled linear.
Error Bars	Specifies whether error bars are to be displayed in the *Ratio Bars* chart. Error bars show ratio errors, normalized ratio errors, or scaled ratio errors as displayed in the result table. For detailed information, see section *Relative quantification analysis*, on page 68. By default, no error bars are displayed.
Display Ratio	Specifies which ratio is to be displayed in the *Ratio Bars* chart: ▶ *Relative* (default value) When this option is selected, the chart shows no bars for the reference genes. ▶ *Normalized* When this option is selected, the chart shows no bars for the run calibrators. ▶ *Scaled* When this option is selected, the chart shows no bars for the study calibrators. For a detailed description of the different ratios, see section *Relative quantification analysis*, on page 68.
Order By	Specifies the order for the dimensions to be displayed. Each bar chart allows ordering by gene, sample, or condition. Within each of these property groups, ordering is additionally allowed by name.

The buttons below the *Order By* area perform the following functions:

Button	Description
Up/Down	Moves the selected property group up or down in the list.
Data	Opens the *Order By Data* dialog box, which allows for ordering the selected property group by name.

7.2.4 Result Table

The result table displays the results of the relative quantification on two tabs:

- *All Data* tab, see below
- *Statistic Data* tab, see section *Statistic Data tab*, on page 166

For detailed information on editing cells and sorting and filtering the table, see section *Working with tables*, on page 90.

B

All Data tab

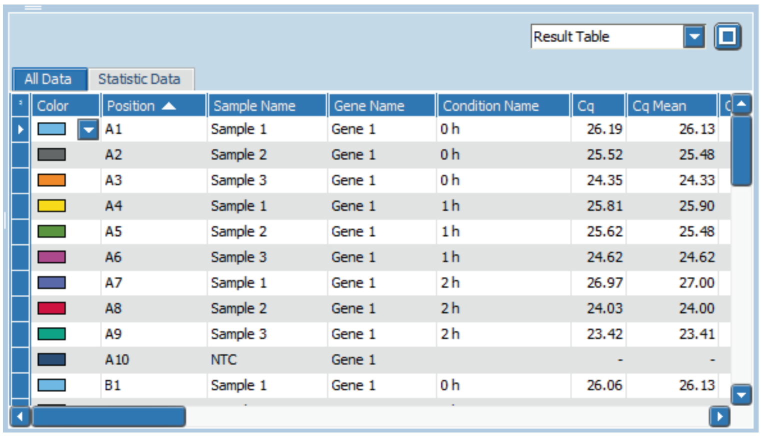


Figure 80: Relative quantification, All Data tab

The columns of the table have the following meanings:

Column	Description
Color	Color coding of the sample. For detailed information on how to change the colors, see section *Editing cells*, on page 93.
Position	Position of the well in the multiwell plate. Each row is allocated a letter from A to H. The wells of a row are numbered from 1 to 12 from left to right.
Sample Name	Name of the sample present in the well.
Gene Name	Name of the gene of interest.
Condition Name	Condition name used for grouping the samples according to different conditions during the experiment run.
Cq	Calculated Cq value. *The Cq is only displayed for samples with a positive call.*
Cq Mean	Calculated mean Cq value for the samples in the corresponding replicate group.
Cq Error	Calculated error (standard deviation) for the samples in the corresponding replicate group. For detailed information, see section *Error calculation*, on page 72.
Excluded	Specifies whether the sample is excluded from the analysis. By default, all samples are included, so all samples are deselected in the exclusion column. Excluding samples can be useful when a sample is clearly an outlier, or if an error has occurred in pipetting or amplification.
Sample Type	Sample type, as defined on the *Sample Editor* tab; for a detailed description of the sample types, see section *Reaction Properties window area*, on page 129.
Sample Type RQ	Specifies whether the sample is used as an unknown sample or as the run calibrator. For detailed information, see section *Relative quantification analysis*, on page 68.
Gene Type	Specifies whether the gene is used as a target or reference gene in the calculation of the ratios. For detailed information, see section *Relative quantification analysis*, on page 68.
Condition Type	Specifies whether the condition is used as the study calibrator for calculating scaled ratios. For detailed information, see section *Scaled ratio*, on page 69.
Replicate Group	Master position of the replicate group the sample belongs to.

Column	Description
Ratio	Concentration ratio of target and reference. When multiple references are defined, the ratio is calculated based on the arithmetic mean of all reference Cq values. For detailed information, see section *Ratio*, on page 68. For reference genes, no ratio values are displayed.
Ratio Error	Ratio error of all target/reference combinations. For detailed information, see section *Error calculation*, on page 72.
Normalized Ratio	Concentration ratio of the target and reference, normalized with the corresponding run calibrator. For detailed information, see section *Normalized ratio*, on page 68. For run calibrator samples, no normalized ratio values are displayed.
Normalized Ratio Error	Normalized ratio error of all target/reference/run calibrator combinations. For detailed information, see section *Error calculation*, on page 72.
Scaled Ratio	Concentration ratio of the target and reference, normalized with the corresponding study calibrator. For detailed information, see section *Scaled ratio*, on page 69. For study calibrator conditions, no scaled ratio values are displayed.
Scaled Ratio Error	Scaled ratio error of all target/reference/study calibrator combinations. For detailed information, see section *Error calculation*, on page 72.
Dye	Name of the associated dye.
Edited Call	Modification status of the sample This check box is selected if an applied threshold has changed the call status of a sample.
Failure	Specifies whether a failure occurred during the experiment run. A failure is automatically raised if one or more of the following criteria are met: ▶ A positive control is negative. ▶ A negative control or a non reverse transcription control is positive. ▶ A standard is negative. ▶ A replicate group contains positive and negative calls. The operator can define additional failure constraints using the *Failure Conditions* dialog box in the sample editor. A failure is raised if one or more of these constraints are met. For detailed information, see section *Failure Constraints*, on page 134. *A failure only means a notification for the operator. The corresponding samples are not automatically excluded from the calculation.*
Slope	Calculated slope value; the slope indicates the maximum fluorescence increase between two acquisitions.
EPF	Calculated endpoint fluorescense (EPF value).
Notes	Description of the sample.
Sample Prep Notes	Notes as specified in imported MagNA Pure 96 sample data files.
Number	Index number of a well. Index numbers are counted sequentially from left to right and from top to bottom.

B

Statistic Data tab

The *Statistic Data* table summarizes all data for samples in replicate groups. The table displays the master positions of replicate groups and all positions not contained in replicate groups:

Column	Description
Color	Color coding of the sample. For detailed information on how to change the colors, see section *Editing cells*, on page 93.
Replicate Group Member	Positions of the replicate group members the sample belongs to.
Replicate Group	Master position of the replicate group the sample belongs to.
Sample Name	Name of the sample present in the well.
Gene Name	Name of the gene of interest.
Condition Name	Condition name used for grouping the samples according to different conditions during the experiment run.
Ratio	Concentration ratio of target and reference. When multiple references are defined, the ratio is calculated based on the arithmetic mean of all reference Cq values. For detailed information, see section *Ratio*, on page 68. For reference genes, no ratio values are displayed.
Ratio Error	Ratio error of all target/reference combinations. For detailed information, see section *Error calculation*, on page 72.
Normalized Ratio	Concentration ratio of the target and reference, normalized with the corresponding run calibrator. For detailed information, see section *Normalized ratio*, on page 68. For run calibrator samples, no normalized ratio values are displayed.
Normalized Ratio Error	Normalized ratio error of all target/reference/run calibrator combinations. For detailed information, see section *Error calculation*, on page 72.
Scaled Ratio	Concentration ratio of the target and reference, normalized with the corresponding study calibrator. For detailed information, see section *Scaled ratio*, on page 69. For study calibrator conditions, no scaled ratio values are displayed.
Scaled Ratio Error	Scaled ratio error of all target/reference/study calibrator combinations. For detailed information, see section *Error calculation*, on page 72.
Cq Mean	Calculated mean Cq value for the samples in the corresponding replicate group.
Cq Error	Calculated error (standard deviation) for the samples in the corresponding replicate group. For detailed information, see section *Error calculation*, on page 72.
Sample Type	Sample type, as defined on the *Sample Editor* tab; for a detailed description of the sample types, see section *Reaction Properties window area*, on page 129.
Sample Type RQ	Specifies whether the sample is used as an unknown sample or as the run calibrator. For detailed information, see section *Relative quantification analysis*, on page 68.
Gene Type	Specifies whether the gene is used as a target or reference gene in the calculation of the ratios. For detailed information, see section *Relative quantification analysis*, on page 68.
Condition Type	Specifies whether the condition is used as the study calibrator for calculating scaled ratios. For detailed information, see section *Scaled ratio*, on page 69.
Excluded	Specifies whether the sample is excluded from the analysis. By default, all samples are included, so all samples are deselected in the exclusion column. Excluding samples can be useful when a sample is clearly an outlier, or if an error has occurred in pipetting or amplification.
Dye	Name of the associated dye.
Failure	Provides an option showing whether a failure has occurred.

Column	Description
Position	Position of the well in the multiwell plate. Each row is allocated a letter from A to H. The wells of a row are numbered from 1 to 12 from left to right.
Number	Index number of a well. Index numbers are counted sequentially from left to right and from top to bottom.

7.2.5 Standard Curves

The display of the standard curves in relative quantification analysis corresponds to their display in absolute quantification analysis. For detailed information, see section *Standard Curves*, on page 157.

7.2.6 Melting Peaks

A melting peaks chart plots the first negative derivative of the fluorescence decrease (-dF/dT) and displays the melting temperatures of the samples as peaks. A melting peaks graph is only available if a melting program has been performed. For detailed information, see section *Tm calling*, on page 74.

7.2.7 Heat Map

The display of the heat maps in relative quantification analysis corresponds to their display in absolute quantification analysis (Exception: the *Concentration* option is not available for relative quantification). For detailed information, see section *Heat Map*, on page 153.

7.3 Endpoint genotyping

Endpoint genotyping is performed to measure the plateau intensities of two dyes associated with the genotype of a given sample. The plateau in dye intensity is measured as endpoint fluorescence (EPF). To call a genotype for a given sample, the EPF values of a pair of genes are compared. By default the tab for viewing an endpoint genotyping analysis is called *Endpoint Genotyping*.

For endpoint genotyping analysis, it is essential to define identical gene names for both dyes. In the case of different gene names, no endpoint genotyping analysis is possible.

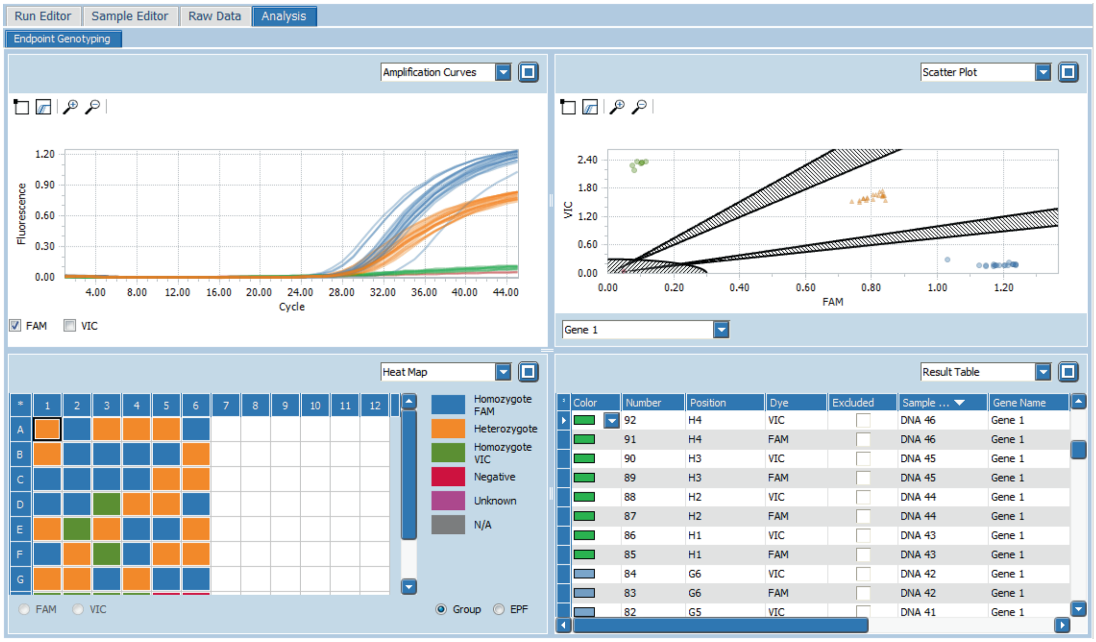


Figure 81: Endpoint Genotyping tab

The views selection list in the sections of the *Analysis* tab provides the following data:

- *Amplification Curves;* see section *Amplification Curves*, on page 152.
- *Scatter Plot*; see section *Scatter Plot*, on page 171.
- *Heat Map*; see section *Heat Map*, on page 173.
- *Result Table*; see section *Result Table*, on page 174.

7.3.1 Endpoint Genotyping Settings

The *Endpoint Genotyping Settings* dialog box allows operators to specify the analysis-specific settings. The dialog box is accessed via the *Analysis Settings* command on the analysis shortcut menu or by clicking the *Analysis Settings* icon in the tool bar.

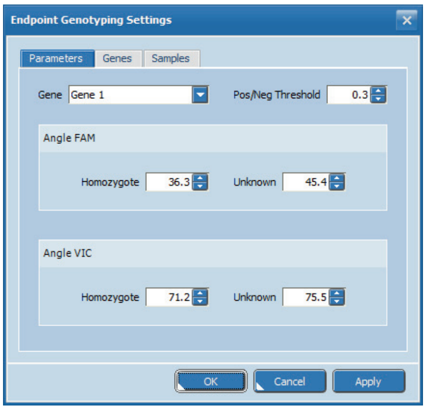


Figure 82: Endpoint Genotyping Settings dialog box

Parameters tab

The samples are classified into different genotypes by their position relative to thresholds. The settings on the *Parameters* tab allow for specifying these thresholds manually. It is also possible to specify the thresholds using the sliders in the scatter plot. For detailed information, see section *Genotypes*, on page 171.

Parameter		Description
Gene		Name of the gene the thresholds are to be applied to.
Pos./Neg. Threshold		Specifies the threshold for negative calls. In the scatter plot, this threshold is displayed as a radius. Any points within this radius of the origin of the graph will be classified as *Negative*. Default: 0.3
Angle <dye1>	*Homozygote*	Specifies the area for samples that are homozygous for *<dye1>*.
	Unknown	Specifies the area for unknown samples in relation to *Homozygote* for *<dye1>*.
Angle <dye2>	*Homozygote*	Specifies the area for samples that are homozygous for *<dye2>*.
	Unknown	Specifies the area for unknown samples in relation to *Homozygote* for *<dye2>*.

B

Genes tab

The *Genes* tab allows for removing genes from the analysis. Removed genes are no longer displayed in tables and charts of the corresponding analysis.

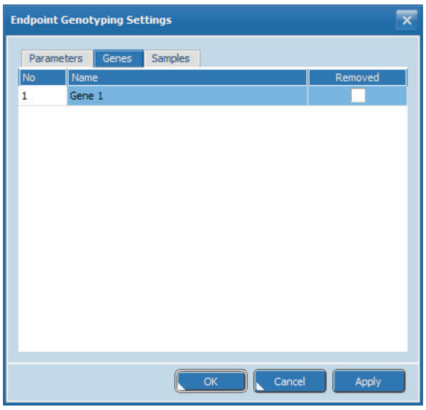


Figure 83: Endpoint Genotyping Settings dialog box, Genes tab

Samples tab

The *Samples* tab allows for removing samples from the analysis. Removed samples are no longer displayed in tables and charts of the corresponding analysis.

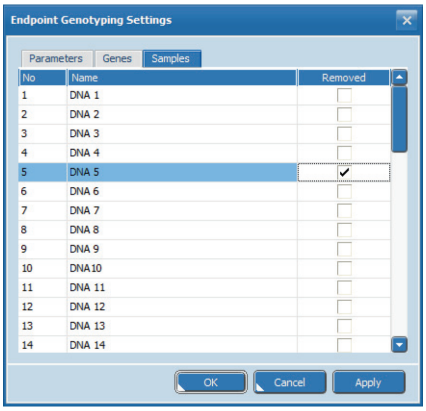


Figure 84: Endpoint Genotyping Settings dialog box, Samples tab

7.3.2 Amplification Curves

The display of the amplification curves in endpoint genotyping analysis corresponds to their display in absolute quantification analysis. For detailed information, see section *Amplification Curves*, on page 152.

7.3.3 Scatter Plot

The *Scatter Plot* chart displays the endpoint fluorescence of the two selected dyes (representing the two alleles). Each point represents a sample, whose x-coordinate is the endpoint fluorescence level of <*dye1*>, and whose y-coordinate is the endpoint fluorescence level of <*dye2*>. The software applies the dye with the lower wavelength to the x-axis, the higher wavelength to the y-axis.

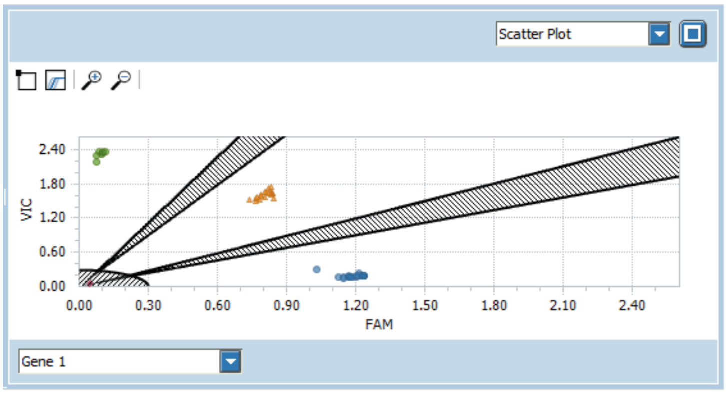


Figure 85: Scatter Plot

The points in the scatter plot are clustered according to the intensity distribution of the two dyes:

- The top left of the plot is for samples that emit a dominant fluorescence signal with the filter combination selected for <*dye2*>.
- The middle is for samples that emit a dominant fluorescence signal with both filter combinations.
- The bottom right is for samples that emit a dominant fluorescence signal with the filter combination selected for <*dye1*>.
- The bottom left, near the origin, consists of samples that emit a weak or no fluorescence signal.

For detailed information on selecting and deselecting, zooming, and using the graphs shortcut menu, see section *Working with graphs*, on page 97.

Gene selection

The scatter plot is displayed gene-specifically, that is, according to the selected gene. This selection specifies the gene that the threshold and angle settings are to be applied to. For detailed information on specifying the thresholds and angle sliders, see below.

Genotypes

The scatter plot provides sliders to define the areas where no clear identification of the genotypes is expected and thus to manually group the samples:

- A radius slider to determine the threshold for negative calls. Any points within this radius of the origin of the graph will be classified as *Negative*.
- Two angle sliders to determine the areas for samples that are homozygous for <*dye1*> or for <*dye2*>.
- Two angle sliders to determine the areas for unknown samples.

B

The slider settings on the scatter plot correspond to the threshold and angle settings in the *Endpoint Genotyping Settings* dialog box.

The points are displayed differently for each genotype:

- *Homozygote: <dye1>* has a blue dot.
- *Homozygote: <dye2>* has a green dot.
- *Heterozygote* has an orange triangle.
- *Negative* samples have a red rectangle.
- *Unknown* samples have a magenta triangle.

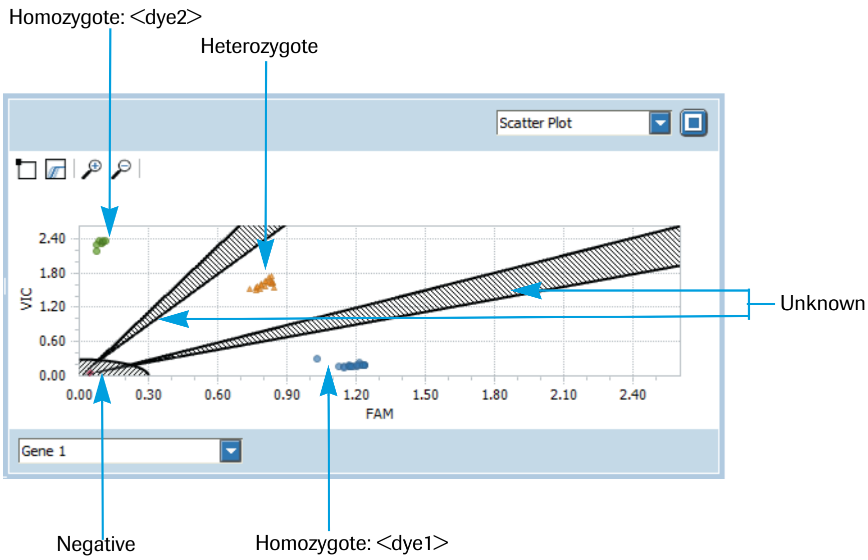


Figure 86: Scatter Plot, genotypes

For detailed information on specifying genotype groups in the graph, refer to the *LightCycler® 96 System User Training Guide*.

Exclude samples

To exclude single samples from the analysis, the scatter plot provides the *Exclude Sample* command on the shortcut menu for each sample.

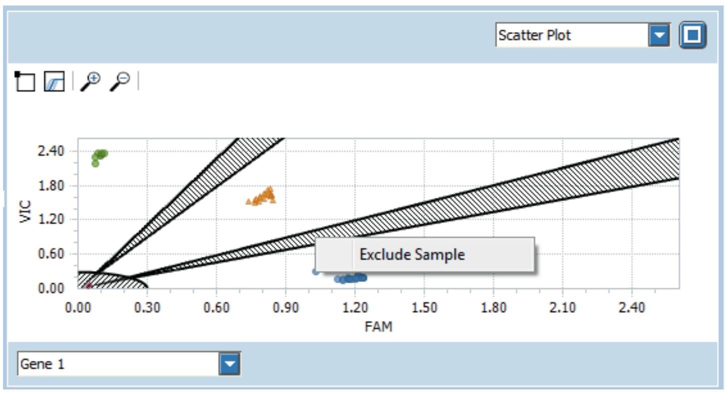


Figure 87: Scatter plot shortcut menu

7.3.4 Heat Map

The heat map shows an image of the multiwell plate used in the experiment for the specified gene. Hovering the cursor over a well will display a tooltip with the properties of the sample.

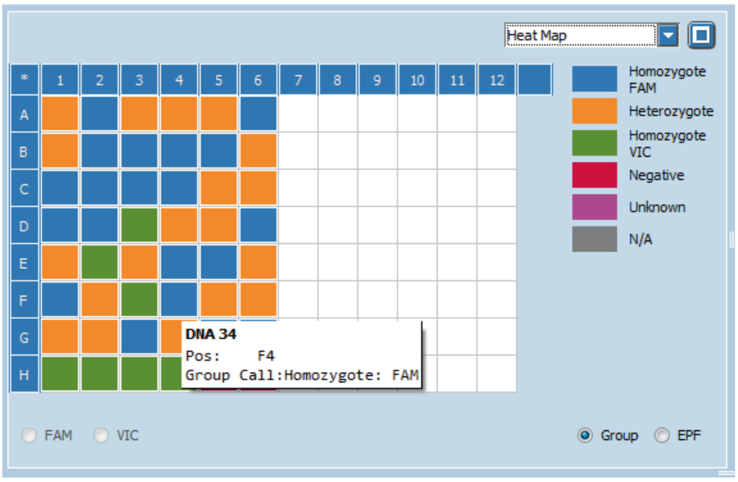


Figure 88: Endpoint genotyping, Heat Map

The heat map has the following options for displaying values:

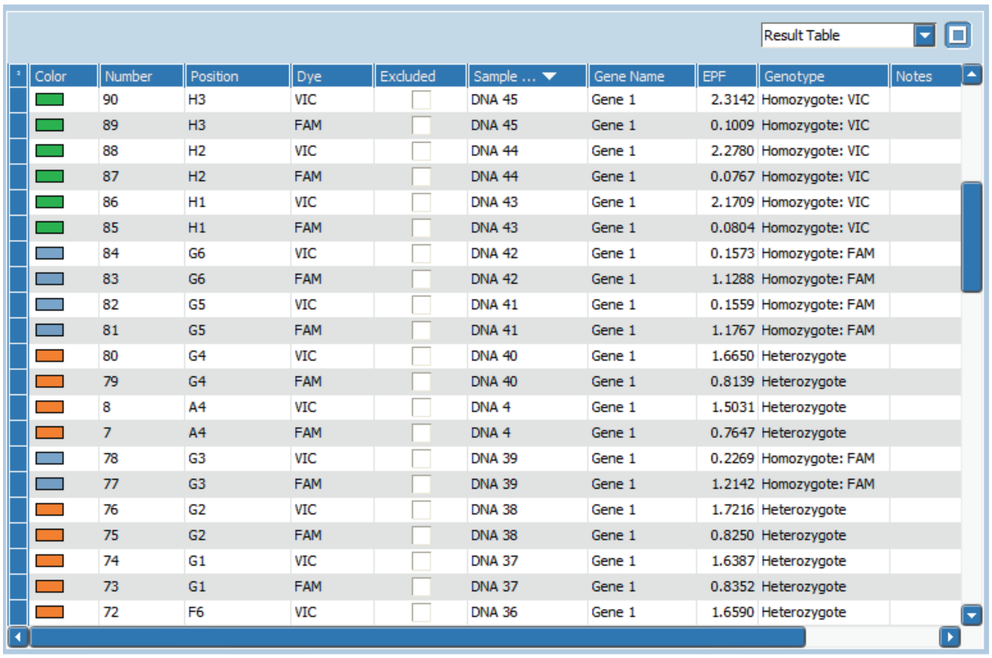
Option	Description
Group	Genotype the sample is assigned to, according to the threshold and angle settings. ▶ Blue: *Homozygote <dye1>* ▶ Orange: *Heterozygote* ▶ Green: *Homozygote <dye2>* ▶ Red: *Negative* ▶ Magenta: *Unknown* ▶ Gray: N/A (not available)
EPF	Endpoint fluorescence values of the samples as a continuous spectrum from red (largest EPF value) to blue (smallest EPF value); each well is colored according to the EPF value called for a particular dye in that well.
<dye>	▶ For the *Group* heat map: A combined result of both dyes is displayed. The dye selection cannot be changed. ▶ For the *EPF* heat map: Dyes assigned in the corresponding detection format. The LightCycler® 96 Application Software provides a heat map for each filter combination. For detailed information on how to define a detection format, see section *Detection Format*, on page 126.

A heat map only displays the samples contained in the sample list. Samples not contained in the sample list (that is, deactivated wells) are displayed in white and samples excluded from calculation are displayed in gray.

B

7.3.5 Result Table

The result table displays the results of the endpoint genotyping analysis. For detailed information on editing cells and sorting and filtering the table, see section *Working with tables*, on page 90.



Result Table

Color	Number	Position	Dye	Excluded	Sample ...	Gene Name	EPF	Genotype	Notes
	90	H3	VIC		DNA 45	Gene 1	2.3142	Homozygote: VIC	
	89	H3	FAM		DNA 45	Gene 1	0.1009	Homozygote: VIC	
	88	H2	VIC		DNA 44	Gene 1	2.2780	Homozygote: VIC	
	87	H2	FAM		DNA 44	Gene 1	0.0767	Homozygote: VIC	
	86	H1	VIC		DNA 43	Gene 1	2.1709	Homozygote: VIC	
	85	H1	FAM		DNA 43	Gene 1	0.0804	Homozygote: VIC	
	84	G6	VIC		DNA 42	Gene 1	0.1573	Homozygote: FAM	
	83	G6	FAM		DNA 42	Gene 1	1.1288	Homozygote: FAM	
	82	G5	VIC		DNA 41	Gene 1	0.1559	Homozygote: FAM	
	81	G5	FAM		DNA 41	Gene 1	1.1767	Homozygote: FAM	
	80	G4	VIC		DNA 40	Gene 1	1.6650	Heterozygote	
	79	G4	FAM		DNA 40	Gene 1	0.8139	Heterozygote	
	8	A4	VIC		DNA 4	Gene 1	1.5031	Heterozygote	
	7	A4	FAM		DNA 4	Gene 1	0.7647	Heterozygote	
	78	G3	VIC		DNA 39	Gene 1	0.2269	Homozygote: FAM	
	77	G3	FAM		DNA 39	Gene 1	1.2142	Homozygote: FAM	
	76	G2	VIC		DNA 38	Gene 1	1.7216	Heterozygote	
	75	G2	FAM		DNA 38	Gene 1	0.8250	Heterozygote	
	74	G1	VIC		DNA 37	Gene 1	1.6387	Heterozygote	
	73	G1	FAM		DNA 37	Gene 1	0.8352	Heterozygote	
	72	F6	VIC		DNA 36	Gene 1	1.6590	Heterozygote	

Figure 89: Endpoint genotyping, Result Table

The columns of the table have the following meanings:

Column	Description
Color	Color coding of the sample. For detailed information on how to change the colors, see section *Editing cells*, on page 93.
Number	Index number of a well per dye. Index numbers are counted sequentially from left to right and from top to bottom.
Position	Position of the well in the multiwell plate. Each row is allocated a letter from A to H. The wells of a row are numbered from 1 to 12 from left to right.
Dye	Name of the associated dye.
Excluded	Specifies whether the sample is excluded from the analysis. By default, all samples are included, so all samples are deselected in the exclusion column. Excluding samples can be useful when a sample is clearly an outlier, or if an error has occurred in pipetting or amplification.
Sample Name	Name of the sample present in the well.
Gene Name	Name of the gene of interest.
EPF	Endpoint fluorescense value of the corresponding dye.
Genotype	Genotype the sample is assigned to, according to the threshold and angle settings.
Notes	Description of the sample.
Sample Prep Notes	Notes as specified in imported MagNA Pure 96 sample data files. Operators can edit the displayed text.

B

7.4 T_m calling

T_m calling is performed on the negative derivative of the melting curve of an amplicon. When working with intercalating fluorescent dyes, it is often useful to have both a quantification analysis of amplification during the cycling program, and a melt analysis. By default the tab for viewing a melting curve analysis is called *Tm Calling*.

A T_m calling analysis can only be created if a melting program has been performed.

If multiple melting programs with acquisitions are defined, the 'Melting Selection' dialog box opens for selecting the melting program for result calculation. For detailed information, see section Adding a new analysis, on page 147.

Figure 90: Tm Calling tab

The views selection list in the sections of the *Analysis* tab provides the following data:

- *Melting Curves*; see section Melting Curves, on page 178.
- *Melting Peaks*; see section Melting Peaks, on page 178.
- *Heat Map*; see section Heat Map, on page 179.
- *Result Table*; see section Result Table, on page 180.
- *Amplification Curves*; see section Amplification Curves, on page 152.
  This option is only available if an amplification program has been performed.

7.4.1 Melting Analysis Parameters

The *Melting Analysis Parameters* dialog box allows operators to specify the analysis-specific settings. The dialog box is accessed via the *Analysis Settings* command on the analysis shortcut menu or by clicking the *Analysis Settings* icon in the tool bar.

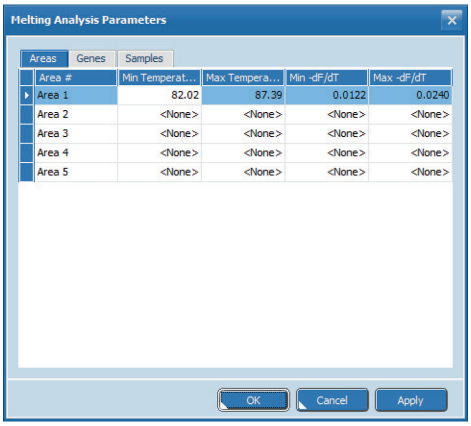


Figure 91: Melting Analysis Parameters dialog box

Areas tab

The settings on the *Areas* tab allow for manually specifying areas where melting peaks are to be called. An area is displayed as a rectangle which represents a temperature range and a fluorescence threshold. It is also possible to specify the areas using area marking in the melting peaks graph. For detailed information, see section *Melting peak areas*, on page 179.

The tab shows a table for editing the area settings:

Column	Setting
Area #	Number of the area. The LightCycler® 96 Application Software allows five areas to be defined in one graph.
Min Temperature	Lowest value of the temperature range, that is, the left border of the area.
Max Temperature	Highest value of the temperature range, that is, the right border of the area.
Min -dF/dT	Lowest value of the fluorescence range, that is, the bottom border of the area.
Max -dF/dT	Highest value of the fluorescence range, that is, the top border of the area.

Genes tab

The *Genes* tab allows for removing genes from the analysis. Removed genes are no longer displayed in tables and charts of the corresponding analysis.

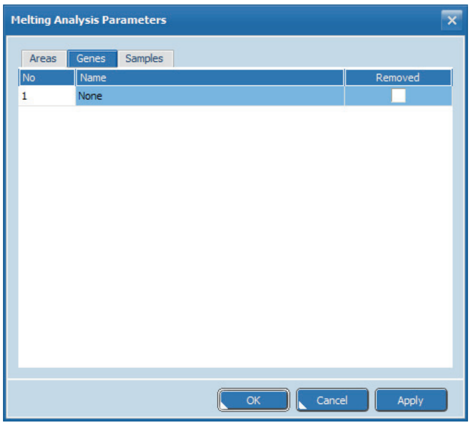


Figure 92: Melting Analysis Parameters dialog box, Genes tab

Samples tab

The *Samples* tab allows for removing samples from the analysis. Removed samples are no longer displayed in tables and charts of the corresponding analysis.

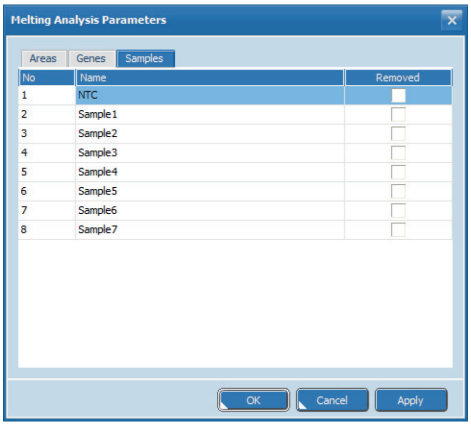


Figure 93: Melting Analysis Parameters dialog box, Samples tab

7.4.2 Melting Curves

Melting curves show the raw fluorescence intensity against the temperature in °C. For detailed information on selecting and deselecting curves, zooming, and using the graphs shortcut menu, see section *Working with graphs*, on page 97.

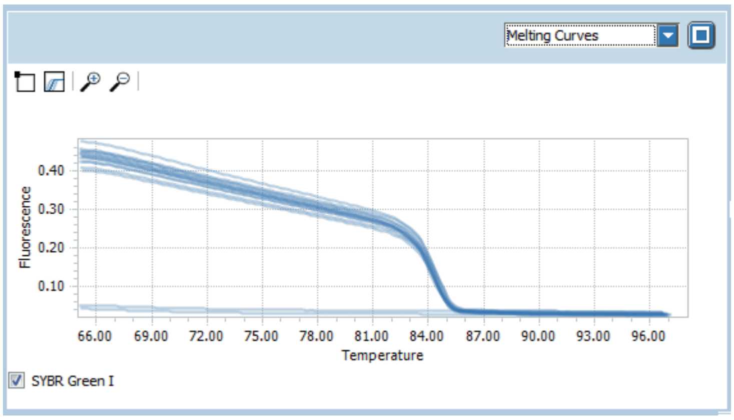


Figure 94: Melting Curves graph

7.4.3 Melting Peaks

The melting peaks graph displays the first negative derivative of the fluorescence with respect to the temperature in the melting program (-dF/dT). For detailed information on selecting and deselecting curves, zooming, and using the graphs shortcut menu, see section *Working with graphs*, on page 97.

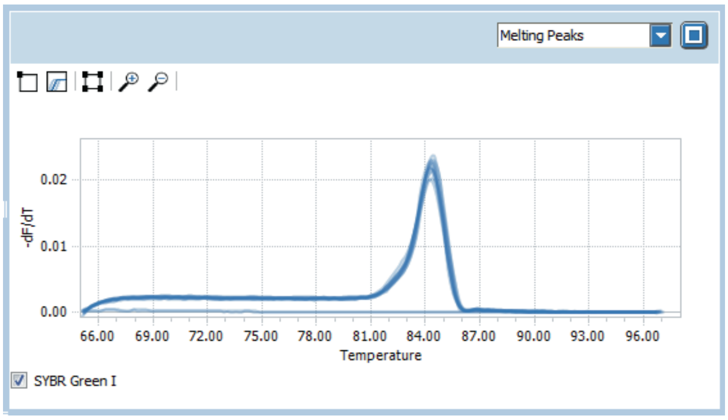


Figure 95: Melting Peaks graph

B

Melting peak areas

The melting peaks graph provides a function to mark areas where melting peaks are to be called. An area is displayed as a rectangle which represents a temperature range and a fluorescence threshold. The area settings on the melting peaks graph correspond to the settings in the *Melting Analysis Parameters* dialog box.

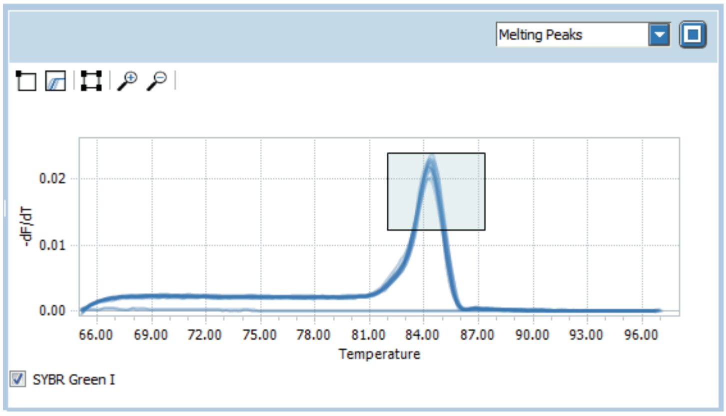


Figure 96: Melting Peaks graph, peak areas

For detailed information on specifying melting peak areas in the graph, refer to the *LightCycler® 96 System User Training Guide.*

7.4.4 Heat Map

A heat map shows an image of the multiwell plate used in the experiment for the specified channel. Hovering the cursor over a well will display a tooltip with the properties of the sample.

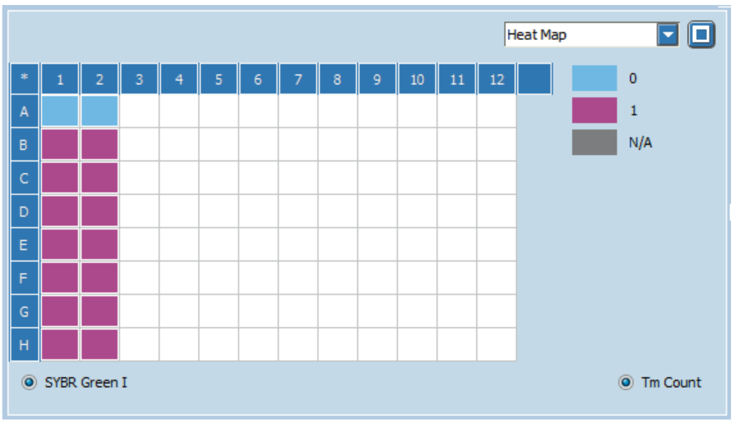


Figure 97: T_m calling, Heat Map

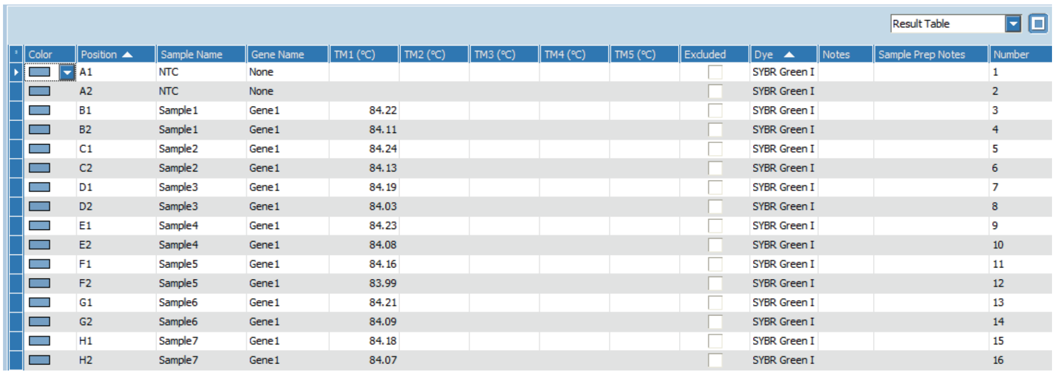
The heat map has the following options for displaying values:

Option	Description
<dye>	Dye assigned in the corresponding detection format. If you have specified a dual color experiment, the LightCycler® 96 Application Software provides a heat map for each filter combination. For detailed information on how to define a detection format, see section *Detection Format*, on page 126.
Tm Count	Number of T_ms called for a sample. The following values are possible: ▶ Light blue: Zero T_ms ▶ Magenta: One T_m ▶ Mauve: Two T_ms ▶ Dark red: Three T_ms ▶ Dark gray: Four T_ms ▶ Light red: Five T_ms ▶ Light gray: N/A (not available)

A heat map only displays the samples contained in the sample list. Samples not contained in the sample list (that is, cleared wells and removed samples and genes) are displayed in white and samples excluded from calculation are displayed in gray.

7.4.5 Result Table

The result table displays the results of the T_m calling analysis. For detailed information on editing cells and sorting and filtering the table, see section *Working with tables*, on page 90.


Result Table

Color	Position	Sample Name	Gene Name	TM1 (°C)	TM2 (°C)	TM3 (°C)	TM4 (°C)	TM5 (°C)	Excluded	Dye	Notes	Sample Prep Notes	Number
	A1	NTC	None							SYBR Green I			1
	A2	NTC	None							SYBR Green I			2
	B1	Sample1	Gene1	84.22						SYBR Green I			3
	B2	Sample1	Gene1	84.11						SYBR Green I			4
	C1	Sample2	Gene1	84.24						SYBR Green I			5
	C2	Sample2	Gene1	84.13						SYBR Green I			6
	D1	Sample3	Gene1	84.19						SYBR Green I			7
	D2	Sample3	Gene1	84.03						SYBR Green I			8
	E1	Sample4	Gene1	84.23						SYBR Green I			9
	E2	Sample4	Gene1	84.08						SYBR Green I			10
	F1	Sample5	Gene1	84.16						SYBR Green I			11
	F2	Sample5	Gene1	83.99						SYBR Green I			12
	G1	Sample6	Gene1	84.21						SYBR Green I			13
	G2	Sample6	Gene1	84.09						SYBR Green I			14
	H1	Sample7	Gene1	84.18						SYBR Green I			15
	H2	Sample7	Gene1	84.07						SYBR Green I			16

Figure 98: T_m calling, Result Table

The columns of the table have the following meanings:

Column	Description
Color	Color coding of the sample. For detailed information on how to change the colors, see section *Editing cells*, on page 93.
Number	Index number of a well per dye. Index numbers are counted sequentially from left to right and from top to bottom.
Position	Position of the well in the multiwell plate. Each row is allocated a letter from A to H. The wells of a row are numbered from 1 to 12 from left to right.
Dye	Name of the associated dye.
Excluded	Specifies whether the sample is excluded from the analysis. By default, all samples are included, so all samples are deselected in the exclusion column. Excluding samples can be useful when a sample is clearly an outlier, or if an error has occurred in pipetting or amplification.
Sample Name	Name of the sample present in the well. *The result table displays no rows for cleared wells, that is, deactivated wells. For detailed information on clearing wells, see section Clear Wells/Set to Default, on page 136.*
Gene Name	Name of the gene of interest.
TM1 (°C) to *TM5 (°C)*	Temperature of the corresponding melting peak maximum according to the area settings in the melting peaks graph. The value is calculated from the maximum call of all corresponding acquisitions. Where no peak is present, the cells are blank.
Notes	Description of the sample.
Sample Prep Notes	Notes as specified in imported MagNA Pure 96 sample data files.

7.4.6 Amplification Curves

The display of the amplification curves in a T_m calling analysis corresponds to their display in absolute quantification analysis. An amplification curves graph is only available if an amplification program has been performed. For detailed information, see section *Amplification Curves*, on page 152.

7.5 Exporting analysis results

To store the results of an experiment or to transfer the results to other software programs, the operator must export the result files. The LightCycler® 96 Application Software supports result export for a successfully completed and calculated experiment. Exporting a file does not remove the data from the software, but copies the data and stores it in the specified location.

You can export the following data:

- Result graphs; see section *Graphs shortcut menu*, on page 99.
- Result tables; see section *Exporting the result table* below.

Additionally, you can create a result file with result table data collected from multiple experiment files. For details, see section *Exporting multiple result data* below.

7.5.1 Exporting the result table

The exported data contain the currently displayed result table, including the header line and the experiment file name. The result table is exported to a tab-delimited text file (.txt).

- If a filter definition is used to reduce the number of displayed rows, only the filtered data are exported.
- Hidden columns are not exported to the result file.

To export the result table

1	Open the corresponding result table.
2	Modify the view of the table according to your needs.
3	Right-click the table and choose *Export to File* on the shortcut menu. The *Save As* dialog box opens.
4	Navigate to the corresponding location. *By default, the default experiment directory is displayed. This directory can be specified in the 'Default Directory' dialog box. For detailed information, see section Preferences, on page 119.*
5	Enter a name for the text file.
6	Click *Save*.

7.5.2 Exporting multiple result data

The LightCycler® 96 Application Software provides a batch export tool that allows the operator to create a result file with result table data collected from multiple experiment files.

The batch export function exports the collected result table data to a tab-delimited text file (.txt). This file contains all result data, including the header rows, the experiment name and plate ID for each sample. You can open this file using Microsoft Excel.

For detailed information on the batch export function, see section *Result Batch Export*, on page 116.

Chapter C

LightCycler® 96 Instrument Software

C

LightCycler® 96 Instrument Software

1 Overview

The LightCycler® 96 Instrument Software provides all functions for configuring and controlling the LightCycler® 96 Instrument:

- Managing experiments; see section *Overview tab*, on page 199.
- Specifying the temperature profile and the dye-specific parameters for an experiment run; see section *Run Editor tab*, on page 203.
- Monitoring an experiment run; see section *Raw Data tab*, on page 212.
- Configuring the instrument; see section *Utilities tab*, on page 214.

After the experiment run, the raw data gathered by the software must be transferred to the application software for analysis. For a detailed description of the LightCycler® 96 Application Software, see chapter *LightCycler® 96 Application Software*, on page 77.

The LightCycler® 96 Instrument Software is installed on the LightCycler® 96 Instrument which may be connected to a network. Please be aware that such connection may have an adverse effect on the product's integrity, for example, through infection with malicious code (viruses, Trojan horses, etc.) or access by unauthorized third parties (such as intrusion by hackers). Roche therefore highly recommends protecting the product against such risks by taking appropriate and state-of-the-art action.

C

C

1.1 The main window

The figure below shows the main window of the LightCycler® 96 Instrument Software (in this example, the *Run Editor* tab is shown). The main window contains the following areas, described below:

- Status bar
- Working window area with working window area tabs representing the main software functions
- Global action bar
- Alarms window area

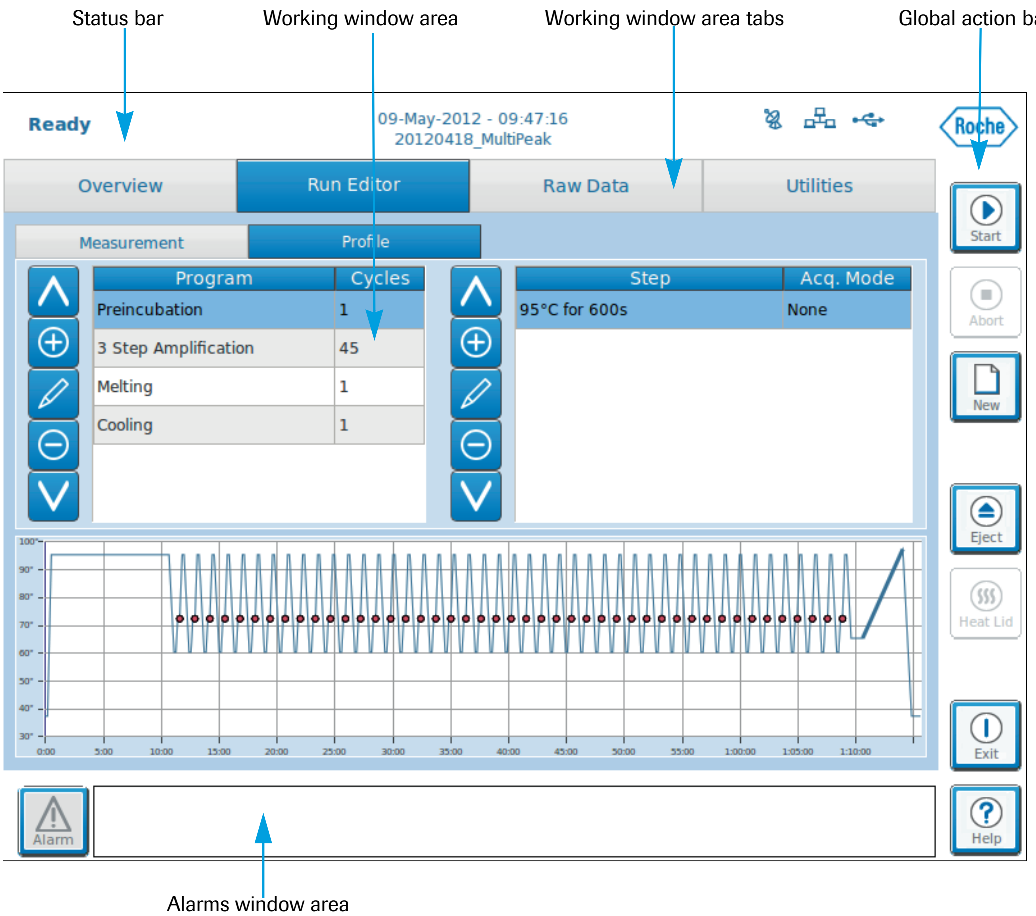


Figure 99: LightCycler® 96 Instrument Software main window

1.1.1 Status bar

The status bar displays the following information:

Field/Icon	Description
<status>	Current instrument status: ▶ *Startup* Startup of the instrument hardware. ▶ *Initializing* Initialization of the instrument hardware. ▶ *Ready* The block cycler cover has reached the target temperature and the instrument is ready to start a run. ▶ *Running: <experiment name>* The specified experiment is running. ▶ *Standby* The heater of the block cycler cover is switched off. ▶ *Lid Reheating* The block cycler cover is heating up after a standby. ▶ *Aborting* The current experiment run is being aborted. ▶ *Error* A hardware or software error has occurred which renders the instrument in a non-functional state. ▶ *Activating Transportation Lock* The instrument is preparing the transportation lock. ▶ *Transportation Lock* The instrument is ready for transport.
<date - time> *<experiment name>*	Current date and time. Name of the currently selected experiment.
	An experiment is currently being saved, removed, synchronized, or otherwise remotely processed. *As long as this icon flashes, the instrument should not be switched off.*
	The instrument is connected to an Ethernet network.
	A USB drive is connected to the instrument.
	Remote monitoring is activated. For detailed information, see section *Remote Monitoring*, on page 219.
	The Axeda client is activated. For detailed information, see section *Axeda client*, on page 220.

1.1.2 Working window area tabs

The LightCycler® 96 Instrument Software provides the complete workflow via the tabs in the working window area:

Tab	Description
Overview	Provides a list of the experiments available on the instrument and the USB drive (if connected). For detailed information, see section *Overview tab*, on page 199.
Run Editor	Provides the following functions: ▶ Defining the measurement settings for the experiment ▶ Defining the temperature and cycling sequence for the experiment For detailed information, see section *Run Editor tab*, on page 203.
Raw Data	Provides the run progress, temperature profile, and raw fluorescence data collected during an experiment run. For detailed information, see section *Raw Data tab*, on page 212.
Utilities	Provides utilities for managing the instrument, for example software update, log file export, or network configuration. For detailed information, see section *Utilities tab*, on page 214.

1.1.3 Global action bar

The buttons in the global action bar provide access to general software functions. Their availability depends on the current status of the instrument. The buttons provide the following actions:

Button	Function	Description
	Start	Only enabled in *Ready* status: Starts the experiment selected on the *Overview* tab. For detailed information, see section *Overview tab*, on page 199.
	Abort	Only enabled while a run is being processed: Aborts the currently executed run. You are prompted to confirm the abort.
	New	Only enabled if less than 50 experiments are saved on the instrument: Opens the *Create New Experiment* dialog box. For detailed information, see section *Experiments*, on page 193.
	Eject	Only enabled in the following instrument states: ▶ *Initializing* ▶ *Standby* ▶ *Ready* ▶ *Error* Pushes the loading module forward. The operator can open the loading module completely using the recessed grip. For detailed information, see section *Loading module*, on page 30. *The loading module may only be opened using the 'Eject' button and when the button is enabled. Otherwise the instrument changes to the 'Error' state and has to be rebooted.*
	Heat Lid	Only enabled in *Standby* status: Starts heating of the block cycler cover. After heating, the instrument changes to the *Ready* status.

Button	Function	Description
	Exit	Not available while a run is being processed: Opens the *Exit Options* dialog box for shutting down or rebooting the LightCycler® 96 Instrument Software. For detailed information, see section *Exiting the software*, on page 198.
	Help	Opens the help browser of the LightCycler® 96 Instrument. For detailed information, see section *Help browser*, on page 229.

1.1.4 Alarms window area

The alarms window area displays the unconfirmed error and warning messages.

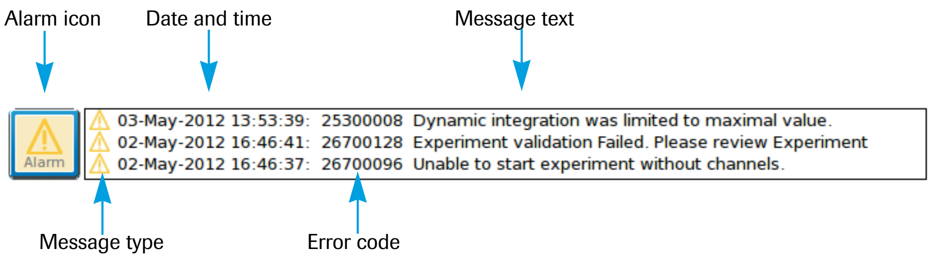


Figure 100: Alarms window area

The alarms window area contains the alarm icon on the left and the message field.

Alarm icon

The color of this icon changes depending on the severity of the alert. It reflects the highest level of any unconfirmed alarm displayed in the alarms window area.

Icon	Function	Description
		There are no unconfirmed alarms.
	Warning	There are unconfirmed alarms at *Warning* level. The system may continue working, but not with full performance, or may run into problems later.
	Error	There are unconfirmed alarms at *Error* level. The system will stop performing some actions if the operator does not intervene.

Message field

By default, the last three alarms are shown in the message field.

- Choosing the alarm icon displays the complete list of alarms; see section *Alarm history*, on page 226.
- Choosing a single message displays the corresponding detailed information; see section *Detailed information*, on page 228.

Each message contains the following information:

- The message type, specifying the alarm level: *Warning* or *Error*
- The date and time when the error occurred
- The error code of the message
- The message text

For detailed information on displaying message details, confirming messages, and deleting messages, see section *Alarms and messages*, on page 225.

C

1.2 General software conventions

1.2.1 Buttons

Standard buttons

The LightCycler® 96 Instrument Software uses the following standard buttons in dialog boxes:

Button	Description
Ok	Closes the dialog box and applies the settings to the corresponding parameters.
Cancel	Closes the dialog box and discards the settings.
Close	Closes the displayed information dialog box.
	Changes to the previous/next possible value for the input field.
	▶ In an input field: Increases the selected value. ▶ In a list or table: Changes to the previous entry. ▶ On a text page: Scrolls up in the text. ▶ On the *Profile* tab of the run editor: Moves the selected item up one place.
	▶ In an input field: Decreases the selected value. ▶ In a list or table: Changes to the next entry. ▶ On a text page: Scrolls down in the text. ▶ On the *Profile* tab of the run editor: Moves the selected item down one place
	Pencil button, update button, and tools button: Each button opens a dialog box which allows for specifying the corresponding additional values.

1.2.2 Input fields

The LightCycler® 96 Instrument Software provides several options for entering data into input fields:

Input Field	Description
Text field	Choose the field itself to open the corresponding dialog box for changing the value.
Text field with left and right arrows	Specify the corresponding previous or next value using the left and right arrows.
Text field with a list	Choose the down arrow next to the field and select a value from the pull-down list.
Numeric values field with up and down arrows	Specify a value using the up and down arrows.

1.2.3 Working with tables

The LightCycler® 96 Instrument Software provides functions to be used in all tables displayed in the user interface. This section describes functions that are shared between the different tables.

To select and deselect items

1. Choose a row in a table to select the corresponding item. The selected item is highlighted.
   Choosing another row deselects the previous row.

To sort tables

1. Choose the header of a column to sort the table by the column values.
   Choosing the header several times toggles the sort order between descending and ascending.

1.3 Experiments

The information provided in the experiment definition controls the LightCycler® 96 Instrument during an experiment run. The experiment definition specifies the target temperatures and hold times of the thermal block cycler, the number of cycles being executed, and other parameters. As the experiment progresses, the software gathers temperature and fluorescence data from the instrument and displays it on the *Raw Data* tab.

For starting an experiment run, the experiment must be available on the instrument. Operators can specify an experiment definition as follows:

- On the instrument using the LightCycler® 96 Instrument Software.
- On a computer using the LightCycler® 96 Application Software. In this case, the experiment must be transferred to the instrument for the run. For detailed information on the application software, see chapter *LightCycler® 96 Application Software*, on page 77.

After the experiment run, the raw data gathered by the software on the instrument must be transferred back to the application software for analysis. For detailed information on how to send an experiment to the instrument or retrieve it to the computer, see section *Instrument Manager*, on page 110.

1.3.1 Creating an experiment

Before a LightCycler® 96 Instrument run can be started, a new experiment has to be created. The operator has the following options for creating a new experiment:

- Generating a completely new experiment
- Using an existing experiment as a template
- Using a predefined Roche template

Creating an experiment is done in the *Create New Experiment* dialog box. You access the dialog box using the *New* button in the global action bar.

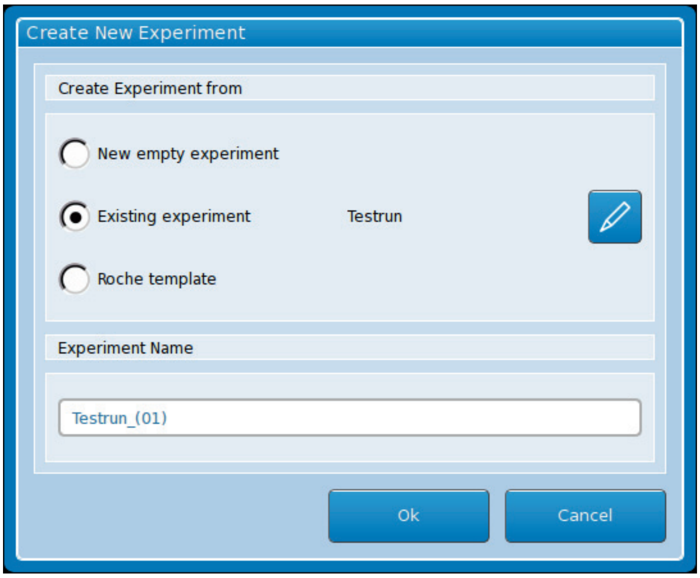


Figure 101: Create New Experiment dialog box

Create Experiment from window area

The *Create Experiment from* window area provides the following options:

Option	Description
New empty experiment	Creates a new, empty experiment.
Existing experiment	Opens the specified experiment as a template.
Roche template	Opens the specified Roche template.

Experiment Name window area

The *Experiment Name* window area provides the input field for the experiment file name. Choosing the text field opens the keyboard dialog box for specifying the name.

Figure 102: Keyboard dialog box

To generate a new experiment

1. In the global action bar, choose *New*. The *Create New Experiment* dialog box opens.
2. Choose the *New empty experiment* option to create a new, empty experiment. The default name for the new experiment is provided in the *Experiment Name* window area as *New_Experiment_(<no>)*.
3. In the *Experiment Name* window area, choose the text input field. The *Alpha Numeric Keyboard* dialog box opens.
4. Specify the name for the new experiment and close the dialog box with *OK*.
5. Choose *OK*.
   The LightCycler® 96 Instrument Software
   - Adds the new experiment to the list on the *Overview* tab.
   - Opens the *Measurement* tab in the *Run Editor* for the new experiment.

To use an existing experiment as a template

To create a new experiment from an existing one (that is, to copy all settings of an experiment), the operator must open the experiment file as a template. In this case, the raw data of the experiment is deleted. The experiment run settings and the sample editor settings of the experiment are provided for editing.

When an existing experiment with analyses is used as a template in the LightCycler® 96 Instrument Software, the analyses and their settings are lost. The analysis settings are only retained if the experiment is generated in the LightCycler® 96 Application Software.

1. In the global action bar, choose *New*. The *Create New Experiment* dialog box opens.
2. Choose the *Existing experiment* option.
3. Choose the pencil button next to the option. The *Experiments list* dialog box opens showing the experiment files available on the instrument.
4. Choose the experiment and close the dialog box with *OK*. The default name for the new experiment is provided in the *Experiment Name* window area as *Existing_Experiment_(<no>)*.
5. In the *Experiment Name* window area, choose the text input field. The *Alpha Numeric Keyboard* dialog box opens.
6. Specify the name for the new experiment and close the dialog box with *OK*.
7. Choose *OK*.
   The LightCycler® 96 Instrument Software
   - Adds the new experiment to the list on the *Overview* tab.
   - Opens the *Measurement* tab in the *Run Editor* for the new experiment.
8. Change all settings according to your needs.
   When detection format settings are changed (for example, the dye type in the same channel), all gene-specific settings (that is, gene name and concentration) are set to their default values.

To use a Roche template

Roche provides a number of predefined experiments as templates. An experiment template contains the temperature profile and the dye-specific parameters for an experiment.

1. In the global action bar, choose *New*. The *Create New Experiment* dialog box opens.
2. Choose the *Roche template* option.
3. Choose the pencil button next to the option. The *Experiments list* dialog box opens showing the Roche templates available on the instrument.
4. Choose the template and close the dialog box with *OK*. The default name for the new experiment is provided in the *Experiment Name* window area as *Template_(<no>)*.
5. In the *Experiment Name* window area, choose the text input field. The *Alpha Numeric Keyboard* dialog box opens.
6. Specify the name for the new experiment and close the dialog box with *OK*.
7. Choose *OK*.
   The LightCycler® 96 Instrument Software
   - Adds the new experiment to the list on the *Overview* tab.
   - Opens the *Measurement* tab in the *Run Editor* for the new experiment.
8. Change all settings according to your needs.

1.3.2 Opening an experiment

Experiments can be opened at any time before, during, or after the experiment run and during execution of another experiment.

The LightCycler® 96 Instrument Software allows for opening and editing an experiment while another experiment is running.

To open an experiment

1. On the *Overview* tab, select the experiment to be opened.
   - This experiment is then available on the *Run Editor* tab.
   - The experiment is executed when you choose the *Start* button in the global action bar.
   - If the experiment is running or completed, the temperature profile and the collected raw data are displayed on the *Experiment* tab.

1.3.3 Saving an experiment

The LightCycler® 96 Instrument Software automatically saves all changes in an experiment file. The experiment file is saved according to its original location:

- On the LightCycler® 96 Instrument.
- On the USB drive.
- On both media, if the operator has synchronized the storage locations.
  For detailed information on synchronizing, see section Control bar, on page 202.

For safety reasons, operators should regularly download the experiment data from the instrument using one of the following functions.

- *On the LightCycler® 96 Instrument: Synchronizing the storage locations; see section Control bar, on page 202.*
- *In the LightCycler® 96 Application Software: Retrieving the experiment from the instrument; see section Instrument Manager, on page 110.*

1.4 Import, export, and file transfer options

The following figure shows the input and output data flow offered by the LightCycler® 96 Instrument Software architecture. The data to be imported and exported is described below.

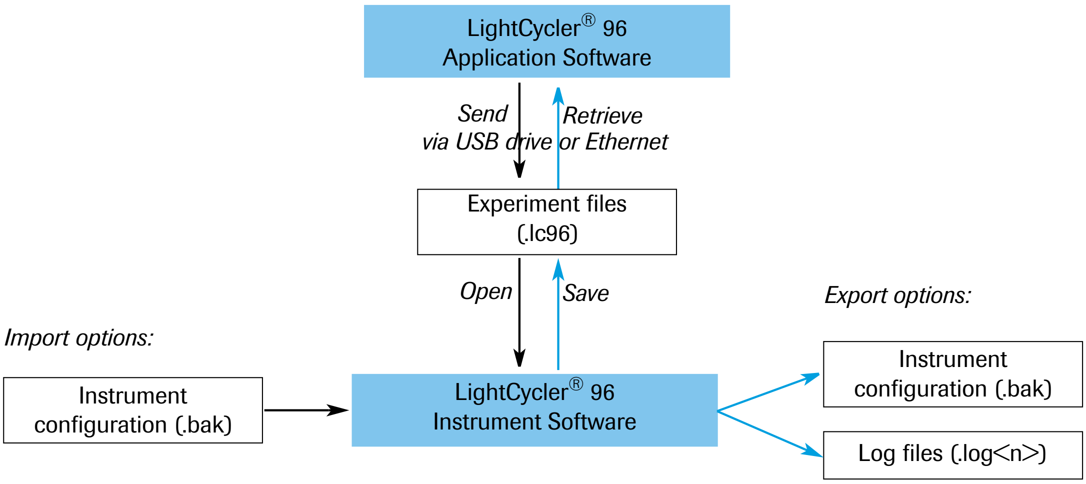


Figure 103: LightCycler® 96 Instrument Software input and output data flow

1.4.1 Import data

To restore the configuration of the LightCycler® 96 Instrument, the following data can be imported into the LightCycler® 96 Instrument Software:

Data	File Format	Description
Instrument configuration	.bak	Configuration settings and experiment files, saved during a system backup of the instrument. For detailed information, see section *Backup and restore*, on page 222.

1.4.2 Export data

To store the configuration or the log files of the instrument or to transfer the data for further analysis, the corresponding files must be exported. The LightCycler® 96 Instrument Software provides export functions for the following data:

Data	File Format	Description
Instrument configuration	.bak	Configuration settings and experiment files, packed and saved as a backup file (.bak) on a connected USB drive. For detailed information, see section *Backup and restore*, on page 222.
Log files	.log	Log files containing all error and operation logs. For detailed information, see section *Export Logfiles*, on page 223.

1.5 Exiting the software

The *Exit* button in the global action bar opens the *Exit Options* dialog box which provides options for shutting down and for rebooting the LightCycler® 96 Instrument Software.

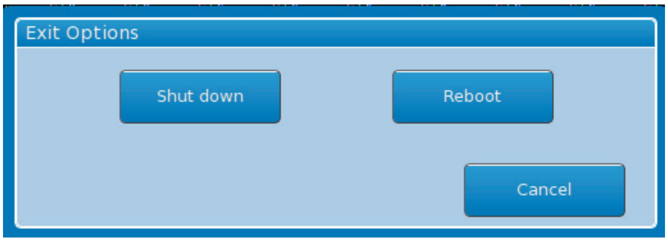


Figure 104: Exit Options dialog box

Button	Description
Shut down	Shuts down the LightCycler® 96 Instrument Software. *After shutting down the instrument software, the operator must switch off the LightCycler® 96 Instrument using the mains power switch on the back of the instrument.*
Restart	Shuts down and reboots the LightCycler® 96 Instrument Software.
Cancel	Closes the *Exit Options* dialog box without shutting down the software.

2 Overview tab

The *Overview* tab provides a list of the experiments available on the instrument and the USB drive (if connected), the status of each experiment, and the memory usage on the instrument.

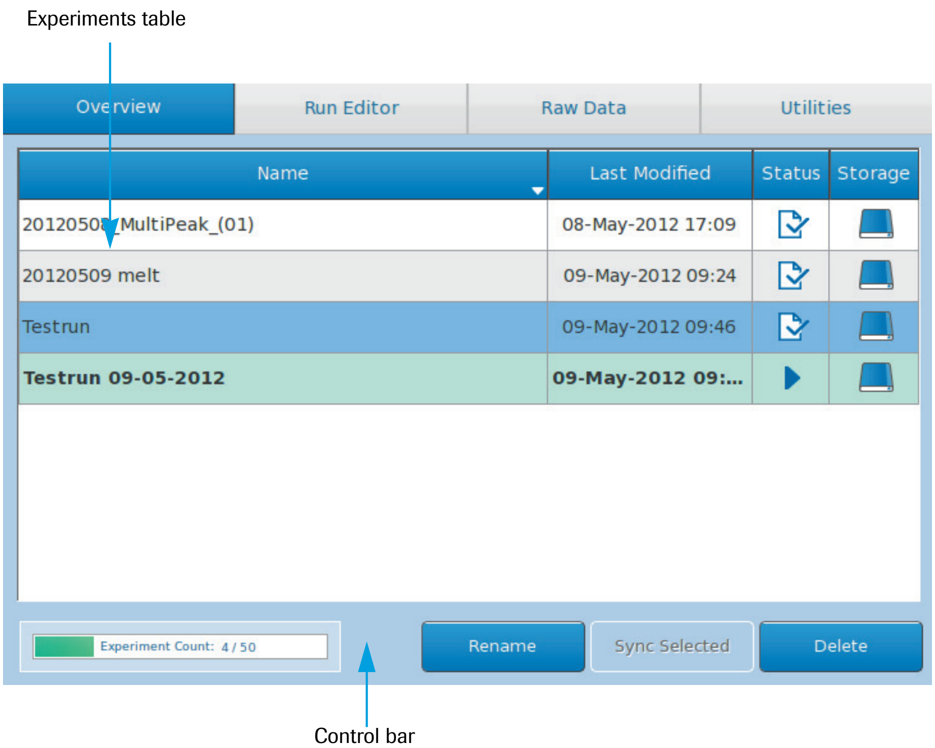


Figure 105: Overview tab

2.1 Experiments table

The experiments table shows all experiments available on the instrument and on a connected USB drive:

- Currently running experiments are displayed with a bold font and a light green background. A running experiment cannot be renamed, deleted, or synchronized. For detailed information, see section *Control bar*, on page 202.
- The currently selected experiment is displayed with a blue background. The selected experiment does not need to be the currently running experiment. For detailed information, see section *Selecting an experiment*, on page 201.
- While an experiment is being saved, removed, synchronized, or otherwise remotely processed, it is displayed in grey and cannot be renamed, deleted, or synchronized. For detailed information, see section *Control bar*, on page 202.

Each experiment has the following properties:

Column	Description
Name	Name of the experiment file.
Last Modified	Date and time of the last modification.
Status	Status of the experiment. For detailed information on the various states, see section *Status*, on page 200.
Storage	Location of the experiment file. For detailed information on the location icons, see section *Storage*, on page 200.

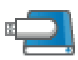
Status

The software displays the following states for an experiment:

Icon	Status	Description
	Ready	Non-executed experiment.
	Running	Currently running experiment.
	Executed	Successfully executed experiment.
	Aborted	Aborted experiment.

Storage

The *Storage* column shows where the experiment file is located:

Icon	Storage	Description
	Instrument	The experiment is stored on the instrument.
	USB	The experiment is stored on the connected USB drive.
	Synchronized	The experiment is synchronized, that is, stored on both locations. For detailed information, see section *Control bar*, on page 202.

The software provides the *Experiment has conflicted states* dialog, if the status of an experiment is different on the connected USB drive and the instrument:

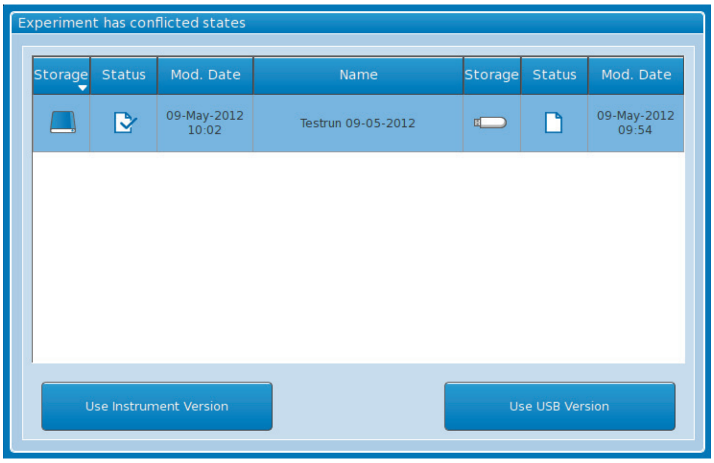


Figure 106: Experiment has conflicted states dialog box

The dialog box provides the following information for both locations:

Parameter	Description
Name	Experiment name.
Storage	Storage location; for detailed information on the icons, see section *Storage* above.
Status	Status of the experiment on the corresponding location; for detailed information, see section *Status* above.
Mod. Date	Date of the last modification on the corresponding location.

The buttons provide the following functions:

Button	Description
Use Instrument Version	Overwrites the experiment file on the USB drive with the experiment file version on the instrument.
Use USB Version	Overwrites the experiment file on the instrument with the experiment file version on the USB drive. You are prompted to confirm the action.

Selecting an experiment

Selecting an experiment in the list opens the experiment file:

- This experiment is then available on the *Run Editor* tab.
- The experiment is executed when you choose the *Start* button in the global action bar.
- If the experiment is running or completed, the temperature profile and the collected raw data are displayed on the *Raw Data* tab.

2.2 Control bar

The control bar on the *Overview* tab provides the following functions:

Experiment Count bar

The *Experiment Count* bar shows the total sum of experiments saved on the instrument and on a currently connected USB drive.

The LightCycler® 96 Instrument memory provides space for a maximum of 50 experiments. If while connecting a USB drive, the total number of 50 experiments is exceeded, a warning is displayed.

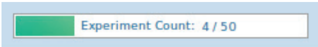


Figure 107: Overview tab, Experiment Count bar

Buttons

The buttons on the *Overview* tab perform the following functions:

Button	Description
Rename	Opens the *Rename Experiment* dialog box to specifiy a new name for the selected experiment. For detailed information, see section *Rename Experiment dialog box* below.
Sync Selected	Synchronizes the storage locations: If the selected experiment is located on the instrument, it is transferred to the USB drive and vice versa.
Delete	Deletes the selected experiment. The operator is prompted to select the location of the experiment to be deleted.

Rename Experiment dialog box

The *Rename* button in the control bar opens the *Rename Experiment* dialog box for specifying a name for the selected experiment:

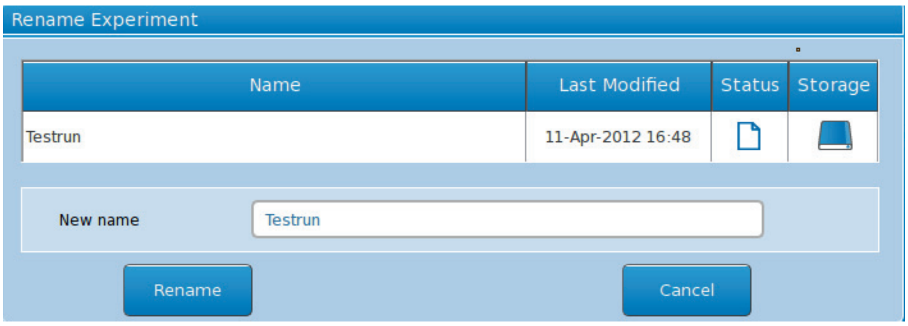


Figure 108: Rename Experiment dialog box

The dialog box provides the following options:

Parameter	Description
Table	Properties of the experiment as displayed on the *Overview* tab. For detailed information, see section *Experiments table*, on page 199.
New Name	Input field for the new experiment name. Choosing the text field opens the *Set new name* dialog box for specifying the name.

3 Run Editor tab

On the *Run Editor* tab, the dye-specific parameters for an experiment run and the temperature profile are specified (in this example, the *Measurement* tab is shown).

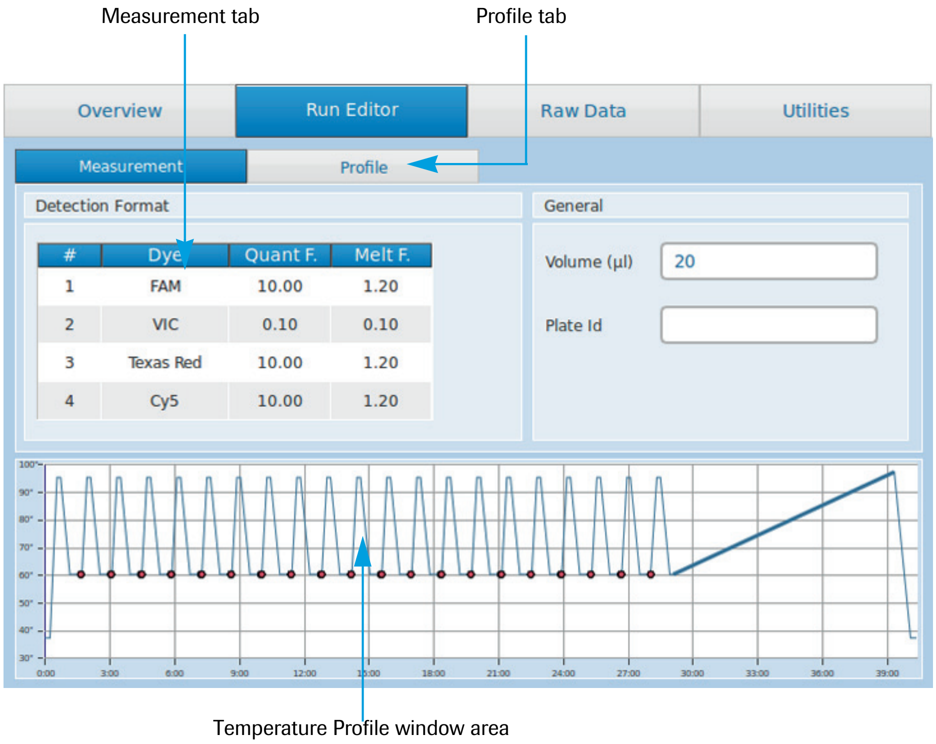


Figure 109: Run Editor, Measurement tab

For a new experiment, this tab shows no data. For detailed information on how to create a temperature profile and set the dye-specific parameters, refer to the *LightCycler® 96 System User Training Guide*.

3.1 Measurement tab

The *Measurement* tab displays the dye-specific settings for an experiment run. The operator specifies the settings in the *Detection Format* dialog box. For detailed information, see section *Detection format*, on page 204.

Setting	Parameter	Description
Detection Format	*Dye*	Name of the dye.
	Quant F.	Only available if the integration time mode is set to *Dynamic*: Multiplication factor to be applied to the filter combination for a quantification program. The *Quant Factor* represents the fold signal stroke from the initial background fluorescence to the plateau phase.
	Melt F.	Only available if the integration time mode is set to *Dynamic*: Multiplication factor to be applied to the filter combination for a melting program.
	Integration T.	Only available if the integration time mode is set to *Manual*: Acquisition time of the CCD camera.

Setting	Parameter	Description
General	*Volume [µl]*	Reaction volume to be used in the experiment. The LightCycler® 96 Instrument supports reaction volumes from 10 to 50 µl. *As the LightCycler® 96 Instrument does not validate the sample volume, the operator must ensure that the specified sample volume matches the volume pipetted into the wells of the multiwell plate.*
	Plate Id	Plate ID; saved to the experiment file for identification of the experiment. When using the external handheld barcode scanner, the *Plate Id* field displays the barcode of the loaded multiwell plate. Alternatively, operators can edit the plate ID manually.

Detection format

For an unprocessed experiment the software provides a pencil button in the *Detection Format* window area which allows for specifying the dye-specific settings:

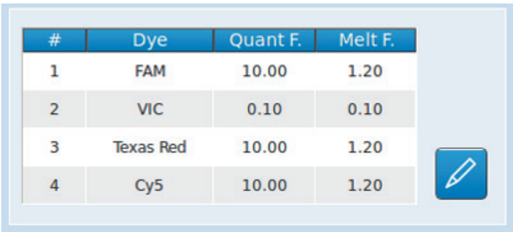


Figure 110: Measurement tab, Detection format window area

The pencil button opens the *Detection Format* dialog box. By setting the detection formats, the operator chooses the filter combinations suitable for the experiment. A detection format specifies one or more excitation-emission filter combinations. For detailed information, see section *Detection channels*, on page 38.

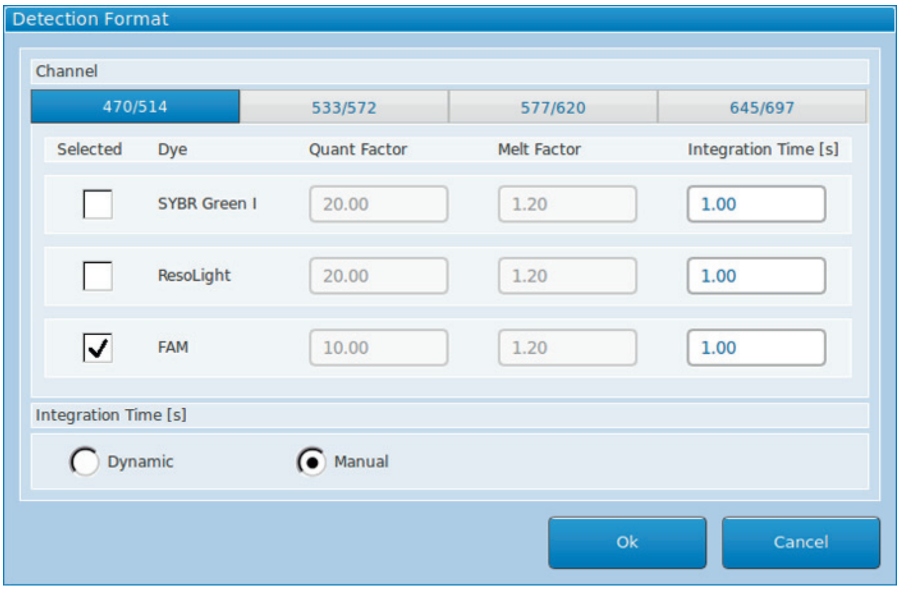


Figure 111: Detection Format dialog box

The dialog box provides a tab for each channel. Each tab shows the following settings:

Column	Description	Possible values
Selected	Specifies whether the channel is to be used by selecting the corresponding dye. *Only one dye can be selected per channel. The software automatically deselects a checkbox when you try to select more than one dye in the same channel group.* *'SYBR Green I' and 'ResoLight' cannot be combined with any dye of another channel.*	
Dye	Name of the dye.	
Quant Factor	Only available if the integration time mode is set to *Dynamic*: Multiplication factor to be applied to the filter combination for a quantification program. The *Quant Factor* represents the fold signal stroke from the initial background fluorescence to the plateau phase.	1.0 to 500
Melt Factor	Only available if the integration time mode is set to *Dynamic*: Multiplication factor to be applied to the filter combination for a melting program.	1.0 to 500
Integration Time [s]	Only available if the integration time mode is set to *Manual*: Acquisition time of the CCD camera.	0.1 to 10 sec

Depending on the integration time mode chosen for the detection format, the dialog box enables the following settings:

Mode	Description	Setting
Dynamic	The integration time is set automatically based on the fluorescence of the individual plate.	Melt Factor
		Quant Factor
Manual	The integration time is set manually.	Integration Time

3.2 Profile tab

All temperature profiles are comprised of programs, which are run by the instrument in the order they are displayed on the *Profile* tab. Each program performs a role within the experiment and can be specified separately.

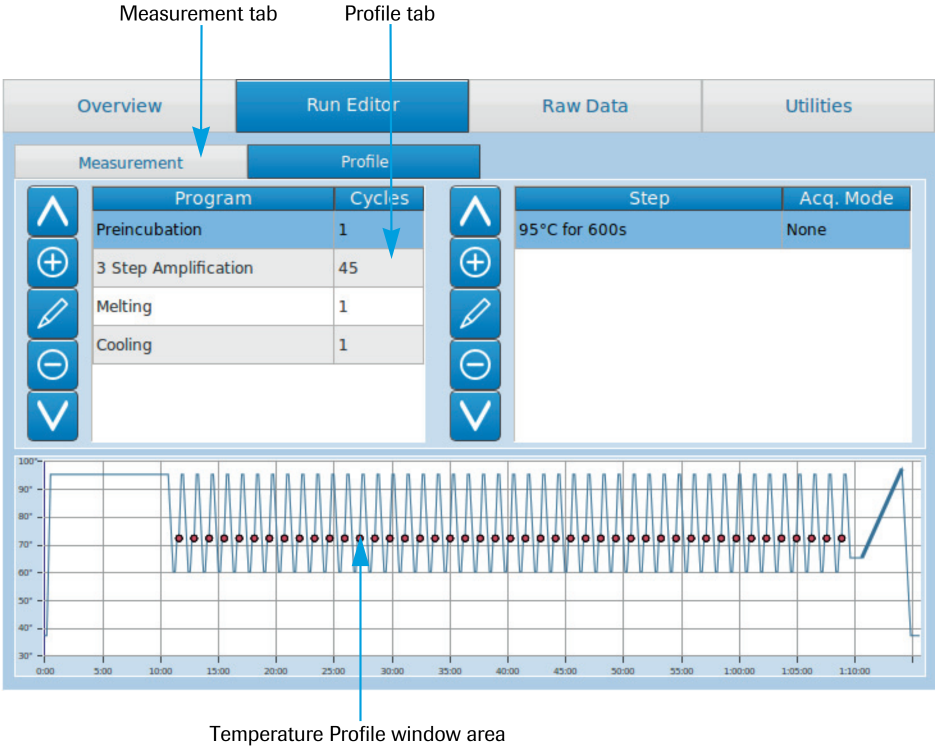


Figure 112: Run Editor, Profile tab

3.2.1 Programs window area

The programs are displayed in a list and have the following properties. The LightCycler® 96 Instrument Software derives these properties from the settings in the *Edit Program Settings* dialog box. For detailed information, see section *Program settings*, on page 208.

Parameter	Description
Program	Name of the program.
Cycles	Specifies how many times the cycle is to be repeated, for example, 1 or 45 times.

Programs list buttons

The programs list can be edited with the following buttons:

Button	Function	Description
	Up	Moves the selected program up one place. If there is no selected program, or the selected item is first in the list, this button is disabled.
	Add	Opens the *Add New Program* dialog box which allows for adding a new program to the list. The new program is added to the end of the list.
	Edit	Displays the *Program Settings* window area which allows for editing the selected program. For detailed information, see section *Program settings*, on page 208.
	Remove	Deletes the selected program from the list. If no program is selected, this button is disabled.
	Down	Moves the selected program down one place. If there is no selected program, or the selected item is last in the list, this button is disabled.

3.2.2 Adding a new program

The button in the *Programs* list opens the *Add New Program* dialog box, which allows for selecting a new program and adding it to the *Programs* list.

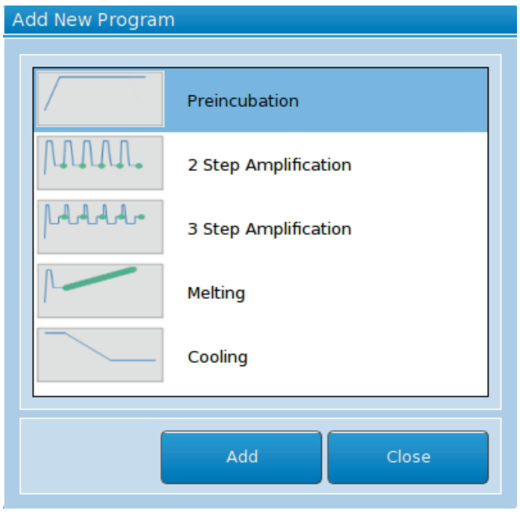


Figure 113: Add New Program dialog box

The following programs are available:

Program	Description
Preincubation	Holds a specified temperature for a defined time.
2 Step/3 Step Amplification	Cycling program; defines a program of the experiment where the instrument will repeatedly heat and cool to a defined series of temperatures. Each repeat is called a cycle. The touchdown function for amplification programs allows the operator to specify that one of the stages of each cycle will have its target temperature modified as the cycling proceeds. This allows for the early cycles of a PCR to have a higher annealing temperature specified, leading to more specific amplification. For detailed information, see section *Step settings*, on page 210.
Melting	Defines a program where the instrument will ramp to an initial temperature, then ramp to a final temperature. While ramping to the final temperature, optical acquisitions will be made continuously. These can then be analyzed to yield melting peaks.
Cooling	Defines a program where the instrument will cool down to a final temperature and then hold the specified temperature for a defined time.

It is not necessary to add a separate cooling program at the end of the run. At the end of each run, the samples are automatically cooled to +37°C.

Program settings

The pencil button in the programs list opens the *Program Settings* window area on the *Profile* tab, which displays the program settings for the selected program and allows for editing a program as long as no run has been performed. Every change is displayed immediately in the *Temperature Profile* window area.

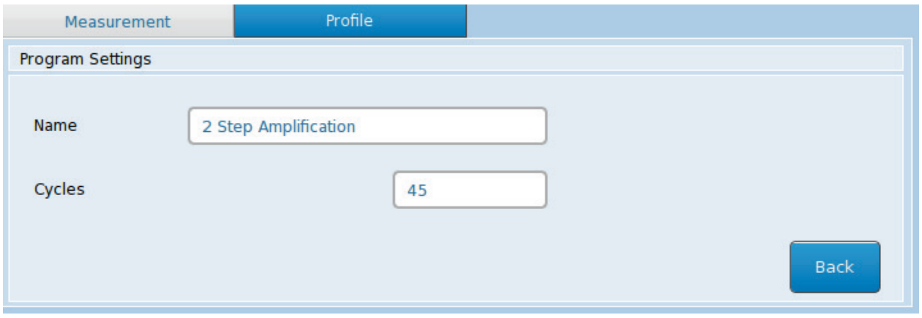


Figure 114: Program Settings dialog box

The *Program Settings* window area provides the following settings for each program:

Parameter	Description
Name	Name of the program.
Cycles	Specifies how many times the cycle is to be repeated, for example, 1or 45 times.
Back	Closes the *Program Settings* window area and displays the programs list with the changed settings.

3.2.3 Steps window area

A program consists of one or more steps, which are run by the instrument in the order they are displayed in the *Steps* window area. A step specifies the following data:

- The temperatures used by the instrument
- The time for which these temperatures are to be held
- The rate at which to heat and cool
- The points at which the instrument will acquire optical data

The minimum experiment definition has one program with one cycle and one valid step.

If multiple amplification programs with acquisitions are defined, only the first amplification program is considered for result calculation and chart display.

The LightCycler® 96 Instrument adjusts the temperature between the steps automatically, cooling or heating up to meet the temperature specified for the next step.

The steps are displayed in a list and have the following properties. The LightCycler® 96 Instrument Software derives these properties from the settings in the *Step Settings* dialog box (see below).

Parameter	Description
Step	Target temperature and duration in seconds for which the temperature is to be held.
Acq. Mode	Acquisition mode: *None*, *Single*, or *Continuous*.

Steps list buttons

The steps list can be edited using the buttons to the left of the list:

Button	Function	Description
	Up	Moves the selected step up one place. If there is no selected step, or the selected item is first in the list, this button is disabled.
	Add	Adds a new step to the list. The new step is added to the end of the list.
	Edit	Displays the *Step Settings* window area which allows for editing the selected program. For detailed information, see section *Step settings*, on page 210.
	Remove	Deletes the selected step from the list. If no step is selected, this button is disabled.
	Down	Moves the selected step down one place. If there is no selected step, or the selected item is last in the list, this button is disabled.

Step settings

The pencil button in the steps list opens the *Step Setting* window area on the *Profile* tab, which displays the step settings for the selected step and allows for editing the steps as long as no run has been performed. Every change is displayed immediately in the *Temperature Profile* window area.

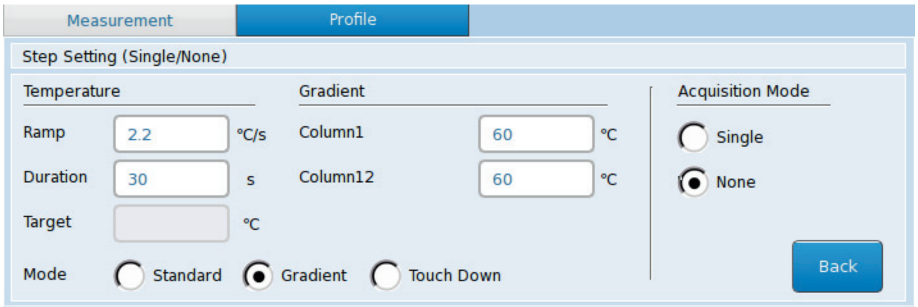


Figure 115: Run Editor, Step Setting window area with Gradient option

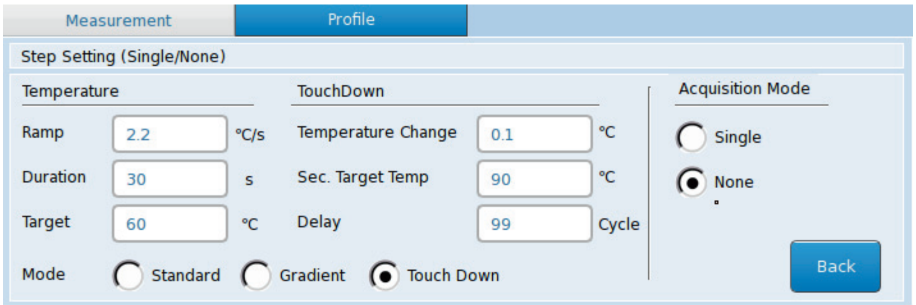


Figure 116: Run Editor, Step Setting window area with Touch Down option

The *Step Setting* window area provides the following settings for each step, depending on the selected program:

Setting	Parameter	Description
Temperature	*Ramp*	Rate of temperature change in °C per second, which the LightCycler® 96 Instrument uses for heating or cooling until the defined temperature is reached.
	Duration	Duration in seconds for which the temperature is to be held.
	Target	Temperature in °C, which is to be held for a defined time.
Gradient		Only available in *Gradient* mode: Temperature grading used by the LightCycler® 96 Instrument for heating the different Peltier elements in the thermal block cycler. Operators can specify temperature gradients from 98°C to 37°C.
	Column1	Maximum temperature for the gradient. This temperature is applied to the leftmost column of the multiwell plate.
	Column12	Minimum temperature for the gradient. This temperature is applied to the rightmost column of the multiwell plate.

Setting	Parameter	Description
Touchdown		Only available in *Touch Down* mode: Enables/disables the touchdown function.
	Temperature Change	Rate of temperature change in °C per cycle, at which the touchdown phase proceeds to the final phase temperature.
	Sec. Target Temp	Second target temperature to be reached by the last cycle of the program. This temperature is used to change the target temperature of a segment during the amplification reaction.
	Delay	Number of cycles after which the temperature change is first applied.
Acquisition Mode	*Single*	Only available for amplification programs: Acquires fluorescence data once only, when the temperature target is reached and the hold time completed.
	Continuous	Not available for amplification programs: Number of optical acquisitions to be performed. The fluorescence data are acquired continuously until the temperature target is reached.
	None	No fluorescence data are acquired.
Mode		Only available for amplification programs: Enables/disables the *Standard*, *Gradient* or *Touch Down* option.
Back		Closes the *Step Setting* window area and displays the programs list and the steps list with the changed settings.

3.3 Temperature Profile window area

The *Temperature Profile* window area provides a summary of the programs selected for the experiment and their temperature and time settings.

When starting an experiment run, the operator must ensure that the correct temperature profile is used.

4 Raw Data tab

The *Raw Data* tab shows the temperature profile and the raw fluorescence data collected during an experiment run in real time. The horizontal and the vertical scaling of the charts changes according to the running experiment. The raw data of an experiment contain all instrument corrections but no color compensation, drift, or background correction (in this example, the temperature profile graph is shown).

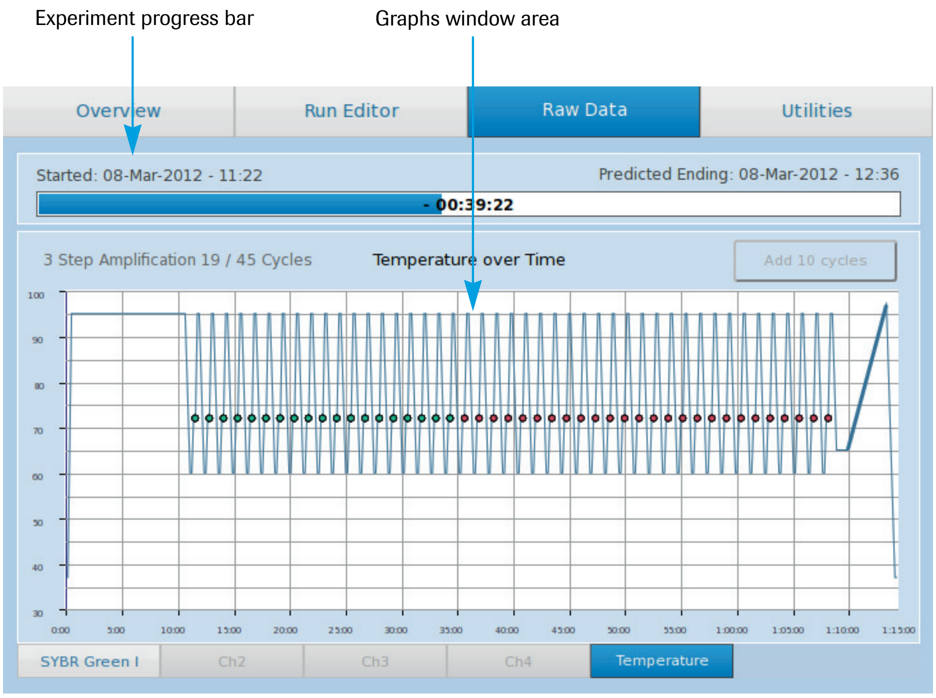


Figure 117: Raw Data tab, temperature profile

4.1 Experiment progress bar

The experiment progress bar provides the following information:

- The start time of the experiment.
- The end time of the experiment. During an experiment run, this field displays the predicted end time.
- During an experiment run, the progress bar displays the run progress in percent and the predicted time the experiment run will take.

4.2 Graphs window area

Depending on the selected tab, the graphs window area displays the following information:

Temperature tab

The *Temperature* tab provides a summary of the programs selected for the experiment and their temperature and time settings; see *Figure 117* above.

The green dots in the temperature profile indicate the already processed cycles of the program.

<dye> tabs

The *<dye>* tabs provide the fluorescence curves, that is, the fluorescence intensity against the time in hours, minutes, and seconds for the entire run. Depending on the dyes selected in the *Detection Format* dialog box, different dye-specific tabs are available.

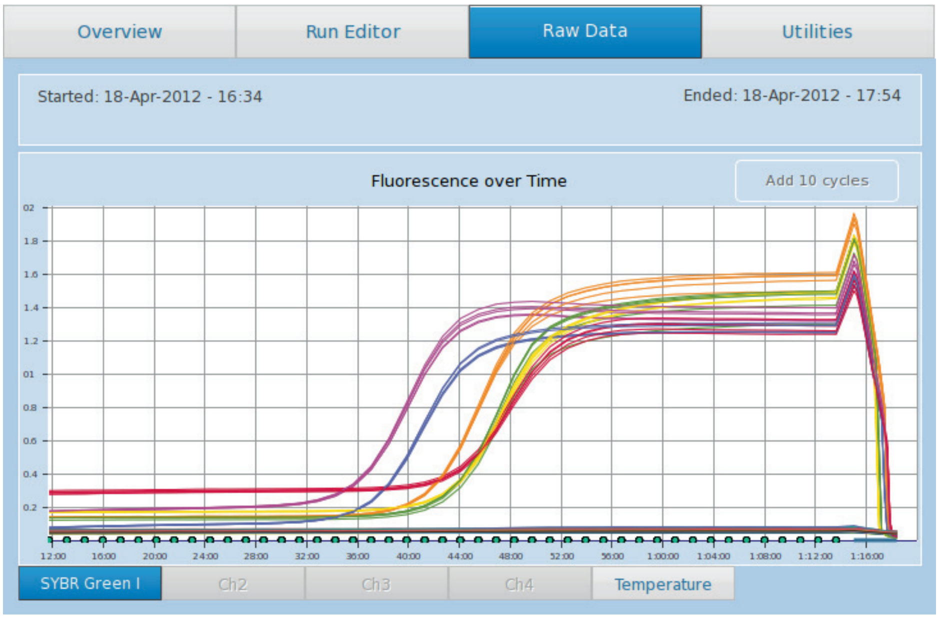


Figure 118: Raw Data tab, fluorescence curves

Add 10 cycles

Choosing this button adds 10 cycles to a running experiment. The software adds 10 cycles to the currently running program.

'Add 10 cylces' can only be performd during an amplification program, and not in a meling program.

5 Utilities tab

The *Utilities* tab provides functions for managing the instrument (in this example, the *Instrument* tab is shown).

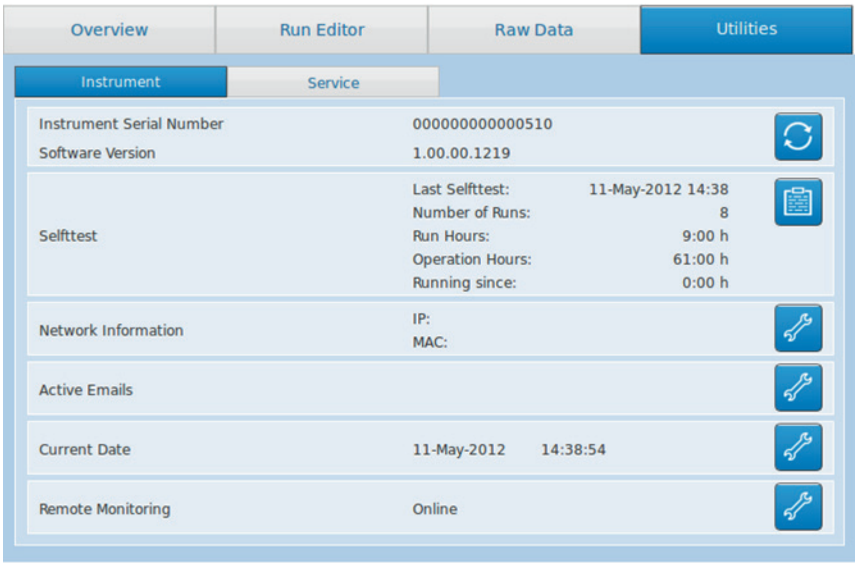


Figure 119: Utilities, Instrument tab

5.1 Instrument tab

The *Instrument* tab displays the following configuration settings for the LightCycler® 96 Instrument and provides the associated configuration functions. Each function is accessed via the button next to the corresponding field:

Setting	Description
Instrument Serial Number	Serial number of the instrument.
Software Version	Version number of the currently installed LightCycler® 96 Instrument Software; the update button opens the *Software Update* dialog box, which allows for installing a software update from a connected USB drive. For detailed information, see section *Installing a LightCycler® 96 Instrument Software update*, on page 58.
Selftest	Information on the last self test of the instrument; the button opens the *Self Test Report* dialog box, which displays detailed information. For detailed information, see section *Self test*, on page 215.
Network Information	IP address of the instrument in the network; the tools button opens the *Network configuration* dialog box, which allows for configuring the LightCycler® 96 Instrument in the network. For detailed information, see section *Configuring the LightCycler® 96 Instrument in the network*, on page 49.
Active Emails	The tools button opens the *Email Configuration* dialog box, which allows for setting up email notifications on different instrument states. For detailed information, see section *Active Emails*, on page 215.
Current Date	Current date and time; the tools button opens the *Date/time configuration* dialog box, which allows for setting the date and time. For detailed information, see section *Current Date*, on page 218.

Setting	Description
Remote Monitoring	Current remote monitoring status; the tools button opens the *Configure Remote Monitoring* dialog box, which allows for configuring the settings for remote monitoring of the instrument. For detailed information, see section *Remote Monitoring*, on page 219.

5.1.1 Self test

The button next to the *Selftest* field opens the *Self Test Report* dialog box, which displays detailed information on the last self test of the instrument. The self test is performed during initialization of the instrument.

The self test information is not exported to a log file and cannot be printed.

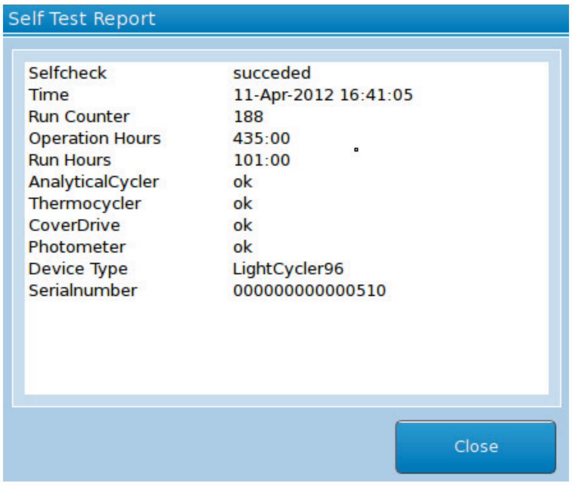


Figure 120: Self Test Report dialog box

5.1.2 Active Emails

The tools button next to the *Active Emails* field opens the *Email Configuration* dialog box, which provides the following functions:

- Defining up to three email addresses
- Activating or deactivating these email addresses
- Enclosing experiment files to email

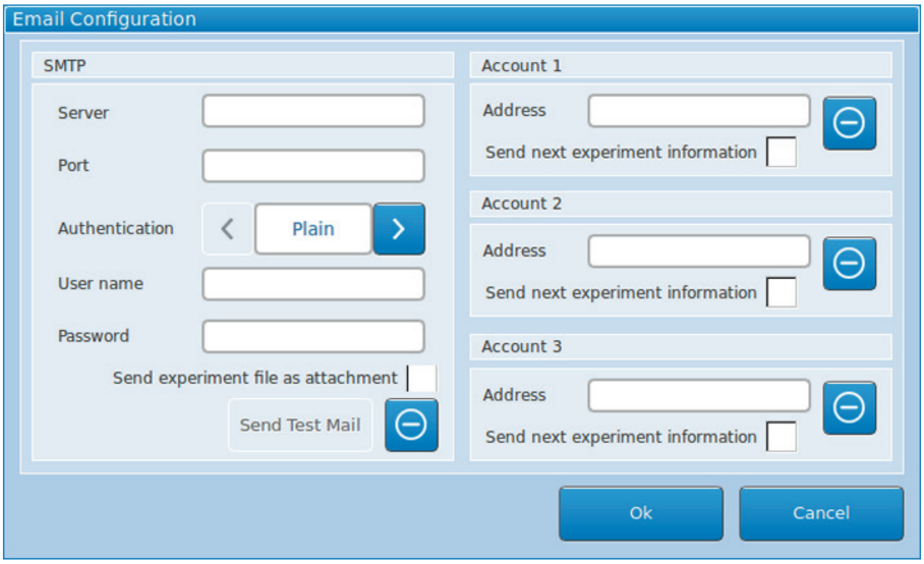


Figure 121: Email Configuration dialog box

Setting	Parameter	Description
SMTP	*Server*	Name or IP address of the SMTP server to be used for sending emails.
	Port	Port number of the SMTP server
	Authentication	Authentication type to be used for the user account on the SMTP server: ▶ *Plain*: User name and password are transferred unencrypted. ▶ *Login*: User name and password are transferred unencypted, but in two steps ▶ *None*: No authentication is necessary.
	User name	User name for the account. The software uses this user name as sender identification for the instrument emails.
	Password	Password to be used for the user account.
	Send experiment file as attachment	Specifies whether the experiment file is to be attached to the email notification when the experiment run is finished.
	Send Test Mail	Sends a test email to the specified addresses. *The button next to the 'Send Test Mail' button resets all fields in the 'SMTP' window area.*

Setting	Parameter	Description
Account <n>	*Address*	Email address of the recipient. Operators can specify up to three recipients, using the fields in the *Account 1*, *Account 2*, and *Account 3* window areas. *The button next to the 'Address' field deactivates the corresponding email address and resets the fields.*
	Send next experiment information	Activates the corresponding email address; when activated, an email notification containing the status of the experiment is sent to the email address when the next experiment run is successfully finished or if it has been aborted. *After an experiment run, the activated email accounts are automatically deactivated.*

5.1.3 Current Date

The tools button next to the *Current Date* field opens the *Date/time configuration* dialog box, which allows for setting the current date and time.

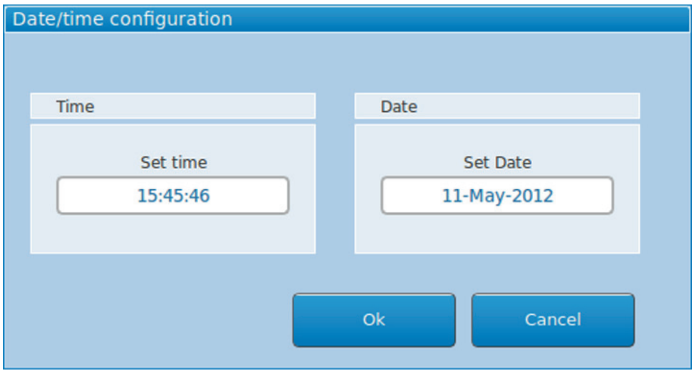


Figure 122: Date/time configuration dialog box

Choosing the input fields opens the *Set time* or *Set date* dialog box, allowing the operator to specify the current time and date.

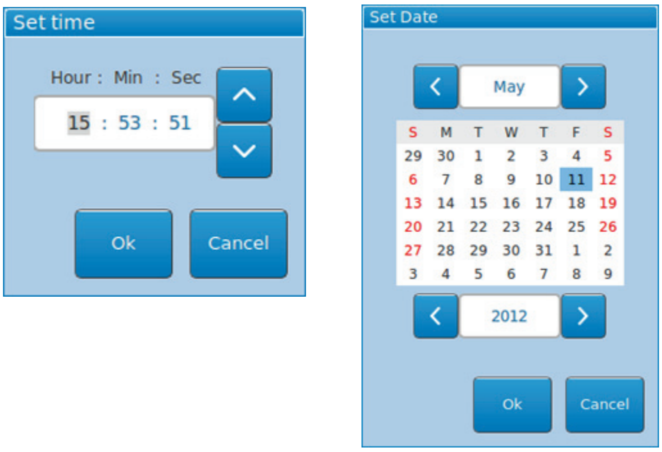


Figure 123: Set time and Set date dialog boxes

5.1.4 Remote Monitoring

The remote monitoring function allows remote monitoring of the LightCycler® 96 Instrument by an operator. For detailed information, see section *Instrument Manager*, on page 110.

The tools button next to the *Remote Monitoring* field opens the *Configure Remote Monitoring* dialog box.

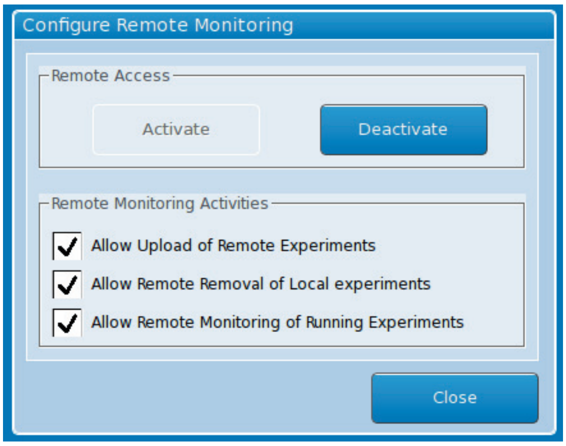


Figure 124: Configure Remote Monitoring dialog box

Setting		Description
Remote Access	*Activate/Deactivate*	Enables/disables remote monitoring of the instrument and sending or receiving of experiment files.
Remote Monitoring Activities	*Allow Upload of Remote Experiments*	Allows for sending experiment files from the LightCycler® 96 Application Software to the instrument. For detailed information, see section *To send an experiment to an instrument*, on page 114.
	Allow Remote Removal of Local Experiments	Allows for deleting experiment files remotely controlled from the instrument by the LightCycler® 96 Application Software. For detailed information, see section *To retrieve an experiment from an instrument*, on page 114.
	Allow Remote Monitoring of Running Experiments	Allows for monitoring an experiment run in the LightCycler® 96 Application Software. For detailed information, see section *Online Monitoring tab*, on page 115.

5.2 Service tab

The *Service* tab provides the following functions:

- Setting up the Axeda client; see section *Axeda client*, on page 220.
- Backing up and restoring the configuration settings of the instrument; see section *Backup and restore*, on page 222.
- Exporting log files; see section *Export Logfiles*, on page 223.
- Preparing the instrument for transportation; see section *Prepare Instrument for Transportation*, on page 224.

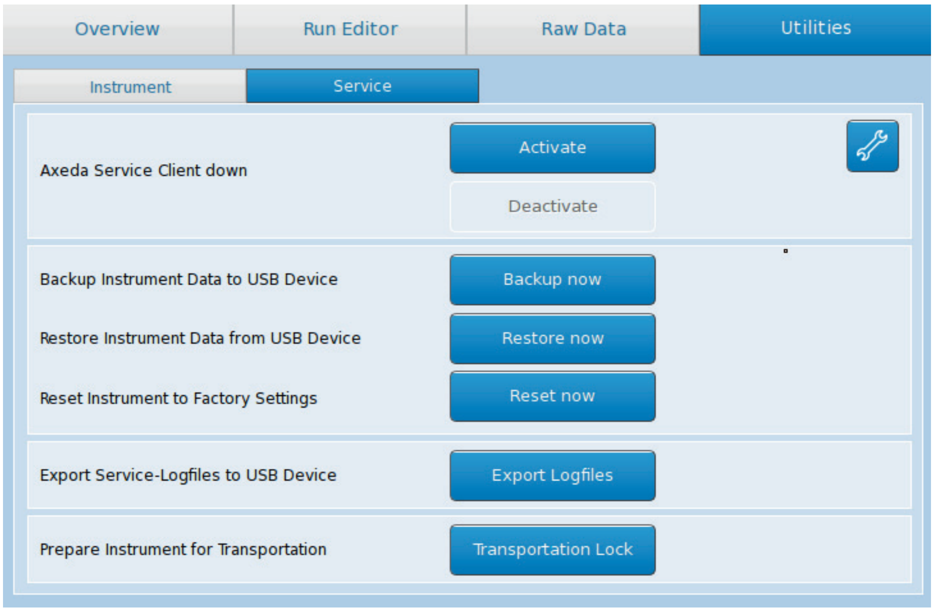


Figure 125: Utilities, Service tab

5.2.1 Axeda client

The Axeda client installed on the LightCycler® 96 Instrument enables an operator to allow remote access by a Roche field service engineer. For detailed information, see section *Remote service*, on page 251.

Button	Description
Activate	Activates the Axeda client; remote access to the instrument is allowed. The operator can continue working without restrictions.
Deactivate	Deactivates the Axeda client; remote access to the instrument is not possible.
Tools button	Opens the *Axeda Client Configuration* dialog box, which allows for setting up the Axeda client. For detailed information, see section *Axeda client configuration* below.

Axeda client configuration

The tools button next to the *Axeda Service Client up/down* field opens the *Axeda Client Configuration* dialog box, which allows for setting up the Axeda client.

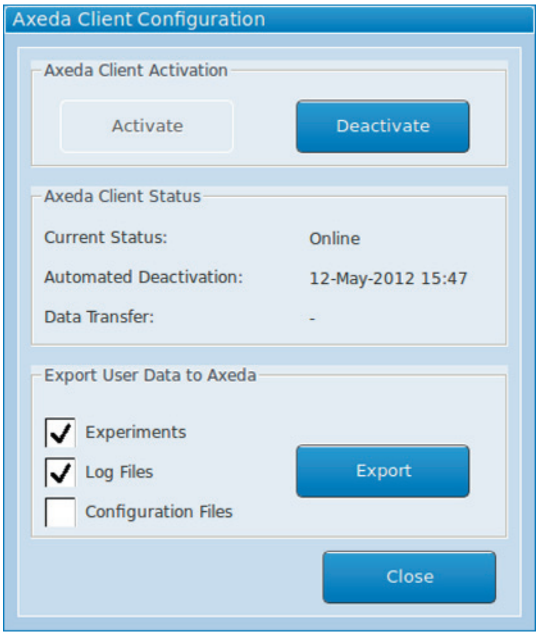


Figure 126: Axeda Client Configuration dialog box

Setting		Description
Axeda Client Activation	*Activate*	Activates the Axeda client; remote access to the instrument is allowed. The operator can continue working without restrictions.
	Deactivate	Deactivates the Axeda client; remote access to the instrument is not possible.
Axeda Client Status	*Current Status*	Current status of the Axeda client: *Online* or *Offline*.
	Automated Deactivation	Date and time of the last automated Axeda client deactivation. *The Axeda client is automatically deactivated after 24 hours. If the 24 hours have not expired yet, the Axeda Client is restarted at the next reboot of the instrument.*
	Data Transfer	Date and time of the last data transfer.
Export User Data to Axeda	*Experiments/ Log Files/ Configuration Files*	Specifies which data are to be exported for access by the Roche field service engineer.
	Export	Exports the specified data.

5.2.2 Backup and restore

The backup/restore function allows for saving the instrument configuration to a connected USB drive and restoring the configuration if necessary.

Backup Now

The *Backup Now* button opens the *System Backup* dialog box for specifying the data to be saved:

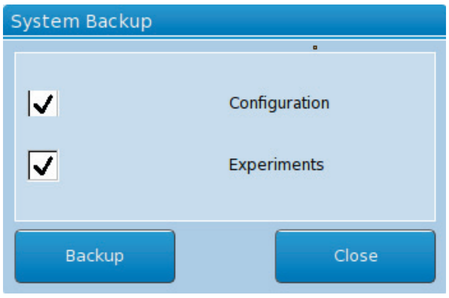


Figure 127: System Backup dialog box

Setting	Description
Configuration	The backup saves the following settings: ▶ Network and email configuration ▶ Axeda client configuration ▶ Remote monitoring settings ▶ Date and time
Experiments	The backup saves all experiment files.

Choosing *Backup* packs the backup files and saves them to the USB drive.

Restore Now

The *Restore Now* button opens the *System Restore* dialog box for specifying the backup file to be restored:

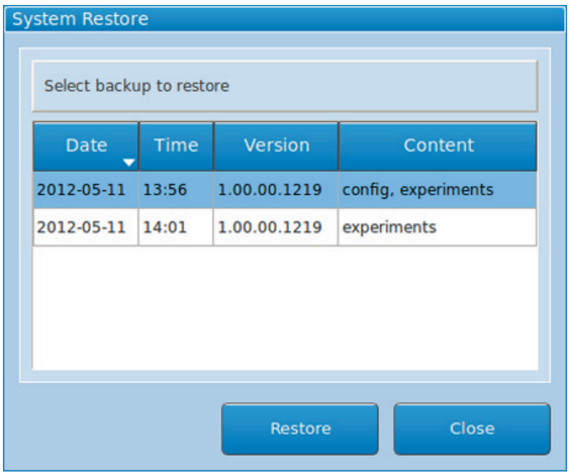


Figure 128: System Restore dialog box

The list in the dialog box provides the date and time of all backup files available on the USB drive.

Restoring the configuration is only possible when the backup before has been completed successfully.

Choosing *Restore* restores the configuration settings and experiment files from the backup directory on the connected USB drive.

Reset Now

The *Reset Now* button reloads the current software version onto the instrument:

- All configuration settings are reset to factory settings.
- All experiment files are deleted.

5.2.3 Export Logfiles

The *Export Logfiles* button opens the *Log Files Export* dialog box, which allows for exporting the instrument log files to a connected USB drive.

Figure 129: Log Files Export dialog box

Choosing *Export* packs the log files to a .zip archive with a time stamp and saves them to the USB drive.

Log files

The LightCycler® 96 Instrument Software creates four different types of log files:

Log file type	Description
AnalyticalCycler.log	Instrument control software debug messages
DataReduction.log	Measured raw data fluorescence values
Support.log	Alarm messages displayed in the instrument software

The log files are saved as **.log<n>* files on a rotational basis, where *<n>* represents the log file counter. The software stores up to 10 files of the same log file type, then the existing log files are overwritten.

For detailed information on error messages and corrective actions, see chapter *Troubleshooting*, on page 241.

5.2.4 Prepare Instrument for Transportation

The *Prepare Instrument for Transportation* button locks the loading module for transport. The operator is prompted to confirm that the transport locking device is inserted. For detailed information on the transport locking device, see section *Assembling the instrument*, on page 44.

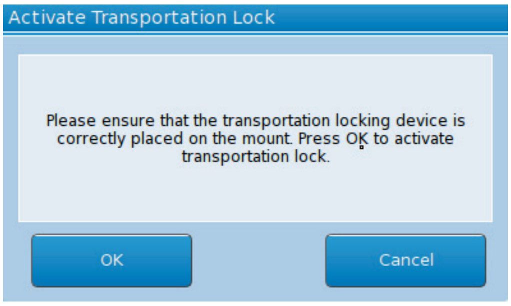


Figure 130: Activate Transport Lock dialog box

6 Alarms and messages

All messages, that is, software, data, and instrument messages, are logged in a message table. They are displayed in the alarms window area.

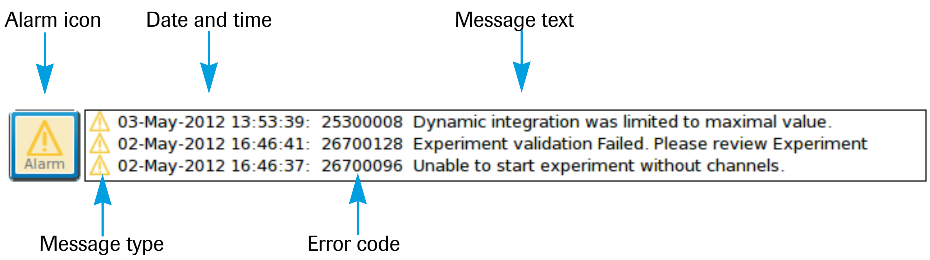


Figure 131: Alarms window area

Each message contains the following information:

- The message type: *Warning* or *Error*
- The date and time when the error occurred
- The error code of the message
- The message text

Displaying and confirming messages

The LightCycler® 96 Instrument Software provides the following options for viewing messages and alarms in general or in detail:

- The alarms window area in the main window; see section *Alarms window area*, on page 189.
- The *unconfirmed Alarms* tab in the *Alarm History* dialog box which displays all unconfirmed messages; see section *unconfirmed Alarms tab*, on page 226.
- The *confirmed Alarms* tab in the *Alarm History* dialog box which displays all confirmed messages; see section *confirmed Alarms tab*, on page 227.
- The *Information* dialog box which displays the details for a selected message; see section *Detailed information*, on page 228.

6.1 Alarm history

Choosing the alarm icon in the alarms window area opens the *Alarm History* dialog box.

unconfirmed Alarms tab

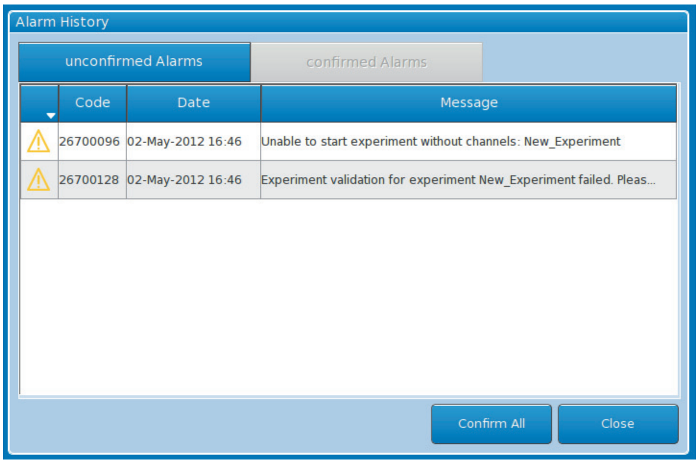


Figure 132: Alarm History dialog box, unconfirmed Alarms tab

The table provides the following information:

Column	Description
Alarm icon	Severity of the alarm; operators can sort the log report by the severity of the alarms.
Code	Error code of the message.
Date	Date and time the message occurred.
Message	Message text.

- For detailed information on how to work with tables, see section *Working with tables*, on page 192.
- For detailed information on error messages and corrective actions, see chapter *Troubleshooting*, on page 241.

To confirm all unconfirmed messages

1. Choose the alarm icon in the message area. The *Alarm History* dialog box opens.
2. Open the *unconfirmed Alarms* tab.
3. Choose *Confirm All*.
   - The messages are removed from the list.
   - The dialog box closes.
   - The alarm icon in the message area changes color to the highest level of any unconfirmed alarm.

confirmed Alarms tab

The *confirmed Alarms* tab displays a summary of all confirmed messages.

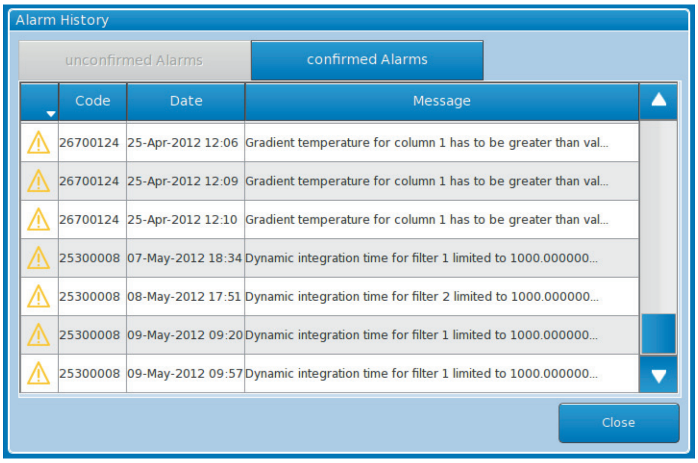


Figure 133: Alarm History dialog box, confirmed Alarms tab

The table provides the following information:

Column	Description
Alarm icon	Severity of the alarm; operators can sort the log report by the severity of the alarms.
Code	Error code of the message.
Date	Date and time the message occurred.
Message	Message text.

- For detailed information on how to work with tables, see section *Working with tables*, on page 192.
- For detailed information on error messages and corrective actions, see chapter *Troubleshooting*, on page 241.

6.2 Detailed information

Choosing a message in the alarms window area or in the *Alarm History* dialog box opens the *Information* dialog box, which displays the details for a selected message:

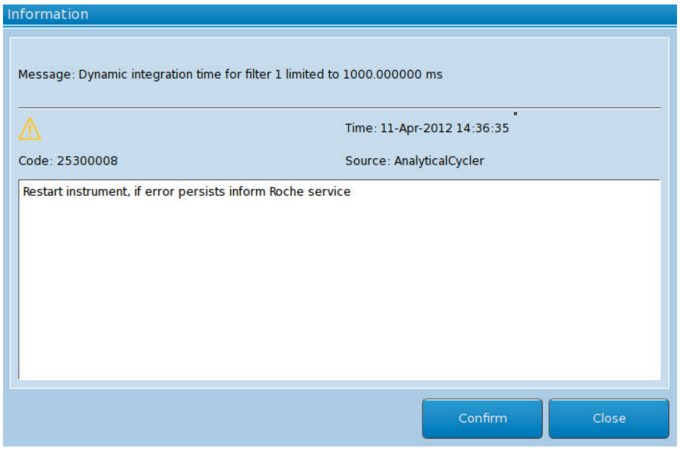


Figure 134: Information dialog box

To confirm a single message

1	Choose the corresponding message in the alarms window area or in the *Alarm History* dialog box. The *Information* dialog box opens, displaying detailed information for the selected message.
2	Choose *Confirm*. ▶ The dialog box closes. ▶ The message is removed from the alarms window area. ▶ The alarm icon in the message area changes color to the highest level of any unconfirmed alarm.

7 Help browser

The *Help* button in the global action bar opens the help browser of the LightCycler® 96 Instrument. The help browser provides information on the currently open tab of the LightCycler® 96 Instrument Software (in this example, the *Overview* tab help text is shown).

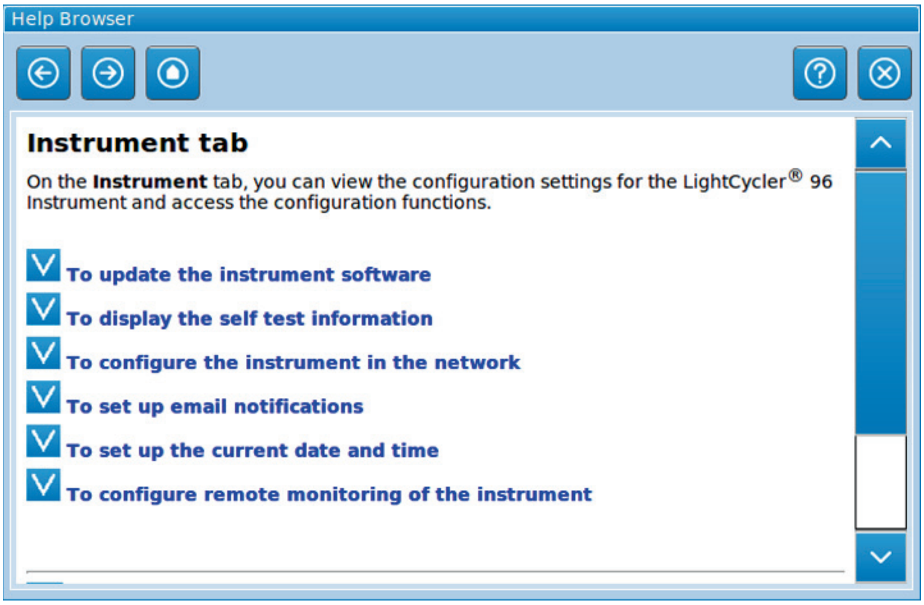


Figure 135: Help browser

The buttons in the help browser provide the following functions:

Button	Description
	Changes to the previous visited page.
	Changes to the next visited page.
	Opens the table of contents.
	Opens the "Trademarks and Licenses" text.
	Closes the help browser.

Links

Links in the help text are indicated by an arrow button. The help browser provides two types of links:

Link	Description
 **Bold continuous text**	An arrow pointing right indicates a link to a related topic. The bold text shows the link target. Choosing the button switches to the other text.
 **Bold continuous text**	An down arrow indicates hidden text. The bold text shows the subkect of the hidden text. ▶ Choosing the button displays the hidden text. ▶ Choosing the button again hides the displayed text.

C

Chapter D
Cleaning and care

D

D

Cleaning and care

This chapter provides basic cleaning instructions and describes how to exchange fuses and dust filters of the LightCycler® 96 Instrument.

1 General maintenance

The LightCycler® 96 Instrument is maintenance-free.

Precautions

The area around the LightCycler® 96 Instrument should be checked regularly to ensure that the air flow is unrestricted and that books, papers, or other items are not interfering with the air flow. For detailed requirements, see section *Installation requirements*, on page 42.

2 Cleaning instructions

Caution!
Do not clean the LightCycler® 96 Instrument when it is plugged in.

Caution!
Do not pour fluids into the loading module, thermal block cycler, or the interior of the instrument.

Caution!
As with all potentially biohazardous specimens, universal safety precautions should be taken when handling and processing samples. Spills should be immediately disinfected with an appropriate disinfectant solution to avoid contamination of laboratory personnel or equipment. Handling and disposal of infectious material should be performed according to local safety guidelines.

General cleaning

Regular cleaning of the LightCycler® 96 Instrument and accessories is not required.

Use only the detergents recommended below for cleaning.

To clean the housing

1	Switch off the instrument.
2	Clean the housing of the instrument with a mild commercial detergent.
3	If necessary, use 70% ethanol to disinfect the instrument housing. *Do not aim sprays directly at the instrument, as malfunctions of the electronics may occur.*

D

To clean the touchscreen

1. Switch off the instrument.
2. Wet a soft, lint-free or microfiber cloth with distilled water or disinfectant.
3. Wring out as much as you can. Make sure the cloth is damp but not wet.
4. Wipe the screen in a gentle motion to remove dust, oil, or fingerprint smudges.

   Do not spray cleaners directly onto the touchscreen.

To clean the thermal block cycler

1. Switch off the instrument.
2. Clean the thermal block cycler with a mild commercial detergent.
3. If necessary, use 70% ethanol to disinfect the thermal block cycler.

   Do not aim sprays directly at the instrument, as malfunctions of the electronics may occur.

To clean the multiwell plate mount

1. Eject the loading module and switch off the instrument.
2. Pipette 125 µl of 70% ethanol or isopropanol into each well.

   Do not aim sprays directly at the instrument, as malfunctions of the electronics may occur.

   Make sure that no liquid passes between mount and cover, as malfunctions of the electronics may occur.

   Take care not to destroy the block coating, for example, by scrubbing with sharp edged or pointed objects.
3. After waiting 15 minutes pipette up and down several times.
4. Remove the liquid.
5. Let the multiwell plate mount dry before using it again.
6. Close the loading module and switch on the instrument.

D

3 Unlocking the loading module

The loading module of the LightCycler® 96 Instrument is locked after loading the multiwell plate and during the subsequent experiment run. It is unlocked when the experiment run is finished.

If the loading module is stuck, for example, after an experiment run, the operator can unlock it using the hexagon socket screw on the back of the instrument.

Hexagon socket screw

Figure 136: LightCycler® 96 Instrument back view with hexagon socket screw

To unlock the loading module

1. Switch off the instrument and unplug the mains power cable.
2. On the back of the instrument, release the cover of the hexagon socket screw.
3. Use a 5.0 mm hex key to unlock the loading module:
   To open the loading module, turn the hex key counter-clockwise.

The hex key is not part of the LightCycler® 96 System Package.

▶ ▶ ▶

4	Turn the hex key clockwise, to close the loading module again.
5	Restart the instrument to verify that the loading module is in the correct position.

D

4 Exchanging the ventilation dust filters

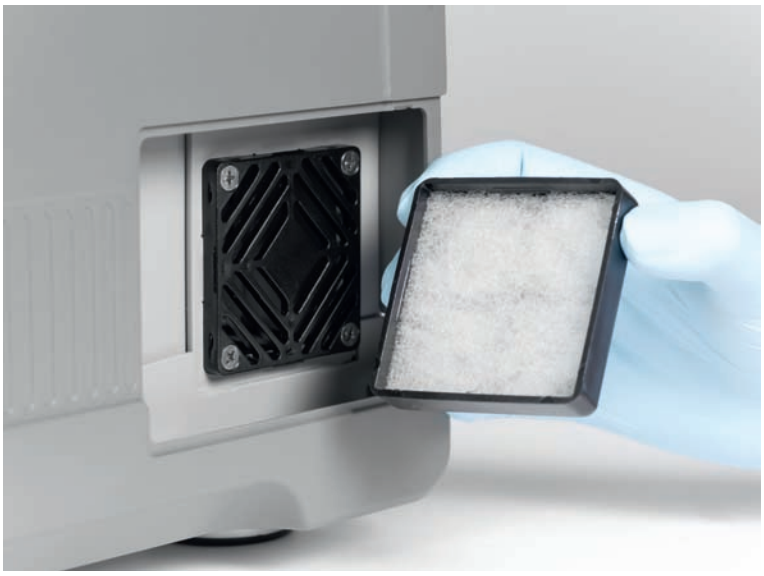
The electronic rack of the LightCycler® 96 Instrument is cooled by ventilation. The ventilation inlet is located in the lower right corner of the right side of the instrument. To avoid any contamination of the instrument interior by dust particles, this ventilation inlet is fitted with a dust filter. Two replacement dust filters come with the LightCycler® 96 System Package.

The dust filter should be checked and cleaned or exchanged when dusty. You can order replacement dust filters from your local Roche Diagnostics representative.

To exchange the ventilation dust filter

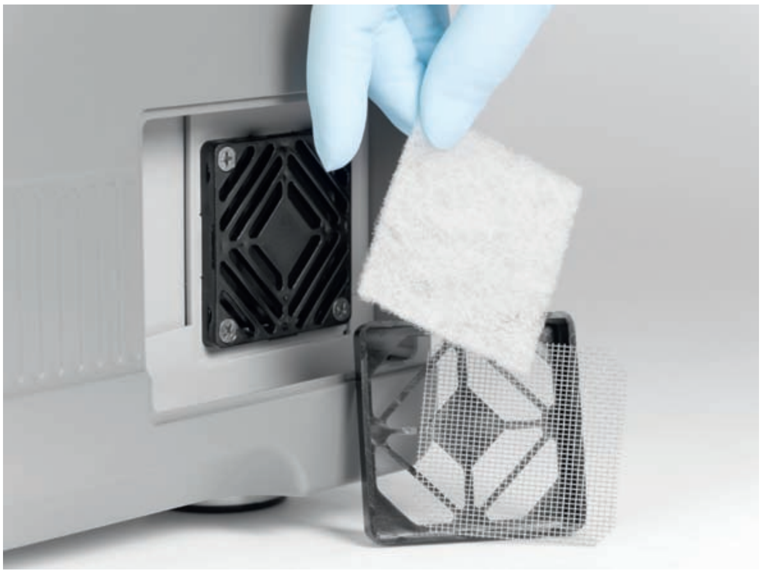
1. Remove the cover of the ventilation inlet.

2. Remove the ventilation dust filter carrier.



D

3 Remove the used dust filter from the carrier and insert a new filter.



4 Replace the dust filter carrier on the ventilation inlet.

5 Close the cover of the ventilation inlet.

D

5 Handling fuses

The LightCycler® 96 Instrument contains two types of fuses:

- Three circuit breakers on the back of the instrument.
- Two electrical backup fuses for the mains power supply (115 V/230 V).

Circuit breakers

The circuit breakers on the back of the instrument can have the following states:

Circuit breaker status	Description
Switch in place	The fuse is working properly.
Switch tripped	The fuse has blown and the circuit is interrupted. The operator can close the circuit by resetting the switch.

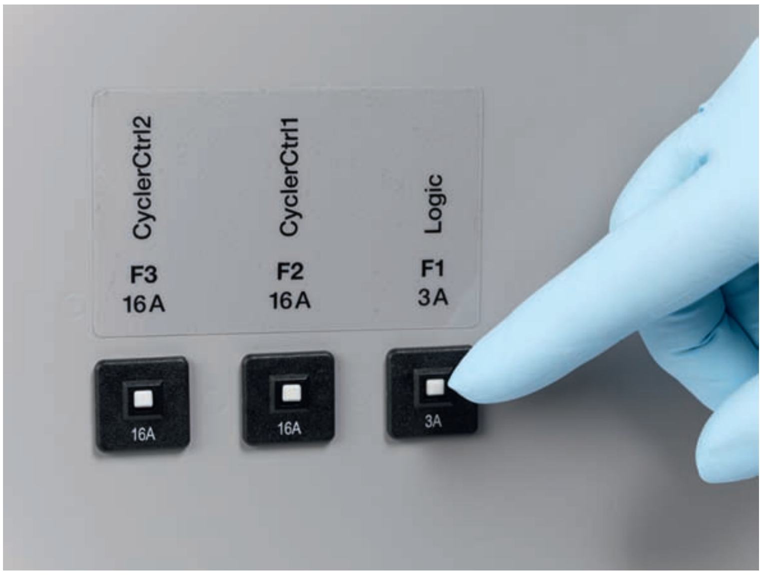


Figure 137: Circuit breakers

Electrical fuses

These fuses must be exchanged by the operator when they are blown. The LightCycler® 96 System Package includes a pack of replacement fuses.

1. Disconnect the instrument.
2. Remove the fuse holder from its fuse chamber.

3. Exchange the blown fuse with a replacement fuse.
4. Place the fuse holder back in the chamber.

Chapter E
Troubleshooting

E

E

Troubleshooting

This chapter provides the messages from the LightCycler® 96 Instrument together with possible causes and corrective actions. It also describes how to use the remote service of the LightCycler® 96 Instrument Software.

1 System messages and errors

The messages and errors which may potentially occur on the LightCycler® 96 Instrument are listed in the tables below. For each message, the probable cause and corrective action typically required for solving the problem are shown. Contact your local Roche Diagnostics representative for troubleshooting assistance.

Data obtained from a run where a system message appeared should be evaluated carefully. If the validity of the results is doubtful, repeat the run.

The LightCycler® 96 Instrument Software issues the following message types:

- Information to support the operator. These messages are not displayed in the message window area and need not to be confirmed. They are only written to the log file for later analysis.
- Warnings which do not stop the task completing. The system may continue working, but not with full performance, or may run into problems later.
- Errors which stop the task completing. The system will stop performing some actions if the operator does not intervene.

Warnings and errors are displayed in the message area of the status bar. For detailed information on displaying and confirming messages, see section *Alarms and messages*, on page 225.

Error code	Module	Type	Message text	Corrective action
17100256	SMStage	Error	Parameters out of range.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
17100272	SMStage	Error	The port is not mapped on the specified device.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
17100512	SMStage	Error	The specified port is already created.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
17100513	SMStage	Error	The command can't be performed because the port was not created.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
17100514	SMStage	Error	The specified input port is used.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
17100545	SMStage	Error	The command can't be performed, because the SM is not initialized.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.

Error code	Module	Type	Message text	Corrective action
17100551	SMStage	Error	Initposition is out of range.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
25700003	Analytical Cycler	Warning	Dynamic integration time for filter <name>[1] limited to <nr>[2] ms.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
26400273	CoverDrive	Error	Screw on the back was released.	The operator can unlock the loading module using the hexagon socket screw on the back of the instrument. In this case the loading module cannot be driven by the motor; see section *Unlocking the loading module*, on page 235.
26400274	CoverDrive	Error	Cover drive over press with <nr>[2] steps.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
26400278	Optics Control	Error	LED temperature is too high.	▶ Switch off the instrument and cool it down. ▶ Check whether the room temperature is within the specification; see section *Environmental parameters*, on page 25. ▶ If the error persists, inform your local Roche representative.
26400279	Optics Control	Error	LED temperature measuring not possible because of NTC circuit overload.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
	Optics Control	Error	PCB Optics Control Read/ Write Error.	▶ Restart the instrument. ▶ Export the log files; see section *Export Logfiles*, on page 223. ▶ Perform a reset to factory settings; see section *Backup and restore*, on page 222. ▶ If the error persists, inform your local Roche representative.
26700001	Optics Control	Error	Photometer has unknown datastructure.	▶ Restart the instrument. ▶ Export the log files; see section *Export Logfiles*, on page 223. ▶ Perform a reset to factory settings; see section *Backup and restore*, on page 222. ▶ If the error persists, inform your local Roche representative.

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Error code	Module	Type	Message text	Corrective action
26700002	Optics Control	Error	Error while comparing checksum of PCB Optics Control.	▶ Restart the instrument. ▶ Export the log files; see section *Export Logfiles*, on page 223. ▶ Perform a reset to factory settings; see section *Backup and restore*, on page 222. ▶ If the error persists, inform your local Roche representative.
26700003	Optics Control	Error	Timeout while controlling Thermocycler.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
26700004	Optics Control	Error	Error during communication with Thermocycler (CSM).	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
26700005	Optics Control	Error	Error during communication with Thermocycler (FWR).	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
26700006	Message Broker	Error	Hardware error received from firmware.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
26700007	Message Broker	Error	Transit error information to parent module.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
26700010	Message Broker	Error	Unhandled error.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
26700011	Message Broker	Error	Generic error sent by a csm.	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
26700012	Instrument software	Error	Could not load and parse experiment: <name>[a].	▶ Check the experiment file. ▶ Call your local Roche representative.
26700013	Instrument software	Error	Error saving lc96 file to <name>[1].	Inform your local Roche representative.

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Error code	Module	Type	Message text	Corrective action
26700017	Instrument software	Error	Error saving <name>[1].	Inform your local Roche representative.
26700018	Instrument software	Error	Error saving <name>[1].	Inform your local Roche representative.
26700019	Instrument software	Error	Error saving <name>[1].	Inform your local Roche representative.
26700020	Instrument software	Error	Conflict for experiment container <name>[1] was not resolved.	Inform your local Roche representative.
26700021	Instrument software	Error	Could not resolve conflict.	Inform your local Roche representative.
26700022	Instrument software	Error	File could not be removed: <name>[1].	Inform your local Roche representative.
26700023	Instrument software	Error	File could not be opened: <name>[1].	▶ Open the experiment with the LightCycler® 96 Application Software. ▶ Inform your local Roche representative.
26700024	Instrument software	Error	Cannot read file as dom document: <name>[1].	Inform your local Roche representative.
26700025	Instrument software	Error	Could not write: <name>[1].	Inform your local Roche representative.
26700026	Instrument software	Error	Could not create temporary directory <name>[1].	Inform your local Roche representative.
26700027	Instrument software	Error	Error unzipping: <name>[1].	Inform your local Roche representative.
26700028	Instrument software	Error	Cannot open lc96 main xml file: <name>[1].	Inform your local Roche representative.
26700029	Instrument software	Error	Cannot read lc96 from main xml file as dom document: <name>[1].	Inform your local Roche representative.
26700030	Instrument software	Error	Cannot open lc96 extension file: <name>[1].	Inform your local Roche representative.
26700031	Instrument software	Error	Cannot read lc96 extension as dom document: <name>[1].	Inform your local Roche representative.
26700032	Instrument software	Error	Cannot write Experiment-Measurements: <name>[1].	Inform your local Roche representative.
26700033	Instrument software	Error	Error while writing experiment definition: <name>[1].	Inform your local Roche representative.
26700034	Instrument software	Error	Error while writing experiment definition: <name>[1].	Inform your local Roche representative.
26700035	Instrument software	Error	Error deleting temporary file for clean overwrite: <name>[1].	Inform your local Roche representative.
26700036	Instrument software	Error	Could not write hashkey <name1>[1] to file <name2>[1].	Inform your local Roche representative.

Error code	Module	Type	Message text	Corrective action
26700037	Instrument software	Error	Could not create archive: <name>[1].	Inform your local Roche representative.
26700038	Instrument software	Error	Could not add path to archive: <name>[1].	Inform your local Roche representative.
26700039	Instrument software	Error	Error while closing archive: <name>[1].	Inform your local Roche representative.
26700040	Instrument software	Error	No experiment element found: <name>[1].	Inform your local Roche representative.
26700041	Instrument software	Error	No channel or programs defined in the experiment.	The minimum experiment definition must have one channel, one program with one cycle, and one valid step.
26700042	Instrument software	Error	Error unzipping archive: <name>[1].	Inform your local Roche representative.
26700043	Instrument software	Error	Could not create temporary directory: <name>[1].	Inform your local Roche representative.
26700044	Instrument software	Error	Could not create temporary directory: <name>[1].	Inform your local Roche representative.
26700045	Instrument software	Error	Error extracting archive: <name>[1].	Inform your local Roche representative.
26700046	Instrument software	Error	Error closing archive: <name>[1].	Inform your local Roche representative.
26700047	Instrument software	Error	Main XML file is not present in the archive: <name>[1].	Inform your local Roche representative.
26700048	Instrument software	Error	Extension XML file is not present in the archive: <name>[1].	Inform your local Roche representative.
26700049	Instrument software	Error	Wrong hashkey (<name1>[1]) found. Expected: <name2>[1] in file <name3>[1]	Inform your local Roche representative.
26700050	Instrument software	Error	Error unzipping: <name>[1].	Inform your local Roche representative.
26700051	Instrument software	Error	Can not open file: <name>[1].	Inform your local Roche representative.
26700052	Instrument software	Error	Can not read lc96 as dom document: <name>[1].	Inform your local Roche representative.
26700053	Instrument software	Error	Can not open file: <name>[1].	Inform your local Roche representative.
26700054	Instrument software	Error	Can not read lc96 as dom document: <name>[1].	Inform your local Roche representative.
26700055	Instrument software	Error	Experiment file %2 has more than one run definition (found: <name>[1]).	Inform your local Roche representative.
26700056	Instrument software	Error	No experiment definition in extension file: <name>[1].	Inform your local Roche representative.

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Error code	Module	Type	Message text	Corrective action
26700057	Instrument software	Error	Dye setting definition missing in the extension file: <name>[1].	Inform your local Roche representative.
26700058	Instrument software	Error	Unexpected data node count: <name1>[1] in file <name2>[1].	Inform your local Roche representative.
26700059	Instrument software	Error	No run node found in: <name>[1].	Inform your local Roche representative.
26700060	Instrument software	Error	No experiment node found: <name>[1].	Inform your local Roche representative.
26700061	Instrument software	Error	Step count doesn't match in <name1>[1], expected <name2>[1], found <name3>[1].	Inform your local Roche representative.
26700062	Instrument software	Error	Error while creating new experiment <name>[1].	Inform your local Roche representative.
26700063	Instrument software	Error	Error while copying <name>[1].	▶ Create a new experiment. ▶ Inform your local Roche representative.
26700064	Instrument software	Error	Error while loading template <name>[1].	Inform your local Roche representative.
26700065	Instrument software	Error	Newly created container has empty experiment... <name>[1].	Inform your local Roche representative.
26700066	Instrument software	Error	Error loading: <name>[1]. Experiment will be removed from overview.	Inform your local Roche representative.
26700067	Instrument software	Error	Trying to remove '<name>[1]' but did not find it on the file system.	Inform your local Roche representative.
26700070	Instrument software	Error	Error opening drawer...	▶ Restart the instrument. ▶ If the error persists, inform your local Roche Diagnostics representative.
26700071	Instrument software	Error	Error aborting experiment...	Inform your local Roche representative.
26700072	Instrument software	Error	Instrument could not start experiment.	Inform your local Roche representative.
26700073	Instrument software	Error	Error saving experiment at run start.	Inform your local Roche representative.
26700074	Instrument software	Error	No experiment selected to load...	Select an experiment to be loaded.
26700075	Instrument software	Error	No experiment selected to load...	Select an experiment to be loaded.
26700076	Instrument software	Error	Error while saving experiment <name>[1].	Inform your local Roche representative.
26700077	Instrument software	Error	Error while adding 10 cycles to current loaded experiment...	Inform your local Roche representative.

Error code	Module	Type	Message text	Corrective action
26700078	Instrument software	Error	Error while adding 10 cycles to current loaded experiment...	Inform your local Roche representative.
26700079	Instrument software	Error	Software update Target was found: <name>[1] but directory on the instrument is not writable.	Inform your local Roche representative.
26700080	Instrument software	Error	Software update Target was not found: <name>[1].	Connect the USB drive with the correct software package.
26700081	Instrument software	Error	Instrument could not execute experiment: <name>[1].	Inform your local Roche representative.
26700082	Instrument software	Error	Could not save experiment to xml: <name>[1].	Inform your local Roche representative.
26700083	Instrument software	Error	Instrument could not abort current experiment.	Inform your local Roche representative.
26700087	Instrument software	Error	Cannot read Boundaries as dom document: <name>[1].	Inform your local Roche representative.
26700088	Instrument software	Error	This is not Boundaries file: <name>[1].	Inform your local Roche representative.
26700089	Instrument software	Error	Predefined programs file doesn't exists: <name>[1].	Inform your local Roche representative.
26700090	Instrument software	Error	Cannot open Predefined programs file: <name>[1].	Inform your local Roche representative.
26700091	Instrument software	Error	Cannot read Predefined programs as dom document: <name>[1].	Inform your local Roche representative.
26700092	Instrument software	Error	Error deleting temporary file for clean overwrite: <name>[1].	Inform your local Roche representative.
26700094	Instrument software	Error	Could not merge experiment <name>[1] from usb-device with instrument, since experiment is running on the instrument.	▶ Remove the USB drive. ▶ Wait till the run is completed.
26700097	Instrument software	Warning	Failed to start remote monitoring server:	▶ Check the network connection. ▶ Call your local Roche representative.
26700102	Instrument software	Error	Error while saving Experiment <name>[1].	Inform your local Roche representative.
26700104	Instrument software	Warning	Unable to start processed experiment. Experiment has to be unprocessed: <name>[1].	Use a non-executed experiment.
26700105	Instrument software	Warning	Experiment <name>[1] has no planned measurements.	Check the experiment settings.
26700106	Instrument software	Warning	Unable to start experiment without programs: <name>[1].	Check the experiment settings.
26700107	Instrument software	Warning	Unable to start experiment without channels: <name>[1].	Check the experiment settings.

Error code	Module	Type	Message text	Corrective action
26700108	Instrument software	Warning	Unable to start experiment without steps: <name>[1].	Check the experiment settings.
26700109	Instrument software	Error	Unable to connect to email server: <name>[1]. (Error: <nr>[2]).	▶ Check the network cable; see section *Installing the LightCycler® 96 Instrument*, on page 42. ▶ Check the network configuration; see section *Configuring the LightCycler® 96 Instrument in the network*, on page 49. ▶ Check the email configuration; see section *Active Emails*, on page 215. ▶ Inform your local Roche representative.
26700110	Instrument software	Error	Unable to login to email server. (Error: <nr>[2]).	Check the email configuration; see section *Active Emails*, on page 215.
26700111	Instrument software	Error	Error while sending mail: <name>[1].	▶ Try again. ▶ Check the network connections; see section *Configuring the LightCycler® 96 Instrument in the network*, on page 49. ▶ Check the email configuration; see section *Active Emails*, on page 215. ▶ Inform your local Roche representative.
26700113	Instrument software	Warning	Wrong or empty server, login or password.	Check the email configuration; see section *Active Emails*, on page 215.
26700114	Instrument software	Warning	No Email Recipients to send mail.	Check the email configuration; see section *Active Emails*, on page 215.
26700116	Instrument software	Warning	More than one run definition (<name1>[1]) in experiment file <name2>[1]. Only first one will be used.	Check experiment settings.

a. <name> here represents a placeholder for the name provided in the message on the LightCycler® 96 Instrument touchscreen.

2. <nr> here represents a placeholder for the number provided in the message on the LightCycler® 96 Instrument touchscreen.

2 Remote service

The LightCycler® 96 Instrument Software includes the Axeda client, which enables an operator to allow remote access by a Roche field service engineer via the Axeda web portal. The application provides a data export function to a shared export folder.

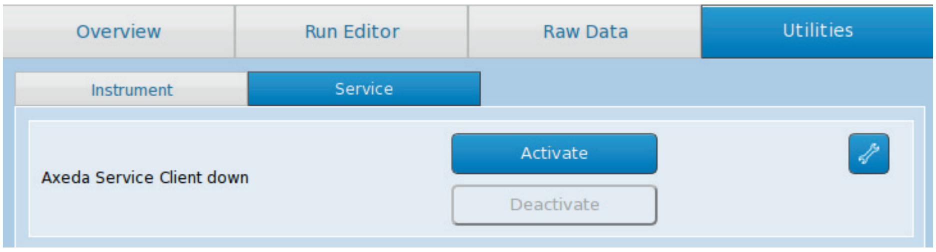
The Axeda client is preinstalled on the LightCycler® 96 Instrument, but has to be configured by a Roche field service engineer before it can be activated. The application is only active after the operator has manually started it. The operator can continue working without restrictions, even if the client is enabled.

The application provides secure connectivity to Roche only. It does not connect to any non-Roche systems.

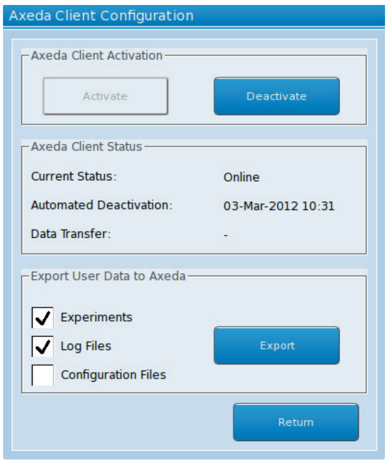
To configure the Axeda client

The Axeda client should only be activated when this is requested by a Roche field service engineer.

1. Switch on the instrument.
2. On the touchscreen, open the *Utilities* tab.
3. Open the *Service* tab.



4. To activate the Axeda client, perform one of the following steps:
   - Next to the *Axeda Service Client down* field, choose *Activate*.
     To configure the Axeda client, proceed with step 6.
   - Choose the tools button. The *Axeda Client Configuration* dialog box opens.
5. To activate the Axeda client, choose *Activate*.



   Proceed with step 7.
6. To configure the Axeda client, choose the tools button. The *Axeda Client Configuration* dialog box opens.

▶ ▶ ▶

7	Choose the files to be exported to the shared export folder. For detailed information on the configuration parameters, see section *Axeda client*, on page 220.
8	The export the files to the shared export folder, choose *Export*.
9	Choose *Return* to close the dialog box.

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Chapter F
Appendix

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Appendix

1 Ordering information

Roche Applied Science provides a large selection of isolation reagents and systems for life science research. For a complete overview of related products and guides, please visit our Special Interest Site for Real-Time PCR Systems at *www.lightcycler96.com*.

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