Roche Applied Science

LightCycler® 480 Instrument Operator's Manual

Software Version 1.0

Prologue

I. Revision History

Version	Revision Date
1.0	September 2005

Questions or comments regarding the contents of this manual can be directed to the address below or to your Roche representative.

Roche Diagnostics GmbH
Roche Applied Science
Customer Support
Nonnenwald 2
82372 Penzberg, Germany

Every effort has been made to ensure that all the information contained in the *LightCycler® 480 Instrument Operator´s Manual* is correct at the time of printing.

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 Instrument Center AG Forrenstrasse CH-6343 Rotkreuz Switzerland
	Distribution	Roche Diagnostics GmbH Sandhofer Straße 116 D-68305 Mannheim Germany
	Distribution in USA	Roche Diagnostics 9115 Hague Road PO Box 50457 Indianapolis, IN 46250 USA

III. Declaration of Conformity

CE	The instrument meets the requirements laid down in Council Directive 89/336/EEC relating to "Electromagnetic Compatibility" and Council Directive 73/23/EEC relating to "Low Voltage Equipment". The following standards were applied: IEC/EN 61326 (EMC) and IEC/EN 61010-1 (Safety).
c UL US	UL 61010-1 Electrical Equipment for Measurement, Control and Laboratory Use; Part 1: General Requirements CAN/CSA-C22.2 No. 61010-1 (Second Edition) – Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use; Part 1: General Requirements

IV. Warranty

Information on warranty conditions are specified in the sales contract. Contact your Roche representative for further information.

Any unauthorized modification of the instrument entails the invalidity of the guarantee and service contract.

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VI. Intended Use

The LightCycler® 480 Instrument is intended for performing rapid, accurate polymerase chain reaction (PCR) in combination with real-time, online detection enabling absolute or relative quantification of a target nucleic acid, as well as post-PCR analysis of the amplified nucleic acid by melting curve analysis.

The LightCycler® 480 Instrument is intended for **general laboratory use** and must be used exclusively by laboratory professionals trained in laboratory techniques and having studied the instructions for use of this instrument. **The LightCycler® 480 Instrument is not intended for use in diagnostic procedures.**

VII. License Statements for the LightCycler® 480 Instrument

This LightCycler® 480 Real-Time PCR System is an Authorized Thermal Cycler. Its purchase price includes the up-front fee component of a license under the non-U.S. counterparts of U.S. Patents Nos. 4,683,202, 4,683,195 and 4,965,188 owned by F. Hoffmann-La Roche Ltd. ("Roche"), covering the Polymerase Chain Reaction (PCR) process, to practice the PCR process for internal research and development using this instrument. The running royalty component of that license may be purchased from Applied Biosystems or obtained by purchasing Authorized Reagents. This instrument is also an Authorized Thermal Cycler for use with applications licenses available from Applied Biosystems. Its use with Authorized Reagents also provides a limited PCR license in accordance with the label rights accompanying such reagents. Purchase of this product does not itself convey to the purchaser a complete license or right to perform the PCR process.

This LightCycler® 480 Real-Time PCR System is a real-time thermal cycler licensed for use in research under U.S. Patent No. 6,814,934 and corresponding claims in its non-U.S. counterparts, and under one or more of U.S. Patents Nos. 5,038,852, 5,656,493, 5,333,675, or corresponding claims in their non-U.S. counterparts, owned by Applera Corporation. No right is conveyed expressly, by implication or by estoppel under any other patent claim, such as claims to apparatus, reagents, kits, or methods such as 5' nuclease methods. This instrument is for research use only. For further information on purchasing licenses other than for *in vitro* diagnostics, contact the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Parts of the Software used for the LightCycler® 480 System are licensed from Idaho Technology Inc., Salt Lake City, UT, USA.

The LightCycler® 480 Instrument and LightCycler® 480 Basic Software is covered in-part by US 5,871,908, co-exclusively licensed from Evotec OAI AG

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IX. Preamble

Before setting-up operation of the LightCycler® 480 Instrument, it is important to read this Operator´s Manual thoroughly and completely. Non-observance of the instructions contained in this manual could entail safety hazards.

X. Usage of the LightCycler® 480 Instrument Operator's Manual

This Operator´s Manual assists with operating the LightCycler® 480 Instrument. It contains the following chapters:

Chapter A Overview contains a short introduction to the operating mode of the LightCycler® 480 Instrument and describes the system´s specifications.

Chapter B System Description contains instructions on the installation of the LightCycler® 480 Instrument and a description of the system´s components and consumables.

Chapter C Operation describes the operating procedures for the LightCycler® 480 Instrument.

Chapter D LightCycler® 480 Basic Software contains instructions for programming LightCycler® 480 Instrument runs and performing data analysis.

Chapter E Maintenance describes the maintenance procedures that are required for the LightCycler® 480 Instrument.

Chapter F Appendix contains the Index and Ordering Information.

XI. Conventions Used in this Manual

Text Conventions

To impart information that is consistent and memorable, the following text conventions are used in this Operator´s Manual:

Numbered Listing	Steps in a procedure that must be performed in the order listed.
Italic type, blue	Points to a different chapter in this Operator´s Manual which should be consulted.
Italic type	Describes how to proceed when operating the LightCycler® 480 Basic Software.
Asterisk (*)	Denotes a product available from Roche Applied Science.

Symbols

In this Operator's Manual symbols are used as an optical signal to point out important things.

Symbol	Heading	Description
	WARNING	This symbol is used to indicate that noncompliance with instructions or procedures could lead to physical injury or even death or could cause damage to the instrument. Consult the Operator´s Manual.
	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.
	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.

The following symbols appear on the instrument

	MANUFACTURER OF DEVICE	On the instrument type plate.
	CE MARK	The CE mark on the instrument type plate expresses conformity with requirements of the directives relevant for this instrument.
	cUL MARK	On the instrument type plate
	CONSULT THE OPERATOR'S MANUAL	On the instrument type plate
	HOT SURFACE	1. On the margin of the multiwell plate loader. 2. On the surface of the block cycler cover. 3. On the surface of the block cycler unit. 4. On the Xenon lamp unit.
	BIO HAZARD	On the margin of the multiwell plate loader.

XII. Warnings and Precautions

The LightCycler® 480 Instrument must only be used by trained and skillful personnel.

It is essential that the following safety information required for installation and operation of the LightCycler® 480 Instrument are carefully read and observed. Please assure that this safety information is accessible for every employee working with the LightCycler® 480 Instrument.

Handling Requirements

The LightCycler® 480 Instrument is an electromechanical instrument. There is a potential danger for the user of an 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® 480 Instrument is connected to the mains.
- ▶ Never touch switches or power cord with wet hands.
- ▶ Do not open the housing while the LightCycler® 480 Instrument is connected to the main power supply.
- ▶ Never clean the instrument without turning the instrument power switch off and disconnecting the power cord.
- ▶ Users may replace fuses and the Xenon lamp if they follow the procedures described in in this Operator's Manual. Any other electrical modification is not allowed and could render the warranties on the LightCycler® 480 Instrument null and void.
- ▶ Only authorized service personnel are allowed to perform service or repairs required for this unit.

- ▶ Do not open the block cycler compartment during operation.
- ▶ Always wear safety goggles and gloves when dealing with toxic, caustic or infectious materials.

- ▶ Although working with highly purified nucleic acids, please regard for your own safety 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 spreading contamination to laboratory personnel or equipment.
- ▶ Please refer to Section *Maintenance* to find instructions for cleaning the LightCycler® 480 Instrument.

- ▶ The multiwell plate holder, the thermal block cycler, the block cycler cover, and the Xenon lamp are hot while the instrument is operating.

General Precautions

	The LightCycler® 480 Instrument PC and other PCs running LightCycler® 480 Software are not designed to be connected to a network. The connection to networks contains an inherent risk to be infected through viruses and worms as well as targeted attacks through malicious attackers through the network. In the event that you connect to a network, Roche shall not be liable for any kind of damages.
	Microsoft Office and Norton Antivirus software are tested not to interfere with LightCycler® 480 Software and LightCycler® 480 software modules. Any other additional software must not be installed on the LightCycler® 480 Instrument PC. Installation of any other additional software on the Light-Cycler® 480 Instrument PC presents the risk of interference with LightCycler® 480 Basic Software and LightCycler® 480 software modules, and could affect result security.
	**Anti-virus software is not provided.** Therefore, it is essential to take precautions to ensure that any software loaded onto the LightCycler® 480 Instrument PC is virus-free.
	It is not allowed to connect two LightCycler® 480 Instruments to one data station.
	Make sure the main switch is freely accessible.
	Incorrect location can cause incorrect results and damage to the equipment parts. Follow the installation instructions carefully. Moving the instrument must be performed only by qualified Roche Service personnel.
	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 a fire. Keep the cover closed while the instrument is connected to the mains and do not use sprays in the vicinity of the LightCycler® 480 Instrument. During firefighting operations, disconnect the LightCycler® 480 Instrument from the mains.
	Do not manipulate the instrument.

Electrical Safety

The LightCycler® 480 Instrument is designed in accordance with Protection Class I (IEC). The chassis/housing of the instrument is connected to protection earth (PE) by means of a cord. For protection against electrical shock hazards, the instrument must be directly connected to an approved power source such as a three-wire grounded receptacle for the 230 V line. Where an ungrounded receptacle is encountered, a qualified electrician must replace it with a properly (PE) grounded receptacle in accordance with the local electrical code. An extension must not 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 user attempt to modify or deliberately defeat the safety features of this instrument. If the power cord becomes cracked, frayed, broken, or otherwise damaged, it must be replaced immediately with the equivalent part from Roche Diagnostics.

Warnings should be consulted as regards interactions and non-recommended functions. The likely scope for misuse must be considered. It is advisable to draw attention to the likely consequences.

XIII. Disposal of the Instrument

The instrument must be treated as biologically contaminated-hazardous waste. Decontamination (*i.e.*, a combination of processes, including cleaning, disinfection and/or sterilization) is required before reuse, recycling or disposal.
Dispose the instrument according to local and/or labor regulations.
For more information contact your local Roche Support personnel.

Overview

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Chapter A • Overview

Short introduction to the operating mode of the LightCycler® 480 Instrument and description of system specifications

A

A	Overview	Page

Overview

1. Introduction

The LightCycler® 480 System enables you to perform real-time, online PCR combined with rapid cycling of up to 96 or 384 samples (depending on the thermal block cycler installed). Results can be quantified and analyzed simultaneously by monitoring fluorescence during nucleic acid amplification. The optical detection system enables the performance of multiplex PCR applications with sequence-specific detection using various available probe formats (*e.g.,* HybProbe probes, SimpleProbe probes, hydrolysis probes, or any other probe format; fluorophores must match excitation and emission filters of the LightCycler® 480 Instrument). Sequence-independent, online detection in monoplex PCR applications can be performed using SYBR Green I dye. Melting curve analysis allows genotyping (single nucleotide polymorphism detection) (when using sequence-specific fluorophore-labeled probes, *i.e.*, HybProbe or SimpleProbe probes) or PCR product characterization (when using SYBR Green I dye). For more details on the methodology and data analysis, refer to Section *LightCycler® 480 Basic Software*.

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2. Specifications of the LightCycler® 480 Instrument

A summary of the LightCycler® 480 Instrument specifications is given below.

The LightCycler® 480 Instrument is equipped with a block cycler unit accommodating either 96- or 384-well format:

LightCycler® 480 Instrument, 96-wells	Cat. No. 04 640 268 001
LightCycler® 480 Instrument, 384-wells	Cat. No. 04 545 885 001

2.1 General

Dimensions	57.4 × 58.8 × 49.7 cm (W × D × H)
Weight	55 kg
Power supply/consumption	200 – 240 Vac 50/60 Hz 1500 VA
Noise level	< 60 dB (A)
Protection class	I
Installation/overvoltage category	II
Electromagnetic emission	Class B

2.2 Environmental Parameters

Temperatures allowed during transportation/storage/pakkaging	-25°C to +60°C
Relative humidity allowed during transportation/storage/packaging	10% to 95%, no condensation
Temperatures allowed during operation	+15°C to +32°C
Relative humidity allowed during operation	Max. 80% at 32°C, no condensation Min. 30%, at +15°C to +32°C
Altitude/pressure allowed during operation	0 – 2000 m above sea level 80 – 106 kPa

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2.3 Interfaces

The LightCycler® 480 Instrument provides the following external interfaces:

Interface	Device
IEEE1394	Interface to CCD camera (for service only)
PS/2	External bar-code scanner through keyboard wedge
LAN 10/100 Base T	Connection to data station for instrument control and data transfer

2.4 Sample Capacity

Number of samples per run	96 or 384
Sample volume	▶96-well thermal block cycler: 10 – 100 µl ▶384-well thermal block cycler: 3 – 20 µl

2.5 Shipping

The LightCycler® 480 Instrument is shipped in a palletized Styrofoam container encircled by a cardboard box.

The original shipping container must be transferred unopened to the installation site. On delivery, carefully inspect the containers. Make a note of any indications of physical damage, and record your observations in the accompanying shipping documents. It is essential that you report any suspected damage immediately to Roche Diagnostics and to the shipping agent before accepting the unit.

Use only the original packaging for transportation or relocation of the LightCycler® 480 Instrument.

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2.6 Data Station

A fully equipped data workstation is delivered by Roche with the LightCycler® 480 Instrument.

The LightCycler® 480 Basic Software will be installed on the data workstation only when the system is installed by a Roche Diagnostics service engineer at your site.

The data station complies with the requirements of the following European Directives:

- Low Voltage Equipment 73/23/EEC
- Electromagnetic Compatibility 89/336/EEC

In addition (for customers in the USA) the data station is certified by Underwriters Laboratories Inc., USA, with respect to electrical and mechanical safety. Consequently the data station is marked with a UL and a CE mark.

By using special software (laboratory information management system, LIMS) it is possible to access the LightCycler® 480 data station by remote control and to combine it, for example, with an automated robotic plate-loading system. To enable this functionality, you must install and activate (by obtaining a valid software user license) the optionally available LightCycler® 480 LIMS/Bar-Code Module. Contact your Roche representative for more information.

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3. Specifications of the Detection Unit

3.1 Excitation

Type	Xenon lamp (Osram XBO R 100W/45 OFR)
Wavelength	435 - 630 nm
Luminous intensity	10 µW/mm²
Wattage	100 W
Lifetime	> 500 h

3.2 Detector

Type	Cooled monochrome CCD camera.
Resolution	1024 × 1344 pixel
Wavelength	490 - 680 nm
Integration time	10 ms to 10 s
Integration time selection	Dynamic or manual
Sensitivity	< 0.4 nmol/l fluorescein, typically 0.1 nmol/l (20 µl reaction volume)
Reproducibility	CV ≤0.15% (50 nmol/l fluorescein)
Crosstalk well-to-well	< 0.2% optically < 0.02% with software correction

3.3 Filter

	Bandpass	Half Band Width (HBW)
Excitation wavelengths (nm)	450 nm 483 nm 523 nm 558 nm 615 nm	30 nm 35 nm 20 nm 30 nm 30 nm
Detection wavelengths (nm)	500 nm 533 nm 568 nm 610 nm 640 nm 670 nm	20 nm 20 nm 20 nm 20 nm 20 nm 20 nm

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4. Specifications of the Thermal Block Cycler

Temperature control	Peltier-based heating and cooling
Temperature range	37 – 95°C
Heating rate	4.8 °C/s
Cooling rate	2.5 °C/s

5. Specifications of the Multiwell Plate Bar-Code Scanner

The multiwell plate bar-code scanner is an integral part of the block cycler unit. It is used for automated identification and identifier (ID) tracking of PCR multiwell plates. During plate loading, the linear bar-code present on the LightCycler® 480 Multiwell Plates is scanned.

To use the multiwell plate bar-code scanner, you must obtain the LightCycler® 480 LIMS/Bar-Code Module with a valid user license. Contact your local Roche representative for details.

Supported bar-code types	▶ Code 39 (250 - 500 µm; Code with Checkdigit, min. code length = 2) ▶ Code 2 of 5 (250 - 500 µm; Code with Checkdigit, min. code length = 2) ▶ Code 128 (250 - 500 µm; min. code length = 2)
Maximum label size	68.0 × 6.5 mm

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6. Specifications of the Handheld Bar-Code Scanner

You can purchase a handheld bar-code scanner for the LightCycler® 480 Instrument as an optional accessory:

LightCycler® 480 Bar-Code Scanner	Cat. No. 04 710 606 001

The handheld bar-code scanner is connected to the data workstation via the keyboard.

Use the handheld bar-code scanner to scan information from bar codes into text input fields of LightCycler® 480 Basic Software.

Note that the type of handheld bar-code scanner is subject to change without notice. The specifications listed below apply to the type provided at the time of publishing of this Operator´s Manual.

Interface

Keyboard Wedge of PC AT & PS/2

Supported bar-code types

- Code 39 (250 - 500 µm; Code with Checkdigit, min. code length = 2)
- Code 2 of 5 (250 - 500 µm; Code with Checkdigit, min. code length = 2)
- Code 128 (250 - 500 µm; min. code length = 2)

System Description

B

Chapter B • System Description
Instructions for the installation of the LightCycler® 480 Instrument and a description of system components and consumables

B

System Description

1. System Package

The table below lists all components delivered with the LightCycler® 480 System package. Use this list to check the completeness of all components.

After opening, check for damage that occurred in transit. Report any visual damage to your local Roche Diagnostics representative.

Quantity	Component
1	LightCycler® 480 Instrument, 96-wells or LightCycler® 480 Instrument, 384-wells
1	LightCycler® 480 data workstation (incl. monitor)
1	LightCycler® 480 accessory package
1	LightCycler® 480 Operator's Manual
1	LightCycler® 480 Basic Software 1.0 installation CD-ROM
10	LightCycler® 480 Multiwell Plate 384
10	LightCycler® 480 Multiwell Plate 96
1	Cable Mains Power (EU)
1	Cable Mains Power (US)
1	LAN Cable (3 m)
1	Xenon Reflector Lamp
4	Ventilation Dust Filters
1	Sealing Foil Applicator
1	Protective Cap Lens
1	Protective Cap CCD Camera
1	Protective Cap Light Guide
10	Fuse 5×20 1.6AT 250V ULR/IEC
10	Fuse 5×20 3.15AT 250V ULR/IEC
10	Fuse 5×20 8AT 250V ULR
10	Fuse 5×20 T 10A H 250V ULR/IEC
10	Fuse 5×20 16AT 250V ULR

2. Installation

2.1 Installation Requirements

- ▶ Place the LightCycler® 480 Instrument on a level surface in the upright position.
- ▶ Do not place the LightCycler® 480 Instrument next to instruments that cause vibration, electromagnetic interference, or have high inductance (*e.g.*, refrigerators, centrifuges, or mixers).
- ▶ Peripheral instruments connected to the LightCycler® 480 Instrument must meet the IEC 950 (UL 1950) standard.
- ▶ All plugs used with the LightCycler® 480 System (instrument, data workstation, monitor) should have the same phasing to prevent switch-on peaks and electronic noise generated by other instruments or by the power supply itself.
- ▶ Use only the power cables and LAN connector supplied with the system package.
- ▶ Do not place the instrument in direct sunlight or close to radiators or heating devices.

The LightCycler® 480 Basic Software is installed by a Roche Diagnostics service engineer.

2.2 Space and Power Requirements

Place the LightCycler® 480 Instrument on a site that can support the following instrument requirements:

Dimensions	The LightCycler® 480 Instrument is 57.4 cm wide, 58.8 cm long and 49.7 cm high.
Weight	The LightCycler® 480 Instrument has a weight of approximately 55 kg.
Power	▶ The LightCycler® 480 Instrument operates at 200 – 240 V (50/60 Hz). ▶ The instrument can be connected to a single-phase or dual-phase supply only. The mains current consumption capacity must not be exceeded. ▶ There are no special provisions for protective 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.* *Do not under any circumstances attempt to modify or deliberately override the safety features of this system.* ▶ The LightCycler® 480 Instrument uses 1500 VA maximum. The data workstation consumes approximately an additional 500 VA. *Depending upon the quality of electrical grounding of the local mains, an uninterruptable power supply (UPS) could be required. An UPS is not provided with the LightCycler® 480 Instrument. We recommend that you contact a local supplier that can provide a UPS in accordance with the electrical requirements specifications. If the instrument will be connected to a UPS, the following requirement should be met: "Online / Direct Mode".*

Ventilation

There are no specific ventilation requirements other than to ensure the following:

- ▶ The ventilation inlet of the electronic rack (1) is not obstructed. The ventilation inlet is located in the upper left corner of the back instrument panel. There should be a horizontal gap of at least 4 cm between this inlet and any surrounding wall, partition or other obstacle.
- ▶ The ventilation outlets (2) of the thermal block cycler are not obstructed. These outlets are located in the lower right corner of the back instrument panel and the lower left corner of the left instrument panel. Do not place anything in front of these outlets.

Figure 1: Ventilation inlet (1) and outlet (2) at the rear side of the instrument

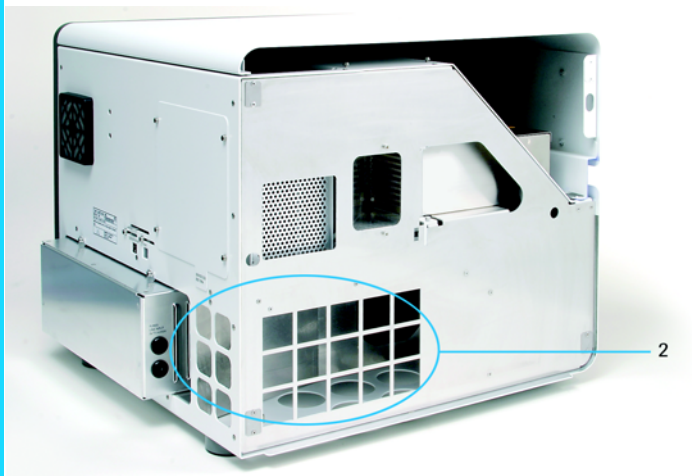

Figure 2: Ventilation outlet (2) at the rear and left side side of the instrument (left side panel dismantled)

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Ventilation	▶ The ventilation inlet of the thermal block cycler (3) and the ventilation inlet of the power box (4) are not obstructed. These inlets are located in the lower right side of the instrument.  **Figure 3: Ventilation inlets at the right side of the instrument**
Access	▶ A gap of 4 cm is recommended between the back of the instrument and the wall, to allow access to the power supply fan filter and the electronic rack. ▶ A minimum clearance of 40 cm must be available on the right side of the instrument to allow access to the block cycler unit and electronic rack fan filter, and to enable safe ejection and loading of the multiwell plate loader. ▶ A gap of 20 cm is required on the left side of the instrument to allow access to the instrument side cover. ▶ A gap of 5 cm is required above the instrument cover to allow the cover to be lifted.

2.3 Environmental Requirements

The LightCycler® 480 Instrument has been designed to safely operate within specifications according to CE and UL certified technical standards at ambient room temperatures between 15°C and 32°C, relative humidity between 30% and 80% (no condensation) and at an altitude up to 2000 meters above sea level (850 – 1050 hP). Atmospheric conditions should conform to Pollution Degree II.

⚠ *Environmental conditions that exceed these specifications could result in instrument failure or could cause incorrect test results.*

⚠ *Keep the instrument in a dry place. Moisture could cause malfunction.*

2.4 Installation of the LightCycler® 480 Instrument

The original shipping containers must be transferred unopened to the installation site. The LightCycler® 480 Instrument should be unpacked and installed only by authorized Roche Diagnostics service personnel. In this case, you need take no further action until the arrival on site of authorized Roche Diagnostics service personnel. Should this not be possible, follow these steps to install the instrument successfully:

► Unpack the instrument:

Carefully inspected the container for damage. Report any damage to your local Roche Diagnostics office before accepting the unit.

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

To carry the instrument, place your hands under the base of the instrument. For this purpose, the instrument base plate provides four recessed carrier grips.

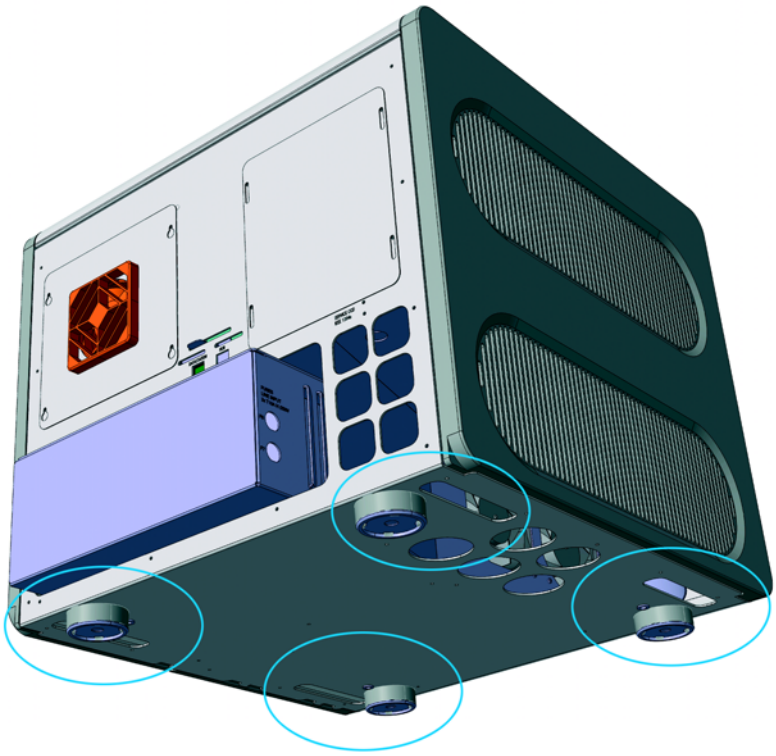


Figure 4: Scheme showing the location of carrier grips at the instrument base plate.

Allow sufficient space to the left, right and behind the instrument to ensure sufficient cooling of the electronic components (for details, see Section *Space and Power Requirements*). Ensure that there is absolutely nothing placed below the base or behind the rear of the LightCycler® 480 Instrument (*e.g.*, paper, plastic film, etc.). For details, see Section *Space and Power Requirements*.

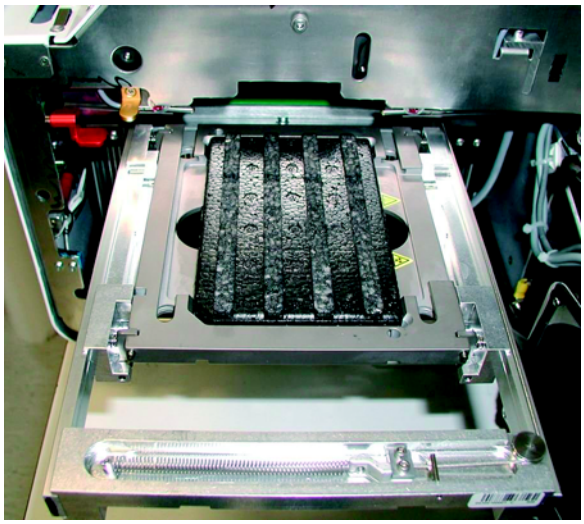
Failure to provide the proper ventilation space could cause damage to the instrument due to overheating.

► Remove the transport locking device:

The transport locking device is a foam part that is inserted into multiwell plate loader to prevent it from unwanted movement during transport.

Remove the transport locking device during the installation process before the instrument is switched on. Otherwise, the block cycler door will be locked and the multiwell plate loader will be deadlocked due to motor movement.

1. To remove the transport locking device, open the block cycler door and manually pull out the multiwell plate loader. Remove the transport locking device, pull back the drawer.



Do not discard the transport locking device. Keep it in the accessories box.

2. Pull out the thermal block cycler. Remove the multiwell plate that is placed on the plate mount. Pull back the thermal block cycler and close the door.

► **Connect the power cable:** The LightCycler® 480 Instrument runs off a single-phase mains supply. Connect the instrument to the mains using the cable supplied. The main voltage input circuits are located at the lower back of the instrument.

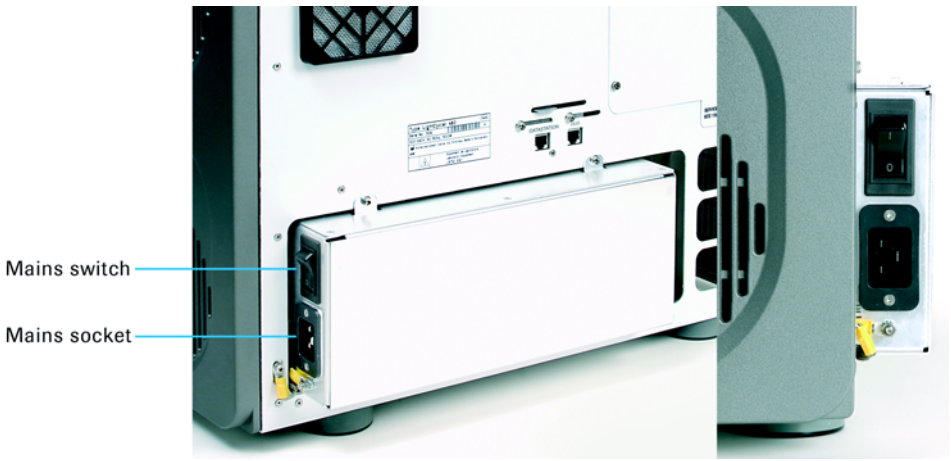


Figure 5: Location of mains switch and socket at the instrument's power box.

The LightCycler® 480 Instrument is delivered with a 2 m long, standard detachable power supply cord. There are two versions of the power cord, one for North America and one for Europe.

Do not touch mains cables when your hands are wet. Do not attempt to connect or disconnect either of the mains 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.

► Unpack the components of the LightCycler® 480 data workstation (*i.e.*, computer, keyboard, mouse, and monitor).

► Place the data station components beside the LightCycler® 480 Instrument and connect the power cables.

We recommend placing the data workstation to the left of the LightCycler® 480 Instrument to provide free access to the multiwell plate loader and the block cycler door which are located in the right instrument panel. Also, power outlets for all data station components should be easily accessible. Use a multiple-outlet distributor plug.

Ensure that the PC and monitor have been set to the correct voltage.

Follow the same precautions regarding handling of power cables as stated above.

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► **Connect the network cable:** The LAN (10/100 Base T) interface with the LAN mode switch is located in the center of the instrument back panel.

Use the LAN cable provided with the instrument. The LAN cable must not be longer than 3 m.

Figure 6: LAN interfaces and LAN mode switch at the rear side of the instrument

The LightCycler® 480 Instrument can communicate with the data workstation in two different ways. The mode that is used is defined by the setting of the LAN mode switch:

LAN Communication Mode	LAN Mode Switch Setting	LAN Interface Used
DATASTATION The LightCycler® 480 Instrument is connected directly (peer-to-peer) to the data workstation using the supplied LAN network cable.	Left	Left
HUB The LightCycler® 480 Instrument is connected indirectly to the data station via a network hub.	Right	Right

In combination with LightCycler® 480 Basic Software Version 1.0, use only the DATASTATION communication mode.

► Plug the network cable into the corresponding LAN (10/100 BT) interface on the back of the PC.

► **(Optional) Connect the handheld bar-code scanner:** Connect bar-code scanner and keyboard using the Y-interface cable. Connect the lilac-colored keyboard plug with one of the paired connectors of the Y-cable. Connect the free paired connector of the Y-cable to the lilac-colored interface at the back of the PC.

► Connect mouse, monitor and printer (optional) to the back of the computer. The connectors are shaped to ensure connection only in the proper orientation. For more information on connecting the printer to the PC, refer to the manual that is delivered with the printer. The connectors are color-coded for easy matching (mouse=green, monitor=blue, printer=purple). Ensure that the color of the connector matches the color of the plug.

A printer is not included in the LightCycler® 480 System package.

3. System Description

3.1 Description of the LightCycler® 480 Instrument

The LightCycler® 480 Instrument is a rapid thermal block cycler with integrated real-time, online detection capabilities. This set-up enables homogeneous PCR to be performed, *i.e.*, simultaneous amplification and detection of target nucleic acids. Detection of target nucleic acid is performed by adding either a fluorescent double-stranded-DNA-specific dye or sequence-specific oligonucleotide probes labeled with fluorophores. Both approaches allow measuring the generation of PCR products during amplification, the basis of quantitative PCR (qPCR). Post-PCR analysis of previously generated PCR products by melting curve analysis is either used for PCR product characterization or detection of mutations (*i.e.*, single nucleotide polymorphisms). The possibility to freely combine five excitation and six emission filters allows analysis of signals from multiple dyes in multiplex PCR assays. For details on available detection formats, see Section *Real-Time PCR Detection Formats for the LightCycler® 480 System*.

The main building blocks of the LightCycler® 480 Instrument are the following:

- **Block cycler unit**, including exchangeable thermal block cycler with block cycler cover (available in two versions: for 96- or 384-multiwell PCR plates), ventilation, multiwell plate loader, and multiwell plate bar-code scanner

- **Detection unit** consisting of the following
  - **Lamp unit**, housing the Xenon excitation lamp
  - **Optics unit,** including the liquid light guide, emission and detection filters wheel, and the CCD camera

All components are assembled on the instrument chassis and shielded by the instrument housing.

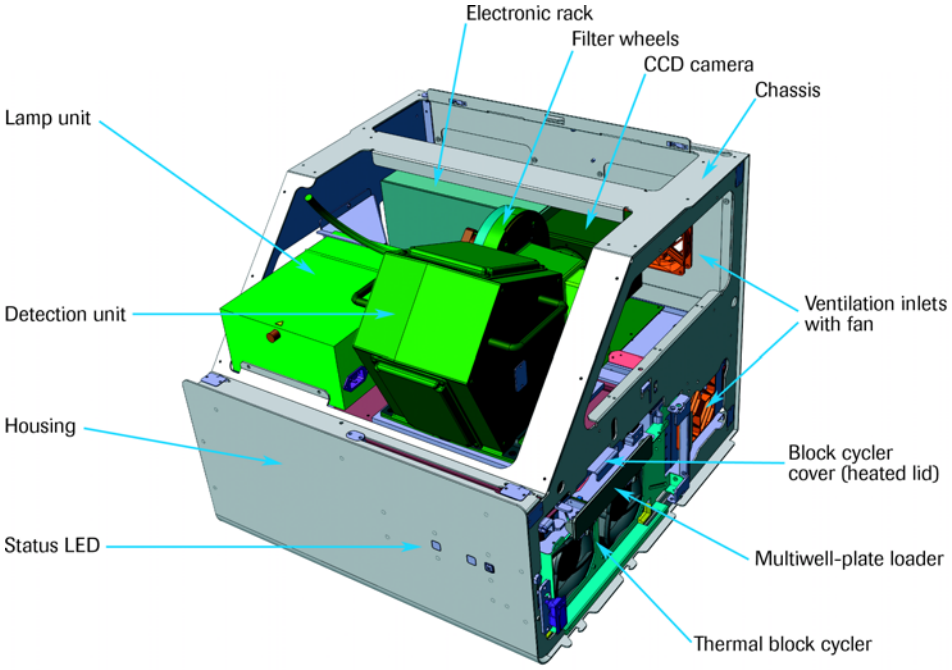

Figure 7: Scheme showing the main building blocks of the LightCycler® 480 Instrument.

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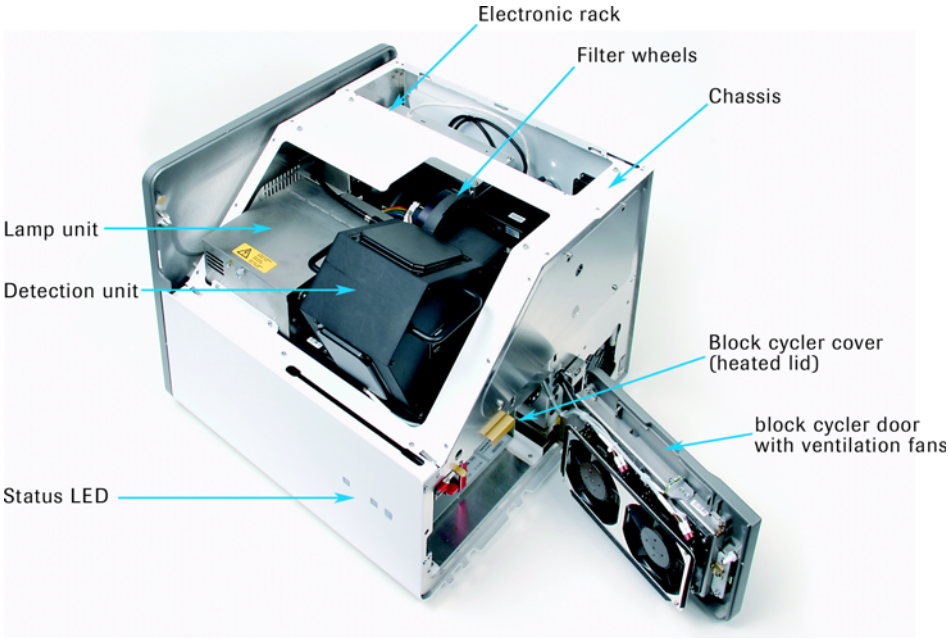

Figure 8: Main building blocks of the LightCycler® 480 Instrument (instrument cover removed)

The front of the LightCycler® 480 Instrument provides two status LEDs which inform the user of the hardware status. The push button for opening and closing the multiwell plate loader is located next to the two LEDs. The instrument cover can be lifted and moved to the right from the front to access the internal instrument components. For instance, the instrument cover must be lifted to exchange the Xenon lamp. (For details about exchanging the Xenon lamp, see Section *Maintenance*.)

Figure 9: Front view of the LightCycler® 480 Instrument

The right side of the instrument provides the PCR multiwell-plate loading mechanism as well as access to the thermal block cycler through the block cycler door. The multiwell plate loader is ejected and retracted by pressing the push button on the front of the instrument. (For more information about loading a multiwell plate, see Section *Preparing and Starting a LightCycler® 480 Instrument Run*.) If you use both available block cycler versions (*i.e.*, for 96- and 384-well PCR plates), open the block cycler door to exchange the thermal block cycler and its corresponding heated block cycler cover. (For more details about exchanging the thermal block cycler, see Section *Exchanging the LightCycler® 480 Thermal Block Cycler*.)

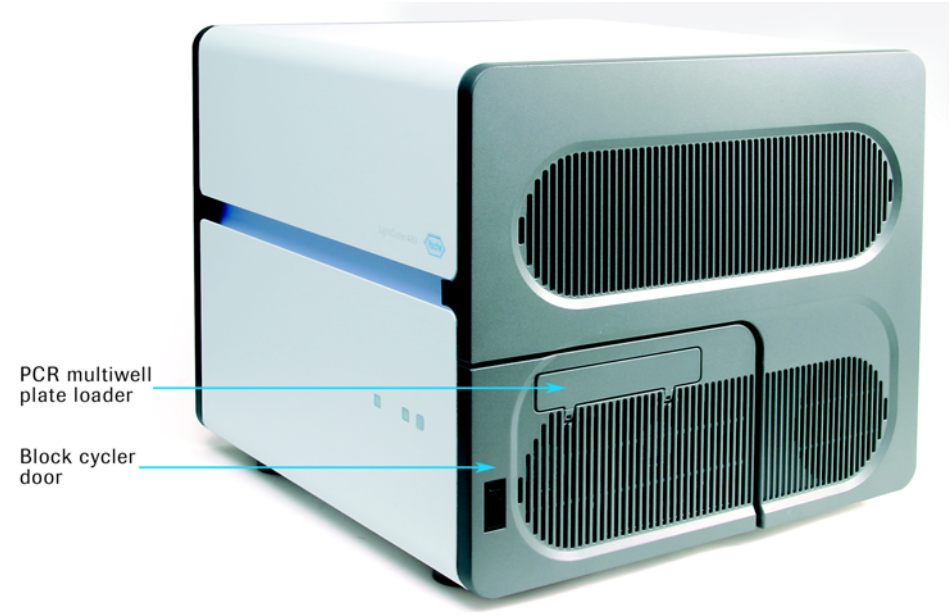

Figure 10: View of the right side of the instrument

Figure 11: View of an ejected multiwell plate loader

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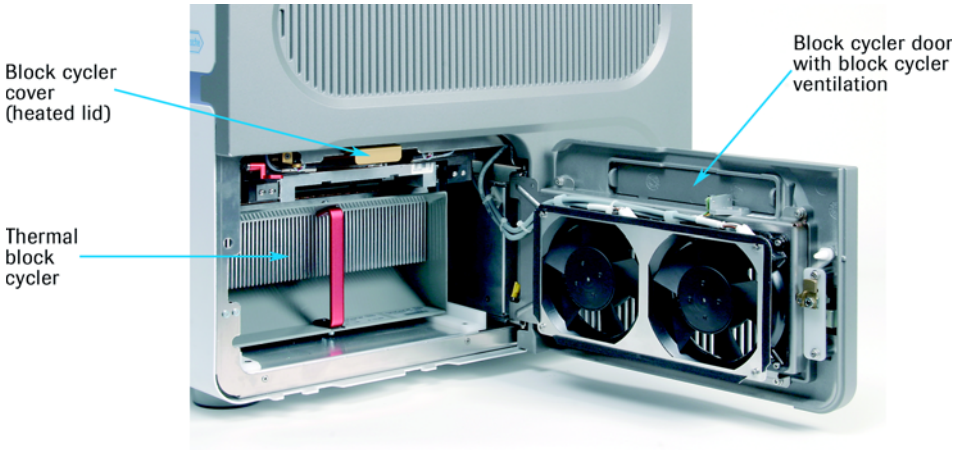

Figure 12: Block cycler door opened allowing a view of the inserted thermal block cycler and block cycler cover

The back of the instrument houses the power box with instrument mains and mains switch and the LAN interface required for connecting the LightCycler® 480 Instrument to the data workstation. (For details about instrument mains and LAN connection, see Section *Installation*.) The ventilation inlet (for the instrument electronic rack; equipped with a dust filter) and ventilation outlet (for the thermal block cycler and power box) are located on the back of the instrument.

Figure 13: View of the back of the LightCycler® 480 Instrument

The LightCycler® 480 Basic Software is an integral part of the system and controls both the PCR process (including detection) and the successive data analysis and data output. (LightCycler® 480 Basic Software is described in detail in Section *LightCycler® 480 Basic Software*.)

The LightCycler® 480 Instrument works in combination with specially designed PCR reagent kits. Optimal performance of the system is achieved only by using the LightCycler® 480 Instrument in combination with the dedicated LightCycler® 480 reagent kits and LightCycler® 480 disposables.

3.2 Description of the Block Cycler Unit

The block cycler unit consists of the following main components:

- Thermal block cycler, which includes the multiwell plate mount, Peltier elements, Therma-Base, cooling elements (heat sink) and electronics interface
- Block cycler cover (heated lid)
- Multiwell plate loader
- Multiwell plate detector
- Multiwell plate bar-code scanner
- Block cycler door with fans

The thermal block cycler with accompanying block cycler cover is available in two versions: one for 96-multiwell plates, one for 384-multiwell plates.

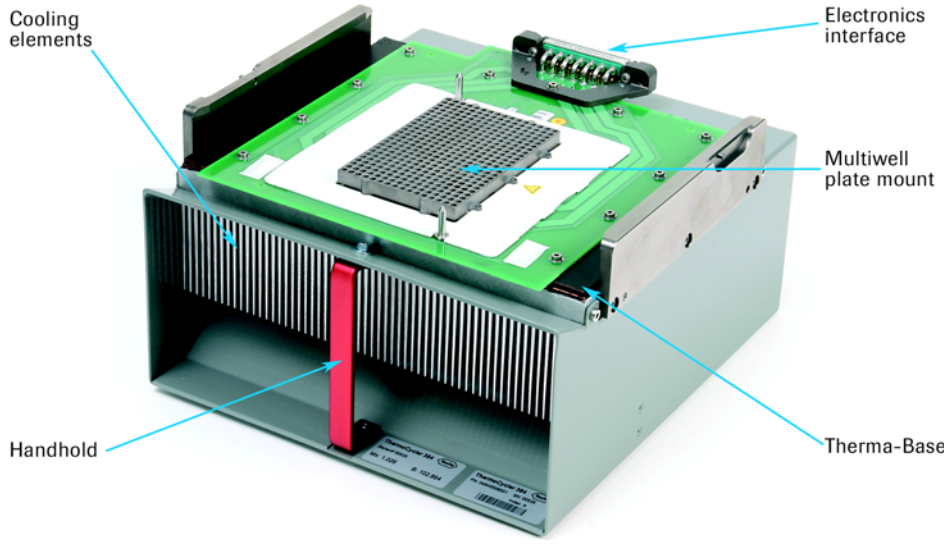

Figure 14: Thermal block cycler of the LightCycler® 480 Instrument (384-multiwell PCR plate version)

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Each version of the thermal block cycler has a matching block cycler cover. The lid has 96 or 384 pinholes (depending on the version) allowing fluorescence detection by the detection unit through the closed lid during cycling. During cycling the block cycler cover is pressed onto the PCR multiwell plate and heated to 100°C. This heating minimizes evaporation of the reaction mixture during thermocycling, and, therefore, it is not necessary to cover the reaction mixture with oil or wax.

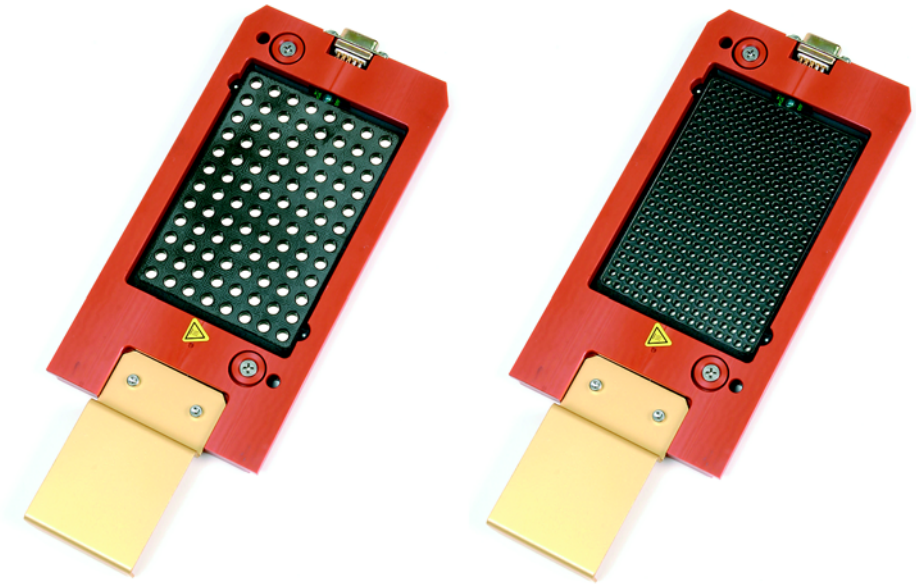

Figure 15: Top view of the 96-well (left) and 384-well (right) block cycler cover

The thermal block cycler is driven by six Peltier elements. In combination with an improved heat-transfer technology (Therma-Base), this enables PCR to be completed in less than 40 minutes (384-multiwell plate). Therma-Base is a heat equalizer which moves a concentrated heat load and distributes it to a surface area many times greater than is possible using conventional cyclers, thus reducing the overall component temperature and ensuring homogeneous heat transfer.

The block cycler unit is easily accessible through the block cycler door in the right instrument panel. The thermal block cycler, including the block cycler cover, is provided in a storage box. This box takes up the block cycler and cover enclosed in the loading device, which is used to facilitate block exchange. (For full details about exchanging the thermal block cycler and block cycler cover, see Section *Exchanging the LightCycler® 480 Thermal Block Cycler*.) Using the loading device, the thermal block cycler can be exchanged within minutes.

To cool the thermal block cycler during operation, the block cycler door in the right instrument panel is equipped with two high-efficiency fans. The air flow is guided through the instrument and expelled at the back on the right side.

Once the appropriate thermal block cycler is installed, the only manual handling step the user must perform is loading and removal of the PCR multiwell plate. The PCR multiwell plate is taken up by the multiwell plate loader, which moves the plate into the block cycler unit and places it on the multiwell plate mount of the thermal block cycler. The loader is moved out from and into the instrument by pressing the push button on the front of the instrument. A built-in plate detector checks whether the correct multiwell plate type (96- or 384-well) has been inserted properly. Furthermore, the built-in multiwell plate bar-code scanner can be used to read the plate ID bar code label. (This optional function is available only when the LightCycler® 480 LIMS/Bar-Code Module has been installed and activated. The scanned plate ID is automatically transferred to LightCycler® 480 Basic Software.) The PCR multiwell plate is adjusted on the thermal block cycler to ensure that the reaction wells are centered exactly under the pinholes in the block cycler cover. In addition, the detection unit optimizes its position relative to the block cycler cover using fluorescent markers present on the lid surface. By this means, emitted fluorescent light is efficiently detected by the detection unit.

The LightCycler® 480 Instrument can also be connected to a LIMS system via the LightCycler® 480 LIMS/Bar-Code Module which then controls opening and closing of the multiwell plate loader to perform automated loading.

Both versions of the thermal block cycler are available as individual accessories (including block cycler cover, storage box, and loading station):

LightCycler® 480 Block Kit 96	Cat. No. 04 643 640 001
LightCycler® 480 Block Kit 384	Cat. No. 04 643 631 001

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3.3 Description of the Detection Unit

The detection unit consists of two main components:

- **Lamp unit** containing the excitation light source (Xenon reflector lamp)
- **Optics unit** consisting of the liquid light guide with light pipe, the emission filters wheel, the excitation filters wheel, and the CCD camera with camera optics

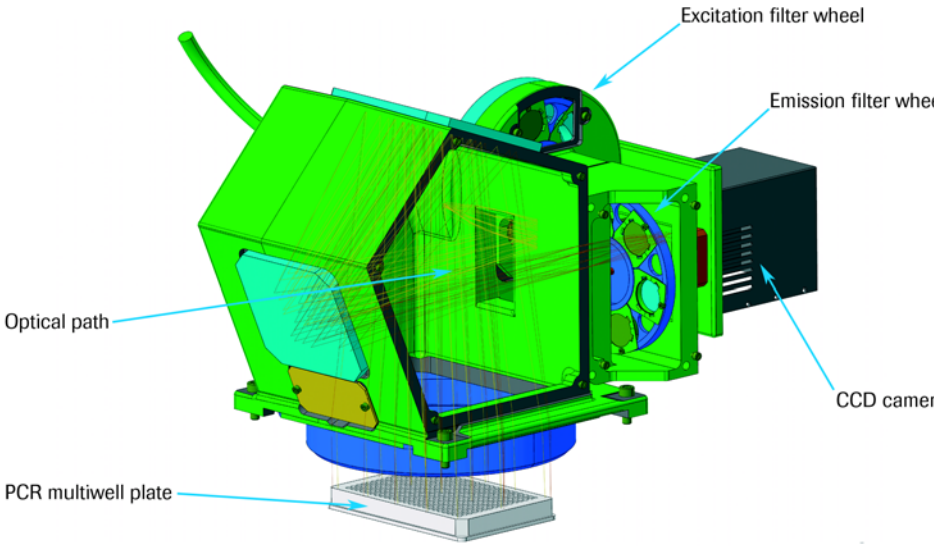

Figure 16: Schematic overview of the LightCycler® 480 detection unit

The LightCycler® 480 Instrument uses a Xenon reflector lamp as excitation light source. The lamp emits light in a broad wavelength range from 430 to 630 nm, making it possible to use various different fluorophores. The lamp requires a prewarming phase of approximately two minutes to reach full intensity. The lamp is shut off automatically after 30 minutes of instrument inactivity. This delay ensures that no additional prewarming is needed for a subsequent run that is performed within a short time of an initial run. The Xenon lamp has a lifetime of approximately 500 hours. Light intensity is measured automatically by the instrument, and the user is informed when the intensity falls below a minimum limit ensuring sufficient excitation efficiency. The Xenon lamp can be exchanged manually by the user: Operation by a Roche service engineer is not necessary. (For details about exchanging the Xenon lamp, see Section *Maintenance*.)

The light emitted by the Xenon lamp is passed to the optics unit through a liquid light guide. A light pipe at the end of the light guide generates a uniform illumination and converts it from a round to a rectangular profile to match the shape of the PCR multiwell plate. The actual wavelength used for excitation of fluorophores in the amplification reaction is determined by the chosen excitation filter. Excitation filters are located in a revolving filter wheel, which is driven by a stepper motor with six filter positions. Five of these positions are equipped with excitation filters (for details, see Section *Specifications of the Detection Unit*), while the sixth position is used for taking a "dark picture." This dark picture is taken every time the instrument is restarted and is used for dark correction of the pictures taken during a LightCycler® 480 instrument run.

After passing the excitation filter, the light is projected to the PCR multiwell plate through a large field lens that efficiently collects rays also from lateral wells of the plate. The optics module adjusts the light beams so that each reaction well in the PCR multiwell plate is illuminated with exactly the same slight angel (2°) through the pinholes of the block cycler cover. In the same way, fluorescent light emitted by the excited fluorophores in the amplification reaction is passed vertically into the optics module. This ensures that (1) there are no shading effects within the plate wells and (2) there are absolutely 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 light emitted by the PCR multiwell plate again enters the optics module and is then guided through a second filter wheel carrying six emission filters (for details, see Section *Specifications of the Detection Unit*.) Finally, the fluorescent signals are detected by the CCD camera. At the heart of the camera lays a cooled CCD chip with a resolution of 1024 × 1344 pixels. Temperature of the chip is controlled to be constantly +10°C to minimize generation of stray electrons which otherwise would contribute to thermal noise. Acquisition time of the CCD camera is adjusted either manually or dynamically (*i.e.*, the integration time is adjusted by signal dynamic to ensure an optimal ratio to the signal strength) by the instrument software. Before measurements are transferred to the LightCycler® 480 data workstation and software, further corrections and data reduction are performed.

In parallel to the fluorescent signals emitted by the PCR multiwell plate, a reference channel is measured. Using this reference channel, the intensity of the Xenon lamp is measured and the values are used to compensate for possible intensity fluctuations that could influence the intensity of fluorescent signals. This measure guarantees the lowest possible intra- and inter-assay variance.

Because excitation and emission filters are placed on filter wheels, detection of dye-specific fluorescence cannot be performed simultaneously in multicolor assays. Rather, one detection "channel" is measured after the other. Time required to switch the filter position is less than 0.65 seconds. The recommended combinations of excitation and emission filters for the dyes used in LightCycler® 480 real-time PCR assays are predefined in the LightCycler® 480 Basic Software as "detection formats". Because only those signals specific for the selected filter combinations are measured, always make sure to use appropriate detection formats.

3.4 Description of the Detection Channels

The five excitation filters and six emission filters of the LightCycler® 480 Instrument can be freely combined to enable optimal excitation of fluorophores and exact measurement of emitted fluorescence signals. The table below shows excitation–emission filter combinations recommended for fluorophores used in various different real-time PCR detection formats:

Fluorophore	Excitation Filter	Emission Filter	Detection Format
LightCycler® Cyan 500	450	500	Hydrolysis Probes (Reporter)
SYBR Green I	483	533	SYBR Green I
Fluorescein (Fluos/FAM)	483	533	Hydrolysis Probes (Reporter) HybProbe Probes (Donor) SimpleProbe Probes
HEX (VIC)	523	568	Hydrolysis Probes (Reporter) HybProbe Probes (Acceptor)
LightCycler® Red 610	558	610	Hydrolysis Probes (Reporter) HybProbe Probes (Acceptor)
LightCycler® Red 640	558	640	Hydrolysis Probes (Reporter) HybProbe Probes (Acceptor)
Cy5	615	670	Hydrolysis Probes (Reporter) HybProbe Probes (Acceptor)

These excitation–emission filter pairs can either be used singly in mono-color applications or in successive combination for multicolor applications. You will find suitable filter pair combinations used in either mono- or multicolor applications as predefined detection formats in LightCycler® 480 Basic Software (*e.g.*, the "Multi Color HybProbe" detection format combines the Red 610, Red 640 and Cy5 filter pairs). For details, see Section *Detection Formats*.

The LightCycler® 480 Instrument can simultaneously detect signals from two or more dyes, which make it possible to obtain more information from a single reaction. The channels chosen for analysis depend on the fluorescent dyes used in the experiment. In a multicolor reaction, the wavelengths of light emitted by the dyes overlap, causing one channel to pick up signals from more than one dye. This so-called cross-talk can cause misleading data. "Color compensation" is required to correct for this bleed-over between channels in multicolor experiments. For details on how to perform color compensation on the LightCycler® 480 Instrument, see Section Color Compensation Analysis.

For multicolor hydrolysis probe assays, it is strongly recommended to use dark quencher dyes (i.e., dye molecules which efficiently quench the fluorescence of a FRET reporter dye without emitting fluorescence themselves) (For details see section Monitoring PCR with Hydrolysis Probes.)

3.5 LightCycler® 480 Disposables

Specially designed PCR multiwell plates in 96- and 384-well format are available for the LightCycler® 480 Instrument. Well geometry and material of these plates are optimized to ensure best heat transfer, a prerequisite for specific PCR and fast cycling rates. Furthermore, these plates generate minimal autofluorescence which is important to achieve a good signal-to-noise ratio in detection. The multiwell plates come together with matching self-adhesive sealing foils.

Use only the PCR multiwell plates recommended in this Operator´s Manual. The block cycler unit of the LightCycler® 480 Instrument has an in-built plate type detector that detects and distinguishes the LightCycler® 480 Multiwell Plates.

Both multiwell plate types have two notches on each of their long sides to allow handling of the plates by robotic loading instruments.

The multiwell plates carry a bar code label (Code 128, 8 characters) on the long side at row A. This bar-code label represents a running plate ID that can be read by the Multiwell Bar-Code Scanner.

Figure 17: LightCycler® 480 Multiwell Plate 96 (left) and Multiwell Plate 384 (right)

LightCycler® 480 Multiwell Plate 96	50 plates with 50 sealing foils	Cat. No. 04 729 692 001
LightCycler® 480 Multiwell Plate 384	50 plates with 50 sealing foils	Cat. No. 04 729 749 001

Before loading the PCR multiwell plate into the LightCycler® 480 Instrument, it has to be sealed properly with the self-adhesive sealing foil. Sealing the plate is crucial to eliminate evaporation at high temperatures. Use only the recommended foil.

The sealing foils are also available separately.

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Figure 18: LightCycler® 480 Sealing Foil

LightCycler® 480 Sealing Foil	5 × 10 foils	Cat. No. 04 729 757 001

3.6 LightCycler® 480 PCR Reagents

The LightCycler® 480 PCR Kits are specifically designed to yield the highest specificity and sensitivity.

The LightCycler® 480 PCR kits feature the following:

- Minimum PCR optimization
- Increased reproducibility and reliability
- Hot-start enzyme for excellent sensitivity, specificity, and robustness
- Short enzyme activation times for fastest results
- Ready-to-use reagents for greatest convenience

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Kit	Application	Cat. No.
LightCycler® 480 SYBR Green I Master 1 kit (5 × 100 reactions, 20 µl each)	qPCR and product characterization by melting curve analysis using the SYBR Green I detection format	04 707 516 001
LightCycler® 480 Probes Master 1 kit (5 × 100 reactions, 20 µl each)	qPCR using hydrolysis probe format and other probe-based detection formats	04 707 494 001
LightCycler® 480 Genotyping Master 1 kit (4 × 96 reactions, 20 µl each)	qPCR and genotyping by melting curve analysis	04 707 524 001

3.7 Additional Equipment Required

The following additional equipment is required to perform real-time PCR assays with the LightCycler® 480 System:

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

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3.8 Real-Time PCR Detection Formats for the LightCycler® 480 Instrument

3.8.1 Overview

The LightCycler® 480 Instrument makes use of fluorescent dyes for online, real-time monitoring of both the generation of PCR products during cycling and melting of PCR products in post-PCR melting curve analysis. Fluorescence signals measured during cycling are correlated with the amount of PCR product in the reaction, allowing the calculation of input copy number of the target nucleic acid (possible both with sequence-independent and sequence-specific detection formats). Fluorescence measurements taken during post-PCR melting curve analysis are either used for PCR product characterization (in sequence-independent detection formats) or genotyping (in sequence-specific detection formats). For maximum flexibility, the LightCycler® 480 Instrument supports several fluorescent analysis formats and can use a broad range of probes and dyes:

- Sequence-Independent Detection Assays
  Rely on fluorophores that bind to all double-stranded DNA (dsDNA) molecules regardless of sequence; for example SYBR Green I.

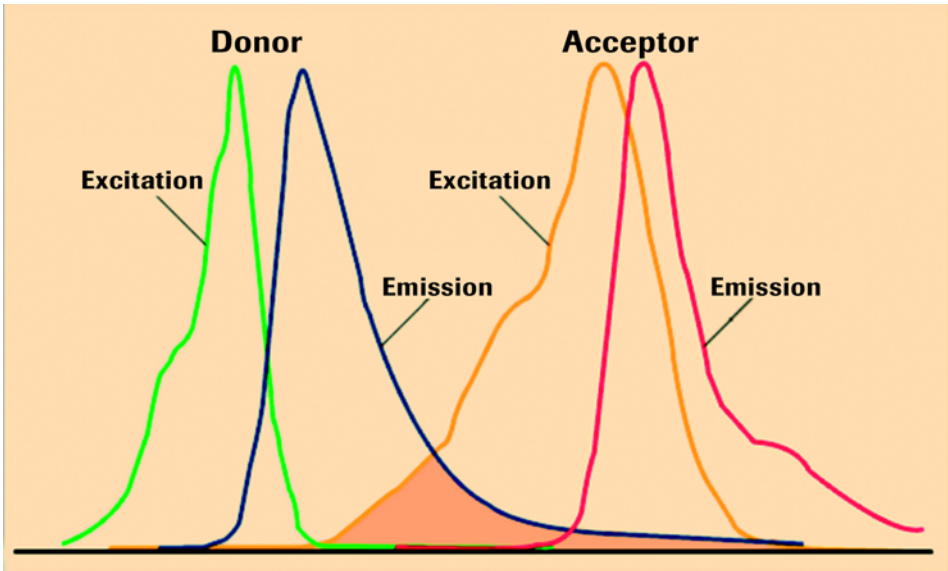
- Sequence-Specific Probe Binding Assays
  Rely on fluorophores coupled to sequence-specific oligonucleotide probes that hybridize to their complementary sequence in target PCR products:
  - Single-labeled probes (SimpleProbe chemistry)
  - Hybridization probes (HybProbe chemistry)
  - Hydrolysis probes (5´-nuclease assay)

Other assay formats may also be adapted for real-time PCR on the LightCycler® 480 Instrument. For example, adaptable probe formats include Bi-Probes (iFRET-Probes), Molecular Beacons and Scorpions. However, it is essential that any fluorescent dyes used in an analysis be compatible with the optical unit of the LightCycler® 480 Instrument.

HybProbe and hydrolysis probe chemistries use the fluorescence resonance energy transfer (FRET) principle, which is based on the transfer of energy from one fluorophore (the donor) to another adjacent fluorophore (the acceptor).

The following are primary conditions for FRET:

- Donor and acceptor molecules must be close to each other
- Excitation spectrum of the acceptor must overlap fluorescence emission spectrum of the donor
- Dipole orientations of donor and acceptor must be approximate parallel



The donor dye is excited by the light source of the LightCycler® 480 Instrument by choosing an excitation filter that matches the dye´s absorption maximum (*e.g.*, 483 nm for fluorescein). This wavelength excites certain electrons in the donor molecule from ground level to a higher energy level. This energy is be released by the following

- Emitting fluorescent light of different, longer wavelength
- Transfer of energy to the acceptor dye (*e.g.*, LightCycler® Red 640). When the energy is released, the electrons return to ground level. By transferring energy to the acceptor molecule, fluorescence of the donor itself is quenched.

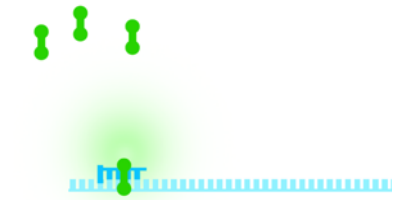
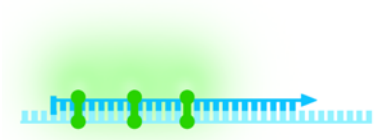
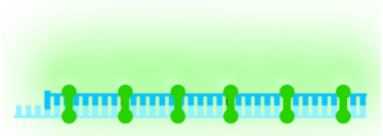
The FRET process can be used in various ways to generate a sequence-specific signal during PCR. While hydrolysis probe chemistry is based on quenching the fluorescence of the donor dye (the acceptor dye is thus called quencher), the HybProbe chemistry uses the fluorescence emission of the acceptor dye.

B

3.8.2 Monitoring PCR with the SYBR Green I Dye

Generation of PCR products can be detected by measurement of the SYBR Green I fluorescence signal. SYBR Green I intercalates into the dsDNA helix. In solution, the unbound dye exhibits very little fluorescence; however, fluorescence (measured at 530 nm) is greatly enhanced (100-fold) upon binding to DNA due to conformational changes. Therefore, during PCR, the increase in SYBR Green I fluorescence is directly proportional to the amount of dsDNA generated. Since SYBR Green I dye is very stable it is the reagent of choice when measuring total DNA quantity.

The following are the basic steps of DNA detection by SYBR Green I during real-time PCR on the LightCycler® 480 System:

1		During annealing, PCR primers hybridize to the target DNA strand and form small regions of dsDNA where SYBR Green I intercalates: the fluorescent signal slightly increases.
2		In the elongation phase, more dsDNA is formed and more SYBR Green I dye can intercalate: the fluorescent signal increases.
3		At the end of the elongation phase, all DNA has become double-stranded and the maximum amount of SYBR Green I is intercalated. The fluorescence is measured (530 nm) at the end of each elongation phase.

Since SYBR Green I binds to any dsDNA, the SYBR Green I format cannot discriminate between different dsDNA species. The specific product, non-specific products and primer–dimers are detected equally well. Any double-stranded PCR artifact contributes to signal intensity, which could result in overestimation of the concentration of the target sequence.

To determine whether only your desired PCR product has been amplified, you can perform a melting curve analysis after PCR. PCR product characterization by melting curve analysis is based on the fact that each particular double-stranded DNA molecule has its characteristic melting temperature Tm, at which 50% of the DNA is double-stranded and 50% is melted, *i.e.*, single-stranded. The most important factors that determine the thermal stability of dsDNA are length and GC content of the molecule.

During a melting curve run, the reaction mixture is slowly heated up to 95°C, which causes melting of dsDNA and a corresponding sharp decrease of SYBR Green I fluorescence when the temperature reaches the T_m of a PCR product present in the reaction. The LightCycler® 480 Instrument continuously monitors fluorescence over temperature transitions. In melting curve analysis using LightCycler® 480 Basic Software these data are then displayed as a melting curve chart (fluorescence [F] vs temperature [T]). The T_m of a PCR product present in the reaction can be estimated from the inflection point of the melting curve. But the T_m is more easily discerned using the LightCycler® 480 Basic Software Tm Calling Analysis module by plotting a derivative melting curve (-dF/dT) where the center of a melting peak corresponds to the point of inflection.

If the PCR generated only one amplicon, melting curve analysis will show only one melting peak. If primer–dimers or other non-specific products are present, they will be shown as additional melting peaks. Checking the T_m of a PCR product can thus be compared with analyzing a PCR product by length in gel electrophoresis.

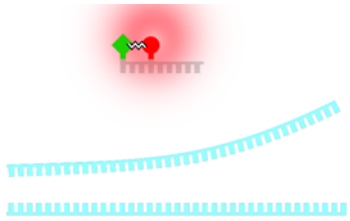
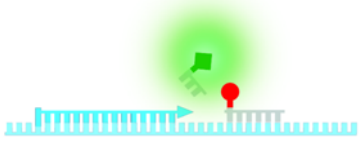
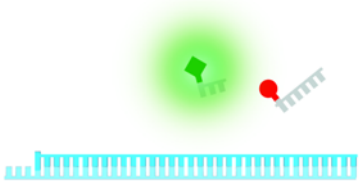
B

3.8.3 Monitoring PCR with Hydrolysis Probes

Hydrolysis probe assays can technically be described as homogeneous 5´-nuclease assays, since a single 3´-non-extendable (due to phosphorylation) 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 (fluorescent quenching takes place via FRET). During PCR, the 5´-nuclease activity of the polymerase cleaves the hydrolysis probe, separating the reporter and quencher. In the cleaved probe, the reporter is no longer quenched and emits a fluorescent signal when excited.

The LightCycler® 480 Instrument can detect hydrolysis probes that are *e.g.*, labeled with the reporter dyes LightCycler® Cyan 500, FAM, HEX, LightCycler® Red 610, LightCycler® Red 640, or Cy5. Hydrolysis probes can be used separately or in combination, which permits either single- or multicolor detection.

For multicolor hydrolysis probe assays, it is strongly recommended to use dark quencher dyes (i.e., dye molecules which efficiently quench the fluorescence of a FRET reporter dye without emitting fluorescence themselves). Roche Applied Science recommends the use of BHQ-2 (quenching range 550 – 650 nm) for all hydrolysis probe reporter dyes listed above. Alternatively, DABCYL (quenching range 380 – 530 nm) can be used for quenching LightCycler® Cyan 500, FAM, or HEX.

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, displaces 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 fluorescent light that can be measured by one detector channel of the LightCycler® 480 Instrument. Thus, the increase in fluorescence from the reporter dye directly correlates to the accumulation of released reporter dye molecules (and thus indirectly to the amount of PCR products). As with SYBR Green I, the fluorescent signal of the reporter dye is measured at the end of the elongation phase.

In the hydrolysis probe format, melting curve analysis cannot be performed, because generation of the fluorescent signal does not depend on the hybridization status of the probe but on digestion of the probe. Therefore, this type of assay requires a different experimental approach for genotyping.

For a digestible hybridization complex to form correctly, the hydrolysis probe must anneal to the target before primer extension. The T_m of the probe should be only slightly higher than the T_m of the PCR primer, so the hybridization complex is stable.

Two principle PCR run programs are possible for hydrolysis probe assays. Both two-step and three-step PCR programs will provide suitable experimental results. Higher $MgCl_2$ amounts in the amplification solution might be necessary to ensure stable hybridization of the hydrolysis probe and favor the hydrolysis event. The amplicon should be short (up to 150 bp) and when performing a two-step PCR program the temperature for annealing and elongation should be around 60°C.

The LightCycler® 480 Instrument in combination with the LightCycler® 480 Probe Master is fully compatible with the 165 pre-validated, dual-labeled (labeled 5´-terminal with fluorescein/FAM and 3´-proximal with a dark quencher dye) real-time PCR probes of the Universal ProbeLibrary for gene expression analysis. The probes are available either as single probes or as organism-specific Universal ProbeLibrary Sets containing 90 pre-validated probes. Each Universal ProbeLibrary Set is able to detect at least 95 – 99% of all transcripts for a given organism (currently Universal ProbeLibrary Sets are available for human, primates, mouse, rat, C. elegans, Arabidopsis, and Drosophila). For detailed information, visit http://www.universalprobelibrary.com.

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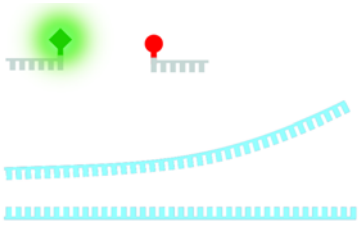
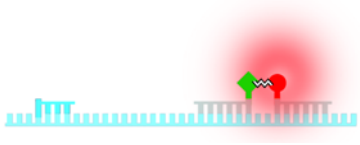
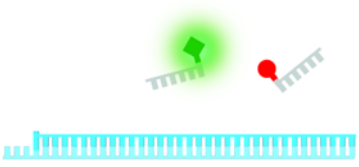
3.8.4 Monitoring PCR with HybProbe Probes

In the HybProbe detection format, two specially designed, sequence-specific oligonucleotide probes labeled with different fluorescent dyes, called donor and acceptor, hybridize to the target sequences on the amplified DNA fragment in a head-to-tail arrangement, thereby bringing the two dyes into close proximity. The donor dye (*e.g.*, fluorescein) is excited by choosing the appropriate excitation filter (483 nm). When the two dyes are close to each other, the energy emitted by the donor dye excites the acceptor dye (*e.g.*, LightCycler® Red 640) attached to the second HybProbe oligonucleotide, which then emits fluorescent light at a different wavelength. The amount of fluorescence emitted is directly proportional to the amount of target DNA generated during PCR.

The LightCycler® 480 Instrument can detect HybProbe probes that are labeled with the acceptor dye LightCycler® Red 610, LightCycler® Red 640, or Cy5. These labeled HybProbe probes can be used separately or in combination, which permits either single- or multicolor detection. Use fluorescein (FLUOS) as donor dye. Note, that the donor-acceptor combination FLUOS-HEX is not suitable as the spectra of both dyes are too close to each other.

1		In the example shown, the HybProbe donor probe is labeled with fluorescein at the 3´-end, while the HybProbe acceptor probe is labeled at the 5´-end with a LightCycler® Red dye (the acceptor probe is also 3´-phosphorylated to prevent extension). (Note that this set-up might also be reversed: acceptor probe labeled at the 3´-end and donor probe labeled at the 5´-end.) Hybridization does not occur during the denaturation phase of PCR. Since the distance between the unbound probes prevents energy transfer between the two dye labels, no fluorescence will be detected from the acceptor dye during this phase.
2		During the annealing phase, the two probes hybridize to the amplified DNA fragment in a close head-to-tail arrangement.The donor dye is excited by the light source of the instrument, which causes it to emit energy. Instead of being emitted as fluorescent light, the emitted energy excites the acceptor dye. The fluorescence emitted by the acceptor dye is measured at the end of each annealing step, when the fluorescence intensity is highest.
3		After annealing, the temperature is raised and the HybProbe probes are displaced during elongation. At the end of this step, the PCR product is double-stranded and the displaced HybProbe probes are again too far apart to allow FRET to occur.

The HybProbe detection format is suited for both sequence-specific detection in qPCR and genotyping (SNP detection). SNP detection using HybProbe probes is based on melting curve analysis. At temperatures below the T_m of the oligonucleotides, the HybProbe pair binds to the complementary template and this brings the anchor probe in close proximity with sensor probe, hence, producing FRET. As the temperature is raised, the probes will melt at their corresponding T_ms and no longer produce FRET. This melting coincides with a drop in fluorescence signal. The temperature at which the sensor probe melts will be dependent on the underlying sequence. Thus, if a SNP exists on the sensor-probe binding region, the complex is destabilized and melts at a lower temperature compared to the perfect match.

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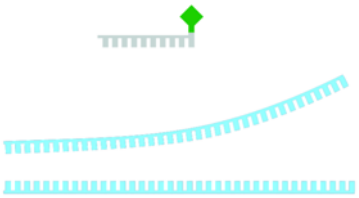
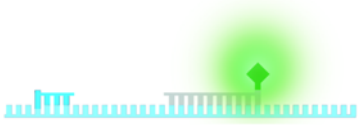
3.8.5 Genotyping with SimpleProbe Probes

SimpleProbe probes are a special type of hybridization probe. These probes differ from HybProbe probes in one important way: Instead of two probes working together, only a single probe is needed. This single probe hybridizes specifically to a target sequence that contains the SNP of interest. Once hybridized, the SimpleProbe probe emits a greater fluorescent signal than it does when it is not hybridized to its target. As a result, changes in fluorescent signal depend solely on the hybridization status of the probe. The SimpleProbe detection format is not based on the FRET principle.

Typically a SimpleProbe probe is designed to specifically hybridize to a target sequence that contains an SNP of interest. Once hybridized to its target sequence, the SimpleProbe probe emits more fluorescence than it does when it is not hybridized. As a result, changes in fluorescence are based solely on the hybridization status of the probe. SimpleProbe probes are an excellent tool for SNP genotyping and mutation detection because they readily identify wild type, mutant, and heterozygous samples with only a single short probe.

A SimpleProbe probe can be labeled at either terminally (the 3´- or 5´-end) or internally (*e.g.*, by using SimpleProbe 519 Labeling Reagent*). If a SimpleProbe probe is free in solution, emission of the reporter dye is quenched by a specific, non-fluorescent quencher. When the probe hybridizes to its target, quenching is reduced and the reporter dye, when excited by the excitation channel of the LightCycler® 480 Instrument, emits fluorescence. However, even when the probe is not hybridized, background fluorescence is detectable at 530 nm, resulting in a low signal-to-noise ratio.

For SNP analysis, the LightCycler® 480 Instrument monitors the melting behavior of the SimpleProbe probe. By measuring the fluorescence, the instrument can detect melting of the probe–target hybrids as the temperature increases. The more stable the hybridization between SimpleProbe probe and target sequence, the higher the melting temperature. Mutations, such as SNPs, weaken the stability of SimpleProbe probe binding.

1		During the denaturation phase no hybridization takes place, thus, only a low fluorescence background is detected at 530 nm.
2		During the annealing phase, the probe hybridizes to the amplified DNA fragment and is no longer quenched. Fluorescein, when excited by the light source of the LightCycler® 480 Instrument, emits green fluorescent light which is measured only at the end of each annealing step at maximum intensity.
3		During the subsequent elongation step, the SimpleProbe probe is displaced.
4		At the end of the elongation step, the PCR product is double stranded and the displaced SimpleProbe probe is again quenched.

Operation

C

Chapter C • Operation
Description of the operating procedures for the LightCycler® 480 Instrument

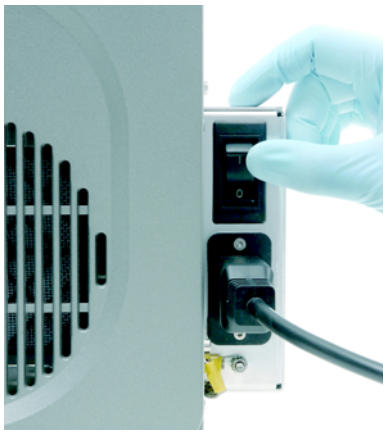
C

Operation

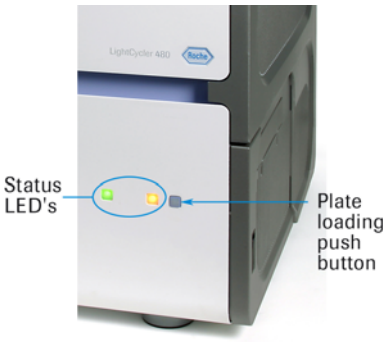
1. Introduction

Prior to starting operation, review Sections *Overview* and *LightCycler® 480 Basic Software* to verify the identification and location of the LightCycler® 480 Instrument components and to become familiar with the software.

2. System Start-Up

1. 

The mains switch is located on the left side of the power box at the back of the instrument. Press | to switch on the LightCycler® 480 Instrument.



Two status LEDs are located at the front of the LightCycler® 480 Instrument.

During instrument operation, the status LEDs function as described in the table below:

Color of Left LED	Color of Right LED	Indication
Orange (flashing)	Orange (flashing)	Instrument is initializing.
Green	Orange	Instrument is turned on. Instrument status is ready. No plate loaded.
Green	Orange (flashing)	Plate is loading.
Green	Green	Instrument is turned on. Instrument status is ready. Plate is loaded.
Green (flashing)	Green (flashing)	Instrument is running.

2. Turn on the data workstation.
3. Start the operating system, Windows XP.
4. Start the LightCycler® 480 Basic Software. Details on working with the LightCycler® 480 Basic Software are described in Section *LightCycler® 480 Basic Software*.

3. Preparing and Starting a LightCycler® 480 Instrument Run

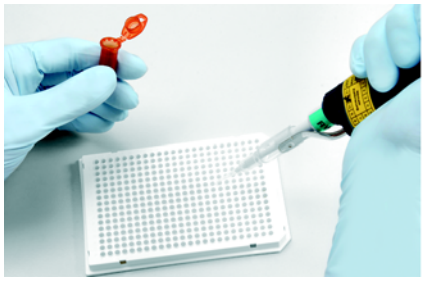
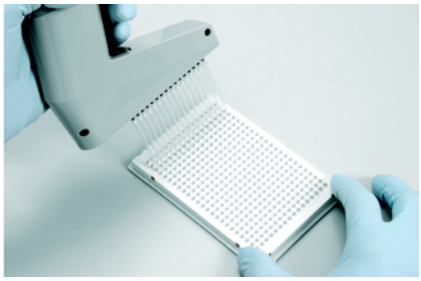
The procedure described below represents a typical example. Other approaches (e.g. distributing replicates of a master mix already containing template DNA) are possible.

1. Program the experimental protocol and define the sample numbers, names, etc. For detailed instructions, see Section *LightCycler® 480 Basic Software*.

2. Prepare a master mix that contains all of the reaction reagents except the DNA template in a 1.5 ml or 0.5 ml reaction tube.

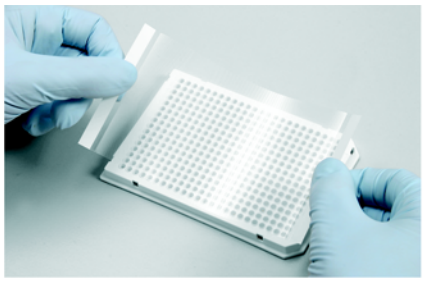
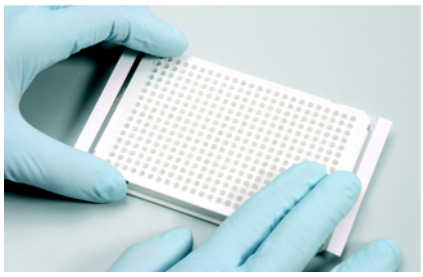
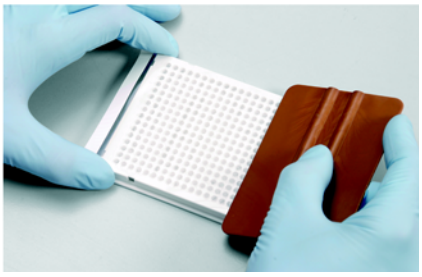
   Follow the protocols provided in the Instruction Manual of the LightCycler® 480 reagent kit. Use only reaction volumes recommended for the type of PCR multiwell plate in use. Use dark reaction tubes to avoid bleaching of fluorescent dyes by ambient light.

3. Pipette the reagent mix into the wells of the LightCycler® 480 Multiwell Plate. Robotic or parallel (8 or 16 tip) pipetting is helpful when processing a large number of samples.

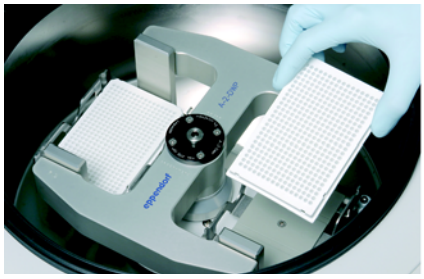
4. Add the DNA template to each well.

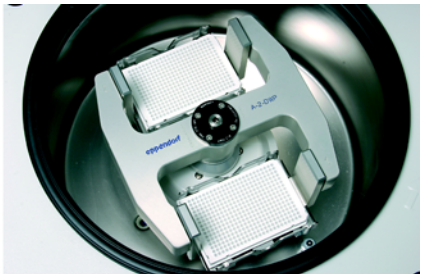
5. Seal the plate properly with a LightCycler® 480 Sealing Foil by pressing it firmly to the plate surface using your hand or a scraper (*e.g.,* the Sealing Foil Applicator provided with the instrument).

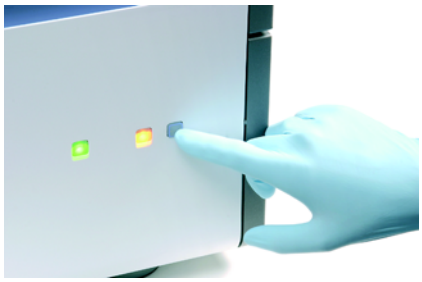
Sealing the plate is crucial to eliminate evaporation at high temperatures.

6 Place the multiwell plate in a standard swing-bucket centrifuge, containing a rotor for multiwell plates with suitable adaptors. Balance it with a suitable counterweight (*e.g.*, another multiwell plate). Centrifuge the plate at 1,500 × g for two minutes. Check the wells for bubbles, and repeat if necessary.



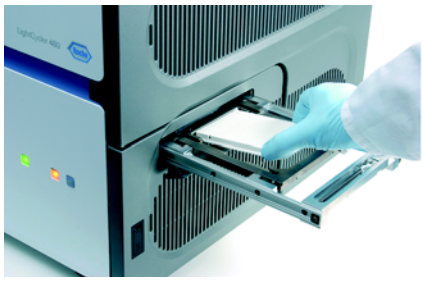


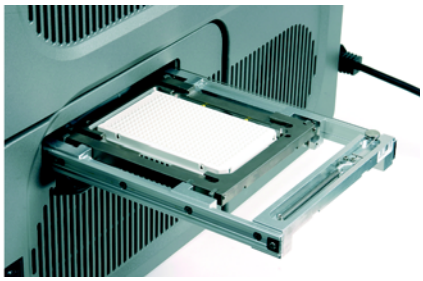
7 ▶To load the prepared multiwell plate into the LightCycler® 480 Instrument, press the push button on the front of the instrument (located next to the instrument status LEDs):



▶The multiwell plate loader extends out of the right side of the instrument:

8 Place the multiwell plate into the loading frame of the loader with the flat edge pointing towards the instrument. (The short plate edge with beveled corners points away from the instrument.)





9 Press the plate loading push button again to retract the loader with the inserted multiwell plate into the instrument. You are now ready to start the run.

10 When the run is finished, open the plate loader again to remove the PCR multiwell plate.

Directly after completion of a run, the multiwell plate loader may be hot enough to cause an immediate burn. Wait an appropriate time period to let the loader cool down. Always include a final cooling step in your LightCycler® 480 instrument run protocol.

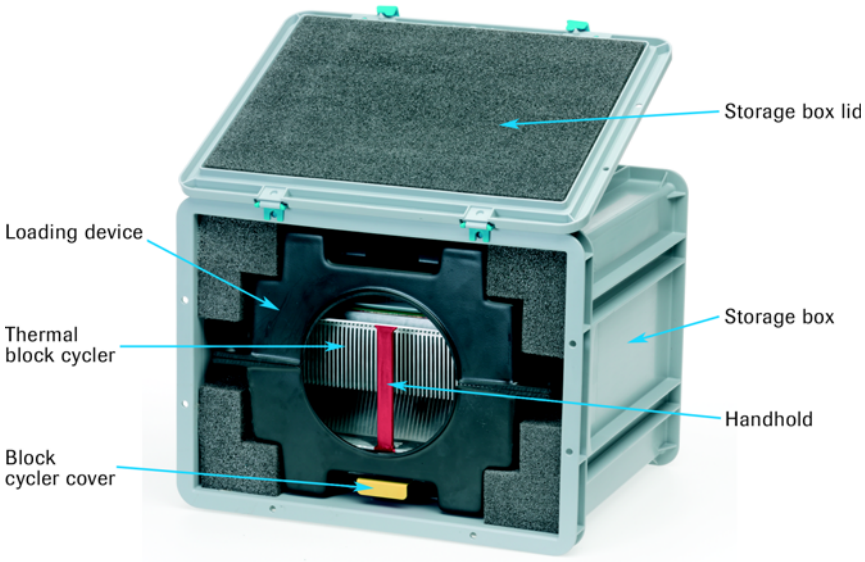
4. Exchanging the LightCycler® 480 Thermal Block Cycler

The LightCycler® 480 Instrument is available in two versions, one with a thermal block cycler for the LightCycler® 480 Multiwell Plate 96-wells, the other for the LightCycler® 480 Multiwell Plate 384-wells. You can purchase each version of the thermal block cycler as an exchangeable accessory (LightCycler® 480 Block Kit 96 or 384). If both thermal block cycler versions are available to you, you can change the thermal block cycler manually depending on which multiwell plate type you want to use. The table below describes how to exchange the thermal block cycler.

Turn off the LightCycler® 480 Instrument before attempting to exchange the thermal block cycler.

Before exchanging the thermal block cycler, make sure you have waited an appropriate period of time (approximately 20 minutes) after a LightCycler® 480 instrument run to allow the thermal block cycler and block cycler cover to cool. Directly after completion of a run, both thermal block cycler and block cycler cover are hot enough to cause an immediate burn. Always include a final cooling step in your LightCycler® 480 instrument run protocol.

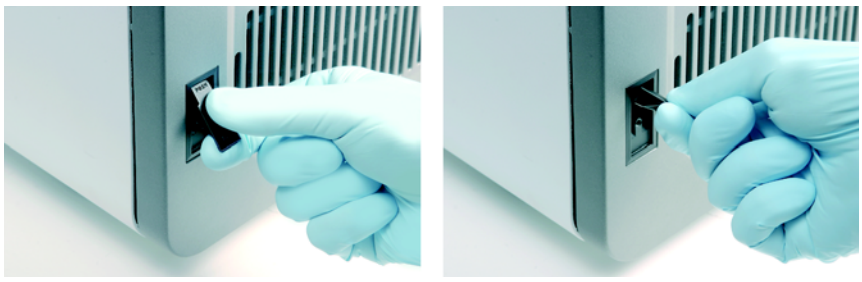
1 The LightCycler® 480 thermal block cycler is provided in a storage box, which protects the accessory from damage occurring during transport. The storage box takes up the thermal block cycler assembled together with the block cycler cover within the block cycler loading device.



2 Open the storage box lid. Grasp the block cycler handhold and pull the block cycler together with the loading device out of the box.

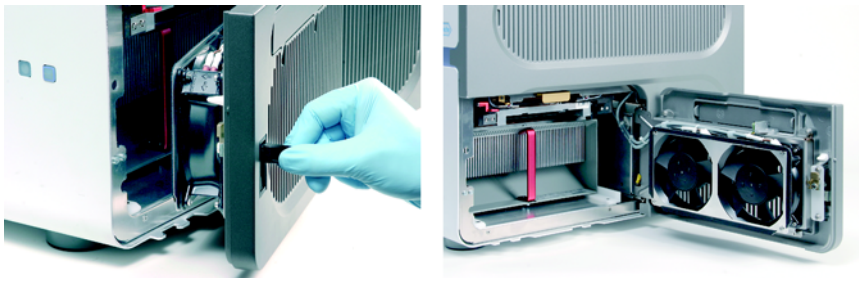
The loading device consists of two identical halves: During block exchange, the upper half is removed and used to take up the thermal block cycler currently installed in the instrument (see below). A retainer located in the bottom is used to take up the disassembled block cycler cover.

3 Open the block cycler door in the right instrument panel:
▶Lift the clip in the door on the right side (1), and turn it clockwise (2).



▶While holding the door clip in the turned position, open the cycler door at the back.

You can open the door only if the instrument is turned off.



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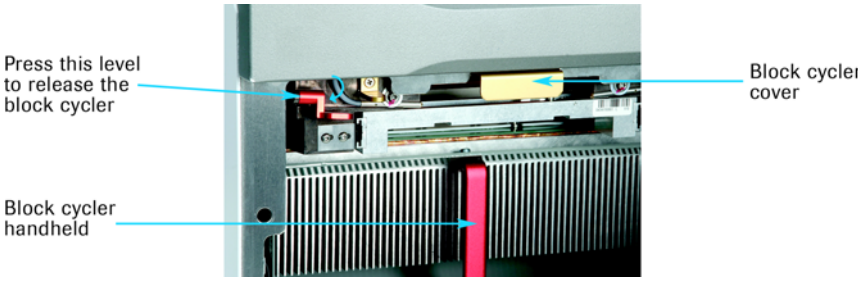
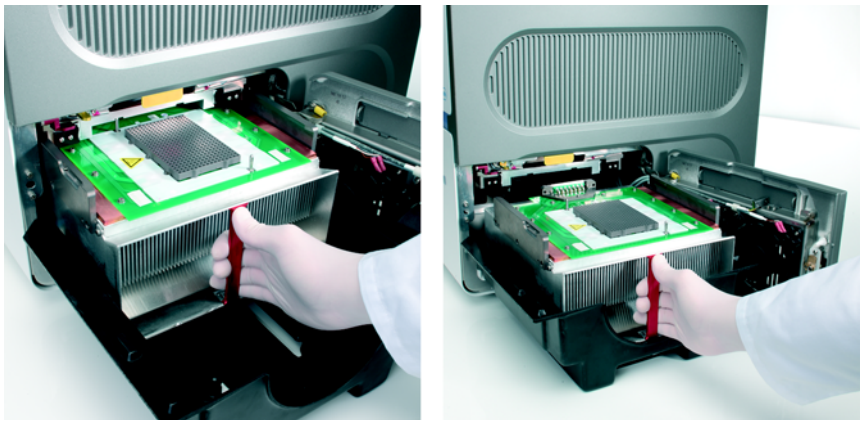
4 Remove the upper half of the loading device and place it upside-down, with its open side pointing towards the instrument, in front of the opened block cycler compartment:

5 ▶While pressing down the red-labeled lever located in the upper left corner of the block cycler compartment opening, grasp the block cycler at its handheld. Use your thumb to apply pressure on the instrument chassis to release the block cycler.



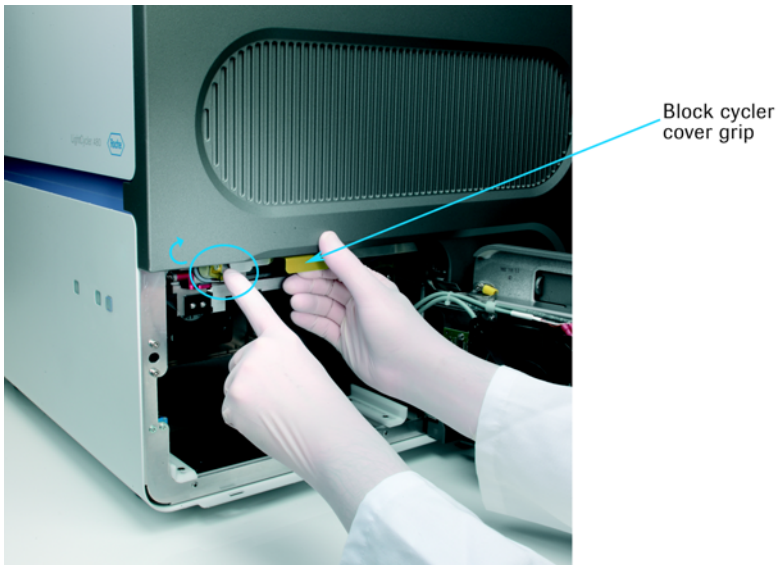
▶Pull the block cycler out of its compartment and onto the provided loading device:



▶Put aside the removed block cycler placed in the loading device.

6 The block cycler cover must be removed after the block cycler has been removed:

► While pushing the small golden lever located between the block-release lever and the block cycler cover to the left, grasp the grip of the block cycler cover. The block cycler cover is released.



► Once released, you can easily pull the block cycler cover out of the compartment.

► Insert the block cycler cover into the retainer provided in the bottom of the loading device.

7 Load the new thermal block cycler into the empty compartment by following the steps described above in reversed order:

▶ Inserting the block cycler cover: While pushing the small golden lever located between the block-release lever and the block cycler cover to the right, push the block cycler cover into its carrier at the upper side of the block cycler compartment.

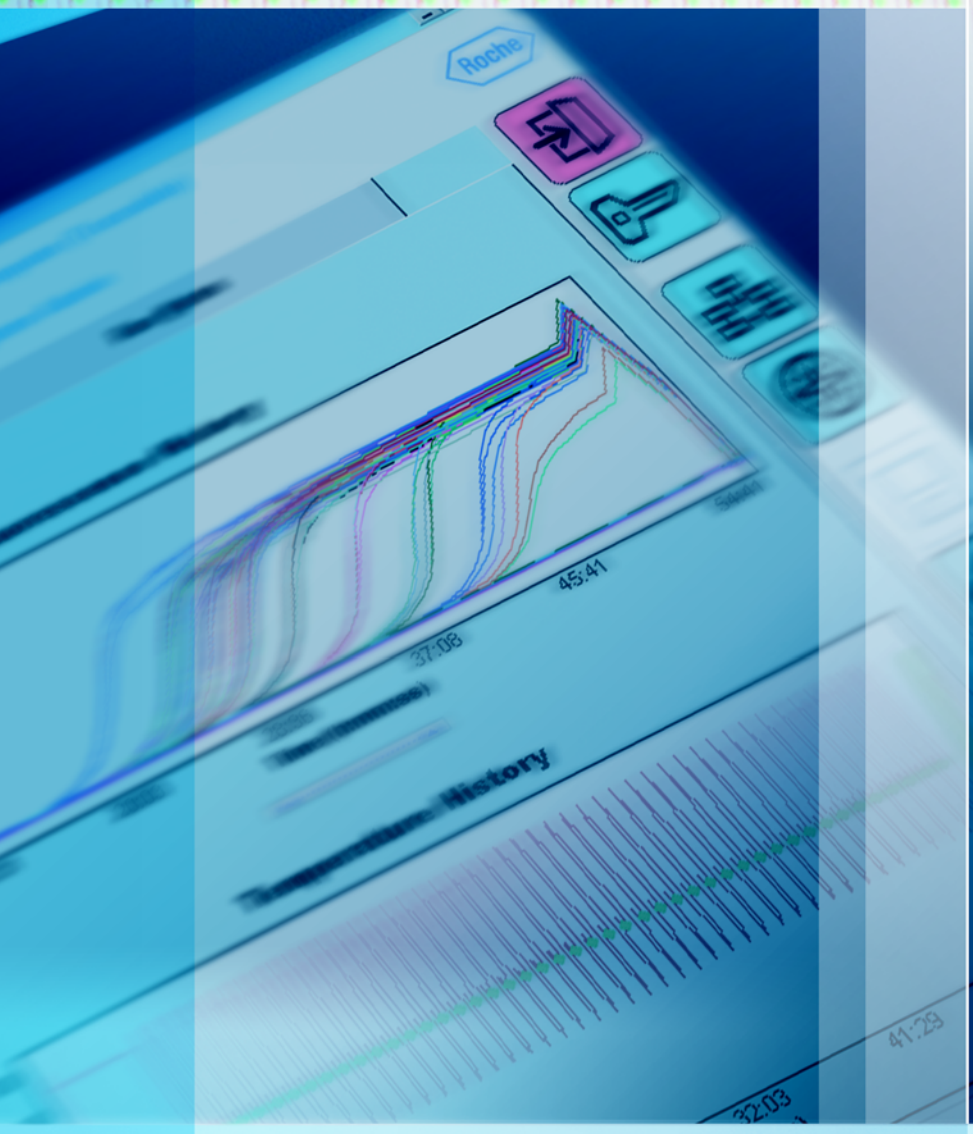
▶ Insert the new block cycler: Follow Steps 5 and 4 above exactly in reversed order.

▶ Finally, after pushing the block cycler back into its compartment, close the block cycler door and secure it by pressing down the door clip.

When you turn on the LightCycler® 480 Instrument again, the new thermal block cycler is automatically detected and identified by the LightCycler® 480 Basic Software.

LightCycler® 480 Basic Software

Software Version 1.0



D

Chapter D •
LightCycler® 480 Basic Software
Instructions for programming LightCycler® 480 Instrument runs and data analysis

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D

Software

1. Overview of LightCycler® 480 Basic Software

LightCycler® 480 Basic Software controls the LightCycler® 480 Instrument using information you provide in an experiment protocol. LightCycler® 480 Basic Software includes the LightCycler® 480 Basic Software application, a database with an audit trail, and the database object server (called "Exor4"), which communicates with the database. The software needs to be installed in a local configuration.

In this configuration all software components are preinstalled on the LightCycler® 480 data station connected to the LightCycler® 480 Instrument. Each configuration (instrument and connected computer) functions as an independent system with its own database and its own set of user accounts. The location and destination folder of the database engine and database files is usually predefined for the installation process.

All data gathered by the LightCycler® 480 System are stored in the database to guarantee security for the data and data integrity. No manipulation of stored data and no access to raw data are possible. Analysis and editing of data can only be done within the LightCycler® 480 Basic Software. To view experiment information in LightCycler® 480 Basic Software, the experiment file must first be stored in a LightCycler® 480 Basic Software database.

This chapter provides a general introduction to LightCycler® 480 Basic Software. Further, it describes all user interface elements and software modules available on all software screens. This chapter includes the following topics:

- Starting the LightCycler® 480 Basic Software
- Understanding the main window
- User interface conventions
- Exporting and importing files and objects

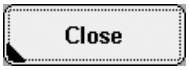
D

1.1 General LightCycler® 480 Basic Software User Interface Conventions

The user interface of the LightCycler® 480 Basic Software displays some common elements (*i.e.*, buttons) with a defined functionality which you will find on nearly all software screens:

Button	Function
	Confirms an entry or action. Proceeds to the next screen, dialog, or step. Corresponds to pressing the <Enter> key.
	Aborts an entry or action. Closes the selected screen or dialog. Corresponds to pressing the <Esc> key.
	Adds an item or object.
	Removes an item or object.
	Edits an item or object.
	Exits LightCycler® 480 Basic Software.

Furthermore, general button design conventions imply the function behind each button by using specific button indicators. The table below explains the conventions for button indicators:

Button	Marking	Behavior
	Black triangle in lower left corner	Completes the action and closes the window or dialog.
	Black triangle in upper right corner	Opens a secondary dialog.
	No black triangle	Performs a specified action in the current window.
	White background, dotted inside border	Button is selected.
	Button combined with arrow-down symbol	Signifies a multi-select button.

Placing the mouse pointer over an icon or button displays a short description of its function and its keyboard shortcut (if available).

1.2 Starting the LightCycler® 480 Basic Software

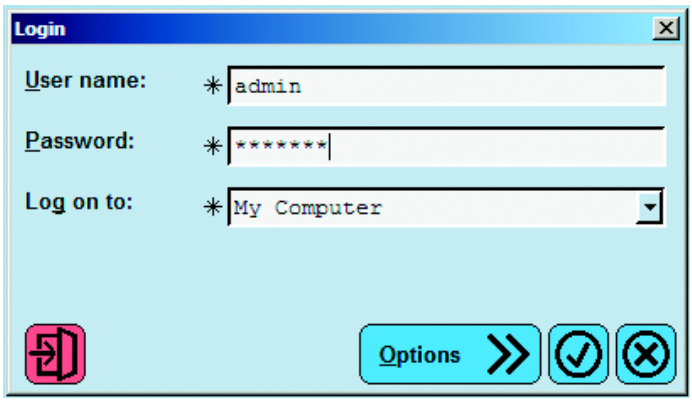
Follow the steps below to start and log onto the LightCycler® 480 Basic Software.

Run the LightCycler® 480 Basic Software at a screen solution of 1280 × 1024 pixels or higher. Note that the software will run under a screen resolution as low as 1024 × 768. However, some software features will be restricted at this lower resolution (for example, some buttons will overlap).

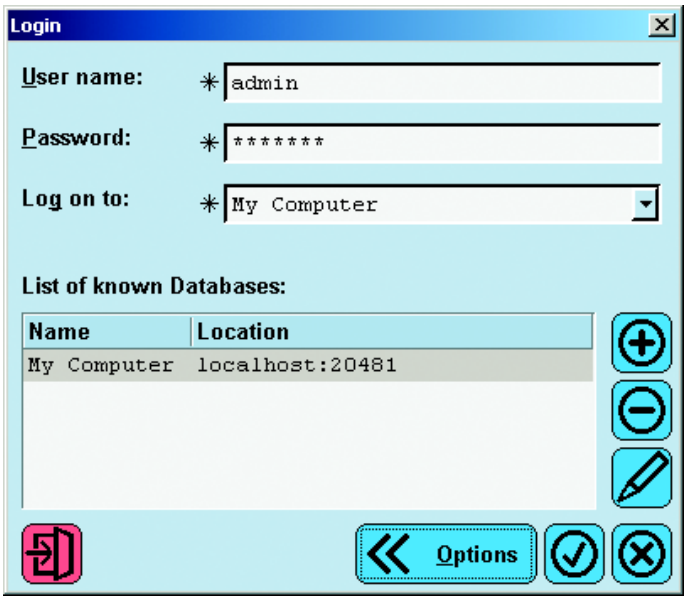
To start the LightCycler 480 Basic Software:

1. Double-click the LightCycler® 480 Basic Software icon on the desktop.

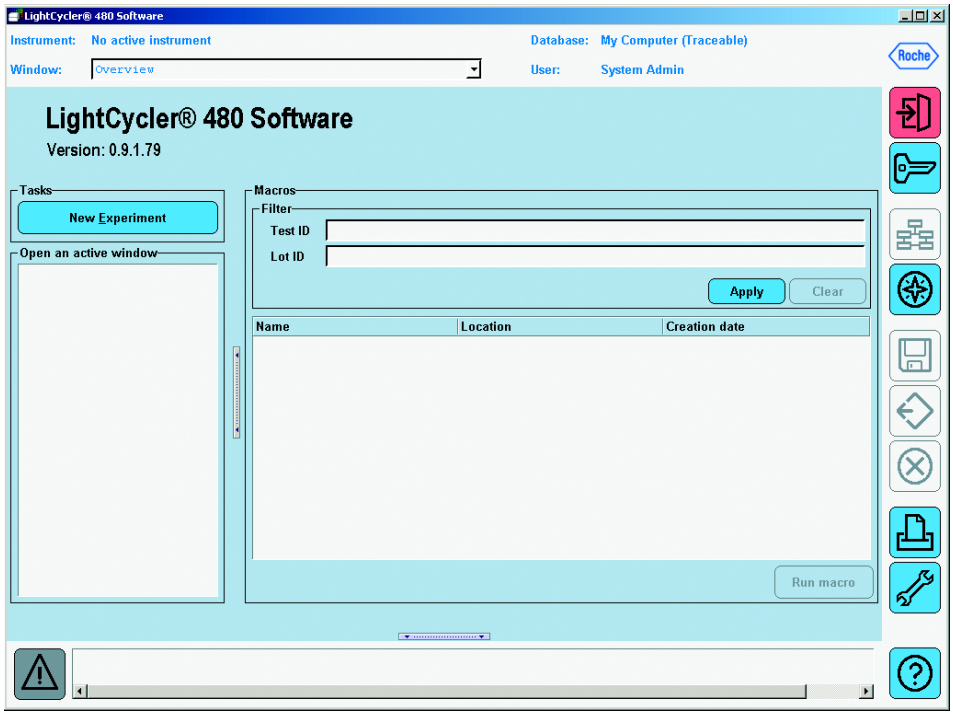
2. In the Login dialog box, type your user name and password.
   The initial password for the admin user is LightCycler480.



3. In the *Log on to* field, the last database selected on this computer is displayed by default.
   If several databases are available and you want to log onto a different database than the one displayed in the Log on to field, select the Options button. The Login dialog box expands to show a selection list of all available databases. Select a database from the list.



4 Click to proceed with the login.
The application now displays the LightCycler® 480 Basic Software *Overview* window containing *Tasks* and *Open an active window* area on the left, a *Macros* area in the middle, and the *Global action* bar on the right. Starting from the *Overview* window, you can create a new experiment, open a macro, or switch to other software modules such as the *Navigator* or the *Tools* section.

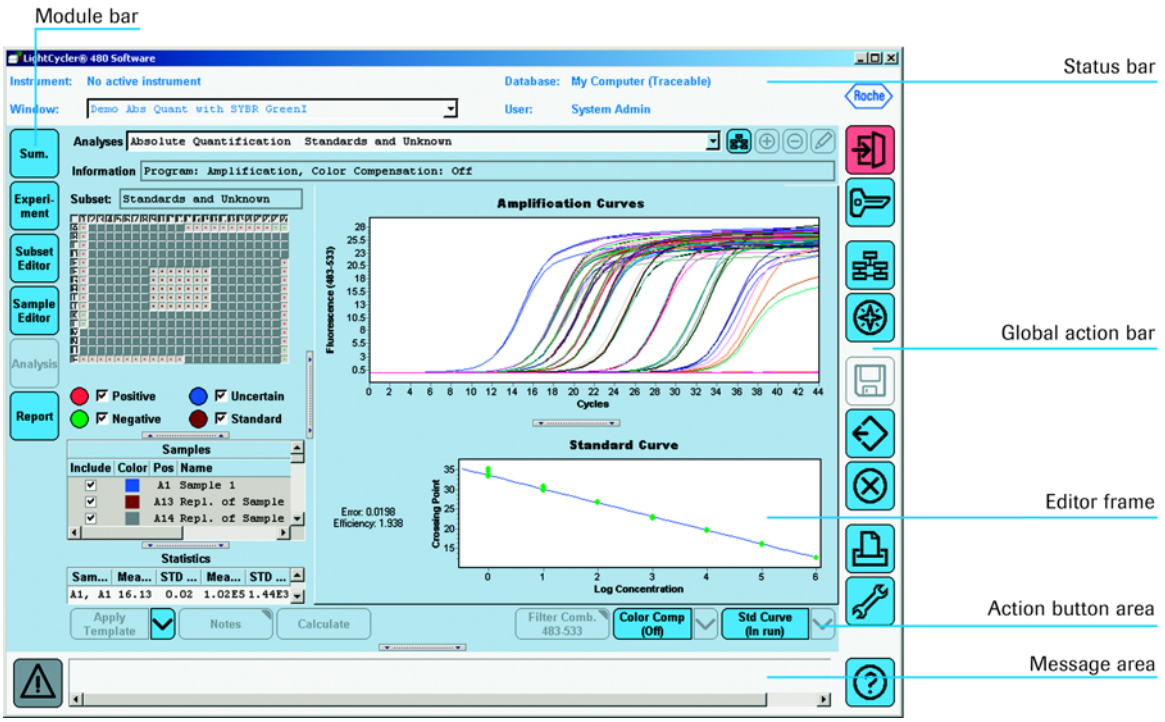


Area	Function
Tasks	Click *New Experiment* to launch the *Run* module. For more information about using the *Run* module, see Section *Programming an Experiment*.
Open an active window	Switch between opened windows by selecting one in the *Open an active window* area. After starting the software, this area will be empty. Names of active software windows are added during operation.
Macros	Select a macro from the list and start it or enter a *Test ID* and/or *Lot ID* to screen the database for a matching macro.
Global action bar	Availability of the buttons in the *Global action* bar depends on the active window currently opened. For more information, see Section *Understanding the LightCycler® 480 Basic Software Main Window.*

1.3 Understanding the LightCycler® 480 Basic Software Main Window

The figure below illustrates the LightCycler® 480 Basic Software main window (as an example, an *Absolute Quantification Analysis* window is shown, but the following description applies to all windows). The main window contains the following areas, which are described below:

- Status bar
- Module bar
- Global action bar
- Editor frame
- Message area



Status Bar

This area displays information about the currently active object and allows you to select an object to view from a list of currently open objects.

Field	Function
Instrument	Displays name and status of the connected instrument. The following instrument states are possible: Not Connected, Connected, Initializing, Standby (MWP loaded), Standby (no MWP), Running, and Error.
Window	Displays a pull-down menu listing all currently open windows. Use the menu to switch between windows.
Database	Displays the name and type of the database to which you are connected.
User	Displays the name of the user who is currently logged on to the database.

Global Action Bar

The *Global action* bar displayed on the right side of the screen contains buttons used for general software functions. Availability depends on the active window currently opened. The following actions are connected to these buttons:

Button	Function
	*Exit:* Clicking this button exits the application.
	*Log Off:* Clicking this button automatically logs you off the current database and lets you log onto another database.
	Clicking this button switches the view to the *Overview* window.
	Clicking this button displays the *Navigator* window. The Navigator is described in detail in Section *Selection and Navigation Features.*
	*Save:* Clicking this button saves changes applied to the currently opened object.
	*Export:* Clicking this button exports the currently opened object to a file. Export is described in detail in Section *Exporting and Importing Files and Objects*.
	*Close:* Clicking this button closes the selected object.
	*Print:* Clicking this button prints the currently viewable screen.
	*Tools:* Clicking this button opens the *Tools* window, where you can change your password, create and edit users, groups and roles, edit the system settings, view the database status, manage instrument information, and define your filter combination selection. The *Tools* dialog is described in detail in Section *Administrative Tools*.

Module Bar

The *Module* bar, displayed on the left side of the screen, has six permanent buttons. The following actions are connected to these buttons:

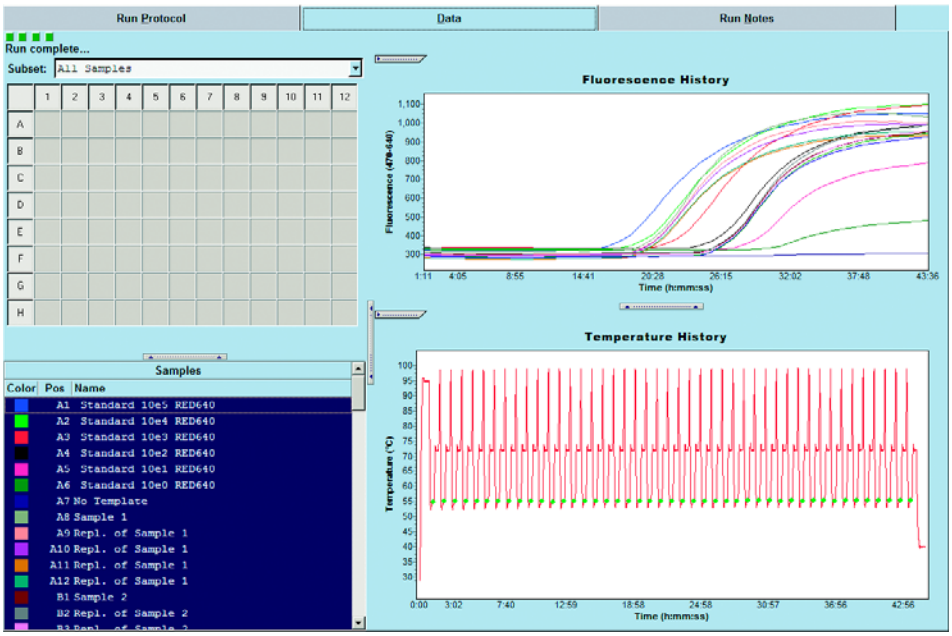
Button	Function
Sum.	Clicking this button opens the *Summary* module of the experiment. This module contains information about the experiment (such as name, date, and owner as well as the filter combinations), displays the change log, and lets you save an experiment as a macro.
Experi-ment	Clicking this icon opens the *Run* module, which includes the details of the experiment protocol, charts of experiment data, and notes entered by the person running the experiment. How to program and run an experiment is explained in detail in Section *Programming and Running an Experiment*.
Subset Editor	Clicking this icon opens the *Subset Editor*, which allows you to group samples into subsets for analysis and for reports. How to create and edit subsets is explained in detail in Section *Working with Subsets*.
Sample Editor	Clicking this icon opens the *Sample Editor*, which is used to define sample information needed for the experiment. How to enter and edit sample information is explained in detail in Section *Entering Sample Information*.
Analysis	Clicking this icon opens the *Analysis* module. If no analysis is yet opened, this will bring you to the *Analyses Overview* window. Here you can either create new analyses or open already existing ones. Each newly created analysis for an experiment is added to the list of analyses and can be selected in the corresponding drop-down list displayed in the *Analysis* module. If an analysis is already open, the corresponding window will be brought to the front. How to perform analysis is explained in detail in Sections *Overview of Experimental Analysis*, *Performing Absolute Quantification Analysis*, *Performing Melting Curve Analysis* and *Performing Color Compensation Analysis*.
Report	Clicking this icon opens the *Report* module which allows you to define the content of a report and to view and print the report.  *You must first save an experiment before this button becomes active.*

To use the Module bar:

Click an icon to open the related experiment module or use the keyboard shortcut for the icon, <Ctrl-Shift-*n*>, where *n* is the number of the icon counting from the top. For example, the *Experiment* icon is always second in the list of icons, so to open the *Experiment* module, press <Ctrl-Shift-2>. (Placing the mouse pointer over an icon displays a short description of the icon function.)

Editor Frame

The frame is the central area where the modules are displayed. The *Editor* frame may contain several sections (see the figure below as an example) that can be resized individually. You can resize a section by dragging the splitter bar on the border between two sections to hide or show the section. The arrows on the splitter bar indicate which area of the *Editor* frame will be affected. Clicking a splitter bar will hide the corresponding area.



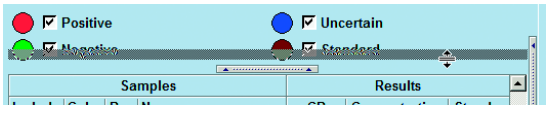
Resizing Window Sections

To hide or display a section:

- ▶ Click the splitter bar on the border.
- ▶ Click the splitter bar again to redisplay the section.

To resize an area:

- ▶ Place the cursor over the section border until the cursor changes to a double-pointed arrow.
- ▶ Click and drag the border to the location you want.

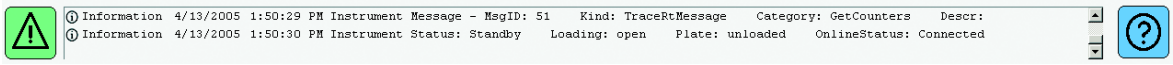


Action Button Area

The *Action button* area displays buttons unique to the currently active window which are used to perform actions on that window. Action buttons vary with the active window you currently have opened (*e.g.*, the *Action button* area displayed below is unique for the *Navigator* window.)

Message Area

The *Message* area displays status messages, errors and warnings.



The *Message* area consists of the following parts:

- *Alarm* icon on the left . The color of this icon changes depending on the severity of the alert:
  - Green = normal
  - Yellow = warning
  - Red = alarm condition

- Text field in the middle. The text field displays messages, including the type, date and time of message and the message text.
  Double-click a message entry to display detailed information.

- O*pen About box* button . Clicking this button opens the program's *About* box, which contains links to the online version of the LightCycler® 480 Instrument Operator's Manual and displays the software version and copyright information about the software.

D

1.4 Selection and Navigation Features

This section describes the object selection, navigation, and query elements of the LightCycler® 480 Basic Software:

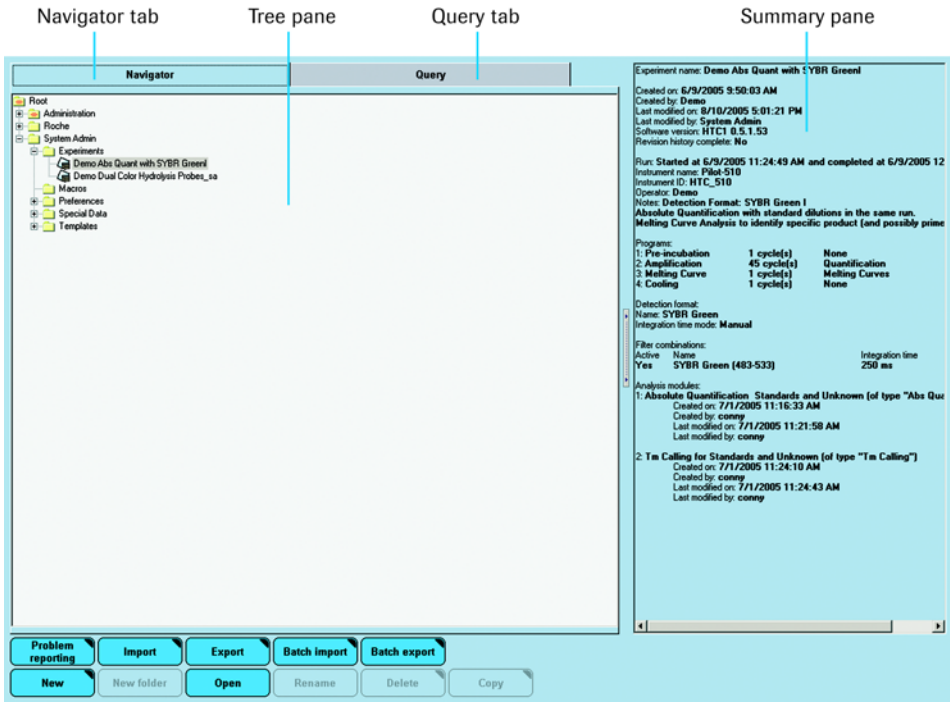
- Navigator
- Query
- Sample Selector and Sample table

1.4.1 The Navigator

The *Navigator* window provides access to items stored in the LightCycler® 480 database. Items include experiments, user accounts, instrument, macros, etc. The Navigator allows you to open experiments and related items (such as preferences, macros, special data) as discrete objects. All items in the Navigator are organized in folders in a tree-like structure (similar to Windows Explorer) and are sorted alphabetically within their folders. You can expand and contract folder views and highlight the object you want to select. In addition, you can use the *Query* tab to search for specific LightCycler® 480 Basic Software objects in the database by entering search parameters.

The *Navigator* window is structured into four areas:

- Tree pane
- Object summary pane
- Navigator controls
- Query tab



Tree Pane

The Navigator *Tree* pane displays a hierarchical tree view of the objects stored in the currently active database. The top object in the tree is always "Root". The tree is used in a similar manner as for Windows Explorer.

The Navigator *Tree* pane always includes the following default folders and objects:

- User folders (including the *System Admin* folder and folders for each user account). Each user folder contains default subfolders, such as a folder for experiments.

- Roche folder that contains experiments, templates, and macros from Roche that can be used by anyone with access to LightCycler® 480 Instrument software.

  The Roche folder contains some useful standard objects:

  - *Three demo experiments (Abs Quant with SYBR Green I, Dual Color Hydrolysis Probes, T_m Calling with HybProbe probes) in the Experiments subfolder*
  - *Four demo run templates in the* Templates *subfolder*
  - *Two predefined subsets (Checkerboard and Quadrant) in the Subsets subfolder*
  - *One color compensation object*

  To modify a Roche object, you must first copy it to your own user folder.

- Administration folder that contains objects for user groups, user roles, user accounts, and security policies.

To show or hide items under a folder, double-click the folder name or click the plus (+) or minus (–) sign next to the folder.

The Navigator is similar, but not identical to the Windows Explorer of your computer. The Navigator displays data that are stored in a database not in the Windows file system.

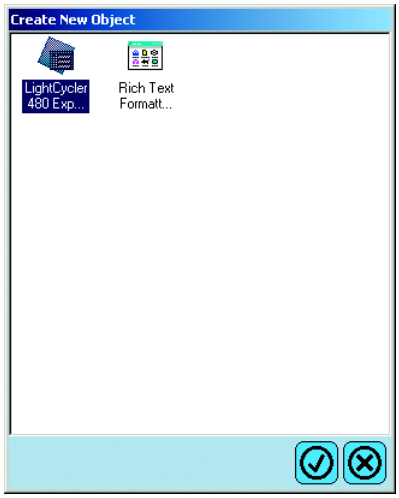
Summary Pane

The Navigator *Object Summary* pane displays experiment summary data if the currently selected object is an experiment.

Navigator Controls

In combination with the *Tree* pane, the *Navigator* control buttons allow you to work with objects in the database and to import and export objects.

Button	Function
Problem reporting	Clicking this button opens a dialog that allows you to save an experiment object together with an event log of the instrument and (optional) descriptive user notes in one file (*.ipr). This file can then be sent to the Technical Services Department of Roche Applied Science for troubleshooting purposes. For more information, see Section *Diagnostic Tools*.
Import	Clicking this button opens a standard Windows file browser that allows you to select the type of data files to be imported and the location from which they are to be imported. The following file types are supported: ▶ ATF files: "ATF" is an abbreviation for a set of files, including .abt (program and sample data), .tem (temperature data), and .flo (fluorescence data) files. All three types of the files are needed for each experiment. (ATF files originate from LightCycler® Software Version 3.5.3 or lower.) ▶ Object files: XML (Extensible Markup Language, *.ixo) object files
Export	Clicking this button opens a standard Windows file browser that allows the user to select the location to which the object data is to be exported and to name the exported object. Object data can be exported as an .ixo file of the selected object (which exports both the experiment and recorded instrument-related information).
Batch import	Clicking this button opens the *Batch import* wizard that lets you import all experiment files in a directory at one time. For more information about batch import, see Section *Exporting and Importing Files and Objects*.
Batch export	Clicking this button opens the *Batch export* wizard that lets you export all experiment files in a directory at one time. For more information about batch export, see Section *Exporting and Importing Files and Objects*.

New	Clicking this button opens the *Create New Object* dialog containing icons for each object type you can create. The icons displayed depend on the user's role.  You can either double-click an icon to create a new instance of the selected icon, or select the icon and click . *The option Rich Text Formatted document allows you to make notes and save them in the LightCycler® 480 database.*
New folder	Clicking this button creates a new folder object under the currently selected folder in the tree.
Open	Clicking this button opens the selected object in the *Editor* frame.
Rename	Clicking this button activates the object name in the tree so you can edit the name.
Delete	Clicking this button deletes the selected object (*i.e.,* removes the object from the database). *By default, LightCycler® 480 Basic Software installs a database with an audit trail. It is not possible to delete experiment objects from an audit trail database.*
Copy	Clicking this button creates a copy of the selected object. You can copy an item from another user's folder into your own folder or subfolder. (However, your user role may limit which other users' folders are visible to you.) Once the item is in your own folder, it becomes your item and you can modify it as necessary (subject to the limitations of your user role). *LightCycler® 480 Basic Software by default installs an Exor database with an audit trail. It is not possible to copy experiment objects in an audit trail database.* To copy items from another user's folder: 1. Right-click the item you want to copy in the *Navigator*, and select *Copy*. A small *Navigator* dialog opens. 2. Select a target folder, enter a new name for the item if desired, and click *OK*.

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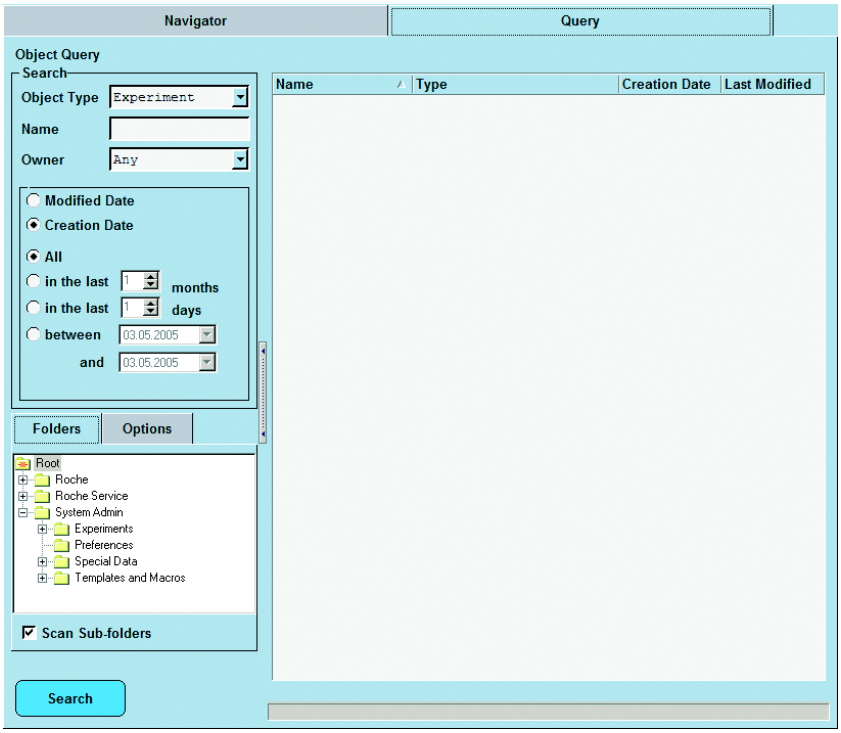
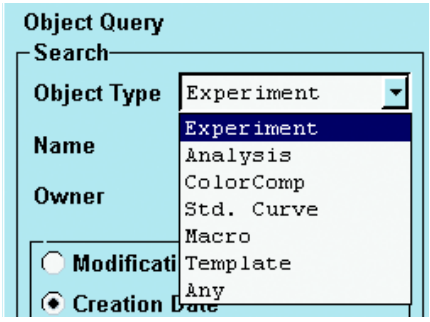
1.4.2 The Query Tab

LightCycler® 480 Basic Software includes a query tool you can use to retrieve experiments and other objects stored in the LightCycler® 480 Basic Software database. The query tool is accessible via the Navigator in form of the *Query* tab.



To create and execute a query:

1. Select the *Query* tab on the *Navigator* window.
2. In the *Object Type* box, select the type of object to be retrieved:



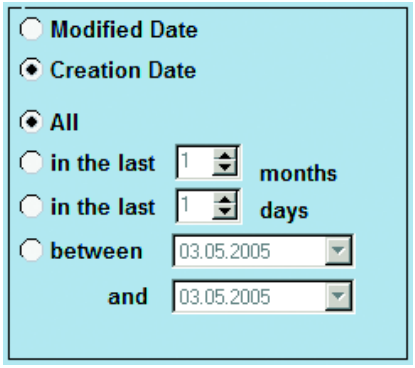
3. (Optional) Enter the name of the item to be retrieved or the owner of the item, if known.

   You may use the wildcard "" to search for any character string.*

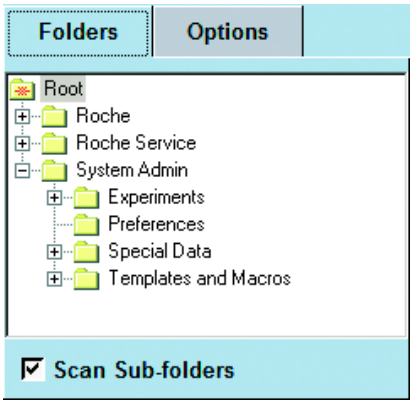
4 Select *Modified Date* or *Creation Date* to specify which date you want to use in the query.

The Modified Date and the Creation Date choices are mutually exclusive (i.e., you can search for one or the other, but not both).

Select a date range for the search. You can specify the number of months or days before the current date or you can select a beginning and ending date in the past.



5 For any possible object type, you can also select a target folder from the *Folders* tab. Tick the *Scan subfolders* box to include all subfolders within a directory in the search:



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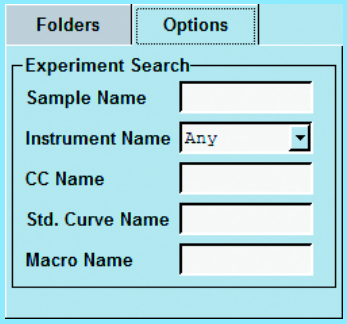
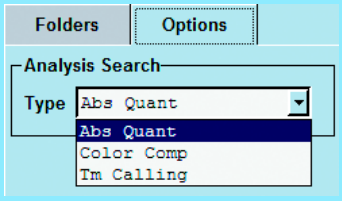
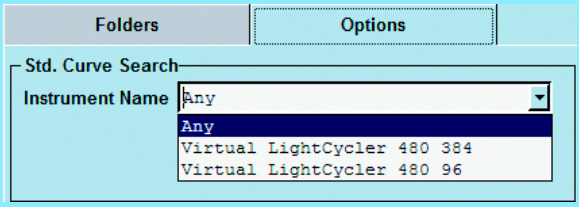
6 For some object types, additional search options can be selected from the *Options* tab:

Object Type	Search Options
Experiment	
Analysis	 The list of available analysis types depends on the installed LightCycler® 480 Basic Software modules.
Color Comp Std. Curve	 You can search for a CC object according to the instrument that created it.

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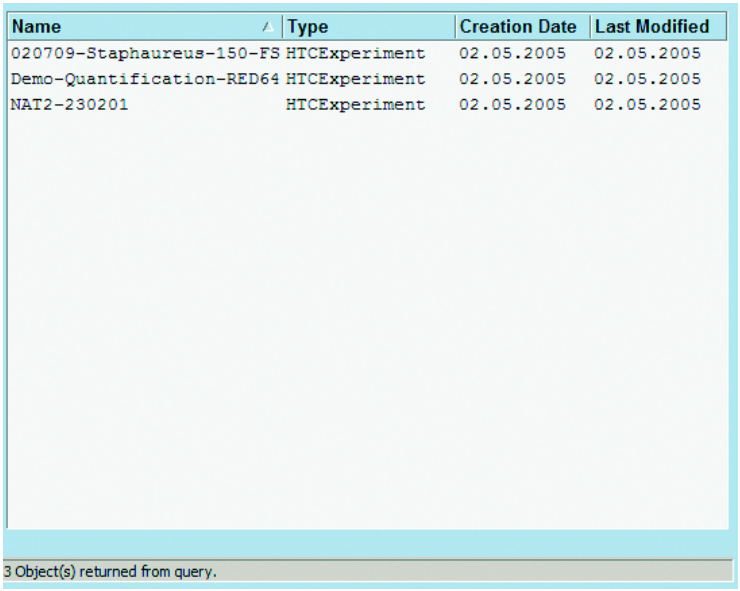
7 ▶Click the *Search* button. Results are displayed in the pane to the right of the search criteria.



▶The results include the following:
- ▶ Object name
- ▶ Object type
- ▶ Date the object was created
- ▶ Date the object was last modified

▶You can sort the result list in ascending or descending order by clicking the corresponding column head.

▶When you select an object in the list, the full path to the object is displayed in the *Status* bar at the bottom of the *Results* pane.

If an error message is displayed stating that the query engine needs to be updated, you must update the database. If you have Local Administrator privileges, see "Updating the database" in Section Administrative Tools for instructions. Otherwise, see your system administrator.

8 To open an object, double-click the object name.

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1.4.3 Sample Selector and Sample Table

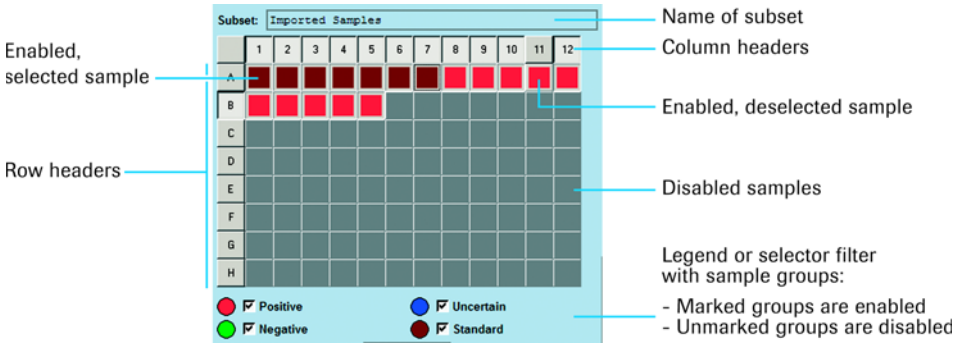
The *Sample Selector* and the *Sample* table are displayed on many windows (*e.g.*, most windows connected to analyses) in the LightCycler® 480 Basic Software and are used to select the samples to be displayed in the corresponding analysis charts or to include or exclude samples from analysis.

Sample Selector

The *Sample Selector* allows you to easily select individual samples or groups of samples for display in an analysis chart. The *Sample Selector* includes a multiwell plate (MWP) image with selectable wells and a legend showing selectable sample groups.

Samples in the MWP image can be enabled or disabled by selecting or deselecting a sample group in the legend. A disabled sample is colored dark-grey and exhibits no response when clicked. Samples in the MWP that do not belong to the subset chosen for analysis are disabled by default and cannot be changed. Which sample groups are available in the legends depends on the analysis type.

When enabled, a sample may be either selected or deselected. A selected sample is displayed as a pressed button, and the button for a deselected sample is displayed as not pressed. Only selected samples are displayed on the corresponding analysis chart.



You can set the selection status of enabled samples in the MWP image as follows:

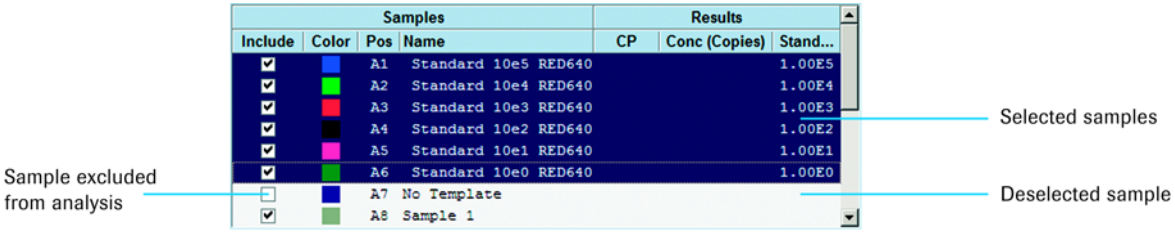
- Clicking a sample toggles the selected status.
- Click and drag on a selected well to deselect all wells in the drag region. Conversely, click and drag on a deselected well to select all wells in the drag region. In other words, the selection status of the region is determined by toggling the status of the first well clicked.
- Click row or column headers to select or deselect the corresponding rows or columns.
- A *Selector Filter* may be included within the legend (depending on the type of analysis) to set the selection status of the samples. In the example above, the *Selector Filter* corresponds to the result calls (Positive, Negative, Standard, Uncertain) of an Absolute Quantification analysis.

Sample Table

The *Sample* table displays the well coordinates of the samples in the MWP and the color which represents a sample in the analysis charts (defined by the sample preferences). Use the *Sample* table to select samples for display in an analysis chart or to include/exclude a sample from analysis.

The sample color in the Sample table always refers to the color in a chart or data display, and never to the color in the MWP image. Colors in the MWP image refer to the sample groups in the Sample Selector legend only.

Only samples that are enabled and selected in the Sample Selector are displayed in the *Sample* table. Other information (in additional columns) may be added to a *Sample* table according to the context of the screen (*e.g.*, results such as CP and concentration for quantification analyses). If there are more samples than can be displayed, a scroll bar is added.



You can select one or all of the samples in the *Sample* table for display in an analysis chart, but you cannot change any of the information or the sort order of the samples. Selected samples are highlighted.

To add or remove samples from the selection in the *Sample* table, use the standard windows shift-click and ctrl-click features.

Further, samples can be included into or excluded from analysis. To include a sample, mark the *Include* box in the left table column. Status of the *Include* box is changed by double-clicking or by pressing the <Space> key. Using the *Include* option, you can, for instance, decide which standards are used to calculate the standard curve in Absolute Quantification analysis.

After you have changed the include status of a sample, you must always recalculate the analysis.

1.5 Exporting and Importing Files and Objects

To view experiment information in LightCycler® 480 Basic Software, the experiment file must be stored in a LightCycler® 480 Basic Software database. Therefore, if experiment files are currently stored on a hard drive or other data carriers, you must import the files into the database.

Importing a file does not remove it from the original location; it copies the file into the database so you can view the information in LightCycler® 480 Basic Software. For example, you need to import files in these cases:

- If you want to move a LightCycler® 480 Basic Software experiment file from one database to another; you must export the file from the first database to a new location, such as your hard drive, and import the file into the second database.
- If you want to view and analyze experiment files from LightCycler® 4.0/4.05 Software.

Using the *Import* Navigator Control button, you can import the following:

- **ATF**: Experiment file from LightCycler® Software 3.5.3 or later; you can import all the FLO files from an entire directory at one time using the batch import tool, described below. (Importing FLO files automatically imports the corresponding ABT and TEM files.)

Raw data imported from ATF files will be associated with the "ATF" detection format. This format will be mapped to one excitation (470) and six emission filters (defined as 530, 555, 610, 640, 670, and 710). If the imported raw data was generated using the continuous mode setting, no conversion will be performed. The acquisition per °C will be set to a fixed value of "5". Other mode settings will be ignored.

- **IXO**: Experiment file from LightCycler® 480 Basic Software and LightCycler® Software 4.0/4.05; you can import all the IXO files from an entire directory at one time using the batch import tool, described below.

When importing IXO files from LightCycler® Software 4.0 or 4.05, only raw data are imported into the LightCycler® 480 database. Any other objects included in the IXO file (e.g., analyses) are not imported.

IXO files that do not have checksums will not be imported and an error message will be generated.

For imported experiments, an "Imported Samples" subset is generated automatically.

To store experiment objects or templates outside the LightCycler® 480 Basic Software database or to transfer objects between databases, you must export the LightCycler® 480 Basic Software files. Exporting a file does not remove the object from the database, but instead copies the file into XML format and stores it at the location you designate. The exported file has an .ixo file extension. In addition, you may save LightCycler® 480 Basic Software chart data in raw data format.

IXO files exported from LightCycler® 480 Basic Software cannot be imported into LightCycler® Software 4.05.

Read the sections below to learn how to import and export LightCycler® 480 files individually or by directory (batch import or export).

Exporting Individual LightCycler® 480 Basic Software Objects

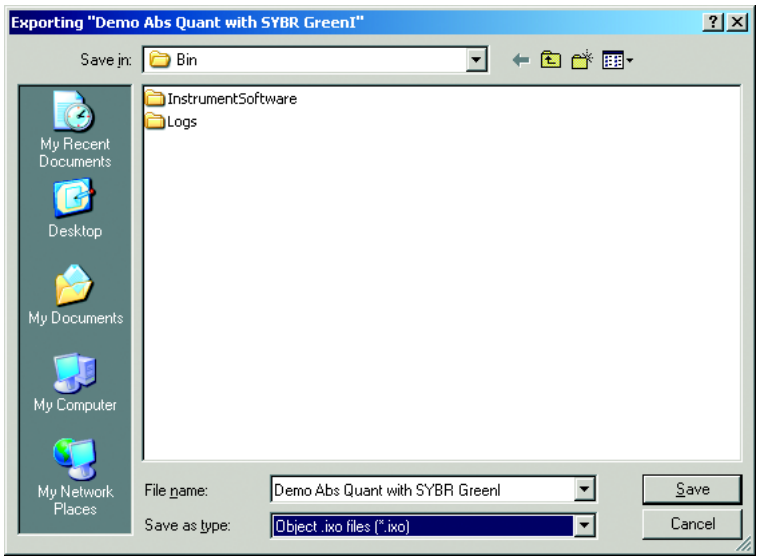
Individual LightCycler® 480 Basic Software objects or data can be exported both from the *Navigator* or when opened in the LightCycler® 480 Basic Software main window.

1. Use one of the following options to export a LightCycler® 480 object:
   ▶Select it in the *Navigator*
   ▶Open it in the main window

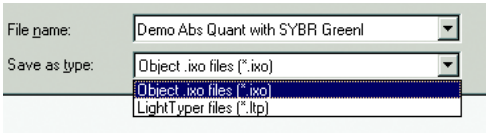
2. ▶When working in the *Navigator*, click the Export button.

   ▶When working on the main window, click the button.

3. The Windows file selection dialog opens. Browse to select a file destination.



4. From the *Save as type* drop-down list, select your preferred export file format (*.ixo or *.ltp):



5. Enter a file name, and click *Save*.

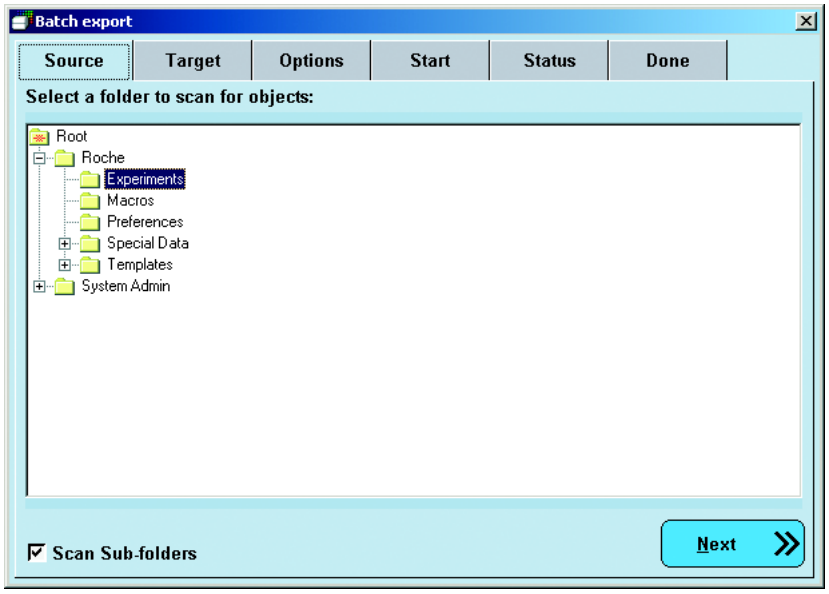
Exporting Multiple Experiment Files Simultaneously

LightCycler® 480 Basic Software includes a batch export tool that lets you export all experiment files in a directory at one time. Follow the instructions below to export a directory of experiment files.

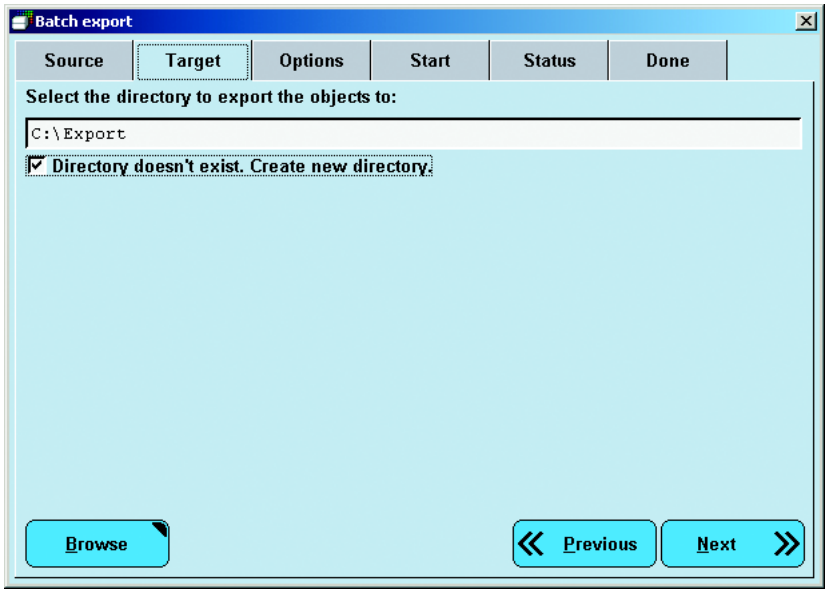
Batch export is only possible from the Navigator.

Batch export is performed using a wizard. You can move from a wizard step to the previous or next step by clicking the corresponding button. Note that the Next button will only be available when you have provided the settings required for the current tab.

1. Click the Batch export Navigator Control button. The *Batch Export* wizard opens. On the *Source* tab of the wizard, select a source folder in the currently open database. Tick the *Scan Sub folders* option to include all subfolders within the source directory.

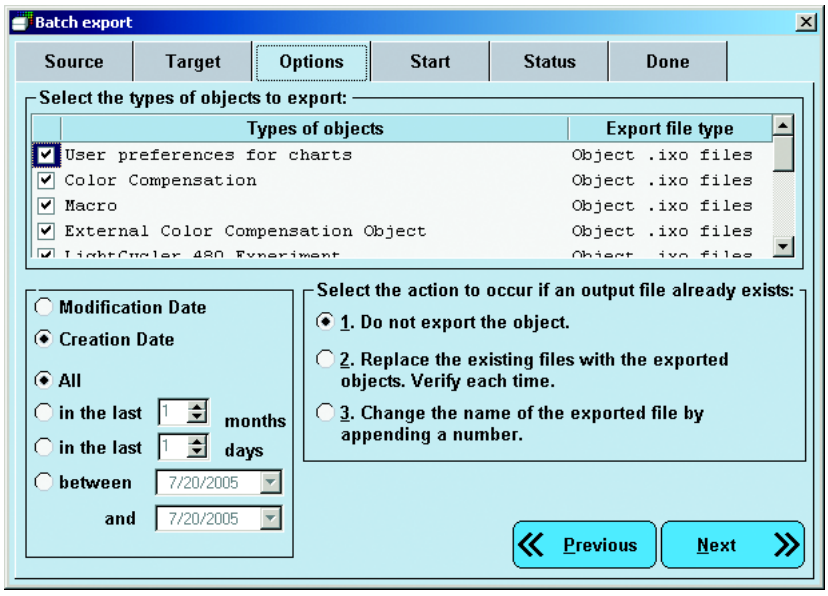


2 On the *Target* tab, select a destination directory. Click the *Browse* button to browse for a destination folder. Alternatively, type the path of the destination directory directly into the input field. If the specified directory does not exist yet, tick the box beneath the input field to create it.



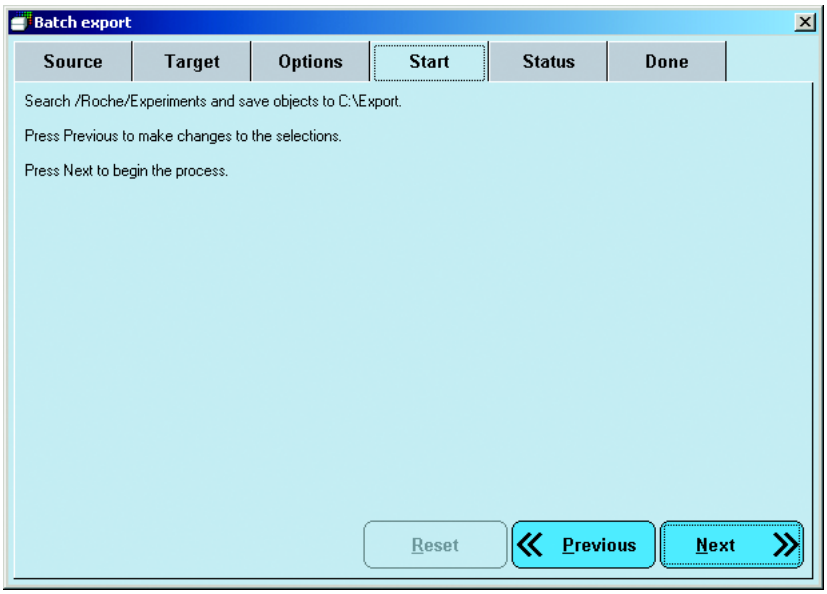
3 On the *Options* tab, you can set various export options.

- ▶Select the type or types of objects to export from the folder
- ▶Limit the set of objects being exported to those with a certain creation date or modified date. The date range may be specified as one of the following
  - All
  - In the last n months
  - In the last n days
  - From a begin date to an end date specified by the user
- ▶Specify the action to take if a file already exists with the same name as an object being exported. The possible actions are as follows:
  - Do not export
  - Replace an existing file after confirmation by the user
  - Save as a new file by appending a number to the file name

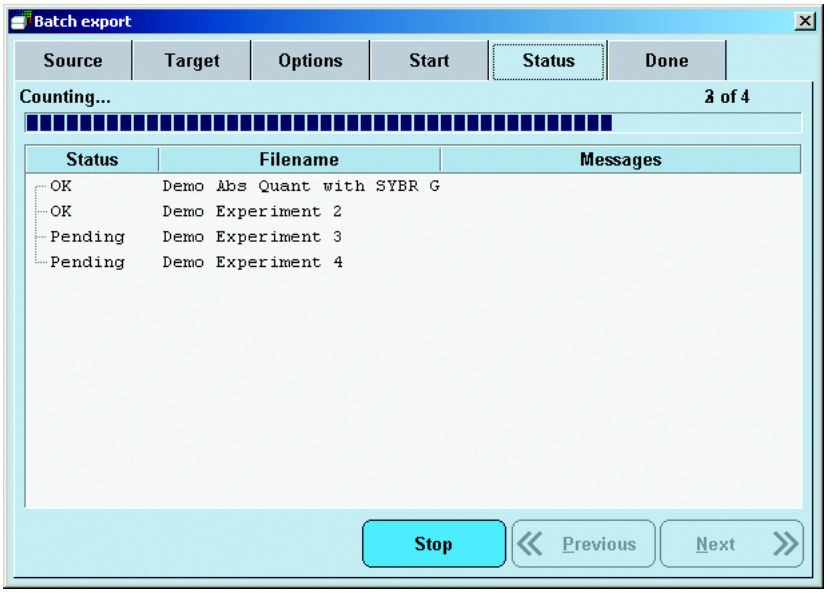


4 On the *Start* tab, you can review your settings and start the export process.

The Reset button on the Start tab is active only after an export is complete. Clicking the Reset button resets the results of the previous export so the export can be repeated.



5 On the *Export status* tab, you can view the status of the export process. While the export is in progress, the *Stop* button is active. You can abort the export by clicking the *Stop* button.



6 The *Done* tab displays a summary of the batch export. Click *Done* to close the wizard.

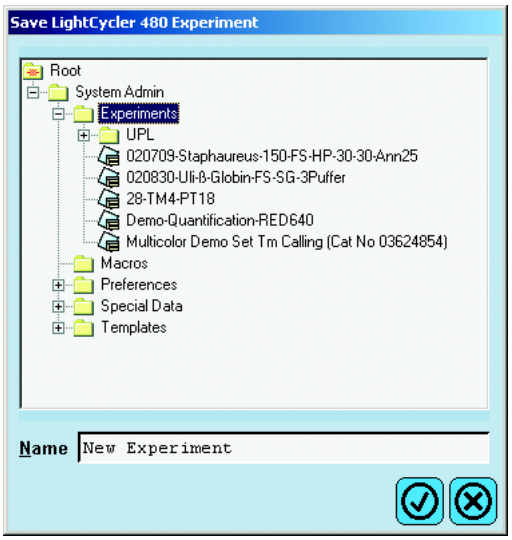
Importing Individual Files

1. From the Navigator Controls select *Import*, and select the type of file you want to import:
   ▶ For FLO, ABT, TEM files, select *ATF Files*
   ▶ For LightCycler® 480 or LightCycler® Software 4.0/4.05 files, select *Object .ixo Files*

2. Find and select the file you want, and click *Open*. The file is imported and is opened in the main window.

   To select multiple files, press the <Ctrl> key while clicking the file names.

3. To save the imported file as an object in the current LightCycler® 480 database, click the *Save* button. Navigate to a location in the database to save the object and enter a new object name, and click *OK*.



Import of LightCycler® Software 4.0/4.05 experiments which include color compensation (CC) data requires importing the respective color compensation experiment separately and creating a new color compensation object.

Importing Multiple Files Simultaneously

LightCycler® 480 Basic Software includes a batch import tool that lets you import all experiment files in a directory at one time.

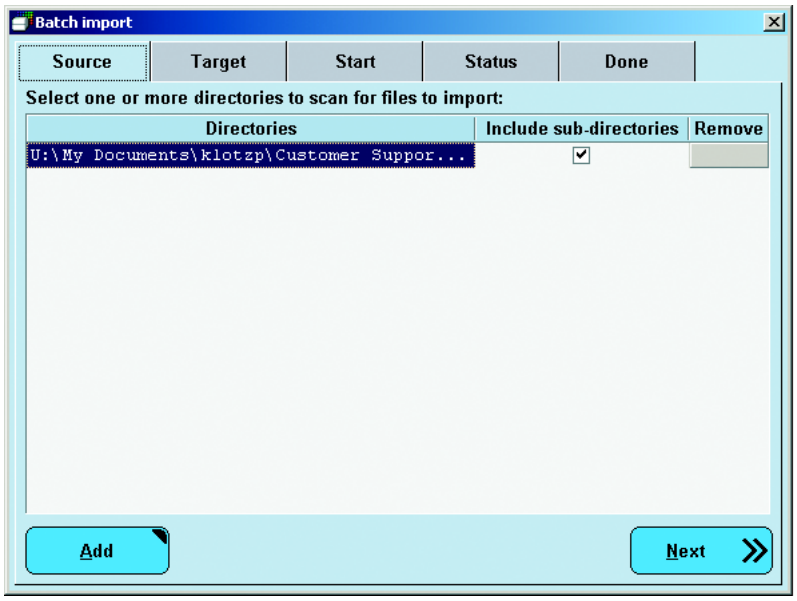
For batch import of FLO files, the directory must contain the corresponding ABT and TEM file for each FLO file or the FLO file cannot be imported.

Batch import is only possible from the Navigator.

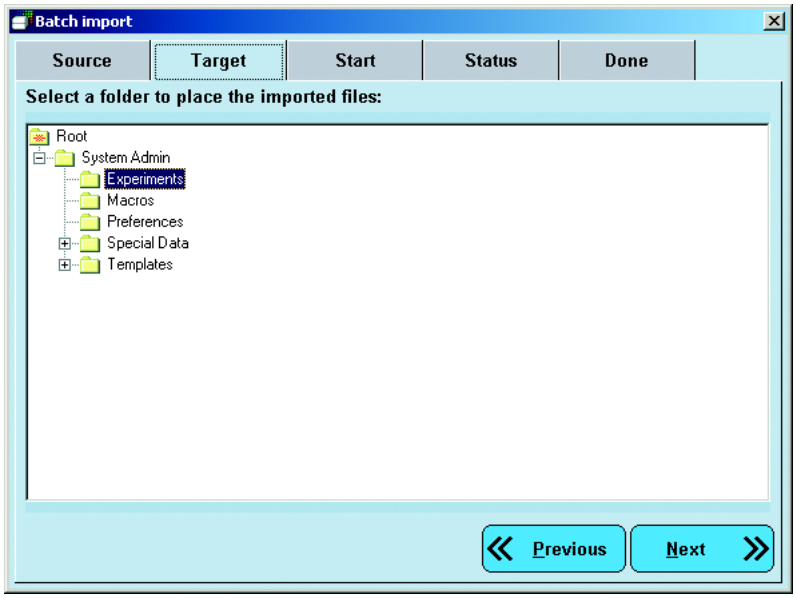
Batch import is performed using a wizard. You can move from a wizard step to the previous or next step by clicking the corresponding button. Note that the Next button will only be available when you have provided the settings required for tab.

Follow the instructions below to import a directory of experiment files and to repeat an import if necessary.

1. Click the Batch import Navigator Control button. The *Batch Import* wizard opens. On the *Source* tab of the wizard, select a source folder on a data carrier. To add source directories to the list, click the *Add* button. Tick the *Include sub-directories* box to include all subfolders within the source directory. Click *Remove* to delete the directory entry from the list.



2. On the *Target* tab, select a destination directory in the currently open database using the location selector.



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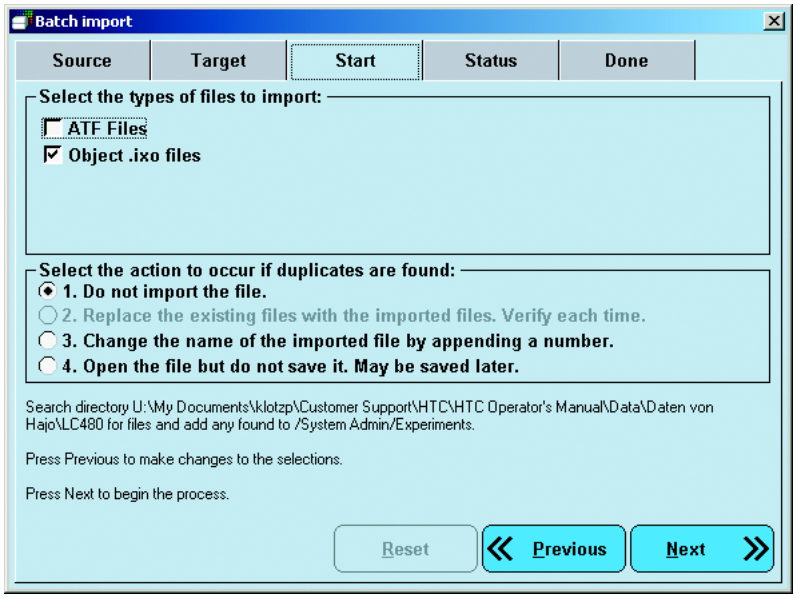
3 On the *Start* tab, you can set various import options and start the import process.



The following options are available:

- ▶Choose whether to import ATF or IXO files (or any combination) from the directories.
- ▶Specify the action to take if a file already exists with the same name as an object being exported. The possible actions are
  - Do not export
  - Replace an existing file after confirmation by the user
  - Save as a new file by appending a number to the file name

Click the *Next* button to start the export process.

The Reset button on the Start tab is active only after an import is complete. Clicking the Reset button resets the results of the previous import so the import can be repeated.

4 On the *Status* tab, you can view the status of the import process. While the import is in progress, the *Stop* button is active. You can abort the import by clicking the *Stop* button.

5 The *Done* tab displays a summary of the batch import. Click *Done* to close the wizard.

2. Programming and Running an Experiment

LightCycler® 480 Basic Software controls the LightCycler® 480 Instrument using information provided in the experiment protocol. During an experiment run, the protocol defines the instrument's temperatures and hold times, the number of cycles being executed as well as other parameters. As the experiment progresses, the software gathers temperature and fluorescence data from the instrument and displays it on the *Data display* tab. After the experiment is finished, you can save the experiment data and use the LightCycler® 480 Basic Software analysis modules to analyze results.

This chapter explains how to perform the following:

- Program an experiment
- Run an experiment
- Define detection formats

2.1 Programming an Experiment

Programming an experiment consists of defining the cycles of heating and cooling to be performed by the instrument and providing general setup information. For you to be able to create an experiment protocol, your user account must have the Expert User or Local Administrator role. For more information about privileges associated with each user role, see Section *Managing User Access*.

You can program an experiment only if an instrument is installed in the LightCycler® 480 Basic Software. To allow programming in offline mode, LightCycler® 480 Basic Software offers two virtual LightCycler® 480 Instruments (in either 96 or 384 version). If you should need to program an experiment while not being connected to a real instrument, select one of the virtual instruments on the Instruments dialog and set it as the default. (For details on how to install an instrument into LightCycler® 480 Basic Software, see Section Administrative Tools. An instrument does not need to be connected for you to be able to program an experiment.)

Follow the general steps below to program an experiment. Detailed information for each step follows the general procedure.

To program a new experiment:

1. Click *New Experiment* in the *Tasks* area of the *Overview* window or switch to the *Navigator* window and click *New*. Select the icon for a new LightCycler® 480 Instrument experiment and click

2. In the *Setup* area of the *Programs* tab, specify the following setup parameters:

   - ▶Detection Format
   - ▶Block Type (determined by active instrument, display only)
   - ▶Plate ID, (optional)
   - ▶Reaction Volume

3. Choose a *Detection Format* from the pull-down list and modify the settings for the available detection formats using the *Customize* option, if needed. For more information, see Section *Setting Detection Formats*.

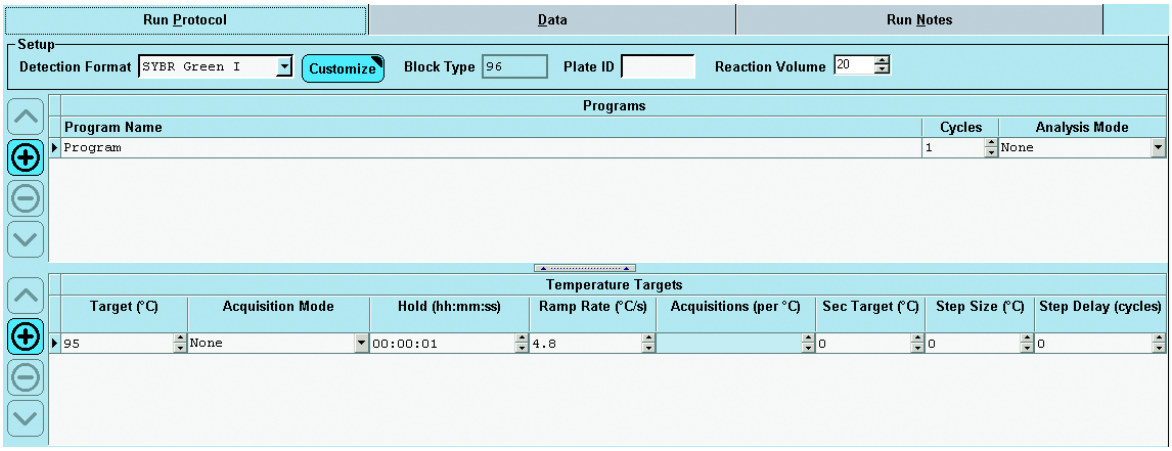
4. Enter a PCR multiwell plate identifier into the *Plate ID* field, either manually or using a handheld bar-code scanner. If you have installed and activated the optional LIMS/Bar-Code Module (laboratory information management system), the Plate ID is scanned automatically by the built-in bar-code scanner of the LightCycler® 480 Instrument during loading of the multiwell plate.

   If you have entered a Plate ID manually into the Plate ID field and the internal bar-code scanner reads a different bar code from the multiwell plate, the software prompts you to overwrite the Plate ID. If you answer with No, the experiment will not start.

5. Select a *Reaction Volume* in µl. The possible range of reaction volume depends on the type of block cycler installed:
   - ▶96-well thermal block cycler: 10 – 100 µl
   - ▶384-well thermal block cycler: 5 – 20 µl

   It is possible to use a sample volume as small as 3 µl. However, this volume is not recommended due to the risk of evaporation and subsequent failure of the experiment. If you want to use this low sample volume, take care to ensure that no evaporation of the reaction mix occurs (e.g., by overlaying it with mineral oil).

6 In the *Programs* and *Temperature Targets* section, click ⊕ to add as many additional programs or temperature targets as needed for the protocol (the first program is always provided by default). For each program row, specify the *Program Name*, *Cycles*, *Analysis Mode,* etc. (For more information, see Section *Defining Programs and Temperature Targets*.)



7 Alternatively, you can apply an experiment template as follows:
a) Click *Apply Template* to display the *Apply Template* dialog box.
b) Select a *Template* from the list, and click ✓.
The template settings are applied to the new experiment protocol.
Modify setup parameters, experiment programs and temperature targets as needed.

8 (Optional) In the *Module* bar, click *Sample Editor* to define sample information. For a detailed description, see Section *Sample Editor.*

9 (Optional) In the *Module* bar, click *Subset Editor* to define sample subsets. For details, see Section *Working with Subsets*.

You are not required to define sample and subset information before the run. You can define this information during or after a run, if desired.

10 Prepare the plate and load it into the instrument as described in Section *Operation.*

11 Click *Start Run*.

The Start Run button is only available if a multiwell plate has been loaded.

12 The *Save Experiment* dialog is displayed. Enter a name for the experiment and select a folder to save it.

2.1.1 Setting Detection Formats

By setting the detection formats, you choose the filter combinations suitable for your experiment.

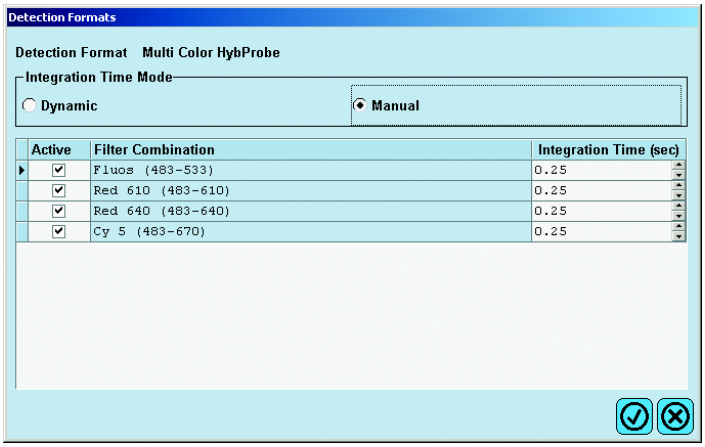
The *Detection Formats* dialog box is displayed when you click *Customize* in the *Setup* area of the *Programs* tab. This dialog box allows you to modify the settings for the available detection formats. The modifications you enter here apply only to the current experiment.

You cannot change or customize the detection format definition after the run has started. If you start a run with a detection format that is not suitable for your experiment, the run is lost because no utilizable data will be generated.

To set the detection formats:

1. In the *Setup* area, select a detection format from the pull-down menu. For more information about pre-defining detection formats, see Section *Administrative Tools*.

2. To modify the settings for the available detection formats, click *Customize*. The *Detection Formats* dialog box opens.



3. Select an integration time mode:
   ▶ Dynamic: No further input is required
   In this mode, LightCycler® 480 Basic Software uses the Melt and Quant Factor defined for the filter combination to extrapolate the final fluorescence intensity based on the initial value at the beginning of the reaction. The calculated final fluorescence is used to set the optimum integration time.
   (For details, see Section Administrative Tools.)
   ▶ Manual: Specify an integration time for each filter combination. The manual integration time is specified in seconds, within the range of 0.01 to 10 seconds.

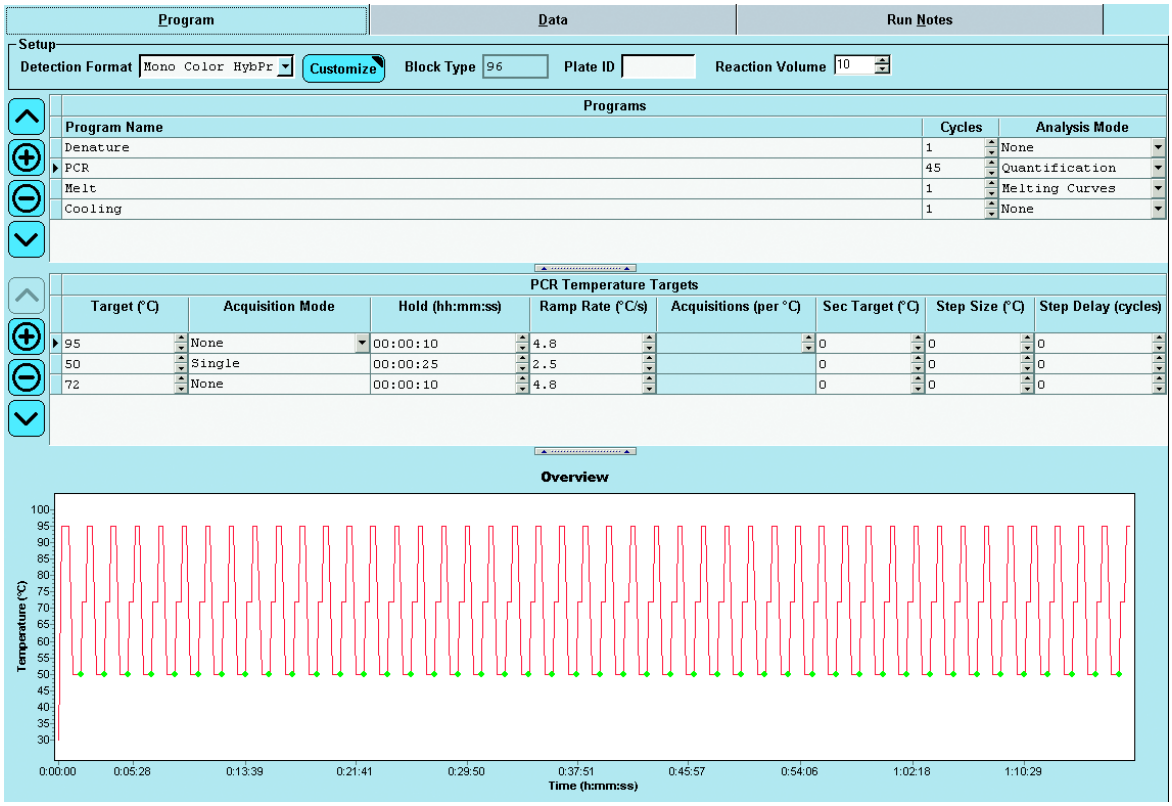
4. Select the filter combinations for the selected detection format. By default all filter combinations are selected; clear the checkbox to deselect a filter combination, if needed.
   For example, if you select the Multicolor HybProbe detection format, which includes four filter combinations, but wish to run an experiment using Red 610- and Red 640-labeled probes only, deselect the Fluos and Cy5 filter combination. Although it would not be detrimental to your experiment to use the default settings, applying filter combinations that are not required will result in a longer measuring time.
   The Fluos filter combination in the Multicolor HybProbe detection format is only required for a color compensation experiment.

5. Click to apply the selected filter combinations to your experiment.

2.1.2 Defining Programs and Temperature Targets

Each experiment protocol has one or more programs. Each program can be executed for multiple cycles. A program consists of one or more temperature targets. A temperature target specifies the target temperature which is to be reached, the duration for which the temperature is applied, the speed with which the temperature is reached, and other parameters. You define programs and their temperature targets in the *Programs* tab of the *Run* module.

The example below includes four programs: Denat, PCR, Melt and Cooling. When the PCR program (45 cycles) is selected in the *Programs* table, the temperatures appear automatically in the *PCR Temperature Targets* table below.



To define programs and temperature targets:

1. In the *Programs* section of the *Programs* tab, click ⊕ to add a new program.
A default program named "Program" is added, containing one default temperature target.
You can add up to 99 programs.

2. Edit the default values for the following program parameters, clicking the <Tab> key on your keyboard to move from one column to the next. (If you make a mistake, click ⊖ to delete the program and start over.)

Parameter	Description/Instruction	Valid Values
Program Name	The name of the program. Click in the *Program Name* box, and enter a new name.	Any alphanumeric string.
Cycle	The number of times the program should be repeated. Enter a value or select it by clicking the up and down arrows.	1 – 99 cycles
Analysis Mode	The type of analysis expected for this program (if any). Select an analysis mode from the pull-down list. *The Analysis Mode defines which parameters can be selected under Temperature Targets (e.g., if you select Quantification it is not possible to select the Continuous Acquisition Mode.)*	**None:** No analysis is expected **Quantification:** A quantification analysis is expected **Melting curves:** A melting curve analysis is expected Color Compensation: A color compensation analysis is expected

3. In the *Temperature Targets* section, edit the default values for the following temperature parameters for the first temperature target.

Parameter	Description/Instructions	Valid Values
Target	The target temperature. Enter a temperature.	37°C to 99°C, default is 95°C
Acquisition Mode	The frequency with which fluorescence data is acquired Select an acquisition mode from the pull-down list.	**None:** No fluorescence data is acquired **Single:** Acquires fluorescence data once at the end of this temperature segment in each cycle This is the typical setting for quantification. **Continuous:** Acquires fluorescence data continuously. This is the typical setting for a melting curve or a color compensation analysis.
Hold	The length of time to hold the target temperature in hours:minutes:seconds format. Enter a hold time.	00:00:00 – 12:00:00

Parameter	Description/Instructions	Valid Values
Ramp Rate (°C/s)	The rate at which the instrument heats up or cools down to the target temperature. Enter a ramp rate. *The maximum ramp rate depends on the block cycler type installed.*	▶Heating up: 1.0 – 4.8 °C/s ▶Cooling down: 1.0 – 2.5 °C/s *If you are cooling down to a target temperature <50°C, use a ramp rate of 2.0°C or less.*
Acquisitions (per °C)	The number of data measurements taken per °C. Only available in the *Continuous* acquisition mode.	1 to 100, default is 5 *The optimal acquisition rate for melting curve analysis has to be determined empirically for each assay and can range from 1 to 5 acquisitions/°C.*
Sec Target (°C)	A second target temperature to be reached by the last cycle of the program. Use this feature to change the target temperature of a segment during the amplification reaction. Enter a temperature.	Default: 0°C (no second target temperature) Valid range: 37°C – 99°C
Step Size (°C)	The number of degrees to change the temperature with each cycle to reach the secondary target. Enter a step size.	Default: 0°C (no step) Valid range: 0.1°C – 20°C
Step delay (cycles)	The number of cycles before the step size is applied for the first time.	Default: 0 (begins with the first cycle) Valid range: 0 – 99

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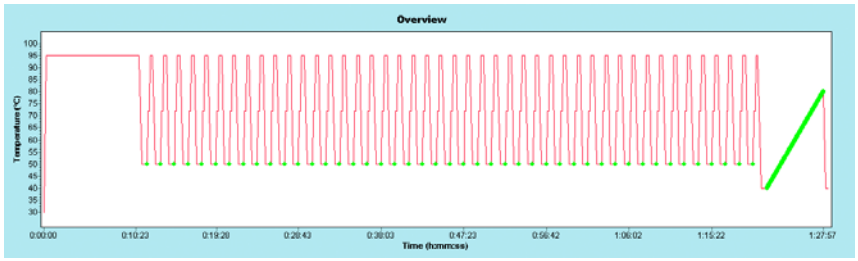
4 Click ⊕ to add another temperature target to the current program, and enter parameter values. Repeat to define as many temperature targets as you need for the current program.

You can add up to 99 temperature targets.

5 Repeat Steps 1 – 4 to create additional programs and their temperature targets.

To reorder the programs or temperature targets, select the item you want to move, and click the up ^ or down v arrow to move the item up or down in the list. To delete an item, select the item, and click ⊖.

6 View the *Overview* section to see a graphical representation of the complete experimental protocol you have defined. Use this chart to see whether the experiment protocol reflects the time and temperature cycles you want and modify the programs and temperature targets as needed.



Declaration of time is estimated.

7 Click 💾 in the *Global action* bar to save the protocol. Navigate to a location to save the protocol, enter a protocol name, and click ✓.

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2.1.3 Customizing the Online Data Display

When the experiment runs, the data being collected are displayed in charts on the *Data* tab of the *Run* module. Three chart types are available:

- Fluorescence History: Fluorescence for selected samples for a selected filter over time
- Temperature History: Temperature and data acquisition points during the experiment run
- Exposure History: Integration time displayed versus acquisition number

You can change any of the type of existing charts and modify the display options.

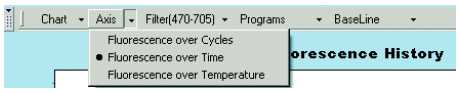
You can customize the online data display while the experiment is running.

To customize the online data display:

1. In the *Run* module, select the *Data* tab, and click  above the chart you want to change. The chart's options toolbar is displayed, containing a chart menu. Fluorescence charts contain additional options.

2. To change the chart type, select the new chart type from the *Chart* menu:
   - Fluorescence History: Displays fluorescence versus time, cycles or temperature
   - Temperature History: Displays temperature versus time
   - Exposure History: Displays integration time versus acquisition number

3. To modify display options for the *Fluorescence History* chart:
   - Select the axis type (the type of data represented by the X axis), the filter combination, and the program containing the data you want see. Additionally select or deselect *BaseLine* subtraction.



2.2 Running an Experiment

After you have defined setup parameters (programs and temperature targets) and the online data charts, you are ready to run the LightCycler® 480 Instrument experiment.

To start an experiment run:

1. Prepare a LightCycler® 480 Instrument run as described in Section *Operation.*
2. Load the plate into the LightCycler® 480 instrument as described in Section *Operation.*
3. Click *Start Run* on the *Run Protocol* tab.

   You can only start an experiment run when an instrument is connected. The Start Run button is only available if a multiwell plate has been loaded.
4. You are prompted to save the experiment. Enter an experiment name and browse to a folder where you want to save the experiment.

   If you close the dialog without saving the experiment, the run will not start.
5. A status bar indicates the progress of the running experiment.

6. As the experiment progresses, the *Messages* area displays messages indicating the progress of the run and any errors encountered during the run. Returned sample data is displayed in the charts on the *Data* tab.
7. To view data for specific samples, select one or more samples in the sample list. If needed, you can modify chart settings during the experiment run. For more information, see Section *Customizing the Online Data Display*.
8. (Optional) You can apply a color compensation object after an experiment run. Use the *Color Comp* multi-select button to turn color compensation on or off and to select a color compensation object.

Color Comp Off	No color compensation object has been selected. Select this if you do not want to use color compensation.
In Use	Allows you to select an object from the *Selected Color Compensations* list. This list displays all selected (*i.e.,* previously applied) color compensation objects. The compensated filter combinations are displayed in brackets after each name.
In Database	Allows you to select an object from the *Available Color Compensations* list. This list displays all available color compensation objects. For each object, the list includes the name, creation date, path and filter combinations that can be compensated.

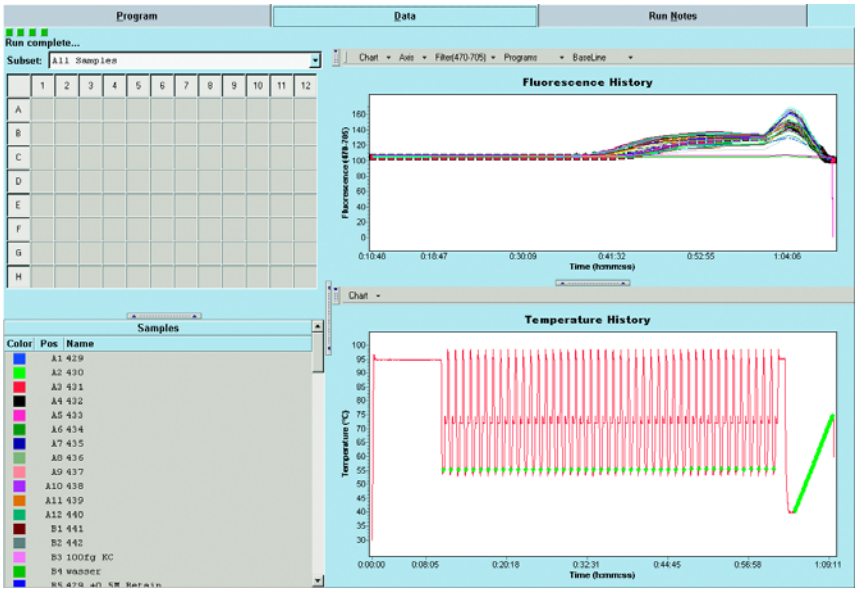
9 (Optional) To adjust or stop the program during the run:
► Click *End Program* to stop the current program and skip to the next program in the experiment protocol.

Performing this task ensures, that the data are complete and an analysis can be performed.

► Click + *10 Cycles* to add 10 cycles to the current program.
Click *Abort Run* to stop the run. (The *Abort Run* button replaces the *Start Run* button during the run.)

Performing this task results in incomplete data, no analysis can be performed.

10 When the experiment is finished, a status message displays “Run complete”.



11 Click *Sample Editor* in the *Module* bar to open the Sample Editor, and complete sample information, if necessary. For more information, see Section *Entering Sample Information*.

You can enter or modify the sample information at any time before, during or after the experiment is completed. We recommend that you enter the sample information before running the experiment.

During a run, temporary backup data for the current experiment is saved to the user’s file system. If the run finishes and has saved the data in the database without an error, these temporary backup data are deleted. If the connection between the application and the instrument is temporarily interrupted, the software will download data automatically from the instrument after the connection is reestablished. The maximum length of a timeout is 7 minutes. If the timeout is exceeded, the run is considered as prematurely terminated, and a warning is generated. If backup or instrument data exist, the data will be automatically recovered upon your next login or start of a new run if a corresponding experiment is found by the software. If no corresponding experiment is found, the software prompts you to confirm the deletion of the data.

2.3 Entering Sample Information

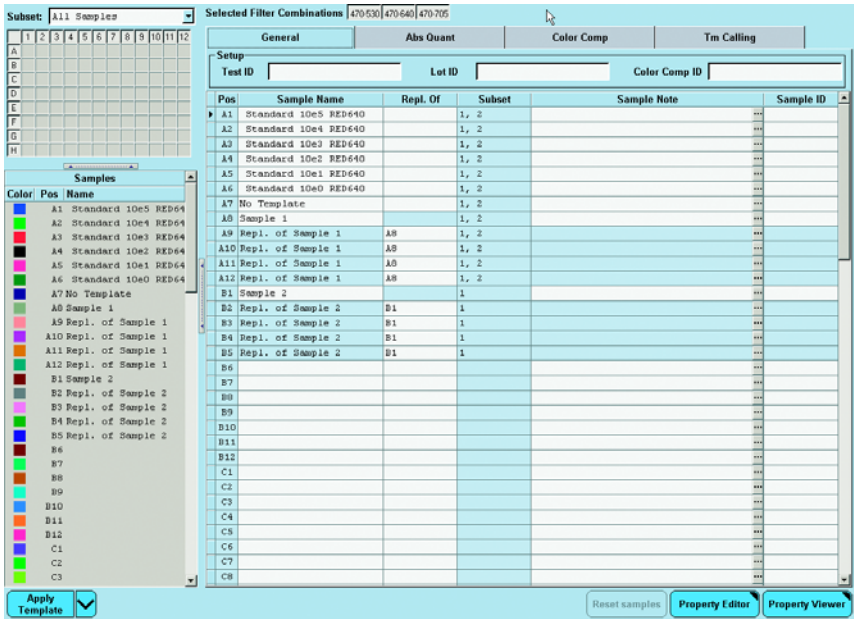
Use the LightCycler® 480 Basic Software *Sample Editor* to record information about the samples in the experiment. You can enter sample information before, during or after an experiment is completed.

Editable fields are displayed as white, and non-editable fields are displayed as pale blue. Only entries that are not replicates may be edited.

Any sample designated as a replicate is automatically renamed "Repl. of S," where "S" is the sample name of the source sample. All information on the analysis tabs for a replicate will duplicate that of the source sample.

To enter sample information:

1. In the *Module* bar, click *Sample Editor*. The *Sample Editor* opens.
2. The *Sample Editor* window is displayed with an image of your MTP on the upper left side of the window and a sample list below the MTP image. The Sample Editor provides four tabs: One tab (*General*) for entering general sample information and three tabs (*Abs Quant, Color Comp, Tm Calling*) for entering analysis-related sample information.
3. Highlighting one or more samples in the *Sample* table of the *Sample Selector* displays rows for the samples in the *General* and analysis tabs. For details, see Section *Sample Selector and Sample Table*.
4. On the *General* tab, you can enter general sample information.



5 In the *Subset* box, select a predefined subset of samples to display or select *All Samples* to display all samples on the MTP. For more information on defining subsets, see Section *Working with Subsets*.

6 In the *Selected Filter Combinations* area, select the filter combinations to be used in the experiment.

7 (Optional) The *Setup* area allows you to specify the catalog number and lot number for the Roche assay used in the experiment and to specify a color-compensation ID. You can scan a bar code to enter the catalog and lot numbers or enter the numbers manually.

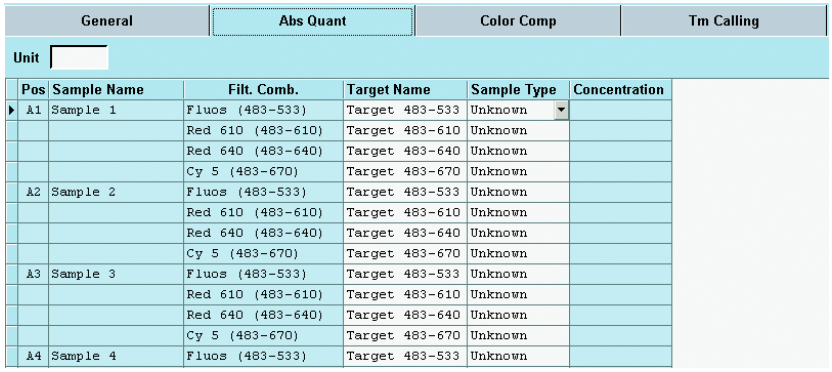
8 Enter the following information for each sample:

Parameter	Description	Valid Values
Sample Name	The name of the sample	Alphanumeric characters; spaces allowed (maximum 25 characters) *You can import a sample list from e.g. an Excel sheet by using the Windows <Ctrl-C>/<Ctrl-V> copy and paste function.*
Repl. of	When the sample is a replicate, the position number of the original sample	The number of another sample that is not specified as a replicate (you cannot create a replicate of a replicate). Enter the position number, *e.g.*, „A5“.
Subset	Number of the subset to which the sample belongs	Display only
Sample Note	Any additional information about the sample	Alphanumeric characters
Sample ID	Enter an ID for the sample type or sample material (required for optional statistic analysis software module)	Alphanumeric characters; spaces allowed (maximum 11 characters)

Changing a sample name on the General tab affects the active experiment only. The default sample names are determined by the value entered for “Sample Name” in Sample Preferences.

D

9 On the *Abs Quant* tab, you can enter sample information required for Absolute Quantification analysis.

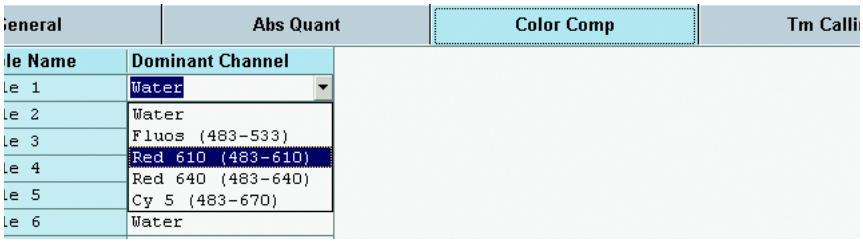


Pos	Sample Name	Filt. Comb.	Target Name	Sample Type	Concentration
A1	Sample 1	Fluos (483-533)	Target 483-533	Unknown	
		Red 610 (483-610)	Target 483-610	Unknown	
		Red 640 (483-640)	Target 483-640	Unknown	
		Cy 5 (483-670)	Target 483-670	Unknown	
A2	Sample 2	Fluos (483-533)	Target 483-533	Unknown	
		Red 610 (483-610)	Target 483-610	Unknown	
		Red 640 (483-640)	Target 483-640	Unknown	
		Cy 5 (483-670)	Target 483-670	Unknown	
A3	Sample 3	Fluos (483-533)	Target 483-533	Unknown	
		Red 610 (483-610)	Target 483-610	Unknown	
		Red 640 (483-640)	Target 483-640	Unknown	
		Cy 5 (483-670)	Target 483-670	Unknown	
A4	Sample 4	Fluos (483-533)	Target 483-533	Unknown	

Enter the following information for each sample:

Parameter	Description	Valid Values
Filter Combination	Displays the filter combinations selected for the run.	Display only
Target Name	Enter a name for each target that is detected by the corresponding filter combinations.	Alphanumeric characters
Sample Type	Select a sample type from the list: ▶ Unknown ▶ Standard	
Concentration	Enter the concentration for samples of type „Standard“. *The unit of the concentration value is defined by the entry in the Unit field above the Sample table (e.g., „copies“).*	Alphanumeric characters

10 On the *Color Comp* tab, you can enter sample information required for color compensation.

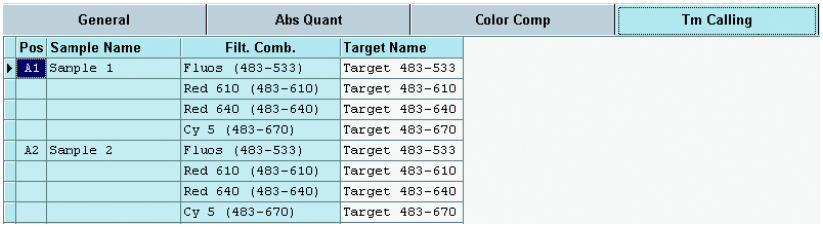


Enter the following information for each sample:

Parameter	Description	Valid Values
Dominant Channel	Select the emission filter combination used for the dye in this well. Select “Water” for the well used as blank, *i.e.*, the one containing no dye.	Filter combination depending on the settings in the *Color Compensation* protocol.

D

11 On the *T_m Calling* tab, you can enter sample information required for T_m calling.



General	Abs Quant	Color Comp	Tm Calling

Pos	Sample Name	Filt. Comb.	Target Name
A1	Sample 1	Fluos (483-533)	Target 483-533
		Red 610 (483-610)	Target 483-610
		Red 640 (483-640)	Target 483-640
		Cy 5 (483-670)	Target 483-670
A2	Sample 2	Fluos (483-533)	Target 483-533
		Red 610 (483-610)	Target 483-610
		Red 640 (483-640)	Target 483-640
		Cy 5 (483-670)	Target 483-670

Enter the following information for each sample:

Parameter	Description	Valid Values
Filter Combination	Displays the filter combinations selected for the run.	Display only
Target Name	Enter a name for each target that is detected by the corresponding filter combinations.	Alphanumeric characters

12 If you need to start over, click *Reset Samples*. You are prompted to confirm resetting the values. Resetting the samples resets all sample information to the default values and resets any analysis-specific Sample Editor tabs to their default values.

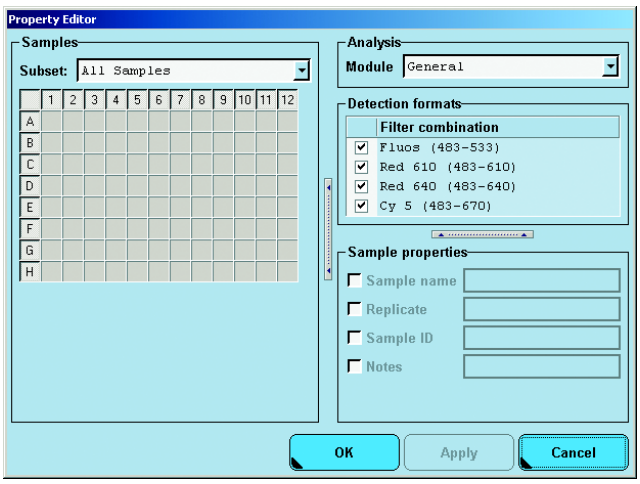
13 When finished, click  in the *Global action* bar to save the sample information with the experiment.

14 To print out your sample loading list, click the *Print* button in the *Global action* bar.

Using the Property Editor

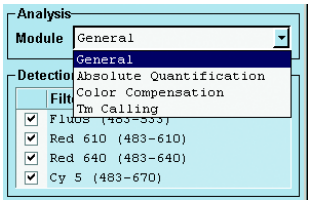
The Property Editor enables you to specify general and analysis-specific properties for a subset of samples simultaneously instead of specifying information for every sample individually.

1. Click *Property Editor* on the *Sample Editor* window. The *Property Editor* window opens:

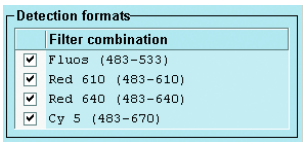


2. Select a subset from the list of subsets in the *Samples* area. Optionally modify the selected wells in the MTP image.

3. Select a Sample Editor module type (corresponding to the tabs displayed in the *Sample Editor* window) from the drop-down list in the *Module* field.



4. Select one or more filter combinations for the samples.



5. The Property Editor displays the property fields for the selected Analysis module type. Check the properties to change and set the property values, and click *Apply*. Clicking *Apply* assigns the settings to the selected samples, but does not save settings. The property values are saved only when the experiment is saved.

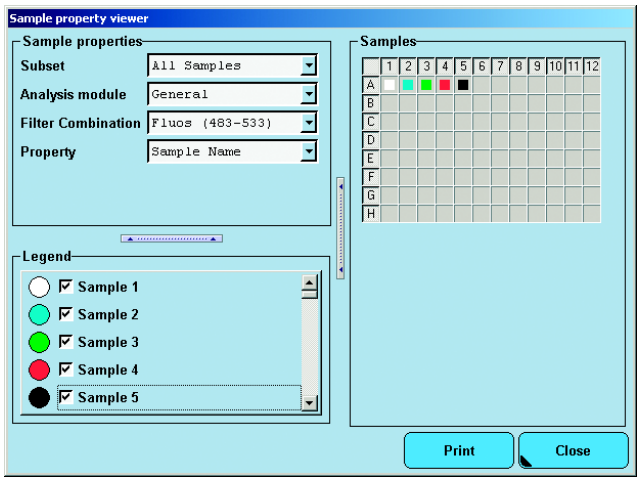
6. Repeat Steps 1 – 4 to set properties for other samples.

7. When finished, click either *OK* or *Cancel*.
OK saves any settings that have not been applied and closes the dialog box.
Cancel closes the dialog box without saving any settings that have not been applied.

Using the Property Viewer

The *Property Viewer* enables you to view the analysis properties for the samples in a particular subset. Specify an analysis property value and see which wells in the plate have been assigned that value.

1. Click *Property Viewer* on the *Sample Editor* window. The *Property Viewer* window opens:



2. Select a sample Subset, an Analysis module type, and a Filter Combination. *Property* displays a drop-down list of all properties for the selected Analysis module type.

3. Select a property: The *Legend* displays a list of colors, checkboxes, and values for each value of the selected property in the sample set.

4. In the *Legend*, select the value or values for the property: The plate image displays colors and icons indicating which samples have the specified property values.

5. Click *Print* to print the *Sample property viewer* window.

D

3. Overview of Experimental Analysis

LightCycler® 480 Basic Software includes analysis modules that can be used to analyze experiment results in various ways. To analyze an experiment, you must add one or more of the analysis modules to the experiment, after the run has finished.

The following analysis modules are available:

- Absolute Quantification:
  Calculates the concentration of target DNA in unknown samples, based on the concentration of standard samples
- T_m Calling:
  Calculates the melting temperatures and melting peaks of target DNA
- Color Compensation:
  Generates color-compensation data that can be applied to a multicolor experiment or to an analysis to compensate for overlap between fluorescence channels

Additional, optional analysis modules will be available in the future (e.g., Relative Quantification, Absolute Quantification with Internal Controls, Qualitative Analysis, and Genotyping). Please check http://www.roche-applied-science.com/lightcycler480 for the latest information.

This chapter explains the general steps required to perform any analysis and presents tips on how to use an analysis window. Subsequent chapters explain in detail how to perform each type of analysis.

3.1 Overview of Analysis Steps

The general steps required to add an analysis module and perform an analysis are described below. The steps are the same for any analysis type. For specific information about setting parameters for each type of analysis, see the section about the specific analysis.

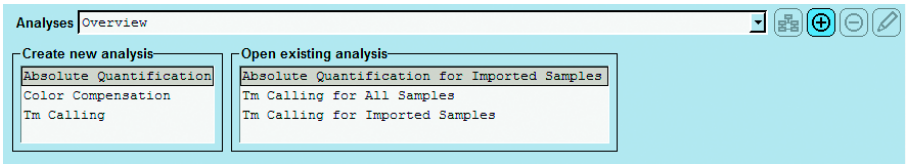
To perform an analysis:

1. Open the experiment you want to analyze in the LightCycler® 480 Basic Software main window.

2. In the *Module* bar, click *Sample Editor*. If you have not already entered general sample information, select the *General* tab, and select the filter combinations used in the experiment and enter information to identify the samples.

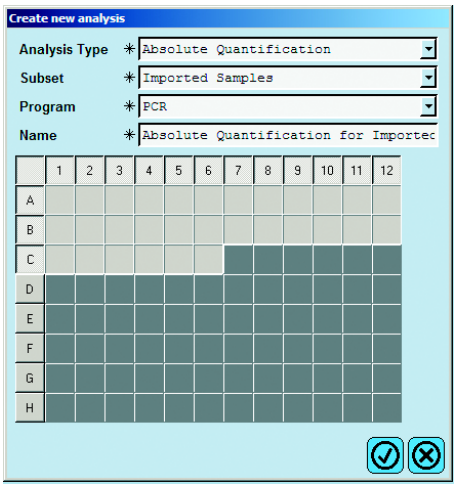
3. Select the analysis-specific tab in the *Sample Editor*, and enter sample information for the analysis.
   In the analysis-specific tab, each sample position includes a row for each filter combination. For example, if you selected three filter combinations, there are three rows for each sample position on the analysis-specific tab. You can enter sample information in each filter combination row. The kind of information you can enter in each analysis-specific tab depends on the type of analysis. For details, see Section *Entering Sample Information*.

4. Click *Analysis* on the LightCycler® 480 Basic Software *Module* bar. The *Analysis Overview* window opens.
   The *Analysis Overview* window displays the *Create new analysis* list and *Open existing analysis* list (if an analysis was created before).



5 Select the analysis type from the *Create new analysis* list.
The *Create new analysis* dialog opens. Here you can again define the analysis type and select an analysis subset. If your experimental protocol should contain several programs that are suited for the selected analysis type, select one from the *Program* list. If desired, you can change the analysis name (the default name is "*analysis type* for *subset name*"). Click *OK*.

You cannot make changes to an analysis subset after an analysis is created using the subset.



6 Enter or adjust analysis parameters, then click *Calculate*. For more information, see sections about individual analysis.

If you change information in the Sample Editor (except sample name, sample note or target name) after performing the analysis, you must recalculate the analysis results using the updated values from the Sample Editor. In this case, the Calculate button becomes active again.

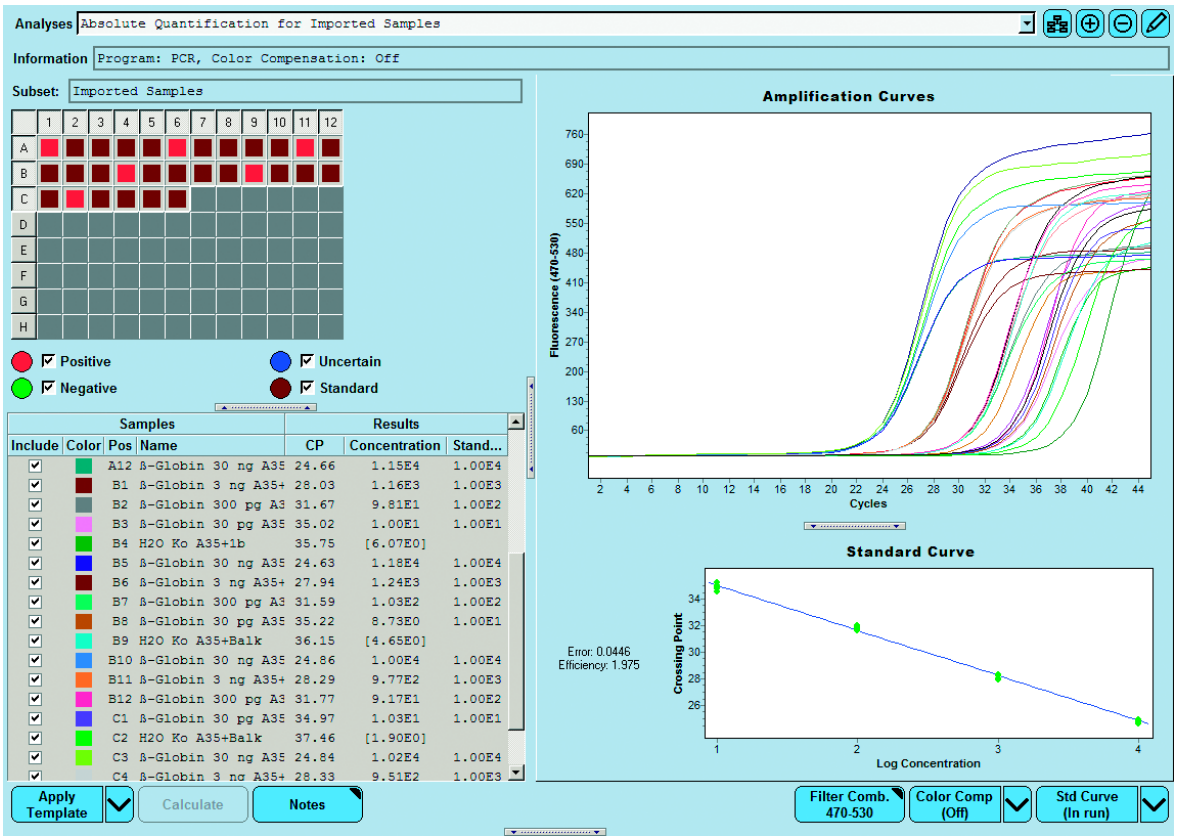
7 You can add more than one analysis to an experiment, including multiple instances of the same analysis type: Click the "+" button in the *Analysis* toolbar.

Repeat Steps 3 – 6 to add additional analysis modules.

8 Click *Save* in the *Global action* bar to save the analysis results as part of the experiment. See the next section for general information about using the analysis window.

3.2 Using the Analysis Window

The following figure illustrates a typical analysis window; in this case, for an Absolute Quantification analysis. The *Analysis* toolbar is at the top, the *Action button* area for the analysis is at the bottom, the list of experiment samples is on the left, and the areas of the window containing analysis charts are on the right.



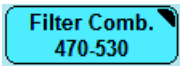
3.2.1 Selecting Filter Combination and Color Compensation

To perform an analysis, you must specify the filter combination you want to analyze and apply color compensation (if appropriate).

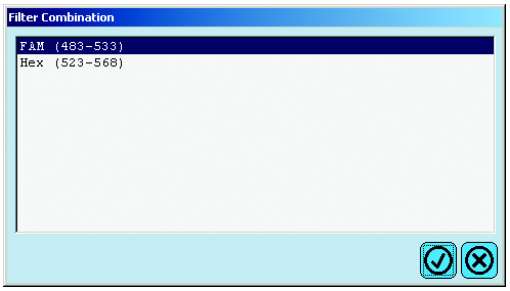
Use the buttons on the analysis' *Action button* area, shown below, to make the necessary selections. (The *Tm Calling* and *Color Compensation* modules have slightly different options. For more information, see the sections related to these modules.)

Use the buttons as follows:

Filter Combination

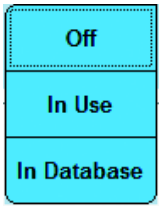


Use *Filter Comb.* to select the fluorescence channel you want to analyze. A list of all filter combinations for which data was gathered for the experiment is displayed:



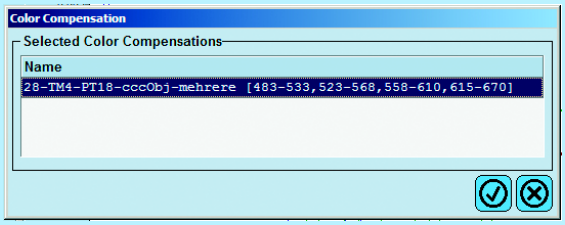
After selecting a filter combination from the list, the button displays the selected filter combination.

Color Compensation



▶ Use the *Color Compensation* multi-select button to turn color compensation on or off and to select a color compensation object:

Off	**Select *Off* if you do not want to use color compensation.**
In Use	Allows you to select an object from the *Selected Color Compensations* dialog.



This dialog displays all selected (*i.e.*, previously applied) color compensation objects. The compensated filter combinations are displayed in brackets after each name.

In Database	Allows you to select an object from the *Available Color Compensations* dialog. This dialog displays all color compensation objects available in the database matching the instrument's serial number and selected filter combination. For each object, the list includes the name and path.

Color compensation is always applied to the filter combination that is selected by the Filter Combination button.

For more information, see Section *Performing Color Compensation Analysis*.

Additional buttons may be displayed, depending on the analysis type.

3.2.2 Working with Samples in the Analysis

An analysis module always displays a list of samples on the left. After analysis calculations are complete, results for the samples are displayed in columns to the right of the sample names. The analysis module also displays charts of sample data. For detailed information, see Section *Sample Selector and Sample Table*.

Selecting Samples to Include in Result Calculations

Select the checkbox next to a sample name to generate analysis results for the sample. By default, all samples are checked at the beginning of an analysis. Double-click a sample checkbox to deselect or reselect it. To check or uncheck a group of samples simultaneously, highlight the range of samples, and press the <Space> bar. This toggles the check marks on or off in all the selected sample boxes.

Selecting Samples to View in Charts

Samples are color-coded. To find a sample in a chart, note the color of the sample in the sample list, and look for the color on the chart. Alternatively, place the mouse pointer over a line on a chart to display a small box containing the name of the sample represented by the line:

When you highlight a sample name in the sample list, data from the selected sample is displayed in the analysis charts. By default, all samples are selected when you first open the analysis window.

To select samples:

- ▶ To select one sample, highlight the sample name in the sample list.
- ▶ To select multiple samples, press the <Ctrl> key while clicking the sample names.
- ▶ To select a contiguous set of samples, click the first sample name, and press the <Shift> key while clicking the last sample name in the set.
- ▶ To select all samples, press <Ctrl-A>.

The analysis graphs are redrawn using the selected samples.

Copying Sample Information

After an analysis is complete, you can copy sample names and results from the analysis window and paste the text into other software programs.

To copy sample names and results:

- ▶ Select one or more sample rows to copy, and press <Ctrl-C>.
- ▶ Open the program into which you want to paste the copied text (*e.g.*, Microsoft Excel), and press <Ctrl-V>.

3.2.3 Working with Charts

To zoom a view of an analysis chart, place the cursor above and to the left of the area you want to enlarge. Click and drag the mouse pointer down and to the right. The mouse pointer draws a rectangle. The area within the rectangle is enlarged to fill the window. To restore the chart to its original size, click and drag the mouse pointer up and to the left. (Do this only once to restore the chart.) For more details, see Section *Working with Charts*.

3.2.4 Adding Analysis Notes

You can add analysis notes to the analysis.

1	To open the *Analysis Notes* window, click the *Notes* button in the *Action* bar.
2	Enter your analysis notes.
3	Click *Save* on the *Global action* bar to save the notes with the experiment.

3.2.5 Removing or Renaming an Analysis

You can remove or rename analyses saved with your experiment if your user account has the Expert User or Local Administrator role. You may also be able to remove or rename analyses stored with experiments of other users, depending on the access privileges associated with your user account. For more information about access privileges, see Section *Managing User Access*.

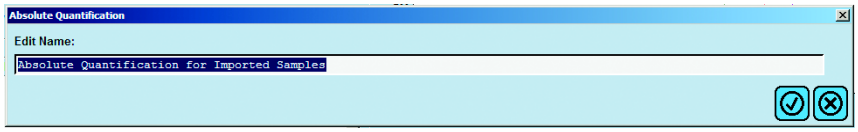
To remove an analysis from an experiment:

1. Select an analysis from the *Analyses* bar.
2. Click the *Remove Item* button in the *Analysis* toolbar.
3. You are prompted to confirm your choice.
4. Click *Yes* to remove the analysis.
   Click *Save* to save the experiment without the analysis.

To rename an analysis:

You can rename the analysis associated with an experiment. Renaming is helpful if you have more than one analysis of the same type associated with the experiment.

1. Select an analysis from the *Analyses* bar.
2. Click the *Rename Item* button in the Analysis toolbar. The *Edit Name* dialog opens:



3. Enter a new name, and click *OK*.

   Names must be unique in the database.

4. Performing Absolute Quantification Analysis

An Absolute Quantification analysis can be performed on any experiment with an amplification program. An Absolute Quantification analysis uses sample "crossing points" (CPs) to determine the concentration of target DNA in unknown samples after amplification. For information about CPs, see the next section.

For analysis of quantification data, the absolute quantification module considers only fluorescence values measured in the exponentially growing phase of the PCR amplification process. This phase is termed the "log-linear" phase because the points making up this exponential curve are converted to a linear curve upon logarithmic plotting.

Kinetic quantification, which is possible only with real-time PCR, allows the course of a PCR to be visualized as a curve similar to a population growth curve, i.e., one that contains an initial lag phase, an exponential (log-linear) phase, and a final plateau phase. The initial lag phase or background phase lasts until the fluorescence signal from the PCR product is greater than the background fluorescence of the probe system. The exponential log phase begins when sufficient product has accumulated to be detected above background and ends when the reaction enters the plateau phase and the reaction efficiency falls.

For Absolute Quantification analysis, serial dilutions of an external standard with a predefined known concentration are used to create a standard curve. The standard dilutions are amplified in separate wells but within the same LightCycler® 480 Instrument run (external standards).

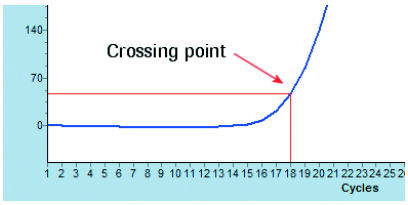
The determination of unknown sample concentration involves determination of the CP value.

4.1 Understanding Sample Crossing Points

In an amplification reaction, the cycle at which the fluorescence of a sample rises above the background fluorescence is called the CP of the sample. The CP of a sample appears as a sharp upward curve on the experiment's fluorescence chart. The CP is the point at which amplified product is first visible in the data. For visualization of PCR products, the number of product molecules must exceed the detection limit of the reaction (at CP, approximately 10^{11} to 10^{12} product molecules are present in the reaction).

A sample's CP depends on the initial concentration of DNA in the sample. A sample with a lower initial concentration of target DNA requires more amplification cycles to reach the CP. A sample with a higher concentration requires fewer cycles. How CP values are used in a quantification analysis depends on the type of analysis.

LightCycler® 480 Basic Software uses an automated method to identify the CP of a sample as the point where the sample's fluorescence curve turns sharply upward. This turning point corresponds to the maximum of the second derivative of the curve. Thus, this method is called "2nd Derivative Maximum Method". The great advantage of this method is that it requires little user input. You just have to specify the type of standard curve (in-run or external) for the experiment, and the software handles all other analysis calculations.



The 2nd Derivative Maximum Method is based on the fact that the fluorescence signal is increasing at an ever-increasing rate in the exponential part of the reaction. This acceleration of the fluorescence signal slows as the reaction begins to enter the plateau. Therefore, the cycle where the second derivative is at its maximum should always be near the log-linear portion of the reaction.

With the 2nd Derivative Maximum Method, the fluorescence at the CP is usually different from one sample to the next. Unlike calculation methods based on thresholds (where the samples are compared at identical fluorescence values), this method rejects the idea that samples with the same fluorescence have the same DNA concentration. Instead, this method posits that the shape of the amplification curve is a better guide to the concentration of the PCR product, and the CP should be at the maximum acceleration, even if the fluorescence levels between curves are different. When using the 2nd Derivative Maximum Method, correction of baseline fluorescence is not necessary.

To handle complex fluorescence data curves, LightCycler® 480 Basic Software takes into account effects and artifacts such as spikes in the log-linear phase of the curve, noisy plateaus, or curves where the plateau phase has not yet been reached. Additionally, no CP values are displayed for curves which exhibit backgrounds that slowly increase or decrease or are noisy, unless the curves reach considerable values of fluorescence.

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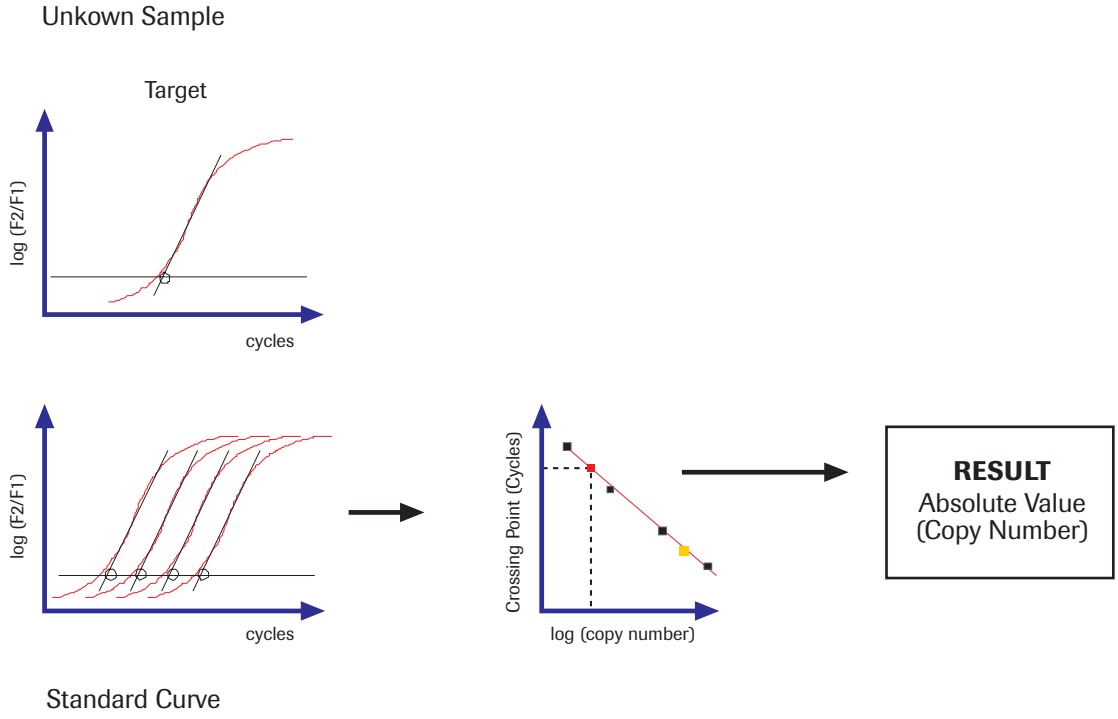
4.2 Understanding the Role of Standard Curves

In an Absolute Quantification analysis, a standard curve is used to determine the concentration of unknown samples. In a standard curve, the concentrations of standard samples are plotted against the CPs of the samples. The X axis represents the log of the initial target concentration, and the Y axis represents CP in cycles. The standard curve is a non-linear (polynomial) regression line through these plotted data points.



The concentrations chosen for the standard curve should fall within the expected concentration range of the target. Typically a standard curve is set up with at least five samples, which are prepared by serial dilution.

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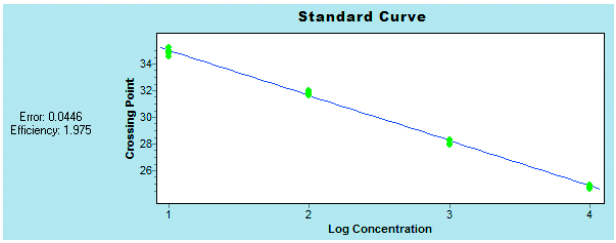
The slope of the standard curve indicates how quickly DNA concentration can be expected to increase with the amplification cycles. The slope of a standard curve is also referred to as the „efficiency“ of the amplification reaction. A perfect amplification reaction would produce a standard curve with an efficiency of "2", because the amount of target DNA would double with each amplification cycle (according to the formula $T_n = T_0 \times 2^n$; T = number of amplified target molecules at cycle n, T_0 = initial number of target, n = number of amplification cycles). The slope of the standard curve can be easily converted into efficiency with the following formula: E= 10 e (-1/slope). LightCycler® 480 Basic Software automatically calculates the efficiency and displays it on the analysis window. In reality, reactions often exhibit an efficiency lower than two. The reaction in the example shown below has an efficiency of 1.975.



The "Error" value (mean squared error of the single data points fit to the regression line), given on the left side of the standard curve, is a measure of the accuracy of the quantification result based on the standard curve (an acceptable value should be < 0.2).

By determining where the CP of an unknown sample falls on the standard curve, the software can determine the initial concentration of target DNA in the sample.

CP data from the standards will be used to convert CP data from the unknowns into concentrations. For these conversions to be valid, the amplification efficiencies of the standards and the samples must be identical. If you use homologous standards, you can usually achieve identical amplification efficiencies easily. If possible, PCR standards should be either linearized plasmid DNA carrying the cloned target sequence or purified PCR products. Always use highly purified templates to ensure absence of contaminants (nucleotides, primers and salt) which can interfere with PCR.

4.3 Providing the Standard Curve

To provide the standard curve for an Absolute Quantification analysis, you can choose one of the following:

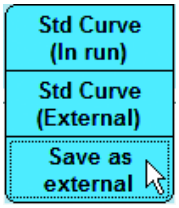
- Include external standards in the experiment: The standard dilutions are amplified in separate wells but within the same LightCycler® 480 Instrument run. The external standards are used to calculate an "in-run" standard curve.
- Use a previously saved standard curve (called an external standard curve). An external standard curve can be loaded into experiments that do not have a standard curve, thus allowing quantitative analysis of those runs. This is especially suitable for applications where the same parameter is analyzed in multiple runs.

One sample (or replicates of this sample) of known concentration must be included in every experiment. This sample should be designated as a standard and should fall within the range of the imported standard curve. The detection format, the analysis mode, and the color compensation data (if any) used for the run must be the same as those used for the imported standard curve.

For the use of the external standard curve to be valid, PCR amplification must be highly reproducible and reaction conditions must be constant for all experiments. Run tests to ensure stable PCR efficiency and use replicate samples (especially for low concentrations) to create the standard curve. Also, include a previously quantified sample in each analyzed run, to verify that the calculated values are reproducible.

To save a standard curve:

1. Perform an amplification experiment containing the standards you want. Alternatively, use an existing experiment that includes standards you want to use.
   The experiment containing the standards must use the same parameters and conditions as the experiment to which the curve will be applied, including the same detection format, concentration units, analysis mode, and color compensation data (for multicolor experiments).
   You can generate several standard curve files from one experiment by choosing different signal channels or analysis modes each time you save the standard curve.
2. Add an *Absolute Quantification* analysis module to the experiment.
3. In the *Abs Quant* tab of the Sample Editor, select *Standard* as the sample type for each standard sample and specify the standard concentration.
4. In the analysis module, calculate the analysis. Tick the checkboxes of the standard samples you want to use in the standard curve. See the next section for detailed information about performing an *Absolute Quantification* analysis.
5. From the *Action button* area of the analysis window, select *Save as external*.



6. Navigate to a location to save the curve, enter a file name, and click *OK*.

You can use the saved external standard curve in other quantification analyses for experiments that have the same experiment parameters as those used to create the standard curve.

To use an external standard curve:

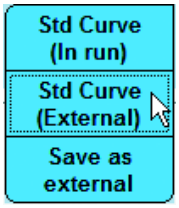
1. Perform an amplification experiment containing one standard or use an existing experiment that includes one standard.

   The standard sample should fall within the range of the imported standard curve.

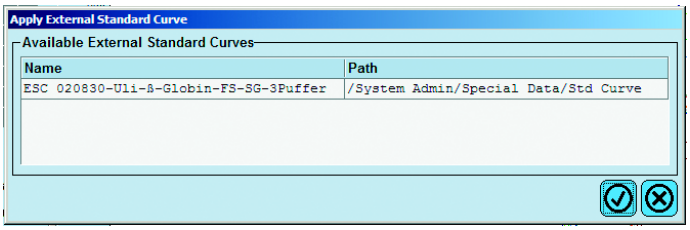
2. Add an *Absolute Quantification* analysis module to the experiment.

3. In the *Abs Quant* tab of the Sample Editor, select *Standard* as the sample type for the standard sample and specify the standard concentration.

4. From the *Action button* area of the analysis window, select *Std Curve (External)*.

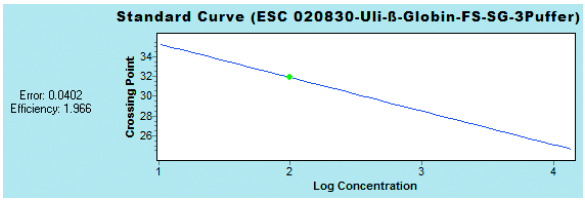


5. The *Apply External Standard Curve* dialog opens. Select an appropriate external standard curve object from the list:



6. In the analysis module select the checkbox of the standard sample in the *Sample* list and perform the analysis. Click *Calculate*.

7. In the *Standard Curve* chart, only the single standard sample included in your experiment is displayed. The name of the external standard curve in use is shown above the chart.



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4.4 Using the Absolute Quantification Method

To perform an absolute quantification analysis:

1. Using LightCycler® 480 Basic Software, create and run a quantification experiment or open an existing experiment.
2. In the *Sample Editor*, enter general sample information on the *General* tab, and select the filter combinations used in the experiment.
3. On the *Abs Quant* tab of the *Sample Editor*, enter sample information as follows:

Parameter	Description
Target Name	Enter a name for each target that is detected by the corresponding filter combinations.
Sample Type	Select a sample type from the list: ▶ Unknown ▶ Standard
Concentration	Enter the concentration for samples of type „Standard“. *The unit of the concentration value is defined by the entry in the Unit field above the Sample table (e.g.,“copies“).*

4. Click *Analysis* on the *Module* bar.
5. From the *Create new analysis* list, select *Absolute Quantification*.
   In the *Create New Analysis* dialog, select an analysis subset and a quantification program in the experiment (typically there is only one quantification program, which is selected by default). Click *OK*.
6. If this is a multiplexed experiment, click the *Filter Comb.* button to open the *Filter Combination* dialog. Select the filter combination for the targets you want to analyze.
7. ▶ If you included standards in the experiment, select the checkbox next to each standard you want to include in the standard curve. (Double-click the box to select or clear it.) Select *Std Curve (In Run)* in the *Action button* area.
   ▶ If you did not include standards in the experiment, select *Std Curve (External)* in the *Action button* area. Find and select the standard curve you want to use, and click *OK*.

   The external standard curve must be from an experiment that has the same detection format, filter combination, and color compensation settings as the current experiment. The external curve and current experiments can be generated on different block types (96, 384). If you want to use an external standard, you must include one of the standard concentrations in the new experiment as a reference. The software calculates the concentration for each sample in the sample list, based on where each sample's crossing point correlates to the standard curve.
8. By default all samples are included in result calculations; to remove a sample from result calculations, double-click the checkbox next to the sample name to clear the checkbox or press the <Space> key. Click *Calculate*.
9. To view amplification curves for one or more samples, highlight the sample names in the sample list.
10. To view analysis results, click and drag the left border of the chart section to the right to display all the result data. Results include the *Sample Selector* with *Selector Filter*, the *Sample* table, and the *Statistics* table (only if the experiment has sample replicates).

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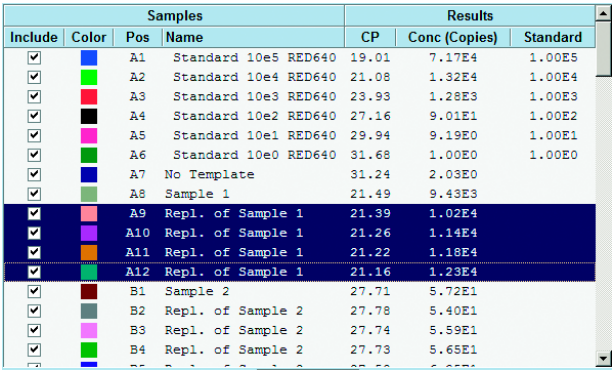
11 The *Sample* table of an Absolute Quantification analysis displays the following results:



Samples				Results		
Include	Color	Pos	Name	CP	Conc (Copies)	Standard
☑		A1	Standard 10e5 RED640	19.01	7.17E4	1.00E5
☑		A2	Standard 10e4 RED640	21.08	1.32E4	1.00E4
☑		A3	Standard 10e3 RED640	23.93	1.28E3	1.00E3
☑		A4	Standard 10e2 RED640	27.16	9.01E1	1.00E2
☑		A5	Standard 10e1 RED640	29.94	9.19E0	1.00E1
☑		A6	Standard 10e0 RED640	31.68	1.00E0	1.00E0
☑		A7	No Template	31.24	2.03E0	
☑		A8	Sample 1	21.49	9.43E3	
☑		A9	Repl. of Sample 1	21.39	1.02E4	
☑		A10	Repl. of Sample 1	21.26	1.14E4	
☑		A11	Repl. of Sample 1	21.22	1.18E4	
☑		A12	Repl. of Sample 1	21.16	1.23E4	
☑		B1	Sample 2	27.71	5.72E1	
☑		B2	Repl. of Sample 2	27.78	5.40E1	
☑		B3	Repl. of Sample 2	27.74	5.59E1	
☑		B4	Repl. of Sample 2	27.73	5.65E1	

Result	Description
CP	The crossing point of the sample.
Conc (Unit)	The calculated concentration of the sample, based on where the crossing point lies on a standard curve of crossing points versus concentration *Unit* is defined by the input (*e.g.*, „copies“) into the *Unit* field of the *Abs Quant* tab on the *Sample Editor*.
Standard	This value is specified in the *Abs Quant* tab of the *Sample Editor*.

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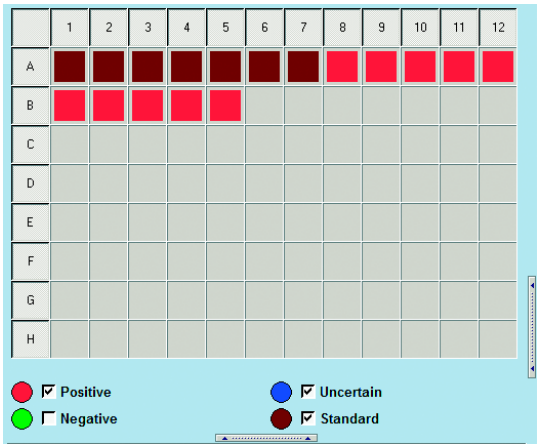
12 The legend of the *Sample Selector* can be used as a selector filter that enables you to select samples depending on the result call: Positive, Negative, Uncertain, and Standard.



Select the Positive, Negative, Uncertain, and Standard checkboxes to select the wells with the matching result type in the *Sample Selector* and to highlight them in the color shown in the legend.
The following group results and statistics are appended to the *Sample* table (when the experiment has sample replicates):

Statistic	Description
Samples	The sample numbers in the replicate group.
Mean Cp	The mean value of the crossing points for the samples in the group.
STD Cp	Standard deviation of the crossing points.
Mean conc.	The mean of the concentrations for the samples in the group.
STD conc.	Standard deviation of the concentrations.

Negative samples are excluded from the statistics calculation.

If you select a replicate set in the Statistics table, the curves for all the samples in the replicate set are displayed in the associated chart.

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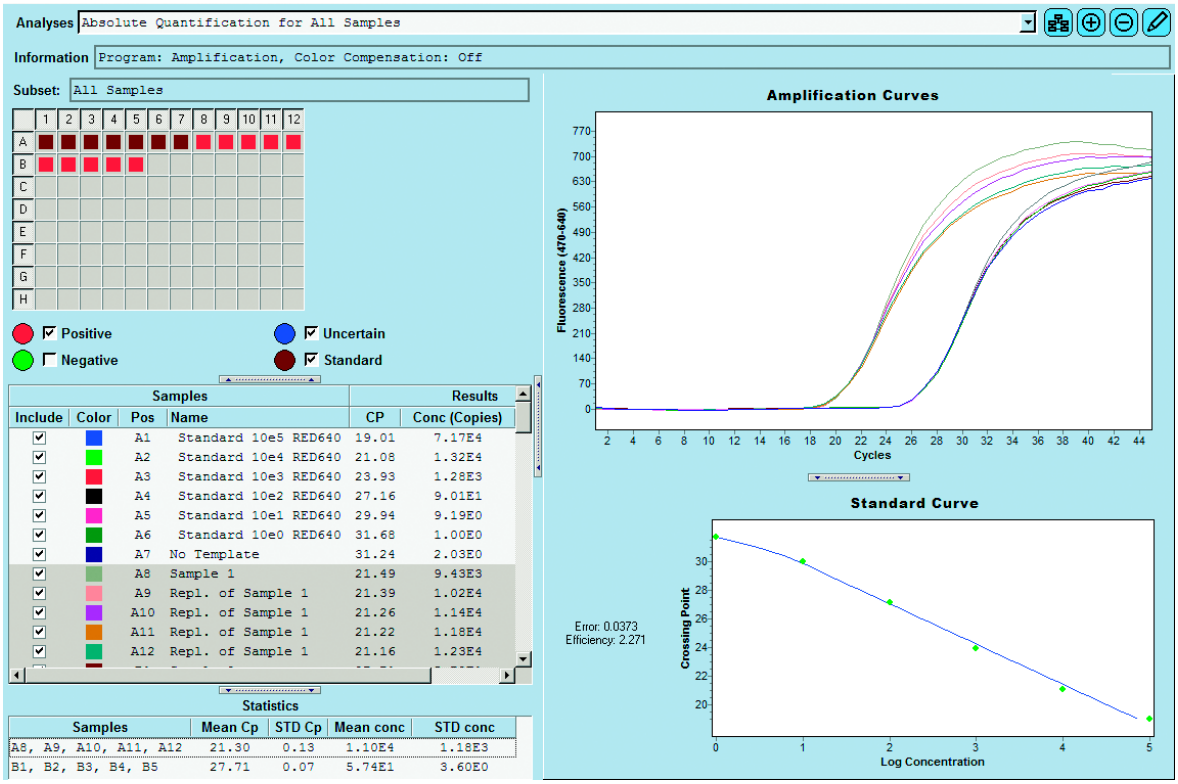
An Absolute Quantification analysis is shown below. Results are calculated for all 17 samples in the experiment. Sample curves are displayed for the 10 highlighted samples. The standard curve is generated from the samples that are checked and that are labeled as standards in the *Sample Editor*.



5. Performing Melting Curve Analysis

This chapter explains how to use melting temperature profiles to identify DNA products and to genotype samples.

5.1 Using Melting Curve Profiles to Identify DNA Products and Genotypes

The temperature at which a DNA strand separates or melts when heated can vary over a wide range, depending on the sequence, the length of the strand, and the GC content of the strand. For example, melting temperatures can vary for products of the same length but different GC/AT 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 hybrids and is, thus, the basis for probe-based genotyping analysis: A perfectly matched (*e.g.*, wildtype-specific) probe will melt at a higher temperature than the mismatched probe bound to a target sequence carrying a single-base mutation.

The purpose of Melting Curve analysis is to determine the characteristic melting temperature of the target DNA and to identify or genotype products based on their melting temperature.

To analyze sample melting temperature profiles, the fluorescence of the samples must be monitored while the temperature of the LightCycler® 480 Instrument thermal block cycler is steadily increased. As the temperature increases, sample fluorescence decreases. In case of the double-stranded DNA-specific dye SYBR Green I, this is due to the separation of the DNA strands and consequently the release of SYBR Green I molecules. For single-labeled probes and hybridization probes, this is due to the separation of target-probe hybrids resulting either in quenching of the reporter dye or in the spatial separation of the dye molecules. Both lead to a consequent drop in fluorescence.

The LightCycler® 480 Instrument T_m Calling analysis software module calculates for each sample the melting temperature, the melting peak, height, width and the area under each melting peak. Use the T_m Calling analysis to identify characteristic melting profiles of DNA products or target-probe hybrids.

5.1.1 Defining a Melt Program

A melting temperature analysis can be performed on any experiment that includes a melt program. A melt program is usually performed after amplification of the target DNA. A typical melt program includes three segments:

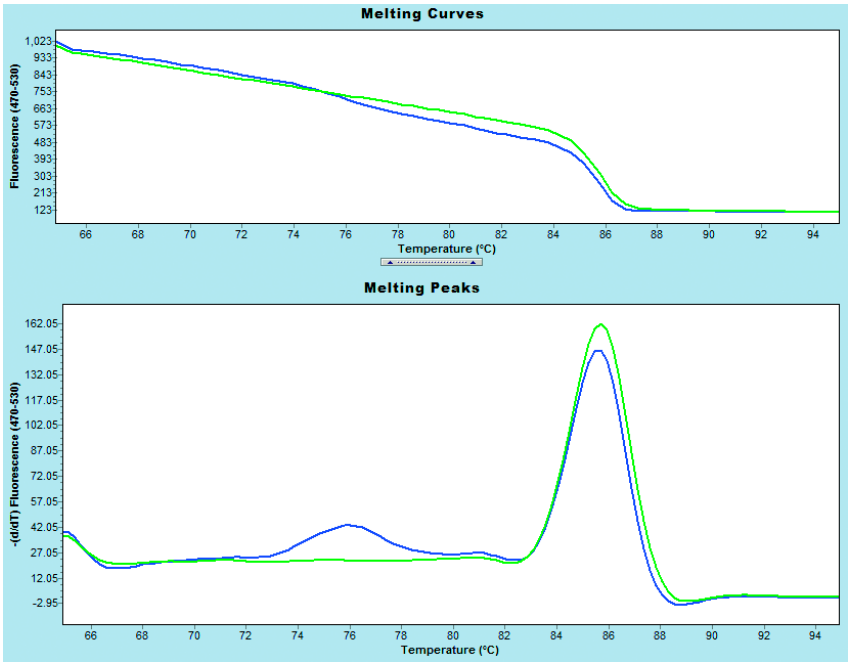
- The samples are rapidly heated to a temperature high enough to denature all DNA molecules.
- The samples are cooled to below the annealing temperature of the target DNA.
- The samples are slowly heated while measuring sample fluorescence as the target DNA melts.

5.1.2 Content of a Melting Temperature Analysis

A melting temperature analysis uses the fluorescence measurements of the melt program to determine the melting temperature of each sample. The melting temperature (or T_m) of a sample is defined as the point at which half of the DNA has melted or half of the probes have melted off the DNA.

The analysis displays a *Melting Curves* chart of sample fluorescence versus temperature. The chart shows the downward curve in fluorescence for the samples as they melt. 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.

The following figure shows a melting curve chart and a melting peak chart from a T_m Calling analysis of a SYBR Green I experiment as an example how melting temperature analysis can be used for DNA product characterization:



Both samples analyzed show a prominent and sharp peak from a DNA product having a T_m of 86°C, representing the desired PCR product. The blue sample shows in addition a weak and broad peak with T_m 77°C, representing an unspecific by-product such as primer–dimers.

5.2 Performing T_m Calling Analysis

You can perform a T_m Calling analysis on any experiment that includes a melt program. During a melt program, the decrease in fluorescence of the samples is monitored while the temperature is steadily increased to melt the DNA or melt probes off the target strands.

The reasons for the decrease of fluorescence during melting are different for the various detection formats:

- In the case of the SYBR Green I dye, this is due to the separation of DNA strands and consequently the release of SYBR Green I molecules. As SYBR Green I only fluoresces 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 single-stranded.
- In case of hybridization probes, the separation of target–probe hybrids results in the spatial separation of the fluorescence resonance energy transfer (FRET) partners and in a drop of fluorescence from the reporter dye at a certain temperature. The melting temperature, or T_m, is defined as the point at which half the probes have melted off their target DNA sequence.
- In case of single-labeled probes, fluorescence is emitted from the reporter dye when the probe is hybridized to its target sequence. Fluorescence is quenched when the probe is free floating in solution. Again, the melting temperature, or T_m, is defined as the point at which half the probes have melted off their target DNA sequence.

After amplification in a hydrolysis probe PCR assay, all probes are digested. Thus, melting curve analysis cannot be performed.

The analysis displays a chart of the melting curves of samples which shows the drop in fluorescence. The software also charts the first negative derivative of the melting curves, which displays the melting temperatures of the samples as peaks. When sample melting temperatures are displayed as peaks, it is easier to discern small differences in the melting profiles of the samples.

The analysis result data includes the melting temperature of each sample as well as area under each peak.

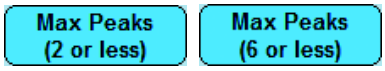
The T_m Calling analysis uses automated algorithms to find the peak areas and melting temperatures. Additionally, you can also determine melting temperatures and peak areas manually.

The automatic Tm Calling algorithm requires that you start the melt program at least 7°C before and end it at least 3°C after the expected Tm value.

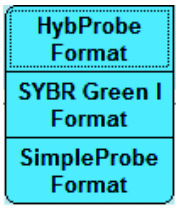
To perform automated T_m Calling analysis:

1. Create and run an experiment or open an existing experiment that contains a melt program.
2. In the *Sample Editor*, enter general sample information on the *General* tab, and select the filter combinations used in the experiment.
3. On the *Tm Calling* tab of the *Sample Editor*, enter sample information as follows:

Parameter	Description
Target Name	Enter a name for each target that is detected by the corresponding filter combinations.

4. Click *Analysis* on the *Module* bar.
5. From the *Create new analysis* list, select *Tm Calling*. In the *Create New Analysis* dialog, select an analysis subset and a program in the experiment (typically there is only one program, which is selected by default). Click *OK*.
6. If this is a multiplexed experiment, click the *Filter Comb.* button to open the *Filter Combination* dialog. Select the filter combination for the targets you want to analyze.
7. 

   Decide the minimum number of melting peaks you expect. You can choose between *Max Peaks (2 or less)* or *Max Peaks (6 or less*). Select *2 or less* for a typical genotype experiment. Choose *6 or less* for curves with many peaks.
   Click the *Max Peaks* button in the *Action button* area to toggle between the two options.

   When using the SYBR Green I Format (see next step), it is recommended to apply the Max Peaks (2 or less) option only.
   We recommend that melting peaks have at least a ΔT_m of 4°C to achieve a good resolution.
8. 

   Select the detection format that was used in your experiment from the *Format* multi-select button. You can choose between *HybProbe Format, SYBR Green I Format,* and *Simple Probe Format*. Click the arrow-down button and make your selection.

   Selecting one of the format options changes the analysis algorithm according to the specific demands of the detection chemistry.
9. By default, all samples are included in result calculations; to remove a sample from result calculations, double-click the checkbox next to the sample name to clear the checkbox or press the <Space> key.
10. Click *Calculate* to complete the T_m Calling analysis.
11. To view analysis results, click and drag the left border of the chart section to the right to display all the result data. Results include the *Sample Selector* with *Selector Filter* and the *Sample* table.

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12 Use the checkboxes in the Display area above the *Sample* table to select which type of result you want to display in the table:

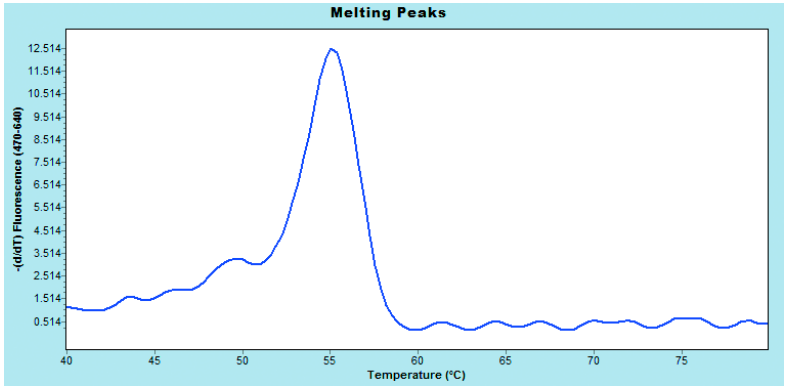
The *Sample* table of an T_m Calling Analysis displays the following results:

Samples				Melting Peaks							
Include	Color	Pos	Name	Tm1	Area1	Width1	Height1	Tm2	Area2	Width2	Height2
☑		A1	H2O Ko 1a+b Kit	76.62	3.72	81.52	21.89	85.63	2.82	430.43	152.70
☑		A2	ß-Globin 30 ng 1a+	85.75	2.63	438.37	166.84				
☑		A3	ß-Globin 3 ng 1a+b	85.71	2.60	412.59	158.82				
☑		A4	ß-Globin 300 pg 1a	85.65	2.60	384.84	147.76				
☑		A5	ß-Globin 30 pg 1a+	78.07	3.14	76.08	24.22	85.43	2.85	371.92	130.60
☑		A6	H2O Ko 1a+b Kit	85.66	2.44	374.69	153.85				
☑		A7	ß-Globin 30 ng 1a+	85.70	2.70	450.24	166.75				
☑		A8	ß-Globin 3 ng 1a+b	85.63	2.65	427.21	161.18				
☑		A9	ß-Globin 300 pg 1a	85.57	2.64	401.66	152.06				

Result	Description
Tm1	The melting temperature for the first peak in the sample.
Area1	The area under the first peak.
Width1	The width of the first peak.
Height1	The height of the first peak.
Tm2	The melting temperature for the second peak for the sample, if any.
Area2	The area under the second peak.
Width2	The width of the second peak.
Height2	The height of the second peak.

If there are additional peaks and the appropriate setting is chosen, the results display them as Tm3, Area3, etc. (Only in Max Peaks (6 or less) mode.)

Sometimes there is a shoulder visible on the side of a peak. If you want to see the result data for these shoulder peaks, select Shoulders from the Display area. To hide them, deselect Shoulders.
In the example below, there is a shoulder at 50°C.



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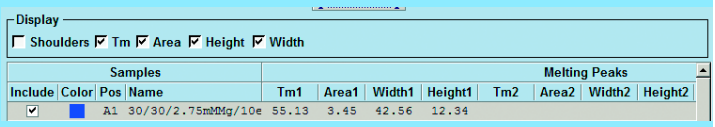
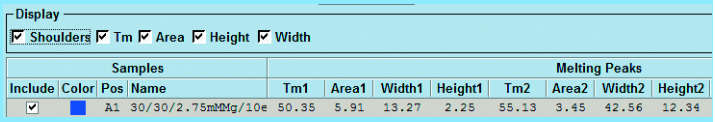
Result data for this shoulder is only displayed if *Shoulder* is selected:

Shoulders deselected

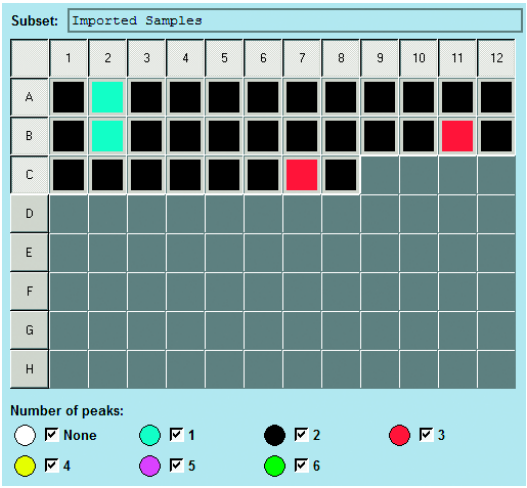


Shoulders selected



Note that the shoulder data are now displayed in the columns for the first peak, while the data of the main peak are displayed in the columns for the second peak. This is because peak data is sorted ascending by Tm automatically.

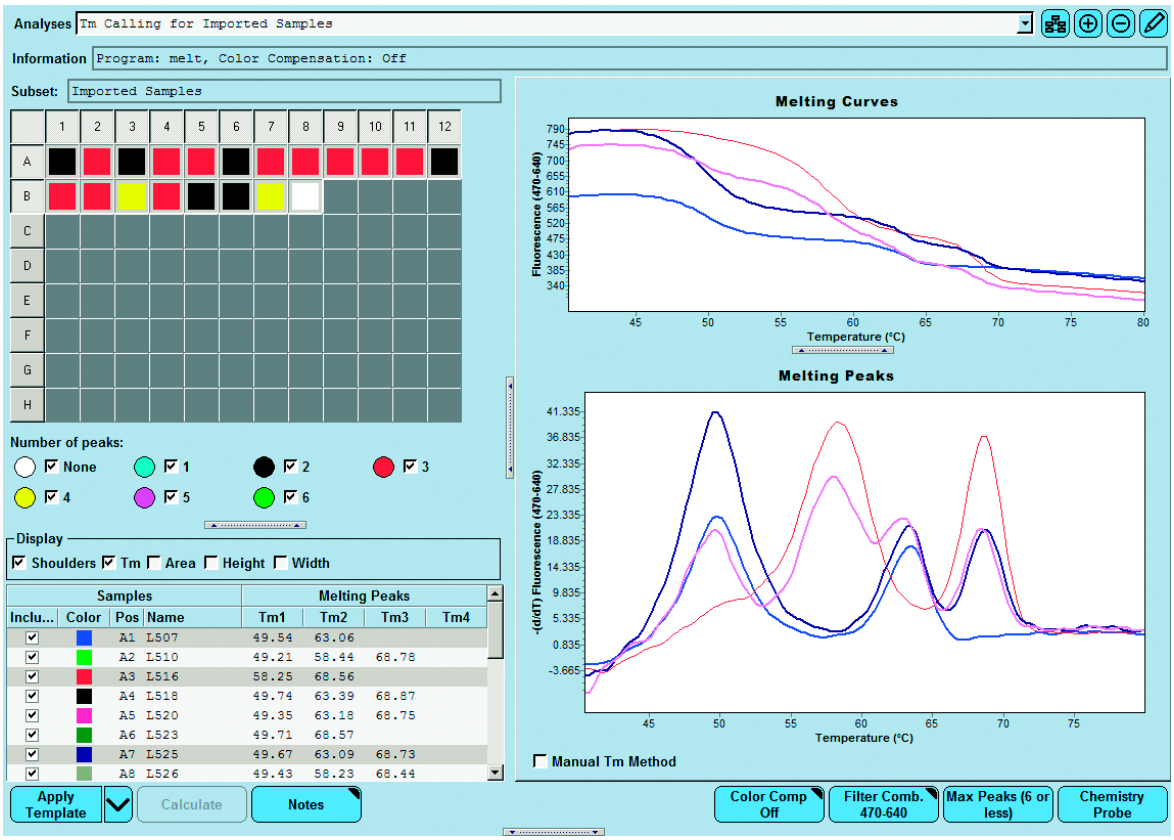
The legend of the *Sample Selector* can be used as a *Selector Filter* that enables the selection of samples depending on the result call, *i.e.*, the number of peaks found.



Select the *Number of peaks* checkboxes to select the wells with the matching result type in the *Sample Selector* and to highlight them in the color.

5

A T_m Calling analysis of a HybProbe assay is shown below. Results are calculated for all 20 samples in the experiment. Sample curves are displayed for the four highlighted samples: Two samples (orange, blue) have two melting peaks, one sample (dark blue) has three peaks, and one sample (pink) has four peaks. Each peak represents a different genotype of the target gene.



D

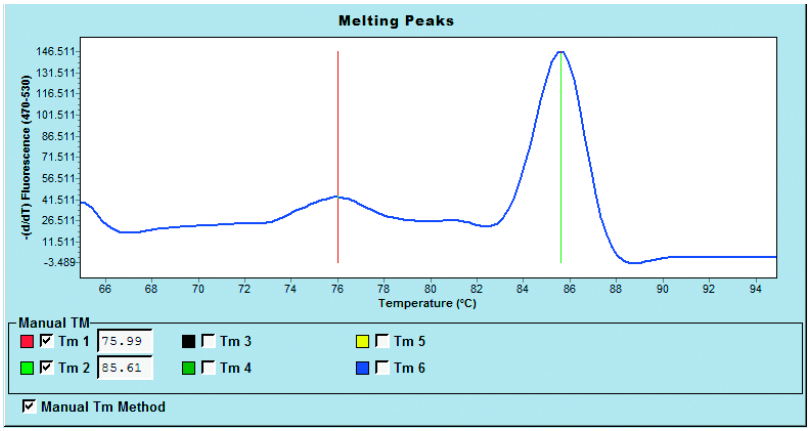
To perform manual T_m Calling analysis:

1. Create and run an experiment or open an existing experiment that contains a melt program. Perform an automated T_m Calling Analysis.

   The manual T_m method becomes active only after the T_m Calling analysis has been calculated.

2. Select one or more samples from the sample list to edit their calculated melting peaks.

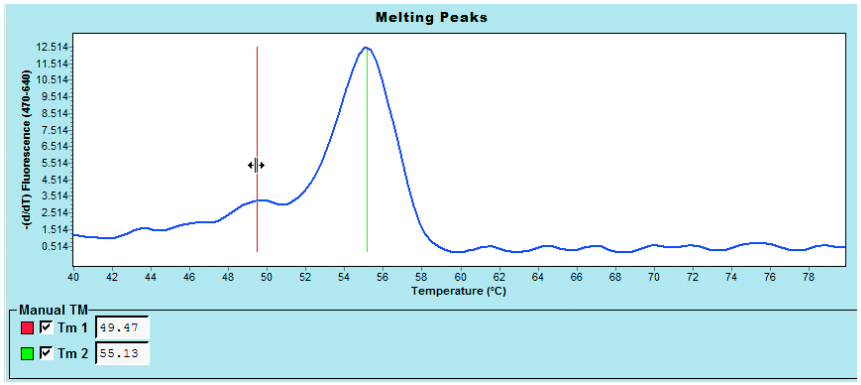
3 Select the *Manual Tm Method* checkbox.
Depending on the *Max Peaks* setting, two or six T_m checkboxes are displayed below the *Melting Peaks* chart.



Indicators are located at the automatically calculated Tm values. If multiple samples are highlighted in the sample list, indicators of the first sample in the selected sample list are displayed.

4 Click a T_m checkbox to display a manual T_m indicator and a T_m field. The color of the indicator matches the selected T_m checkbox.

5 To change a T_m value, place the cursor on the vertical T_m indicator. The cursor changes to a double-pointed arrow. Click and drag the line to the desired T_m value.



The T_m value is displayed in the T_m field to the right of the T_m checkbox. The areas, heights and widths of the corresponding T_m are recalculated and displayed in the sample list for this sample.

In the Sample table, manually edited T_m values are indicated by a prefixed asterisk.

It is possible to have both automatically generated and manually generated T_m values in the same analysis (but not for the same samples).

During the manual Tm editing it is not possible to change the Show Shoulders display.

6 To restore the original (automatically calculated) T_m values, deselect *Manual Tm Method*, and click the *Max Peaks* button twice, followed by *Calculate*.

6. Performing Color Compensation Analysis

The LightCycler® 480 System is able to simultaneously detect and analyze more than one fluorescent signal in each reaction. By this means, different target sequences can be detected in one reaction. Due to overlap of the emission spectra of the dyes, one filter combination picks up signals from a dye measured by another channel, a phenomenon called "crosstalk". Although each emission filter is optimized for a specific emission maximum, all fluorescent dyes currently available have emission spectra with long "tails," leading to this spectral overlap. This bleed-over of fluorescence signal can result in data that cannot be interpreted. To correct the crosstalk, color compensation can be applied before data analysis. When color compensation is activated, LightCycler® 480 Basic Software algorithms use the data from a so-called color compensation (CC) object to compensate for the fluorescence crosstalk.

Color compensation is only necessary when you run an experiment where you want to detect two or more different dyes in one reaction. Color compensation is not required when the experiment uses only a single dye.

You can easily correct the spectral overlap described above by calibrating the instrument. During a calibration run, the LightCycler® 480 Instrument measures the fluorescence of each dye and generates an instrument-specific color compensation object. Later, the LightCycler® 480 Basic Software automatically uses this so-called CC object to reassign the fluorescence in each channel to the appropriate dye. The net result is detection of only one dye signal in each channel.

A CC object can only be applied to experiments that were run on the same LightCycler® 480 Instrument it was created on.

To use color compensation, you must perform the following steps:

- Run a color compensation experiment on the instrument where you are going to perform the multiplexed experiments. The color compensation experiment gathers the data needed to compensate for the fluorescence bleed-over. From the color compensation experiment, create a color compensation object. For more information, see Section *Performing a Color Compensation Experiment*.

- Apply the color compensation information when you perform the experiment or when you analyze the experiment. For more information, see Section *Performing a Color Compensation Experiment*.

6.1 Performing a Color Compensation Experiment

The temperature profile used in a color compensation protocol always includes a heating, cycling, temperature gradient and cooling program. The cycling program mimics a typical PCR, including data acquisition. Instead of running a separate color compensation experiment, you can also run the color compensation reactions in parallel to your experimental samples. In this case, apply the appropriate experimental PCR protocol, but always add a temperature gradient or melting curves program.

For hydrolysis probe color compensation runs it is obligatory to perform a real PCR, as cleavage of the probe by the Taq DNA polymerase during cycling is required to generate a fluorescent signal.

For the following dye combinations used in dualcolor hydrolysis probe assays, no color compensation is required:
Cyan 500 (450-500) - Red 610 (558-610)
Cyan 500 (450-500) - Red 640 (558-640)
Cyan 500 (450-500) - Cy 5 (615-670)
FAM (483-533) - Red 610 (558-610)
FAM (483-533) - Red 640 (558-640)
FAM (483-533) - Cy5 (615-670)
HEX / VIC (523-568) - Cy5 (615-670)

The data required for color compensation are taken from the temperature gradient program. In this program, after a brief denaturation (95°C), the protocol slowly increases the temperature from 40°C to an end temperature that should be approx. 5°C above the temperature the fluorescent signal is usually measured during an experimental run. During the temperature gradient fluorescence is measured at a rate of 1 acquisition/°C. If you run HybProbe probe color compensation reactions in parallel to experimental samples you can also apply a melting curves program used for later T_m Calling analysis.

The optimal acquisition rate for melting curve analysis has to be determined empirically for each assay and can range from 1 to 5 acquisitions/°C.

After the calibration run, the LightCycler® 480 Basic Software saves the data generated as a normal experimental file. For these data to be used for color compensation, you must first convert the data of the temperature gradient or melting curves program into a CC object and save it separately.

For both HybProbe and hydrolysis probes it is sufficient to generate one color compensation object per dye combination used irrespective of the specific probe sequence applied in your experiments.

To run a color compensation experiment:

1. Prepare a minimum of 3 to 5 replicate color compensation reactions for each fluorescence dye you need to compensate in your experiment, including a blank (*i.e.*, set up mono color reactions not the multi color reactions you want to run with your experimental samples).
   - ▶ For a HybProbe color compensation experiment use dilutions of the same fluorescent probes that you are going to use in your multicolor experiments per reaction (*i.e.*, do not use the HybProbe probe pairs but the individual probe oligonucleotides). The final concentration of Fluos-labeled HybProbe donor probes should be 0.2 – 0.3 µM; the final concentration of the HybProbe acceptor probes should be 1 µM.
   - ▶ For a hydrolysis probe color compensation experiment set up and run complete amplification reactions according to your experimental protocol. Each reaction (except the Blank) should contain one of the hydrolysis probes that will go into your multicolor hydrolysis probe assay. Use the same hydrolysis probe concentration as in your experiment.

   Setting up and performing an amplification reaction using hydrolysis probes requires the cleavage of the hydrolysis probe and the release of the reporter dye from the quencher. Otherwise, no fluorescence signal required for color compensation will be generated.

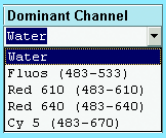
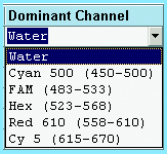
   For the blank (the sample designated "Water"), you can use water, but it is better to use a buffer (e.g., 50 mM Tris, pH 8.3, with 0.25 mg/ml BSA). This buffer is also suitable for diluting and storing the primers and probes.

2. Open LightCycler® 480 Basic Software and, using the same program settings you plan to use for your multicolor experiment, create a color compensation experiment that has one temperature gradient program with the following segments:
   Segment 1: 95ºC for 0 seconds at 4.8ºC/sec.
   Segment 2: 40ºC for 30 seconds at 2.5ºC/sec.
   Segment 3: XºC for 0 seconds at 1 acquisition/°C, continuous; X = experimental measurement temperature +5°C
   For the temperature gradient program, select *Color Compensation* in the *Analysis Mode* field.

   In case a melting curve analysis is performed in parallel wells of the multiwell plate in the same run, select Melting Curves in the Analysis Mode field. It is possible to perform a color compensation analysis from a melting curve program, but it is in turn not possible to perform a T_m calling analysis from a color compensation program.

3. In the *Sample Editor*, enter the following information in the *General* tab, if needed:
   Test ID: String that identifies the probe combination used for the color compensation experiment.
   Lot ID: String that identifies the reaction mix used for the color compensation experiment.
   Color Comp ID: String that identifies the color compensation object to an experiment kit wizard. Especially when working with the LightCycler® 480 LIMS/Bar-Code Module, this ID number is highly convenient for the correct identification of color compensation objects in combination with macros. If you saved a macro with the "smart select color compensation" option, you can select the appropriate CC object, when starting the macro remotely via LIMS, by submitting the Color Comp ID.)

4 In the *Color Comp* tab of the *Sample Editor*, enter sample information as follows:

Column Name	Valid Values	Description
Dominant Channel	▶ Multi Color HybProbes  ▶ Multi Color Hydrolysis Probes 	The channel used for the dye in this well Select the appropriate filter combination for each additional well.

5 Pipette the reactions into the multiwell plate.

6 When the experiment is finished, click *Analysis* on the *Module* bar.

7 From the *Create new analysis* list, select *Color Compensation*. In the *Create New Analysis* dialog, select an analysis subset and a program in the experiment (typically there is only one program, which is selected by default). Click *OK*.

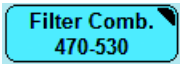
8 Click *Calculate* on the *Action button* area to perform the color compensation analysis.

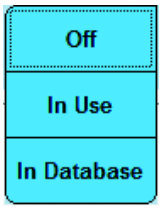
9 Click *Save CC Object*. By default, the *CCC folder* in your *Special Data* folder is selected as location.

You can now apply the color compensation data to another experiment. For more information, see the following section.

To apply color compensation:

1 First, select the filter combination you want to display and compensate by using the *Filter Combination* button.



2 

▶ To apply color compensation during an experiment run, click the arrow-down button. Select between the options *In Use* or *In Database*.
▶ To apply color compensation to an analysis, add the analysis module, and click the arrow-down button. Select between the options *In Use* or *In Database*.

3 Select the color compensation object you want to apply, and click *OK*.

After you select an object for an analysis, the object name is added to the Color Compensation menu for all analysis modules added to this experiment.

4 The experiment or the analysis charts are redrawn using the compensated data. Note that the *Color-Compensation* button label now says "(On)."

7. Working with Templates and Macros

Templates and macros provide convenient ways to speed up the process of creating an experiment.

A template is based on an individual item, such as a protocol or a sample list containing the information you want to use.

A macro is a collection of templates; one for each portion of an experiment, along with a program (the actual macro) that automatically applies the templates and runs the experiment.

This chapter explains how to create templates and macros, how to apply templates and run macros, and how to add macro buttons to the main window.

7.1 Creating and Using Templates

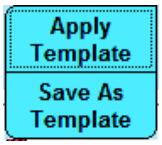
Templates provide a convenient way to speed up the process of creating and analyzing an experiment. A template is based on an individual item, such as a predefined subset or sample table, that includes all the information you want to use in your experiment. For example, you cannot rerun an experiment protocol, but you can save the protocol from an existing experiment as a template and apply this template to a new experiment. Subset, sample, report and analysis templates can be used in a similar manner. Using the template function, it is possible to save all settings made in an *Editor* frame to a database object as a template object and, subsequently, to apply these settings to a different, matching database object. This allows preferences that replace existing user preferences to be stored and applied.

Templates generated with a 384 MWP block type cannot be applied to an experiment run with the 96 block type and vice versa.

See the table below for object types which allow saving and applying of templates:

Object Type	Parameters Included in the Template	Comment
Run	▶ Programs and temperature targets ▶ Detection format ▶ Selected filter combinations (in the customize window) ▶ Integration time mode and integration time if manual is selected ▶ Plate ID ▶ Reaction volume ▶ Block type	*LightCycler® 480 Basic Software comes with four demo run templates (located in the Roche folder under Templates/Run Templates)*
Subsets	▶ Availability of the subset for analysis ▶ Availability of the subset for report ▶ List of included wells ▶ The subset ID ▶ Subset name ▶ Block type	*A subset template cannot be applied after an analysis has been created.* *LightCycler® 480 Basic Software comes with two demo subset templates (checkerboard and quadrant; located in the Roche folder under Templates/Subset Templates)*
Sample	A sample template includes block type, active filter combinations, and a list of samples. For each sample, the template includes all sample properties including ▶ Replicate info ▶ Sample note ▶ Sample ID ▶ Absolute Quantification analysis sample type and concentration for active channels ▶ Color compensation dominant channel ▶ T_m Calling analysis target name for each active channel	*To apply a sample template, the filter combinations in the template must match the filter combinations in the experiment.*
Report	A report template includes the section selection setting on the *General* tab and the detailed subsection requirements on the *Details* tab.	*Does not include any visual settings, such as the page number or current magnification.*
Analysis	▶ Notes ▶ Filter combination ▶ Color compensation object, ▶ External standard curve (quantification only) ▶ Format setting (T_m Calling) ▶ Peak mode (T_m Calling)	▶ A quantification analysis template does not include the positive/negative filter states nor the sample include/exclude status. ▶ A T_m Calling analysis template does not include the peak number filter states nor the sample include/exclude status. ▶ If you create a template from an analysis that has color compensation, the template will include these color-compensation data. ▶ A T_m calling analysis template cannot be saved and applied with the manual T_m method setting.

Whenever an object that can be used or can be saved as a template is open in the LightCycler® 480 Basic Software, the *Template* button is active.



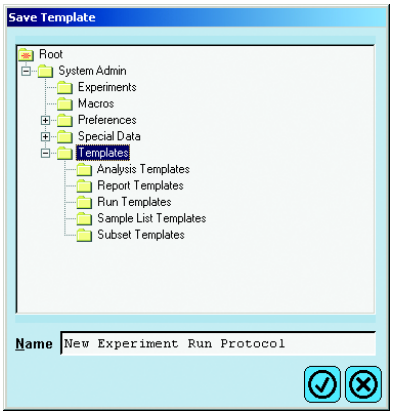
The Template button allows to select and apply a template to the currently open object and to save the currently open object as a template.

A template can only be created from an existing object that is open and active in the software. It is not possible to create a template from an object that is not open and active.

To create a template:

1. Open or create the object to be used as template.
2. Click *Save as Template* to open the *Template* dialog box.
3. Select a location to save the template and enter a name for the template in the *Name* field.

   The default template name is the name of the current experiment, plus the object type. The default storage location for all templates is the current user's preference setting for the Template and Macros folder.



4. Click *Save*.
   Result: The template is saved and the dialog box closes.

   After a template has been saved to the database, you can edit (from the navigator) only the template name and notes of the template object.

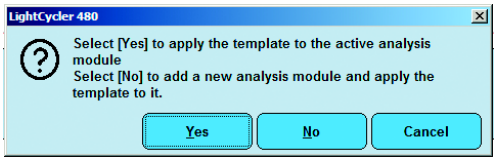
To apply a template:

1. Open the object to which you want to apply the template.
2. Click *Apply Template* to open the *Template* dialog box. The dialog box displays a tree list of all templates for which you have access.
3. Highlight a template in the list, and click *Open*. The dialog box closes and the template is applied to the currently open object.

To apply any template, the block type in the template must match the block type used by the experiment.

You can still manually change an object after a template has been applied.

If you apply a template to an analysis that does not match the analysis type of the template, a new analysis screen of the matching type is opened automatically. If an analysis template is applied from an analysis of the same type, a dialog asks whether you wish to apply the template to the current analysis or to create a new analysis:



7.2 Creating and Using Macros

While a template is based on an individual item, such as a protocol or a sample list that includes the information you want to apply to another experiment, a macro is a collection of templates. Macros automate the entire process of running an experiment, including setting up the experiment protocol, entering sample information, running the experiment, performing analyses and generating reports. You can create an experiment macro to automate the process of running a frequently used experiment protocol.

The macro functionality is required when operating the LightCycler® 480 Basic Software via a LIMS client: The LIMS client can execute a LightCycler® 480 Instrument run remotely by starting a macro.

Read this section to learn more about the following topics:

- Creating experiment macros
- Selecting and running an experiment macro

To create an experiment macro:

1	Open the *Summary* pane of the experiment you want to use as the basis for the macro.
2	Click *Save as macro* in the *Action* bar of the *Summary* pane to open the *Save macro* dialog box.
3	Enter a name for the macro and browse to a location where you want to save it, and click *OK*. (The default location for saving the macro is the user's *Macros* directory and the default name for a macro is "Macro" followed by the experiment name. You can, however, specify a different name and location.)

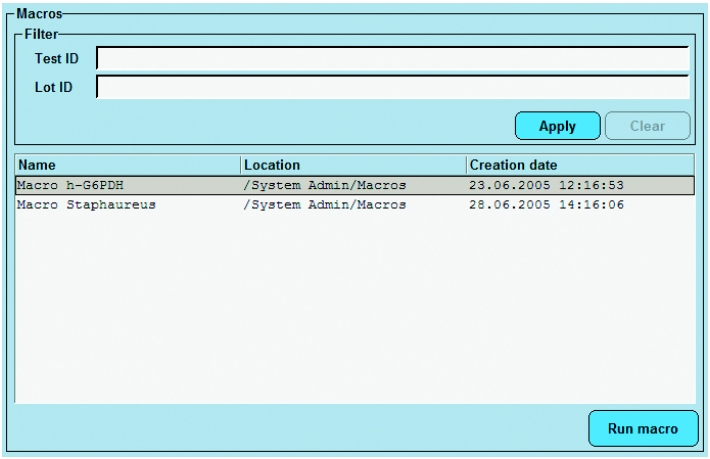
Once you have saved a macro, you can only edit the macro name and notes of the saved macro object in the navigator.

When you create a macro from an experiment with color compensation, you can select whether to incorporate the CC object into the macro or use smart color compensation. Smart color compensation autoselects a CC object from the CC objects being available in the database. The selection filters are: (1) CC object generated with the same instrument, (2) CC object having the same Color Comp ID as the macro (if Color Comp ID is specified), and (3) most recently created CC object matching criteria (1) + (2).
This option is possible only when the optional LightCycler® 480 LIMS/Bar-Code Module is installed and activated by purchasing an appropriate licensing key.

To run an experiment macro:

1. Select the *Overview* window. In the *Macros* area, a list of all macros is displayed for which the user has rights to execute. Select a macro from the list, and click *Run macro*.

   The Run Macro button is only active when LightCycler® 480 Basic Software is connected to an instrument.



2. (Optional) Enter a Test ID number and click *Apply* to filter the macro list by Test ID, and select a macro returned by the software.

3. (Optional) Enter a Lot ID number and click *Apply* to filter the macro list by Lot ID, and select a macro returned by the software.

4. The *Save Experiment* window opens. Name your experiment and browse to a location to store it, and click *OK*.
   Once the macro has been started, it performs the following steps:
   ▶ Applies all templates for the run, including run, sample information and subsets.
   ▶ Starts the run.
   ▶ Automatically saves the experiment at the end of the run.

   The macro does not perform data analysis after the run. This option is possible only when the optional LightCycler® 480 LIMS/Bar-Code Module is installed and activated by purchasing an appropriate licensing key.

8. Working with Subsets

The standard way to use LightCycler® 480 Basic Software is to simultaneously analyze all the samples on the PCR multiwell plate. Alternatively, you can define subsets of the samples for separate analysis of each subset. This is useful if, for example, you want to use one section of a multiwell plate to test for one quantification target gene, and another section of the same plate to test for a different target.

You can also define different sample sets to include in a report. Reports are based on report subsets; a subset can be defined as both an analysis and a report subset. In this case, the analysis subset and the report subset include the same samples. Alternatively, reports can contain a different set of samples from those contained in the analysis subsets.

For example, each column in a multiwell plate can be used to analyze a different SNP, while each row in the plate can represent a different sample. In this case, an analysis subset can be used for each column and a report subset for each row.

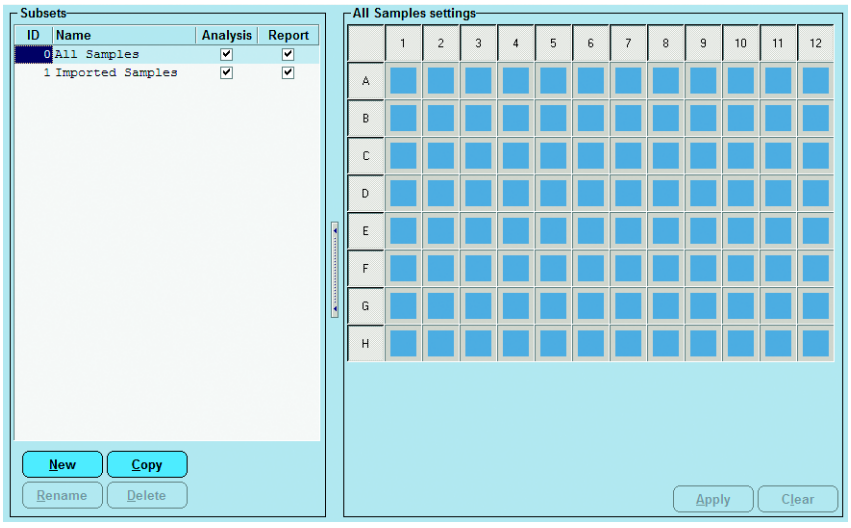
The same samples may be assigned to multiple subsets of either type (analysis or report).

The *Subset Editor* is used to create, modify, and delete subsets. Subsets are applied using the subset drop-down lists on the *Data* tab, *Sample Editor*, analysis and report modules.

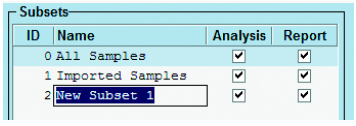
To create a subset:

1. Open the experiment for which you want to define subsets (or create a new experiment).

2. Click *Subset Editor* in the *Modules* bar. The *Subsets* window opens:



3. Click *New* to activate a new row in the list of subsets or click *Copy* to create a new subset based on the currently selected one.

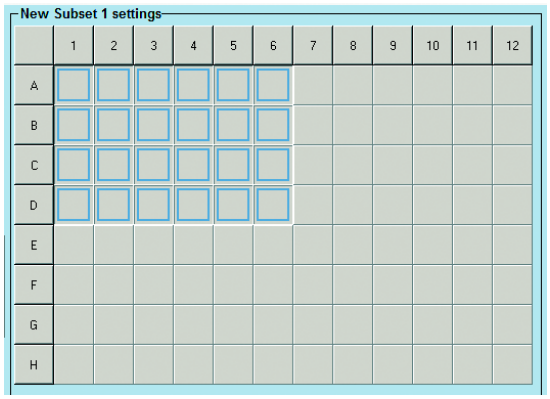


An ID number is automatically assigned to the new row. “Analysis” and “Report” are selected by default as the subset types. The *Name* column is automatically selected. The ID number is displayed in the *Sample Editor*.

4 Define the subset as follows:
▶ Select the subset type boxes for the new subset (Report, Analysis, or both)
▶ Type a name for the subset

A subset name can have up to 25 characters.

▶ In the multiwell plate image, select the well positions for the samples to be included in the subset: Click individual positions to select samples, or click column and row buttons to select columns or rows of samples. Alternatively, you can drag the mouse pointer over an area of the multiwell plate image to select all samples in this area simultaneously. During dragging, selected wells are displayed in dark blue.



▶ Click *Apply* to add the selected well positions to the subset.
▶ Click *Clear* to cancel the selection.

5 When finished, click *Save* in the *Global action* area to save the experiment with the new subset.

Subsets are experiment-specific. You can exchange subsets between experiments by using the template function.

To modify or rename a subset:

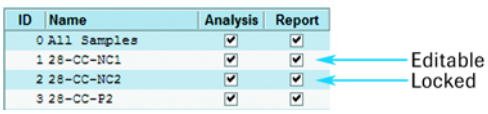
1 Open the experiment containing the subset you want to modify.

2 Click *Subset Editor* in the *Modules* bar. The *Subsets* window opens.

3 In the subset list, highlight the subset you want to modify.

4 Modify the subset as follows:
▶ Select or clear the subset type boxes as needed (Report, Analysis, or both).
▶ Modify the subset name as needed by clicking *Rename* or clicking the subsets name.
▶ Select well positions and click *Apply* to add or remove them from the subset (depending on whether they were unselected or selected before).
▶ Click *Clear* to select all well positions to remove.
▶ Click *Delete* to remove a subset.

A subset cannot be modified, renamed, or deleted if it has been used in an analysis. Locked subsets are highlighted in the Subset list.



5 When finished, click *Save* in the *Global action* bar to save the experiment with the modified subset.

9. Working with Charts

LightCycler® 480 Basic Software generates many kinds of charts during and after an experiment run and as part of an experiment analysis. Charts allow you to view the following types of information:

- Program time, temperature cycles and acquisition points
- Data gathered from an experiment
- Information and results charted by analysis modules

You can print charts, export charts to various graphic formats, and copy and paste chart images and chart data. The charts include zoom and pan functions, so you can enlarge details of a chart and move the chart left or right. Right-clicking a chart displays a context menu with some or all of the available chart options. At a glance, the context menu provides the following options:

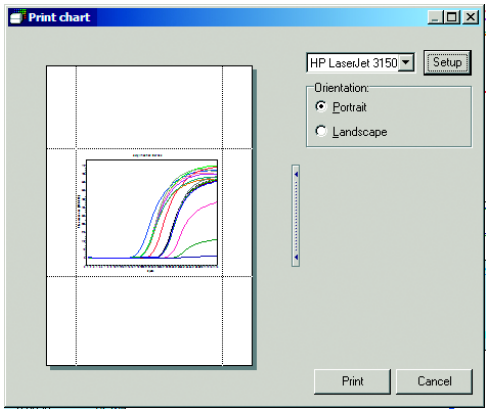
Option	Description
Chart Preferences	Opens a *Chart Preferences* editor. For more information, see Section *Overriding Default Chart Preferences*.
Sample Preferences	Opens a *Sample Preferences* editor. For more information, see Section *Overriding Default Chart Preferences*.
Load Sample Preferences	Opens a Navigator so the user can select a *Sample Preferences* object to load. For more information, see Section *Using Sample Preferences*.
Save Sample Preferences	Opens a *Save* dialog so the user can save sample settings from the current experiment as a *Sample Preferences* object.
Clear Sample Preferences	Restores sample settings from the current default *Sample Preferences* object.
Print	Opens a *Print* dialog used to print the chart.
Export	Opens an *Export* dialog box used to export the chart image and its data.
Copy to Clipboard	Copies chart data to the clipboard.
Show Legend	Displays a legend on the current chart.

9.1 Printing, Exporting, and Copying Charts

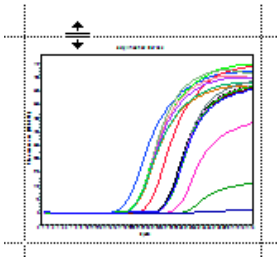
You can print any chart displayed in LightCycler® 480 Basic Software. You can also export the chart image and the chart data separately or copy and paste the image and the data separately into other programs.

To print a chart:

1. Display the chart you want to print.
2. Right-click within the chart boundaries, and select *Print*. A setup window is displayed.

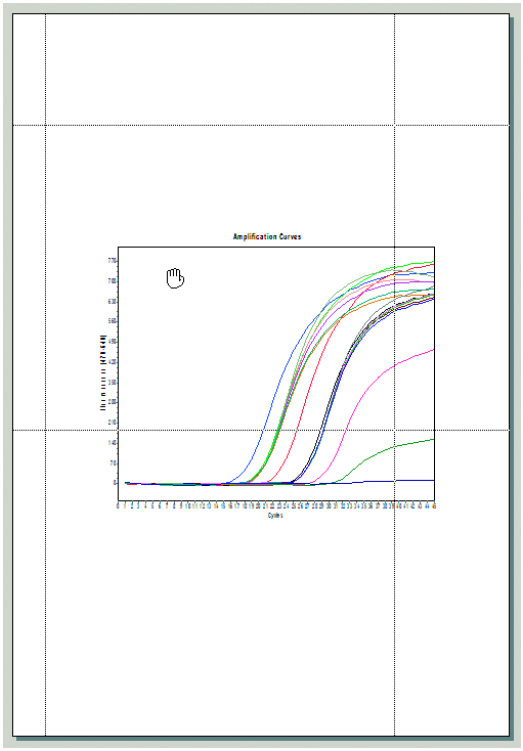


3. To change the graph margins, and therefore the size of the graph, click and drag the gray margin lines that surround the graph image. Note that the mouse pointer changes its appearance to indicate that you can now drag the margin line.

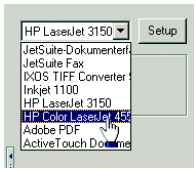


You can resize the dialog box to make it easier to adjust the graph margins.

4 To change the position of the graph on the page, position the mouse pointer over the graph. The pointer will change to a hand. Click and drag the graph margins to a new position. Release the left mouse key and the graph is placed at the new position.



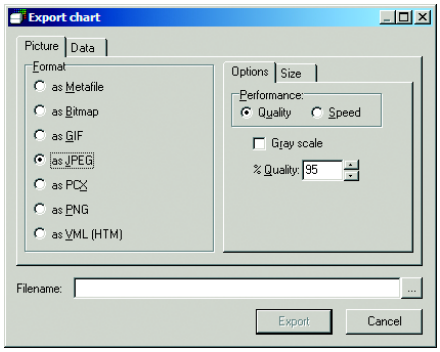
5 If necessary, select a printer from the drop-down list. The list displayed depends on the printers installed on your data station. Your default Windows printer is selected by default.



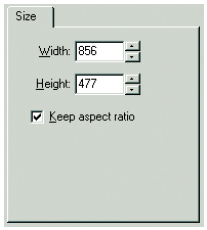
6 To change printer configuration options, click *Setup*. A standard Windows printer setup dialog box is displayed. Enter the necessary information, and click *OK*. Select the paper orientation (portrait or landscape) and click *Print*.

To export a chart image:

1. Display the chart you want to export.
2. Right-click within the chart boundaries, and select *Export*. An *Export* dialog box opens.



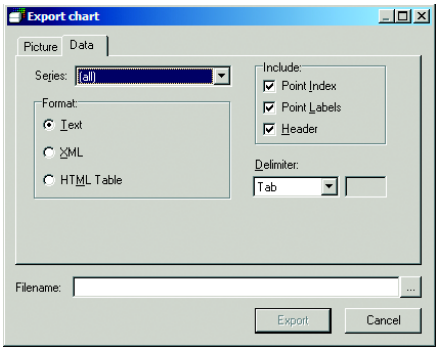
3. On the *Picture* tab, under *Format*, select the graphic format to be used for exporting the chart.
4. If an *Options* tab is displayed (on the right), select conversion options as needed. (The tab is not displayed for all graphic formats. If the tab is displayed, the options vary, depending on the format you selected.)
5. To change the size of the exported image, select the *Size* tab, and enter the new width and height values. Select *Keep aspect ratio* if you want to maintain the proportions of the chart.



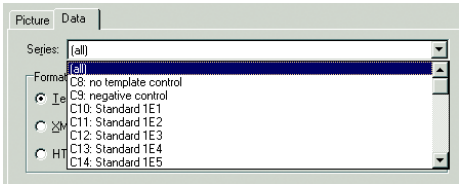
6. Click the ... button (to the right of the *Filename* box), to open a *Select output file* dialog box. Browse to the location where you want to save the exported chart image, enter a name for the image, and click *Save*.
7. Click *Export* to export the chart.

To export chart data:

1. Display the chart containing the data you want to export.
2. Right-click within the chart boundaries, and select *Export*. An *Export* dialog box opens.
3. Select the *Data* tab.



4. In the *Series* box, select the data item you want to export. The items vary, depending on the type of chart.



5. In the *Include* box, select the text labels to export with the data.
6. In the *Format* box, select a format for the exported data. You can choose a format: Plain text, XML, and HTML table.
7. If you selected *Text* as the format, select a delimiter in the *Delimiter* box. You can choose between space, tab, comma, or a custom delimiter. Enter a custom delimiter into the field right of the *Delimiter* box.
8. Click the ... button (to the right of the *Filename* box), to open a *Select output file* dialog box.
9. Browse to the location where you want to save the exported data, enter a name for the data file, and click *Save*.
10. Click *Export* to export the data.

To copy a chart image or chart data:

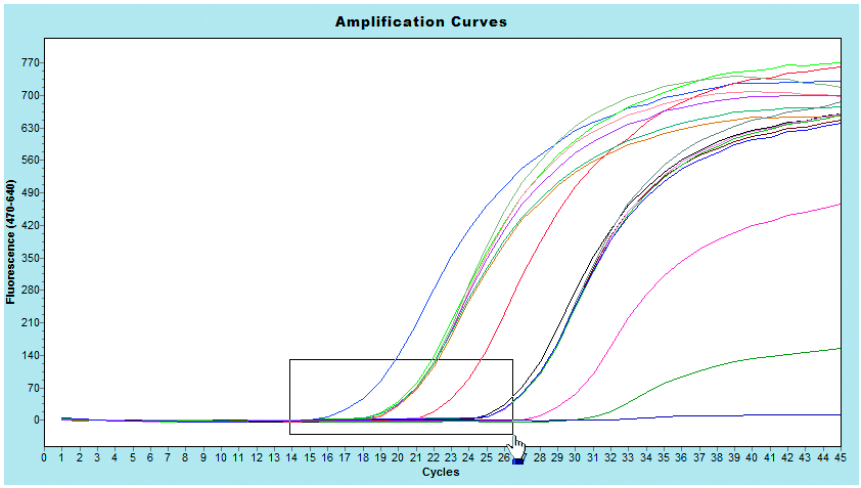
1. Display the chart you want to copy.
2. Right-click within the chart boundaries, and select *Copy to clipboard*. The chart is saved as a bitmap and the data is saved as text.
3. To paste the chart image, open a graphics application, such as Paint, and press <Ctrl-V>.
4. To paste the chart data, open a text editor, such as Notepad, and press <Ctrl-V>.

9.2 Zooming and Panning to View Chart Details

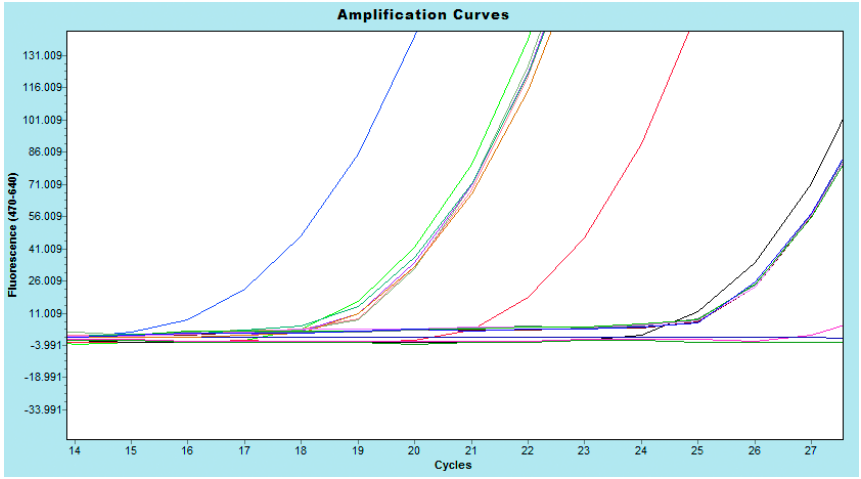
You can enlarge a portion of a chart as many times as necessary to view important details. If you use a three-button mouse, you can shift the chart in any direction to view details that are outside the window. Follow the procedures below to enlarge chart details (zoom) or to move a chart (pan).

To zoom:

1. Place the cursor above and to the left of the chart area you want to enlarge.
2. Click and drag the mouse pointer down and to the right. (The pointer changes to a rectangle.)



Release the mouse button when the rectangle covers the area you want to enlarge. The area within the rectangle is enlarged to fill the work pane.



3. Repeat Step 2 as often as necessary until the chart details are as large as you want.
4. To restore the chart to its original size, click and drag the mouse pointer up and to the left. (You need to do this only once to restore the chart to its original size.)

To pan:

If you want to see portions of the chart that are not displayed in the window, use the middle mouse button to click the chart. Drag the chart until the portion you want to see is in view.

You may be able to configure a two-button mouse so that clicking both buttons at the same time is equivalent to clicking the middle button of a three-button mouse. See your system administrator or refer to the device driver instructions that came with your mouse.

10. Generating Reports

After you analyze an experiment, you can generate an analysis report containing general experiment information and analysis results. You can customize the report to include any of the following:

- Experiment summary information (such as name and date)
- Experiment protocol
- Sample information
- Instrument information
- Change history
- Analysis results and other analysis items, such as statistics and settings (the actual analysis items you can include vary by analysis type)

You can arrange the order of items in the report and print the report.

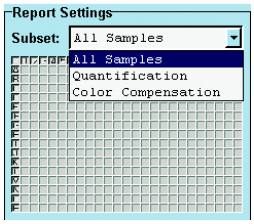
To generate and print a report:

1. Open an experiment that includes one or more analysis modules or save your currently opened experiment.

2. Click the *Report* button in the *Module* bar. The *Report* window opens in the work pane.
   The left side contains the *Report Settings*.

   You must first save an experiment before the Report button becomes active.

3. First, select a report subset from the *Subset* list:

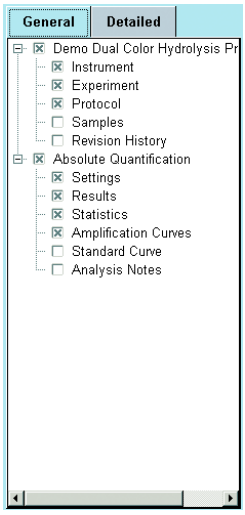


   You can only choose from report subsets, not from analysis subsets.

4 The *General* and *Detailed* tab provide a list of available report items, with some items selected by default. The order of items in the list indicates the order of items in the report.
The list of report sections on the *General* tab includes the following:
- ▶ General information section labeled with the experiment name
- ▶ Analysis sections for each type of analysis performed on the samples in the selected subset

An analysis section is not included in the list unless that analysis has been performed on at least one sample in the selected report subset.

The list of report sections on the *Detailed* tab includes the following:
- ▶ General information section labeled with the experiment name
- ▶ Analysis sections for each analysis performed on any of the samples in the subset; there will be multiple analysis sections of the same type if more than one analysis of the same type has been performed on the samples



5 Click items in the list to include or exclude them. If you click a section item, all corresponding subsections are automatically selected.

Note that changes made to settings on the General tab override the current settings on the Detailed tab for the same sections or subsections.

6 To change the location of an item in the report, click and drag the item to a new location in the list on the *Detailed* tab. You can drag both sections and subsections.

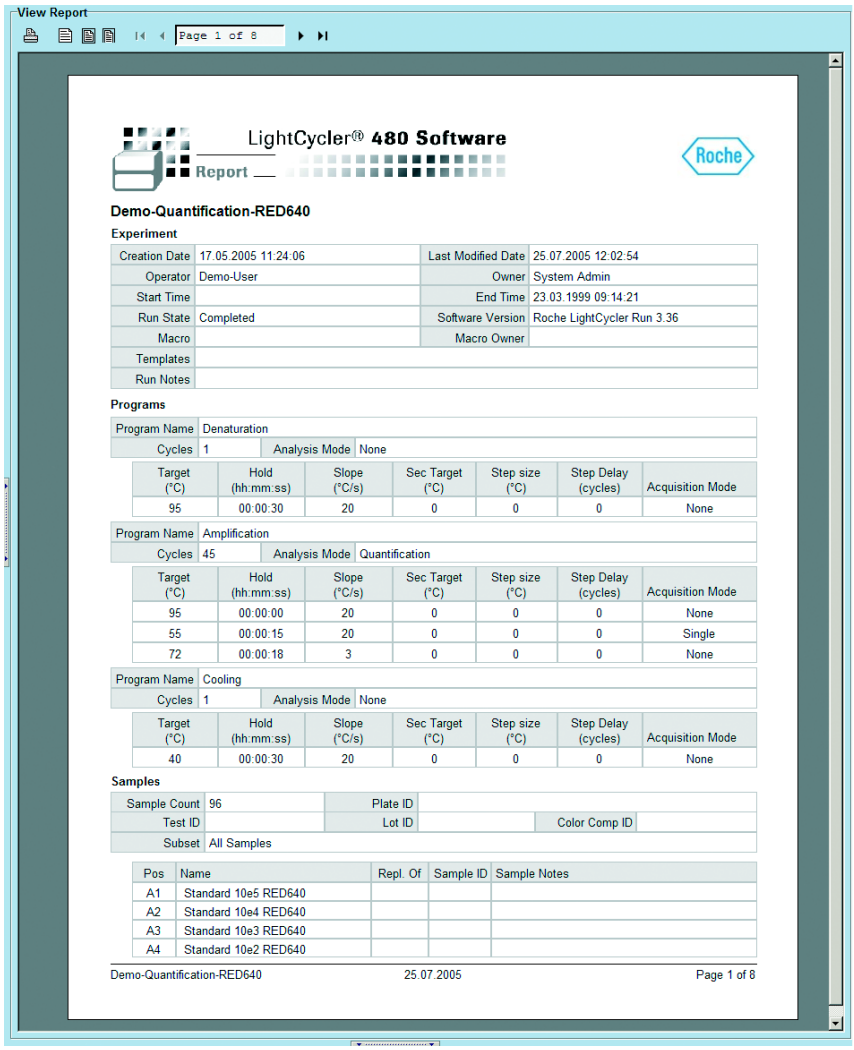
You cannot change the order of items on the General tab.

You cannot drag an item into a category where it does not belong. For example, you cannot drag results from a melting temperature analysis into an absolute quantification analysis.

7 Click *Save* in the *Global action* bar to save the changed report settings together with the experiment. You will be prompted to enter change notes. The saved report settings will be used the next time you generate a report.

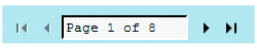
8 If you saved changed settings and want to restore the default settings click *Default Settings*. Close the *Report* window and reopen it. The default settings will be restored.

9 Click *Generate* to view the report:



You can hide the Roche logo that appears on the report using the Report Settings tab on the Tools window. (For more details, see Section Administrative Tools.)

10 To see additional pages of the report, use the page forward and backward controls:



11 To change the scale of the report within the window, click one of the following buttons:

▶The first button displays the report at its printed size.
▶The second button fits the entire report page into the window.
▶The third button fits the width of the report page into the window.

12 To print the report, click the printer symbol at the top of the report window.

11. Working with Preferences

LightCycler® 480 Basic Software provides the following preferences you can use to customize charts and samples and to set various default options:

- **Chart preferences** determine the default appearance and content of your charts.
- **Sample preferences** determine the default sample names in the *Sample Editor* and the default colors and line styles of samples in charts.
- **User preferences** determine default import and export directories and other settings.

Preferences items are located in the *Preferences* folder in the user's folder in the LightCycler® 480 Basic Software *Navigator*. When you open a preferences item, a window opens in the main window to allow you to set the preference options.

You can have multiple chart and sample items, each with different settings. You can specify which item will be default.

Form preferences contains information about the last screen settings and cannot be edited.

If all the preference items of a particular type, such as chart, are deleted, LightCycler® 480 Basic Software creates new default preference items the next time you log on.

This chapter explains how to do the following:

- Use each type of preference
- Create multiple instances of preference items and specify an instance as the default for that preference type

11.1 Using Chart Preferences

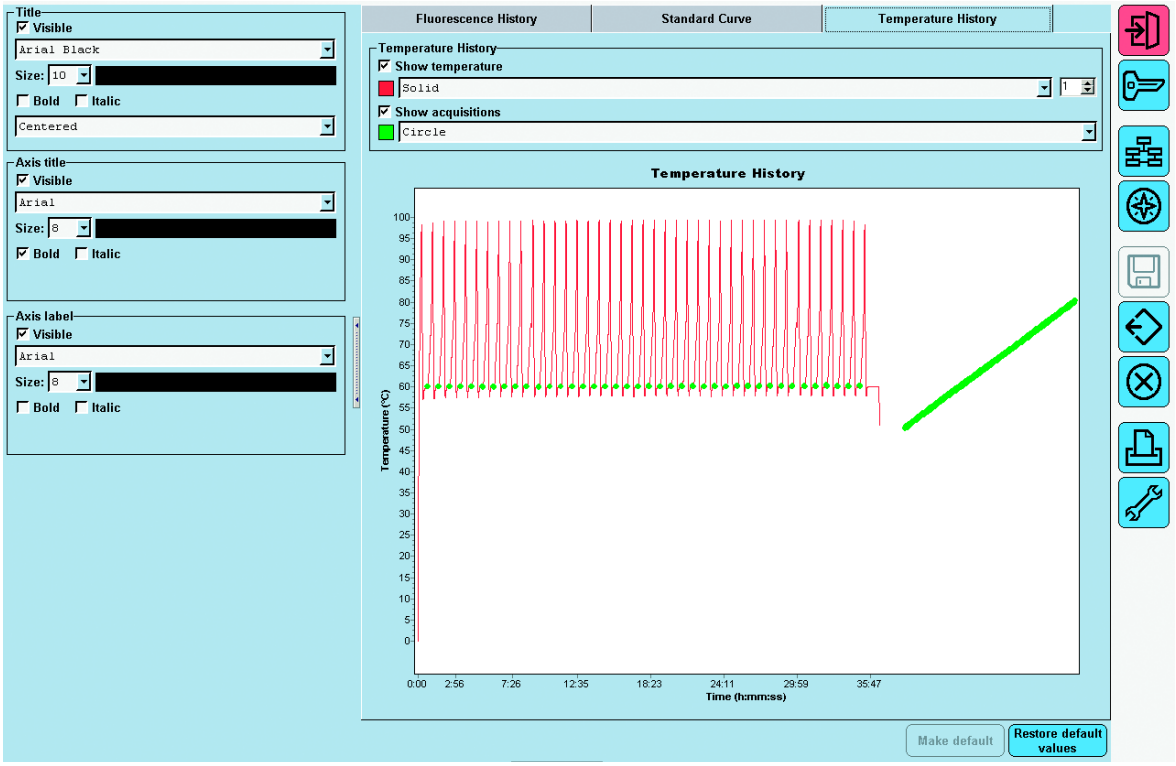
Your user account includes the *Chart preferences* item, which determines the default appearance and content of your charts. You can change the default chart settings as needed.

You can also save a modified version of a chart preference item with a new name and apply the preferences in place of the default. You can have as many different chart preference items as you want, each one defining a different look and feel for your charts. For more information, see Section *Creating a Separate Preferences Item and Making It the Default*. You can override the current chart preferences for individual charts, analyses, or experiments; for more information, see Section *Overriding Default Sample Preferences*.

If you need to undo your changes and restore the previous values for the selected level at any time, click Restore default values.

To open the chart preferences item:

1. In your user folder in the LightCycler® 480 Basic Software *Navigator*, open the *Preferences* subfolder.
2. Double-click *Chart preferences*. The *Chart preferences* window opens in the main window.
3. Using the *Chart preferences* window, you can customize the following chart settings:
   ▶ Chart heading and label styles (using the three sections on the left).
   ▶ Content and appearance of specific types of charts (using the tabs on the right).



There are no chart preference settings for the Exposure History Chart.

11.1.1 Specifying Chart Heading and Label Styles

Use the following three boxes on the window to modify headings and labels:

Title	Specifies the appearance of chart titles.
Axis title	Specifies the appearance of the text below the X axis and to the left of the Y axis on the charts.
Axis label	Specifies the appearance of the measurement values on an axis, such as the times on the X axis of a fluorescence chart.

Each of the three sections has the same format options, except that the title section includes an option for title position.

To specify heading and label styles:

1	In the appropriate section, select or deselect the *Visible* checkbox to include or exclude this type of text on charts.
2	Specify the text appearance as follows: ▶ Select the typeface from the pull-down list in the first box. ▶ Select the type size from the pull-down list in the *Size* box or enter a value. ▶ To change the text color, click the colored bar to the right of the *Size* box to display a color palette. Select the color you want, and click *OK*. ▶ To make the text bold or italic, select the *Bold* or *Italic* checkbox (or both). ▶ (Title only) To position the chart title, select a position from the pull-down list in the last box (left or right justified, centered).
3	Click *Save* in the *Global action* bar to save your settings.

11.1.2 Specifying the Content of Fluorescence Charts

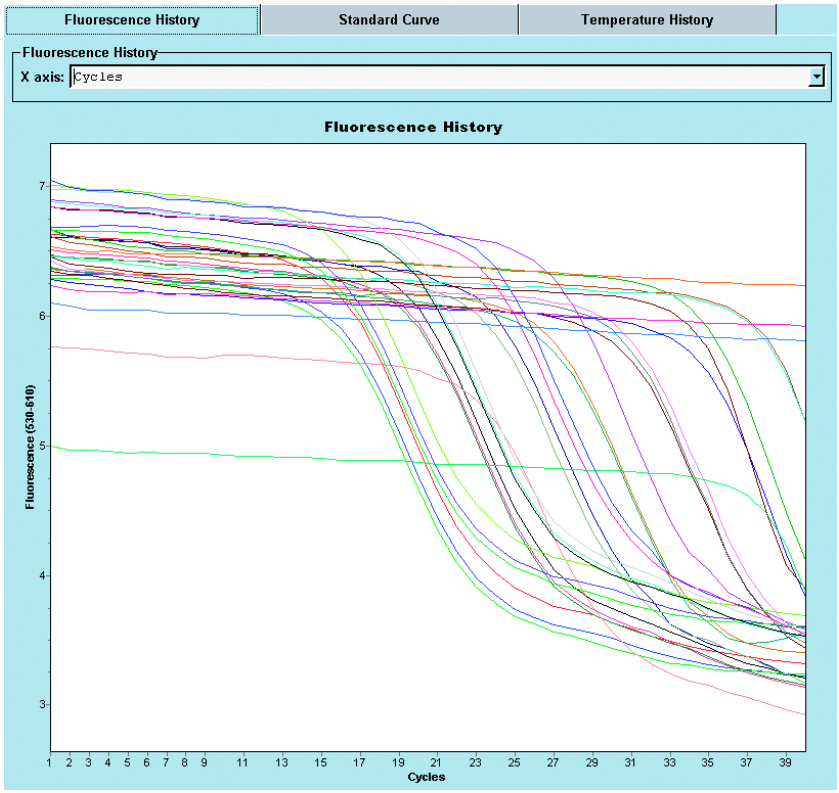
The *Fluorescence* tab of the *Chart preferences* window controls the default appearance of the fluorescence data displayed in the *Fluorescence History* chart. The *Fluorescence History* chart is displayed on the *Data* tab of the *Experiment* module.

- The default axis value for the *Fluorescence History* chart that plots fluorescence versus time, cycles, or temperature.
- The default channels for the *Current Fluorescence* bar chart that displays the level of fluorescence for each sample and each channel at a particular acquisition point.

Both charts are displayed on the *Online Data Display* tab of the *Run* module and on the *Raw Data* tab of the *Summary* module.

To specify fluorescence chart information:

1. Select the *Fluorescence* tab (if not already selected).
2. In the *Fluorescence History* box, select a value for the X axis from the pull-down list.



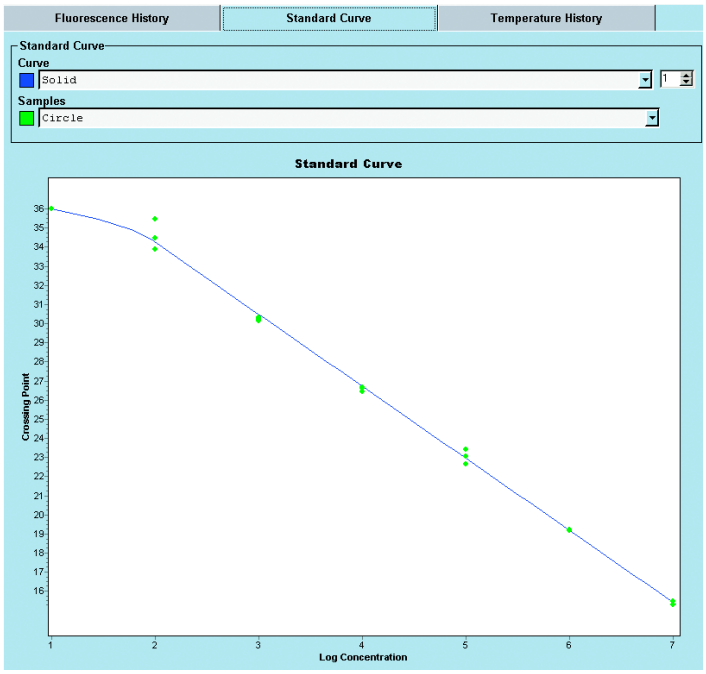
3. Click *Save* in the *Global action* bar to save your settings.

11.1.3 Specifying the Appearance of Standard Curve Charts

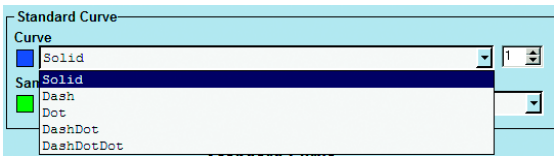
The *Standard Curve* tab of the *Chart preferences* window controls the appearance of the standard curve charts in quantification analyses. You can specify the appearance of the curve and the sample points from which the curve is derived.

To specify the appearance of the standard curve and sample points:

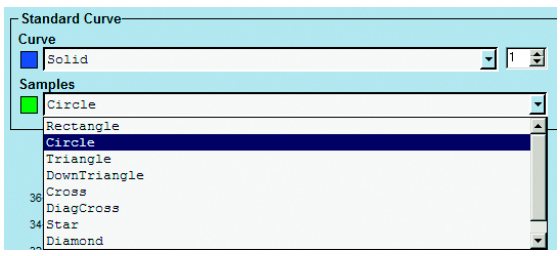
1. Select the *Standard Curve* tab.



2. The appearance of the curve can be modified as follows:
   - ▶ To specify line color, click the colored box under *Curve* to display a color palette, select a color, and click *OK*.
   - ▶ To specify line style, select a style from the pull-down list.
   - ▶ To specify line width, enter or select a value.



3. The appearance of the sample points can be modified as follows:
   - ▶ To specify point color, click the colored box under *Samples* to display a color palette, select a color, and click *OK*.
   - ▶ To specify point style, select a style from the pull-down list.



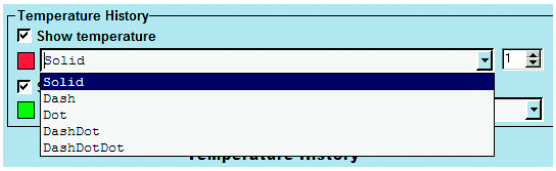
4. Click *Save* in the *Global action* bar to save your settings.

11.1.4 Specifying the Content and Appearance of the Temperature Chart

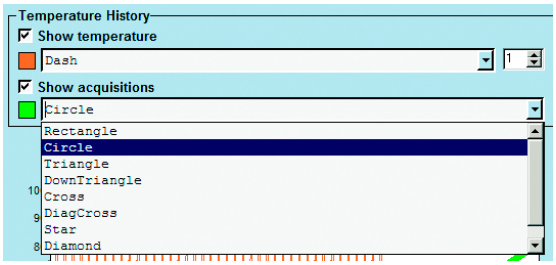
The *Temperature* tab of the *Chart preferences* window controls the appearance of the *Temperature History* chart, which displays temperature readings and fluorescence acquisition points. The chart is displayed on the *Run Protocol* tab (where it is labeled "Overview") and on the *Data* tab

To specify content and appearance of the temperature chart:

1. Select the *Temperature* tab.
2. To include or exclude temperature readings, select or deselect the *Show temperature* box.
3. The appearance of the temperature lines on the chart can be modified as follows:
   ▶ To specify the *line color*, click the colored box under *Show temperature* to display a color palette, select the color you want, and click *OK*.
   ▶ To specify the *line style*, select a style from the pull-down list.
   ▶ To specify the *line width*, enter or select a value.



4. To include or exclude fluorescence acquisition points, select or clear the *Show acquisitions* box.
5. To specify the appearance of the acquisition points on the chart:
   ▶ To specify the *point color*, click the colored box under *Show acquisitions* to display a color palette, select the color you want, click *OK*.
   ▶ To specify a point style, select a style from the pull-down list.



6. Click *Save* in the *Global action* bar to save your settings.

11.1.5 Overriding Default Chart Preferences

In some cases, you may want an individual chart, analysis, or experiment to use different chart settings from the defaults you specified in the *Chart preferences* item. You can override the default settings at the following levels:

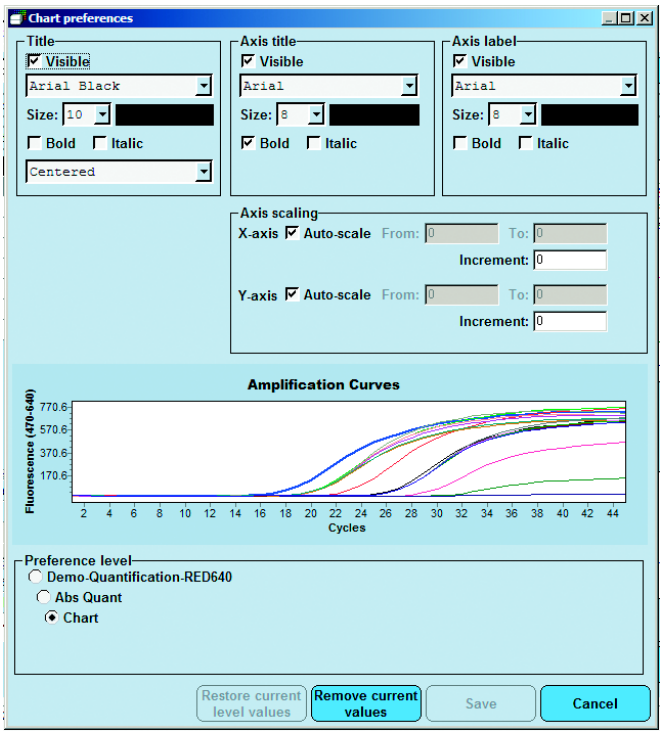
- An individual chart
- All charts within an analysis
- All charts within an experiment

When you specify custom settings at any of these three levels, the new settings override the default values for the charts at that level.

To override chart preferences:

1. Open the experiment and right-click the chart you want to modify.
   To override preferences for all charts within an analysis or within an experiment, right-click any chart in the analysis or experiment.

2. Select *Chart Preferences*.
   A *Chart preferences* dialog box opens that contains options for the chart type similar to those in the *Chart preferences* window, described above. However, the dialog box includes an additional option for setting the chart X- and Y-axis scale.



3 Before you make any changes, you must select the level at which to apply the changes.
In the *Preference level* area, select one of the following:
- ▶ Experiment name to apply the settings to all charts in the current experiment
- ▶ Analysis name to apply the settings to all charts in the current analysis (you must have opened the menu from an analysis chart for this option to be available)
- ▶ *Chart* to apply the settings only to the current chart

The settings can be saved for only one level at a time. That is, if you make changes at the chart level, select the analysis level and make more changes and click Save, only the changes for the analysis level are saved. The settings for a higher level do not override settings saved at a lower level. For example, if you change the title color to blue at the chart level for a standard curve and save the setting, and subsequently change the title color to green at the experiment level and save the setting, the standard curve title remains blue; it does not change to green.

4 Change text settings in the *Title*, *Axis title*, and *Axis label* boxes, as needed.
For more information, see Section *Specifying Chart Heading and Label Styles*.

5 Change the chart-specific settings (if any) in the box below the *Title* section.
The options displayed depend on the type of chart that was active when you opened the dialog box. For some chart types, there are no chart-specific settings.

6 In the *Axis scaling* area, set the range of units for the X axis and Y axis and the increment size.
- ▶ If you select 0 as the increment size, the increment size is determined automatically.
- ▶ If you set an increment size so small that the labels overlap, the setting is ignored and the increment size is determined automatically.

7 If you need to undo your changes and restore the previous values for the selected level, click *Restore current level values*.

8 When finished, click *Save*.

Example of overriding chart preferences:

1	Right-click the amplification curve chart in an *Absolute Quantification analysis* module and select *Chart Preferences*.
2	The *Chart* level is selected by default; deselect *Auto-scale*, set the scaling range from 1 to 50, and save settings.
3	Reopen *Chart Preferences*, select the *Abs Quant* (analysis) level, deselect *Auto-scale*, set the scaling range from 1 to 100, and save settings. Result: The *Amplification Curve* chart now has a scaling range of 1 to 50. The scaling range for all other charts in the analysis module (*e.g.*, the *Standard Curve* chart) is now 1 to 100.
4	Reopen *Chart Preferences* (the *Chart* level is selected by default), click *Remove Current Values*, and save settings. Result: The amplification curve chart now has a scaling range of 1 to 100. The settings for the chart level were removed, so the value for the level above (the analysis level) is applied.
5	Reopen *Chart Preferences* (the *Chart* level is selected by default), and set the scaling range from 1 to 150, click *Restore Current Level Values*, and close the dialog box. Result: The amplification curve chart scaling range remains at 1 to 100, because the 1 to 150 setting was not saved. When you click *Restore Current Level Values*, the previously saved setting (1 to 100) was restored.

11.2 Using Sample Preferences

You can modify the appearance of sample lines and points on LightCycler® 480 Basic Software charts in two ways:

- Modifying the sample preferences that apply to all experiments; you can modify the default sample preferences item or create multiple versions of the default item and apply the version you want
- Overriding the sample preferences for the current experiment; you can override preferences for multiple samples in the experiment or for an individual sample on a chart

11.2.1 Modifying the Sample Preferences for All Experiments

Your user account includes the *Sample preferences* item, which determines the default sample names and the appearance of sample lines and points on all LightCycler® 480 Basic Software charts. You can change the settings in the sample preferences item as needed. You can also save multiple versions of the sample preferences item and apply the preferences you want.

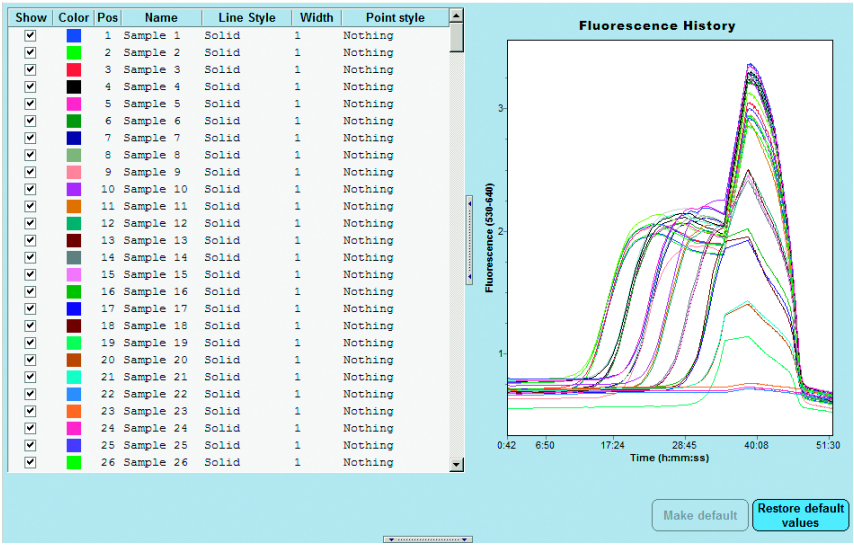
To modify default sample preferences:

As you follow Steps 3 – 8 below, remember that you can change settings for a contiguous group of samples at one time. To change settings for a group:

- *Select the item you want to change for the first sample in the group.*
- *Hold down the <Shift> key, and select the same item for the last sample in the group.*
- ***Press the <F2> key.***
- *Change the value for the last sample and press the <Return> key.*

The changes are applied to all the selected samples.

1. In your user folder in the LightCycler® 480 Basic Software Navigator, open the *Preferences* subfolder, and double-click *Sample preferences*.
   The *Sample Preferences* window opens in the work pane.

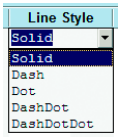


2. To include or exclude a sample in charts, select or deselect the corresponding *Show* checkbox.

3. To change the default color for a sample, click the colored square next to the sample name in the sample list to open a color palette.
   Select a color, and click *OK*.
   Result: The sample color appears next to the sample name in LightCycler® 480 Basic Software analysis results and is used for sample lines and points in the charts.

4. To change the default sample name, click the sample name, and type a new name.
   The default sample name is applied to new experiments; existing experiments are not affected.

5. To change the line style used for the sample, click in the *Line Style* column, and select a new style from the pull-down list.
   For example, you can select a dashed line instead of a solid line.



6. To change the line width, click in the *Width* column, and enter a new width.

7. If you prefer to see a sample line as a string of measurement points, click in the *Point Style* column, and select a style from the pull-down list. (If you prefer solid lines, leave the *Point Style* set to “Nothing.”)

8. Click *Save* in the *Global action* bar to save your settings.

   If you need to undo your changes and restore the previous values for the selected level at any time, click Restore default values.

11.2.2 Overriding Default Sample Preferences

At times you may want to change the appearance of samples in just one experiment or chart, without changing the defaults applied to all experiments. Or you may want to change settings for a chart and save the settings as a sample preferences item that can be applied to other experiments.

The following options are available at the experiment level:

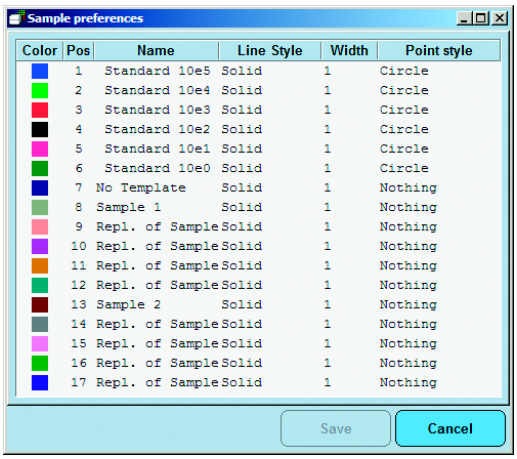
- Use a Sample Preference Editor to change the appearance of multiple samples in the experiment
- Apply an existing sample preferences item to the experiment
- Change the appearance of an individual sample line
- Save experiment settings as a sample preferences item
- Clear any changes and reapply the default sample preferences

You cannot change the sample names or positions in an existing experiment.

To use the Sample Preference Editor to change multiple samples:

Your settings are applied to all the charts in the current experiment, but do not affect other experiments or the default sample preferences settings. The settings are saved with the experiment.

1. Open the experiment for which you want to modify the samples.
2. Right-click an experiment chart that contains sample information.
3. Select *Sample Preferences*.
4. Modify the settings, and click *Save*.



5. Modify the settings as you would if you were modifying the default sample preferences item. For more information, see Section *Modifying the Sample Preferences for All Experiments*.
6. Click *Save* in the *Global action* bar to save the experiment with the new settings.

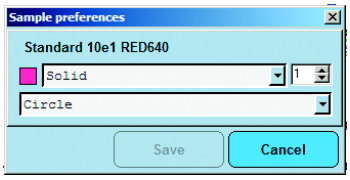
To apply a sample preference item to an experiment:

The sample preferences in a sample preferences item can be applied to individual experiments.

1. Open the experiment for which you want to modify the samples.
2. Right-click any of the experiment charts that contains sample information.
3. Select *Load Sample Preferences*.
4. Select the sample preferences item from the navigator, and click *Open*.
   The settings are applied to all charts in the experiment.
5. Click *Save* on the *Global action* bar to save the experiment with the new settings.

To modify an individual sample line:

1. Open the experiment for which you want to modify the samples.
2. Make sure a chart is displayed that contains the line you want to change. In an analysis module, select the sample in the sample list to display the corresponding line in an analysis chart.
3. Move the mouse pointer over the line until the pointer changes to a hand, and right-click the line. A small dialog box opens containing settings for the line.



4. To change the line color:
   ▶Click the colored box to open a color palette.
   ▶Select a new color, and click *OK*.
5. To modify the line style, width and measurement symbol, select the values from the pull-down lists. Click *Save*. The line is changed in all charts in the current experiment.
6. Click *Save* to save the experiment with the new settings.

11.3 Creating a Separate Preferences Item and Making It the Default

You can create multiple chart or sample preference items and specify which item is to be used as the default. You can change the default designation whenever you need to. If you delete all instances of a preference item, such as all chart preference items, the software creates a new default item the next time you log in. The settings are the application defaults.

To create a separate preferences item and make it the default:

1	In your user folder in the LightCycler® 480 Basic Software *Navigator*, open the *Preferences* subfolder.
2	Select the default item for the preference type you want (*Sample preferences* or *Chart preferences*) and copy it: Navigate to a location to save the item, enter a name for the new preference item, and click *Save*.
3	Open the preferences item and modify the preferences, as described in the previous sections.
4	To specify this sample preferences item the default, click *Make Default* in the *Preferences* editor pane. *You can also specify the default preferences item using options in User Preferences. For more information, see Section Specifying User Preferences.*

11.4 Specifying User Preferences

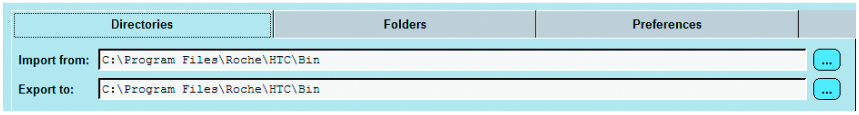
User preferences specify the following:

- Default directories for importing files and exporting LightCycler® 480 Basic Software files
- The default database folders in which to save LightCycler® 480 Basic Software items, such as experiments, macros, and queries
- Chart and sample preference items to apply as the default, when multiple instances of a preferences item are available

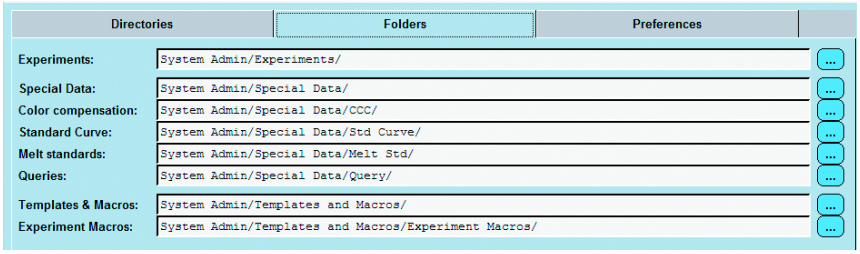
To specify user preferences:

1. In your user folder in the LightCycler® 480 Basic Software *Navigator*, open the *Preferences* subfolder, and double-click *User preferences*.

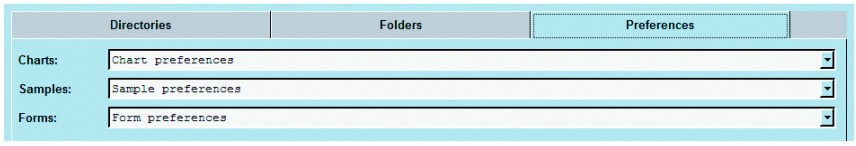
2. To specify import/export directories, select the *Directories* tab (if not currently selected).
In each box, type a directory path on your local computer or click the ... button, navigate to a location on your local computer or the network, and click *OK*.



3. To specify default folders, select the *Folders* tab.
In each box, type the path for the folder location in the Navigator or click the ... button to navigate to a location on your local computer or the network.
Select a folder, and click *OK*.



4. To specify a preferences item as the default, select the *Preferences* tab.
In each box, select the preference item to specify it as the default for the preference type.



5. Click *Save* on the *Global action* bar to save the experiment with the new settings.

These settings will be applied next time you open an experiment.

12. Administrative Tools

Administrative Tools are accessible via the *Tools* dialog. These tools allow you to perform the following:

- Manage user access, which includes managing user passwords, user and group accounts and general system settings
- Define settings for the report
- View database status and manage the database
- Manage the connection settings to LightCycler® 480 Instruments and view the operation log
- Define detection formats

Access to the Administrative Tools modules is dependent on your user role.

Open the *Tools* dialog by clicking the button. The *Tools* dialog has a *Navigator* on the left that lists the available options and an *Editor* pane on the right.

12.1 Managing User Access

To use the LightCycler® 480 Basic Software, you must have a user account in the LightCycler® 480 Basic Software database. User accounts have different levels of access to the software, depending on the role assigned to the account and the groups to which the account belongs.

This section explains the function of user accounts, roles and groups and explains how to manage these aspects using the LightCycler® 480 Basic Software *User Management* tool. The chapter also explains how to change a user password. Read this chapter if you are responsible for creating or modifying user accounts or if you want to understand the privileges associated with your account. Read the section on passwords if you need to change your password.

Your own user account must have the Local Administrator role to use the User Access tool.

12.1.1 Understanding User Accounts

A user account provides access to the LightCycler® 480 Basic Software. The user account specifies the user's login name and password and defines the user's level of access to the software.

When you create a user account, you must assign it a role. The role determines the tasks the user can perform using the software. For more information, see Section *Understanding Roles*. You can also add a user account to one or more groups. Users in the same group have access to the objects belonging to group members. For more information, see Section *Understanding Groups*.

Each user account has a default folder in the LightCycler® 480 Basic Software *Navigator* labeled with the user's full login name, with several default subfolders. The user's default folder and subfolders cannot be deleted, renamed, or moved. However, each user can create additional folders underneath the default folders.

A user called "admin" (for System Administrator) is created automatically when LightCycler® 480 Basic Software is installed. The admin user has the Local Administrator role and is used to create other user accounts. The admin account cannot be edited or disabled. Once a user account has been created, it cannot be edited or deleted. A user account can, however, be made inactive by the Local Administrator. An inactive user account cannot have a role and cannot be assigned to a group.

12.1.2 Understanding Groups

A group is a collection of user accounts. The members of a group have access to objects belonging to any group member. For example, a group member can open any experiment belonging to another member.

Users can belong to more than one group. A user has access to another user's objects when both users are members of at least one group, regardless of membership in other groups. For example, if user Bob belongs to Groups A and B, while user Susan belongs to Groups B and C, both Bob and Susan have access to each other's objects because both are members of Group B. The level of access a user has to objects belonging to others is determined by the user role assigned to the user account. For more information about user roles, see the next section.

12.1.3 Understanding Roles

Each user account is assigned to one and only one role. The role determines the user's privileges. There are three roles:

- Expert User
- Local Administrator

Roles cannot be created or deleted, but certain access privileges can be enabled or disabled for each role. For more information, see Section *Working with Roles*.

It is possible to have multiple Expert Users and Local Administrators.

12.1.4 Privileges of the Expert User Role

Expert Users can perform the following:

- Change their password
- Use the *Run programming* module to create and execute experiments, including the following:
  - Use the Sample Editor, Property Editor, and Property Viewer
  - Use the Subset Editor
  - Add an analysis to an experiment and edit all analysis settings
  - Apply and create templates
- Create all other objects and open, copy, execute, modify and move any of their objects; for experiments, this includes modifying sample information, the sample count (before the run begins), adding an analysis to the experiment, including and excluding samples from the analysis, and using the analysis toolbar to change any of the analysis settings
- Delete their objects and non-default folders, if enabled by the Local Administrator (only possible if the folder does not contain any objects)
- Rename their non-default folders and objects
- Open, copy, and execute objects owned by the Local Administrator
- Open, copy, and execute objects owned by other Expert Users who are members of the same group
- View and copy items from the *Roche* folder
- Create and execute queries
- Change their preference settings
- Manage detection formats when enabled by administrator
- Access the instrument tool and add an instrument

Expert Users cannot do the following:

- Create, delete, move, modify, or rename objects belonging to the Local Administrator or other Expert Users
- See the folders or objects belonging to Expert Users who are not members of the same group
- Copy, delete or rename experiment objects (including their own experiment objects)
- Delete, move, copy, or rename default folders (including their own folders)

12.1.5 Privileges of the Local Administrator Role

Local Administrators can perform the following:

- Use the *Run programming* module to create and execute experiments
- Create and use existing templates and macros to execute experiments and analyze results
- Create all other objects and open, copy, execute, modify, delete, and move any of their own objects (modify rights include modifying sample information, adding an analysis to the experiment, and using the analysis toolbar to change any of the analysis settings)
- Open, execute and copy items belonging to other Local Administrators
- Open items in the *Roche* folder
- Create system folders that are owned by the Local Administrator but can be read by all users
- Create, open, copy, execute, modify, delete and move objects in folders belonging to Expert Users
- Use the *User Access* tool to manage users and groups; for more information, see Section *Managing Users, Groups, and Roles*
- Maintain the database (update, reindex and cleanup)
- Activate the following access privileges for roles:
  - For Roche Users: The ability to access objects owned by the Local Administrator
  - For Expert Users: The ability to delete non-traceable objects the user owns and to edit detection formats

Local Administrators cannot do the following:

- Modify or move objects in the *Roche* folder
- Move, delete or modify objects owned by other Local Administrators; for example one administrator cannot copy objects into another administrator's folder
- Delete, move, copy or rename default folders (including their own folders)

12.1.6 User Access to Objects

The access rights to certain objects (experiments, folders, templates, preferences) in the LightCycler® 480 database are defined by the user role. Access to each kind of object is regulated by specific kinds of permissions. The following table lists user access rights to database objects:

For an **experiment**, there are four kinds of permissions:

- Read — View the experiment in the navigator, open the experiment, and export the experiment to a file
- Move — Move the experiment from one folder to another
- Modify — Make changes to experiment
- Execute — Execute the experiment on an instrument

Owner	Type of User	Rights
Both	Experiment Owner	Read, Move, Modify, Execute
Admin	Administrator in same group	Read
	Administrator not in group	Read
	Expert user in same group	Read
	Expert user not in group	Read
Expert	Administrator in same group	Read, Move, Modify, Execute
	Administrator not in group	Read, Move, Modify, Execute
	Expert user in same group	Read
	Expert user not in group	None

For a **folder**, there are six kinds of permissions:

- ▶ Read — View the folder and its contents in the navigator
- ▶ Delete — Delete an empty folder
- ▶ Copy — Make a copy of the folder in another location (copies tree but not objects)
- ▶ Write — Save a new object to the folder or create sub-folder
- ▶ Move — Move the folder from one parent folder to another
- ▶ Rename — Change the name of a folder

Owner	Type of User	Rights
Both	Folder Owner	Read, Delete, Copy, Write, Move, Rename
Admin	Administrator in same group	Read, Delete, Copy
	Administrator not in group	Read, Delete, Copy
	Expert user in same group	Read, Copy
	Expert user not group	Read, Copy
Expert	Administrator in same group	Read, Delete, Copy, Write, Move, Rename
	Administrator not in group	Read, Delete, Copy, Write, Move, Rename
	Expert user in same group	Read, Copy
	Expert user not group	None

For a **template**, there are six kinds of permissions:

- ▶ Read — View and open in the navigator, apply to experiment, and export
- ▶ Edit — Open and edit notes and template type only
- ▶ Delete — Delete the template
- ▶ Copy — Make a copy of the template in another location
- ▶ Move — Move the folder from one parent folder to another
- ▶ Rename — Change the name of a folder

Owner	Type of User	Rights
Both	Template Owner	Read, Edit, Delete, Copy, Move, Rename
Admin	Administrator in same group	Read, Delete, Copy
	Administrator not in group	Read, Delete, Copy
	Expert user in same group	Read, Copy
	Expert user not group	Read, Copy
Expert	Administrator in same group	Read, Edit, Delete, Copy, Move, Rename
	Administrator not in group	Read, Edit, Delete, Copy, Move, Rename
	Expert user in same group	Read, Copy
	Expert user not group	None

For a **preference**, there are five kinds of permissions:

- ▶ Read — View in the navigator
- ▶ Edit — Open in navigator and edit
- ▶ Delete — Delete the preference
- ▶ Copy — Make a copy of the preference in another location
- ▶ Move — Move the preference from one parent folder to another

Owner	Type of User	Rights
Both	Preference Owner	Read, Edit, Delete, Copy, Move
Admin	Administrator in same group	Read, Delete, Copy
	Administrator not in group	Read, Delete, Copy
	Expert user in same group	Read, Copy
	Expert user not group	Read, Copy
Expert	Administrator in same group	Read, Edit, Delete, Copy, Move
	Administrator not in group	Read, Edit, Delete, Copy, Move
	Expert user in same group	None
	Expert user not group	None

12.1.7 Managing Users, Groups, and Roles

The *User Access* tool allows you to perform the following:

- Create, modify, enable, or disable user accounts
- Assign roles to user accounts and change role assignments (you cannot create, modify, or delete roles)
- Create or delete user groups and assign users to groups

You must have the Local Administrator role to use the *User Access* tool.

To open the User Access tool:

- From the *Tools* navigator, open *User Access*, and select *Users and Groups*.
- The *Users* tab is selected by default.

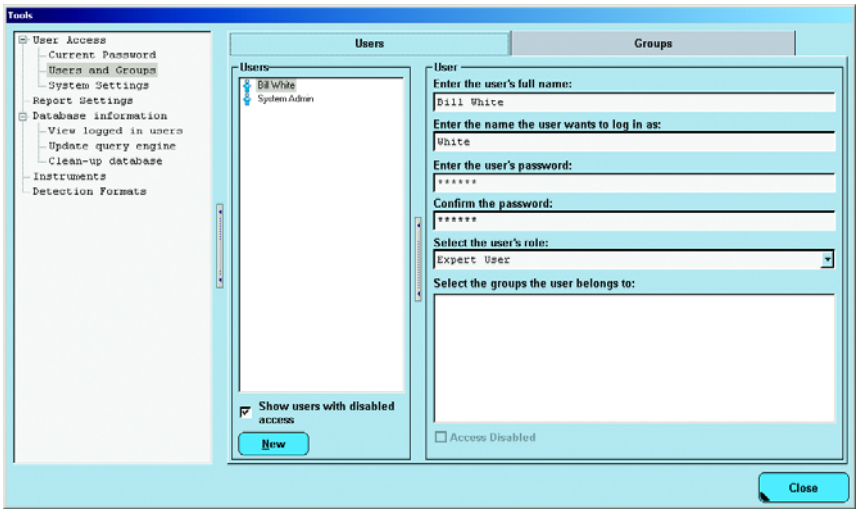
Working with Users

When working with the traceable (audit trail) LightCycler® 480 database, a user account can never be deleted, only disabled. In addition, the user's full name, login name and role cannot be changed.

As an alternative to the procedure described below, you can always access the User window directly by double-clicking a user object in the Navigator.

To create a new user account:

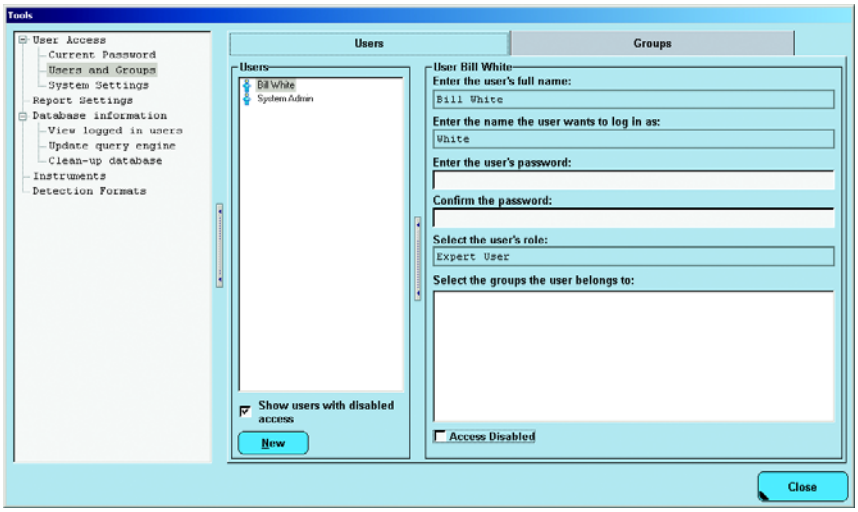
1. In the *User Access* tool, click *Users and Groups*. Select the *Users* tab (if not already selected).
2. Click *New*.
3. Enter the user's full name, login name and password, and select a role for the user.



4. Check each group to which the user should be added. If no groups have been created, you can add the user when you create the group.
5. Click the user name in the *Users* list to confirm your input.
   Result: A default folder for the new user is added to the *Navigator*, and the user name is added to the list of users in the *\Administration\Users* folder in the *Navigator*.

To edit, enable, or disable a user account:

1. In the *User Access* tool, click *Users and Groups*. Select the *Users* tab (if not already selected).
2. Select the user name in the *Users* list. Information about the selected user is displayed:



3. The only information you can change is the password and group membership.
4. To disable the user account, select the *Access Disabled* checkbox; to reactivate a disabled account, clear the *Access Disabled* checkbox.
   You cannot disable the System Admin account.
5. When finished, click *Close*.

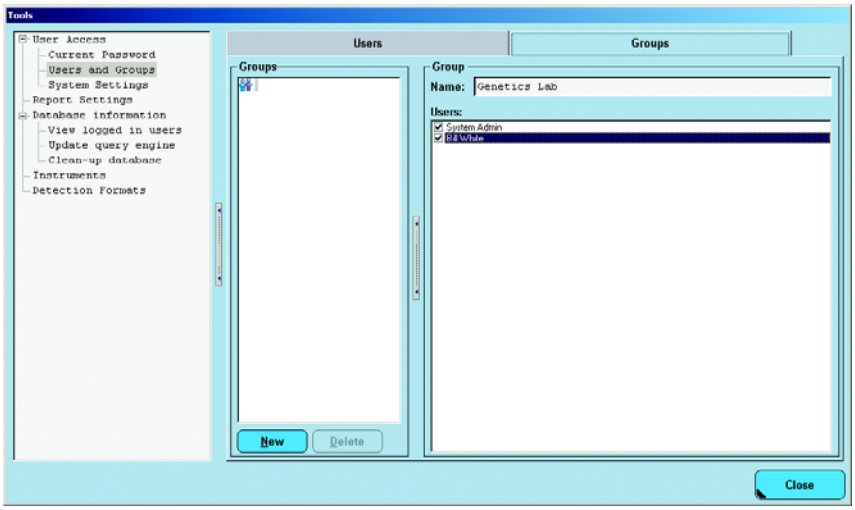
Working with Groups

When working with the traceable (audit trail) LightCycler® 480 database, a user account can never be deleted, only disabled. In addition, the user's full name, login name and role cannot be changed.

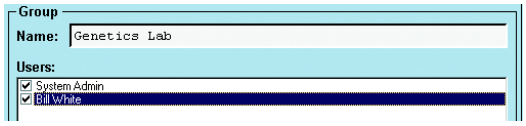
As an alternative to the procedure described below, you can always access the Group window directly by double-clicking a group object in the Navigator.

To create a new group:

1. In the *User Access* tool, click *Users and Groups*. Select the *Groups* tab (if not already selected).



2. Click *New*.
3. Type a name for the group in the *Group Name* box.
4. To add users to the group, check the names of the users you want to add.



You can create a group without adding users.

5. Click the empty group icon that was created in the *Groups* list to confirm your input.
6. If you did not add users to the group, a message asks you to confirm saving the group without users. Click *Yes*.

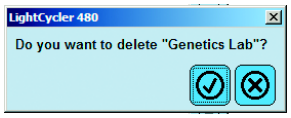
Result: The group is added to the *Groups* folder in the *Navigator*.

To edit a group:

1. In the *User Access* tool, click *Users and Groups*. Select the *Users* tab (if not already selected).
2. Select the group name in the *Groups* list.
3. Change the group name or select and deselect members of the group. You can also assign a group to a user when you edit the user account. For more information, see Section *Managing Users, Groups, and Roles*.
4. Click the group name in the *Groups* list to confirm your changes.

To delete a group:

1. In the *User Access* tool, click *Users and Groups*. Select the *Users* tab (if not already selected).
2. Select the group name in the *Groups* list.
3. Click *Delete*.
4. A message asks you to confirm the deletion of the group. Click *Yes*.



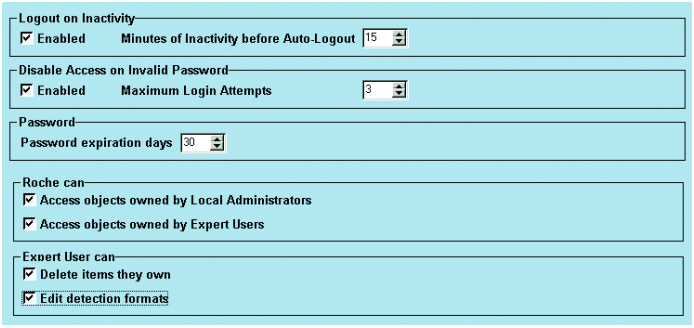
Result: The group is deleted from the *Groups* folder in the *Navigator*.

12.1.8 Working with Roles

You cannot create or delete roles, and you cannot change a user's role assignment. You can modify certain access rights associated with the Expert User and Roche User roles. You cannot modify access rights of the Local Administrator role. You can also specify the period of inactivity before a user is automatically logged off as well as the maximum login attempts before an access is disabled on entering an invalid password.

To set conditions for access and modify a role's access rights:

1. In the *User Access* tool, click *System Settings*. The available options are displayed in the *Editor* pane:



The following options are available:

Name	Description
Enable Auto Logout	When selected, causes users to be automatically logged out after a designated period of inactivity.
Minutes Inactivity	The period of inactivity [1 – 1000 min.] after which auto logout occurs.
Disable Access…	When selected, causes a user account to be disabled after a designated number of unsuccessful login attempts.
Maximum Login Attempts	Specifies the number of unsuccessful login attempts [1 – 5] that causes automatic logout.
Enable Password Expiration	When selected, causes passwords to expire after the designated number of days.
Password Expiration Days	Specifies the number of days [1 – 100000] before a password expires.
Roche user can access objects owned by Local Administrators	When selected, allows Roche Users to access objects owned by Local Administrators.
Roche user can access objects owned by Expert Users	When selected, allows Roche Users to access objects owned by Expert Users.
Expert user can delete items they own	When selected, allows Expert Users to delete their own objects.
Expert user can edit detection formats	When selected, allows Expert Users to edit detection formats.

2. Select or deselect the available options.
3. When finished click *Close*.

12.1.9 Changing Your Password

When you are assigned an account on the LightCycler® 480 Basic Software, you receive an initial password, which you can use to log onto the LightCycler® 480 Basic Software the first time (you will be prompted to change your initial password upon your first login). You can change your password whenever you want.

To change your password:

- ▶ In the *User Access* tool, select *Current Password*.
- ▶ Enter your current password in the *Old Password* field.
- ▶ Enter the new password in the *New Password* field and again in the *Confirm Password* field.
- ▶ Click *OK*.

When the entries in the New Password and Confirm Password fields do not match, the OK button is not active.

The password must contain at least six characters; one character must be a number and one character must be upper case. Passwords are case-sensitive! Remember the password or keep it in a secure place. Do not share your password with others!

12.2 Report Settings

Here you can define whether the Roche logo should appear on the report.

12.3 Database Information

The *Database Information* window allows you to:

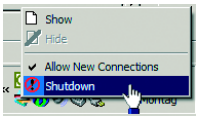
- Display the users logged onto the current database
- View status of the database engine; allows updating if out of date
- Perform a batch export, optionally deleting the exported objects

Tab	Usage
View Logged in Users	Displays a list of the currently logged-on users.
Update Query Engine	Displays the status of the database query engine. If updating the database is necessary, the *Update* button is active. *Updating the database might be necessary after you updated to a new LightCycler® 480 Basic Software version.* *Reindex button: When the database increases in size the time to access your data might also increase. Use the reindex function to optimize the organization of the data in the database thus minimizing the access time to your data.*
Clean-up Database	Provides the option to create an empty copy of the database. This is useful if you want to create a new database with the same structure (*i.e.*, users, groups, and folders) and basic content as the current database. *Database clean-up is similar to a batch export with the difference that multiple database objects are exported and deleted from the database the same time.*

Although the Logged in Users and Query Engine options can be accessed by both Expert Users and Local Administrators, the Clean-up option can be accessed only by Local Administrators.

To clean-up the database:

1. Shutdown LightCycler® 480 Basic Software and Exor4. To shutdown Exor, right-click the Exor icon in the Windows taskbar, and select *Shutdown*.

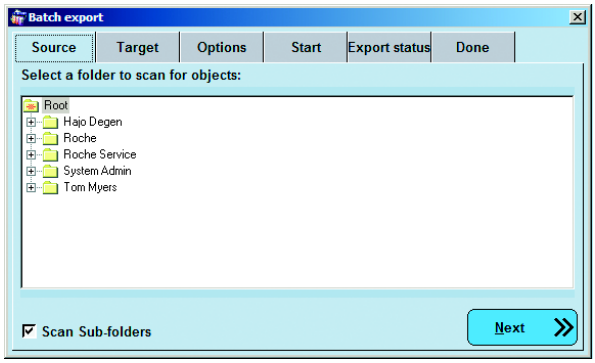


2. In Windows Explorer, open the database directory (C:\Program Files\Roche\Exor4\Data). Make a copy of the XDMS_T.IB file.

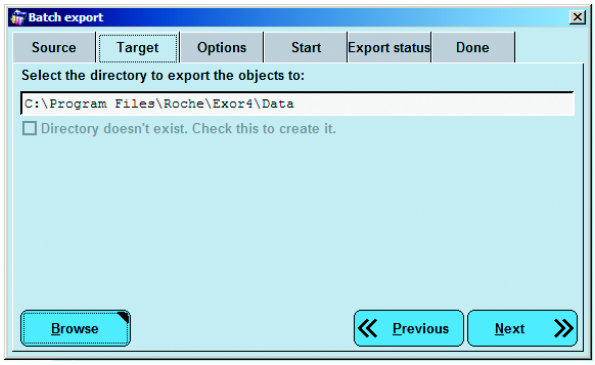
3. Start Exor4 and LightCycler® 480 Basic Software again.

4. Open the *Tools* window, select *Database Status* and the *Clean-Up* option.
Result: The *Clean-up* wizard opens.

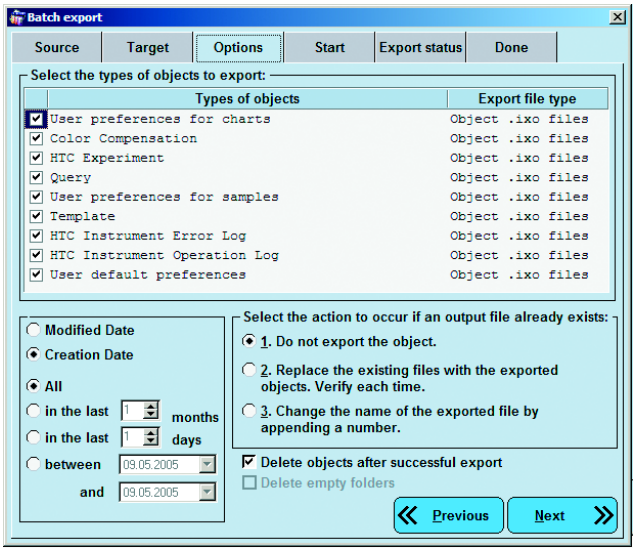
5. First, select the source. If you want to clean-up the complete database, select *Root*. Select the *Scan Sub-folders* box to include all subfolders.



6. Select the target directory to which you want to export the database objects.



7 On the *Options* tab, select all database object types you want to export and delete from the currently installed database:



All other options are identical to the *Batch Export* wizard. (For details, see „*Exporting Multiple Experiment Files Simultaneously*“ under Section *Exporting and Importing Files and Objects*.)

8 Proceed to the next steps of the wizard to complete the database clean-up.

9 Shutdown LightCycler® 480 Basic Software and Exor4 again.

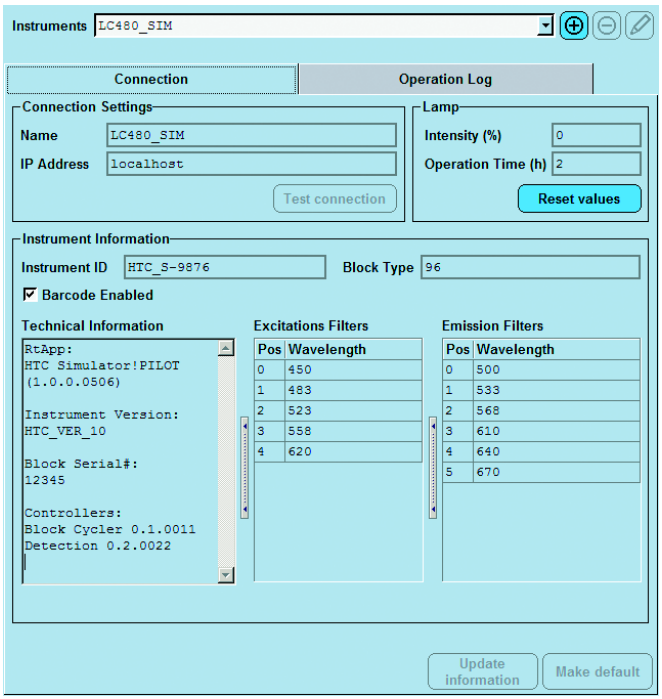
10 Use the *CompactIB* tool to compress the database file and recover space. Start the tool by selecting the entry from the Roche program group in the Windows Start menu.

12

D

12.4 Instruments

The *Instrument* window allows you to view information for the currently active instrument and to change the active instrument.



The *Instrument* window has the following control elements and input fields:

▶ Connection tab

Name	Description
Instruments	Select the instrument to view
Make Default	Set the selected instrument the active instrument
Update Information	Read information from the instrument and update the instrument information
New	Create a new instrument object and activate the connection settings fields
Delete	(Inactive) You cannot a delete an instrument object
Edit	(Inactive) You cannot edit an instrument object
Instrument Information	Display the following: ▶Instrument ID and block size ▶Version information ▶Excitation and emission filters

By default, two virtual LightCycler® 480 Instruments (in either 96 or 384 version) are installed in LightCycler® 480 Basic Software. The virtual LightCycler® 480 Instruments are required to enable programming in offline mode, since you can program an experiment only if an instrument is installed in the LightCycler® 480 Basic Software. When you need to program an experiment while a real instrument is not connected, select one of the virtual instruments on the Instruments dialog and set it as the default. For details, see below.

► Connection Settings area

Name	Description
Instrument name	Name of the currently selected instrument
Address	IP address or name of the selected instrument
Test Connection	Option to open a connection to the selected instrument

►Instrument Information area

Name	Description
Instrument ID	Serial number of the connected instrument
Block Type	Type of block cycler installed in the connected instrument (96 or 384)
Barcode Enabled	Enables the internal bar-code scanner used to read a multiwell plate ID. *This option is possible only when the optional LightCycler® 480 LIMS/Bar-Code Module is installed and activated by purchasing an appropriate licensing key.*
Technical Information	Version of the current instrument firmware
Excitation Filters	List of excitation filters available in the instrument
Emission Filters	List of emission filters available in the instrument

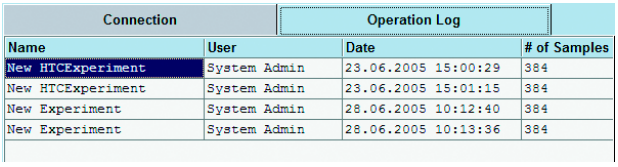
►Lamp area

Name	Description
Lamp Intensity	Intensity of the instrument lamp as a percentage value, read from the instrument
Operation Time	Total time (in hours) the Xenon lamp has been operated, read from instrument
Reset Values	Set lamp intensity to its initial value and operation time to 0. *Select the Reset Counter button only after you exchanged the Xenon lamp (For details, see Section Exchanging the Xenon Lamp.) After the counter is reset, the instrument reads and saves the intensity of the lamp as the starting intensity value. During operation, the instrument compares this saved value to the actual lamp intensity to determine the loss of lamp intensity. When the lamp intensity reaches 50% of its starting intensity, you will be informed. Selecting the Reset values button without inserting a new lamp would cause the instrument to save a wrong starting intensity value.*

► Operation log

To view the Operation log:

1. In the *Instruments* section of the *Tools* window, select the instrument name from the *Instruments* list.
2. Select the *Operation Log* tab.
3. The *Operation Log* displays a list of the runs performed on the instrument. The log includes basic information about each run, such as the run name, user, date and number of samples (96 or 384).



12.4.1 Defining an Instrument

To define and connect a LightCycler® 480 Instrument follow the steps described below:

1. Open the Tools dialog by clicking the button.
2. From the *Navigator* of the *Tools* dialog select *Instruments*.
3. Next to the Instruments selection box click .
4. Enter a name for the LightCycler® 480 Instrument and click *OK*.
5. On the *Connection* tab enter "192.168.95.41" as *IP Address*.
6. (Optional) Click on *Test Connection* to test the IP address.
7. Click on *Update information*.
8. Click on *Make default* to make this instrument your default instrument.
9. Click *Close*.

12.5 Detection Formats

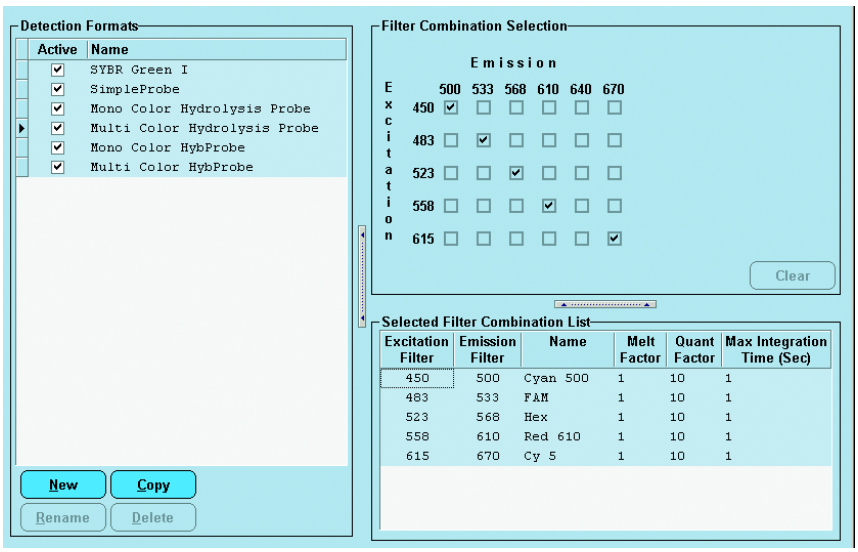
A detection format specifies one or more excitation–emission filter combinations. The *Detection Format* tab is used to define detection formats and to specify which detection formats are active (*i.e.*, available to be selected for an experiment). When programming a new experiment, you can choose detection formats to be used during the run from detection formats previously defined on the *Detection Format* tab. If a detection format contains more than one filter pair, you can choose which of the combinations is actually applied during a run by using the *Customize* option, (For details, see Section *Programming and Running an Experiment*.)

The following default detection formats are available:

Detection Format	Filter Combination Name	Excitation Filter	Emission Filter
SYBR Green I	SYBR Green I	483	533
SimpleProbe	SimpleProbe	483	533
Mono Color Hydrolysis Probe	FAM	483	533
Multi Color Hydrolysis Probe	Cyan 500	450	500
	FAM	483	533
	Hex	523	568
	Red 610	558	610
	Cy 5	615	670
Mono Color HybProbe	Red 640	483	640
Multi Color HybProbe	Fluos	483	533
	Red 610	483	610
	Red 640	483	640
	Cy 5	483	670

Default detection formats cannot be edited.

The *Detection Format* tab includes a *Detection Format* list, a *Filter Combination Selection* area, and a *Selected Filter Combination List*.



The *Detection Format* list is used to manage detection formats. It has the following control elements:

Name	Usage
Active	Select the *Active* box to make the detection format listed on the corresponding row available for use in the software.
Name	Click the name entry of a detection format to change the name.
New	Activate the next available row in the *Detection Format* list to enter a name for a new format.
Copy	Copy the selected detection format.
Rename	Activate the currently selected detection format name for editing.
Delete	Delete the currently selected detection format.

Detection formats that are displayed as shaded in the Detection Format list cannot be edited (e.g., all Roche default detection formats).

For the detection format selected in the *Detection Format* list, the *Filter Combination* selection area displays a grid of emission and excitation filter names, with a checkbox for each possible combination. Select checkboxes for the emission–excitation combinations to include them in the currently selected detection format.

Valid (i.e., selectable) filter combinations are those for which emission wavelength minus excitation wavelength is ≥ 40.

Details of the selected emission–excitation filter combinations are displayed in the *Selected Filter Combination List* below the *Filter Combination* selection area:

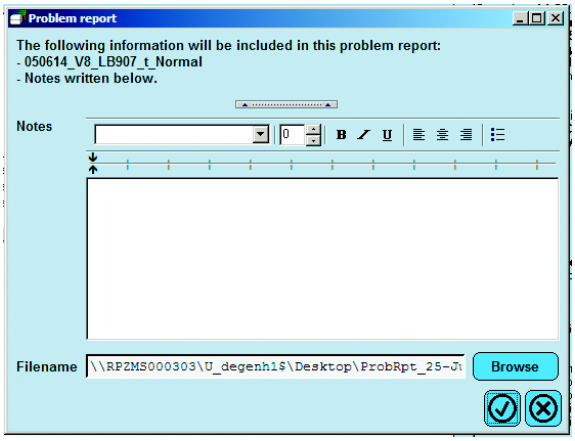
Name	Description
Excitation Filter	Display the excitation filter value
Emission Filter	Display the emission filter value
Name	Enter a name for the filter pair
Melt Factor	Multiplication factor to be applied to the filter pair for melt analysis (when the dynamic integration time mode is selected on the *Customize Detection Formats* dialog).
Quant Factor	Multiplication factor to be applied to the filter pair for quantification analysis. The Quant Factor represents the fold signal stroke from the initial background fluorescence to plateau phase (when the dynamic integration time mode is selected on the *Customize Detection Formats* dialog).
Maximum Integration Time	The maximum integration time that can be used for this pair when the dynamic integration mode is selected in the *Detection Format Definitions* dialog box. (For details, see Section *Running an Experiment*.)

13. Diagnostic Tools

LightCycler® 480 Basic Software includes the *Instrument Problem Report* as a diagnostic tool to monitor and report LightCycler® 480 Instrument performance: In case of an instrument problem you can export an *Instrument Problem Report* (*.ipr) and forward it to your support representative. The *Instrument Problem Report* object contains error log and operation log information as well as the experimental data of the source experiment.

To export an instrument problem report:

1. Select an experiment object in the *Navigator* window.
2. In the *Action* bar of the *Navigator*, click the *Problem Reporting* button.
3. The *Problem report* window opens.



4. Enter additional notes describing the problem into the *Notes* field.
5. In the *Filename* field, a default file destination and name is listed. Click *Browse* if you want to use a different file destination or name.
6. Click the *OK* button to confirm your entries and save the Problem Report as *.ipr file.

14. Installation and Maintenance of LightCycler® 480 Basic Software

The LightCycler® 480 Basic Software consists of the application, a database, and a database object server (called “Exor4”), which communicates with the database. Follow the instructions below to install the LightCycler® 480 Basic Software on your local computer.

During installation of the complete LightCycler® 480 System, LightCycler® 480 Basic Software is usually installed by a Roche service engineer.

Read this chapter to learn more about the following topics:

- Installing LightCycler® 480 Basic Software
- Saving an existing database and installing additional databases
- Removing LightCycler® 480 Basic Software
- Using the FLEXNet License Manager

To be able to run LightCycler® 480 Basic Software you must have a valid software user license file from Roche Applied Science installed. Usually, the license file is generated and installed by a Roche specialist during installation of the complete system. In case a Roche specialist is not available for software installation, please follow the steps below to obtain the license file:

(1) Determine the MAC address (alternatively physical or ethernet address)) of your data workstation:

- *Open the Windows Start menu and select "Run ...".*
- *Type 'cmd" into the Open field. Press "Enter".*
- *The Windows console opens. At the prompt, type the command 'ipconfig /all', then press enter.*
- *The Windows IP Configuration parameters are listed. Find the line beginning with "Physical Address". Write down the number at the end of this line. (It has the following general format: ## - ## - ## - ## - ## - ##-; # being a number or character.)*

(2) Submit the MAC number together with the number appearing on your Software License Certificate (coming together with the software installation CD) to your local Roche office.

(3) Your local Roche office will generate a software license file and send it to you via email.

(4) From your email programm, save the email as plain text (text-only) file to the following location: %programfiles%\Roche\RocheLM\files. Assign a meaningful filename in combination with the file extension .lic. Only files having the extension .lic are recognized by the License Manager software!.

%programfiles% is an environmental variable of Windows XP which stands for the location of the default install directory for applications. The actual name of the install directory depends on the language version of your Windows XP Professional installation. To determine the name of the install directory, type "echo %programfiles%" at the cmd prompt. This will return the path of the install directory (e.g., for a standard installation in combination with an English version of Windows XP this is “C:\Program Files”).

14.1 Initial Start-Up Procedure

In case you want to install the LightCycler® 480 Basic Software for the first time on a validated PC that has been supplied with your LightCycler® 480 Instrument, please follow the procedure described below.

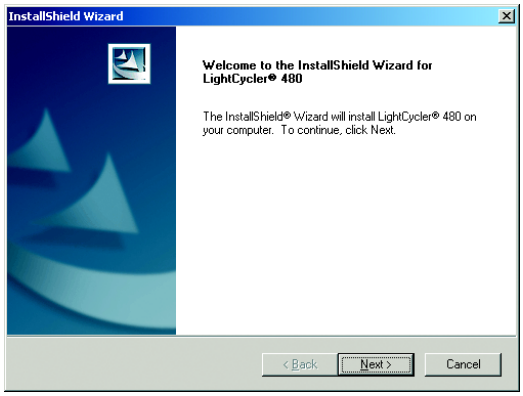
1	Install the LightCycler® 480 Instrument as described in chapter *Installation of the LightCycler® 480 Instrument*.
2	Turn on the PC and wait until the password window appears.
3	Enter login and password: ▶ **Login: lcinstall** ▶ **Password: LCinstall** Click *OK*. *Any unauthorized modification of the operating system entails the invalidity of the guarantee and service contract.*
4	Install the LightCycler® 480 Basic Software as described in chapter *Installing LightCycler® 480 Basic Software* below, then logout.
5	Login on Windows XP as operator: ▶ **Login: operator** ▶ **Password: LC480**
6	Start the LightCycler® 480 Basic Software as described in chapter *Starting the LightCycler® 480 Basic Software*.

14.2 Installing LightCycler® 480 Basic Software

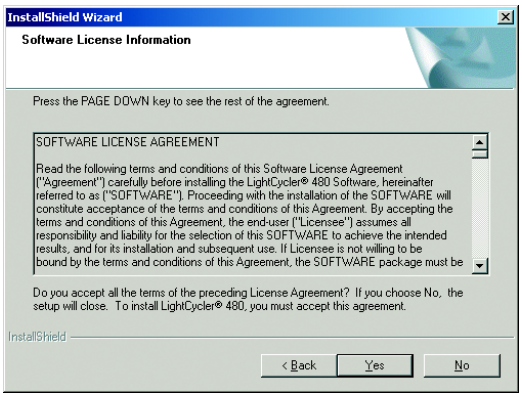
The LightCycler® 480 Instrument is controlled by the LightCycler® 480 Basic Software which is loaded on the data workstation connected to the instrument. The LightCycler® 480 Basic Software operates the LightCycler® 480 Instrument using the information provided with the experiment protocol. Software installation is performed using a self-extracting installation program. To install the software on your local workstation follow the steps below.

To install LightCycler® 480 Basic Software:

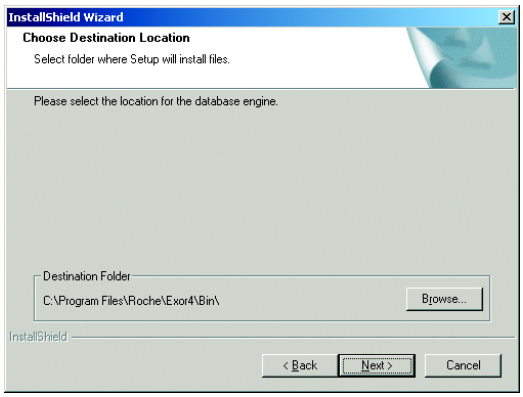
1. Insert the LightCycler® 480 Basic Software CD. If installation does not start automatically, double-click LightCycler480_Software_Setup.exe. The installation process transfers files, extracts the files, and prepares the installation wizard. The *InstallShield* wizard *Welcome* window opens. Click *Next*.



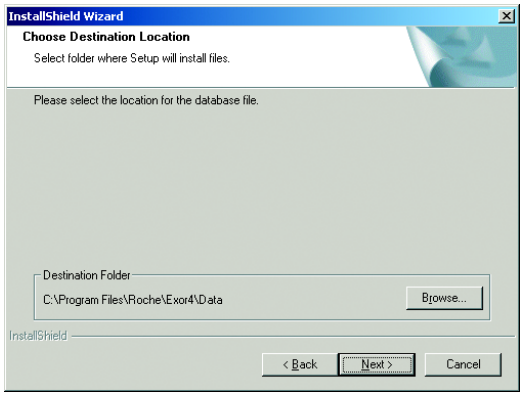
2. You are prompted to agree to the license conditions. Click *Yes*.



3 In the *Database Engine Location* window, use the default settings to install the Exor4 object server or browse to select the location of the database engine. Click *Next*.

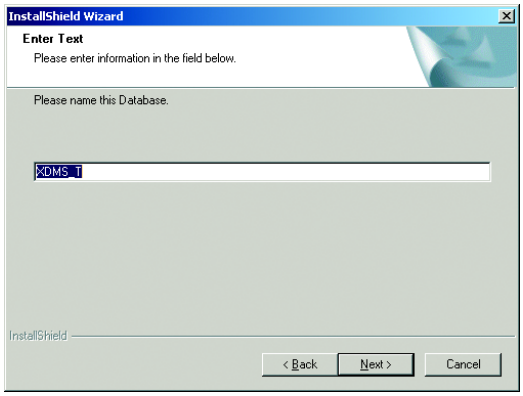


4 In the *Database File Location* window, use the default settings to install the database or browse to select the location of the database file. Click *Next*.

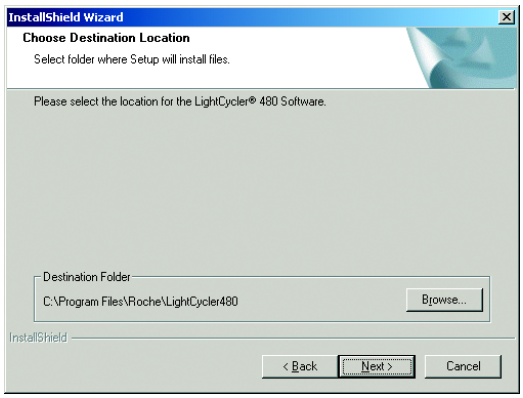


The installation process creates a traceable database with an audit trail. An audit trail is a secure, computer-generated time-stamp which independently records the date, time and names of operator entries and actions that create, modify or delete electronic records. Record changes shall not obscure previously recorded information.

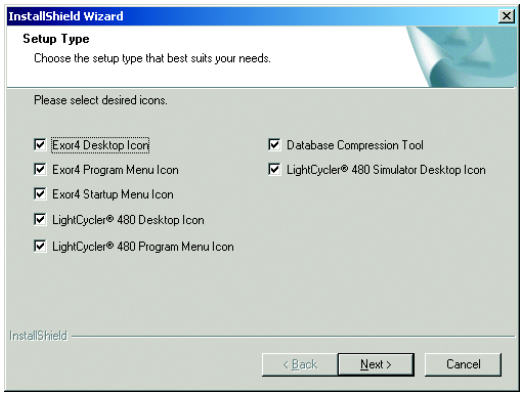
5 The software prompts you to name the new database. Enter a database name or leave the default, and click *Next*.



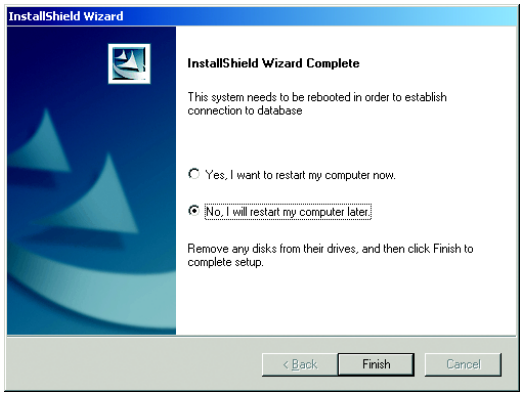
6 Keep the default settings to install the LightCycler® 480 Basic Software or browse to select a location for installation. Click *Next*.



7 Select the location for program icons. These are locations from which the LightCycler® 480 Basic Software can be started. Deselect the icon locations you do not want, and click *Next*.



8 The *Installation Complete* window states that the installation has finished. Restart your computer directly after installation to establish the database connection or delay the restart. Click *Finish*.



14.3 Saving an Existing Database and Installing Additional Databases

Save a copy of your database routinely for backup purposes. Before saving the database, make sure the size of the database is equal to (or less than) 700 MB, the capacity of one CD. To check the size of your database, proceed as follows:

To check the database size:

1	In Windows Explorer, select C:\Program Files\Roche\Exor4\Data (the location of the database corresponds to the setting during installation; it may vary depending on what you entered during installation of the database file. See also Section *Installing LightCycler® 480 Basic Software*).
2	Right-click the database (*.IB) you want to check. From the menu, select *Properties* and read the size from the corresponding menu item.

When you need to compress the database file (e.g., if the database has exceeded the size of 700 MB), you can use the CompactIB tool.

To compress a database file:

1	Shutdown all running database engines by right-clicking the Exor4 icon in the system tray and selecting *Shutdown*.
2	Select the database you want to compress in the Windows Explorer under C:\Program Files\Roche\Exor4\Data (the location of the database corresponds to the setting during installation; it may vary depending on what you entered during installation of the database file. See also Section *Installing LightCycler® 480 Basic Software*).
3	Right-click the database you want to compress. From the menu, select *Properties* and select the *Security* tab. Check the *Allow* box for *Full Control* and *Modify*. Click *OK*.
4	In the *Start* menu, select Programs/Roche/CompactIB.
5	The *Compact Interbase Database File* window opens. In the *Database File to Compact* box, enter the directory of the database or click ... to navigate to a location, and click *Open*.
6	Click *Compact* to start the process.

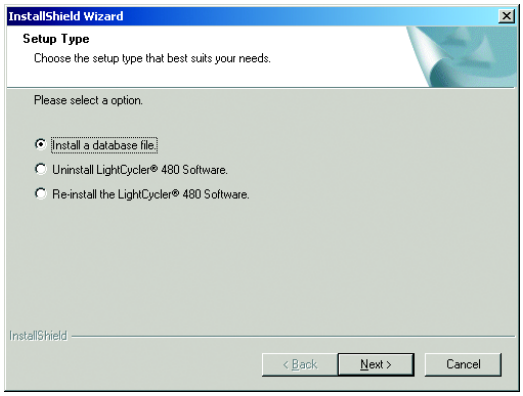
To save a database file:

1	Shutdown the Exor4 by right-clicking the Exor4 icon in the system tray and selecting *Shutdown*.
2	Save the database (*.IB) on a CD by using the CD record software on your PC.

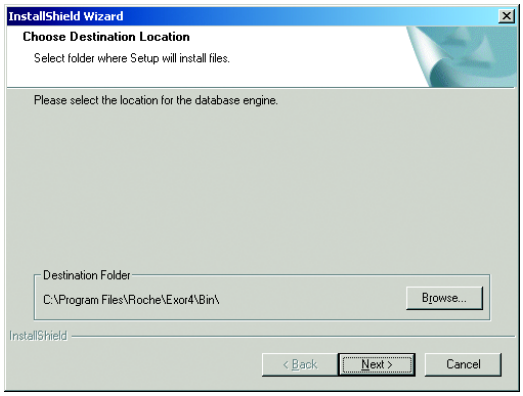
To install additional databases:

If the LightCycler® 480 Basic Software is already installed on your computer, you can use the LightCycler® 480 Basic Software installation utility to install additional databases:

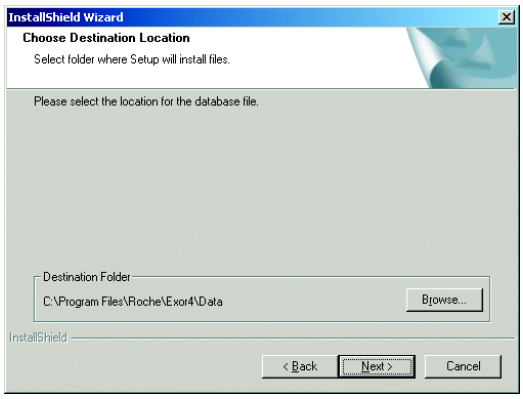
1. Shutdown all running database engines by right-clicking the Exor4 icon in the system tray and selecting *Shutdown*.
2. Insert the LightCycler® 480 Basic Software CD. If installation does not start automatically, double-click LightCycler480_Software_Setup.exe.
3. The *Setup Type* window is displayed. Use the default setting *Install a database file*, and click *Next*.



4. In the *Database Engine Location* window, use the default settings to install the Exor4 object server or browse to select a location of the database engine. Click *Next*.

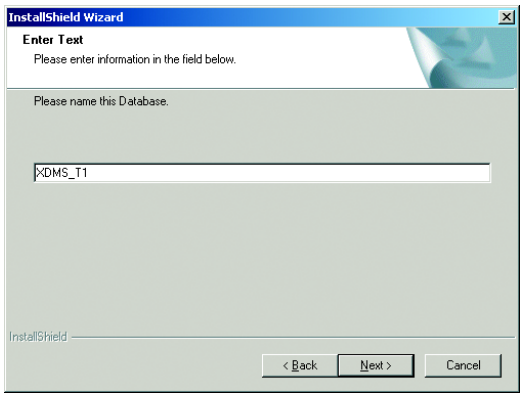


5 In the *Database File Location* window, use the default settings to install the database or browse to select a location for installation. Click *Next*.

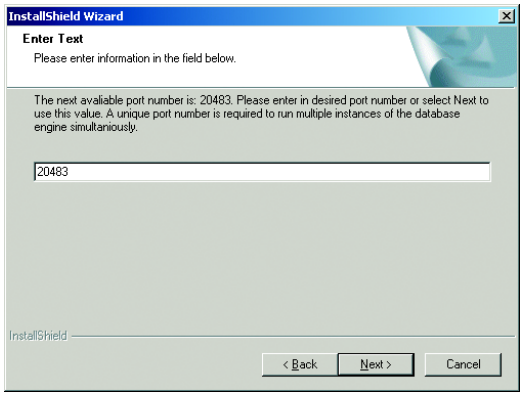


The installation process creates a traceable database with an audit trail. An audit trail is a secure, computer-generated time-stamp which independently records the date, time and names of operator entries and actions that create, modify or delete electronic records. Record changes shall not obscure previously recorded information.

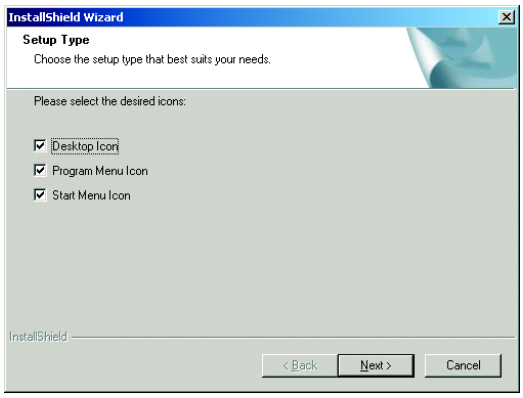
6 The software prompts you to name the new database. Enter a database name, and click *Next*.



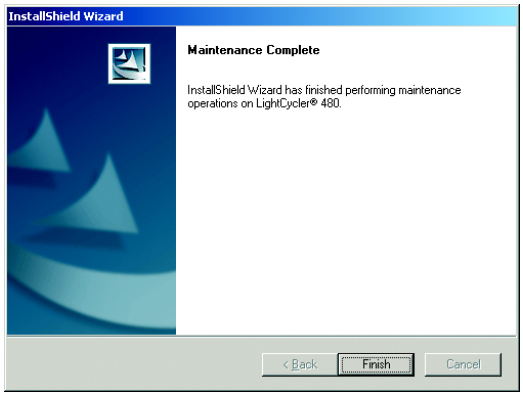
7 The software prompts you to enter a port number for the database. Use the default value or enter a unique port number, and click *Next*.



8 Select the location for program icons. These are locations from which the LightCycler® 480 Basic Software can be started. Deselect the icon locations you do not want, and click *Next*.



9 A message states that the maintenance is complete. Click *Finish*.



The installation process installs another Exor4 icon  *on your desktop. Before you can log onto the new database, you must start the newly installed Exor4 service either by double-clicking the icon on your desktop or by rebooting the system.*

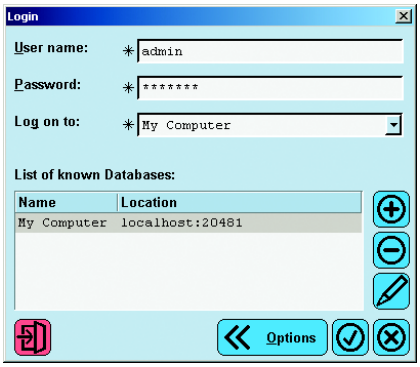
Before defining an experiment using the newly installed database you need to define an instrument. See Section Defining an Instrument for details.

14.4 Logging onto Different Databases

You can log onto an additionally installed database by selecting a previously included database in the *Log on to* pull-down menu.

1. Double-click the LightCycler® 480 Basic Software icon to launch the LightCycler® 480 Basic Software.

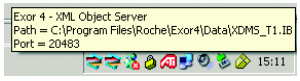
2. A login dialog box opens. Click *Options* to display the list of known object servers.



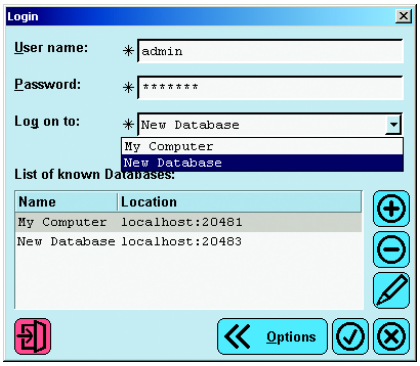
3. Click ⊕. A *Database Properties* window opens.

4. Enter a name for the database and its location. The location is always composed of the word “localhost” and the port number of the database to be integrated, separated by a colon. Click *OK*.

To find the port number for a database, point at the Exor4 icon in the system tray, and read the object server properties, which are displayed as shown below.



5. The database is included in the *List of known Databases* and can be selected in the *Log on to* box. The default password for a newly installed database is LightCycler480.



6. Select a database in the *List of known Databases* and click ⊖ to remove it. Click ✎ to change the name or location of the database.

14.5 Replacing an Existing Database File with a Database File of the Same Name

You can replace an existing database file by a database file of the same name (*e.g.*, after reinstalling LightCycler® 480 Basic Software). No additional Exor4 service is necessary to perform this task.

To replace an existing database file with a database file of the same name:

1	Exit the LightCycler® 480 Basic Software.
2	Shutdown database engine corresponding to the database to be replaced by right-clicking the Exor4 icon in the system tray and selecting *Shutdown*.
3	In Windows Explorer, select the database to be replaced under C:\Program Files\Roche\Exor4\Data (the path corresponds to the default setting during installation; it may vary depending on what you entered during installation of the database file. See also Section *Installing LightCycler® 480 Basic Software*).
4	Right-click the database you want to replace. From the menu, select *Properties* and select the *Security* tab. Check the *Allow* box for *Full Control* and *Modify*. You can now delete the database or store it under a different name.
5	Copy the database file to be restored (*e.g.,* from a CD) into the database directory. *The name of the database must be identical with the name of the deleted database. Rename the database if necessary.*
6	Disable access rights for the database regarding *Full Control* and *Modify*. If you restored the database from a CD, right-click the database file and clear the *Read only* box in the *Properties* menu on the *General* tab.
7	Start the Exor4 service by double-clicking the icon on your desktop.
8	Start the LightCycler® 480 Basic Software.

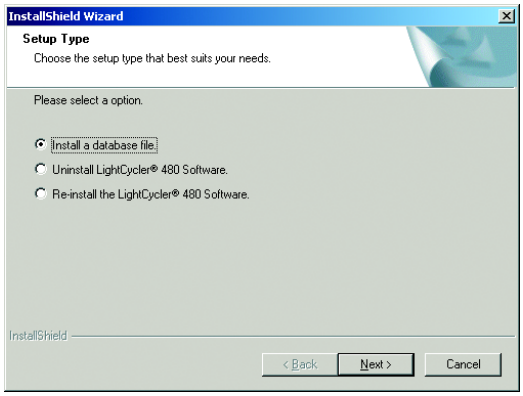
To log onto the restored database you must enter the user name and password for this database.

14.6 Integrate a Restored Database File as an Additional Database in LightCycler® 480 Basic Software

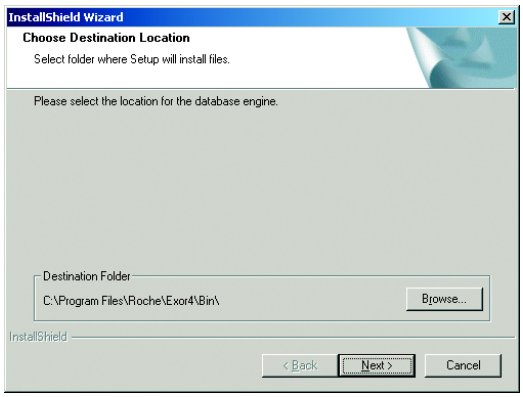
You can integrate an additional database (*e.g.*, a restored database from a CD) in the LightCycler® 480 Basic Software. An additional Exor4 service is necessary to perform this task.

To integrate an additional restored database file in LightCycler® 480 Basic Software:

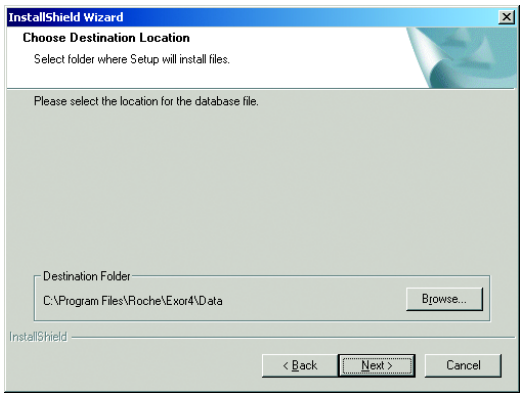
1. Copy the database file (*e.g.,* from a CD) in the database directory. Ensure that database names are unique.
2. Check if the access rights for the database regarding *Full Control* and *Modify* are disabled, and clear the *Read-only* box in the *Properties* menu on the *General* tab, as described above.
3. Shutdown all running database engines by right-clicking the Exor4 icon in the system tray and selecting *Shutdown*.
4. Insert the LightCycler® 480 Basic Software CD. If installation doesn't start automatically, double-click LightCycler480_Software_Setup.exe.
5. The *Setup Type* window is displayed. Use the default setting *Install a database file*, and click *Next*.



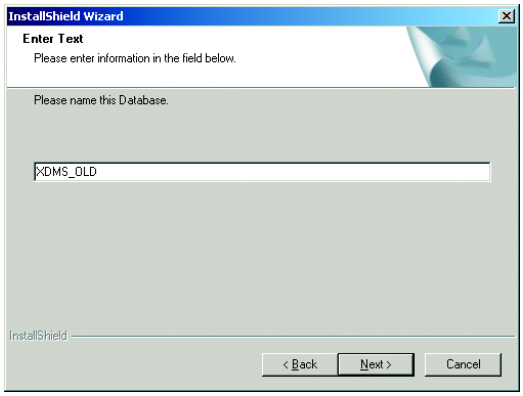
6. In the *Database Engine Location* window, use the default settings to install the Exor4 object server or browse to select a location of the database engine. Click *Next*.



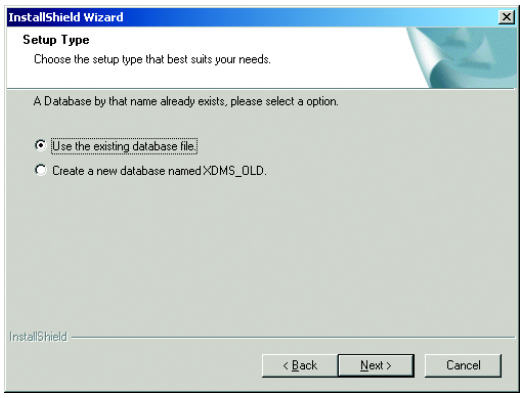
7 In the *Database File Location* window, use the default settings to install the database or browse to select a location. Click *Next*.



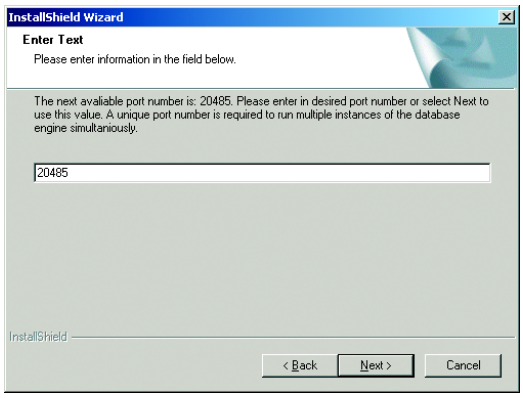
8 The software prompts you to name the database. Enter the name of the database you want to restore, and click *Next*.



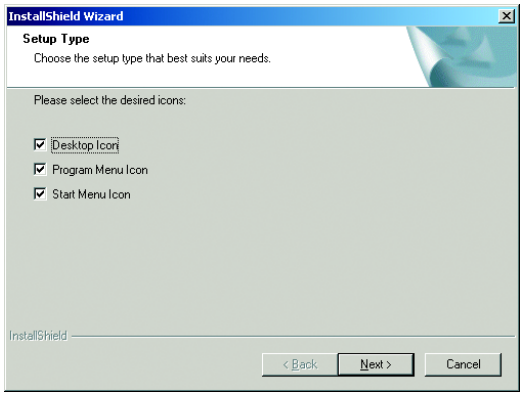
9 Select *Use the existing database file*, and click *Next*.



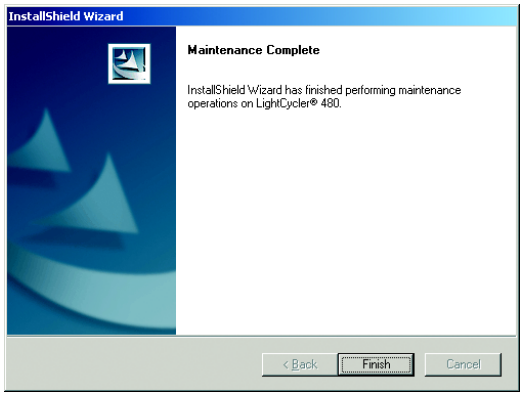
10 The software prompts you to enter a port number for the database. Use the default value or enter a unique port number, and click *Next*.



11 Select the location for program icons. These are locations from which the LightCycler® 480 Basic Software can be started. Deselect the icon locations you do not want, and click *Next*.



12 A message states that the maintenance is complete. Click *Finish*.



13 Start the newly installed Exor4 service by double-clicking the icon on your desktop 

, and start the LightCycler® 480 Basic Software.

14 Integrate the restored database as described in Section *Logging onto Different Databases*.

To log onto the restored database you must enter the user name and password for this database.

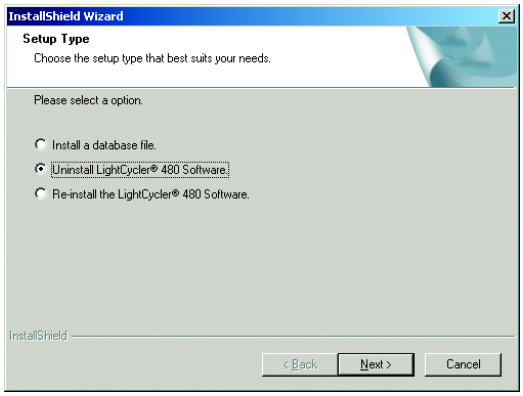
14.7 Removing LightCycler® 480 Basic Software

Follow these steps to uninstall the LightCycler® 480 Basic Software from your local computer.

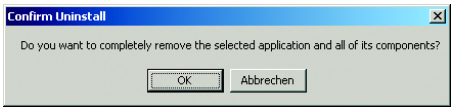
To uninstall LightCycler® 480 Basic Software:

1. Shutdown all running database engines by right-clicking the Exor4 icon in the system tray and selecting *Shutdown*.

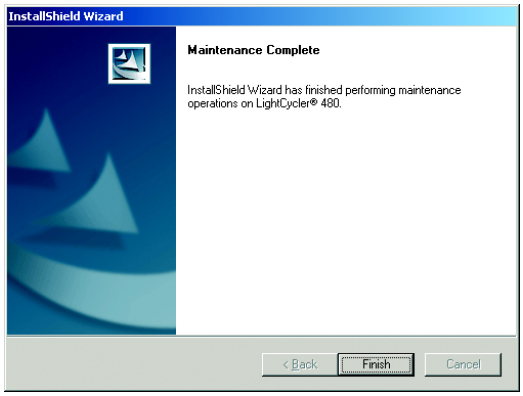
2. Insert the LightCycler® 480 Basic Software CD. If installation doesn't start automatically, double-click LightCycler480_Software_Setup.exe. The *Setup Type* window is displayed. Select *Uninstall LightCycler® 480 Basic Software*.



3. You are prompted to confirm the deletion. Click *OK*.



4. A message states that the maintenance is complete. Click *Finish*.



14.8 The FLEXnet License Manager

FLEXnet is an electronic license manager. You can access the LightCycler® 480 Software only after you have installed FLEXnet and received a valid license file from Roche Diagnostics. Some features of the LightCycler® 480 Software, as for example additional software modules like the LIMS/Bar-Code Module, require a special license and are not part of the standard package.

If you did not receive a license file from Roche Diagnostics for the LightCycler® 480 Basic Software or a software module, please contact your local sales representative.

Depending on your user rights, you might not be able to perform all tasks described in this section. In this case you need to contact your system administrator.

Installation and Setup of the FLEXnet License Manager

The FLEXnet License Manager is automatically installed on the LightCycler® 480 data workstation during installation of the LightCycler® 480 System. The standard installation directory is in the 'RocheLM' subdirectory of the LightCycler® 480 Software installation directory. The standard LightCycler® 480 Software installation directory (in combination with the English version of Windows XP Professional) is 'C:\Program Files\Roche\RocheLM\'.

The following instruction assumes that you have installed the LightCycler® 480 Software in the standard installation directory. If you have installed the LightCycler® 480 Software in a different directory or in combination with a non-English language version of Windows XP Professional, remember to always refer to your installation directory instead.

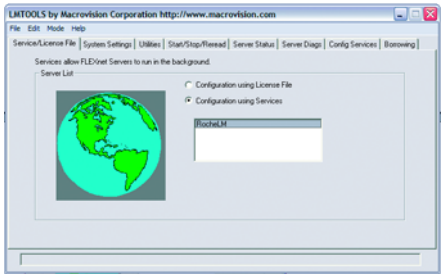
The actual name of the default software install directory of Windows XP Professional depends on the language version of your Windows XP Professional installation. To determine the name of the install directory, type 'echo %programfiles%' at the cmd prompt. This will return the path of the install directory.

Checking for correct setup of FLEXnet License Manager

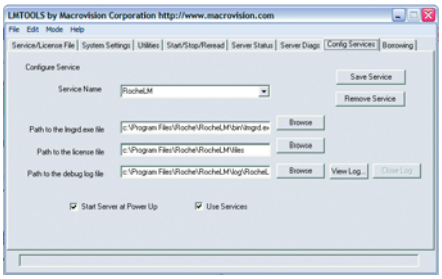
1. In the FLEXnet installation directory (for standard installation: 'C:\Program Files\Roche\RocheLM\bin'), check if the following files are listed:
   - lmgrd.exe
   - lmtools.exe
   - lmutil.exe
   - RASFLEXL.exe

   If one or more of these files is missing, the FLEXnet license manager is not correctly installed.

2. In the FLEXnet installation directory (for standard installation: 'C:\Program Files\Roche\RocheLM\bin'), double-click on LMTOOLS.EXE. This will open the LMTOOLS application to setup and check the License Manager.



3 Click on the 'Config Services' Tab.



If the FLEXnet License Manager has been correctly installed during set-up of the LightCycler® 480 System, the information shown in the screen above will be displayed:
Service Name: 'RocheLM'
Path to the lmgrd.exe file: 'C:\Program Files\Roche\RocheLM\bin\lmgrd.exe'
Path to the license file: 'C:\Program Files\Roche\RocheLM\files'
Path to the debug log file: 'C:\Program Files\Roche\RocheLM\log\RocheLM.log'

If the FLEXnet License Manager has been installed correctly, the checkboxes 'Start Server at Power Up' and 'Use Services' are checked as shown in the screenshot above.

4 Use Windows Explorer to check if the license file you received from Roche Diagnostics is located in the directory Path to the license file: 'C:\Program Files\Roche\RocheLM\files'.) The name of the license file should be 'Roche_license_yyyymmdd.lic' with yyyymmdd representing the date of license generation, *e.g.*, 'Roche_license_20050818.lic'.

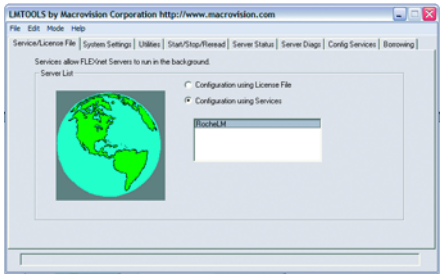
14

D

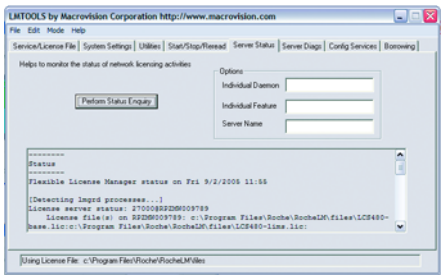
Checking for available licenses in the FLEXnet License Manager

Before checking for available licenses, make sure the FLEXnet License Manager is setup correctly.

1 In the FLEXnet installation directory (for standard installation: 'C:\Program Files \Roche\RocheLM\'), double-click on LMTOOLS.EXE. This will open the LMTOOLS application to setup and check the License Manager



2 Click on the 'Server Status' Tab. In the displayed screen, click on the 'Perform Status Enquiry' button..



The LMTOOLS application will display the 'Status' information of your FLEXnet License Manager and the available licenses for the LightCycler® 480 Software. The text box at the bottom of the window displays the path to the license files. All your licenses that reside in this directory are detected and used by the FLEXnet License Manager. Usually you will have one license file only. You will have more than one license file if you have licensed additional features of the LightCycler® 480 Software, as *e.g.* the LIMS/Bar-Code Module.

The 'Status' window in the middle of the screen displays a message in the following format:

```
--------
Status
--------
Flexible License Manager status on Thu 8/18/2005 23:12

[Detecting lmgrd processes...]
License server status: 27000@rpzmw010317
License file(s) on rpzmw010317: c:\Program Files\Roche\RocheLM\files\
Roche_license_20050818.lic:

rpzmw010317: license server UP (MASTER) v10.0

Vendor daemon status (on rpzmw010317):

RASFLEXL: UP v10.0

Feature usage info:

Users of RUN: (Total of 1 license issued; Total of 0 licenses in use)

Users of TEMPLATE: (Total of 1 license issued; Total of 0 licenses in
use)

Users of REPORT: (Total of 1 license issued; Total of 0 licenses in use)

Users of ABS-QUANT: (Total of 1 license issued; Total of 0 licenses in
use)

Users of TM-CALLING: (Total of 1 license issued; Total of 0 licenses in
use)

Users of COLOR-COMP: (Total of 1 license issued; Total of 0 licenses in
use)
```

On top of the status information window information the computer name is shown, followed by the license file used. After the 'RASFLEXL: UP v10.0' line detailed information about the licenses available on your computer is shown. In the example above, there is one issued license for each RUN, TEMPLATE, REPORT, ABS-QUANT, TM-CALLING and COLOR-COMP module available. All of these 6 licenses are part of the LightCycler® 480 Basic Software and are required to run the software.

In the above example none of the licenses is currently in use ('Total of 0 licenses in use'). You should now start the LightCycler® 480 Software and again press the 'Perform Status Inquiry' button in the LMTOOLS application. Now the displayed message should change to 'Total of 1 licenses in use'.

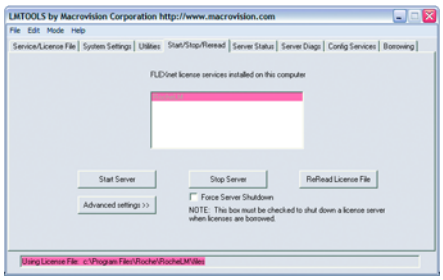
Troubleshooting information for the FLEXnet License Manager

In case you start the LightCycler® 480 Software and receive an error message that no license is available, you need to check the following:.

1. Perform a status inquiry of the FLEXnet License Manager. If no available licenses are displayed but instead the following text is displayed, the FLEXnet License Manager is currently not running.

```
lmgrd is not running: Cannot connect to license server.
The server (lmgrd) has not been started yet, or
the wrong port@host or license file is being used, or the
port or hostname in the license file has been changed.
```

Go to the 'Start/Stop/Reread' Tab and press the 'Start Server' button.



If the FLEXnet License Manager can be started correctly, the text box at the bottom of the window will show the message 'Server Start Successful'. Returning to the 'Server Status' button and pressing 'Perform Status Enquiry' should no display the available licenses.

2. Perform a status enquiry of the FLEXnet License Manager. If no available licenses are displayed but instead the following text is displayed, then the FLEXnet License Manager was not able to find your license file.

```
Error getting status: Cannot find license file.
The license files (or server network addresses) attempted are
listed below.  Use LM_LICENSE_FILE to use a different license file,or
contact your software provider for a license file.
```

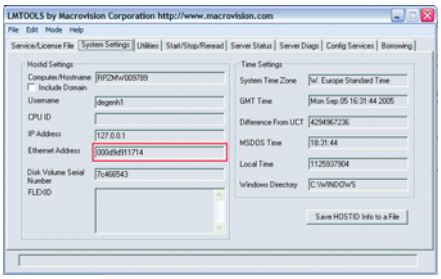
Check for correct setup of the FLEXnet License Manager. If you have located your license file and moved it to the correct directory, go to the 'Start/Stop/Reread' Tab and press the 'ReRead License File' button. Return to the 'Server Status' button, press the 'Perform Status Enquiry' button and check if available licenses are found now.

3. Perform a status enquiry of the FLEXnet License Manager. If no available licenses are displayed but instead the following text is displayed, then the FLEXnet License Manager found a license but which did not match your computer ID.

```
Vendor daemon status (on rpzmw010317):

RASFLEXL: The desired vendor daemon is down.
1) Check the lmgrd log file, or 2) Try lmreread
Feature:       RUN
Vendor:Host:   rpzmw009789
License path:  27000@rpzmw010317
FLEXnet Licensing error:-97,121
```

Note that your license file contains information about your computer. The license needs to be specifically produced for your computer, matching your Ethernet-address (also referred to as MAC-address).
Press the 'System Settings' Tab. The LMTOOLS application will display information about your computer. .



Additionally, open your license file ('Roche_license_yyyymmdd.lic') using a texteditor (*e.g.*, Microsoft Notepad or WordPad):

```
The following license file enables a perpetual license of the LightCycler®
480 Basic Software on the computer with the host id mentioned in the license
file.
The license agreement for this software license can be found in the manual.
The LightCycler® 480 Basic Software license comprises the following modules:
Run, Template, Report, Absolute Quantification, Tm-Calling, Color Compensa-
tion and Macro.
SERVER RPZMW009789 000d9d911714
VENDOR RASFLEXL
USE_SERVER
INCREMENT RUN RASFLEXL 1.0 31-dec-2005 1 HOSTID=000d9d911714 \
   DUP_GROUP=H NOTICE='Licensed to RAS Computer *' SIGN='006F \
   BEAF 4520 1704 6067 367A 4F4B 3B00 8850 D0D4 E685 A247 8AA1 \
   156D 55CF'
INCREMENT TEMPLATE RASFLEXL 1.0 31-dec-2005 1 HOSTID=000d9d91714 \
   DUP_GROUP=H NOTICE='Licensed to RAS Computer *' SIGN='008B \
   16C6 4749 D782 71F0 2027 1557 4000 61A7 CCA6 DC47 792E 4CB7 \
   E8BA D1A8'
INCREMENT REPORT RASFLEXL 1.0 31-dec-2005 1 HOSTID=000d9d911714 \
   DUP_GROUP=H NOTICE='Licensed to RAS Computer *' SIGN='002E \
   B2F5 36D7 8DBC B3B7 FCF8 9D3D 3600 534D 4ED1 03B2 6658 2370 \
   4D3F F529'
INCREMENT ABS-QUANT RASFLEXL 1.0 31-dec-2005 1 HOSTID=000d9d911714 \
   DUP_GROUP=H NOTICE='Licensed to RAS Computer *' SIGN='00B4 \
   A496 C94C 3ADC EFB7 FD27 4C13 9700 13C3 A172 550C 67FE 6499 \
   B216 8BAD'
INCREMENT TM-CALLING RASFLEXL 1.0 31-dec-2005 1 HOSTID=000d9d911714 \
   DUP_GROUP=H NOTICE='Licensed to RAS Computer *' SIGN='0083 \
   2AB9 207F 76E1 5147 B522 C1B0 3B00 A33D E8C3 E8C9 211A 0279 \
   DE98 5D2F'
INCREMENT COLOR-COMP RASFLEXL 1.0 31-dec-2005 1 HOSTID=000d9d911714 \
   DUP_GROUP=H NOTICE='Licensed to RAS Computer *' SIGN='00A8 \
   4723 BC4B 8504 6EE4 8D74 21E2 FC00 EF85 A2F8 1A6F 6BB0 4412 \
   BD7D CB26'
INCREMENT MACRO RASFLEXL 1.0 31-dec-2005 1 HOSTID=000d9d911714 \
   DUP_GROUP=H NOTICE='Licensed to RAS Computer *' SIGN='0048 \
   2A13 5252 E807 44B9 6623 9E96 A000 35C0 A0C4 708C E5BE D6B6 \
   D703 F7C3'
```

Now compare the 'Ethernet Address' displayed in the LMTOOLS window with the 12 digit code at the end of the first line in your license file.

```
SERVER rpzmw010317 001279bfbfa4
```

If these two codes do not match, you do not have the correct license file for your computer installed.

In case no or a dummy Ethernet address is displayed (*e.g.*, 'ffffffffff'), there is a problem with your Network card. In this case you need to contact your system administrator.

Maintenance

E

Chapter E • Maintenance
Description of the maintenance procedures required for the LightCycler® 480 Instrument

Maintenance

1. General Maintenance

The LightCycler® 480 Instrument is maintenance-free.

2. Cleaning Instructions

Never clean the LightCycler® 480 Instrument without turning the instrument power switch off and disconnecting the power cable.

Do not poor fluids into the thermal block cycler, the compartment of the block cycler unit, or the interior of the instrument.

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 spreading contamination to laboratory personnel or equipment. Handling and disposal of infectious material should be performed according to local safety guidelines.

2.1 General Cleaning

Clean the housing of the LightCycler® 480 Instrument, the thermal block cycler, and the block cycler cover with a mild commercial detergent. If necessary, use 70% ethanol for disinfecting the instrument housing, the thermal block cycler, and the block cycler cover.

2.2 Preventive Maintenance

The area around the LightCycler® 480 Instrument should be checked regularly, to ensure that the air flow is unrestricted and that books, papers, or other supplies are not interfering with the air flow. For detailed requirements, see Section *Installation*.

3. Exchanging the Xenon Lamp

Sufficient intensity of the Xenon excitation lamp is a requirement for optimal detection of fluorescent signals during real-time PCR and melting curve analysis. Therefore, the LightCycler® 480 Instrument automatically and continually measures the intensity of the Xenon lamp. If the lamp intensity falls below 50% of its initial intensity, you are automatically warned by the LightCycler® 480 Basic Software that the Xenon lamp must be exchanged. The Xenon lamp is available as an exchange spare part directly from Roche or can be bought by retail. Please contact your local Roche representative for details.

Use only the following Xenon lamp type: XBO R 100W/45 OFR 2×1 (OSRAM)

Never exchange the Xenon lamp without turning the instrument power switch off and disconnecting the power cable. Non-compliance poses the danger of electric shock and damage to the eyes by the bright light of the lamp.

Before exchanging the Xenon lamp, make sure you have waited an appropriate period of time (approximately 20 minutes) after a LightCycler® 480 instrument run to allow it to cool. Directly after completion of a run, the lamp is hot enough to cause an immediate burn.

In its cold state the lamp has a high internal pressure (as much as 20 bar). During operation the internal pressure is around three times higher than in the cold state. The lamp is extremely unlikely to explode but the possibility cannot be entirely ruled out. Therefore, when handling the Xenon lamp always use the protective jacket or cap supplied. When installing the lamp, remove the protective jacket or cap and always take the following precautions: wear goggles and gloves and protect your neck (e.g., with a thick scarf). Take the same precautions when removing the lamp.

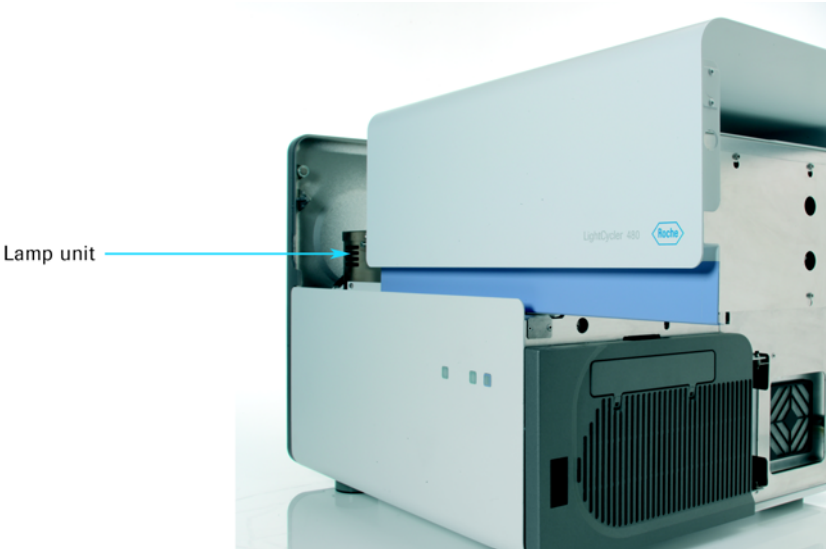
Do not get finger marks, grease, paint or the like on the bulb. Before using the lamp, remove any such marks with isopropanol or ethanol or any other suitable agent that leaves no residues on the bulb.

The Xenon lamp does not contain any materials which are harmful to the environment so they are not subject to special waste disposal regulations. Prior to disposal, the old lamp should be stored in their protective jacket or cap where it cannot be easily accessed. Where possible, the lamp should be disposed off by a specialist waste management company. If this is not possible, put on protective clothing, wrap the lamp completely in leather or thick cloth, smash the lamp, including the discharge tube, with a suitable implement and dispose off the pieces.

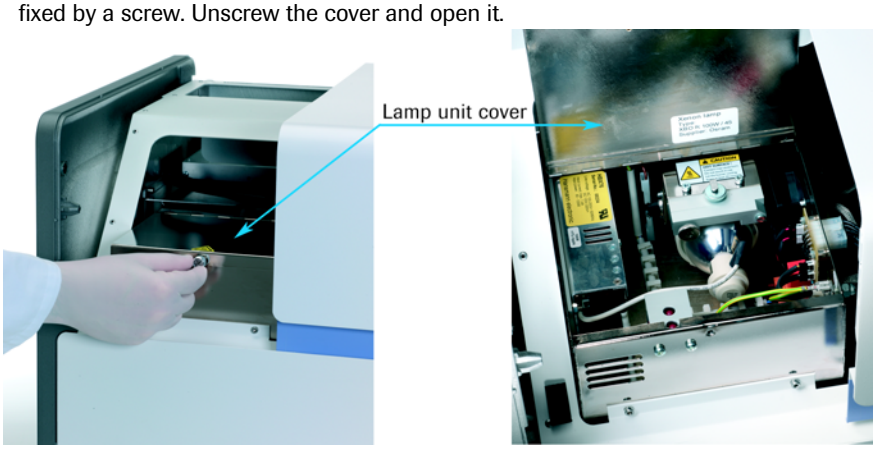
The table below describes how to exchange the Xenon lamp.

1 Remove the right instrument panel.

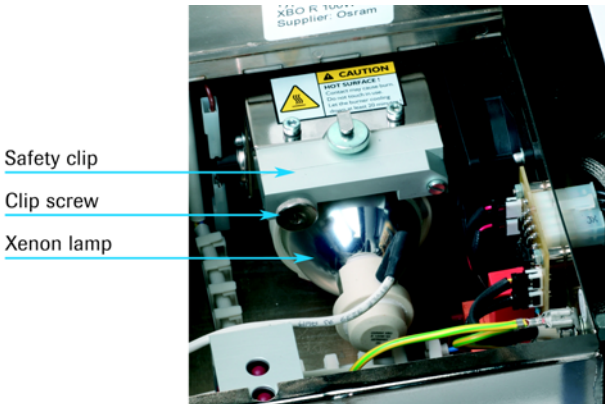
2 Now you can easily move the instrument cover to the right, giving access to the lamp unit.

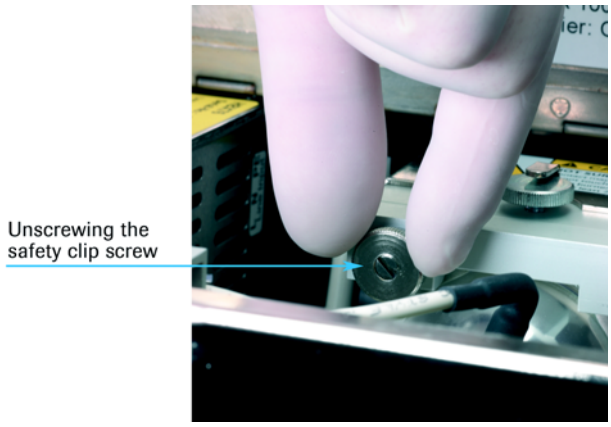


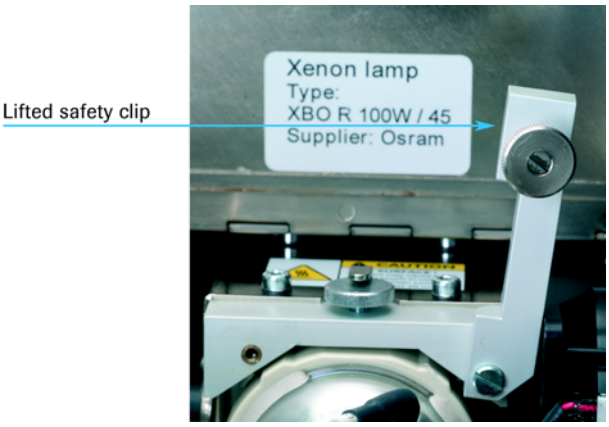
3 To access the Xenon lamp, you have to remove the lamp unit cover first. The cover is fixed by a screw. Unscrew the cover and open it.



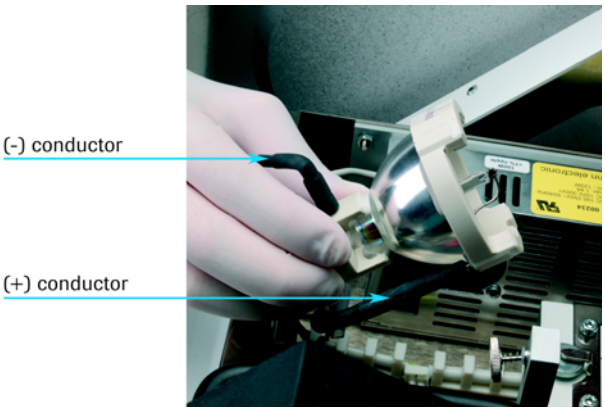
4 The Xenon lamp is fixed by a clip which itself is secured by a screw. Unscrew the clip and open it.



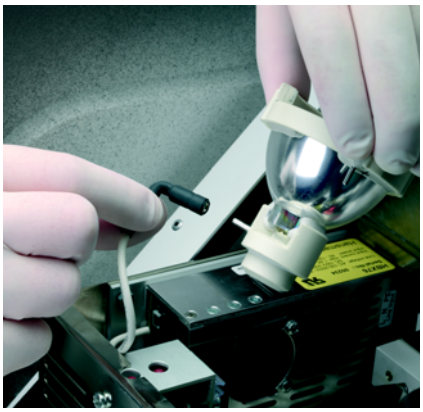




5 Lift the Xenon lamp out of its clamp and remove the two conductors.



6 Install the new Xenon lamp. First attach the (–) conductor, and then the (+) conductor.



7 Complete installing the new Xenon lamp by putting it back into its clamp. Make sure the (–) conductor points upwardly. Follow Steps 4 to 1 above exactly in reversed order.

8 *After you exchanged the Xenon lamp, make sure to reset the lamp counter in the Instruments window of the Tools dialog of the LightCycler® 480 Basic Software (for details, see Section Administrative Tools.) The instrument will read and save the intensity of the lamp as the starting intensity value. During operation, the instrument compares this saved value to the actual lamp intensity to determine the loss of lamp intensity. When the lamp intensity reaches 50% of its starting intensity, you will be informed and prompted to exchange the lamp.*

4. Exchanging the Ventilation Dust Filters

The electronic rack of the LightCycler® 480 Instrument is cooled by ventilation. Two ventilation inlets are located in the lower right corner of the right side of the instrument (right beside the block cycler compartment) and in the back of the instrument. To avoid any contamination of the instrument interior by dust particles, these ventilation inlets carry dust filters.

The dust filters should be exchanged regularly every year.

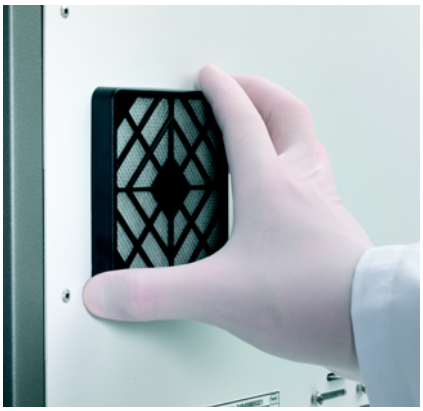
Four exchange dust filters are part of the LightCycler® 480 System package.

The table below describes how to exchange the ventilation dust filters.

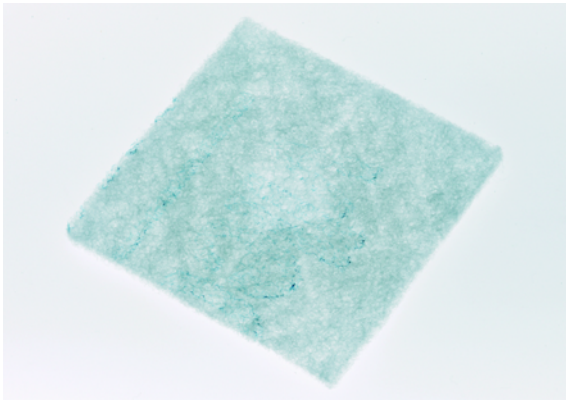
1 Remove the right instrument panel to access the lateral ventilation inlet.

2 Remove the ventilation dust filter carrier.

3 In the same way, remove the dust filter carrier from the rear ventilation inlet.



4 Remove the used dust filters from each carrier and insert new filters.



5 Replace each dust filter carrier on the corresponding ventilation inlet. Replace the right instrument panel.

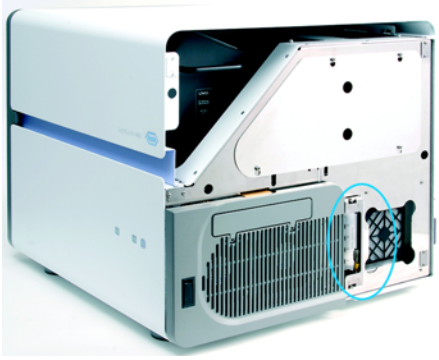
5. Exchanging Fuses

The LightCycler® 480 Instrument contains eight fuse types. Fuses must be exchanged by the user when they are blown. The LightCycler® 480 System package includes 10 replacement fuses for each type.

The following table provides an overview over the types and location of fuses used by the LightCycler® 480 Instrument:

Type	Location	Labeling	Amperage Voltage	Consumer Load
Primary fuses				
High-breaking capacity (T 10A H / 250V)	Left side of power box (when viewed from the instrument front side), instrument exterior 	FUSES LINE INPUT 2 × T10A H / 250V	2 × T10A / 250V	Line input instrument

Type	Location	Labeling	Amperage Voltage	Consumer Load
Secondary fuses				
High- or low-breaking capacity	Right side of power box, instrument interior *To access secondary fuses F1 – F5, remove the right instrument panel.*	F1	T3.15 A / 250V	Detection unit
		F2	T8 A / 250V	Block cycler unit
		F3, F4, F5	T16A / 250V	Thermal block cycler – Peltier elements

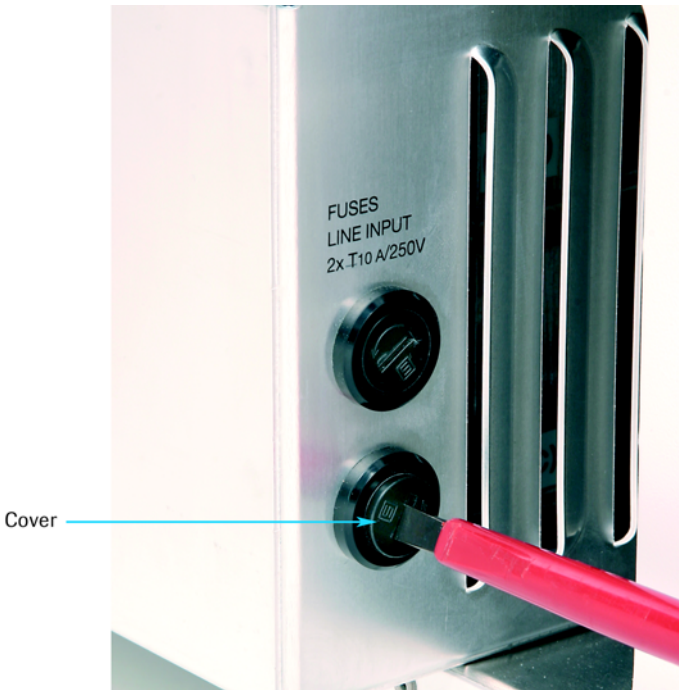


Each of the secondary fuses F1 – F5 has a LED, which indicates its intactness. If a fuse is blown, the corresponding LED will be off.

Type	Location	Labeling	Amperage Voltage	Consumer Load
Xenon lamp fuse				
	Instrument interior, right side of Xenon lamp module, above the lamp's mains socket.  *To access the Xenon lamp fuse, remove the instrument cover.*		2 × T1.6A / 250V	Xenon lamp

To exchange a primary or secondary fuse:

1. Using a screwdriver, unscrew the cover of the fuse chamber:

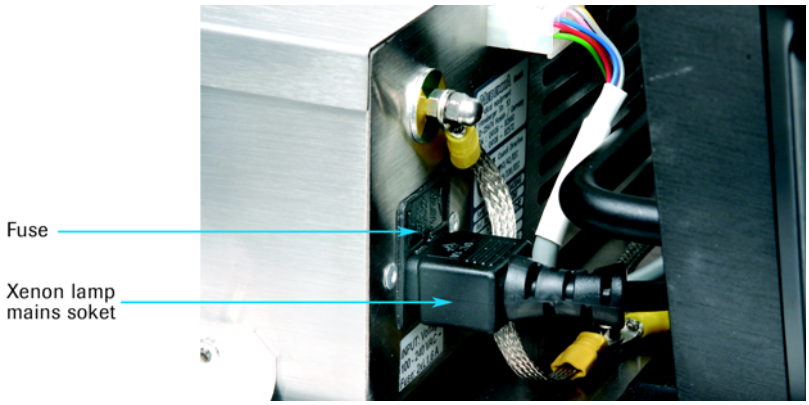


2. Remove the cover together with the fuse attached from the chamber.

3. Exchange the blown fuse with a replacement fuse and place it back in the chamber.
4. Screw up the cover again.

To exchange the Xenon lamp fuse:

1. Remove the instrument cover.
2. The Xenon lamp fuse is located above the mains socket of the Xenon lamp module.

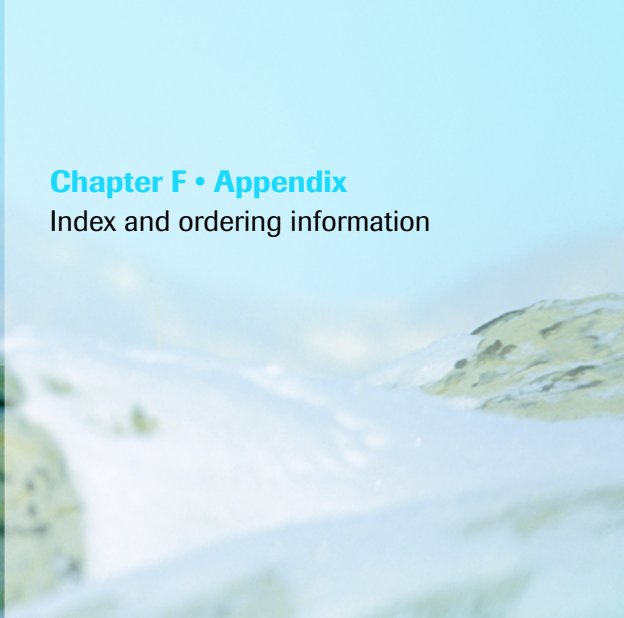


3. Using forceps, press the clamps of the left and right side of the fuse holder inward. Pull the fuse holder out of its chamber.

4. Exchange the blown fuse with a replacement fuse and place the fuse holder back in the chamber.
5. Re-assemble and close the instrument cover.

Appendix

F



Chapter F • Appendix
Index and ordering information

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Appendix

1. Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, http://www.roche-applied-science.com, and visit our special interest site for

- the LightCycler® 480 System:
  http://www.roche-applied-science.com/lightcycler480
- the MagNA Pure System family for automated nucleic acid isolation:
  http://www.maganapure.com
- DNA & RNA preparation – Versatile Tools for Nucleic Acid Purification:
  http://www.roche-applied-science.com/napure

Instruments		
LightCycler® 480 Instrument, 96-well	1 instrument with data workstation and accessories	04 640 268 001
LightCycler® 480 Instrument, 384-well	1 instrument with data workstation and accessories	04 545 885 001
Software		
LightCycler® 480 Basic Software 1.0	1 software package	04 722 205 001
LightCycler® 480 LIMS/Bar-Code Module	1 software package	04 727 886 001
LightCycler® 480 Genotyping Software	1 software package	04 727 860 001
LightCycler® 480 Relative Quantification Software	1 software package	04 727 851 001
Accessories		
LightCycler® 480 Block Kit 96	96-well thermal block cycler unit, including block cycler cover, storage box and loading device	04 643 640 001
LightCycler® 480 Block Kit 384	384-well thermal block cycler unit, including block cycler cover, storage box and loading device	04 643 631 001
LightCycler® 480 Bar-Code Scanner	1 bar-code scanner	04 710 606 001

Disposables		
LightCycler® 480 Multiwell Plate 96	50 plates with 50 sealing foils	04 729 692 001
LightCycler® 480 Multiwell Plate 384	50 plates with 50 sealing foils	04 729 749 001
LightCycler® 480 Sealing Foil	5 × 10 foils	04 729 757 001
PCR Reagents		
LightCycler® 480 SYBR Green I Master	1 kit (5 × 100 reactions, 20 µl each)	04 707 516 001
LightCycler® 480 Probes Master	1 kit (5 × 100 reactions, 20 µl each)	04 707 494 001
LightCycler® 480 Genotyping Master	1 kit (4 × 96 reactions, 20 µl each)	04 707 524 001
LightCycler® 480 Control Kit	3 control experiments	04 710 924 001
Labeling Reagents		
SimpleProbe 519 Labeling Reagent	100 µmol	04 687 132 001
LightCycler® Fluorescein CPG	1 g 5 columns	03 138 178 001 03 113 906 001
LightCycler® Red 640-N-hydroxysuccinimide ester	1 vial	12 015 161 001
LightCycler® Red 610-N-hydroxysuccinimide ester	1 vial	03 561 488 001

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