Roche Applied Science

LightCycler® 2.0 Instrument Operator's Manual

Software Version 4.05

Manual B: for general laboratory use*

* Please note: For *in vitro* diagnostic applications in combination with Roche diagnostic reagent kits use Manual A

Preface

I. Revision History

Version	Revision Date
1.1	October 2003
2.0	June 2005

II. Contact Addresses

	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 Corporation 9115 Hague Road PO Box 50457 Indianapolis, IN 46250 USA

III. Declaration of Conformity

The LightCycler® 2.0 Instrument meets the requirements of the European Directive for *In vitro* diagnostic medical devices 98/79/EC.
Compliance is demonstrated by the following mark:

CE

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.

V. Trademarks

LIGHTCYCLER and MAGNA PURE are trademarks of Roche.

VI. Intended Use

The LightCycler® 2.0 Instrument is intended for performing rapid PCR (Polymerase Chain Reaction) with real-time detection and/or quantification of a target NA (nucleic acid), as well as post-PCR analysis of the amplified NA by melting curve analysis.
Beside its universal application in fields like life science research, food analysis and forensics, the instrument can - in countries where approved for this purpose - also be used in specific diagnostic applications in combinations with LightCycler® reagent kits manufactured by Roche and labeled for diagnostic purposes (according to the workflow described in the package insert of the respective LightCycler® reagent kit).

Please note: The Roche LightCycler® reagent kits for *in vitro* diagnostic applications requiring this instrument are not available in all countries. Any diagnostic use of the LightCycler® 2.0 Instrument in combination with LightCycler® reagents (other than those labeled for diagnostic purposes, manufactured by Roche and recommending the LightCycler® 2.0 Instrument in their instructions of use) is in the sole responsibility of the user and has to be validated by the user, taking into account all relevant national legislation.
Customers using Roche *in vitro* diagnostic LightCycler® kits have to use "Manual A, marked *for in vitro diagnostic use*" (not available in all countries; id. no. 04 624 491 001) while "Manual B, marked *for general laboratory use*" (id. no. 04 624 505 001) has to be used for all other laboratory applications.

The LightCycler® 2.0 Instrument must be used exclusively by laboratory professionals trained in laboratory techniques and having studied the instructions for use of this instrument.

VII. License Statements for the LightCycler® 2.0 Instrument

The LightCycler® 2.0 Instrument is designed for in vitro diagnostic ("IVD") applications. LightCycler® reagent kits manufactured by Roche and labeled for In Vitro Diagnostic Use will provide the user with a license to perform PCR-based IVD testing. For IVD testing performed without an IVD kit from Roche, a separate diagnostic service license is available. For information on acquiring a diagnostic service license, please contact either the PCR Licensing Manager, Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501 USA; Tel: 510-814-2984; Fax: 510-814-2977, or PCR Licensing Manager, Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim, Germany; Tel: +49 (621) 759 28 36; Fax: +49 (621) 759 27 05.

The LightCycler® 2.0 Instrument can also be used in life science research, food analysis, forensics and other laboratory disciplines, where PCR applications and melting curve analysis is required. A license to perform PCR for life science research is obtained as described below. For information on licenses to perform PCR for food analysis, forensics, etc., please contact either the PCR Licensing Managers listed above, or the Director of Licensing at Applied Biosystems, as listed below.

The LightCycler® 2.0 Instrument is an Authorized Thermal Cycler. Its purchase price includes the up-front fee component of a license under the non-U.S. counterparts of United States Patents Nos. 4,683,195, 4,683,202 and 4,965,188 owned by F. Hoffmann-La Roche Ltd, 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. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

No rights are conveyed expressly, by implication or estoppel to any patents on real-time methods, including but not limited to 5' nuclease assays, or to any patent claiming a reagent or kit.

Applied Biosystems does not guarantee the performance of this instrument.

VIII. Software License Agreement

Read this Software License Agreement ("Agreement") before removing the program CD (hereinafter referred to as program media) or product documentation from its protective cover. Removing the program media or product documentation from the protective cover will constitute acceptance of the terms and conditions of this Agreement. By accepting the terms and conditions of this Agreement, the end-user ("Licensee") assumes all responsibility and liability for the selection of this software program, hereinafter referred to as "Software", to achieve the intended results, and for its installation and subsequent use. If Licensee is not willing to be bound by the terms and conditions of this Agreement, the unopened package must be promptly returned to Roche Diagnostics GmbH ("RDG") with a copy of the receipt. The license fee will be refunded upon receipt by RDG of the unopened package.

Program License Agreement

You assume all responsibility and liability for the selection of this software program, hereinafter referred to as **Product,** to achieve your intended results, and for its installation and subsequent use.

Grant of Software License

RDG grants to Licensee a non-exclusive, single-use license to use the Software upon the terms and conditions contained in this Agreement. Licensee may:

1. Use the Software on a single workstation owned, leased or otherwise controlled by Licensee.
2. Make one (1) copy of the Software solely for backup purposes in support of Licensee's use of the Software on a single workstation.
3. Transfer the Software product and assign the rights under this Agreement to another party, provided that the other party agrees in writing to accept the terms and conditions of this Agreement. If the Software is transferred, and the rights under this Agreement are so assigned, Licensee must, at the same time, either transfer Licensee's copy, if any, of the Software to the same party, or destroy any such copy not transferred. In addition, Licensee must ensure that the copyright notice is maintained on the Software transferred and on any copy concurrently transferred.

Licensee may not:

1. Use or copy the Software, in whole or in part, except as expressly provided in this Agreement.
2. Use the Software on more than one workstation at a time.
3. Copy, sell, or otherwise transfer the Software or assign its rights under this Agreement, in whole or in part, to another party, except as specifically set forth in this Agreement.
4. Rent, distribute, license or sublicense the Software.
5. Create derivative works based on Software.
6. Modify, adapt, translate, reverse engineer, decompile or disassemble the Software.

RDG reserves all rights not expressly granted herein, including, but not limited to, the rights to market the Software either directly or through affiliates, distributors and/or third parties.

For further information, please contact:

Roche Diagnostics GmbH
Roche Applied Science
Sandhoferstraße 116
D-68305 Mannheim
Germany

Terms

The Agreement is effective until terminated. Licensee may terminate this Agreement at any time by destroying the Software together with any copy previously made in compliance with the terms of this Agreement and documentation relating to the Software in any form. The Agreement will terminate automatically and without notice from RDG, if Licensee fails to comply with any term or condition of this Agreement. Licensee agrees to destroy the Software and the copy, if any, of the Software upon termination of this Agreement by RDG.

Limited Warranty

The Product is provided as is without warranty of any kind, either expressed or implied, including, but not limited to the implied warranties of merchantability and fitness for a particular purpose. The entire risk as to the quality and performance of the Product is with you as Licensee, should the Product prove to be defective. You assume the entire costs of all necessary servicing, repair, or correction. However, Roche Diagnostics GmbH warrants that the program media on which the software is furnished is free from defects in materials and workmanship under normal use for a period of ninety (90) days from the date of delivery as evidenced by a copy of your receipt. ROCHE DIAGNOSTICS GMBH MAKES NO FURTHER WARRANTIES OR GUARANTEES NOR EXPLICIT NOR IMPLIED.

Warranty Disclaimers

THE WARRANTY SET FORTH IN THE PREVIOUS PARAGRAPH, IS IN LIEU OF ALL OTHER WARRANTIES, EXPRESS OR IMPLIED, ARISING BY LAW, FROM A COURSE OF PERFORMANCE, A COURSE OF DEALING, TRADE USAGE, OR OTHERWISE. RDG AND ANY ENTITY CONTROLLING, CONTROLLED BY OR UNDER COMMON CONTROL WITH RDG ("RDG AFFILIATE") SPECIFICALLY DISCLAIM, WITHOUT LIMITATION, ALL WARRANTIES OF ANY KIND, WHETHER EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, THE IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON-INFRINGEMENT. RDG AND RDG AFFILIATES MAKE NO REPRESENTATION OR WARRANTY AS TO THE SOFTWARE OR AS TO THE RESULTS TO BE ATTAINED BY LICENSEE COMPANY OR ANY THIRD PARTY FROM THE SOFTWARE. LICENSEE ACKNOWLEDGES THAT IT HAS NOT RELIED UPON ANY REPRESENTATIONS OR WARRANTIES MADE BY RDG OR AN RDG AFFILIATE EXCEPT FOR THOSE EXPRESSLY AND SPECIFICALLY SET FORTH IN THIS AGREEMENT.

Limitations of Remedies

RDG's sole liability and your sole remedy shall be:

1. the replacement of the program media not meeting RDG's limited warranty and which is returned to RDG with a copy of your receipt;
2. if RDG is unable to deliver replacement program media which is free of defects in workmanship, you may terminate this Agreement by returning the Product and a copy of your receipt to RDG, and your money will be refunded.

IN NO EVENT WILL RDG OR ANY RDG AFFILIATE (OR THEIR RESPECTIVE OFFICERS, EMPLOYEES, CONSULTANTS, ATTORNEYS OR AGENTS), BE LIABLE FOR ANY SPECIAL, INDIRECT, INCIDENTAL, OR CONSEQUENTIAL DAMAGES (INCLUDING, BUT NOT LIMITED TO, LOST PROFITS, LOST DATA OR INFORMATION, LOSS OF USE OF THE SOFTWARE, BUSINESS INTERRUPTION, LOSS OF BUSINESS REPUTATION OR GOODWILL, OR DOWNTIME COSTS) WHICH THE LICENSEE OR OTHERS MAY INCUR OR EXPERIENCE, DIRECTLY OR INDIRECTLY ARISING OUT OF OR RELATING TO THE SOFTWARE, THIS AGREEMENT, OR THE TERMINATION OF THIS AGREEMENT, EVEN IF RDG OR AN RDG AFFILIATE HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES AND NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE. THE AGGREGATE LIABILITY, ON A COMBINED BASIS, OF RDG AND RDG AFFILIATES (AND THEIR RESPECTIVE OFFICERS, EMPLOYEES CONSULTANTS, ATTORNEYS, AND AGENTS) FOR DAMAGES FOR ANY CAUSE WHATSOEVER DIRECTLY OR INDIRECTLY RELATING TO OR ARISING OUT OF THIS AGREEMENT OR THE SOFTWARE, AND REGARDLESS OF THE FORM OF ACTION, SHALL BE LIMITED TO, AT RDG's OPTION, REPLACEMENT OF THE SOFTWARE OR REFUND OF THE FEES RECEIVED BY RDG OR AN RDG AFFILIATE FROM LICENSEE WITH RESPECT TO THE SOFTWARE.

General Information

You may not sublicense, assign or transfer the license or the Product, in whole or in part, except as expressly provided in this Agreement. Any attempt otherwise to sublicense, assign or transfer any of the rights, duties or obligations hereunder is void.

- This Agreement will be governed by the laws of Germany.
- Should any part of this agreement be declared void or unenforceable by a court of competent jurisdiction, the remaining terms shall remain in full force and effect.
- Failure of RDG to enforce any of its rights in this Agreement shall not be considered a waiver of its rights, including but not limited to its rights to respond to subsequent breaches.

By opening and using this software you acknowledge that you have read this Agreement, understand it, and agree to be bound by its terms and conditions. You further agree that this Agreement is the complete and exclusive statement of the Agreement between you and RDG and supersedes any proposal or prior Agreement, oral or written, any other communications between you and RDG relating to the subject matter of this Agreement.

IX. Preamble

Before setting-up operation of the LightCycler® 2.0 Instrument it is important to read this Operator's Manual thoroughly and completely. Non-observance of the instructions contained in this manual may entail safety hazards.

X. Usage of the LightCycler® 2.0 Instrument Operator's Manual

This Operator's Manual assists with operating the LightCycler® 2.0 Instrument. It contains the following chapters:

Chapter A Overview contains a short introduction in the operating mode of the LightCycler® 2.0 Instrument and describes the system's specifications.

Chapter B System Description contains instructions on the installation of the LightCycler® 2.0 Instrument and a description of the system's components and consumables.

Chapter C Operation describes the operating procedures for the LightCycler® 2.0 Instrument.

Chapter D Software contains instructions for programming LightCycler® runs and data analysis.

Chapter E Maintenance describes the maintenance procedures that are required for the LightCycler® 2.0 Instrument.

Chapter F Troubleshooting lists all LightCycler® system messages, explains their meaning and indicates appropriate measures.

XI. Conventions Used in this Manual

Text Conventions

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

Text Convention	Usage
Numbered Listing	Steps in a procedure that must be performed in the order listed.
Italic Type	- Points to a different chapter in this Operator's Manual which should be consulted. - Describes how to proceed when operating the LightCycler® Software

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 non-compliance with instructions or procedures may 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.
	BIOHAZARD	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.
	Warning On the instrument type plate (see *XII Handling Precautions*).
	Hot surface On the margin of the thermal chamber (see *XII Handling Precautions*).
	The CE mark on the instrument type plate expresses conformity with essential requirements of the directive relevant for this instrument (see *III*).
	cUL mark On the instrument type plate (see Chapter *Overview, 2.2 General specifications*)

XII. Warnings and Precautions

The LightCycler® 2.0 Instrument must only be used by trained and skillful personnel.

It is essential that the following safety precautions required for installation and operation of the LightCycler® 2.0 Instrument are carefully read and observed. Please assure that these safety precautions are accessible for every employee working with the LightCycler® 2.0 Instrument.

Handling Precautions

The LightCycler® 2.0 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.
- ►Never touch switches or power cord with wet hands.
- ►Do not open the housing of the LightCycler® 2.0 Instrument.
- ►Never clean the instrument without turning the instrument power switch off and disconnecting the power cord.

Only authorized service personnel should perform service or repairs required for this unit.

- ►Do not open the thermal chamber during operation.
- ►When programming a run, always include a cooling step (*e.g.,* 40°C for 30 sec) as last cycle program to make sure that the thermal chamber has cooled down upon opening the lid.

- ►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.
- ►Always wear safety goggles and gloves when dealing with toxic, caustic or infectious materials.
- ►Please refer to chapter *Maintenance* to find instructions for cleaning the LightCycler® 2.0 Instrument.

- ►The chamber lid and the sample carousel are hot while the instrument is operating.

The corresponding symbol is attached to the upper margin of the thermal chamber.

General Precautions

The LightCycler® 2.0 Instrument must not be connected to a network, although network hardware is present. 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. Roche is not responsible for any damages caused by connection of the LightCycler® 2.0 Instrument to a network by the customer.

Additional software must not be installed on the LightCycler® 2.0 workstation. Installation of additional software contains the risk to interfere with LightCycler® Software 4.05 and may affect result security.

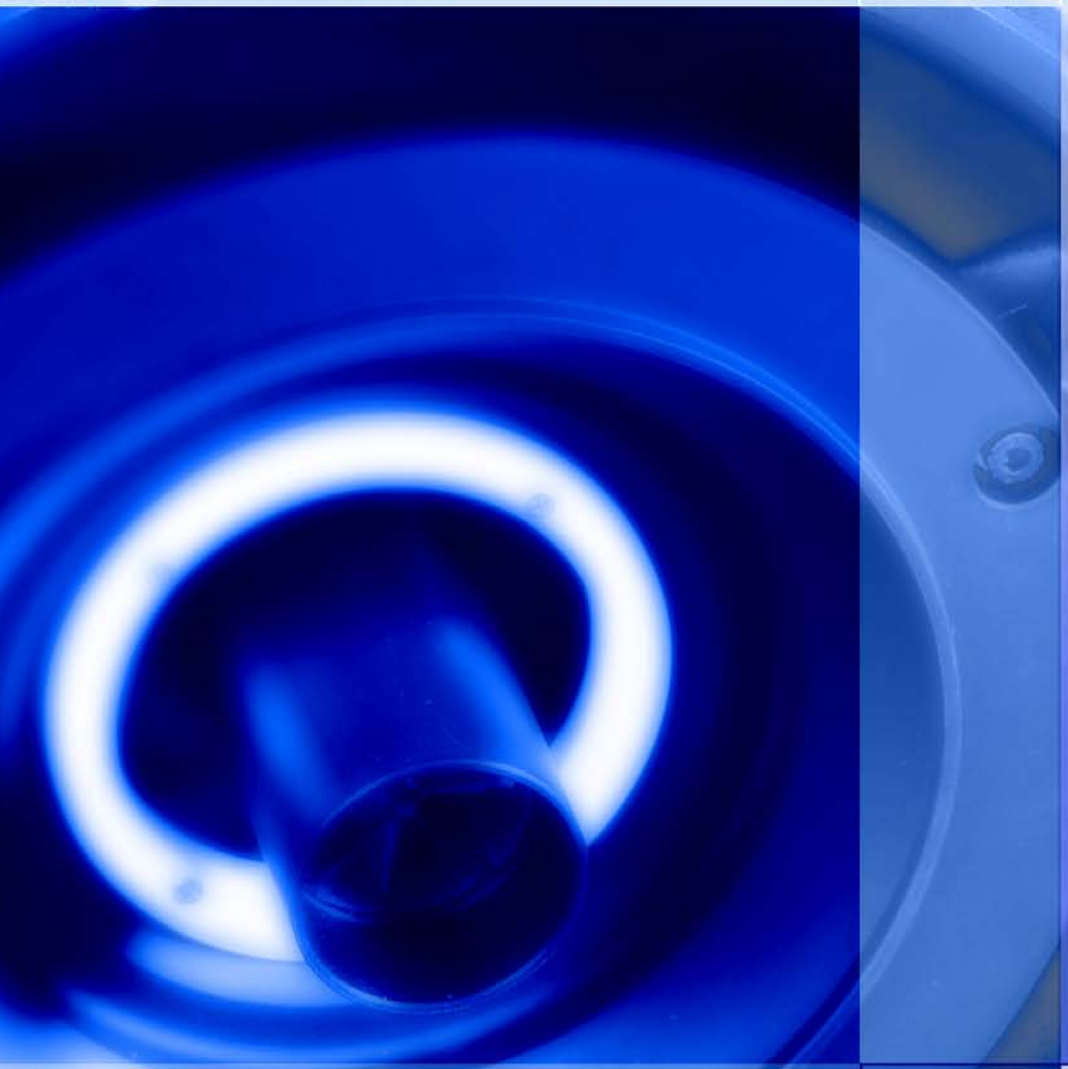
One PC must not be used with 2 LightCycler® Instruments simultaneously.

Do not manipulate the instrument.

Electrical Safety

The LightCycler® 2.0 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 3-wire grounded receptacle for the 115V or 230V 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, may 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.

Overview



A

Chapter A • Overview
contains a short introduction in the operating mode of the LightCycler® 2.0 Instrument and describes the system's specifications.

A

A	Overview	Page

A

Overview

1. Introduction

The LightCycler® 2.0 Instrument enables you to perform rapid PCR. Results can be quantified and analyzed simultaneously by monitoring fluorescence during amplification. Melting curve analysis allows mutation detection and product characterization. For more details on the Methodology and Data Analysis refer to Chapter *Software*.

2. Specifications of the LightCycler® 2.0 Instrument

A summary of the LightCycler® 2.0 Instrument specifications is given below.

2.1 Technical Specifications

Dimensions	28 x 38.5 x 50.5 cm +/- 0.5 cm tolerance (W x H x D)
Weight	Approx. 22 kg
Power supply	115/230 V; 8A; 50-60 Hz
Power consumption	Max. 800 VA
Noise level	< 65 dBA
Heat emission	2900 kJ/h (max.) 2100 kJ/h (average value during operation)
Protection Class	I

2.2 General Specifications

Temperatures allowed during transportation/storage/packaging	-25°C to +60°C; relative humidity: 10% to 95% (no condensation)
Temperatures allowed during operation	+18°C to +30°C; relative humidity: 20% to 80% (no condensation)
Relative Humidity	10% to 95%, no condensation
Altitude/Pressure	0 to 2000 m above sea level 850 – 1050 hP
Safety	Complies with safety standards IEC 61010-1 and IEC 61010-2-101, level of pollution 2, Overvoltage category II, CAN/CSA-C22.2 No. 1010.1-92 as well as UL 61010A-1.
c UL US	The safety mark has been issued by Underwriters Laboratories, Inc. (UL) for Canada and the US.

A

2.3 Sample Capacity

Number of samples per run	32
Sample volume	20 µl, 100 µl

2.4 Shipping

The LightCycler® 2.0 Instrument is transported in a styrofoam container packed in a cardboard box. The container should be carefully inspected for damage. Report any damage to your local Roche Diagnostics office before accepting the unit.

For transportation or relocation of the LightCycler® 2.0 Instrument, only the original packaging shall be used.

2.5 Data Station

A fully equipped data workstation (desktop or notebook version) is delivered by Roche with the LightCycler® 2.0 Instrument.

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 it is possible to access the LightCycler® PC by remote control. Contact your Roche representative for more information.

3. Specifications for the Detection System

3.1 Excitation

Type	High Brightness LED
Wavelength (Peak)	470 nm +/- 10 nm
Wattage at capillary position in the range of 450 nm-500nm	> 0.6 mW
Filter	Interference Filter: Bandpass 470 nm, HBW 40 nm

3.2 Detector

Type	Photohybrid
Resolution	16 bit

3.3 Filter

Detector Channel 1	Interference filter: Bandpass 530 nm, HBW 20 nm
Detector Channel 2	Interference filter: Bandpass 555 nm, HBW 20 nm
Detector Channel 3	Interference filter: Bandpass 610 nm, HBW 20 nm
Detector Channel 4	Interference filter: Bandpass 640 nm, HBW 20 nm
Detector Channel 5	Interference filter: Bandpass 670 nm, HBW 20 nm
Detector Channel 6	Interference filter: Bandpass 710 nm, HBW 40 nm

3.4 Acquisition Time

Acquisition Time for single capillary	≤ 46 ms
Acquisition Time for 32 capillaries	< 6 sec

A

4. Temperature Kinetics for PCR

4.1 General

Temperature range	40°C to 98°C
Accuracy of „Mean Capillary Temperature at Thermal Equilibrium“ [a]	+/-0.4°C
Accuracy of „Displayed Temperature“ with respect to capillary temperature at thermal equilibrium [a]	+/-0.3°C (at 50°C and at 95°C)

a. Excluding error of measurement equipment

4.2 Capillary Heating Rates

Heating rate 40°C to 95°C (non-linear)	20µl: ≤ 15 sec 100µl: ≤ 27.5 sec
Heating rate 50°C to 72°C (non-linear)	20µl: ≤ 8 sec 100µl: ≤ 11 sec
Heating rate 72°C to 95°C (non-linear)	20µl: ≤ 8 sec 100µl: ≤ 11.5 sec

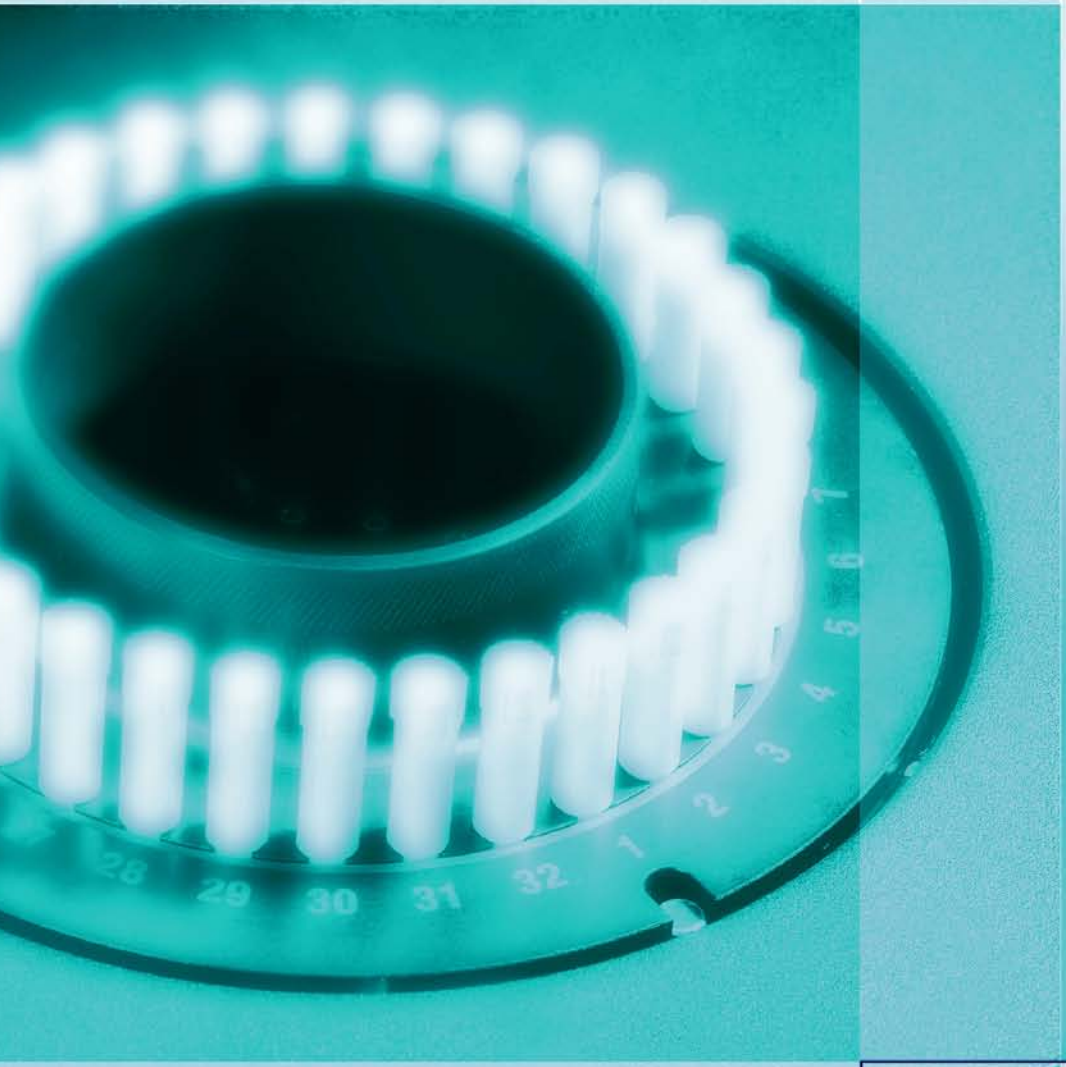
4.3 Capillary Cooling Rates

Cooling rate 95°C to 50°C (non-linear)	20µl: ≤ 15 sec 100µl: ≤ 24 sec

4.4 Temperature Tolerances, Short Term

Precision of capillary temperature over all capillary positions when measured for 30 sec at 95°C	+/-0.3°C (+/-1.0°C)
Precision of capillary temperature over all capillary positions when measured for 30 sec at 70°C	+/-0.15°C (+/-0.5°C)
Precision of capillary temperature over all capillary positions when measured for 30 sec at 50°C	+/-0.3°C (+/-0.8°C)

System Description



B

Chapter B • System Description contains instructions on the installation of the LightCycler® 2.0 Instrument and a description of the system's components and consumables.

B

System Description

1. Unpacking and Installation

The table below lists all components delivered with the LightCycler® 2.0 Instrument. Use this list to check the completeness of all components. Check for damages in transit after opening. Report any visual damage to your local Roche Diagnostics representative.

Do not lift the instrument by the lid handle when unpacking, instead place your hands under the base of the instrument.

1.1 Components of the LightCycler® 2.0 Instrument

Components	Description
System component 1	▶ LightCycler® 2.0 Instrument (Cat.No.: 03 531 414 201)  ▶ LightCycler® 2.0 Sample Carousel (20 µl) (Cat.No.: 03 603 962 001) mounted in LightCycler® 2.0 Instrument 

B

Components	Description
System component 2	▶ 1 box LightCycler® Capillaries (20 µl) (1 box out of Cat.No.: 11 909 339 001)  ▶ LightCycler® 2.0 Sample Carousel (100 µl) (Cat.No.: 03 603 954 001)  ▶ 1 box LightCycler® Capillaries (100 µl) (1 box out of Cat.No.: 03 337 090 001)  ▶ LightCycler® Centrifuge Adapters (Cat.No.: 11 909 312 001)  ▶ 2x LightCycler® Capping Tool (Cat.No.: 03 357 317 001)  ▶ LightCycler® 2.0 Capillary Releaser (Cat.No.: 03 603 920 001) 

Components	Description
System component 2	▶ LightCycler® 2.0 Operator's Manual ▶ LightCycler® Software 4.05 (Cat.No.: 03 604 012 001) ▶ Serial Cable to connect LightCycler® 2.0 Instrument to the Computer  ▶ Power cord (one with German plug and one with US plug) 

A fully equipped data workstation (desktop or notebook version) is delivered with the LightCycler® 2.0 Instrument.

A printer and a barcode reader are provided locally upon request.

B

2. Installation

2.1 Installation Requirements

- Do not place the LightCycler® 2.0 Instrument next to instruments that cause vibration, electromagnetic interference, or have high inductance (*e.g.*, centrifuges or mixers).
- Peripheral instruments connected to the LightCycler® 2.0 Instrument must meet the IEC 950 (UL 1950) standard.
- All plugs used with the LightCycler® 2.0 Instrument (PC, printer, monitor) should have the same phasing in order to prevent switch-on peaks and electronic noise generated by other instruments, or by the power supply itself.
- Use only the power lines and RS232 connector supplied.
- Do not place the instrument in direct sunlight or close to radiators or heating devices.
- Do not use the instrument in an atmosphere where an explosion could occur.

2.2 Space and Power Requirements

Place the LightCycler® 2.0 Instrument on a site that can support the following instrument requirements:

Dimensions	The LightCycler® 2.0 Instrument is 28 cm wide, 50.5 cm long and 38.5 cm high.
Weight	The LightCycler® 2.0 Instrument has a weight of approximately 22 kg.
Voltage requirements	The LightCycler® 2.0 Instrument operates at ▶ 115 V (60 Hz) ▶ 230 V (50 Hz) If the voltage in your country does not meet the voltage requirements, please contact your local Roche representative. The LightCycler® 2.0 Instrument adjusts automatically to the available voltage when the instrument is plugged in. The user does not have to set the instrument to the correct voltage manually. Do not open the LightCycler® 2.0 Instrument housing.
Power consumption	The LightCycler® 2.0 Instrument uses 800 VA maximum. PC and printer consume approximately an additional 500 VA.

2.3 Environmental Requirements

The LightCycler® 2.0 Instrument has been designed to safely operate within specifications according to CE and UL certified technical standards at ambient room temperatures between 18°C and 30°C, relative humidity between 20% and 80% (no condensation) and at an altitude less than 2000 meters above sea level (850-1050 hP). Atmospheric conditions should conform to pollution degree II.

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

2.4 Storage Conditions

Keep the device in a dry place. Moisture could cause malfunction.

2.5 Installation of the LightCycler® 2.0 Instrument

The LightCycler® 2.0 Instrument should be unpacked and installed by your Roche Diagnostics representative. Should this not be possible, follow these steps to install the instrument successfully:

- Unpack the instrument by following the instructions outlined in *Unpacking and Installation*.

- Position the instrument on the workbench in the upright position with the instrument's backside towards the wall. Allow 10 cm space to the left, right and behind the instrument to ensure sufficient cooling of the electronic components. Ensure that there is absolutely nothing placed below the base of the LightCycler® 2.0 Instrument (*e.g.*, paper, plastic film etc.).

⚠ Failure to provide this ventilation space may cause damage to the instrument due to overheating.

- Establish the following electrical connections:
  a) Connect the LightCycler® 2.0 Instrument to the PC using the RS232 cable (serial interface) provided with the system.
  b) It is recommended to connect the LightCycler® 2.0 Instrument, PC, monitor, and printer to the same multiple-outlet distributor plug.

Rear connections of the LightCycler® 2.0 Instrument

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

B

To prevent unwanted removal of the LightCycler® 2.0 Instrument, the instrument can be fixed to an unremovable device like *e.g.,* a lab bench. For this purpose, a key lock for commercially available safety locks is provided on the back of the LightCycler® 2.0 Instrument (see picture). The safety lock is not provided with the system.

3. Starting up the Data Station

3.1 Installation of the Computer

To install the data station, follow the instructions outlined below:

- Connect mouse, keyboard, and monitor to the computer.
- Connect the LightCycler® 2.0 Instrument to the computer with the RS232 cable (serial interface) provided with the system.
- Connect the computer, monitor, and LightCycler® 2.0 Instrument to the same multiple-outlet distributor plug.

The computer is now ready for operation.

3.2 Using the CD-RW Drive

The data station is equipped with a CD-RW drive and a preinstalled software package that allows easy read/write access to standard re-writable CDs. The software package used is Roxio Easy CD Creator and Direct CD. The CD-RW drive is a combined CD-ROM/ CD-writer which allows you to easily transfer LightCycler® data to another PC. To do this a special re-writable CD (CD-RW) is needed. This CD-RW has to be formatted first by performing the following steps:

1	Insert the CD-RW disc.
2	Start the Direct CD software.
3	Follow the guidelines given by the software for formatting a CD-RW.
4	You may enter an identifier for the CD-RW.
5	Choose option for complete formatting.
6	Start the formatting process.
7	Once the formating process is finished, the CD-RW is ready for use in the LightCycler® Workstation's CD-RW drive.
8	For more details check the Help function implemented in the Direct CD software.

Once these Steps have been followed, the CD-RW is formatted in a way, that allows easy copying of data, using standard drag and drop and cut and paste functions of Windows Explorer.

Do not run the CD burning software in parallel to a LightCycler® run.

B

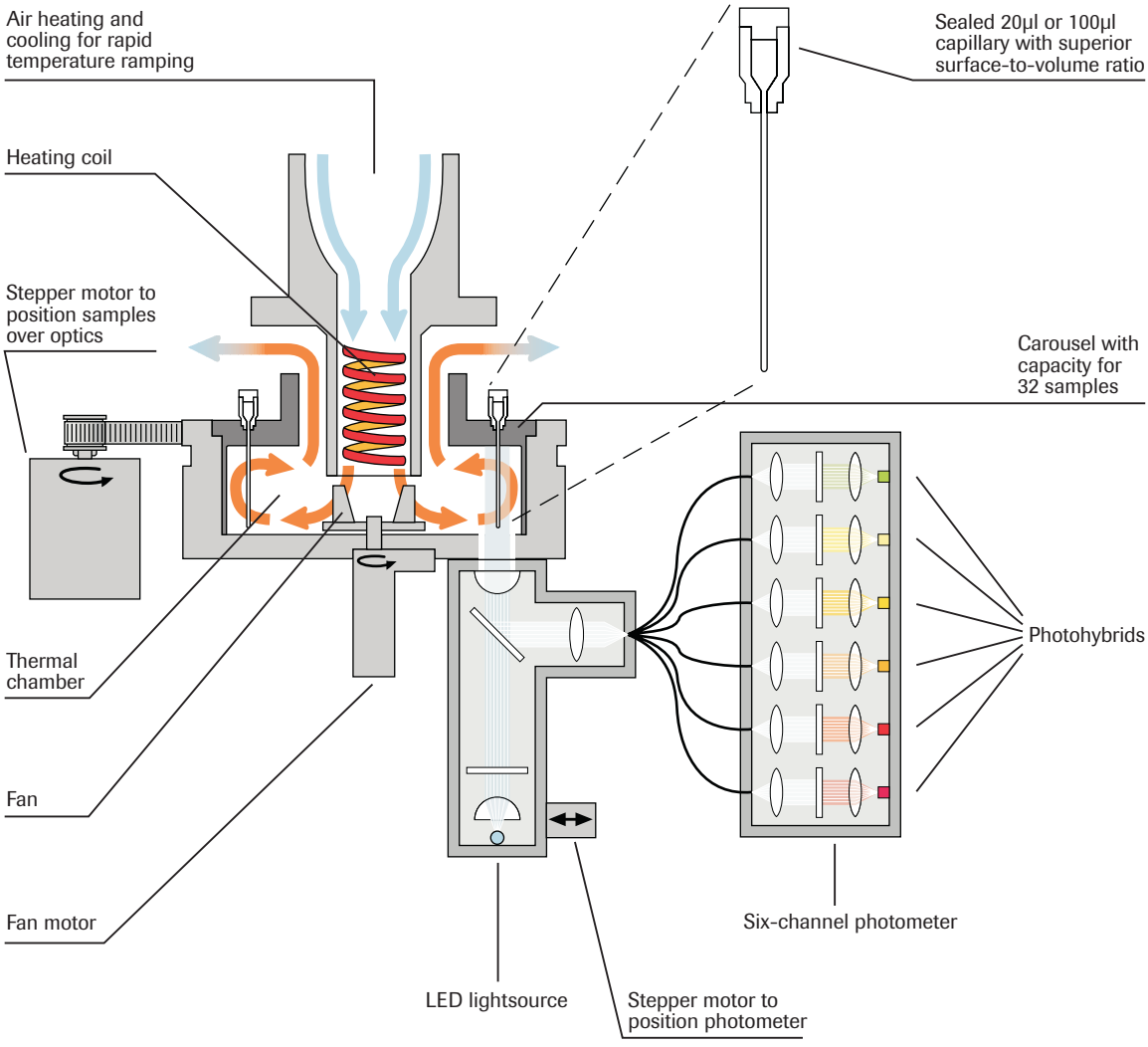
4. System Description

4.1 Description of the LightCycler® 2.0 Instrument

Rapid thermal cycling with the LightCycler® 2.0 Instrument is made possible by the unique design of the instrument. Compared to conventional cyclers the LightCycler® 2.0 Instrument uses air for heating and cooling instead of thermal blocks for the high-speed thermal cycling. Ambient air is drawn into the machine and heated up by a heating coil, which is located in the upper part of the instrument. The lower unit contains the thermal chamber, photometer and drive units.

A fan located within the thermal chamber ensures efficient air circulation and temperature homogeneity during cycling. Varying the voltage supplied to the heating coil regulates the temperature in the thermal chamber. During the heating phase the fan in the thermal chamber operates at low speeds to ensure homogenous distribution of the temperature. During the cooling phase the fan operates at higher speeds so that the capillaries and the heating coil can be cooled efficiently.

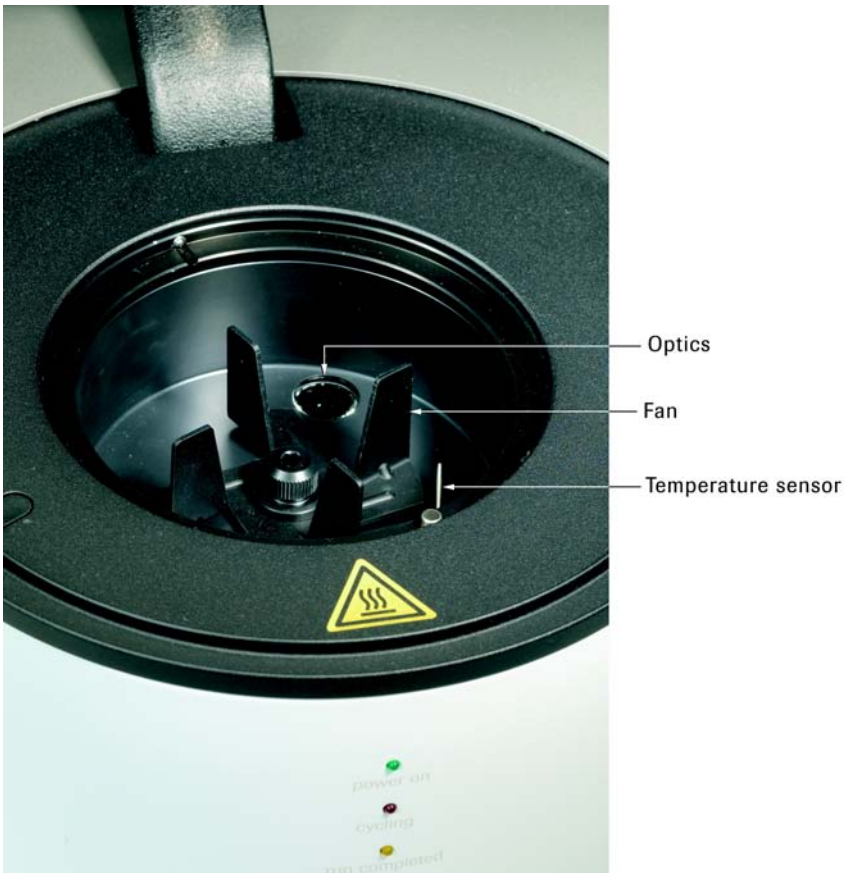
During measurements a stepper motor rotates the sample carousel within the thermal chamber to position the capillary tip precisely at the focal point of the photometer optics. The stepper motor of the sample carousel works with the horizontal photometer stepper motor to achieve optimal positioning of the capillaries into the focus of the photometer.



4.2 Thermal Chamber

Temperatures within the thermal chamber are controlled by an integrated measuring system equipped with a temperature sensor, which is installed near the sample capillaries in the carousel. A blower supplies the thermal chamber with either ambient air or hot air, which has been heated up by the heating coil beforehand. A high-velocity fan, located at the base of the thermal chamber, evenly distributes the incoming air throughout the thermal chamber. Surplus air is exhausted through an air vent.

The use of air as a heat-transfer medium contributes to the high-speed cycling capabilities of the LightCycler® 2.0 Instrument. Thus, heating and cooling in the LightCycler® 2.0 Instrument occurs about ten times faster than in a normal thermal cycler. A typical amplification cycle requires only 50 seconds with 20 µl capillaries and 95 seconds with 100 µl capillaries, which means that an amplification reaction with 40 cycles is usually completed within 35 to 65 minutes.



View into the thermal chamber of the LightCycler® 2.0 Instrument

B

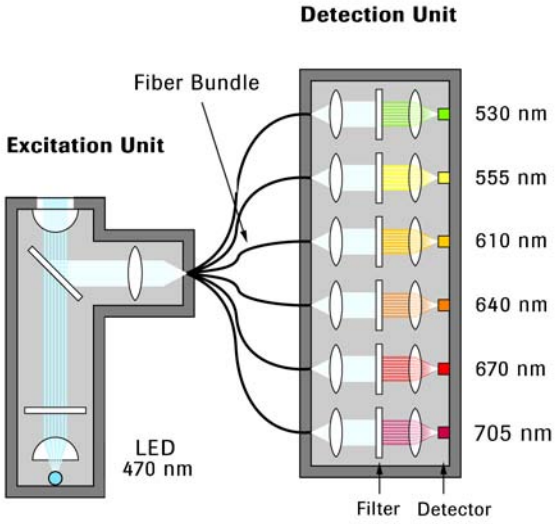
4.3 Photometer

4.3.1 Optics

The photometer, as schematically illustrated below, is composed of two units connected with a light conductor. The excitation unit is equipped with a blue LED light source with a maximum emission of 470 nm. The blue LED serves as the energy source for sample excitation. Light emitted by the blue LED is focussed on the tip of the capillary and hence excites the fluorescent dyes therein. The emitted fluorescent light is reflected back in the photometer with the aid of a dichroitic mirror.

The light conductor divides the fluorescent light into six channels independently from wavelength.

In the detection unit the fluorescent light is collimated with aspherical lenses and a special optical filter system is accountable for the wavelength specific measurements at 530 nm, 555 nm, 610 nm, 640 nm, 670 nm and 705 nm. A second aspheric lens transmits the signal of each detection channel to a photohybrid for final evaluation. The signals of the photohybrids are sent to the LightCycler® 2.0 Instrument via a serial interface.



Schematic of the Photometer Unit

4.3.2 Detection Channels

The six detection channels within the LightCycler® photometer unit permit analyses at certain emission wavelengths, which allow exact measurement of emissions from the fluorophores shown in the table below:

	Excitation channel	Detection channel					
Fluorophore	470 nm	1 530 nm	2 555 nm	3 610 nm	4 640 nm	5 670 nm	6 705 nm
Fluorescein	494 nm	520 nm					
SYBR Green I	494 nm	520 nm					
HEX/VIC			560				
LC Red 610[1]				610			
LC Red 640[2]					640		
LC Red 670[3]						670	
LC Red 705[4]							705

[1] *LC Red 610 = LightCycler® Red-610-N-hydroxysuccinimide ester (Cat:No.: 03 561 488 001)*

[2] *LC Red 640 = LightCycler® Red-640-N-hydroxysuccinimide ester (Cat:No.: 12 015 161 001)*

[3] *LC Red 670 = LightCycler® Red 670-N-hydroxysuccinimide ester*

[4] *LC Red 705 = LightCycler® Red-705-Phosphoramidite*

LC Red 610, LC Red 640, LC Red 670, and LC Red 705, are not excited by the blue LED, but are FRET partners of fluorescein.

HEX/VIC are directly excited by the blue LED, but emission is suppressed by a quencher. Upon removal of the quencher, emission of HEX/VIC can be measured in channel 2.

B

5. Mobile Components and Consumables

In this section you will learn more about the mobile components, consumables and products that are additionally available for use with the LightCycler® 2.0 Instrument.

5.1 LightCycler® 2.0 Sample Carousel

The rotor-like sample carousel is the central element within the thermal chamber. It is available for 20 µl (marked with a brown tag) and 100 µl (marked with a purple tag) glass capillaries. Both have the capacity to hold up to 32 samples. The sample carousel can be taken out of the instrument to be loaded on the workbench. This also allows easy cleaning and decontamination of the rotor and the thermal chamber (for cleaning instructions refer to chapter E *Maintenance*). Furthermore, the use of an additional carousel provides the possibility of preparing new samples while a run is in progress.

The LightCycler® 2.0 Sample Carousel (20 µl) as well as the LightCycler® 2.0 Sample Carousel (100 µl) have an integrated barcode label for easy identification.

The LightCycler® 2.0 Sample Carousel can only be centrifuged in a LightCycler® Carousel Centrifuge 2.0 described later in this chapter.

5.2 LightCycler® Capillaries

The LightCycler® Capillaries (20 µl) just as the LightCycler® Capillaries (100 µl) consist of the glass capillary itself, a plastic reservoir at the top, and a plastic stopper to seal the capillary.

Each LightCycler® Capillary (20 µl) is 45 mm long (without plastic stopper) and has an outer diameter of 1.55 mm.

Each LightCycler® Capillary (100 µl) is 51 mm long (without plastic stopper) and has an outer diameter of 3.175 mm.

Samples are pipetted into the capillary reservoir and then forced into the glass capillary by centrifugation.

High-speed thermal cycling in the LightCycler® 2.0 Instrument is made possible, in part, by the unique design of the capillaries. Their superior surface-to-volume ratio guarantees extremely rapid thermal transfer within the reaction mixture.

B

5.3 LightCycler® 2.0 Capillary Releaser

The LightCycler® 2.0 Capillary Releaser is a tool designed to allow easy release of all capillaries placed into a LightCycler® 2.0 Sample Carousel (for 20 µl or 100 µl capillaries) in one simple step. It can be used independently of the number of capillaries loaded. Refer to section *How to use the Capillary Releaser* for a description of the functionality.

5.4 LightCycler® Capping Tool

The LightCycler® Capping Tool is a tool designed to allow easy sealing of the sample capillaries with their plastic stoppers.

5.5 LightCycler® Sample Carousel O-Ring

The LightCycler® Sample Carousel O-Ring is designed to firmly hold the capillaries. The procedure for changing the O-Ring is described in chapter *Maintenance*.

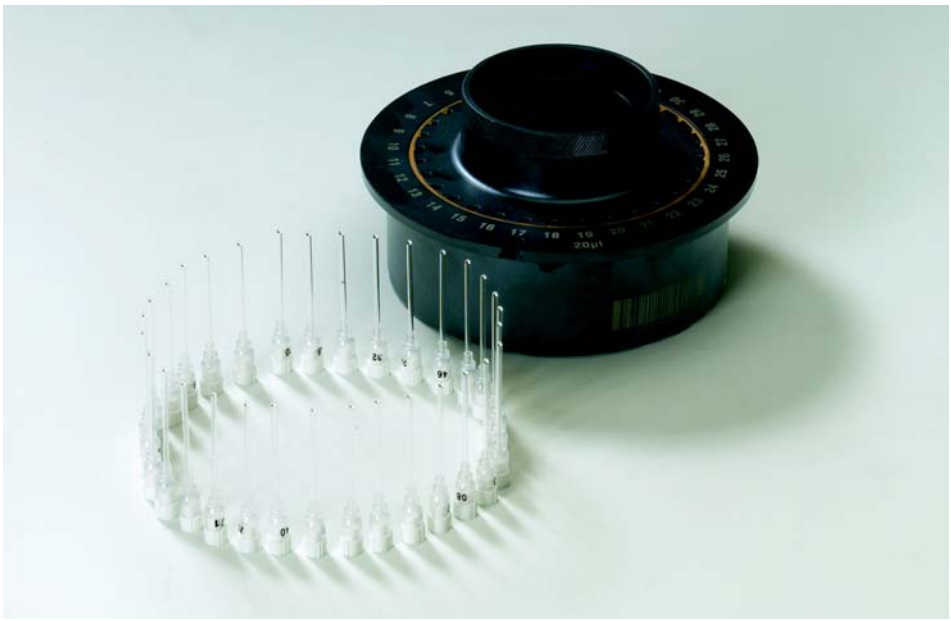
B

5.6 How to Use the LightCycler® Capillary Releaser

The LightCycler® Capillary Releaser is a plastic part, slightly smaller in diameter than the sample carousel. You can use it to easily unload capillaries from the LightCycler® 2.0 Sample Carousel.

Place the loaded carousel on the LightCycler® Capillary Releaser, then push the carousel down with the flat of your hand. The capillaries will be released from the rubber O-ring and held at a slightly elevated position. The capillaries can now be easily removed from the sample carousel.

If the capillaries are simply to be discarded, put the loaded sample carousel onto the table upside down. Then insert the LightCycler® Capillary Releaser into the bottom of the carousel and press down to release the capillaries.



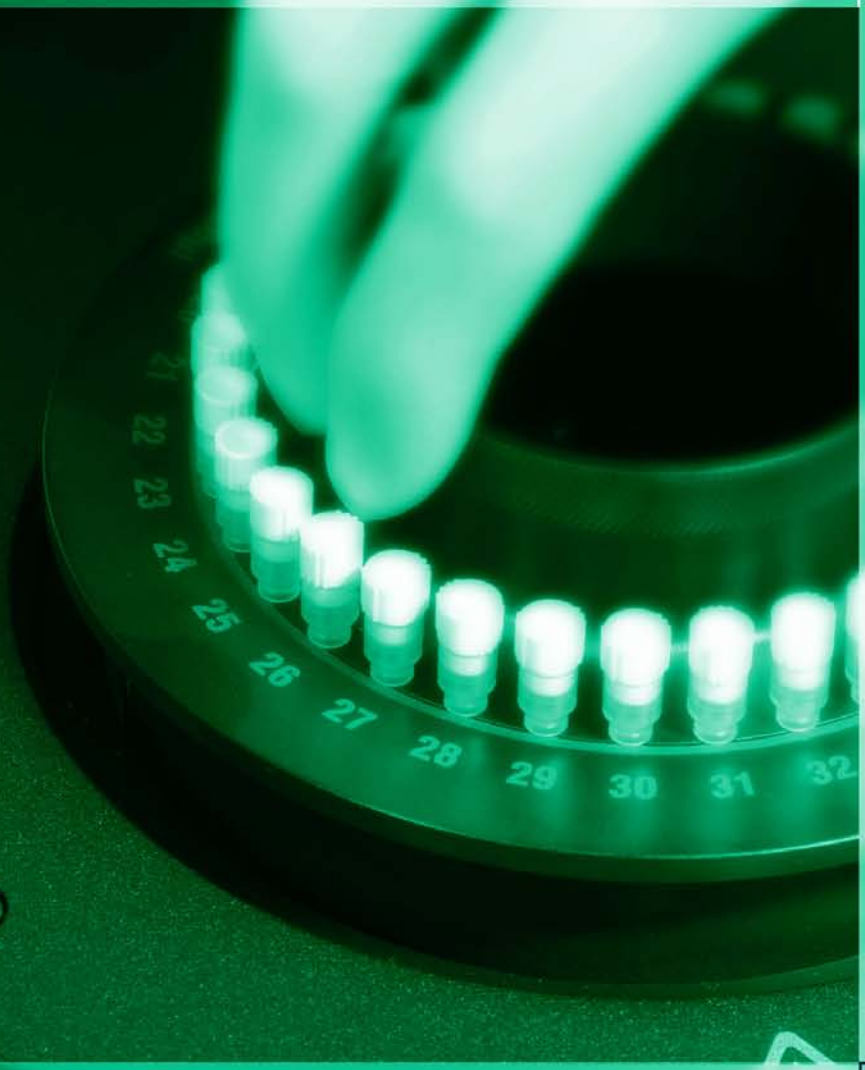
When the sample carousel is lifted, the LightCycler® Capillary Releaser will slide down into the carousel, fully releasing the capillaries from the sample carousel.

B

5.7 LC Carousel Centrifuge 2.0

The LC Carousel Centrifuge 2.0 (Cat. No.: 03 709 582 001 for 230 Volt machine, Cat. No.: 03 709 507 001 for 115 Volt machine) is a specially designed table top centrifuge which allows a convenient spin-down procedure for capillaries which have been loaded directly into the sample carousel after pipetting. The whole carousel is centrifuged and transferred to the LightCycler® 2.0 Instrument. The LC Carousel Centrifuge 2.0 may additionally be required to work with the LightCycler® 2.0 Instrument instead of using LightCycler® Centrifuge Adapters. For details refer to chapter C *Operation*.

Operation



C

Chapter C • Operation
describes the operating procedures for the LightCycler® 2.0 Instrument.

C

Operation

1. Introduction

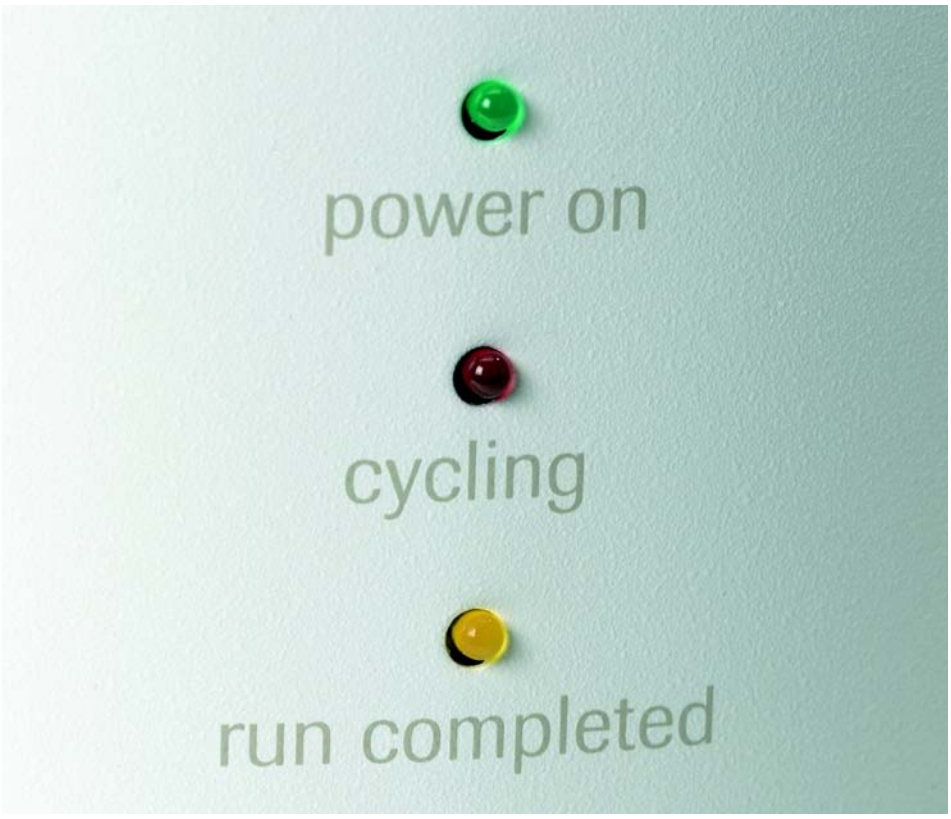
Prior to starting operation, review chapter A *Overview* and chapter D *Software* to verify the identification and location of the LightCycler® 2.0 Instrument components and to become familiar with the software handling.

2. Start-Up

1	Close the LightCycler® 2.0 Instrument lid.
2	Put the LightCycler® **ON/OFF** switch on the back of the instrument in the **ON** position. To locate the **ON/OFF** switch refer to chapter A *Overview*
3	Switch on the PC and printer.
4	Start-up Windows.
5	Start the LightCycler® Software. Details on working with the LightCycler Software 4.05 are described in chapter D *Software*.

2.1 Status LED

Three diodes are located at the front of the LightCycler® 2.0 Instrument. All diodes come on when the instrument is switched on. In this way, the instrument tests for proper functioning of the diodes. During instrument operation, the diodes function as described in the table below.



Position of diode	Color of diode	Label	Function	Indication
Top	Green	Power on	On	Instrument is switched on
			Off	▶ No power ▶ Instrument is defective
Middle	Red	Cycling	On	Instrument is running
			Flashing	Instrument is defective
Bottom	Yellow	Run completed	On	Instrument is not running either because the run is completed or because the run has not yet been started. Lid lock is inactive and lid can be opened.
			Off	Instrument is running. Lid lock is active, lid cannot be opened.

2.2 Lid Lock

The lid is locked automatically when a run starts. It can also be locked by activating the Real Time Fluorimeter. The lid is unlocked after completion of a run or after exiting a run.

Make sure you keep your hands out of the way when the lid is closing to avoid physical injury that might be caused by the upper metal part of the lid lock.

Button for opening the Lid Lock manually

The lid lock is regulated electronically. For manual opening (*e.g.*, if no electricity is available), the lid can be unlocked manually by pressing the hidden button under the front left side of the instrument as indicated in the picture.

If the instrument is unlocked manually during a run, the run will be aborted and all data will be lost.

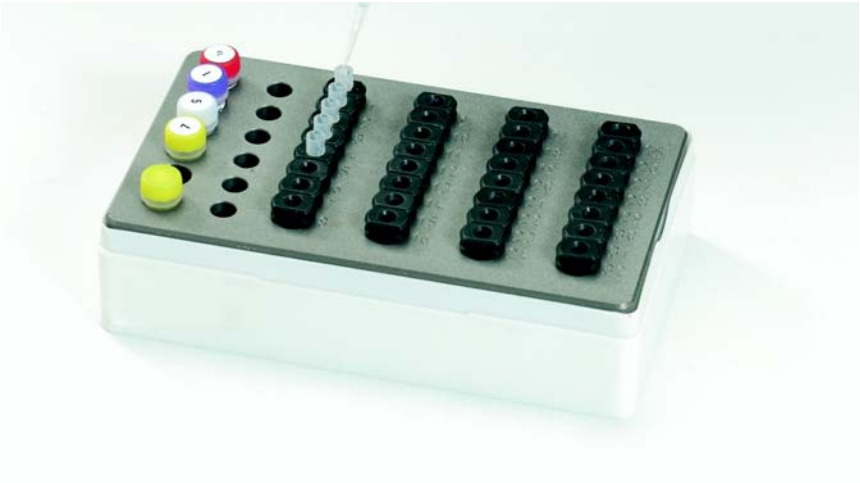
3. Preparing a LightCycler® Run

1. Program the experiment protocol and define the sample numbers, names, etc. Refer to chapter D *Software* for detailed instructions.

2. Prepare a master mix and omit the DNA/RNA. Details on preparing the master mix are described in the corresponding pack inserts.

3. Mix by gentle vortexing

4. Place the capillaries into the LightCycler® Centrifuge Adapters that have been precooled in the cooling block.
Pipette the reagent mix into the plastic reservoir at the top of the capillary.
Add the DNA/RNA template to the capillary.



5 Seal each capillary with a plastic stopper using the LightCycler® Capping Tool. Ensure that each LightCycler® capillary is closed tightly by checking it visually: The lower part of the plastic stopper must be completely inserted into the glass capillary.

6 Place the capillaries in the LightCycler® 2.0 Sample Carousel, keeping the capillaries in an upright position. Make sure that all capillaries are fixed in the optimal position where the O-ring of the LightCycler® 2.0 Sample Carousel covers the lower part of the plastic chamber. Proper positioning can be ensured by lightly pressing the cap until a final "click" is heard as the capillary reaches its final position.

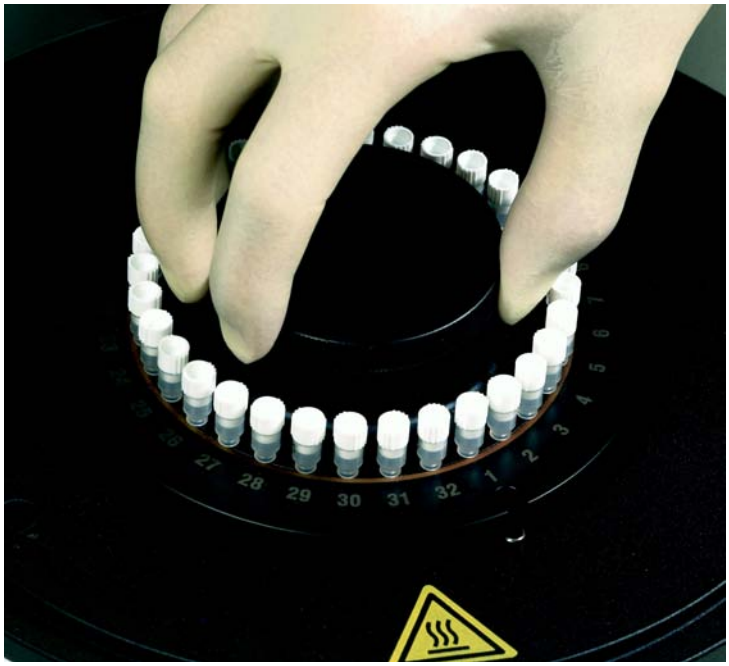
When pressing the capillaries into the LightCycler® 2.0 Sample Carousel, do not press too hard. Do not use LightCycler® capillaries that show slight cracks or cracked slightly when inserted into the LightCycler® 2.0 Sample Carousel. Do not use LightCycler® capillaries that have been dropped. LightCycler® capillaries with slight cracks, possibly invisible, might rupture during a LightCycler® run.

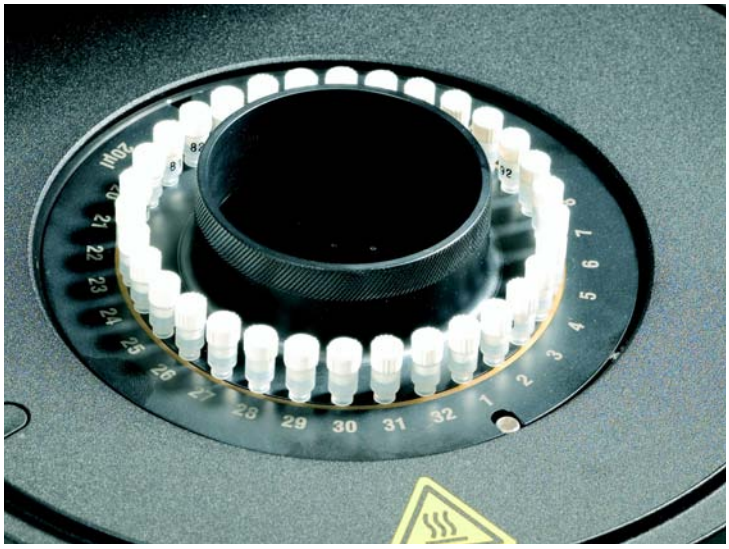
C

7 Put the loaded LightCycler® 2.0 Sample Carousel into the rotor cup and place it in the LC Carousel Centrifuge 2.0. Refer to the LC Carousel Centrifuge 2.0 Operator's Manual for operating instructions.

Alternatively, with the help of the LightCycler® Centrifuge Adapters, you can spin down the reaction mix inside the capillaries in a standard benchtop centrifuge, like the Biofuge 19 from Heraeus Instruments. Centrifuge briefly, at not more than 735 x g. Take care to use only rotors that are designed to hold 2.0 ml reaction tubes.

8 Place the LightCycler® 2.0 Sample Carousel in the LightCycler® 2.0 Instrument. Ensure that the notch below sample position 1 on the LightCycler® 2.0 Sample Carousel locks into position against the pin on the thermal chamber as indicated in the upper picture. Check visually if the carousel is inserted correctly and fits perfectly in the thermal chamber as indicated in the lower picture.





Before the LightCycler® 2.0 Sample Carousel is placed in the LightCycler® 2.0 Instrument ensure that the thermal chamber is clean and free of any items which could interfere with the capillaries during the run. Cleaning instructions are specified in chapter E *Maintenance*.

9 Close the lid. You are now ready to start the run.

Refer to chapter E *Maintenance* for details on subsequent measures if capillary breakage occurs.

4. Abort a Run

Refer to chapter D *Software* for instructions on how to abort a run.

5. Shut-Down

To shut down the LightCycler® 2.0 Instrument proceed as follows:

1	Exit the LightCycler® software and shut-down the computer.
2	Put the LightCycler® **ON/OFF** switch on the back of the instrument in the **OFF** position. To locate the **ON/OFF** switch refer to chapter A *Overview*.
3	Switch off PC and printer.

To ensure that the lid is unlocked, complete or exit a run before shutting-down the LightCycler® 2.0 Instrument.

Software

D

Chapter D • Software
contains instructions for programming LightCycler® runs and data analysis.

D	Software	Page

How to use the Software Part

The following sections summarize which portions of the software chapter you will find most helpful, depending on your responsibilities and the role assigned to your user account. If you are installing the LightCycler® Software 4.05, you must use an account that has the Local Administrator role. If you are using LightCycler® Software 4.05 after it has been installed, see your System Administrator to learn which role is associated with your account.

All users

Refer to the following chapters for general information and for instructions for tasks any user can perform:

Chapter 1 **"Overview of LightCycler® Software 4.05"**
Chapter 3 **"Overview of Experiment Analysis"**
Chapter 9 **"Working with Preferences"**

If your user role is Local Administrator

Refer to the following chapters for information about tasks that require the Local Administrator role:

Chapter 8 **"Using Reports, Charts, Queries and Instrument Tools"**
▶ "Using diagnostic tools"
Chapter 11 **"Managing User Access"**
Chapter 12 **"Installation of LightCycler® Software 4.05"**

If your user account has the Local Administrator role, you have access to all features and functions of LightCycler® Software 4.05. Refer to the rest of the software chapter for information about all the tasks you can perform.

If your role is Expert User

Refer to the following chapters for information about tasks that require at least the Expert User role (Local Administrators can also perform these tasks):

Chapter 2 **"Creating and Running an Experiment"**
Chapter 4 **"Performing Quantification Analyses"**
Chapter 5 **"Performing Qualitative Detection Analysis"**
Chapter 6 **"Performing Melting Curve Analyses"**
Chapter 7 **"Performing Other Analyses"**
Chapter 8 **"Using Reports, Charts, Queries, and Instrument Tools"**
Chapter 10 **"Using Templates and Macros"**
Chapter 12 **"Installation of LightCycler® Software 4.05"**
▶ "Adding a new instrument to the database"

If your role is Standard User

Refer to the following chapters for information about tasks that require the Standard User role (Expert Users and Local Administrators can also perform these tasks):

Chapter 2 "Creating and Running an Experiment."

As a Standard User, you cannot create experiment protocols, but you may be able to modify a protocol or a sample list as you run an experiment macro. Read this chapter to learn how to use the LightCycler® Software 4.05 Run module to create or modify experiment protocols and sample lists.

Chapter 10 "Using Templates and Macros"

- **"Executing an experiment kit macro"**
- **"Adding and deleting macro buttons on the Front window"**

These sections explain how to run predefined macros and how to add or delete macros from the LightCycler® Software 4.05 Front window.

Subchapter summary

The chapters in the software part of this manual cover the following topics:

Chapter 1, "Overview of LightCycler® Software 4.05"– introduces the buttons, menus, and panes of the LightCycler® Software 4.05 window and explains how to use the window.

Chapter 2, "Creating and Running an Experiment"– explains how to define and run experiment protocols.

Chapter 3, "Overview of Experiment Analysis"– describes the analysis modules available in LightCycler® Software 4.05, explains the general procedure for performing any analysis, and explains how to use the analysis window.

Chapter 4, "Performing Quantification Analyses"– explains how to perform each of the quantification analyses.

Chapter 5, "Performing Qualitative Detection Analysis"– explains how to perform a Qualitative Detection analysis, which determines the presence or absence of a target DNA sequence.

Chapter 6, "Performing Melting Curve Analyses"– explains how to perform each of the melting curve analyses.

Chapter 7, "Performing other Analyses"– explains how to perform nucleic acid quantification and how to use color compensation to compensate for multicolor experiments.

Chapter 8, "Using Reports, Charts, Queries, and Instrument Tools"– explains how to use LightCycler® Software 4.05 tools to create reports, customize charts, retrieve items from the database, and monitor the LightCycler® Instrument.

Chapter 9, "Working with Preferences"– explains how to set preferences that determine the format of charts, samples, and windows and how to set default directory paths.

Chapter 10, "Using Templates and Macros"– explains how to create and apply templates to reuse experiment protocols and other items, and how to create and execute macros to automate LightCycler® Software 4.05 procedures.

Chapter 11, "Managing User Access"– describes the different types of user accounts available in LightCycler® Software 4.05 and explains how to set up user accounts and how to change your password.

Chapter 12, "Installation of LightCycler® Software 4.05"– explains how to install LightCycler® Software 4.05, set up initial user accounts, and add a LightCycler® Instrument to a LightCycler® Software 4.05 database.

Software

1. Overview of LightCycler® Software 4.05

This chapter provides a general introduction to LightCycler® Software 4.05. It includes the following topics:

- Starting the LightCycler® Software 4.05
- Understanding the LightCycler® Software 4.05 window
- Opening, creating, and saving objects
- Renaming and deleting objects
- Importing and exporting objects
- Changing your password

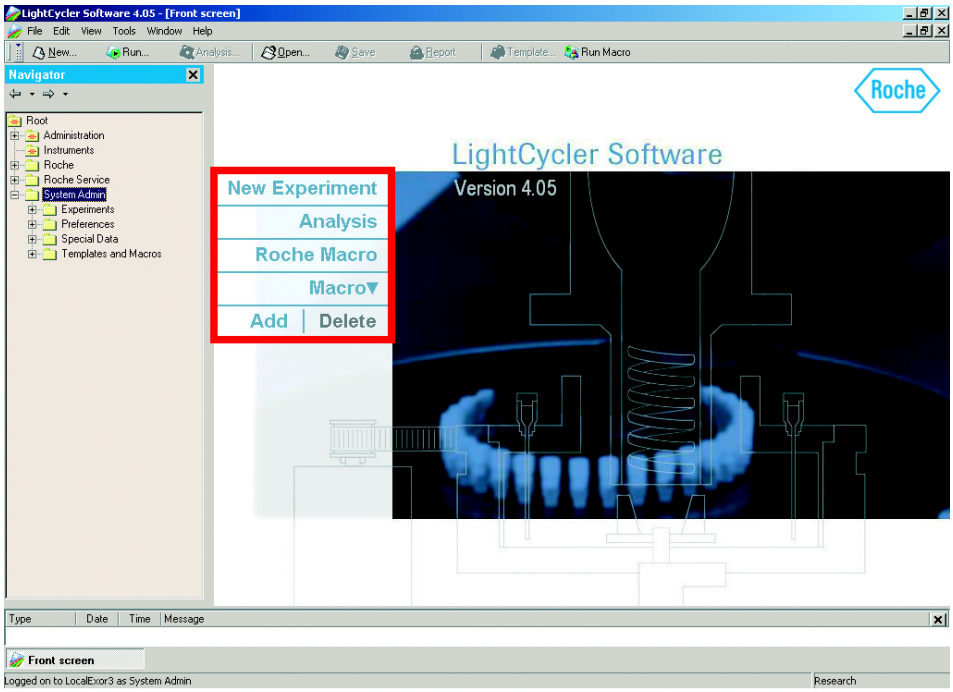
1.1 Starting the LightCycler® Software 4.05

Follow the procedure below to start the software and open a demo experiment. The demo experiment illustrates the features and components of the LightCycler® Software 4.05 described in the rest of this chapter.

To start LightCycler® Software 4.05 and view a demo experiment

1	Double-click the LightCycler® Software 4.05 icon on the desktop.
2	In the *Login* dialog box, type your user name and password. (The initial password for the *admin* user is *LightCycler01*. You will be prompted to change the initial password upon your first login. For more information see *Changing your Password*.)
3	To connect to the database on the local computer, select *LocalExor3* in the *Log on to* box.
4	Click *Login*.

The application displays the LightCycler® Software 4.05 window containing the Navigator on the left and a picture of a LightCycler® Instrument on the right. The activity of the buttons you see on the window depends on the role associated with your user account. If your account has the Standard User role, you can choose an experiment button from the *Macro* list visible on this window to execute predefined experiment protocols. If your account has the Expert User or Local Administrator role, you can also choose the buttons *New Experiment* and *Analysis*.



The headings in the picture are buttons used to perform various tasks. Which buttons are active depends on the role associated with your user account.

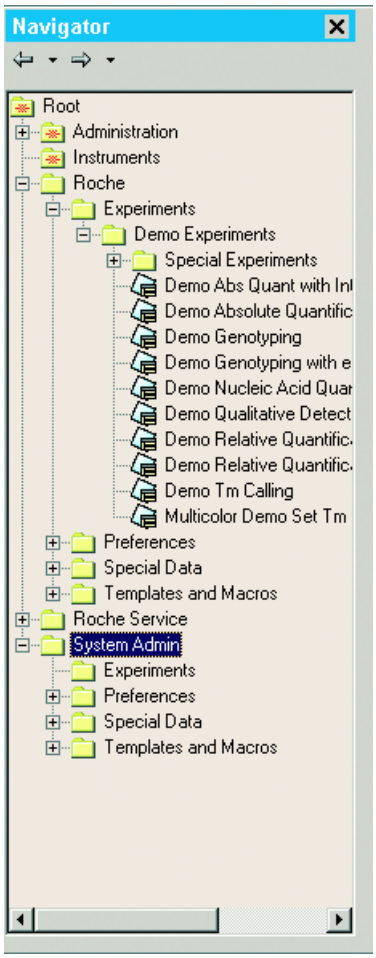
New Experiment launches the Run module. For more information about using the Run module to create experiments see chapter *Creating and Running an Experiment*.

Analysis lets you select an existing experiment to analyze. For more information about analyzing experiments see chapter *Overview of Experiment Analysis*. (Not all user accounts have rights to analyze an experiment. See chapter *Managing User Access* for information about privileges associated with user accounts.)

Roche Macro lets you select a previously installed Roche Macro. For more information about installing and executing Roche Macros see chapter *Using Roche Macros* described in the LightCycler® 2.0 Operator's Manual, Manual A: for in vitro diagnostic use.

Macro displays the protocol window, which contains custom buttons used to execute predefined experiments. For information about creating macros for predefined experiments and linking them to buttons on the front window see chapter *Using Templates and Macros*.

5 To open a demo experiment, in the Navigator, click the plus sign (+) next to each item in the path *Roche\Experiments\Demo Experiments*.



6 Double-click any of the experiment names to open the experiment in the LightCycler® Software 4.05 window. The summary information for the experiment is displayed in the main window.

7 Select the *Raw Data* tab. The tab contains a list of samples and charts of experiment data.

8 To learn more about the LightCycler® Software 4.05 window, refer to the next section.

9 To close an experiment select *Close* from the *File* menu or click the X in the upper right corner of the work pane.

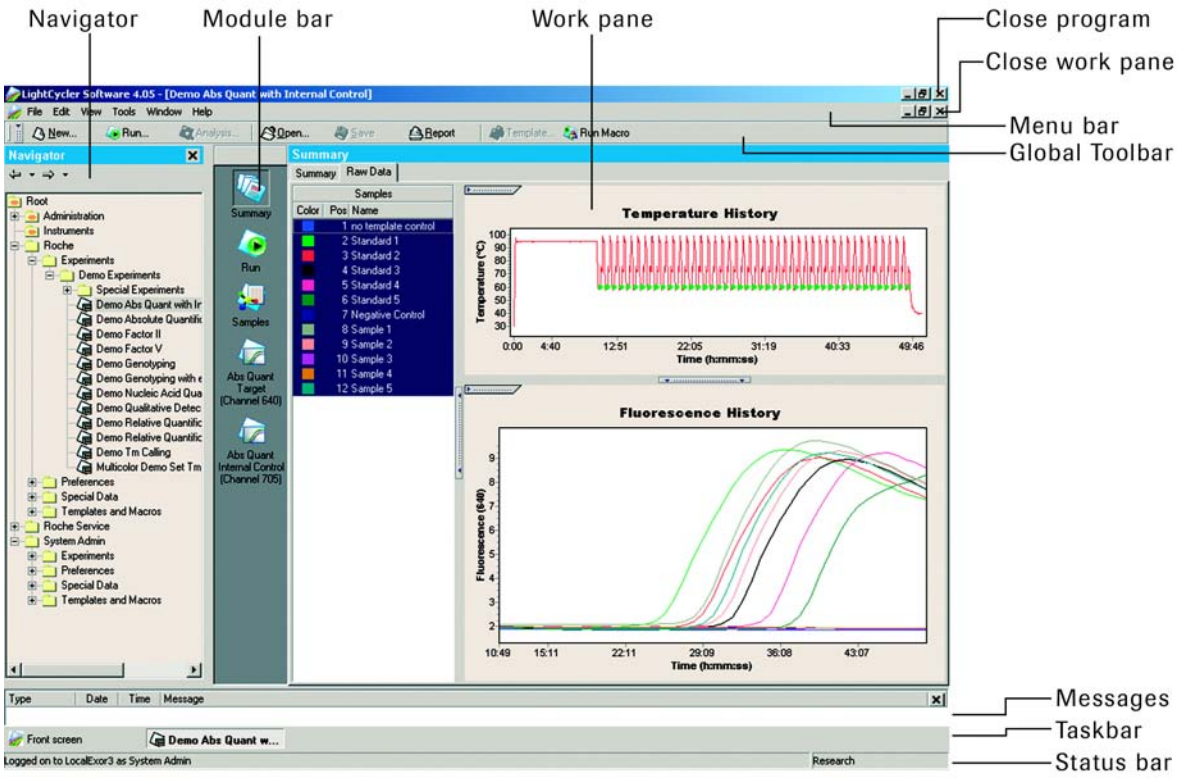
10 To exit the software, select *Exit* from the *File* menu or click the X in the upper right corner of the main window.

D

1

1.2 Understanding the LightCycler® Software 4.05 Window

The figure below illustrates a LightCycler® Software 4.05 window containing information from a demo experiment.



D

The LightCycler® Software 4.05 window contains the following areas:

- The *Navigator* organizes and displays information about users, instruments, and data.
- The M*enu Bar and Global Toolbar* contain menus and buttons for common tasks.
- The *Module Bar* provides access to experiment modules, including protocols, sample information, and analysis modules. The module bar is visible only when the work pane displays experiment information.
- The W*ork Pane* is used to perform the specialized operations of the software, such as programming the experiment, viewing experiment data, or analyzing results. After Login the *Front* window is displayed in this area.
- A *Messages* window displays messages generated from the software during an experiment run as *e.g.*, error messages.
- A T*askbar* and a *Statusbar* are located at the bottom of the window. The taskbar displays icons for all open windows, including ones "hidden" behind the current window. The status bar displays the name of the current user and computer, as well as any status messages generated as you use the software.

The *Global Toolbar*, *Taskbar*, *Statusbar*, *Messages*, *Navigator* and the *Front* window can be independently closed or reopened by deselecting or selecting the area from *View Menu* in the *Menu Bar*. The following sections describe the main areas of the LightCycler® Software 4.05 window in more detail.

1.2.1 The Navigator

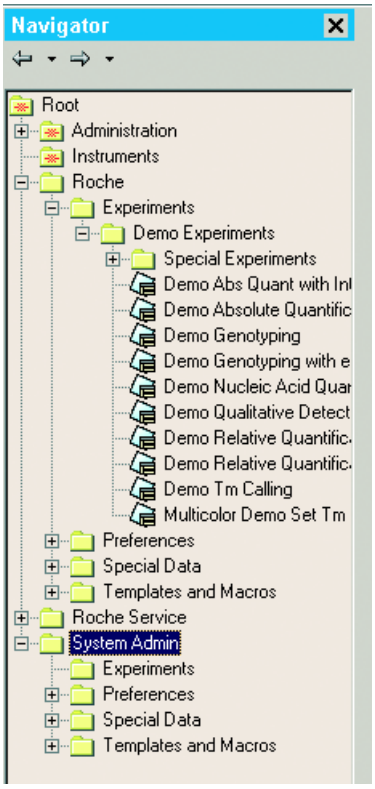
The Navigator on the left side of the LightCycler® Software 4.05 window provides access to items stored in the LightCycler® Software 4.05 database. Items include experiments, user accounts, instruments, macros, and so on. In the Navigator, items are organized in folders in a tree structure.

The Navigator 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.
- A Roche folder that contains experiments, templates, and macros from Roche that can be used by anyone with access to LightCycler® Software 4.05. (To modify a Roche object, you must first copy it to your own user folder.)
- An Instruments folder that contains an object for each LightCycler® Instrument, when the instruments are added to the software.
- An Administration folder that contains objects for user groups, user roles, user accounts, and security policies. The Administration folder is visible only to users with Local Administrator privileges.

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

A typical Navigator is shown below.



- To show or hide items under a folder, double-click the folder name or click the plus (+) or minus (-) sign next to the folder.
- To close the Navigator, deselect Navigator from the *View menu* or click the X in the upper right corner of the Navigator pane. Closing the Navigator causes the work pane to extend the full width of the window.
- To reopen the Navigator from the *View menu*, select Navigator.

1.2.2 The Menu Bar and Global Toolbar

Use the menu bar and global toolbar (along with the Navigator) to create and manage LightCycler® objects. The buttons provide quick access to many commands that are available in the menus.

Using menus

File menu

- Create (*New*), open, save, and close objects
- Log out of the current session
- Import or export files
- Print an experiment report or print the current window
- Exit the software

Edit menu

- Cut, copy, and paste data in the work pane
- Select all items

View menu

- Display or hide the following parts of the LightCycler® Software 4.05 window: the Global Toolbar, Taskbar, Statusbar, Messages and Navigator
- Display the Front window (the window you see when you start LightCycler® Software 4.05) in the work pane

Tools menu

- Change your password
- Open the user management tool (used to create, delete, and edit users, groups, and roles)
- Create, apply and run macros and templates based on an object currently open in the LightCycler® Software 4.05 window
- Manage instrument information
- View database status (for local administrators only)
- Obligate users to enter a Carousel ID in the Policy Editor (for local administrators only)
- Install a Roche Macro

Window menu

- Close, minimize, and arrange windows when multiple windows are open in the work pane
- Select an open window to make it the active window (The bottom of the Window menu displays a list of all currently open windows. The active window is checked.)

Help menu

- Display an online version of the LightCycler® 2.0 Instrument Operator's Manual
- View version and other information about the software, *e.g.* copyright.

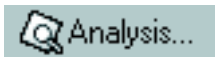
Using toolbar buttons

Use toolbar buttons to perform key LightCycler® Software 4.05 tasks, such as creating a new experiment, opening an existing experiment, saving data, and so on. If a button is inactive (dimmed) it may be because you do not have the necessary permission to perform the task or because the task isn't allowed in the current state. For more information about permissions see chapter *Managing User Access*.

The global toolbar contains the following buttons:

Click *New* to create a new object. A dialog box displays icons for all the objects you can create. Select the type of object you want.

Click *Run* to open the Run module so you can create a new experiment.



Click *Analysis* to add a new analysis module to an existing experiment. A dialog box displays a list of available analyses. The button is enabled only when an experiment is active in the work pane.

Click *Open* to open any object. A navigator opens so you can select the object. (You can also double-click an object in the main Navigator to open it.)

Click *Save* to save any changes you make to an object. The button is enabled only when the object in the active window has been modified.

Click *Report* to open an experiment report window. Choose the information you want to include in the experiment report.

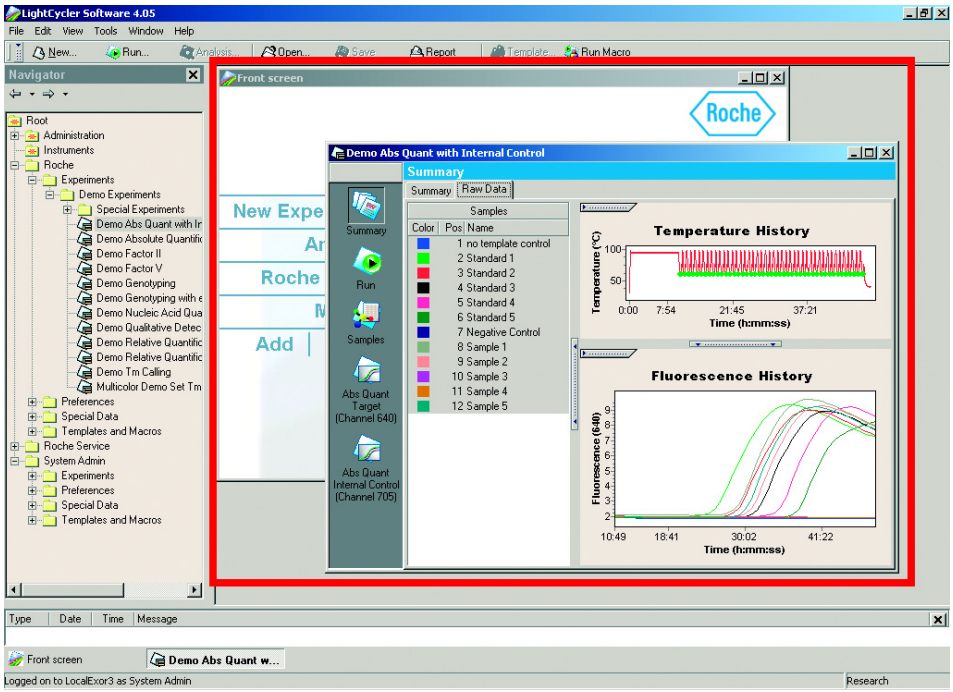
Click *Template* to apply a predefined template to the currently active object. A navigator opens so you can select the template to apply.

Click *Run Macro* to run a predefined macro. A navigator opens so you can select the macro to run.

1.2.3 The Work Pane

The LightCycler® Software Work Pane contains information specific to the operation you are performing. For example, when you run an experiment, the work pane displays a run window that includes a tab for the experiment protocol, a tab that displays the current data being generated, and a tab for experiment notes.

The work pane is designed to allow multiple open windows. A label for each open window is visible in the taskbar at the bottom of the window. The work pane illustrated below displays two windows: the Front window and a window for an experiment.



To view and size windows in the work pane

- Use the mouse pointer to drag a window's edge or corner to resize the window.
- Use the buttons  in the upper right corner of each window to maximize, minimize, restore, or close the window.
- Use the *Window* menu to close, minimize, or arrange the windows.
- Click a label in the taskbar to restore a minimized window or to bring a hidden window to the front.

To size individual sections of Windows

- Drag the horizontal borders between sections to size them.
- Click the handle on the border of a section  to hide or show/ expand the section.

1.2.4 The Module Bar

The module bar contains icons for experiment components. The menu is visible only when an experiment is open. The module bar displays the following icons:

Summary icon opens the Summary module of the experiment, which contains information about the experiment (such as name, date, and owner) and charts of data generated by the experiment.

Run 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.

Samples icon opens the Sample Editor, which contains sample information needed for the experiment and the analyses.

Analysis icons open the associated analysis module. When an analysis is added to an experiment, an analysis icon is added to the module bar. This example shows an icon for an Absolute Quantification analysis.

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 Samples icon is always third in the list of icons, so to open the Sample Editor module, press Ctrl-Shift-3. (Hold the mouse pointer over an icon to see the name of the related module and the keyboard shortcut for opening the module.)
- Click *Analysis* of the toolbar or right-click the module bar and select New Analysis to add an analysis module to an experiment.
- Right-click an analysis icon to display a menu that lets you
  - Remove the current analysis from the experiment.
  - Rename the current analysis. Renaming is helpful if you have more than one analysis of the same type, such as two absolute quantification analyses.

1.3 Opening, Creating, and Saving Objects

You can use the Navigator and the toolbar menus and buttons to open, create, or save LightCycler® objects. (The objects you are allowed to create depend on your user status.)

To open an existing object

Double-click the object name in the Navigator or click Open... on the toolbar or select *Open* from the *File* menu. A small navigator opens in a dialog box. Select the object name, then click *OK*. The information for the object is displayed in the work pane.

To create and save a new object

- Click New... on the toolbar, or from the *File* menu select *New*. A dialog box opens containing icons for LightCycler® objects. Select the icon for the object you want, then click *OK*.

Tip: To create a new experiment, you can also click Run... *on the toolbar or New Experiment on the Front window.*

- Enter information in the work pane to define the object.
- Click Save on the toolbar, or from the *File* menu, select *Save*.

1.4 Copying Items

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). For example, you might want to copy a macro template from the Roche folder into your own Templates folder and then modify it.

To copy items from another user's folder

- Right-click the item you want to copy in the *Navigator*, then select *Copy*. A small navigator dialog opens.
- Select a target folder enter a new name for the item if desired, then click *OK*.

1.5 Renaming and Deleting Objects

Your user role determines which objects you can rename or delete. For more information about user roles see chapter *Managing User Access*.

To rename an object

Right-click the object in the *Navigator*, then select *Rename* or select the object in the Navigator, then press *F2*. The object name becomes an editable text box. Type in the new name of the object.

To delete an object

Right-click the object in the Navigator, then select *Delete*. Or select the object, then press the keyboard *Delete* key. Click *OK* to confirm the deletion.

1.6 Exporting and Importing Files and Objects

To view experiment information in LightCycler® Software 4.05, the experiment file must be stored in a LightCycler® Software 4.05 database. Therefore, if experiment files are currently stored on a hard drive, or a diskette, 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® Software 4.05.

For example, you need to import files in these cases:

- If you want to view experiment files from previous versions of LightCycler® software.
- If you want to move a LightCycler® Software 4.05 experiment file from one LightCycler® Software 4.05 database to another; in this case you must first export the file from the database to a location such as your hard drive, then import the file into the other database.

Using the Import command on the *File* menu, you can import the following:

- ATF (experiment file from LightCycler® software V3.5.3); you can import all the FLO files from an entire directory at one time using the LightCycler® Software 4.05 batch import tool, described below. (Importing FLO files automatically imports the corresponding ABT and TEM files.)
- COF (coefficient files from LightCycler® Relative Quantification Software V1.01)
- IXO (experiment file from LightCycler® Software 4.05)

Read the sections below to learn how to import and export LightCycler® Software 4.05 files individually or by directory.

You can import a MPLC or a LCDA 3.5.3 sample (SAM) file into a new experiment, using the Import SAM button in the LightCycler® Software 4.05 Sample Editor. For more information see chapter Creating and Running an experiment.

D

1.6.1 Exporting Individual LightCycler® Software 4.05 Objects

To store LightCycler® Software 4.05 experiment objects or templates outside the LightCycler® Software 4.05 database or to transfer LightCycler® Software 4.05 objects between databases, you must export the LightCycler® Software 4.05 files. Exporting a file does not remove the object from the database, but copies the file into XML and stores it at the location you designate. The exported file has an .ixo file extension.

To export a LightCycler® Software 4.05 object:

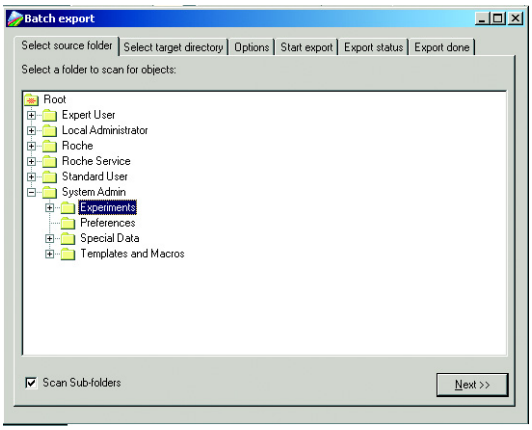
1. In LightCycler® Software 4.05 open the experiment or template you want to export.
   The object must be open in the Work Pane before you can export it.
2. From the *File* menu, select *Export*.
3. Navigate to a location to save the exported file, select *Object .ixo Files* (IXO) as the file type, enter a new file name (or keep the existing name), then click *Save*. The file is saved with an .ixo extension.

1.6.2 Exporting Multiple Experiment Files simultaneously

LightCycler® Software 4.05 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.

To export a directory of experiment files:

1. From the LightCycler® Software 4.05 *File* menu, select *Batch* export. The Batch export dialog box is displayed.



2. Select the folder containing the experiment files. Check the *Scan Sub-folders* box if you want to include the subdirectories of the directory. Click *Next*.

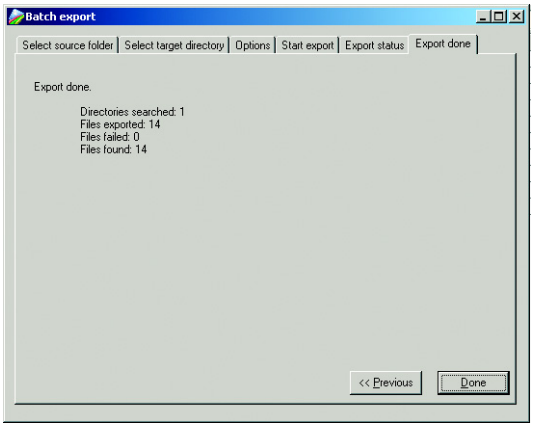
3. In the *Select target directory* tab select the folder in which to store the exported files. You can either enter a directory manually (check the *Directory doesn't exist* box to create a new directory), or use the *Browse* function to search for an existing directory.

4. In the *Options* tab select the types of objects to export.

5. Click *Next* in the *Start export* tab to start the batch export.

6. As the files are exported the *Export status* tab displays status messages. If a file cannot be exported successfully, the word FAILED appears in front of the file path name, and the status message indicates the cause of the failure.

7. When all the files have been exported view the results on the *Export done* tab.



8. If any of the files could not be exported, repeat the export process as follows: Change the export options and parameters on any of the tabs as necessary, then click *Reset* on the *Start export* tab. Press *Next* to repeat the export process.

9. When finished, click *Done* on the *Export done* tab to close the dialog box.

1.6.3 Importing Individual Files

You can import individual LightCycler® Software 4.05 object (IXO) files, as well as FLO (experiment), and COF (coefficient files) files from previous versions of LightCycler® Software. You can also import all the experiment files from an entire directory at one time. After you import a file, you can save it as an object in your LightCycler® Software 4.05 database.

To import an individual file:

1. From the LightCycler® Software 4.05 *File* menu, select *Import*, then select the type of file you want, as follows:
   - For FLO, ABT, TEM files, select *ATF Files*.
   - For COF files, select *COF Std. Curve Files*.
   - For LightCycler® Software 4.05 object files, select *Object .ixo Files*.
2. Find and select the file you want, then click *Open*. The file is imported and is opened in the work pane.

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

   If you import an experiment that includes color compensation, the color compensation data is displayed in the work pane. To see the experiment, click the experiment name in the taskbar.
3. To save the imported file as an object in the current LightCycler® Software 4.05 database, click *Save*.
4. Navigate to a location in LightCycler® Software 4.05 to save the object and enter a new object name, then click OK. If an imported experiment includes color compensation data, the color compensation data is automatically opened with the imported experiment and should be saved by the user in the CCC subfolder of his Special Data folder

D

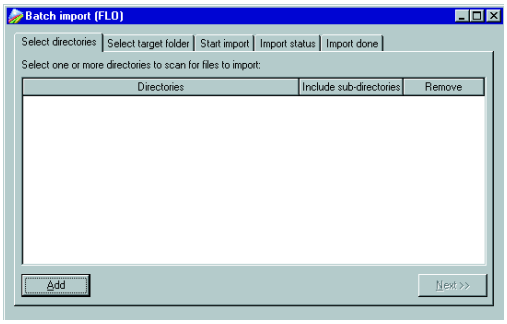
1.6.4 Importing Multiple Experiment Files simultaneously

LightCycler® Software 4.05 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.

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

To import a directory of experiment files:

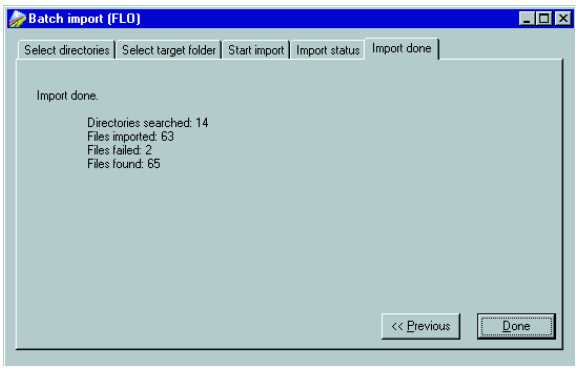
1. From the LightCycler® Software 4.05 *File* menu, select *Batch import*. The Batch Import dialog box is displayed.



2. Click *Add* in the *Select directories* tab to find and select the directory containing the experiment files, then click *OK*. The directory path is added to the window.
3. Check *Include Subdirectories* if you want to include the subdirectories of the directory.
4. Repeat Steps 2 and 3 to add additional directories as needed. Press the *Remove* button to erase a selected directory. When finished adding directories, click *Next*.
5. In the *Select target folder* tab, select the folder in which to store the imported files, then click *Next*.
6. In the upper part of the *Start import* tab select the types of files to import:
   - ATF files: ABT, TEM, FLO files from LightCycler® Software 3.5.3
   - COF Std. Curve Files: coefficient files from LightCycler® Relative Quantification Software V1.01
   - Object .ixo files: experiment files from LightCycler® Software 4.0 or 4.05

   In the lower part of the *Start import* tab select the action to take if an imported file has the same name as an existing file, as follows:
   - Don't do anything. The new file is not imported if there is an existing file of the same name. The word "Failed" appears next to the file name in the status messages on the Status import tab.
   - Replace the existing one with the new one.
   - Change the name of the new one. A number is appended to the end of the file name of the imported file.
   - Don't save but leave open when done. The imported file is opened in LightCycler® Software 4.05, but is not saved in the LightCycler® Software 4.05 database. You can view and then manually save the file, if desired.
7. Click *Next*. As the files are imported, the *Import status* tab displays status messages. If a file cannot be imported successfully, the word FAILED appears in front of the file path name, and the status message indicates the cause of the failure.

8	When all the files have been imported, view results on the *Import done* tab.



9	If any of the files could not be imported, repeat the import process as follows: Change the import parameters on any of the tabs as necessary, then click *Reset*. Select the *Start import* tab, then click *Next* to repeat the import process.
10	When finished, click *Done* on the *Import done* tab to close the dialog box.

To repeat an import:

If any of the files in a directory failed to be imported successfully, you may need to repeat the import after correcting any errors. For example, a FLO file cannot be imported if its corresponding ABT or TEM file is missing. Or you may want to reimport a set of files using different import options.

You can quickly repeat an import as follows:

1	After performing the initial import, click *Previous* twice to return to the *Start Import* tab.
2	Click *Reset*.
3	Clicking *Reset* prepares the software for a new import.
4	(Optional) If desired, select a different import option on the *Start Import* tab.
5	Click *Next* to start the new import.

2. Creating and Running an Experiment

LightCycler® Software version 4.05 controls the LightCycler® 2.0 Instrument using information provided in an experiment protocol. During an experiment run, the protocol controls the instrument's temperatures and hold times, the number of cycles being executed, and so on. As the experiment progresses, LightCycler® Software 4.05 gathers temperature and fluorescence data from the instrument and displays it on the LightCycler® Software 4.05 window. After the experiment is finished, you can save the experiment data and use LightCycler® Software 4.05 analysis modules to analyze results.

This chapter explains how to do the following:

- Create and run an experiment protocol
- Enter sample information

For information about analyzing experiment data see chapter *Overview of Experiment Analysis*.

For you to create an experiment protocol, your user account must have the Expert User or Local Administrator role. If your user account has the Standard User role, you can run macros for predefined protocols. For information about running macros see chapter *Using Templates and Macros*.

2.1 Creating a Protocol and Running the Experiment

Follow the general steps below to create a new experiment protocol and run the experiment. Detailed information for each step follows the general procedure.

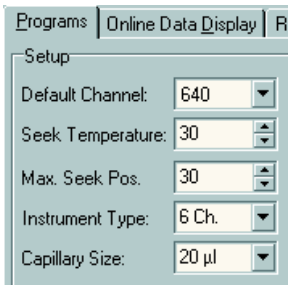
To create a protocol and run the experiment:

1. Turn on the computer and the LightCycler® 2.0 Instrument. Start the LightCycler® Software and log in.

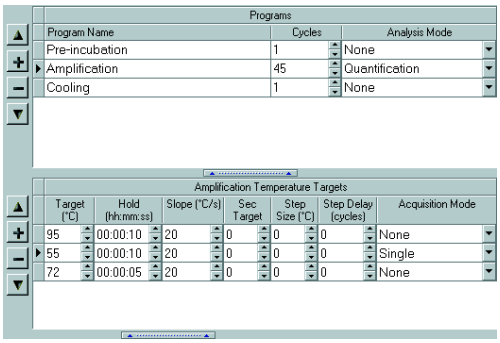
   Ensure that the instrument is running before you start the software.

2. Click *New Experiment* in the work pane or *Run* in the global toolbar. The *Programs* tab of the Run module is displayed.

3. In the *Setup* section of the *Programs* tab, specify general instrument settings. (For more information see *Entering Setup Information* below.)

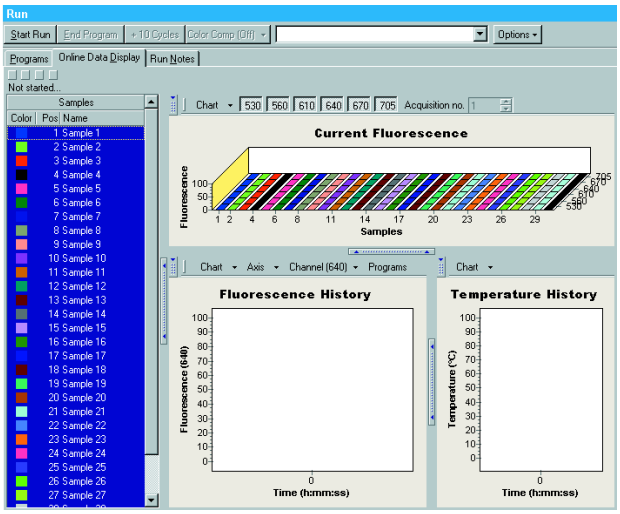


4 In the *Programs* section of the *Programs* tab, define one or more experiment programs. For each program, define temperature targets. (For more information see *Defining Programs and Temperature Targets* below.)



5 (Optional:) Add any additional information in the *Run Notes* tab.

6 Select the charts you want to view as the experiment runs in the *Online Data Display* tab. (For more information see *Customizing the Online Data Display* below.)



7 (Optional:) Perform an instrument self test.

8 Prepare a LightCycler® run as described in chapter *Operation*.

9 Load capillaries into the LightCycler® 2.0 Instrument.

10 Enter sample information. (For more information see *Entering Sample Information* below.)

You can also enter sample information during or after the run.

11 Click *Start Run* to run the experiment, then monitor data as it is gathered from the instrument. (For more information see *Running the Experiment* below.)

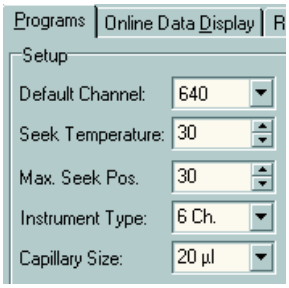
Assure that the Windows screensaver function is turned off before starting a LightCycler® run. Startup of the screensaver during a LightCycler® run may lead to a loss of data.

12 When the experiment is finished, click *Save* to save the experiment data.

Read the following sections for more information about the key steps described above.

2.1.1 Entering Setup Information

The Setup section of the Run module is shown below.



- In the *Default Channel* field, select the default fluorimeter channel used to gather fluorescence data. Data is gathered from all channels, but the selected channel becomes the default channel for which information is displayed during the experiment and in the analysis modules.
- In the Seek Temperature field, enter the temperature the instrument reaches for the seek process.

  In most cases the default temperature of 30°C need not to be changed. For some applications as e.g., a reverse transcription (RT) PCR it might be a good idea to seek at the same temperature that is used for the RT-step.

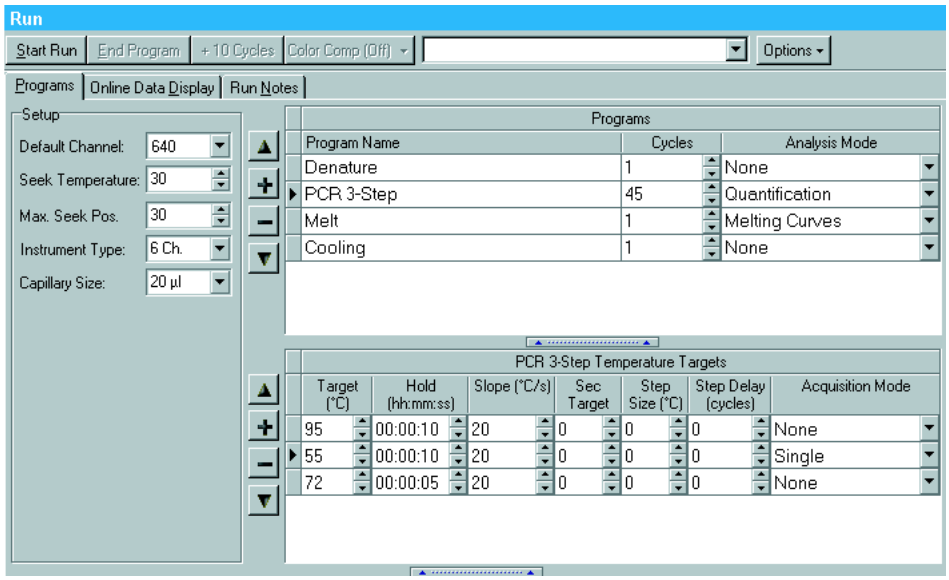
  The instrument is not able to cool below room temperature. If you program a lower temperature, which cannot be reached by the instrument, the seek process will be performed at the lowest possible temperature.
- In the *Max. Seek Pos.* field, enter the highest position number in the sample carousel holding a capillary. After the run begins, the instrument carousel rotates to detect (seek) the number of samples you specify.
- In the *Instrument Type* field, choose the *6 Ch.* Instrument type for your LightCycler® 2.0 Instrument (selected by default). For LightCycler® 1.1 and 1.2 select the *3 Ch.* Instrument type.
- For the 6 Ch. Instrument type a *Capillary Size* field is displayed. Select the capillary size for the experiment.

2

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 a temperature for the LightCycler® 2.0 Instrument, a hold time for the temperature, the speed with which the temperature is reached, and so on. You define programs and their temperature targets in the *Programs* tab of the Run module.

In the example below, there are four programs: Denature, PCR 3-Step, Melt and Cooling. When the PCR 3-Step program is selected (45 cycles) the temperatures appear in the table below, automatically. However temperatures should be selected, as required by the user.



D

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.

2. Edit the default values for the following program parameters, clicking the tab button on your keyboard to move from one column to the next. (If you make a mistake, click (-) to delete the program, then start over.)

Parameter	Description/Instructions	Valid Values
Program Name	The name for the program. Click in the Program Name box, then 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 on the up and down arrows.	1-99 cycles
Analysis Mode	The type of analysis expected for this program (if any). The program will be selected automatically when you add the designated type of analysis module after the experiment has finished running. Select an analysis mode from the pull-down list.	**None:** No analysis is expected. However, if you add an analysis module after the run, the software will automatically select the appropriate program for the analysis. **Melting Curves:** A melting curve analysis is expected. **Quantification:** A quantification 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.

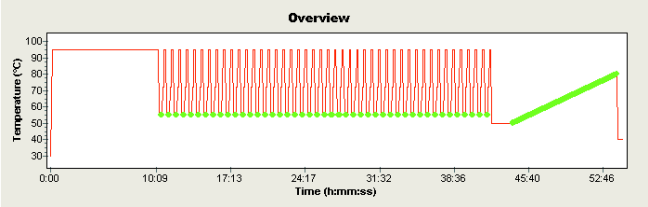
Parameter	Description/Instructions	Valid Values
Target	The target temperature. Enter a temperature.	37°C-98°C
Hold	The length of time to hold the target temperature in hours:minutes:seconds. Enter a hold time.	00:00:00-12:00:00
Slope	The rate at which the program will reach the target temperature, specified as degrees per second. Enter a slope.	0.05°C-20°C per second
Sec. Target	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. (This is useful for performing *touchdown* PCR.) Enter a temperature.	37°C-98°C
Step Size	The number of degrees the temperature will increase after each cycle, in order to reach the secondary target. Enter a step size.	0°C-20°C
Step Delay	The cycle number at which the temperature step up or step down begins. Enter a cycle number.	0-99 cycles
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. The rate depends on the number of samples in the reaction and is fastest for one sample. This is the recommended setting for a melt. The valid range for the Ramp Rate is 0.05°C/s to 0.2°C/s. If you select a value outside this range a default value of 0.05°C/s will be set. **Step:** Acquires fluorescence data at each temperature transition. For example, if 0.5°C/sec is the Slope, fluorescence data is acquired every 0.5°C. This setting can be useful in melts. The valid range for the Ramp Rate is 0.3°C/s to 1.0°C/s. If you select a value outside this range a default value of 0.3°C/s will be set.

4. Click (+) to add another temperature target to the current program, then enter parameter values. Repeat to define as many temperature targets as you need for the current program.

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, then click the up ▲ or down ▼ arrow to move the item up or down in the list.
   To delete an item, select the item, then click (-).

6. Look at the *Overview* section to see a graphical representation of all the programs you have defined.



7. Click *Save* in the global toolbar to save the protocol. Navigate to a location to save the protocol, enter a protocol name, then click *OK*.

2

2.1.3 Customizing the Online Data Display

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

Current Fluorescence: For selected samples, displays fluorescence in each channel at each fluorescence acquisition point

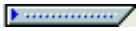
Fluorescence History: For selected samples, displays fluorescence in a user-designated channel over time

Temperature History: Temperature and data acquisition points during the experiment run

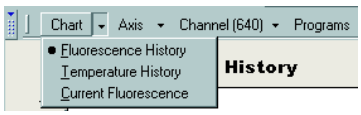
You can change the type of chart displayed and modify display options for the two fluorescence charts.

If you prefer, you can customize the online data display while the experiment is running.

To customize the online data display

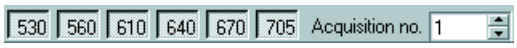
In the *Run* module, select the *Online Data Display* tab, then click  above the chart you want to change. The chart's options toolbar containing a Chart menu, is dlsplayed. Fluorescence charts contain additional options.

To change the chart type, from the *Chart* menu, select the new chart type.

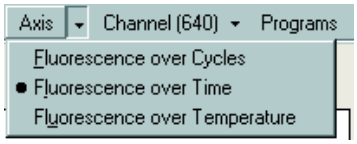


To modify display options for the fluorescence charts:

- For a *Current Fluorescence* chart select the channels to include in the chart. To see the data from a previous acquisition, enter or select an acquisition number in the *Acquisition no.* field.



- For a *Fluorescence History* chart select the *Axis* type (the type of data represented by the X axis), the *Channel*, and the *Program* containing the data you want to see.



D

2.1.4 Performing an Instrument Self-Test

Before you load capillaries into the LightCycler® 2.0 Instrument, you can perform an instrument test that checks all instrument functions, such as heating to the correct temperature and rotating the carousel.

We recommend that you perform an instrument self-test once a day before starting LightCycler® experiments. Always insert an empty carousel when performing an instrument self-test.

To perform the instrument self-test:

- In the *Run* module, click *Options* next to the instrument selection box.
- Select *Run Self-Test*. The instrument performs the test and displays a message that the test passed or failed. Click *OK* to clear the message.
- If the test failed, refer to chapter F *Troubleshooting* for further instructions or contact your local Roche representative, otherwise, run the experiment.

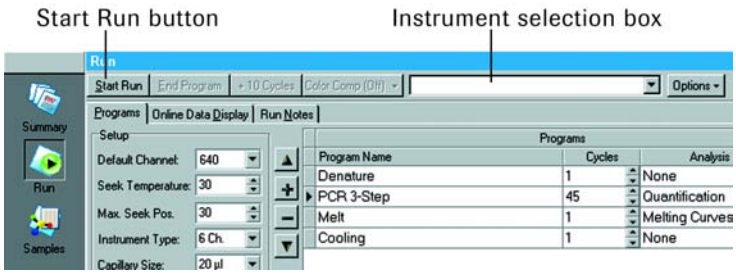
2

2.1.5 Running the Experiment

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

To start an experiment run:

1. Prepare a LightCycler® run as described in chapter C *Operation*.
2. Insert the capillaries into the LightCycler® carousel.
3. In the *Run* module, select an instrument from the instrument selection box. (If only one instrument is connected to the computer, it is selected by default.)



4. Click *Start Run*. The Online Data Display tab displays a status message that indicates the software is connecting to the instrument.

   If for any reason the software cannot find the instrument, see chapter Instrument Cannot be Detected.

5. You are prompted to save the experiment. Enter a name and a folder where you want to save the experiment.
6. Status messages indicate the progress as the instrument performs the following preparatory steps:
   - Sets the seek temperature specified in the program
   - Waits while the LED warms up
   - Seeks the sample positions. The instrument displays a message similar to the following as it locates each sample:
     Position 2 of 32: found "Neg Control"
   - Begins the experiment run. Messages indicate which program, cycle, and segment (temperature target) is being executed, for example:
     Program 2 of 4 (Amplification), cycle 5 of 45, segment 1 of 3.

   As the experiment progresses, the data is displayed in the charts on the *Online Data Display* 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 *Customizing the Online Data Display* above.

D

8 (Optional) To adjust or stop the program during the run:

- Click *End Program* to stop the current run program and skip to the next program in the experiment protocol.
- Click *+10 Cycles* to add 10 cycles to the current program.

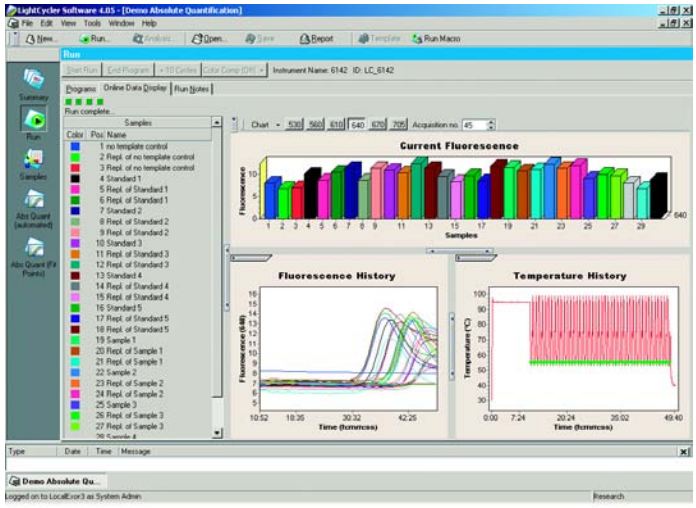
The End Program and the +10 Cycles buttons are inactive during execution of programs with acquisition modes "None", "Continuous" and "Step".

- Click *Abort Run* to stop the run. (The *Abort Run* button replaces the *Start Run* button during run.)

This will lead to a loss of data of the aborted experiment. An aborted run cannot be restarted.

If you abort a run, the instrument may be hot. Before you open the lid, wait until the instrument has cooled down.

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



10 Click *Samples* in the module bar to open the Sample Editor, and complete sample information if necessary.
For more information see *Entering Sample Information* below.

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

2.1.6 Instrument Cannot be Detected

If for any reason the instrument cannot be detected when you start the experiment, a message prompts you to search for the instrument. Follow these steps:

- First determine whether the instrument is running, and turn it on if necessary. The software cannot detect an instrument that has not been turned on.
- Check all connections.
- Click *Yes* in the message box to search for the instrument, then follow the remaining prompts to find and select the instrument.

If the instrument is still not detected, call your local Roche service.

2.2 Entering Sample Information

Use the LightCycler® Software 4.05 Sample Editor to record information about the samples in the experiment. You can enter sample information before, during, or after an experiment. However, you cannot change the number of samples after the experiment run has begun.

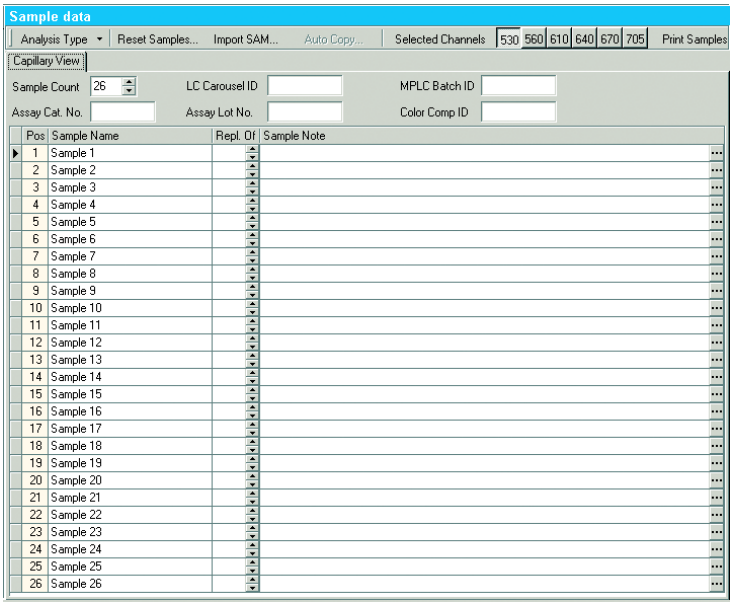
You can also import a SAM file from a MagNA Pure LC purification or an older version of LightCycler® software, if you want to use your usual experiment sample settings.

If you have Standard User privileges you can enter or change sample names, sample notes, and the number of samples (before the run begins). If you have Expert User or Local Administrator privileges, you can use all the features of the Sample Editor described in the steps below.

To enter sample information:

1. Click *Samples* in the module bar to open the *Sample Editor*.

2. The *Capillary View* tab is displayed. The *Sample Count* field defaults to the value you specified in the *Max Seek Pos field* in the Run module. (You cannot change this value after the run has begun.) You can also add Analysis Tabs. For more information about these tabs refer to the appropriate analysis chapters.



3. In the *Selected Channels* area, click the channels to be used in the experiment. For your LightCycler® 2.0 Instrument, six channel buttons are displayed. If you are using a LightCycler® 1.1 or 1.2 Instrument, three channel buttons are displayed.

4. (Optional) Type values in the following boxes to identify the run.
   - LC Carousel ID: A name or other identifier for the carousel used in this experiment
   - MPLC Batch ID: A MagnaPure LC Batch ID
   - Assay Cat. No.: A catalog number for a Roche kit (if using this kit)
   - Assay Lot No.: A chemical manufacturing lot number of a Roche kit
   - Color Comp ID: A color compensation ID used for this experiment

5 Enter the following information for each sample:

Parameter	Description	Valid Values
Sample Name	The name of the sample	Alphanumeric characters; spaces are allowed (the length of sample names is limited to 25 characters)
Repl. Of	The position number of another sample this sample is a replicate of	The number of another sample that is not already a replicate (you cannot create a replicate of a replicate)
Sample Note	Any additional information about the sample	Alphanumeric characters

6 If you need to start over, click *Reset Samples*. You are prompted to confirm resetting the values. Click *OK*. Resetting the samples leaves the same number of samples, but resets the sample names and sample notes to the default values (including the sample names and sample notes of the analysis-specific sample editor tabs).

7 When finished, click *Save* in the global toolbar to save the sample information with the experiment. (Note that this is not possible during run.)

8 To print out your sample loading list, click *Print Samples*.
The print-out contains the following information:
- Assay Cat. No.
- Assay Lot No.
- For each sample:
  - position
  - sample name
  - replicate information
  - sample note

D

To import a SAM file:

1 To import a MagNA Pure LC or LightCycler® SAM file containing the sample information from a MagNA Pure nucleic acid isolation or an older version of LightCycler® software, open the sample editor as described above. Click *Import* SAM in the toolbar of the work pane, find and select the SAM file, then click *Open* or use a barcode reader to scan the *barcode* of your SAM file generated by a MagNA Pure instrument.

Importing a SAM file is only possible before a run has started.

LightCycler® Software 4.05 checks if the information, imported with the SAM file (e.g., sample data, LC carousel ID, MagNA Pure Batch ID) matches with the information entered in the experiment. If this check fails, an error message appears. Follow the instructions displayed.

2 If you need to start over, click *Reset Samples*. You are prompted to confirm resetting the values. Click *OK*. Resetting the samples leaves the same number of samples, but resets the sample names and sample notes to the default values (including the sample names and sample notes from the analysis-specific sample editor tabs).

3 When finished, click *Save* in the global toolbar to save the sample information with the experiment.

3. Overview of Experiment Analysis

LightCycler® Software 4.05 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.

To add an analysis module, you must have either Expert User or Local Administrator privileges. If you have Standard User privileges you can view analyses that have already been added to an experiment, but you cannot add the modules yourself. You can run predefined experiment macros, which may add analysis modules automatically.

The following analysis modules are available:

Absolute Quantification:
Calculates the concentration of target DNA in unknown samples, based on the concentration of standard samples.

Qualitative Detection:
Determines whether a target sequence is present in unknown samples.

Relative Quantification (Monocolor and Dual Color):
Compares the ratio of two sequences in unknown samples to the ratio of the same two sequences in known samples.

Genotyping:
Defines genotype groups based on melting temperature profiles of the unknown samples or based on imported standard profiles.

Tm 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.

Nucleic Acid Quantification:
Calculates the concentration of DNA in unknown samples without amplification by comparing the samples' fluorescence to the fluorescence of known samples using fluorescent dyes such as Pico Green.

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.

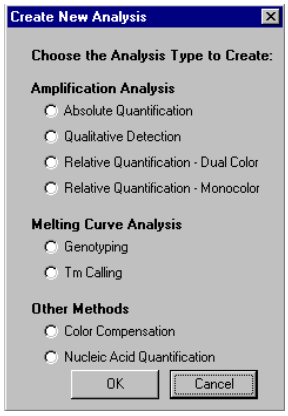
3.1 Overview of Analysis Steps

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 specific analysis sections.

To perform an analysis:

1. Open the experiment you want to analyze in the LightCycler® Software 4.05 work pane.

2. Click *Analysis* on the LightCycler® Software 4.05 toolbar, or right-click the module bar then select *New Analysis*. The *Create New Analysis* dialog box opens.



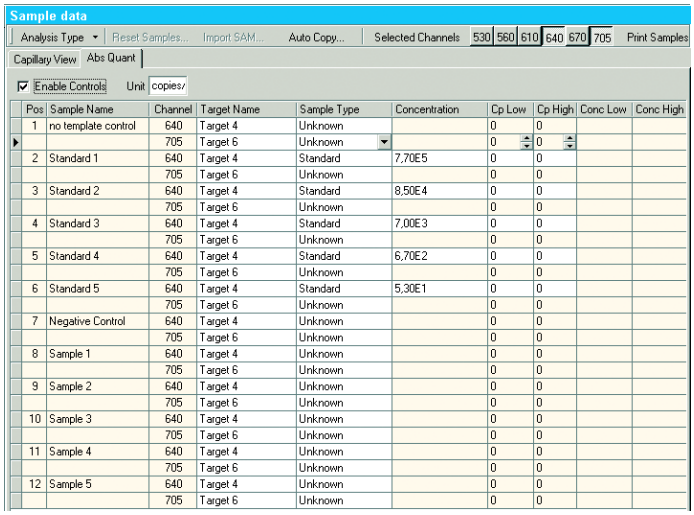
3. Select the analysis type you want, then click *OK*. If you create an analysis that has not existed before, the software prompts you to enter the sample settings before creating the analysis. If you answer with *Yes*, you are taken to the analysis specific sample editor tab to enter the sample settings. No analysis is created in this case. To create an analysis repeat step 2. If you answer with *No* the analysis module is added to the experiment and an icon for the analysis is displayed in the module bar. An analysis-specific tab is added automatically to the Sample Editor to allow for entering analysis-specific information.

   If desired, you can manually add an analysis-specific tab to the Sample Editor before you add the analysis module to the experiment. You might want to do this if you prefer to add all sample information to the experiment at one time, for example before you run the experiment. For more information see Manually adding an analysis-specific tab to the Sample Editor, below.

4. In the *module bar*, click *Samples*. If you have not already entered general sample information, select the *Capillary View* tab, then select the channels used in the experiment and enter information to identify the samples.

3

5 Select the analysis-specific tab in the *Sample Editor*, then enter sample information for the analysis.
In the analysis-specific tab, each sample position includes a row for each channel. That is, if you selected three channels, there are three rows for each sample position on the analysis-specific tab. You can enter sample information in each channel row. The figure below illustrates the Absolute Quantification tab in the Sample Editor. The tab contains fields for sample types and control information specific to an Absolute Quantification analysis.



Pos	Sample Name	Channel	Target Name	Sample Type	Concentration	Cp Low	Cp High	Conc Low	Conc High
1	no template control	640	Target 4	Unknown		0	0		
		705	Target 6	Unknown		0	0		
2	Standard 1	640	Target 4	Standard	7,70E5	0	0		
		705	Target 6	Unknown		0	0		
3	Standard 2	640	Target 4	Standard	8,50E4	0	0		
		705	Target 6	Unknown		0	0		
4	Standard 3	640	Target 4	Standard	7,00E3	0	0		
		705	Target 6	Unknown		0	0		
5	Standard 4	640	Target 4	Standard	6,70E2	0	0		
		705	Target 6	Unknown		0	0		
6	Standard 5	640	Target 4	Standard	5,30E1	0	0		
		705	Target 6	Unknown		0	0		
7	Negative Control	640	Target 4	Unknown		0	0		
		705	Target 6	Unknown		0	0		
8	Sample 1	640	Target 4	Unknown		0	0		
		705	Target 6	Unknown		0	0		
9	Sample 2	640	Target 4	Unknown		0	0		
		705	Target 6	Unknown		0	0		
10	Sample 3	640	Target 4	Unknown		0	0		
		705	Target 6	Unknown		0	0		
11	Sample 4	640	Target 4	Unknown		0	0		
		705	Target 6	Unknown		0	0		
12	Sample 5	640	Target 4	Unknown		0	0		
		705	Target 6	Unknown		0	0		

The kind of information you can enter in each analysis-specific tab depends on the type of analysis.

6 Click the analysis icon in the *module bar* to open the analysis window; perform the analysis by entering or adjusting analysis parameters. For more information, see the individual analysis sections.

If you change information in the Sample Editor (except sample name, sample note or target name) after performing the analysis, the analysis results are recalculated using the updated values from the Sample Editor.

7 Repeat steps 2-6 to add additional analysis modules. You can add more than one analysis to an experiment, including multiple instances of the same analysis type.

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

D

3.2 Tips for Using Analysis-specific Sample Editors

When you add an analysis to a completed experiment, and the analysis is a type you have not yet performed, an analysis-specific tab is automatically added to the Sample Editor.

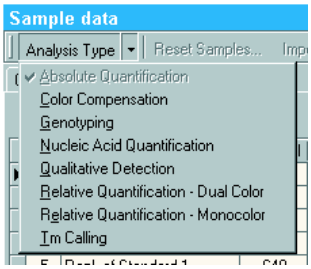
However, you can manually add an analysis-specific tab to the Sample Editor, if desired. For example, you might want to add an analysis-specific tab before running the experiment and then enter both the general sample information and the analysis-specific information while the experiment is running.

You can only add one analysis tab for any individual analysis type, per experiment.

3.2.1 Manually Adding an Analysis-specific Tab to the Sample Editor

To manually add an analysis-specific tab to the Sample Editor:

1. Open the experiment you want to analyze.
2. In the *module bar*, click *Samples*.
3. In the *Sample Editor* toolbar, click *Analysis Type*, then select the analysis type from the menu. Check marks indicate analysis tabs that have already been added to the Sample Editor. The analysis-specific tab you selected is added to the Sample Editor window.

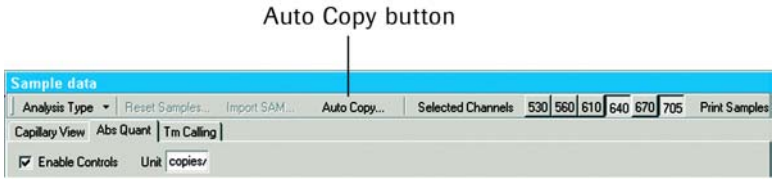


To delete an analysis-specific tab, clear the check mark next to the analysis type. You cannot delete an analysis-specific tab if an analysis module of the same type already exists.

3

3.2.2 Copying Information in the Sample Editor

You can use the *Auto Copy* button to quickly copy sample information in an analysis-specific Sample Editor. For example, if the same internal control is used for each sample in a multicolor experiment, you can enter the information once, then use Auto Copy to copy the information to the appropriate channel for each sample.



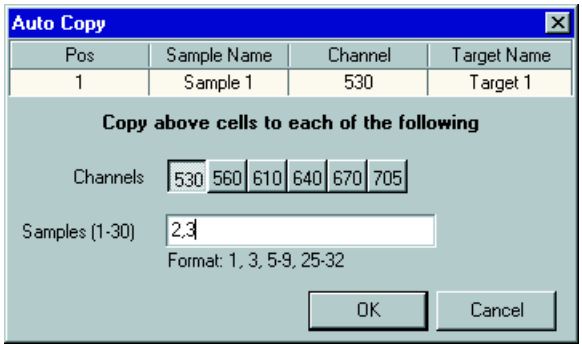
Keep in mind the following restrictions when using Auto Copy:

- The Auto Copy button is available only for the analysis-specific Sample Editor tabs.
- You can copy only the information in the white cells of the sample editor; other cells contain read-only information.
- You can copy information from multiple cells at one time, but the cells must be in the same row; you cannot copy cells from multiple rows.

D

To use Auto Copy:

1. Open the analysis-specific tab of the *Sample Editor*.
2. Hold down the *Shift* key, then click and drag to select the cells you want to copy. (Cells must be in the same row.) After you select cells, the Auto Copy button becomes active.
3. Click *Auto Copy*. The information from the selected cells is displayed at the top of the Auto Copy dialog box.

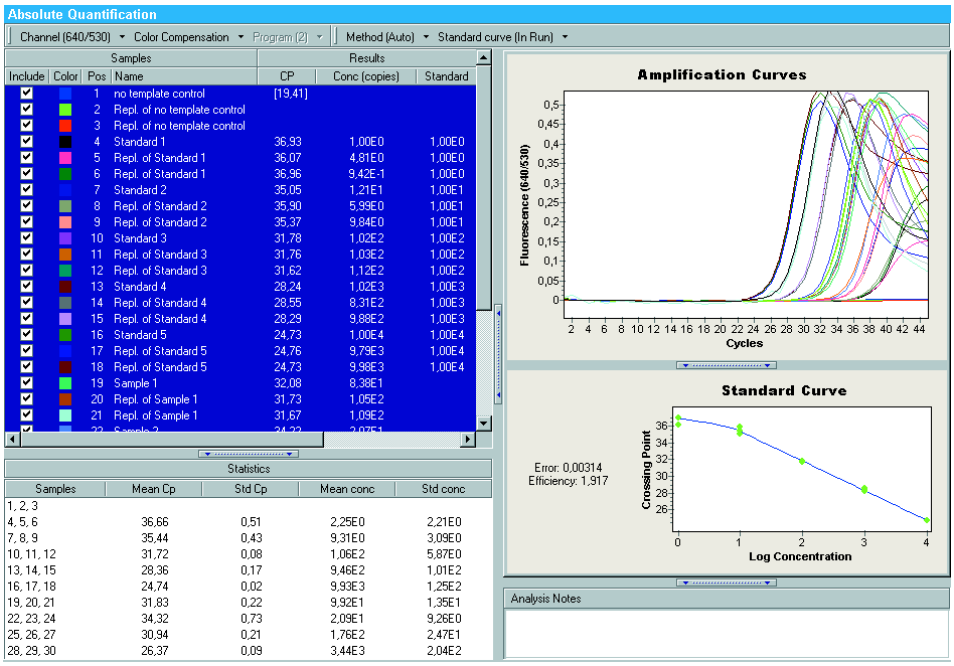


4. Select the channels you want to copy the information to.
5. Enter the sample positions to copy the information to, using any of the following formats:
   An individual sample number or multiple numbers separated by commas (1,3,5)
   A sample number range, separated by a dash (1-5)
   A sample number range from the beginning of the list to a particular number (-5)
   A sample number range from a particular number to the end of the list (5-)
   Any combination of the formats above
6. Click *OK*.
   You are warned that copying cell data may cause existing analysis modules to be recalculated.
7. Click *Yes* to continue. (If you do not want to see the warning again, select *Don't ask this question again*.)
8. The information is copied to the designated channels in the designated sample positions.

3.3 Tips for Using the Analysis Window

The following figure illustrates a typical analysis window, in this case for an Absolute Quantification analysis. A toolbar for the analysis is at the top, the list of experiment samples is on the left, and the areas of the window containing analysis charts and notes are on the right.

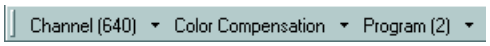
Closing the Navigator expands the Work Pane.



3.3.1 Selecting Channel, Color Compensation and Program

To perform an analysis you must specify the channel you want to analyze, apply color compensation (if appropriate), and select the experiment program you want to analyze (such as a melting curve).

Use the buttons on the analysis module toolbar, shown below, to make the necessary selections. (The Nucleic Acid Quantification module and the Color Compensation module have slightly different options. For more information, see the sections related to these modules.)



If your user account has the Standard User role, this toolbar is not visible in the analysis window because the selections have already been made for the analyses that you can view.

Use the buttons as follows:

Channel: Use *Channel* to select the fluorescence channel you want to analyze.

Color Compensation: Use *Color Compensation* to select a color compensation object to apply to the current analysis. For more information, see chapter *Using Color Compensation*.

Program: Use *Program* to select the portion of the experiment (the experiment program) to analyze.

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

3.3.2 Resizing Window Sections

To hide or display a section:

Click the handle  on the border. Click the handle 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.

3.3.3 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.

Selecting samples to include in result calculations

Select the check box 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's check box to deselect or reselect it. To check/uncheck a group of samples at one time, highlight the range of samples, then press the spacebar. 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, then look for the same color on the chart. Or hold 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 select a sample name (not the check box) 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, click the sample name (not the check box) in the sample list.
- To select multiple samples, press *Ctrl* while clicking the sample names.
- To select a contiguous set of samples, click the first sample name, then press *Shift* 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 , then press *Ctrl-C*.
- Open the program into which you want to paste the copied text, then press *Ctrl-V*.

3.3.4 Working with Charts

To zoom in on 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.)

3.3.5 Adding Analysis Notes

Many analysis modules include a small *Analysis Notes* section.

1. If the *Analysis Notes* section is not visible, click the handle  above the Analysis Notes section heading to display the section.
2. Type in the *Analysis Notes* section.
3. Click *Save* on the LightCycler® Software 4.05 toolbar to save the notes with the experiment.

3.3.6 Removing or Renaming an Analysis

You can remove or rename analyses stored in your own user folder, if your user account has the Expert User or Local Administrator role. You may also be able to remove or rename analyses in other folders, depending on the access privileges associated with your user account. For more information about access privileges see chapter *Managing User Access*.

To remove an analysis module from an experiment:

1. Right-click the analysis module icon in the *module bar*, then select *Remove Analysis*. You are prompted to confirm your choice.
2. Click *Yes* to remove the module.
3. Click *Save* to save the experiment without the analysis.

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

To rename an analysis icon:

1. Right-click the icon in the module bar, then select *Name*.
2. Enter a new name, then click *OK*.

4. Performing Quantification Analyses

A quantification analysis can be performed on any experiment with an amplification program. A quantification analysis uses sample "crossing points" to determine the presence, the concentration, or the relative concentration of target DNA in unknown samples after amplification. For information about crossing points see the next section.

For analysis of quantification data, a 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.

There are three types of quantification analysis:

Absolute Quantification:
Calculates the concentration of target DNA in unknown samples, based on the concentration of standard samples.

Qualitative Detection:
Determines whether a target sequence is present in unknown samples.

Relative Quantification (Monocolor and Dual Color):
Compares the ratio of two DNA sequences in unknown samples to the ratio of the same two sequences in a calibrator.

LightCycler® Software 4.05 also provides a Nucleic Acid Quantification analysis, which is not based on PCR amplification and does not use sample crossing points to determine concentration values, but represents a simple fluorimetric method to quantitate nucleic acids *e.g.,* in eluates after nucleic acid purification. For more information about Nucleic Acid Quantification see chapter *Performing a Nucleic Acid Quantification Analysis*.

4.1 Understanding Sample Crossing Points

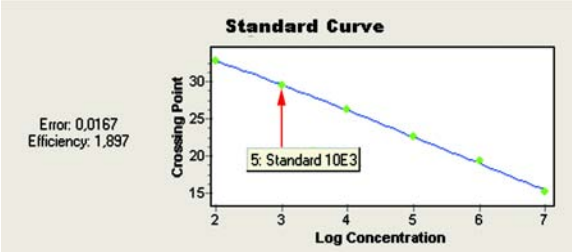
In an amplification reaction, the cycle at which the fluorescence of a sample rises above the background fluorescence is called the "crossing point" of the sample. The crossing point of a sample appears as a sharp upward curve on the experiment's fluorescence chart. The crossing point is the point at which amplified product is first visible in the data.

A sample's crossing point 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 crossing point. A sample with a higher concentration requires fewer cycles. How crossing point values are used in a quantification analysis depends on the type of analysis.

4.2 Understanding the Role of Standard Curves

In an Absolute Quantification or a Relative Quantification analysis, a standard curve is used to determine the concentration or the relative concentration of unknown samples. In a standard curve, the concentrations of standard samples are plotted against the crossing points of the samples. The X axis of the standard curve represents the log of the initial DNA concentration, and the Y axis represents crossing point in cycles. In general, the higher the crossing point, the lower the initial concentration of DNA.

The following figure illustrates a standard curve generated by an Absolute Quantification analysis. In this example, a sample with an initial DNA concentration of 10^3 units has a crossing point of 30 amplification cycles. This means it requires 30 cycles for the initial amount of DNA to be amplified to the point that it is visible above the background fluorescence of the sample.



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 curve. 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. In reality, reactions often have a lower efficiency. The curve above has an efficiency of 1.897.

By determining where an unknown sample's crossing point falls on the standard curve the software can determine the initial concentration of target DNA in the sample.

4.2.1 Providing the Standard Curve or Efficiency Value

To provide the standard curve for an Absolute Quantification analysis, you can:

- Include standards in the experiment
- Use a previously saved standard curve (called an external standard curve)

To provide the standard curve for a Relative Quantification analysis you can:

- Include standards in the experiment
- Use a previously saved standard curve (called an external standard curve)
- Specify an efficiency value of "2"

If you use an external standard curve the used data or value must be generated using the same channel settings, analysis method (for Absolute Quantification), concentration units, and color compensation data (if any), as the experiment you want to analyze. For more information, see the specific analysis sections in the rest of this chapter. For information about saving standard curves for use in other experiments see the following section.

4.2.2 Saving a Standard Curve

This section explains how to generate and save a standard curve as an extra object so the curve can be used for other Absolute Quantification analyses.

To save a standard curve:

1. Perform an amplification experiment containing the standards you want or 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 fluorescence channels, 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 sample concentration.
4. In the analysis module perform either a *Fit Points* or an *Automated* analysis. Select the check boxes 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 *Standard Curve* menu, select *Save as external...*.
6. Navigate to a location to save the curve, enter a file name, then click *OK*.

You can use the standard curve in other quantification analyses for experiments that have the same experiment parameters such as this standard curve.

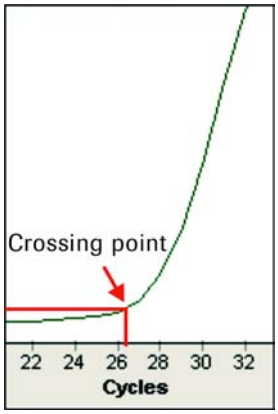
4.3 Performing Absolute Quantification Analysis

An Absolute Quantification analysis determines the concentration of target DNA in unknown samples.

There are two Absolute Quantification analysis methods: Automated (the default) and Fit Points. The algorithm for the Fit Point method is identical to the corresponding algorithm in LightCycler® Software Version 3.5, the algorithm for the Automated method is improved. Both methods use standard curves to calculate unknown sample concentrations, but each method determines a sample's crossing point a different way.

4.3.1 Understanding the Automated Method

The Automated method identifies the crossing point of a sample as the point where the sample's fluorescence curve turns sharply upward. This turning point corresponds to the first maximum of the second derivative of the curve.



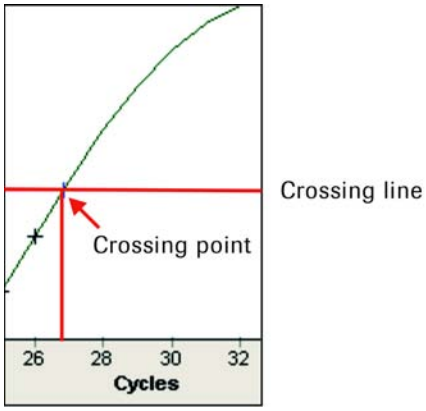
The Automated method requires little user input. You must determine how to provide the standard curve for the experiment; the software handles all other analysis calculations.

To handle complex fluorescence data curves, the software copes with 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 crossing point values are displayed for curves with a slowly increasing, decreasing, or noisy background, unless the curves reach considerable values of fluorescence.

If desired, you can include internal controls in the analysis to help ensure that samples have not been contaminated and that the reaction is performing as it should. For more information see *Using the Automated Method with Controls,* below.

4.3.2 Fit Points Method

The Fit Points method converts a sample's exponential curve to a straight line (a linear curve). The beginning of the line is extended downward until it intersects a horizontal line called the *crossing line*. The intersection of these two lines is defined as the sample's crossing point. The software uses the calculated crossing points of the standard samples to generate the standard curve of crossing point versus sample concentration.



The Fit Points method requires you to perform most of the analysis steps manually. You must adjust all sample curves so that each sample starts with the same baseline fluorescence, then you must eliminate background fluorescence from the data. You choose the number of data points used to generate the linear curves for the samples and adjust the crossing line.

You cannot use internal controls with the Fit Points method.

4.3.3 Comparison of the Two Methods

The following table describes the features and benefits of each Absolute Quantification method.

Method	Features
Automated	Automatic data calculation, no user input Fast calculation High reproducibility Internal controls can be used **Recommended for:** ● All applications ● High throughput with repeating standard curves
Fit Points	Data calculation requires user input for baseline adjustment, noise band setting, choice of fit points. Included for backward compatibility with LightCycler® Software version 3.5.3. **Recommended for:** ● All applications where user input for standard curve calculation is desired ● Runs with few or irregular standards. At least four standards should be available. Fewer standards can create high error rates for the standard curve, although user input can compensate for some errors.

4.3.4 Using the Automated Method

To perform the analysis using the automated method:

1. Using LightCycler® Software 4.05, create and run a quantification experiment or open an existing experiment.
2. Click *Analysis* on the main toolbar.
3. Select *Absolute Quantification*, then click *OK*.
4. In the *Sample Editor*, enter general sample information on the *Capillary View* tab, and select the channels used in the experiment.
5. On the *Abs Quant* tab of the *Sample Editor*, enter sample information as follows:

Column Name	Valid Values	Description
Target Name	Any name	Name of the target for this channel
Sample Type	Unknown Standard	Type of sample in this capillary
Concentration	Any concentration value	Concentration of a standard sample.

6. Click *Abs Quant* in the *module bar* to open the analysis module.
7. If this is a multiplexed experiment, from the *Channel* menu select the channel for the targets you want to analyze.
8. From the *Program* menu, select a quantification program in the experiment (typically there is only one quantification program, which is selected by default).
9. If you included standards in the experiment, select the check box next to each standard you want to include in the standard curve. (Double-click the box to select or clear it.) If you did not include standards in the experiment, from the *Standard curve* menu, select *Use external*. Find and select the standard curve you want to use, then click *OK*.

   The external curve must be from an experiment that has the same channel, mode, and color compensation settings as the current experiment.
   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.

10. 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.
11. To view amplification curves for one ore more samples, highlight the sample names in the sample list.

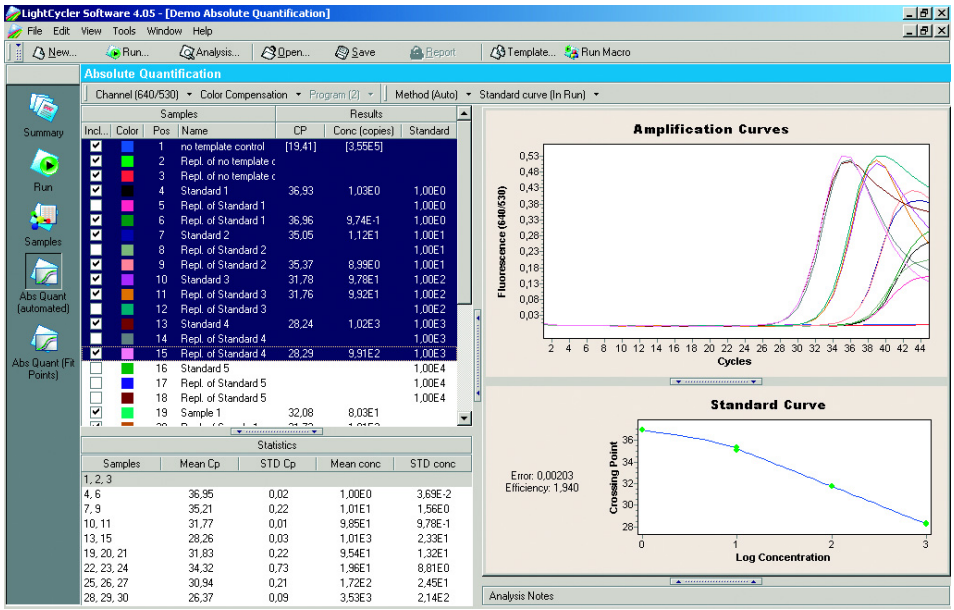
12 To view analysis results, click and drag the left border of the chart section to the right to display all the result data. Results include the following:

Result	Description
CP	The crossing point of the sample.
Conc (copies)	The calculated concentration of the sample, based on where its crossing point lies on a standard curve of crossing points vs. concentration.
Standard	The concentration of a standard sample; this value is specified in the *Abs Quant* tab of the Sample Editor.

13 Appended to the sample list view the following group results and statistics are given:

Statistic	Description
Samples	The sample numbers in the replicate group.
Mean Cp	The mean 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.

An Absolute Quantification analysis is shown below. Results are calculated for the eight samples that are checked in the sample list. Sample curves are displayed for the 15 samples that are highlighted in dark blue. The standard curve is generated from the samples that are checked and that are labeled Standards in the Sample Editor.



Using the automated method with controls

If you use the Automated method, you can include negative, positive, and internal controls in the quantification experiment to help ensure that samples have not been contaminated and that the reaction is performing as it should.

How a negative control is used

A negative control does not contain the target DNA sequence. It is an external control because it is located in a separate capillary from the unknown samples. The negative control must show no amplification.

How a positive control is used

A positive control contains a known concentration of the target DNA sequence. It is an external control because it is located in a separate capillary from the unknown samples. The positive control should show amplification characteristic of the target DNA sequence.

You can specify the concentration range expected for the positive control. In this case, the unknown samples can be called positive or negative only if the positive control's calculated concentration falls within its expected range.

The concentration range helps to ensure that the correct standard curve has been applied to the experiment. If the experiment results show that the positive control's calculated concentration is outside its expected range, then the wrong standard curve may have been applied to the experiment or the standards are not behaving as expected.

If you do not specify a concentration range for the positive control, the control only needs to be positive for a valid run.

How an internal control is used

An internal control is a positive control located in the same capillary as the unknown to which it applies. An internal control is a known DNA sequence, different from the target DNA, that you expect to amplify in a predictable way.

You can specify an expected crossing point range for the internal control. In this case, the associated unknown sample can be called negative only if the internal control is within its expected range. If the internal control performs as expected, it indicates that the reaction in the capillary proceeded correctly.

If you do not specify a crossing point range for the internal control, and the unknown sample is negative, the run will be considered valid if the internal control has any positive crossing point value.

To perform an analysis using controls:

1. Follow steps 1-4 in the procedure for an Automated analysis, above.
2. Select *Enable Controls* on the *Abs Quant* tab of the *Sample Editor*.
3. Enter sample information as described in step 5 in the procedure for an Automated analysis, above, then enter the Cp Low and Cp High information for standards and internal controls or Conc Low and Conc High information for positive controls as follows:

Column Name	Description	Valid Values
Cp Low	The low end of the range in which this sample's crossing point is expected to occur. (For standards or internal controls.)	A crossing point expressed in number of cycles.
Cp High	The high end of the range in which this sample's crossing point is expected to occur. (For standards or internal controls.)	A crossing point expressed in number of cycles.
Conc Low	The low end of the range in which this sample's concentration is expected to occur. (For positive controls only.)	A concentration expressed *e.g.,* in the number of copies.
Conc High	The high end of the range in which this sample's concentration is expected to occur. (For positive controls only.)	A concentration expressed *e.g.,* in the number of copies.

4. Follow steps 6-11 in the procedure for an Automated analysis, above. In the analysis module, be sure to select the channel for the unknown target you want to analyze.

5 To view analysis results, click and drag the left border of the chart section to the right to display all the result data. For an explanation of results, see step 12 in the procedure for an Automated analysis, above.
In addition to the results for a standard Automated analysis, a *Calls* section is added to the results. The Calls section includes the following:

Combined	The sample's result, based on both the sample's own call and the control calls. **Possible results for unknown samples:** Positive: The sample's call is positive and all relevant controls behaved as expected. Negative: The sample's call is negative and all relevant controls behaved as expected. Uncertain: The sample's amplification cannot be determined, perhaps because the amplification curve is not consistent or typical. All relevant controls behaved as expected. Invalid: At least one relevant control did not behave as expected. **Possible results for control samples:** Success: All relevant controls behaved as expected. Failure: At least one relevant control did not behave as expected.
Target	The result for the sample alone. The Call is based on whether amplification is detected for the sample. Positive: The sample shows amplification. Negative: The sample does not show amplification. Uncertain: The sample's amplification cannot be determined, perhaps because the amplification curve is not consistent or typical.
Control	The success or failure of a control sample. Success: All controls related to this sample behaved as expected. Failure: One or more controls related to this sample behaved not as expected.

4.3.5 Using the Fit Points Method

The Fit Points method requires you to manually perform most of the analysis steps. For information about key steps, see the sections that follow the procedure.

To perform the analysis using the Fit Points method:

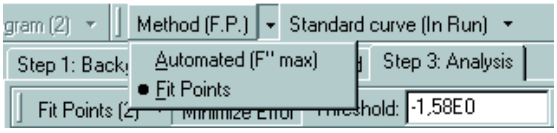
1. Add an *Absolute Quantification* analysis. Specify analysis-specific sample information and define or import standards as described under *Using the Automated method*, above.

   You cannot use internal controls with the Fit Points method. To use Fit Points make sure, that the Enable control checkbox in the Abs Quant tab of the Sample Editor is deselected.

2. If this is a multiplexed experiment, from the *Channel* menu in the analysis module, select the channel you want to analyze.

3. From the *Program* menu, select a quantification program in the experiment (if there is only one quantification program, it is selected by default).

4. From the *Method* menu, select *Fit Points*.



5. Click the sample names to highlight the samples you want to analyze.

6. Select the *Step 1: Background* tab to view the amplification curves for the selected samples. By default, the curves are corrected so that the initial, or *background,* fluorescence is the same for all the samples.

7. (Optional) To remove the background correction from the curves, from the *Background* menu, select *None*. For more information see *Specifying a background adjustment*, below.

8. Select the *Step 2: Noise Band* tab. The jagged lines at the beginning of the curves represent noise.

9. To eliminate the noise from the samples, adjust the *noise band* any of these ways:
   - Drag the red horizontal *noise band* up or down to exclude the noise.
   - Enter a location in the *Noiseband:* box (above the chart) to place the noise band.
   - Click *Automatic* from the *Noiseband method* pull down menu to let LightCycler® Software 4.05 adjust the noise band.
   - For more information see *Setting the noise band*, below.

10. Select the *Step 3: Analysis* tab.

11	Select the check boxes for the standard samples you want to include in the standard curve and for all the unknown samples for which you want to calculate concentrations. (Double-click a check box to either select or deselect it.)
12	From the *Fit Points* menu, select the number of data points (the "fit points") used to generate the log-linear curves for the samples. To view the fit points, from the *Fit Points* menu, select *Show*. For more information see *Selecting the fit points*, below.
13	To determine the crossing threshold for the samples, adjust the *crossing line* any of these ways: ▶Drag the red horizontal *crossing line* up or down. ▶Enter a location in the *Threshold* box (above the chart) to place the crossing line. ▶Click *Minimize Error* to let LightCycler® Software 4.05 adjust the crossing line. ▶For more information see *Adjusting the Crossing Line*, below. The software generates results for the checked samples.
14	Click and drag the left border of the chart section to the right to display all the analysis results. Read the calculated concentrations of the unknown samples from the appropriate column in the sample list. For more information see *Viewing results*, below.

Specifying a background adjustment

Background fluorescence may vary from sample to sample for several reasons, including differences in sample preparation, differences in DNA content from capillary to capillary, or pipetting errors.

The Fit Points method automatically adjusts the background fluorescence for all the samples to a comparable level using the arithmetic method. With this method, the mean value of the 2nd to 6th measured data point for each sample is calculated, then subtracted from each data point.

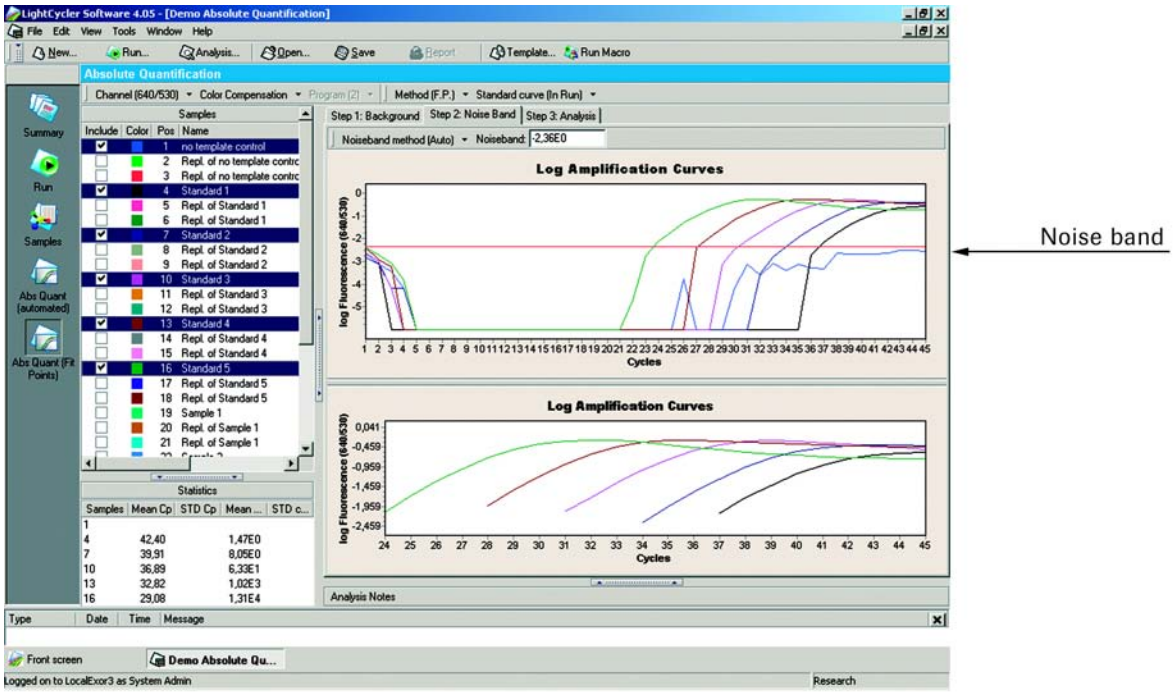
If you want to view the samples without background adjustment, select *None* from the *Background* menu on the *Step 1: Background* tab.

Setting the noise band

The *Step 2 Noise Band* tab displays two graphs. The upper graph shows Fluorescence vs. Cycle Number. The graph contains a red horizontal line (the noise band) to delineate the background noise. The lower graph shows the same curves with the background noise eliminated.

Move the noise band to a position, as low as possible, but as high as necessary, where it clearly crosses all sample curves in the lower part of the log-linear phase, as shown in the screen below.

Only data points above the Noise Band are considered for analysis. Data that fall below the noise band are excluded from analysis.

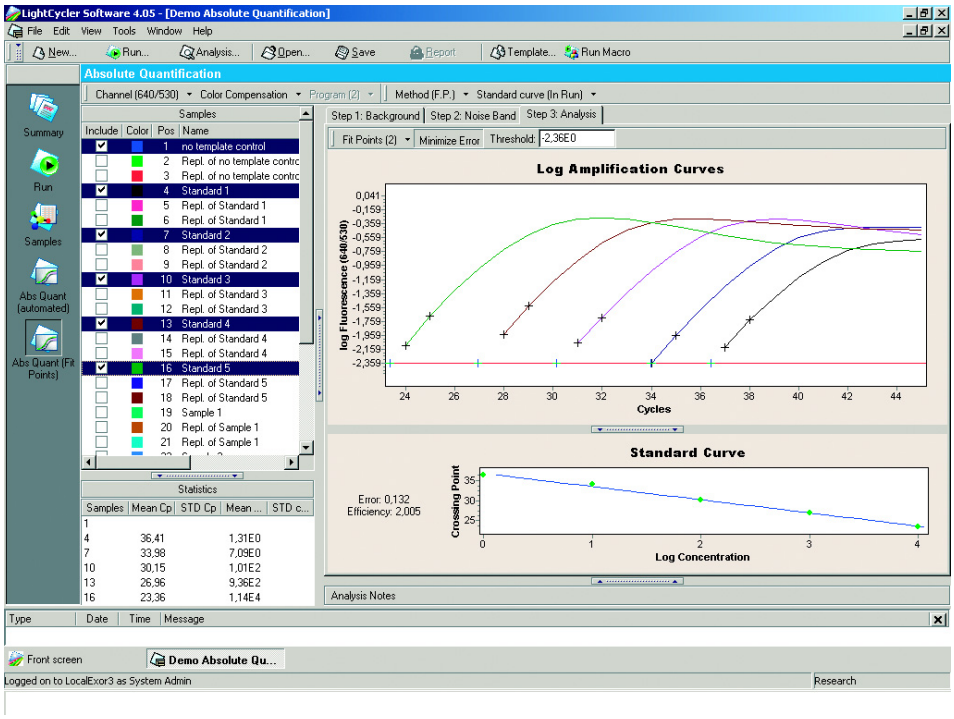


4

Selecting the fit points

The software converts the slope of each curve into a log-linear curve (a straight line).

In the *Fit points* box, specify the number of data points used to calculate the line. Select a limited number of points so that all the points are located on the linear portion of the curve. Begin by selecting 3 or 4 points and then increase the number. More points are better, as long as none of the points are located outside the linear portion of the curve.



D

Adjusting the crossing line

When the software calculates the log-linear lines for each standard sample, it extends the lines so they intersect with a horizontal line called the crossing line. The intersection of each log-linear line with the crossing line represents the crossing point for the sample.

In many cases, the default position of the crossing line is sufficient for quantification. In some cases, you can slightly improve the standard curve error value by manually moving the crossing line up or down. To have the software automatically adjust the crossing line, click *Minimize Error*.

Viewing results

The software generates a standard curve using all the samples that are labeled as Standard in the Sample Editor and that are also checked in the sample list of the analysis module.

Based on the standard curve, the software determines the concentrations of unknown samples. Calculated sample concentrations and crossing points are displayed in the result columns in the analysis module. Only samples with check marks in the sample list are included in the analysis results.

4.4 Performing Relative Quantification Analysis

A Relative Quantification analysis compares two ratios: 1) the ratio of a target DNA sequence to a reference DNA sequence in an unknown sample, and 2) the ratio of the same two sequences in a standard sample called a “calibrator”. The calibrator contains a "typical" ratio of the target and reference sequences, against which the target-to-reference ratio in the unknown sample can be compared. The result is expressed as a normalized ratio, *i.e.* ratio (1) divided by ratio (2).

A Relative Quantification analysis can be performed on an experiment that has an amplification program and that has the appropriate sample types. You can perform a relative quantification analysis on a single channel experiment (Relative Quantification-Monocolor) or on a multichannel experiment (Relative Quantification-Dual Color). If you perform a dual-color experiment, each pair of target and reference samples must be in the same capillary.

A Relative Quantification analysis is based on the assumption that the concentration of DNA at a sample’s crossing point is the same for every sample containing the same target DNA. This is the DNA concentration necessary for the LightCycler® fluorimeter to detect a signal above the background noise.

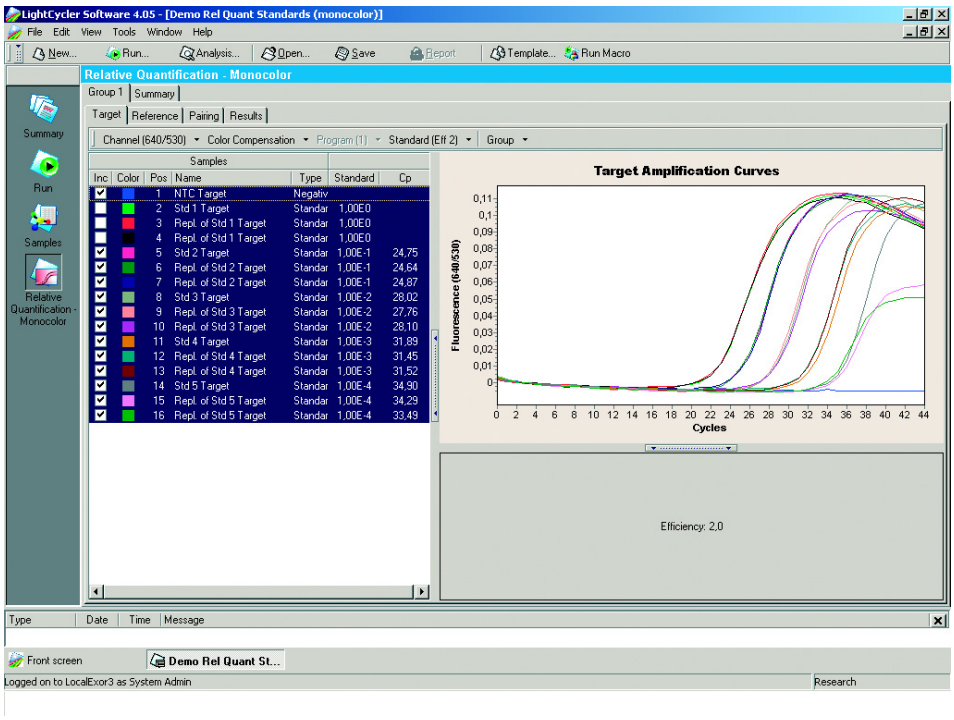
Each sample may require a different number of cycles to reach the crossing point, depending on the initial concentration of DNA in the sample. At the end of the experiment, each sample’s DNA concentration may vary, depending on how many cycles were completed by that sample after the crossing point was reached.

The analysis uses the sample’s crossing point (expressed as a cycle number), the efficiency of the reaction, the number of cycles completed, and other values to determine how much the DNA concentration must have increased for each sample by the end of the amplification. The analysis uses these calculations to compare the samples and generate the ratios. The analysis does not determine the actual concentration of DNA in the samples.

When you perform the analysis, you provide an efficiency value for the reaction by importing a standard curve, including standards in the experiment to determine the efficiency value, or simply specifying an efficiency value.

You can specify crossing point ranges for calibrators. If a calibrator sample’s crossing point is outside the specified range, then a message is displayed that the control failed.

The screen for a Monocolor Relative Quantification analysis is shown below.



The window displays two main tabs:

Group1 Contains four subtabs (Target, Reference, Pairing, Results), used to define the analysis sets and view results. You can add additional Group tabs, if needed. Each Group tab functions as a separate analysis. The results from each group tab can be viewed together on the Summary tab.

Summary Contains two subtabs that summarize results. One subtab lists a summary of all results sets in each group, the other subtab organizes the result sets across groups (if you used more than one Group tab). For more information see *Viewing results*, below.

You perform all the Relative Quantification analysis work in a Group tab, using the four subtabs as follows:

Target Use the Target subtab to select target samples to include in the analysis and to determine the efficiency value for the target samples.

Reference Use the Reference subtab to select reference samples to include in the analysis and to determine the efficiency value for the reference samples. You can import reference information from another experiment, if desired.

Pairing Use the Pairing subtab to select an unknown target/reference pair and a calibrator target/reference pair. The four samples together form a result set.

Results Use the results subtab to view results for each result set. For each set, the software compares the ratio of the unknown target/reference pair to the ratio of the calibrator target/reference pair.

4.4.1 Performing a Relative Quantification-Monocolor Analysis

Before you perform a Relative Quantification-Monocolor analysis, you must decide the following:

- Whether to include the reference samples in the experiment or to run a separate experiment to amplify the references
- How to provide the efficiency value for the targets and for the references:
  - By including standards in the current experiment to generate a standard curve
  - By importing a standard curve (or COF standard curve file when using a corresponding Roche kit)
  - By specifying an efficiency value of 2 (the only efficiency value that can be specified directly)

To perform a Monocolor Relative Quantification analysis, follow the general steps below. Key steps are described in detail in the sections that follow the procedure.

1	If reference samples are to be amplified in a separate experiment, perform the reference experiment.
2	Perform the relative quantification experiment containing the target samples. Enter sample information. (This experiment must also contain the reference samples if you did not perform a separate reference experiment.) For more information see *Performing the relative quantification experiment*, below.
3	Add the *Relative Quantification-Monocolor* analysis module to the target experiment. If reference samples are defined in a separate experiment, select the reference experiment when prompted.
4	On the *Target* tab of the analysis module, specify the target efficiency and select the target samples (both unknowns and calibrators) you want to analyze. For more information see *Setting target efficiency and selecting targets*, below.
5	On the *Reference* tab, specify the reference efficiency and select the reference samples you want to analyze. For more information see *Setting reference efficiency and selecting references*, below.
6	On the *Pairing* tab, select the sets of samples to be compared. For each set, select one unknown target and reference pair and one calibrator target and reference pair. For more information see *Pairing the samples*, below.
7	On the *Results* tab, view the results for each result set. For more information see *Viewing results*, below.
8	To perform an additional analysis, from the *Group* menu, select *Add Group*. Perform the analysis as described in the previous steps. View combined results for all groups on the *Summary* tab. For more information see *Viewing results*, below.

Performing a reference experiment

1	Perform an amplification experiment to amplify the reference samples. Include unknown and calibrator samples in the experiment.
2	You can also include reference negatives and standards, if desired. (Or you can choose to specify standard curve or efficiency for the reference when you perform the relative quantification experiment.)
3	Select the *Samples* module, and enter general sample information in the *Capillary View* tab.
4	From the *Analysis Types* menu, select *Relative Quantification – Monocolor*.
5	In the *Rel Quant Mono* tab of the *Samples* module:
6	Enter a target name for each sample, if desired.
7	Specify the sample type for each sample: reference unknown, reference calibrator, reference negative, or reference standard.
8	Specify the concentration value for each reference standard.
9	Click *Save*, navigate to a location to save the experiment, enter an experiment name, then click *Save*.

Performing the relative quantification experiment

The relative quantification experiment is an amplification experiment containing the target unknowns, calibrators, negatives, and standards (if any). If you did not perform a separate reference experiment, then the relative quantification experiment must also include the reference samples. Follow the steps below to define the samples for the experiment and to add the Relative Quantification – Monocolor analysis module to the experiment.

To perform a relative quantification experiment:

1. Perform an amplification experiment to amplify the target samples. If there is no separate reference experiment, include the reference samples in the experiment.
2. Click *Samples* in the module bar, then from the *Analysis Types* menu, select *Relative Quantification – Monocolor*.
3. Enter sample types and concentrations, as follows:

Column Name	Description	Valid Values
Sample Type	Type of sample	Target Unknown Target Calibrator Target Standard Target Negative Reference Unknown Reference Calibrator Reference Standard Reference Negative
Conc.	Concentration of a standard sample. This field is active only when the sample type is Standard.	Concentration value

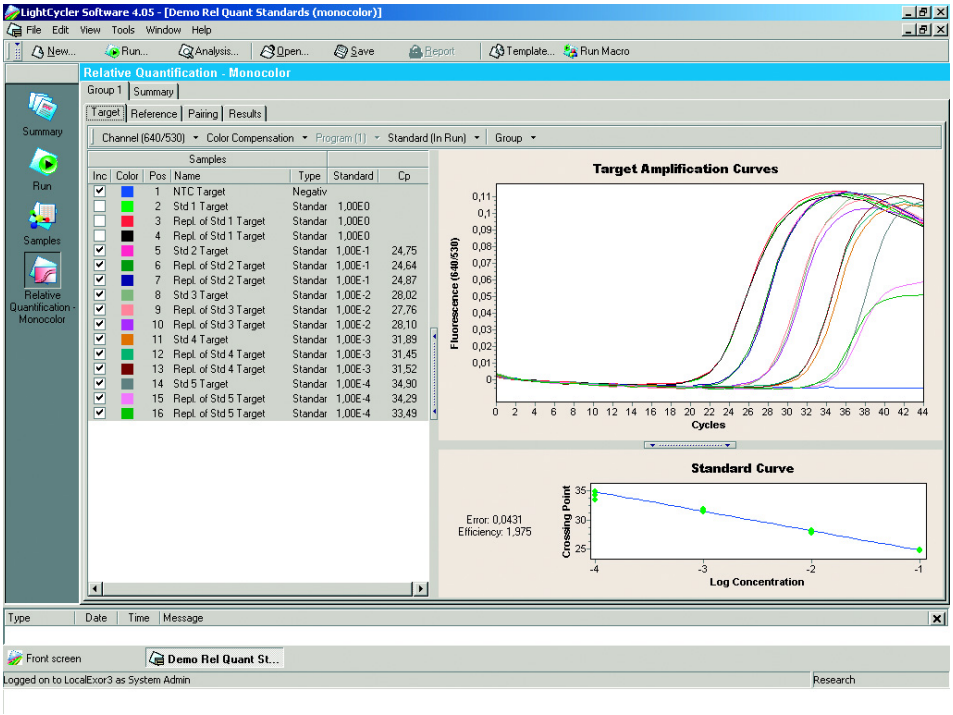
   Specify the reference sample types only if you did not perform a separate reference experiment.
4. Click *Analysis* on the main LightCycler® Software 4.05 toolbar.
5. In the *Create New Analysis* dialog box, select *Relative Quantification-Monocolor*, then click *OK*.
6. If you did not specify reference samples in the *Samples* module, a message prompts you to select a reference experiment. Click *OK* to clear the message.
7. Select the reference experiment, then click *OK*. The imported reference samples are added to the *Reference* tab of the analysis module.

Setting target efficiency and selecting targets

If you specified target standards in the Samples module, the standards are used to generate a standard curve, displayed in the lower chart of the *Target* tab. You can use this standard curve, import an external standard curve, or specify an efficiency value of 2.

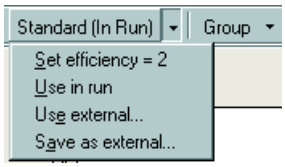
If you did not specify target standards, then the target standards area is gray and displays the message "No Standard Data." In this case, you must import a standard curve or specify an efficiency value.

An example of a standard curve is shown below.



To set target efficiency and select targets:

1. On the *Target* tab click *Channel*, then select the channel to be analyzed.
2. Drag the border on the right side of the samples list to the right, to display the type column.
3. If you included target standards in the experiment, select the check box next to each standard you want to include in the standard curve. Double-click the check boxes to select or deselect them. If you do not want to use standards from the experiment, click *Standard* in the menu bar.



From the standard menu, select one of the following:

Set Efficiency = 2	Select this option to use an efficiency value of 2 in analysis calculations. No standard curve is needed.
Use in run	Use this option to use the standard curve generated from standards in the current experiment.
Use external …	Use this option to import a previously saved standard curve or COF standard curve file. The curve must have been created from an experiment that has the same channel settings and color compensation data (if any) as the current experiment.

(Select *Save as external…* if you would like to save the current standard curve for use with other experiments.)

4. In the *Samples* list, select the check boxes of the target samples you want to make available for result sets. Double-click the check boxes to select or deselect them.

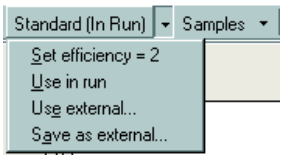
Setting reference efficiency and selecting references

If you specified reference standards in the Samples module or in an imported reference experiment, the standards are used to generate a standard curve, displayed in the lower chart of the *Reference* tab. You can use this standard curve, import an external standard curve, or specify an efficiency value of 2.

If you did not specify reference standards, then the target standards area is gray and displays the message "No Standard Data". In this case, you must import a standard curve or specify an efficiency value.

To set reference efficiency and select references:

1. On the *Reference* tab click *Channel*, then select the channel to be analyzed.
2. Drag the border on the right side of the samples list to the right, to display the type column.
3. If you included reference standards in the experiment, select the check box next to each standard you want to include in the standard curve. Double-click the check boxes to select or deselect them or if you do not want to use standards from the experiment, click *Standard* in the menu bar.



From the standard menu, select one of the following:

Set Efficiency = 2	Select this option to use an efficiency value of 2 in analysis calculations. No standard curve is needed.
Use in run	Use this option to use the standard curve generated from standards in the current experiment.
Use external …	Use this option to import a previously saved standard curve or COF standard curve file. The curve must have been created from an experiment that has the same channel settings and color compensation data (if any) as the current experiment.

(Select *Save as external…* if you would like to save the current standard curve for use with other experiments.)

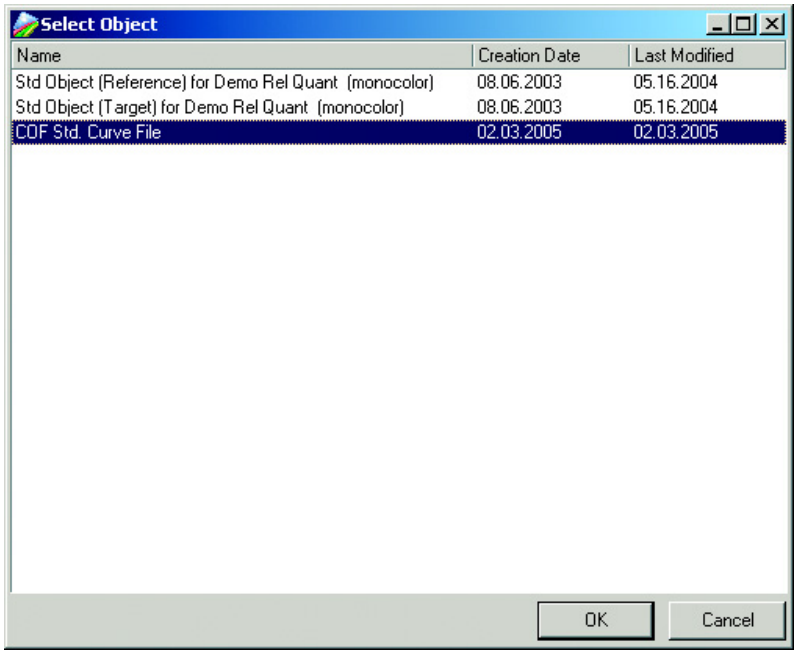
4. In the *Samples* list, select the check boxes of the reference samples you want to make available for result sets. Double-click the check boxes to select or deselect them.

Saving and selecting COF standard curve files

When using a Roche kit designed for Relative Quantification you need to import the corresponding COF (coefficient) standard curve file for performing a Relative Quantification analysis. The COF file includes the lot-specific target and reference standard curve as well as the Correction and Multiplication factors.

To save and select COF standard curve files:

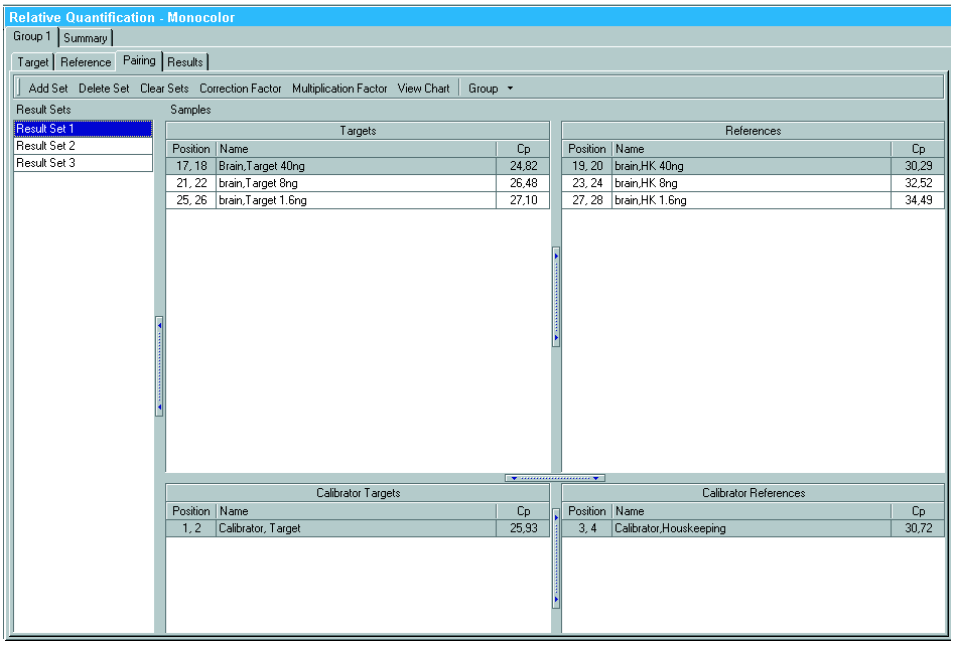
1. Download the COF file and save it locally. Information about where you go to download the COF file is provided in the documents that accompany the kit.
2. In the *File* menu select *Import* and then *COF Std. Curve Files*.
3. Find and select the file you want, and then click *Open*. The file is imported and opened in the work pane.
4. To save the imported COF file as an object in the current LightCycler® 4.05 database click *Save*.
5. Navigate to a location in LightCycler® Software 4.05 to save the object, enter a new object name (or keep the existing name), then click *OK*.
6. To use the COF file in the current experiment, from the *Standard* menu select *Use external…*, then select the object you want to use.



You cannot enter the Fit Coefficients manually

Pairing the samples

Use the Pairing tab to form result sets consisting of an unknown target and reference and a calibrator target and reference. The Pairing tab is shown below with three result sets.



To create result sets:

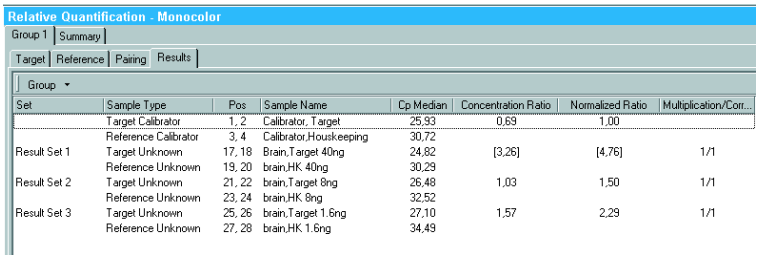
1	Select the *Pairing* tab. The unknown targets and references are listed in the upper panes, the calibrator targets and references are listed in the lower panes.
2	In the upper panes, select the unknown target and unknown reference that form a pair. In the lower panes, select the calibrator target and calibrator reference that form a pair.
3	In the menu bar, click *Add Set*. The set is added to the list of *Result Sets* on the left.
4	To apply a correction factor to the result sets, press the button *Correction Factor* in the upper line of the tab, then type the new value. The correction factor is entered automatically after import of a COF standard curve file. The correction factor adjusts results for any lot-to-lot variations in kit chemistry.
5	To apply a multiplication factor to the result, press the button *Multiplication Factor* in the upper line of the tab, then type the new value. The multiplicaton factor is entered automatically after import of a COF standard curve file.The normalized ratio is multiplied by this value before the result is displayed on the *Results* tab.
6	Create other results sets, as needed.
7	To view graphs for the samples in a result set, select the result set, then click *View Chart*.

Viewing results

You can view results for each group on the group's *Results* tab. You can view results for all groups on the *Summary* tabs.

To view results on the Results tab:

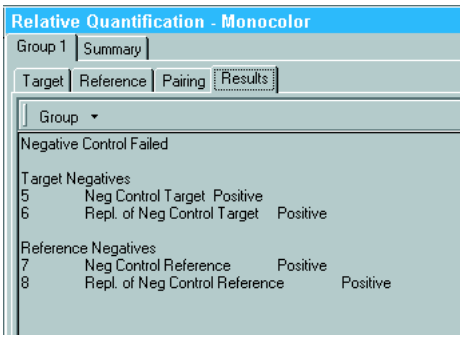
1. Select the *Results* tab to view detailed results for each result set in the group.
2. The *Results* tab is shown below.



Set	Sample Type	Pos	Sample Name	Cp Median	Concentration Ratio	Normalized Ratio	Multiplication/Corr...
	Target Calibrator	1, 2	Calibrator, Target	25,93	0,69	1,00	
	Reference Calibrator	3, 4	Calibrator,Housekeeping	30,72			
Result Set 1	Target Unknown	17, 18	Brain,Target 40ng	24,82	[3,26]	[4,76]	1/1
	Reference Unknown	19, 20	brain,HK 40ng	30,29			
Result Set 2	Target Unknown	21, 22	brain,Target 8ng	26,48	1,03	1,50	1/1
	Reference Unknown	23, 24	brain,HK 8ng	32,52			
Result Set 3	Target Unknown	25, 26	brain,Target 1.6ng	27,10	1,57	2,29	1/1
	Reference Unknown	27, 28	brain,HK 1.6ng	34,49			

For each set, the tab displays sample types, positions, names, the crossing points for each sample, the ratios of targets to references, and the normalized ratio of the unknown pair with the calibrator pair.

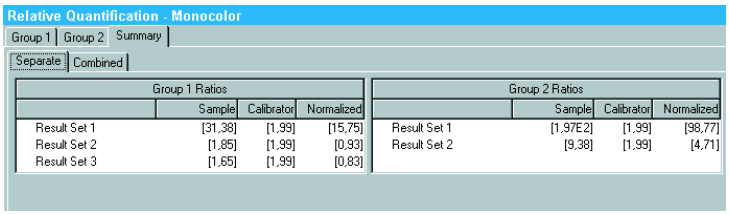
If one or more controls fail, the tab lists the failed controls, as shown below. For each failed control, the tab displays the sample number, name, and the sample result (positive, negative, or indeterminate).



To view results on the Summary tab:

1. Select the *Summary* tab.

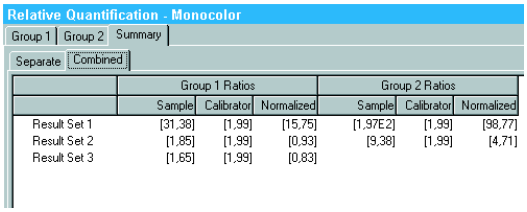
2. Select the *Separate* subtab (shown below) to see result sets listed by group.



For each result set, the tab displays

- *Sample* The ratio of the target/reference pairs
- *Calibrator* The ratio of the calibrator target/reference pairs
- *Normalized* The normalized ratio of the two pairs

3. Select the *Combined* subtab to see the results for all groups together. The *Combined* tab allows you to compare results for result sets across groups. The results for Result Set 1 (from all groups) are displayed on the first line, the results for Result Set 2 (for all groups) on the second line, and so on, as shown below.



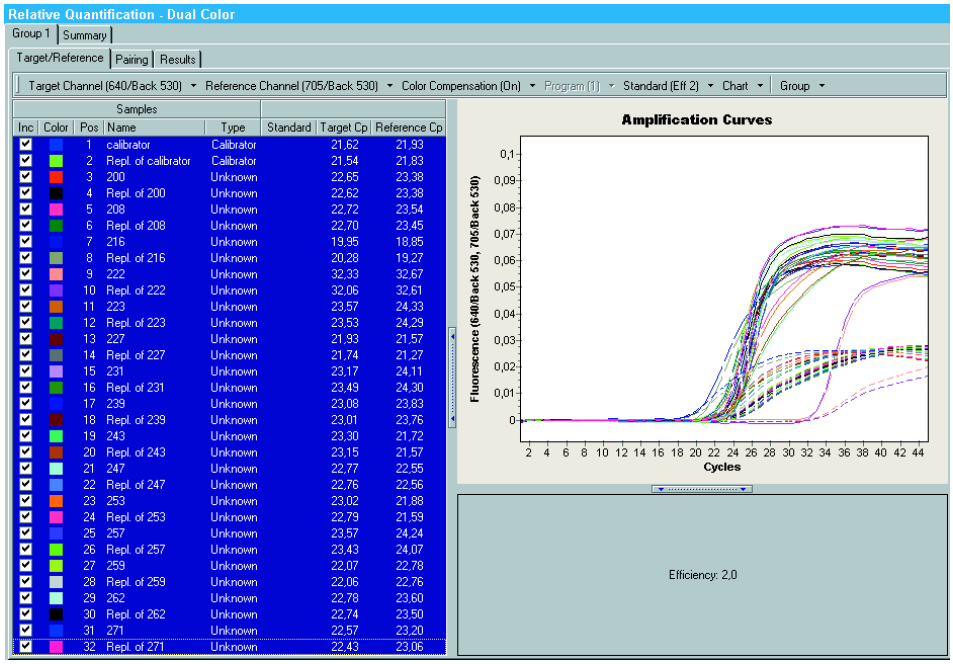
4

4.4.2 Performing a Relative Quantification–Dual Color Analysis

Relative Quantification-Dual Color requires a multicolor reaction in which each target/reference pair are in the same capillary. The targets and references are measured by different fluorescence channels.

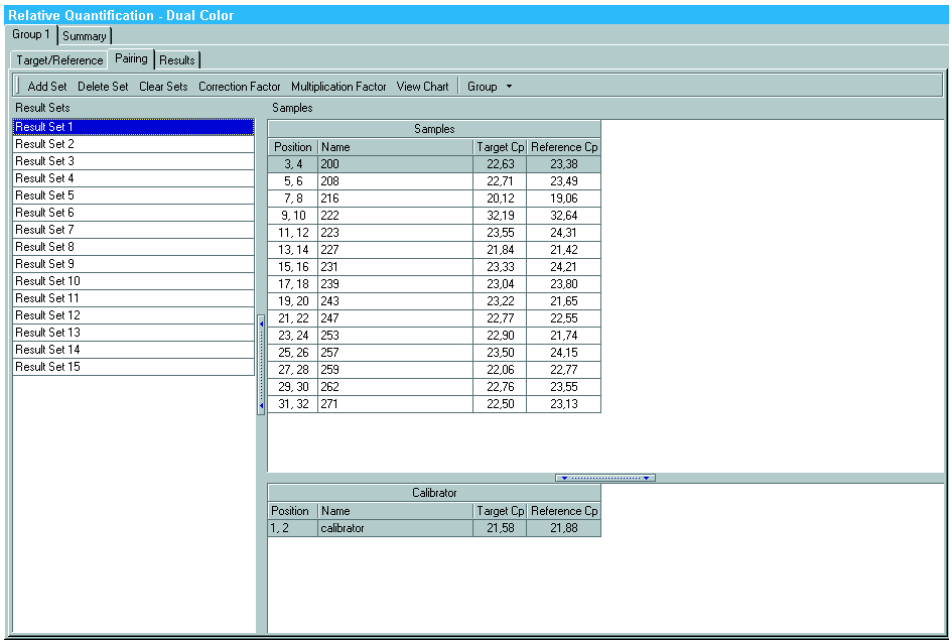
Before performing a Relative Quantification-Dual Color analysis, read the previous sections to understand how to perform a Relative Quantification-Monocolor analysis. The processes are similar. Follow the same general steps as for a Monocolor analysis, but with these differences:

- In the *Rel Quant Dual* tab of the *Sample Editor*, specify a sample type for each sample in each channel. Target and reference samples must be in the same capillary.
- Perform the quantification experiment as a multiplexed experiment, with target/reference pairs in the same capillaries.
- When you add the analysis module, select *Relative Quantification–Dual Color*. When you open the analysis module, note that the target and reference information is combined on one tab, the *Target/Reference* tab.
- On the *Target/Reference* tab of the analysis module:
  - In the menu bar, click *Target Channel*, then select the channel with target data.
  - Click *Reference Channel*, then select the channel with reference data.
  - Click *Chart*, then select which data to display in the charts on that tab (targets, references, or both).
  - Specify the efficiency by using standards from within the experiment, importing a standard curve, or specifying an efficiency of 2. The efficiency applies to both the target and the reference samples.
- The *Target/Reference* tab is shown below.



D

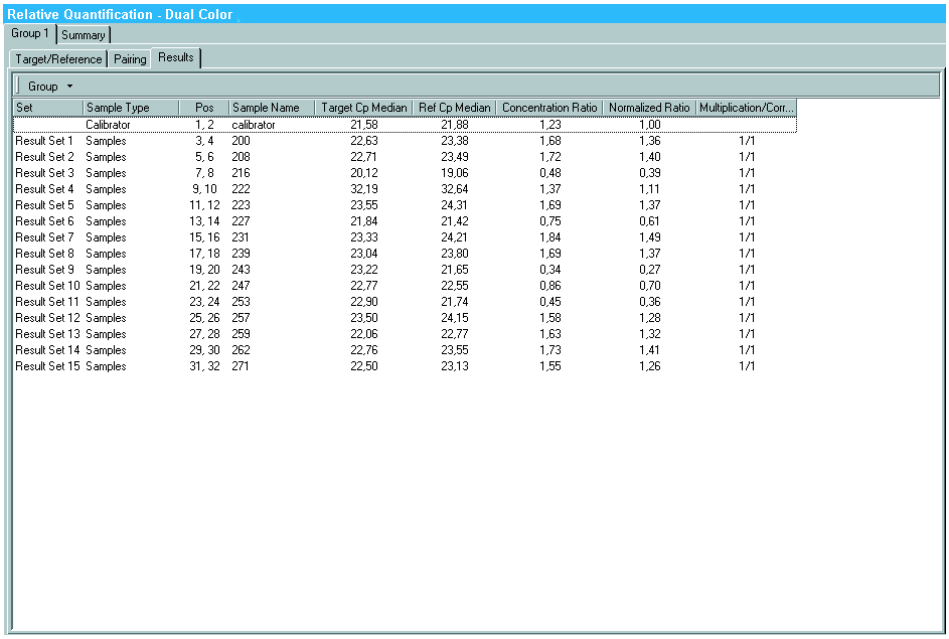
- On the *Pairing* tab, select two samples for each result set (an unknown sample and a calibrator sample); each sample contains both a target and a reference. The *Pairing* tab is shown below.



5

D

- View results on the *Results* tab and the *Summary* tab. Note that each sample row includes a column for the target crossing point and a column for the reference crossing point. The *Results* tab is shown below.



Set	Sample Type	Pos	Sample Name	Target Cp Median	Ref Cp Median	Concentration Ratio	Normalized Ratio	Multiplication/Corr...
	Calibrator	1, 2	calibrator	21,58	21,88	1,23	1,00	
Result Set 1	Samples	3, 4	200	22,63	23,38	1,68	1,36	1/1
Result Set 2	Samples	5, 6	208	22,71	23,49	1,72	1,40	1/1
Result Set 3	Samples	7, 8	216	20,12	19,06	0,48	0,39	1/1
Result Set 4	Samples	9, 10	222	32,19	32,64	1,37	1,11	1/1
Result Set 5	Samples	11, 12	223	23,55	24,31	1,69	1,37	1/1
Result Set 6	Samples	13, 14	227	21,84	21,42	0,75	0,61	1/1
Result Set 7	Samples	15, 16	231	23,33	24,21	1,84	1,49	1/1
Result Set 8	Samples	17, 18	239	23,04	23,80	1,69	1,37	1/1
Result Set 9	Samples	19, 20	243	23,22	21,65	0,34	0,27	1/1
Result Set 10	Samples	21, 22	247	22,77	22,55	0,86	0,70	1/1
Result Set 11	Samples	23, 24	253	22,90	21,74	0,45	0,36	1/1
Result Set 12	Samples	25, 26	257	23,50	24,15	1,58	1,28	1/1
Result Set 13	Samples	27, 28	259	22,06	22,77	1,63	1,32	1/1
Result Set 14	Samples	29, 30	262	22,76	23,55	1,73	1,41	1/1
Result Set 15	Samples	31, 32	271	22,50	23,13	1,55	1,26	1/1

5. Performing Qualitative Detection Analysis

The Qualitative Detection module analyzes unknown samples to detect the presence of a particular target, based on whether the data shows target amplification. The analysis does not quantify the amount of target DNA. A Qualitative Detection analysis can be performed on any experiment containing an amplification program.

A simple qualitative detection experiment contains unknown samples, but no controls. A more complex Qualitative Detection experiment uses positive, negative, internal, or any combination of controls.

The following sections describe how to perform the analysis with or without controls.

5.1 Performing a Qualitative Detection Analysis without Controls

A simple qualitative detection experiment contains unknown samples, but no controls.

To perform the analysis:

1	Open the experiment you want to analyze.
2	Click *Analysis* on the main toolbar.
3	Select *Qualitative Detection*, then click *OK*.
4	In the *Sample Editor*, enter sample information in the *Capillary View* tab.
5	If this is a multicolor experiment, in the *Sample Editor* toolbar, select the channels used in the experiment.
6	In the *Qual Detect* tab of the *Sample Editor*, enter target names for each sample in each channel.
7	Select *Qual Detect* in the *module bar* to open the analysis module.
8	If this is a multicolor experiment: From the *Channel* menu, select the channel for the targets you want to analyze. To apply color compensation, click *Color Compensation*, then select *Select Color Compensation*. Find and select the color compensation object for the current instrument.
9	From the *Program* menu, select a quantification program in the experiment (if there is only one quantification program, it is selected by default).

10 View the detection calls for each sample in the *Target* column, as follows.

Column Name	Description	Possible Values
Target	The sample call, based on whether amplification is detected for the sample.	***Positive*:** The sample shows amplification. ***Negative*:** The sample does not show amplification. ***Uncertain*:** The sample's amplification cannot be determined, perhaps because the amplification curve is inconsistent.

11 (Optional) Click *Advanced* to display a *Results* section that includes the following columns:

Column Name	Description	Possible Values
CP	Crossing point of the sample, in cycles	A positive number with up to two decimal places
Score	A numerical value reflecting whether the target is positive, negative, or indeterminate	A positive or negative number with up to two decimal places, or 0
	***Positive*:** A value of +1 or above ***Negative*:** A value of -1 or below ***Uncertain*:** A value between +1 and -1 *The score does not indicate the confidence level of the call. For example, a score of 1.1 is no more or less positive than a score of 4.1.*	

12 If this is a multicolor experiment, repeat the procedure to analyze the results for another channel.

5.2 Using Controls in a Qualitative Detection Analysis

A more complex Qualitative Detection experiment uses one or more controls. The control reactions provide information about the quality of the template DNA, reagents, and reaction conditions. Controls are expected to behave in a certain way, depending on their type and other user-specified information. If they fail to behave as expected, the unknown sample reactions are considered unreliable.

You can use the following types of controls in a Qualitative Detection analysis:

Positive control Contains the DNA target for which the unknown samples are being analyzed. A positive control is located in a separate capillary from the unknown samples, and its results apply to all the unknowns in the experiment that use the same channel. You can specify a crossing point range for the positive control. If amplification does not occur, or if the crossing point is not within the expected range, then the control fails. In this case, the reaction did not proceed correctly or the sample may be contaminated.

Negative control Does not contain the DNA target for which the unknown samples are being analyzed. A negative control is located in a separate capillary from the unknown samples. A negative control applies to all the unknowns in the experiment that use the same channel. If amplification occurs, then the control fails. In this case, the sample is contaminated, and other unknown samples may also be contaminated.

Internal control Is a special type of positive control, located in the same capillary as the unknown to which it applies. The internal control is a separate DNA sequence that can be expected to amplify predictably. You can specify a crossing point range for an internal control. If the crossing point is not within the expected range for negative samples, then the control fails.

To provide an internal control, you can add a known DNA target to the sample mix, one for which you have a robust assay. An internal control requires multicolor chemistry.

The presence of an internal control in the same capillary as an unknown indicates whether the reaction in the capillary proceeded correctly. If the unknown fails to amplify, and the internal control is not positive or the control's CP is not within the designated range, then the reaction may have failed and the results are unreliable. However, if the unknown fails to amplify, but the internal control amplifies as predicted, then the reaction proceeded correctly.

Circumstances that cause a control to fail

The success or failure of a control affects whether a sample result is valid. A control fails when it does not behave as expected, therefore a control fails in the following circumstances:

- A Negative Control shows amplification and is consequently called positive or uncertain. In this case, samples may be contaminated.
- A Positive Control does not show amplification and is consequently called uncertain or negative or the Positive Control's CP is not within the range designated in the Sample Editor. In this case, the reaction may not have worked correctly or the samples may be contaminated.
- The Unknown is Negative and the Internal Control's CP is not within the range designated in the Sample Editor. The failure of the control indicates that the reaction in the capillary did not work correctly. (If the Unknown is Positive, the control is assumed to succeed.)

How results are displayed

When controls are used, the Qualitative Detection module displays three results for each sample: Combined, Target, and Control, as shown below:

Calls		
Combined	Target	Control
Negative	Negative	Success
Negative	Negative	Success
Positive	Positive	Success
Positive	Positive	Success

Combined result The Combined value is a "Metacall" based on the Target value and the Control value.

Target result The Target result is the call for the sample based on the sample's amplification profile.

Control result The Control result displays the Success or Failure for the combination of controls that apply to the particular sample. All the controls that apply to a particular example are treated together. If any of the controls that apply to the sample fail, the Control column displays "Failure". Controls apply not just to Unknown samples, but to other controls. For example, if an experiment contains two Positive controls, each control applies to the other. A failure of one control causes a failure for the other control.

The amplification curves of the controls that influence the validity of the selected target samples are displayed separately in the work pane.

Possible results for a Control sample

For Control samples (Positive Control, Negative Control or Internal Control), the following results values are possible in each of the result columns:

Combined result is…	**When…**
Success	Control behaves as expected and Control Column shows Success or is empty.
Failure	Control does not behave as expected and Control Column shows Failure.
Target result is…	**When…**
Positive	Control sample shows amplification.
Negative	Control sample does not show amplification.
Uncertain	The control sample's amplification cannot be determined, perhaps because the amplification curve is inconsistent.
Control result is…	**When…**
Success	All other controls that apply to this control succeed.
Failure	One or more controls fail.
no value (empty)	No controls apply to this control. For example, this is an internal control and there are no other controls in the experiment.

Combined and Control consider replicates.

Possible results for an Unknown sample

For Unknown samples, the following results values are possible in each of the result columns:

Combined result is…	**When…**
Positive	Target value is Positive and Control column is Success (or no value).
Negative	Target value is Negative and Control column is Success (or no value).
Uncertain	Target value is Uncertain and Control column is Success.
Invalid	Target value is Positive, Negative, or Uncertain and Control column is Failure.
Target result is…	**When…**
Positive	Sample shows amplification.
Negative	Sample does not show amplification.
Uncertain	The sample's amplification cannot be determined, perhaps because the amplification curve is inconsistent.
Control result is…	**When…**
Success	All controls that apply to this sample succeed.
Failure	One or more controls fail.
no value (empty)	No controls apply to this sample. For example, there are no controls in the experiment or there are internal controls, but not in the same capillary as this sample.

Summary of possible Combined result values for an Unknown sample

For an Unknown sample, the Combined metacall is Positive, Negative, or Uncertain in the following cases; in all other cases, the call is Invalid:

Combined call is…	When Target value is…	And Control value is….
Positive	Positive	Success (for all controls)
Negative	Negative	Success (for all controls)
Uncertain	Uncertain	Success (for all controls)

To determine whether an internal control fails while other controls succeed, you will need to look at the result for the internal control's channel. For more information see *Example of Qualitative Detection results using an internal control that fails*, below.

If all controls succeed, the combined call for an unknown sample is the same as the target value (positive, negative, uncertain). If one control (that applies to the sample) fails, the sample is invalid.

Performing a Qualitative Detection analysis using controls

Follow the steps below to perform an analysis using any or all of the controls described above:

1. Open the experiment you want to analyze.
2. Click *Analysis* on the main toolbar.
3. Select *Qualitative Detection*, then click *OK*.
4. In the *Sample Editor*, enter sample information in the *Capillary View* tab.
5. If this is a multicolor experiment, in the *Sample Editor* toolbar, select the channels used in the experiment.
6. In the *Qual Detect* tab of the *Sample Editor*, select *Enable Controls*.

7 Enter sample information for each channel in each row, as follows:

Column Name	Description	Valid Values
Target Name	Name of the DNA target for this channel for this sample.	Any name
Sample Type	The type of sample.	Unknown Positive Control Negative Control Internal Control
Cp Low	The lowest crossing point value expected from this sample, expressed in cycles. If you specify a value for Cp Low, but no value for Cp High, the control must have a crossing point equal to or greater than the Cp Low value to succeed.	A positive number, with up to two decimal places
Cp High	The highest crossing point value expected from this sample, expressed in cycles. If you specify a value for Cp High value, but no value for Cp Low, the control must have a crossing point equal to or less than the Cp High value to succeed.	A positive number, with up to two decimal places

8 Select *Qualitative Detection* in the *module bar* to open the analysis module.

9 If this is a multicolor experiment:

10 From the *Channel* menu, select the channel you want to analyze.

11 To apply color compensation, click *Color Compensation*, then select *Select Color Compensation*. Find and select the color compensation object for the instrument you are using.

12 From the *Program* menu, select a quantification program for the experiment (if there is only one quantification program, it is selected by default).

13 View the calls for each sample in the *Combined*, *Target*, and *Control* columns of the *Calls* section, as follows.

Column Name	Description	Possible Values
Combined	The sample call, based on both the Target result and the Control result.	**For unknown samples:** ***Positive*:** The sample's call is positive and all controls behaved as expected. ***Negative*:** The sample's Target call is negative and all controls behaved as expected. ***Invalid:*** At least one control failed. ***Uncertain*:** The sample's Target call is uncertain and all controls behaved as expected. **For control samples:** ***Success:*** The control behaves as expected. ***Failure:*** The control does not behave as expected.
Target	The sample call, based on whether amplification is detected for the sample.	***Positive*:** The sample shows amplification. ***Negative*:** The sample does not show amplification. ***Uncertain*:** The sample's amplification cannot be determined, perhaps because the amplification curve is inconsistent.
Control	The success or failure of all the controls for the sample.	***Success:*** All controls behave as expected. ***Failure:*** One or more controls fail.

14 (Optional) Click *Advanced* to display a *Results* section that includes the following:

Column Name	Description	Possible Values
CP	The sample crossing point, in cycles.	A positive number with up to two decimal places
Score	A numerical value reflecting whether the target is positive, negative, or indeterminate. ***Positive***: A value of +1 or above ***Negative***: A value of -1 or below ***Uncertain***: A value between +1 and -1 *The score does not indicate the confidence level of the call. For example, a score of 1.1 is no more or less positive than a score of 4.1.*	A positive or negative number with up to two decimal places, or 0

15 If this is a multicolor experiment, select another channel to analyze.

5.2.1 Example of Qualitative Detection Results when Positive Controls behave as Expected

In this example, a single-channel experiment contains controls in sample positions 1-3. All other sample positions contain unknowns. The controls all behave as expected, that is, they show no amplification for the negatives (sample 1-2) and amplification within the designated range for the positive (sample 3).

The following figure illustrates the results from a Qualitative Detection analysis of the experiment. The Target column and the Control column together determine the metacall value in the Combined column. The results are explained in detail in the following sections.

Qualitative Detection [CC Object from Color Compensation Set (Cat N

Channel (640/Back 530) ▾ Color Compensation (On) ▾ Program (2) ▾ Advanced

Samples				Calls		
Include	Color	Pos	Name	Combined	Target	Control
☑		1	no template control	Success	Negative	Success
☑		2	Negative Control	Success	Negative	Success
☑		3	Positive Control	Success	Positive	Success
☑		4	Sample 1	Positive	Positive	Success
☑		5	Sample 2	Positive	Positive	Success
☑		6	Sample 3	Negative	Negative	Success
☑		7	Sample 4	Positive	Positive	Success
☑		8	Sample 5	Positive	Positive	Success

Results for samples 1-3

Samples 1-3 are designated as negative and positive controls in the Sample Editor. The results for these samples are as follows:

- The *Combined* column displays “Success” because these samples behaved as expected as indicated by the Target column.
- The *Target* column shows “Negative“ for the negative controls, because sample 1-2 showed no amplification, and “Positive” for the positive control, because sample 3 showed amplification.
- The *Control* column shows “Success” because all the controls for these samples behaved as expected. In this case, samples 1-3 are the controls, but because negative and positive controls apply to all samples using the same channel, each control applies to the other control samples, as well.

Results for samples 4-5 and 7-8

Samples 4-5 and 7-8 were designated as unknowns in the Sample Editor. The results for these samples are as follows:

- The *Combined* column is the metacall for these unknown samples. The metacall is "Positive," because the samples showed amplification (as indicated in the Target column) *and* the controls succeeded (as indicated in the Control column).
- The *Target* column shows "Positive" because these unknown samples showed amplification.
- The *Control* column shows "Success" because all the controls for these samples succeeded.

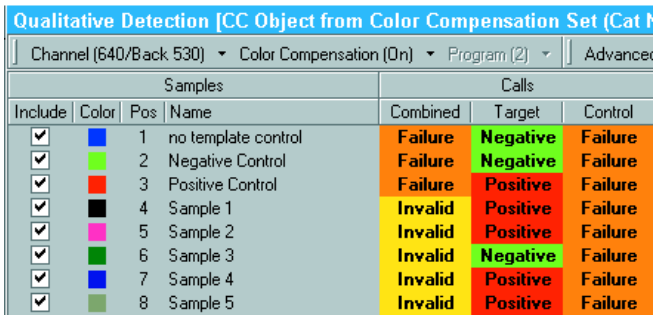
Results for samples 6

Sample 6 was designated as unknown in the Sample Editor. The result for this sample is as follows:

- The *Combined* column shows the metacall for this unknown sample. The metacall is "Negative" because the sample did not show amplification (as indicated by the "Negative" call in the Target column), and the controls succeeded (as indicated in the Control column).
- The *Target* column shows "Negative" because this unknown sample did not show amplification.
- The *Control* column shows "Success" because all the controls (Samples 1-3) succeeded.

5.2.2 Example of Qualitative Detection Results when a Positive Control Fails

In this example, the same positive control sample (sample 3), negative controls and unknowns are defined as in the previous example. However, the crossing point for the positive control (sample 3) does not fall within the CP range designated on the Sample Editor. Therefore, all three, the two negative controls and the positive control fail. The effect of the failed controls on the calls for the samples is described below.



Samples				Calls		
Include	Color	Pos	Name	Combined	Target	Control
☑		1	no template control	Failure	Negative	Failure
☑		2	Negative Control	Failure	Negative	Failure
☑		3	Positive Control	Failure	Positive	Failure
☑		4	Sample 1	Invalid	Positive	Failure
☑		5	Sample 2	Invalid	Positive	Failure
☑		6	Sample 3	Invalid	Negative	Failure
☑		7	Sample 4	Invalid	Positive	Failure
☑		8	Sample 5	Invalid	Positive	Failure

Results for samples 1-3

The positive control sample apply to all other samples, including the other control samples. The results for the control samples are as follows:

- The *Combined* column shows the metacall for these samples. The metacall is “Failure,” because the controls for these samples (as indicated in the Control column) failed.
- The *Target* column shows „Negative“ for the negative controls, because sample 1-2 showed no amplification, and “Positive” for the positive control, because sample 3 showed amplification.
- The *Control* column shows “Failure” because at least one applicable control failed for these samples. In this case, sample 3 is the control, that failed, because its CP did not fall within the CP range designated on the Sample Editor.

Results for samples 4-5 and 7-8

Samples 4-5 and 7-8 are designated as unknowns in the Sample Editor. The results for these samples are as follows:

- The *Combined* column shows the metacall for these unknown samples. The metacall is “Invalid” because the controls for these samples failed (as indicated by the Control column).
- The *Target* column shows “Positive” because these unknown samples showed amplification.
- The *Control* column shows “Failure” because at least one of the applicable controls failed.

Results for sample 6

Sample 6 is designated as unknown in the Sample Editor. The result for this sample is as follows:

- The *Combined* column shows the metacall for this unknown sample. The metacall is “Invalid” because the controls for these samples failed (as indicated by the Control column).
- The *Target* column shows “Negative” because this unknown sample did not show amplification.
- The *Control* column shows “Failure” because at least one of the applicable controls failed.

5.2.3 Example of Qualitative Detection Results Using an Internal Control that Fails.

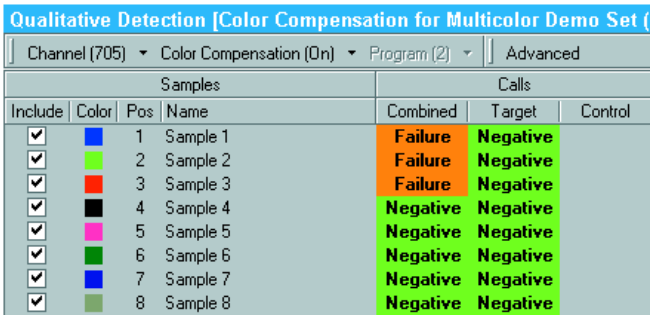
An internal control is located in the same capillary as an unknown, and its results apply only to the unknown in that capillary. Because the internal control is in the same capillary as an unknown, a multicolor reaction is required.

In this example, internal controls are located in samples 1-3 and are measured in channel 705. The unknown samples are located in all the capillaries and are measured in channel 640. There are no Positive Control or Negative Control samples in the experiment.

In this experiment, the internal controls fail because their crossing points are not within the range designated on the Sample Editor, which is expected for an unknown sample showing no amplification.

Viewing results for the controls (channel 705)

In the example below, channel 705 (the control channel) is selected in the channel menu of the analysis module, so the results displayed in the window are those for the internal controls.



Results for samples 1-3

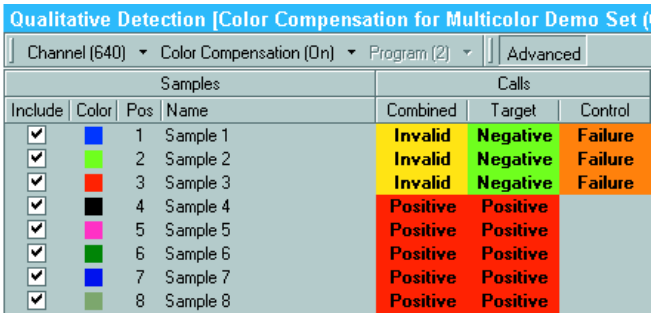
- The *Combined* column displays the metacall for the three control samples. The metacall is "Failure" for each sample because the control did not behave as expected. The control sample showed no amplification as indicated by Negative in the Target column.
- The *Target* column shows "Negative" because the samples did not show amplification in this channel.
- There is no value in the *Control* column because there were no controls that applied to these control samples. Each of these controls was an Internal Control and therefore did not apply to the other samples in the experiment. Each control applied only to the unknown in the same capillary, which was measured in a different channel.

Results for samples 4-8

There were no controls for samples 4-8 and there was no target DNA detected in this channel (705). Therefore, the Control column displays no data, the Target column displays Negative (because no amplification is visible), and the Combined call is Negative.

Viewing results for unknowns (channel 640)

The illustration below shows the results for the Unknown samples measured in channel 640. Note that 640 is selected in the Channel menu. In this channel, all sample positions contain Unknown samples. Samples 1-3 contain internal controls, measured in channel 705.



Results for samples 1-3

- The *Combined* column shows the metacall for these unknown samples. The metacall is "Invalid" because the internal control for each sample failed. To see the control calls, you must select channel 705, as described above.
- The *Target* column shows "Negative" because the unknown samples in this channel did not show amplification.
- The *Control* column shows Failure because each internal control failed.

Results for samples 4-8

- The *Combined* column shows the metacall for these unknown samples. The metacall is "Positive" because the samples showed amplification (as indicated in the Target column), and there are no controls that apply to samples 4-8. The only controls in the experiment are internal controls, which apply only to samples 1-3.
- The *Target* column shows "Positive" because the samples in this channel showed amplification.
- The *Control* column is empty because there are no controls that apply to these samples.

6. Performing Melting Curve Analyses

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

6.1 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. Even single-base differences in heterozygous DNA can result in melting temperature shifts.

Because melting temperatures vary according to these differences, melting temperature profiles can be used to identify and genotype DNA products.

To analyze sample melting temperature profiles, the fluorescence of the samples must be monitored while the LightCycler® temperature is steadily increased. As the temperature increases, sample fluorescence decreases. In the case of SYBR Green I dye, this is due to the separation of double DNA strands and consequently the release of SYBR Green I molecules. For HybProbe probes, this is due to the separation of target-probe hybrids resulting in the spatial separation of the dye molecules and a consequent drop in fluorescence.

6.1.1 Defining a Melting 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:

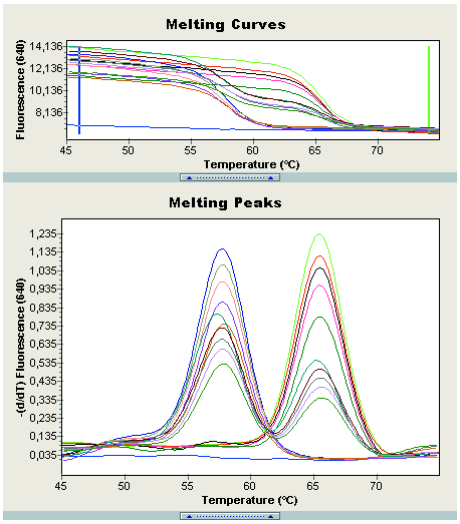
- A segment that rapidly heats the samples to a temperature high enough to denature all the DNA
- A segment that cools the samples to below the annealing temperature of the target DNA
- A segment that slowly heats the samples while measuring sample fluorescence as the target DNA melts

6.1.2 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 Tm) of a sample is defined as the point at which half the probes (or dye) have melted off the DNA.

The analysis displays a melting curve 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 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 illustrates a melting curve chart and a melting peak chart from a melting temperature analysis.



In the melting peak chart, notice that some sample curves include two peaks. These curves indicate heterozygous samples. After DNA amplification, a heterozygous sample contains two DNA sequences, which each melt at a different temperature, producing a two-peak curve for the sample.

6.1.3 Types of Melting Temperature Analysis

LightCycler® Software 4.05 includes two types of melting temperature analysis:

Genotyping analysis Groups samples with similar melting profiles together and identifies each group as a genotype. If desired, you can import melting curves for known genotypes and apply them to the samples, or you can include melting standards in the experiment. Use the Genotyping analysis for genotyping and for mutation detection. For more information see *Performing a Genotyping analysis*, below.

Tm Calling analysis For each sample, calculates the melting temperature, the melting peak, height, width and the area under each peak. Use the Tm calling analysis to identify characteristic melting profiles of DNA products. For more information see *Performing a Tm Calling analysis*, below.

6.2 Performing a Genotyping Analysis

You can perform a Genotyping analysis on an experiment that contains a melt program. The Genotyping analysis module determines the genotypes of unknown samples by analyzing the shapes of the melting curves of all the samples and then grouping curves with similar shapes together. The median curve of each group is defined as the genotype standard for that group. The software compares the melting curves of individual samples to the standard genotype curves.

You can also include standards with known genotypes in the experiment, or import an object containing standards. In these cases the software compares the melting curves of the individual samples to the designated standards.

The presence of a mutation introduces a mismatch that lowers the temperature at which the probe melts off the sequence. The melting temperature shift (ΔT_m) between a normal allele-probe match and a mutated allele-probe mismatch causes different fluorescence profiles, which indicate the presence of the mutation. The difference in melting temperature depends on the type of mismatch, the mismatch position within the probe sequence, and the base pairs immediately adjacent to the mismatch.

6.2.1 Methods Used to Compare Sample to Standard

As the software compares melting curves of individual samples and genotype standards, the software reports two numerical values. These values describe how closely an individual sample curve resembles the genotype standard curves.

- The *Score* measures the similarity between the sample and the standard that is closest to the sample. A Score of 1 indicates a sample whose melting curve is identical to the melting curve of at least one standard. A score of "nearly 0" indicates a sample whose melting curve is unlike the melting curve of any of the standards.
- The *Resolution* measures the dissimilarity between the sample and the second closest standard to the sample. If the Resolution of a sample is nearly equal to the Score of that sample, that sample's melting curve is similar to the melting curve of only one standard. Alternatively, if the Resolution of a sample is nearly 0, that sample's melting curve is similar to the melting curves of two standards.

There are three possible and one impossible combinations of Score and Resolution:

- If the Score and Resolution values are both nearly equal to 1, then the software has found an ideal match. However, the software can still declare a good match (*i.e.*, group the sample with a specific genotype) if the Score is nearly equal to 1 and the Resolution is as low as 0.1.
- If the Score is nearly equal to 1, but the Resolution is less than 0.1, the software has found a good match between the sample and a standard, but the sample is also a good match with another standard. In this case, it is difficult to distinguish between the two genotypes, and the software will classify the sample as an unknown genotype.
- If both Score and Resolution are near 0, then the software cannot classify the sample.
- It is mathematically not possible for Score to be near 0, and Resolution to be near 1.

Meaning of Score and Resolution values for "Negatives" and "Unknowns":

- Negatives: Sample curves are compared to an artificial negative standard (a quadratic polynomial). In this case, both the score and the resolution describe the similarity of the sample to this negative standard. Thus, the Score and Resolution are identical.
- Unknowns: You can specify stringency thresholds for Score and Resolution. If either value is below the threshold you set, the sample is classified as “Unknown.”

It is difficult for the software to determine genotypes if all the melting curves are very similar, or if, collectively, melting curves do not have distinct differences between groups. These situations arise when the melting peaks are small relative to the background, when there are too many genotypes present, or when the probes are old.

6.2.2 Performing the Analysis Using Automatic Grouping

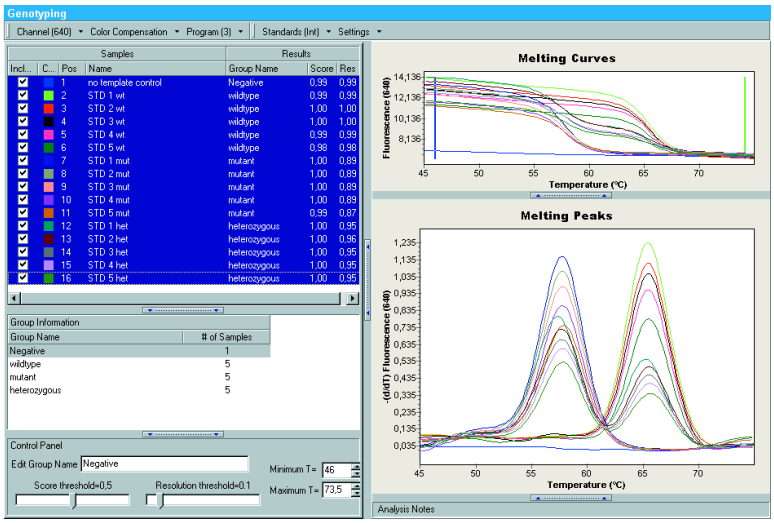
If you do not specify any standard samples in the Sample Editor, the module will use automatic grouping. In automatic grouping, the software analyzes the shapes of all sample melting curves and then groups curves with similar shapes, defining each group as a genotype. Then, the software compares the melting curves of individual samples to the median curve of each group. You can refine grouping parameters as necessary.

To perform automatic grouping:

1	Create and run a mutation detection experiment or open an existing experiment.
2	Click *Analysis* on the main toolbar.
3	Select *Genotyping*, then click *OK*.
4	In the *Sample Editor* enter general sample information in the *Capillary View* tab.
5	In the *Genotyping* tab of the *Sample Editor*, enter sample types (Unknown, Positive Control, or Negative Control). Do not designate any samples as Melting Standards.
6	Select *Genotyping* in the *module bar* to open the analysis module. The Samples list is displayed on the left; by default all samples are selected. Melting curve and melting peak graphs for the samples are displayed on the right.

The software automatically generates genotype groups and assigns genotypes to the unknown samples. The genotype groups are listed in the *Group Information* box below the sample list. In the sample list, the *Group Name* indicates the genotype group each sample is assigned to. The *Score* and *Res* values indicate how well each sample's curve can be matched to the genotype curve.

7 If you are not satisfied with the accuracy of the default automatic grouping and want to separate the melting peaks more precisely, click on *Settings* and select *High Sensitivity*.



8 To view the melting curves and peaks for a particular genotype group, select the group name in the *Group Information* box. Then, the melting curves and peaks in the group are displayed in the graphs portion of the work pane. The samples included in the group are highlighted in the sample list.

9 To rename a group, select a group in the *Group Information* box, then type a new name in the *Edit Group Name* box.

10 To remove a sample from its genotype group, double-click the check box next to the sample name in the sample list.

To refine the automatic grouping:

Groups are recalculated as soon as you change any of the parameters described below.

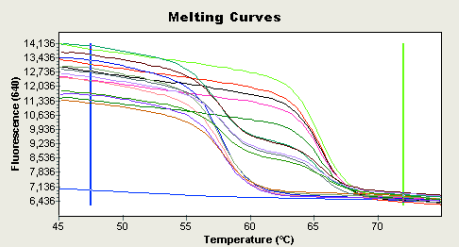
In the *Control Panel* at the bottom of the window, set the following parameters as needed:

Score threshold: Slide the bar to set the minimum score a sample must have to be identified as a genotype. The default value of 0.5 is recommended for initial analysis.

Resolution threshold: Slide the bar to set the minimum resolution a sample must have to be identified as one genotype rather than another. The default value for the Resolution threshold is 0.1.

Set the temperature range for genotype calls by dragging the blue and green vertical lines in the Melting Curve graph. The temperatures are displayed in the *Minimum T* and *Maximum T* boxes.

Genotype calls are made only for melting peaks located within the minimum and maximum temperature range. Make sure the temperature range is wide enough to include all melting peaks in the raw data.



Minimum T The temperature at which the melt analysis should begin. This value should be 5° to 10°C lower than the first melt peak.

Maximum T The temperature at which the melt analysis should end. This value should be 5° to 10°C higher than the last melt peak.

6.2.3 Performing the Analysis Using Standards Included in the Experiment

If standards are included in the experiment, the software compares the melting curves of the unknown samples to the curves of the standard samples to make the genotype calls.

To perform the analysis using standards in the experiment:

1	Create and run a mutation detection experiment or open an existing experiment that includes standard samples.
2	Click *Analysis* on the main toolbar.
3	Select *Genotyping*, then click *OK*.
4	In the *Sample Editor*, enter general sample information in the *Capillary View* tab.
5	In the *Genotyping* tab, select *Melting Standard* in the *Sample Type* column for each standard sample.
6	Enter a genotype name in the *Genotype* column for each standard sample. *You must enter a genotype name in the Genotype column for each melting standard. Enter different names for different melting standards.*
7	Select *Genotyping* in the *module bar* to open the analysis module. The software automatically assigns genotypes to the unknown samples, based on similarity to the melting curves of the standard samples. The genotype groups are listed in the Group Information box below the sample list. In the sample list, the Group Name indicates the genotype group each sample is assigned to. The Score and Res values indicate how well each sample's curve can be matched to the genotype curve.
8	To view the melting curves and peaks in a particular group, select the group name in the *Group Information* box. Then, the melting curves and peaks are displayed in the graphs portion of the work pane. The samples included in the group are highlighted in the sample list.
9	To rename a group, select a group in the *Group Information* pane, then type a new name in the *Edit Group Name* box.
10	To remove a sample from its genotype group, clear the check box next to its name in the sample list. To re-add a sample to its genotype group, select the check box next to its name.

6.2.4 Exporting Genotype Standards

You can export genotypes to be used as standards in other experiments.

To export a genotype:

1	Perform a *Genotyping* analysis, using either of the procedures above.
2	In the *Group Information* box, select the standard you want to export.
3	Click *Standards* and select *Save Standard As External*.
4	Navigate to the location where you want to save the object. (The typical location is *\Special Data\Melt Std* located under your user folder.) Enter a file name, then click *OK*. The standard information is exported and saved.

6.2.5 Performing the Analysis Using Imported Standards

You can import genotype standards into an experiment and use them to determine the genotypes of the unknowns.

To perform an analysis using imported standards:

1	Analyze the experiment using automatic grouping.
2	Click *Standards* and select *Use External Standard*.
3	Find and select the object containing the standards to import, then click *Open*. The imported standards are listed in the Group Information box. The results are recalculated using the imported standards.
4	To recalculate the genotypes using the melt curves from the experiment (that is, without the imported standards), click *AutoGroup*.

6.3 Performing Tm Calling Analysis

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

If SYBR Green I or HybProbe probes are used, the dye separates from the DNA during a melt, resulting in decreased fluorescence as the temperature increases. In the case of the SYBR Green I dye, this is due to the separation of double strands and consequently the release of SYBR Green I molecules. For HybProbe 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 at a certain temperature. The melting temperature, or Tm, is defined as the point at which half the probes have melted off the DNA.

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

The analysis displays a chart of the samples' melting curves 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 each sample's melting temperature and also the size of the area under each sample's melting peak.

The Melting Temperature analysis uses automated algorithms to find the peak areas and melting temperatures. The analysis module includes a *Settings* menu you can use to refine the analysis. Additionally you can edit the automatically calculated Tm results manually.

To perform the analysis:

1. Using LightCycler® Software 4.05, create and run an experiment or open an existing experiment that contains a melt program.
2. Click *Analysis* on the main toolbar.
3. Select *Tm Calling*, then click *OK*.
4. In the *Sample Editor*, enter general sample information in the *Capillary View* tab.
5. (Optional) On the *Tm Calling* tab select one sample as a *Tm Calibrator,* if desired, and specify its characteristic melting temperature. A Tm Calibrator is a sample with a known melting temperature. If you include a Tm Calibrator, the analysis results will be adjusted so that the melting temperature of the Tm calibrator sample matches its expected melting temperature. This will adjust the results for the other samples, as well. You might want to use a Tm Calibrator to adjust for minor differences between instruments.

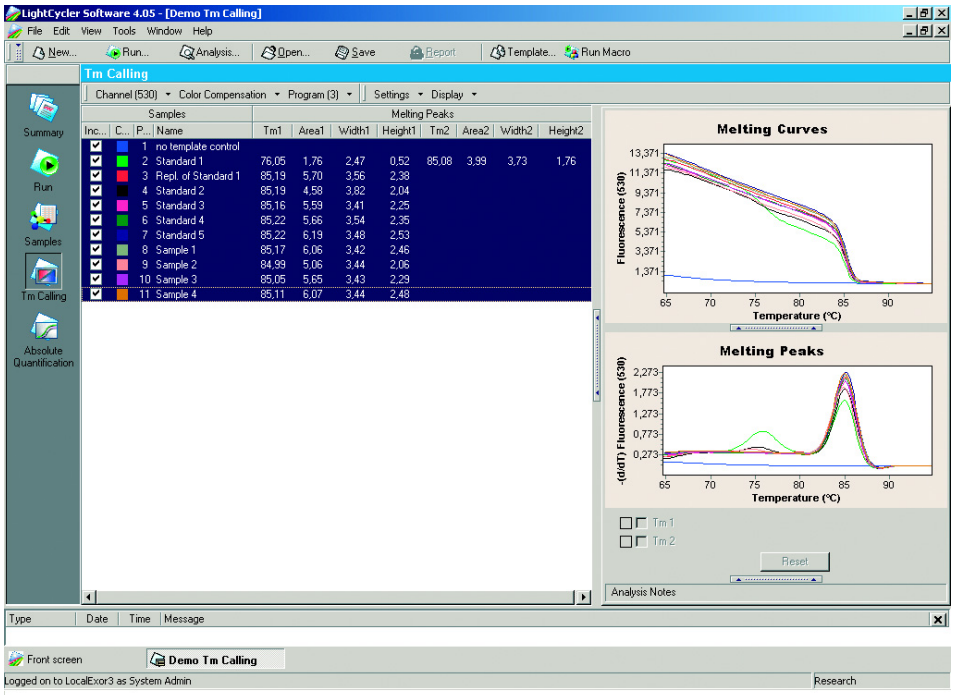
   In very rare cases where peaks contain distinct shoulders, the software may use the shoulder as a calibrator, when the real peak does not match its expected melting temperature. In this case, check the plausibility of the calculated melting temperatures of all samples.
6. In the *module bar*, select *Tm Calling* to open the analysis module.
7. If this is a multiplexed experiment, from the *Channel* menu, select the channel you want to analyze.
8. Click on *Display* and choose which results you want to view by selecting or deselecting the offered parameters. (For a description of the result parameters refer to the table below.)

9. View the results in the work pane, as described below. To view all the columns, drag the divider between the sample list and the charts to the right.

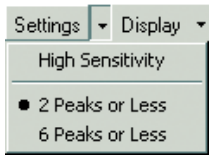
Result	Description
Tm1	The melting temperature for the first peak for the sample
Area1	The area under the first peak.
Width1	The width of the first peak
Height1	The height of the first peak
Tm2	The melting temperature for the second peak for the sample, if any.
Area2	The area under the second peak.
Width2	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, Area 3, and so on. The figure below shows results for a typical melting curve analysis.



To refine the analysis:

You can use the *Settings* and the *Display* menu to modify the specificity of the analysis.



To set an upper limit to the number of peaks found in the sample data, from the *Settings* menu, select *2 Peaks or Less* or *6 Peaks or Less*.

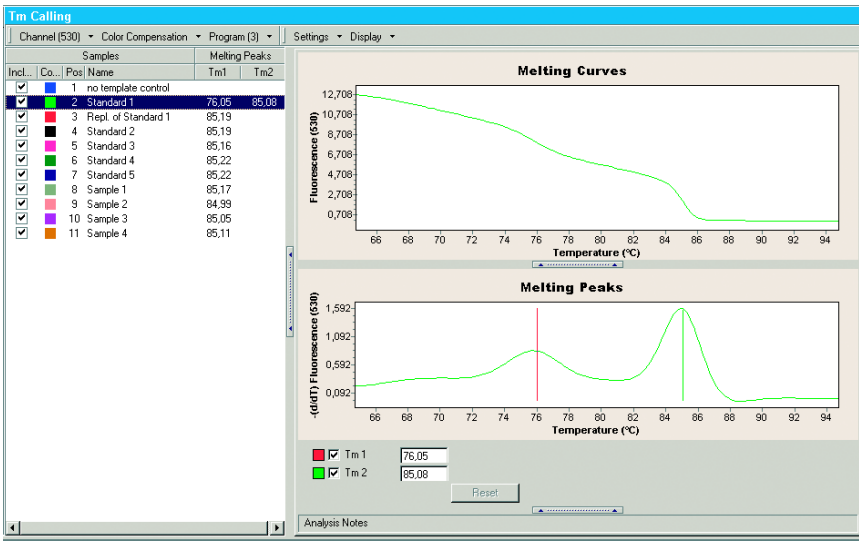
Sometimes there is a shoulder visible on the side of a peak. If you want to see these shoulders in the result data, from the *Display* menu, select *Show Shoulders*. To hide them, deselect Show Shoulders.

The calculation must be complete before Show Shoulders becomes active.

Sometimes there are very small peaks in the data. To calculate values for these minor peaks, from the *Settings* menu, select *High Sensitivity*. When you select High Sensitivity, the Tm and Peak Area values for the major peaks in the data might be smaller than if you do not use High Sensitivity. This is because the values for the major peaks will not include the data from the minor peaks. If you deselect High Sensitivity, then the data from the minor peaks might be incorporated into the results for the major peaks, making the Tm and Peak Area values larger for the major peaks.

To edit Tm calling results:

1. Perform the Tm calling analysis as described above.
2. Select a sample from the sample list to edit its calculated melting peaks.



3. Use the manual Tm sliders in the Melting Peaks chart to edit the Tm values or enter a value for the Tm in the corresponding Tm box. The respective Tm's, areas, heights and widths are recalculated and displayed in the sample list for this sample.
4. The changed results can be saved and then displayed on the report.
5. To remove a calculated Tm from the sample list, click the corresponding Tm check-box in the Melting Peaks chart to clear it.
6. To restore the original (automatically calculated) Tm values click *Reset*.

Tm editing is only possible for single samples.

7. Performing Other Analyses

This chapter explains how to determine nucleic acid concentration based on sample fluorescence, and how to compensate for bleedover between fluorescence channels in a multicolor experiment.

7.1 Performing Nucleic Acid Quantification Analysis

A Nucleic Acid Quantification analysis compares the fluorescence of unknown samples to the fluorescence of known samples without using an amplification reaction.

The analysis is performed on an experiment that measures sample fluorescence at a constant temperature for a specified hold time. The experiment can include multiple cycles, but each cycle uses the same temperature and hold time.

The experiment must include standard samples with known concentrations. The analysis uses the standard samples to generate a standard curve of concentration versus fluorescence. The analysis calculates the concentrations of the unknown samples based upon the location of their fluorescence values on the standard curve. The results are displayed in the sample list.

Prerequisite

You must include two or more standard samples of known concentration in the experiment. The samples are used to generate a standard curve of fluorescence versus concentration.

To perform the analysis:

1. Using LightCycler® Software 4.05, create and run the quantification experiment or open an existing experiment.
2. Click *Analysis* on the main toolbar.
3. Select *Nucleic Acid Quantification*, then click *OK*.
4. In the *Sample Editor*, enter general sample information in the *Capillary View* tab.
5. In the *NA Quant* tab of the *Sample Editor*, specify standards and unknowns as shown in the table below.

Column Name	Valid Values	Description
Target Name	Any name	Name of the target for this channel.
Sample Type	Unknown Standard	The type of sample in this capillary.
Conc.	Any concentration	Concentration of a standard sample.

6. Select *Nucleic Acid Quantification* in the *module bar* to open the analysis module.
7. Select the check boxes for the standards you want to use to generate the standard curve and for the samples you want to analyze. (Double-click a check box to select or clear it.)

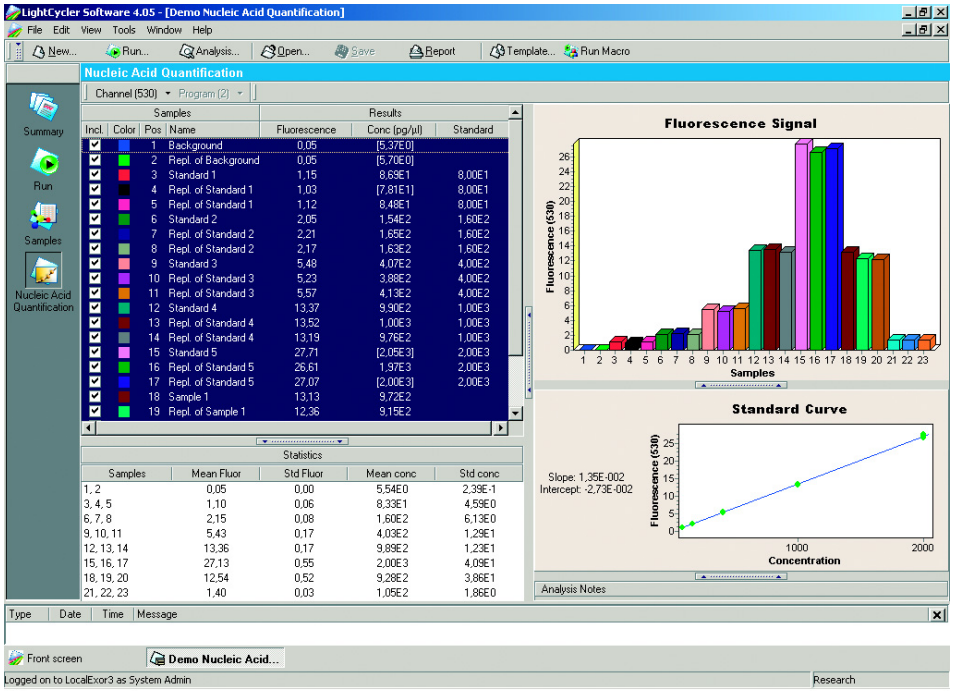
8 To view analysis results, click and drag the left border of the chart section to the right to display all the result data.
Results include the following:

Result	Description
Fluorescence	The fluorescence value.
Concentration	The calculated concentration of the sample (in the units specified in the NA Quant Sample Editor).
Standard	The specified concentration if this is a standard sample; this value is from the NA Quant Sample Editor.

9 Under the sample list view the following statistics for groups of replicate samples:

Statistic	Description
Samples	The samples in the replicate group.
Mean Fluor	The mean fluorescence for the samples in the group.
Std Fluor	The standard deviation for the fluorescence in the group.
Mean Conc.	The mean concentration for the samples in the group.
Std Conc.	The standard deviation for the concentration in the group.

The Nucleic Acid Quantification window is shown below.



7.2 Using Color Compensation

In a multicolor reaction, the wavelengths of light emitted by the dyes overlap, causing one channel to pick up signals from a dye measured by another channel. This bleed-over of fluorescence signal can result in uninformative data.

To correct the bleed-over, you can apply a color compensation object when you run the experiment or when you analyze the data. When you apply a color compensation object, LightCycler® algorithms use the data from the file to compensate for the fluorescence bleed-over. You then see only the specific signals in each channel.

Color compensation is used when you run an experiment that uses two or more different dyes. Typically for dual color applications LightCycler® Red 640 and LightCycler® Red 705 dyes are used.

Color compensation is only necessary when data is extracted from multiple fluorescence channels for analysis. Color compensation is not required when the experiment uses a single channel.

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

7.2.1 Performing a Color Compensation Experiment

A color compensation object always applies to a specific instrument. Therefore, you must run the color compensation experiment on the instrument you are going to use to run the multicolor experiments. To run the experiment, you can use a color compensation kit from Roche (for dual color applications with LightCycler® Red 640 and LightCycler® Red 705), or you can create and run your own color compensation experiment, as described below.

A color compensation object can also be created for a specific assay or chemistry lot number.

To run your own color compensation experiment:

If you are using a Roche kit, please refer to the pack insert of the kit for more details. To create the color compensation object, use the macro for automatic execution of the calibration, which is included in the Roche folder under Templates and Macros/Experiment Macros/Demo Macros/Color Compensation Set Macro (Cat No 02158850). Follow the instructions provided by the Experiment Kit Wizard.

To generate your own color compensation object, follow the general recommendations below.

1. Prepare up to seven capillaries, a blank and the fluorescence dyes you want to use in your experiments. (The number depends on the number of channels you need to compensate). We recommend using the same fluorescence probes that you are going to use in your multicolor experiments.

2. Open LightCycler® Software 4.05 and, using the same program settings you plan to use for your multicolor experiment, create a color compensation experiment that has one melting program with the following segments:
   Segment 1 95°C for 0 sec. at 20°C/sec.
   Segment 2 40°C for 30 sec. at 20°C/sec.
   Segment 3 95°C for 0 sec. at 0.1°C/sec., continuous.
   Select *Color Compensation* in the program's *Analysis Mode* field.
   As an Expert User you can copy the Color Compensation Set Macro to your own user folder and edit the sample information in the Color Comp tab of the Sample Editor. Most importantly, you must define the dominant channels.

3. In the *Sample Editor*, enter the following information in the *Capillary View* tab, if needed:
   Assay Cat No. The assay catalog number.
   Assay Lot No. The assay lot number.
   Color Comp ID A string that identifies the color compensation object to an experiment kit wizard. For more information about experiment kits see *Creating and using experiment kits*.

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

Column Name	Valid Values	Description
Dominant Channel	Water 530 560 610 640 670 705	The channel used for the dye in this capillary. Select "Water" for the first capillary (which should contain only buffer and water). Select the appropriate channel for each additional capillary.

5. Always place the capillary containing buffer or water (the blank) in the first position of the carousel.
   The Mg buffer in the "blank" should be the same as the one used in the assay to which the color compensation data will be applied.

6. Place the capillaries containing the dyes into the carousel in such an order, that the first one in position 2 has the lowest and the last one in position 4-7 the highest wavelength. An example is shown below.
   Capillary 1 A reagent blank (buffer, water)
   Capillary 2 Fluorescein
   Capillary 3 LightCycler® Red 640
   Capillary 4 LightCycler® Red 705

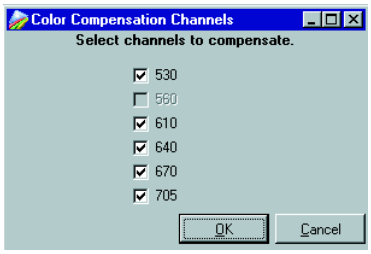
7	When the experiment is finished, select *Color Compensation* from the *Analysis* menu.
8	Select *Color Compensation Analysis* in the *module bar* to open the analysis window.
9	Click *Save CC Object* on the analysis window toolbar, then navigate to a location to save the color compensation object (typically *\Special Data\CCC* under your user name in the Navigator). Enter a name, then click *OK*.

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

7.2.2 Applying Color Compensation

You can apply color compensation before you run the multicolor experiment or when you analyze the experiment.

To apply color compensation:

1	To apply color compensation during an experiment run, click *Color Compensation* in the *Run* module, then select *Select Color Compensation...* . To apply color compensation to an analysis, add the analysis module, click *Color Compensation* in the analysis window, then select *Select Color Compensation...* . A *Select Object* dialog box displays color compensation objects that match the current instrument's ID.
2	Select the color compensation object you want to apply, then 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.*
3	A small dialog opens so you can select the channels to compensate. The number of channels displayed depends on the number of channels used in the color compensation experiment. By default all channels are selected. Channels that are not checked cannot be compensated. 
4	Deselect any channels you do not want to compensate, then click *OK*.
5	The experiment or the analysis charts are redrawn using the compensated data. Note that the Color-Compensation menu label now says "(On)."

8. Using Reports, Charts, Queries and Instrument Tools

In addition to the run and analysis software, LightCycler® Software 4.05 includes the following tools and commands:

- A report generator used to generate reports that include experiment information and analysis results; for more information see *Generating a Report* below.
- A tool to export and print charts to various graphic formats and copy and paste chart images and data; for more information see *Working with Charts* below.
- A query tool used to find objects in the database; for more information see *Using Queries to Find Information* below.
- A fluorimeter used to measure the fluorescence of a sample without cycling; for more information see *Measuring Fluorescence without Cycling* below.
- Instrument and application diagnostic tools, including an instrument self test, error and operation logs. For more information see *Using Diagnostic Tools* below.

8.1 Generating a Report

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
- Analysis results and other analysis items (the analysis items you can include vary by analysis type)

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

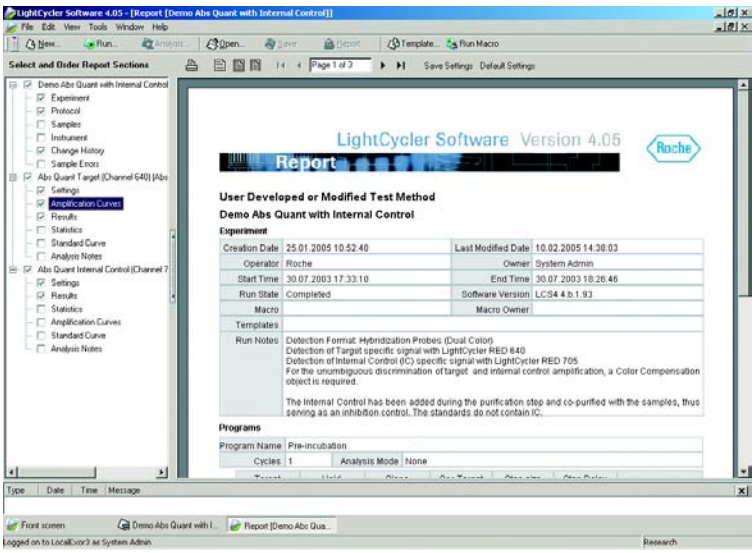
8

D

To generate and print a report:

1. Open an experiment that includes one or more analysis modules.

2. Click Report, or select *Report* from the *File menu*. The report window opens in the work pane. The left side contains 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.



3. Click items in the list to include or exclude them.

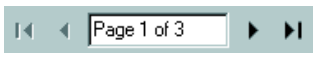
4. To change the location of an item in the report, click and drag the item to a new location in the list.

   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.

5. To save the changed settings for this object click *Save Settings*.

6. If you saved changed settings and want to restore the default settings click *Default Settings*, close the *Report* window and reopen it. The settings will then be the default settings again.

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



8. 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.

9. To print the report, click Print at the top of the report window.

8.2 Working with Charts

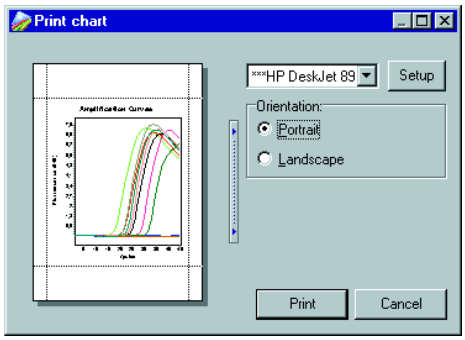
LightCycler® Software 4.05 generates many kinds of charts during and after an experiment run and as part of an experiment analysis. You can print charts, export charts to various graphic formats, and copy and paste chart images and chart data. The charts include a zoom and a pan function, so you can enlarge details of a chart and move the chart left or right.

8.2.1 Printing, Exporting, and Copying Charts

You can print any chart displayed in LightCycler® Software 4.05. 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, then select *Print*. A setup window is displayed.



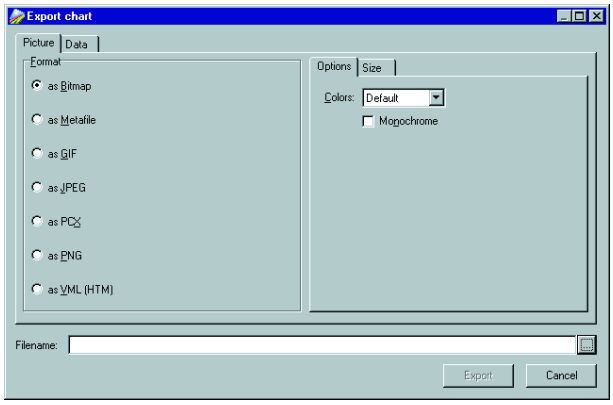
3. To change the graph margins, and therefore the size of the graph, click and drag the gray margin lines that surround the graph image.

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

4. If necessary, select a printer. (Your default Windows printer is selected by default).
5. To change printer configuration options, click *Setup*. A standard Windows printer setup dialog box is displayed. Enter the necessary information, then click *OK*.
6. Select the paper orientation (Portrait or Landscape) and click *Print*.

To export a chart image:

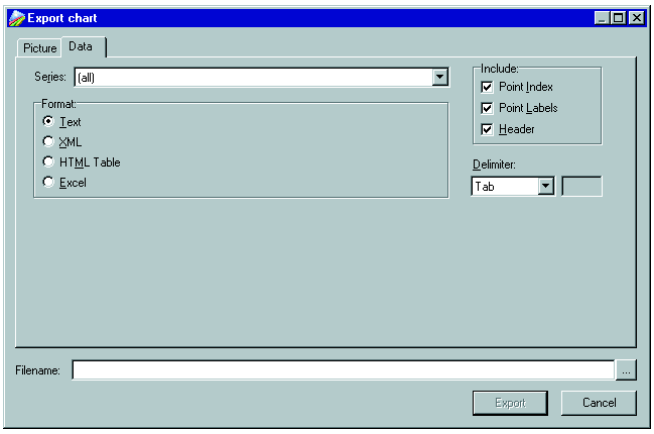
1. Display the chart you want to export.
2. Right-click within the chart boundaries, then select *Export*. An Export dialog box opens.



3. On the *Picture* tab, under *Format*, select the graphic format in which you want to export 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, then enter the new width and height values. Select *Keep aspect ratio* if you want to maintain the proportions of the chart.
6. Click  (to the right of the *Filename* box), to open a *Select output file* dialog box.
7. Browse to the location where you want to save the exported chart image, enter a name for the image, then click *Save*.
8. 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, then 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.
7. If you selected *Text* as the format, select a delimiter in the *Delimiter* box.
8. Click  (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, then 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, then 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, then press *Ctrl-V*.
4. To paste the chart data, open a text editor such as Notepad, then press *Ctrl-V*.

8.2.2 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 3-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 portions of the chart disappear off the window, use the middle mouse button to click the chart, then drag the chart until the portion you want to see is in view.

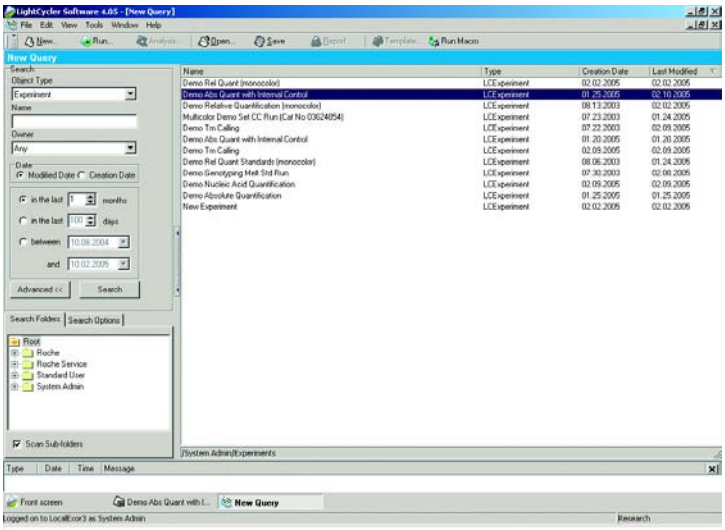
You may be able to configure a 2-button mouse so that clicking both buttons at the same time equals the functionality of a middle button. See your system administrator or refer to the device driver instructions that came with your mouse.

8.3 Using Queries to Find Information

LightCycler® Software 4.05 includes a query tool you can use to retrieve experiments and other objects stored in the LightCycler® Software 4.05 database.

To create and execute a query:

1. Click *New*, select the Query icon, then click *OK*. A query window opens in the LightCycler® Software 4.05 work pane.



2. In the *Object Type* box, select the type of object to look for, such as Experiment.
3. (Optional) Enter the name of the item to search for or the owner of the item, if known.
4. Select *Modified Date* or *Creation Date* to specify which date you want to use in the query.
5. Select a date range. You can specify the number of months or days before the current date to search, or you can select a beginning and ending date in the past.
6. (Optional) To refine the search, click *Advanced*, then enter additional search criteria. The available options in the *Search Options* tab depend on the type of object your are looking for. You can also select a certain folder in the *Search Folders* tab. Check the *Scan Sub-folders* box to include subdirectories of the directory.
7. Click *Search*. Results are displayed 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
   - Path where the object is stored (displayed when object is highlighted in the result list)

   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" below for instructions. Otherwise, see your system administrator.
8. To open an object, double-click the object name.
9. To save the query, click *Save*. A small navigator opens. Select a location, enter a query name, then click *OK*.
10. To close the search window, click *Close* from the *File* menu or click the X in the upper right corner of this window.

To execute a saved query:

1	Find the query you want in the *Navigator*. The default location for saved queries is in the Query folder under the Special Data folder for your user account.
2	Double-click the query name to open the query in the *Query* window.
3	In the *Query* window, click *Search* to execute the query.

8.3.1 Updating the Database

Occasionally, the LightCycler® Software 4.05 database may need to be updated, for example if a LightCycler® Software 4.05 release includes new query search parameters. When this happens, the software displays an error message stating that the query engine needs to be updated.

If your user account is assigned the Local Administrator role, follow the steps below to update the database. Otherwise, see your system administrator.

Prerequisites:

- You must have Local Administrator privileges to update the database.
- There must be no other users using the database.

To update the database:

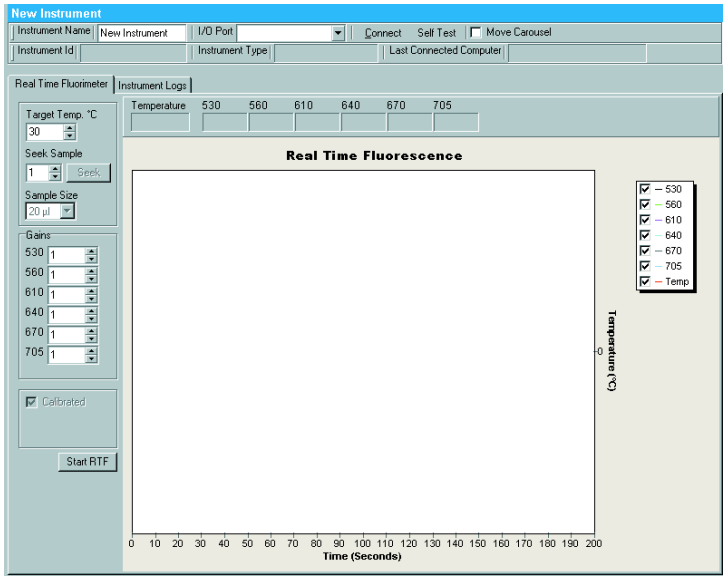
1	From the *Tools* menu, select *Database Status*.
2	Check the list of users in the *Logged In Users* tab. If other users are logged in, you cannot update the database. (The button on the Query Engine tab is inactive.)
3	If no other users are logged in, select the *Query Engine* tab.
4	Click *Update*. You are reminded that the update will take several minutes.
5	Click *Yes* to clear the message and continue. After the database is updated, a message states that the update is complete.
6	Click *OK* to clear the message. The *Query Engine* tab states that the query engine is now up to date. A *Reindex* button is displayed.
7	Click *Reindex*. You are reminded that the reindex will take several minutes.
8	Click *Yes* to clear the message and continue. After the database is reindexed, a message states that the reindex is complete.
9	Click *OK* to clear the message.
10	Close the *Database Status* dialog box.

8.4 Measuring Fluorescence without Cycling

The Real Time Fluorimeter monitors the fluorescence of a sample without running a cycling program. You can use the Real Time Fluorimeter to test the fluorescence of different dyes or to develop novel uses of the instrument, such as measuring the fluorescence of chemicals. Temperature and fluorescence data are displayed in a chart on the Real Time Fluorimeter window.

To use the Real Time Fluorimeter:

1. In the *Navigator*, double-click the instrument name to open the *Instrument* window. The Real Time Fluorimeter tab is active.



2. Place one or more capillary containing the chemistry you want to monitor in the LightCycler® carousel.

3. In the *Target Temp* field, type the temperature at which you want to monitor the fluorescence.

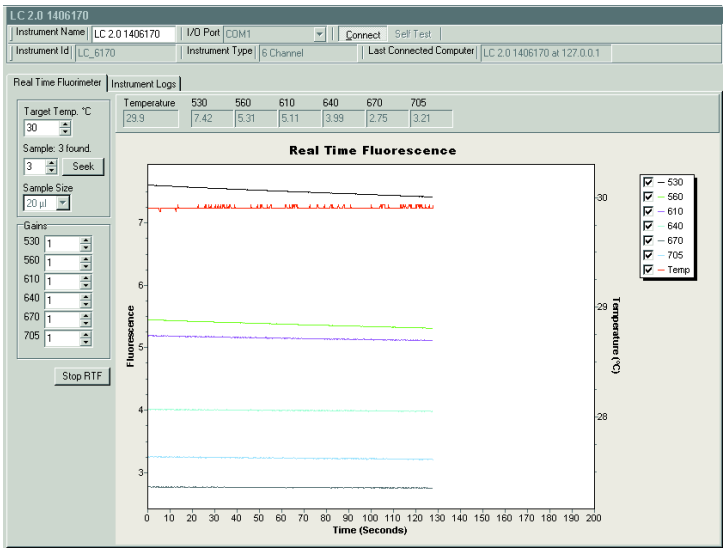
4. In the *Seek Sample* field, enter the carousel position for the sample you want to monitor.

5. Select a sample size.

6. Click *Start RTF*. The Real Time Fluorimeter begins warming the instrument, finds the sample, then starts reading the raw fluorescence output from the fluorimeter for each channel. It plots the temperature and the fluorescence values on the RTF chart.
For sample fluorescence, the Y axis of the RTF chart represents the percentage of maximum fluorescence. For temperature, the Y axis represents degrees Celsius. The X axis represents time in seconds.
A key to the right of the chart indicates the colors assigned to each channel and to the temperature (temperature is always red).

Select or deselect items in the key to add or delete them in the chart.

7 When the temperature of the sample reaches the target temperature, as indicated by the temperature line on the chart, note the fluorescence values for the channel of interest. The current temperature and the fluorescence for each channel are also displayed in fields above the chart.



8 (Optional) In the *Gains* box on the left, adjust the display for each channel to increase or decrease the raw fluorescence signal.

If measuring a dye, be sure to adjust the gains for the appropriate channel. For example, if the dye in the reaction mix is TaqMan, adjust gains in channel 1; if the dye is LightCycler® Red 640, adjust gains in channel 2.

9 To measure fluorescence for a different sample in the instrument, enter the sample position in the *Seek Sample* field, then click *Seek*.

10 (3-channel LightCycler® Instrument only) Select the Calibrated option if you want to set the instrument LED power to the factory-calibrated setting or deselect the Calibrated option to display a slider control; use the slider control to set the instrument LED power as a percentage of maximum LED power.

11 When finished, click *Stop RTF*.

8.5 Using Diagnostic Tools

LightCycler® Software 4.05 includes the following diagnostic tools used to monitor the LightCycler® Instrument's performance:

- A button used to perform an instrument self test
- Instrument logs

8.5.1 Performing an Instrument Self Test

The instrument self test checks all instrument functionality, such as heating to the correct temperature and rotating the carousel.

To perform an instrument self test:

1	In the *Instruments* folder of the *Navigator*, double-click the instrument name to open the *Instrument* window.
2	Click *Self Test* on the instrument toolbar. The software is locked while the self test is performed.

If an error occurs, the self test displays an error message and logs the error to the instrument's error log. See the next section for information about the error log.

If there are no errors, the software displays "Self test passed" when the self test is finished.

Do not start other software applications when performing an instrument self test.

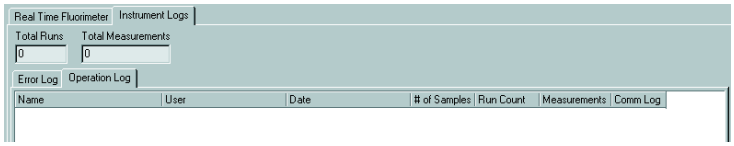
It is recommended to perform an instrument self test once a day before starting the LightCycler® runs. Always insert an empty carousel when performing an instrument self-test.

8.5.2 Viewing the Instrument Logs

LightCycler® Software 4.05 generates an Error Log and an Operation Log, which are both located on the Instrument Logs tab of the Instrument window. You do not need to use these logs in normal instrument operation. In case of an instrument problem you can export a System Query Data object and forward it to your support representative. The System Query Data object contains Error Log and Operation Log information as well as the experimental data of the source experiment.

To view instrument logs:

1. In the *Instruments* folder of the *Navigator*, double-click the instrument name to open the *Instrument* window.
2. Select the *Instrument Logs* tab.
3. The Total Runs and Total Measurements fields at the top of the tab indicate the total runs completed and the total number of individual fluorescence measurements taken since the instrument came from the factory. The fields are illustrated below.

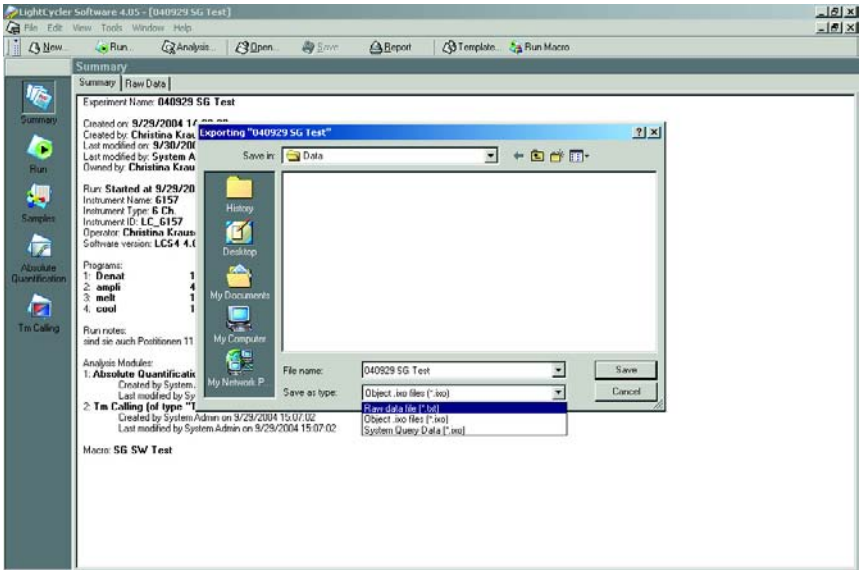


4. Select the *Operation Log* tab or the *Error Log* tab to view the related log. The logs contain the following information:
   - The *Operation Log* displays a list of the last 10 runs on the instrument. The log includes basic information about each run, such as the run name and date. It also lists the total number of runs and the total number of fluorescence measurements on the instrument as of the end of the run. A Comm Log button is displayed at the end of each row. Click *Comm Log* to display a list of all the commands executed in this run.
   - The *Error Log* lists all errors that have occurred on the instrument since the instrument came from the factory. Each error includes an error number, the date, and a remark (the error message). The error log information may be requested by a support representative, if there is an instrument problem.

8.5.3 Exporting a System Query Data Object

To export a System Query Data object:

1. In LightCycler® Software 4.05 open the experiment you want to export.
2. From the *File* menu, select *Export*.
3. Navigate to a location to save the exported file, select *System Query Data* as the file type, enter a file name, then click *Save*. The file is saved with an .ixo extension.



8.5.4 Viewing Application Errors

Error messages generated by LightCycler® Software 4.05 are displayed in a message pane at the bottom of the LightCycler® Software 4.05 window. You can choose to display or hide the messages pane.

To display or hide the messages pane:

- From the *View* menu select or deselect *Messages*.

Each error entry includes the date and time of the error, and the error message.

9. Working with Preferences

LightCycler® Software 4.05 provides the following preferences you can use to customize charts and samples and to set various default options:

Chart preferences determines the default appearance and content of your charts.

Sample preferences determines the default sample names in the Sample Editor and the default colors and line styles of samples in charts.

User preferences determines default import and export directories and other settings.

A set of preferences can be copied (as a separate item) from the Roche folder to one or more user folders in the LightCycler® Software 4.05 Navigator. When you open a preferences item in any folder, a window opens in the work pane to allow you to set the preference options.

You can have multiple chart and sample items, each with different settings. You can then choose the one you want to specify as the 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® Software 4.05 creates a new default preferences item when you log in.

This chapter explains how to

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

9.1 Using Chart Preferences

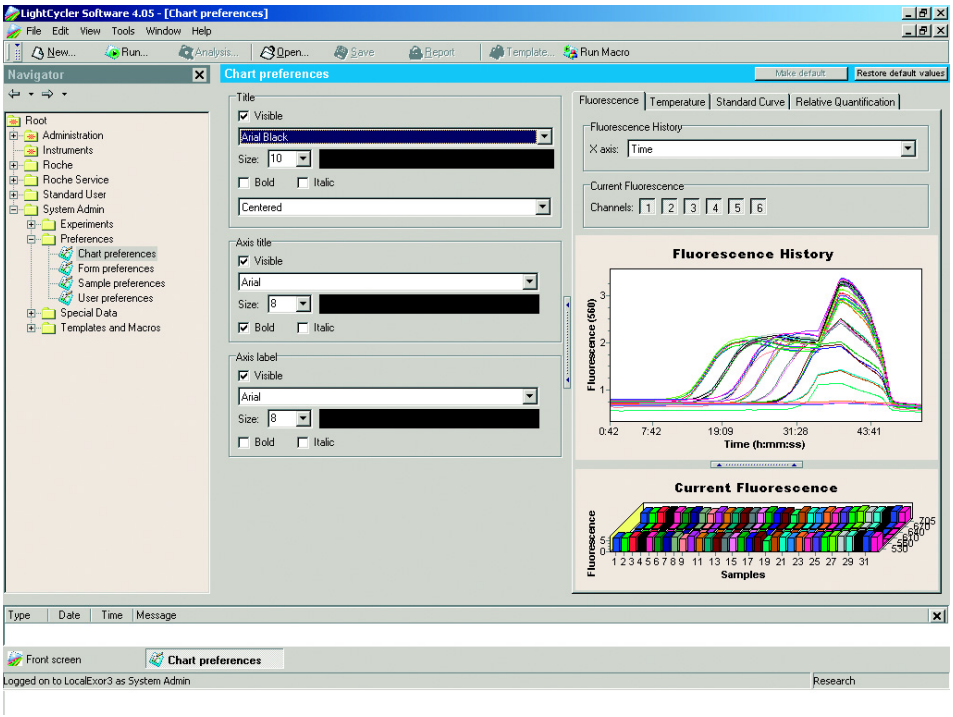
If copied from Roche folder, your user account includes an item called *Chart preferences* that determines the default appearance and content of your charts. You can change the default chart settings as needed.

You can also save a modified version of a chart preferences item with a new name and then apply the preferences in place of the defaults. You can have as many different chart preferences items as you want, each one defining a different look and feel for your charts. For more information see *Creating a Separate Preferences Item and Making it the Default.*

You can override the current chart preferences for individual charts, analyses, or experiments. See *Overriding Default Chart Preferences.*

To open the chart preferences item:

In your user folder in the *LightCycler® Software 4.05 Navigator*, open the *Preferences* subfolder.
Double-click *Chart preferences*. The *Chart preferences* window opens in the work pane.



Using the Chart preferences window, you can customize the following chart settings:

- Chart heading and label styles (using the three sections on the left).
- The content and appearance of specific types of charts (using the tabs on the right).

9.1.1 Specifying Chart Heading and Label Styles

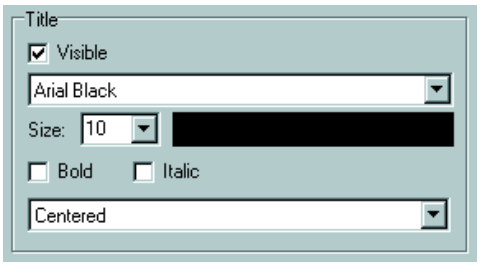
Use the following three boxes on the window to modify headings and labels:

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* check box 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 right of the *Size* box to display a color palette. Select the color you want, then click *OK*.
   - To make the text bold or italic, select the *Bold* or *Italic* check box (or both).
   - (*Title* only) To position the chart title, select a position from the pull-down list in the last box.

3. Click *Save* in the global toolbar to save your settings.

9.1.2 Specifying the Content of Fluorescence Charts

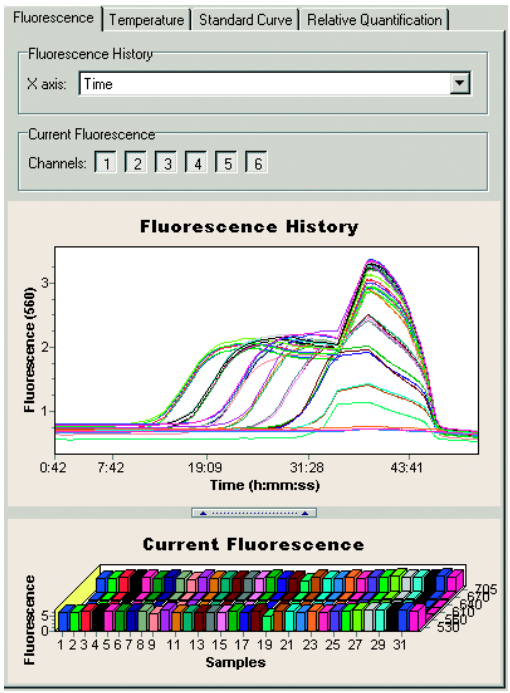
The *Fluorescence* tab of the *Chart preferences* window controls the default appearance of the fluorescence data displayed in these two fluorescence charts:

- The default axis value for the *Fluorescence History* chart that graphs fluorescence vs. 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).



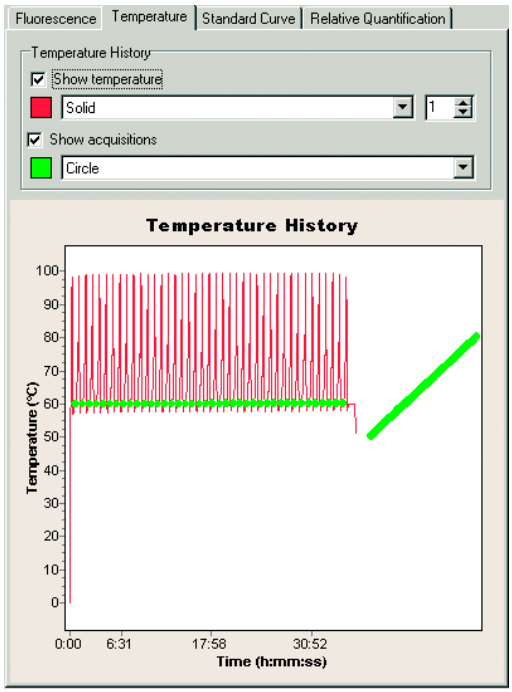
2. In the *Fluorescence History* box, select a value for the X axis from the pull-down list.
3. In the *Current Fluorescence* box, click each channel you want to include in the *Current Fluorescence* bar chart.
4. Click *Save* in the global toolbar to save your settings.

9.1.3 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 *Programs* tab (where it is labeled "Overview"), in the *Online Data Display* tab, and on the *Raw Data* tab of the Summary module.

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. To specify the appearance of the temperature lines on the chart:
   - To specify line color, click the colored box under *Show temperature* to display a color palette, select the color you want, then click *OK*.
   - To specify 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:
   - To specify point color, click the colored box under *Show acquisitions* to display a color palette, select the color you want, then click *OK*.
   - To specify a point style, select a style from the pull-down list.

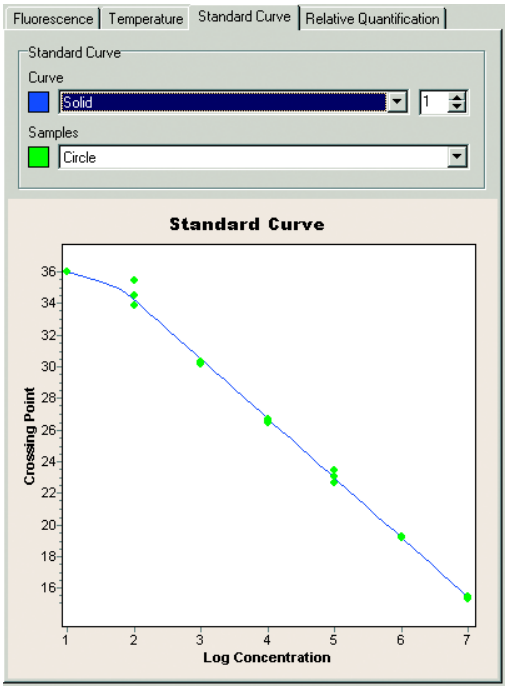
6. Click *Save* in the global toolbar to save your settings.

9.1.4 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. To specify the appearance of the curve:
   - ▶ To specify line color, click the colored box under *Curve* to display a color palette, select the color you want, then click *OK*.
   - ▶ To specify line style, select a style from the pull-down list.
   - ▶ To specify line width, enter or select a value.

3. To specify the appearance of the sample points:
   - ▶ To specify point color, click the colored box under *Samples* to display a color palette, select the color you want, then click *OK*.
   - ▶ To specify point style, select a style from the pull-down list.

4. Click *Save* in the global toolbar to save your settings.

9.1.5 Specifying the Appearance of Relative Quantification Charts

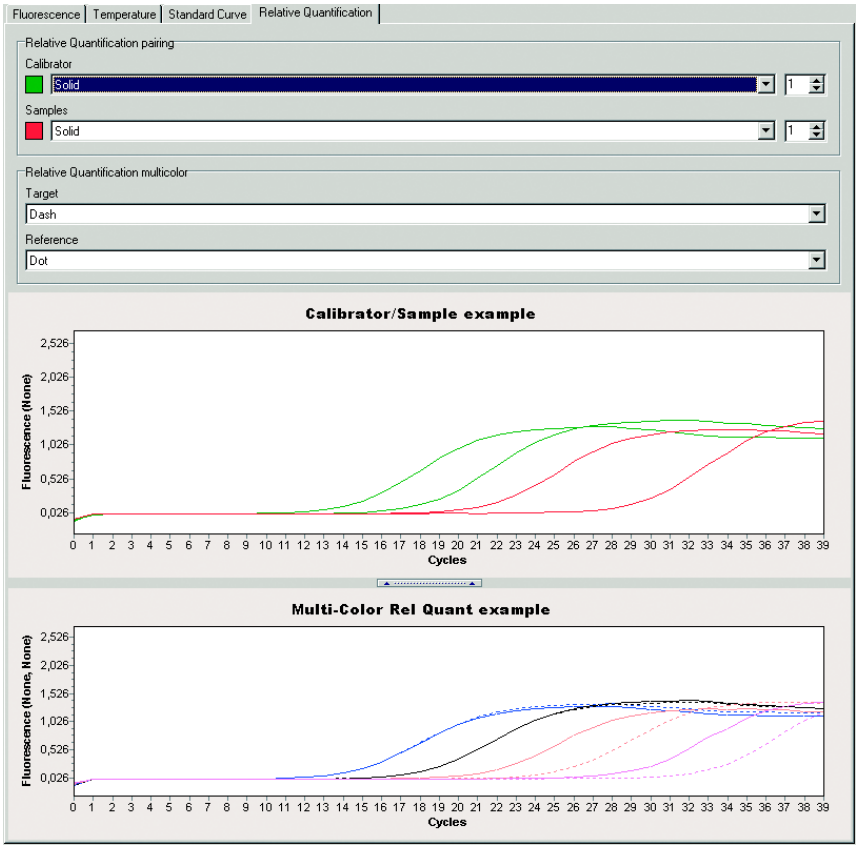
The *Relative Quantification* tab of the *Chart preferences* window controls the appearance of Relative Quantification pairing charts and multicolor Relative Quantification charts.

The *Pairing* chart displays amplification curves for target and reference unknowns and for target and reference calibrators. The curves for the unknowns have a different appearance than the curves for the calibrators. You can customize these settings.

The charts in a multicolor Relative Quantification analysis display amplification curves for target and reference samples that are in the same capillary. These samples have the same sample name and sample color and therefore must be distinguished by different line styles. You can customize these settings. For more information about Relative Quantification analyses see *Performing Relative Quantification analysis*.

To specify the appearance of the Relative Quantification charts:

1. Select the *Relative Quantification* tab.



2. To specify the appearance of the Calibrators in the *Pairing* chart:
   - To specify line color, click the colored box under *Calibrator* to display a color palette, select the color you want, then click *OK*.
   - To specify line style, select a style from the pull-down list.
   - To specify the line width, enter or select a value.

3 To specify the appearance of the *Samples* (the unknowns) in the *Pairing* chart:
- To specify line color, click the colored box under *Samples* to display a color palette, select the color you want, then click *OK*.
- To specify line style, select a style from the pull-down list.
- To specify the line width, enter or select a value.

Make the appearance of calibrators and samples different enough so that you can easily distinguish them. Refer to the sample chart to view your choices.

4 Specify the line styles of references and targets in the multicolor charts as follows:
- Under *Target*, select a line style for targets from the pull-down list.
- Under *Reference*, select a line style for references that is different from the Target style.
- Refer to the sample chart to view your choices.

5 Click *Save* in the global toolbar to save your settings.

9.1.6 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:

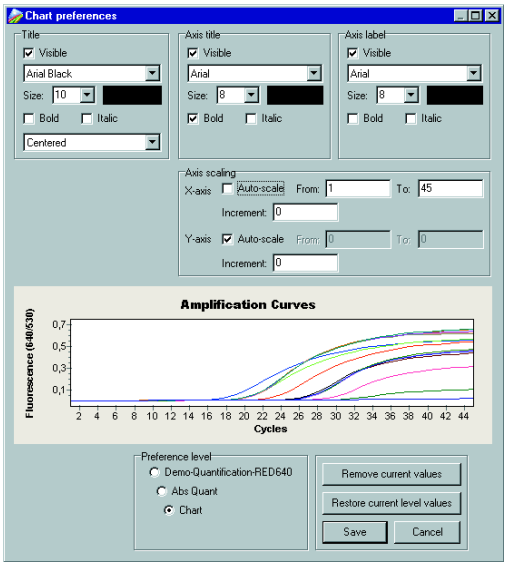
- An individual chart
- All charts within an analysis
- All charts within an experiment

When you specify custom settings at any of these three levels, the new settings take precedence over 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.

4. In the *Preference level* area, select one of the following:
   - Select the experiment name to apply the settings to all charts in the current experiment.
   - Select the 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.)
   - Select *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, then select the analysis level and make more changes, then 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, then 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.

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

6. 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.

7. 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.

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

9. When finished, click *Save*.

9.2 Using Sample Preferences

You can modify the appearance of sample lines and points on LightCycler® Software 4.05 charts in two ways:

- By 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.
- By 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.

You cannot change sample names for an existing experiment.

9.2.1 Modifying the Sample Preferences for all Experiments

If copied from Roche folder, your user account includes an item called *Sample preferences* that determines the default sample names and the appearance of sample lines and points on all LightCycler® Software 4.05 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 then apply the preferences you want.

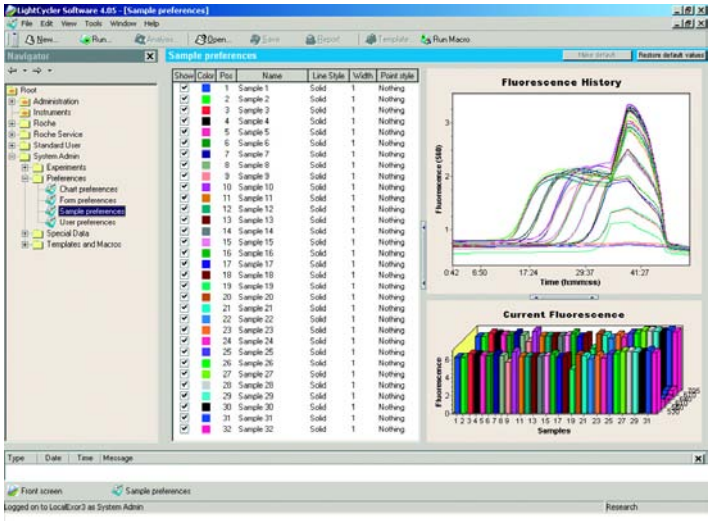
To modify default sample preferences:

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, then select the same item for the last sample in the group.
- Press *F2*.
- Change the value for the last sample and press the *Return* key.

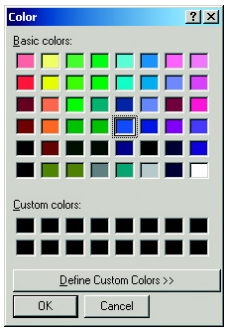
The changes are applied to all the selected samples.

1. In your user folder in the *LightCycler® Software 4.05 Navigator*, open the *Preferences* subfolder, then 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 sample's checkbox.

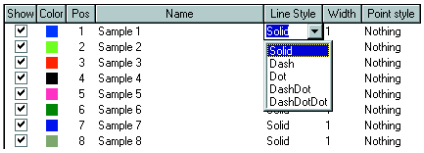
3 To change a sample's default color, click the colored square next to the sample name in the sample list to open a color palette.



4 Select the color you want, then click *OK*.
The sample color appears next to the sample name in LightCycler® Software 4.05 analysis results and is used for sample lines and points in the charts.

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

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



7 To change the line width, click in the *Width* column, then enter a new width.

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

9 Click *Save* in the global toolbar to save your settings.

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

1	In your user folder in the *LightCycler® Software 4.05 Navigator*, open the *Preferences* subfolder, then double-click *Sample preferences*.
2	Modify the preferences, as needed. For more information see *To modify default sample preferences*, above.
3	From the *File* menu, select *Save As*.
4	Navigate to a location to save the item, enter a name for the new preference item, then click *Save*.
5	To make this sample preferences item the default, click *Make Default* in the preferences title bar. *You can also specify the default sample preferences item using an option in the User Preferences. For more information see Specifying User Preferences, below.*

9.2.2 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 then save the settings as a sample preferences item that can be applied to other experiments.

At the experiment level, you can:

- Use a sample preference editor to change the appearance of multiple samples in the experiment.
- Apply an existing sample preferences item to the experiment.
- Changing 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 preference editor to change multiple samples:

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, then click *Save*.
5	Modify the settings as you would if you were modifying the default sample preferences item. For more information see *To modify default sample preferences*, above.
6	Click *Save* in the global toolbar to save the experiment with the new settings.

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.

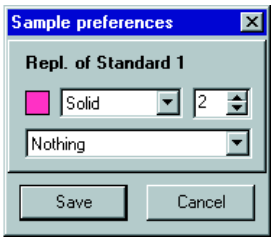
To apply a sample preference item to an experiment:

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, then click *Open*.
   The settings are applied to all charts in the experiment.
5. Click *Save* on the global toolbar 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 sample line.
   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 its line in an analysis chart
2. Move the mouse pointer over the line until the pointer changes to a hand, then right click the line. A small dialog box opens containing settings for the line.



3. To change the line color:
   ▶ Click the colored box to open a color palette.
   ▶ Select a new color, then click *OK*.
4. 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.
5. Click *Save* in the global toolbar to save the changes with the experiment.

To save experiment settings as a sample preferences item:

1	Open the experiment that has the settings you want to save as a sample preferences item.
2	Right-click an experiment chart that contains sample information.
3	Select *Save Sample Preferences*.
4	Navigate to a location to save the sample preferences item, type a name for the item, then click *Save*.

To reapply default sample preferences:

1	Open the experiment for which you want to reapply the default sample preferences.
2	Right-click any of the experiment charts that contains sample information.
3	Select *Clear Sample Preferences*. The samples in the experiment charts are reset to the current default sample preferences.
4	Click *Save* in the global toolbar to save the experiment with the settings.

9.3 Creating a Separate Preferences Item and Making it the Default

You can create multiple chart or sample preference items and then 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 preference item and specify it as the default:

1	In your user folder in the *Navigator*, open the *Preferences* subfolder, then double-click the default item for the preference type you want (*Sample preferences* or *Chart preferences*).
2	Modify the preferences, as described in the previous procedures.
3	From the *File* menu, select *Save As*.
4	Navigate to a location to save the new preference item, type a name for the item, then click *Save*.
5	To make this item the default, click *Make Default* in the preferences title bar. The new default settings are applied immediately and will be applied as the default preferences the next time you log in. *You can also specify the default preference items using options in User Preferences. For more information see Specifying User Preferences, below.*

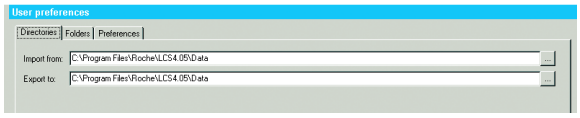
9.4 Specifying User Preferences

User preferences specify the following:

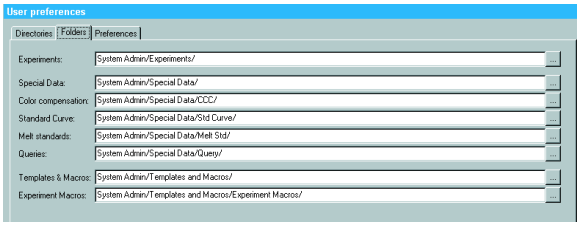
- Default directories to import files from and to export LightCycler® Software 4.05 files to
- The default database folders in which to save LightCycler® Software 4.05 items, such as experiments, macros, and queries
- Which chart and sample preference item to apply as the default, when there are multiple instances of a preferences item.

To specify user preferences:

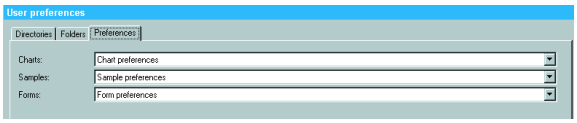
1. Open the *User preferences* item.
2. To specify import/export directories, select the *Directories* tab (if not currently selected).



3. In each box, type a directory path on your local computer or click , then navigate to a location on your local computer or the network, then click *OK*.
4. To specify default folders, select the *Folders* tab.



5. In each box, type the path for the folder location in the Navigator or click to display a *Navigator*. Select the folder you want, then click *OK*.
6. To specify a preference item as the default, select the *Preferences* tab.



7. In each box, select the preference item you want to specify as the default for the preference type.
8. The software prompts you to confirm the modifications when closing the *User preferences* window. Click *Yes* to save your changes.

10. Using Templates and Macros

Templates and experiment kits 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, that has the information you want to use.

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

Your user account must have the Expert User or Local Administrator role for you to create templates or experiment kits. If your user account has the Standard User role, you can execute an existing kit macro to run an experiment. You can also assign macros to buttons on the Front window of the LightCycler® Software 4.05, for easy access. Clicking a button executes the kit macro.

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

10.1 Creating and Using Templates

Templates enable you to quickly reproduce frequently used items such as experiment protocols, reports, and sample lists.

A template is based on an individual item, such as a protocol or a sample list, that has the information you want to use. You apply the template to a blank item to quickly duplicate the information. For example, you cannot rerun an experiment protocol, but you can save the protocol from an existing experiment as a template. To reproduce the experiment, you simply create a new experiment, then apply the template. You can modify the new experiment created from the template, if needed.

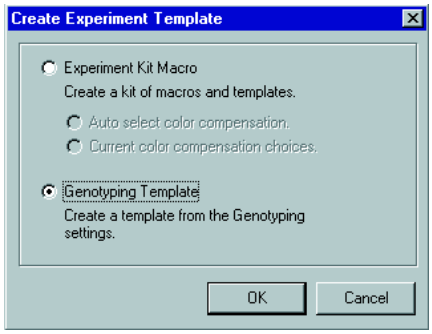
10.1.1 Creating Experiment Module Templates

You can create a template for a single module of an experiment, such as a protocol, a sample list, or an analysis module.

To create an experiment module template:

1	Create the module to be used as the template; be sure the module has all the settings and other information you want to include in the template. Make the module active in the work pane.
2	From the *Tools* menu, select *Create Macro/Kit/Template*.

3. Select the option for the individual module type, then click *OK*.
   In the example below, a Genotyping analysis module is selected for the template.



4. Navigate to the location where you want to save the template (typically under the *Templates and Macros* folder under your user name in the *Navigator*), enter a template name (or accept the default name), then click *OK*.

10.1.2 Creating Report Templates

You can create templates also for reports.

To create the template:

1. Open the report, then select and arrange the order of items you want in the report.
2. From the *Tools* menu, select *Create Macro/Kit/Template*.
3. Navigate to the location where you want to save the template (typically under the *Templates and Macros* folder under your user name in the *Navigator*), enter a template name, then click *OK*.

10.1.3 Applying a Template

Templates created for experiment modules or reports are executed the same way. Follow the instructions below.

The components of a complete experiment template are stored as individual templates in the location you chose for the complete experiment. You can select and apply these individual templates as you would any other experiment module template.

To apply an individual template:

1. Open the experiment module or other item you want to apply the template to.
2. Click *Template*, or from the *Tools* menu, select *Apply Template…*
3. Find and select the individual template you want to apply, then click *OK*.
   After applying the template, modify the item, if needed.
4. Click *Save*.

10.2 Creating and Using Experiment Kit Macro

An experiment kit macro is based on an existing experiment with its run protocol, sample list, and analysis modules and reports. A kit macro includes templates for all the components of the experiment and a program called a wizard used to run the kit macro. When you run the kit macro, the wizard steps you through the process of choosing an instrument, and then applies the experiment protocol and sample templates, starts the experiment run, and after the run is finished, adds the analysis modules to the experiment. The wizard gives you the opportunity to modify the protocol, sample list, and so on, if needed.

A kit macro is stored as a single item in the database. You can export it as a file and then import it into other LightCycler® Software 4.05 databases. For example, you might want to create a kit for a commonly used protocol and then share it with other LightCycler® Software 4.05 users.

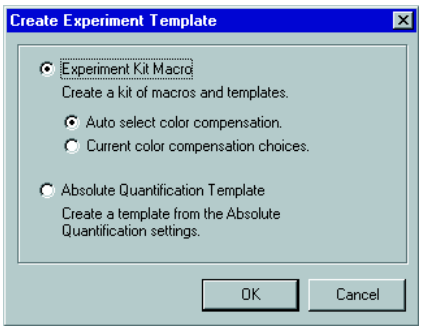
To execute a kit macro you can use the Run Macro command on the toolbar or the Tools menu. You can also link the kit macro with a macro button on the LightCycler® Software 4.05 Front window. Clicking the button executes the kit macro.

10.2.1 Creating an Experiment Kit Macro

To create an experiment kit macro, your user account must have the Expert User or Local Administrator role.

To create an experiment kit macro:

1. Open the existing experiment you want to use for the kit macro.
2. Be sure to include all the components you want in the kit: the experiment programs, a sample list, color compensation (if needed), analysis modules, and reports.
3. With the experiment active in the work pane, from the *Tools* menu, select *Create Macro/Kit/Template*.
4. A dialog box similar to the following is displayed. (The second option depends on which experiment module is currently displayed in the work pane.)



5. Select *Experiment Kit macro* (if not already selected), then click *OK*.
6. If the current experiment uses color compensation, select one of the following options:
   - *Auto select color compensation* The current color compensation data will not be included in the kit macro. When the kit macro is executed, the software will search for the last color compensation data set that matches the current instrument ID and the color compensation ID, entered on the Capillary View tab of the Sample Editor (if any).
   - *Current color compensation choices* The color compensation data for the current experiment will be included in the kit macro.
7. Navigate to the location where you want to save experiment kit macro (typically under the *Templates and Macros* folder under your user name in the *Navigator*).
8. Enter a template name (or accept the default name), then click *OK*. An item for the kit macro is saved at the designated location.

10.2.2 Executing an Experiment Kit Macro

When you execute an experiment kit macro, LightCycler® Software 4.05 automatically launches a wizard to guide you through the process of using the kit macro to run the experiment. As you run the kit macro, you can modify sample information, run parameters, and analysis parameters if needed.

To execute an experiment kit macro:

1. Click *Run Macro* or from the *Tools* menu, select *Run Macro/Kit*.
2. Find and select the kit macro name, then click *Open*.
3. A wizard Welcome window opens.

4. Click *Next*, then follow the wizard prompts to select an instrument, load samples, and run the experiment.

   The wizard provides opportunities for you to modify the experiment protocol and the sample information as e.g., the number of samples before proceeding with the experiment. While you enter your modifications in the work pane the Experiment Kit Wizard window will be open in front of the work pane. You can move it to any area of the screen, if you need to see the whole work pane.

5. When the experiment is finished, the wizard generates the analyses and reports, if these are included in the kit. If a report is displayed, you can modify report selections to change the content of the report. You must close the report to view the experiment analyses.

10.2.3 Modifying a Kit Macro

If you need to modify a kit macro, you must create a new experiment that has the modifications you want, then create a kit macro from the experiment. Save the new kit macro using the name of the old kit macro to replace the old kit macro.

10.2.4 Sharing a Kit Macro with Other LightCycler® Software 4.05 Systems

An experiment kit macro is a convenient way to share an experiment template with others, because you can export the kit macro as a single file. The file has an .ixo extension. The file can then be imported into another LightCycler® Software 4.05 system where it can be executed as a macro or linked to a button on the LightCycler® Software 4.05 Front window. Follow the procedures below to export and import an experiment kit macro.

To export an experiment kit macro:

1	In the *Navigator*, double-click the kit macro name.
2	From the *File* menu, select *Export*.
3	Navigate to a location to export the file, then click *Save*.

To import an experiment kit macro:

1	From the *File* menu, select *Import*.
2	Select *Object .ixo files*.
3	Find and select the IXO file for the kit macro you want, then click *Open*.
4	From the *File* menu, select *Save*.
5	Navigate to a location to save the kit, then click *OK*.

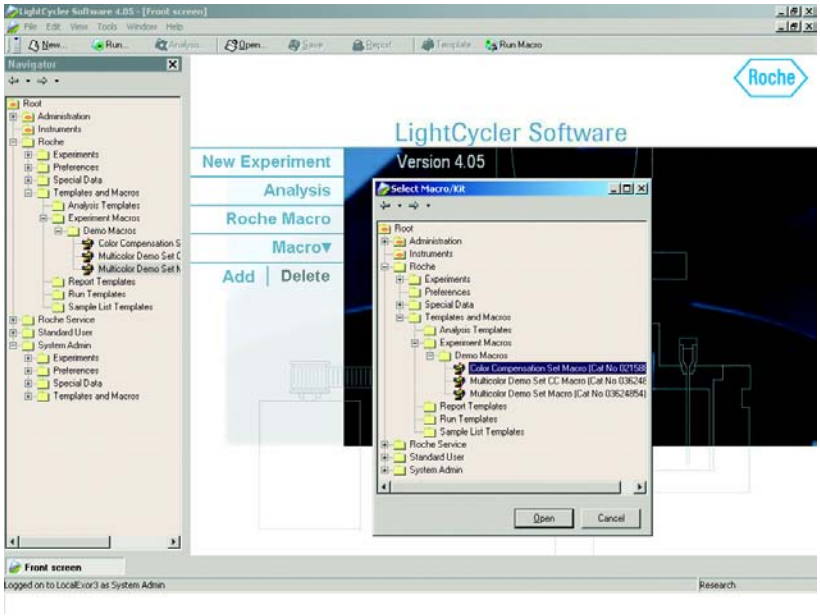
10.3 Adding and Deleting Macro Buttons on the Front Window

If you create experiment kit macros, you can link each kit macro to a macro button on the LightCycler® Software 4.05 Front window. Clicking the button runs the kit wizard.

Follow the instructions below to assign a kit macro to a button on the Front window. You can add and delete macro buttons no matter what role is associated with your user account.

To add a macro button:

1. If the LightCycler® Software 4.05 Front window is not currently displayed, click *Front screen* in the taskbar or select it from the *View* menu to display the window.
2. Click *Add*. If *Add* is not visible on the window, click the arrow next to *Macro* to display *Add / Delete*.
3. The *Select Macro/Kit* dialog box opens, as shown below.



4. Find and select the experiment kit macro you want, then click *Open*.
5. A button labeled with the kit macro name is added to the window below *Add / Delete*.
6. To execute the kit macro, click the button.

To delete a macro button:

1. If *Delete* is not visible below *Macro* on the LightCycler® Software 4.05 *Front* window, click the arrow next to *Macro* and click *Delete*.
   A list of all macro buttons is displayed.
2. Select the button to delete, then click *OK*.
3. The button is deleted from the window.
   Only the macro button is deleted from the window, not the macro itself.

11. Managing User Access

To use LightCycler® Software 4.05, you must have a user account in the LightCycler® Software 4.05 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 chapter explains the function of user accounts, roles, and groups, and explains how to manage them using the LightCycler® Software 4.05 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 for you to use the User Management tool.

11.1 Understanding User Accounts

A user account provides access to the LightCycler® Software 4.05. 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 *Understanding Roles* below. You can also add a user account to one or more groups. Users in the same group have access to each other's objects. For more information see *Understanding Groups* below.

Each user account has a default folder in the LightCycler® Software 4.05 Navigator, 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 System Admin is created automatically when LightCycler® Software 4.05 is installed. The System Admin user has the Local Administrator role and is used to create other user accounts. The System Admin account cannot be edited or disabled, but the default password can be changed.

A user account once created can be made inactive. An inactive user account cannot have a role and cannot be assigned to a group.

11.2 Understanding Groups

A group is a collection of user accounts. The members of a group have access to each other's objects; for example, they can open each other's experiments.

If users belong to more than one group, they need only one group in common to have access to each other's objects. 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 another user's objects is determined by the user's own role. For example, if Bob has the Standard User role, he can open and execute Susan's experiments. If Susan has the Expert User's role, she can open, execute, modify, copy, move, or delete Bob's experiments. See the next section for more information about roles.

11.3 Understanding Roles

Each user account is assigned one and only one role. The role determines the user's privileges. There are four roles:

- Standard User
- Expert User
- Local Administrator
- Roche User

Roles cannot be created or deleted, but certain access privileges can be enabled or disabled for each role. For more information see *Working with Roles* below.

11.3.1 Privileges of the Standard User Role

A Standard User has limited access to the LightCycler® Software 4.05. A Standard User can do the following:

- Use macros to execute and analyze experiments (if the analyses are included in the macros).
- Modify, move, copy, and open or execute the user's own objects. For experiments, this includes modifying sample names, target names, sample notes, analysis notes, and the sample count (before the run begins). The standard user can include or exclude samples from the experiment's analysis only if enabled by the Local Administrator.
- Open or copy objects, and execute macros owned by a Local Administrator or the Roche User.
- Open or copy objects, and execute macros in other Standard Users' and Expert Users' folders, if the other users are members of the same group as the Standard User.

A Standard User cannot do the following:

- Use the Run programming window to create experiment programs from scratch or to refine experiment programs.
- Add analyses to experiments.
- Use the analysis toolbar to modify analysis settings, such as program or color compensation.
- Create macros or templates.
- Modify or move objects belonging to other users.
- View folders or objects belonging to users who are not in the same group as the Standard User.
- Delete, move, copy, or rename default folders (including the user's own default folders).
- Delete any object (including the user's own objects).

11.3.2 Privileges of the Expert User Role

An Expert User can do the following:

- Use the Run programming module to create and execute experiments.
- Create and use templates and macros to execute experiments and analyze results
- Create all other objects and open, copy, execute, modify, and move any of the user's own 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 the user's own objects and nondefault folders, if enabled by the Local Administrator.
- Open, copy, and execute objects owned by the Local Administrator or the Roche User.
- Open, copy, and execute objects owned by other Expert Users, if the other users are in the same group as this user.
- Create, open, copy, execute, modify, delete, and move items in a Standard User's folder, if the Standard User is in the same group as the Expert User.

An Expert User cannot do the following:

- Create, delete, move, modify, or rename objects belonging to the Local Administrator, the Roche User, or to Expert Users.
- See the folders or objects belonging to Standard and Expert Users who are not in the same group as the user.
- Delete, move, copy, or rename default folders (including the user's own folders).

11.3.3 Privileges of the Local Administrator Role

A Local Administrator can do 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 the user's 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 and execute items belonging to other Local Administrators and copy items from Local Administrators.
- Open, execute, and delete items in the Roche folder.
- Create, open, copy, execute, modify, delete, and move objects in folders belonging to Standard and Expert Users.
- Use the User Management tool to manage users and groups; for more information see *Managing Users, Groups, and Roles* below.

The Local Administrator 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 the user's own folders).

11.3.4 Privileges of the Roche User and Roche Role

The Roche User is a special, predefined user account that is assigned the Roche role. Neither the Roche User nor the Roche role is visible in the User Management tool. Only representatives from Roche have the user name and password needed to log in as the Roche User.

The Roche User has access to the Roche folder, which contains objects, such as experiment protocols and standard curves, supplied by Roche.

The Roche Role allows the Roche User to do the following:

- Create, modify, move, execute, copy, and delete items and subfolders in the Roche folder.
- Create, modify, move, execute, copy, and delete subfolders and items belonging to other users, only if enabled by a Local Administrator.

The Roche User cannot do the following:

- Delete, move, copy, or rename the Roche folder or user folders.

11.4 Managing Users, Groups, and Roles

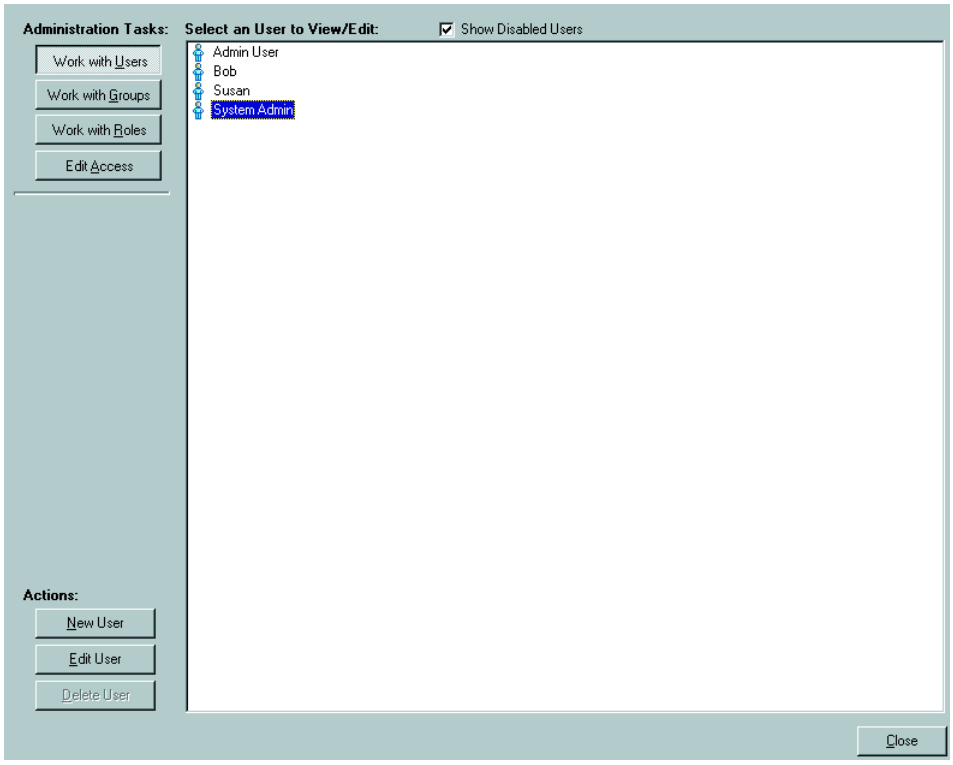
The User Management tool allows you to

- 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 Management tool.

To open the User Management tool:

▶ From the *Tools* menu, select *Manage Users*.
The main window of the User Management tool is shown below. *Work with Users* is selected by default.

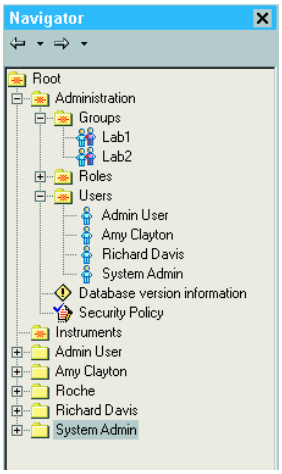


11.4.1 Working with Users

To create a new user account:

1	In the *User Management Tool*, click *Work with Users* (if not already selected).
2	Click *New User*.
3	Enter the user's full name, login name, and password and select a role for the user.
4	Check each group you want to add the user to. If no groups have been created, you can add the user when you create the group.
5	Click *Done*.

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.



In the example above, four user accounts and two groups are listed in the Navigator. The user accounts are

Admin User
Amy Clayton
Richard Davis
System Admin

Note that each user account has its own user folder.

The groups are
Lab1
Lab2

To edit, enable, or disable a user account:

1	In the *User Management Tool*, click *Work with Users* (if not already selected).
2	Select the user's name in the right pane, then click *Edit User*.
3	Change any of the user fields as needed. If you selected System Admin, the only information you can change is the password.
4	To disable the user account, select the *Access Disabled* check box; to reactivate a disabled account, clear the *Access Disabled* check box. You cannot disable the System Admin account.
5	When finished, click *Done*.

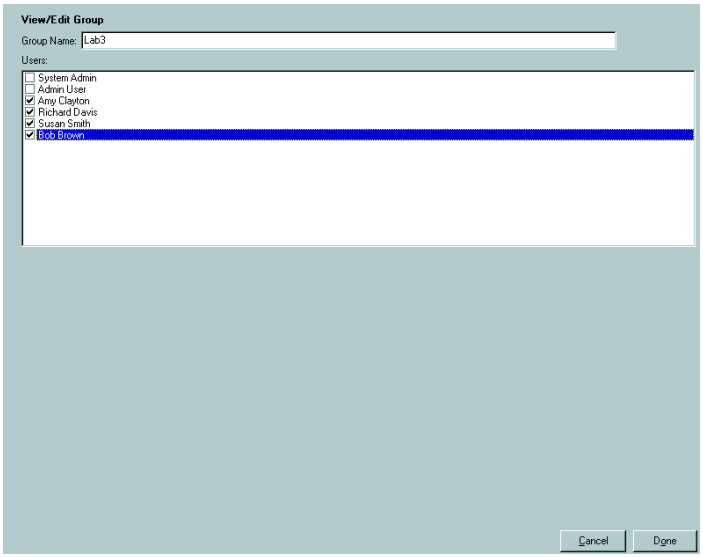
To delete a user account:

1	Disable the user account you want to delete as described above.
2	Right-click the disabled user in the N*avigator* and select *delete* to empty the folder, if the folder contains objects.
3	Select the user's name in the *User Management Tool* and click *Delete User*.

11.4.2 Working with Groups

To create a new group:

1. In the *User Management Tool*, click *Work with Groups*.
2. Click *New Group*.
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 *Done*.
6. If you did not add users to the group, a message asks you to confirm saving the group without users. Click *OK*.

The group is added to the Groups folder in the Navigator.

To edit a group:

1	In the *User Management Tool*, click *Work with Groups*.
2	Select the group name in the right pane.
3	Click *Edit Group*.
4	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. See *Working with Users* above.
5	Click *Done*.

To delete a group:

1	In the *User Management Tool*, click *Work with Groups*.
2	Select the group name in the right pane.
3	Click *Delete Group*. You are prompted to confirm the deletion.
4	Click *Yes* to delete the group.

11.4.3 Working with Roles

You cannot create or delete roles, but you can change a user's role assignment. You can also modify certain of the access rights associated with the Standard User, Expert User, and Roche User roles. You cannot modify access rights of the Local Administrator role. You can also determine the time when a user is automatically logged off and determine the maximum login attempts before access is disabled on entering an invalid password.

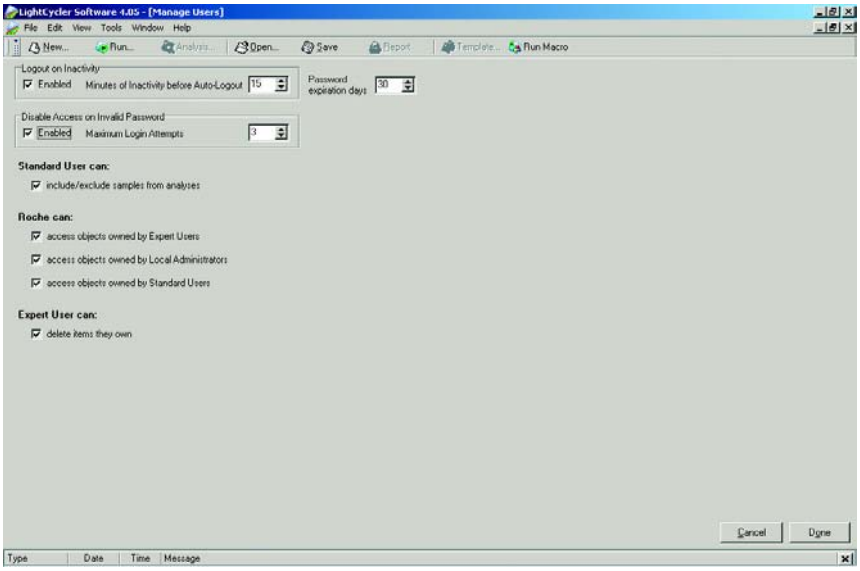
To change a role assignment:

1	In the *User Management Tool*, click *Work with Roles*. Roles are displayed in the work pane.
2	Click the + next to a role to display a list of users assigned to the role.
3	To change a user's role, select the user's name, then drag the user name to a new role.
4	Click *Done*.

You can also change a user's role assignment when you edit the user account. See *Working with Users* above.

To modify a role's access rights:

1. In the *User Management Tool*, click *Edit Access*. The access window displays the available options. Initially, all options are selected.



2. In the *Logout on Inactivity* area, enter a value in the *Minutes of Inactivity before Auto-Logout* box or keep the default. Deselect the *Enabled* box to disable the Auto-Logout function.
3. In the *Disable Access on Invalid Password* area, enter a value for the *Maximum Login Attempts* or keep the default. Deselect the "Enabled" box to disable this function.

   Exceeding the maximum login attempts blocks the access to your account. Contact your system administrator.

4. Enter a value in the *Password expiration days* box or leave the default.
5. Select or deselect access rights for each role.

To unblock a user account:

1. In the *User Management Tool*, click *Work with Users* (if not already selected).
2. Select the disabled user's name in the right pane, and then click *Edit User*.
3. Clear the *Access Disabled* check box, and then click *Done*.

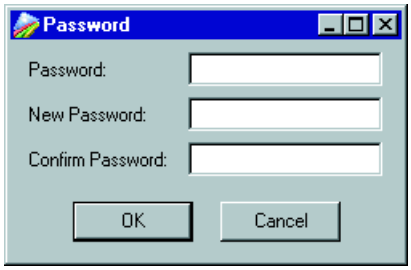
You need local administrator rights to unblock a user account.

11.5 Changing your Password

When you are assigned an account on the LightCycler® Software 4.05 system, you receive an initial password you can use to log on to LightCycler® Software 4.05 the first time. You should change this initial password as soon as possible.

To change your password:

- From the *Tools* menu, select *Change Password*. The Password dialog box is displayed.



- Enter your current password in the *Password* box.
- Enter the new password in the *New Password* box and again in the *Confirm Password* box. Click *OK*.

The password must contain at least six characters and contain one number and one upper case character.

Passwords are case-sensitive.

Remember the password or keep it in a secure place. Do not share your password with others.

12. Installation of LightCycler® Software 4.05

The complete LightCycler® software version 4.05 includes the LightCycler® Software 4.05 application, a database, and an object server. If you have received a license to install the LightCycler® Software 4.05 also on a second or several other local computers *e.g.*, for data analysis, follow the installation instructions below.

This chapter explains how to install LightCycler® Software 4.05. It includes the following topics:

- Overview of software installation
- Hardware and software requirements
- Installing LightCycler® Software 4.05
- Saving an existing database and installing additional databases
- Removing LightCycler® Software 4.05
- Changing the default administrator password and creating user accounts

12.1 Overview of Software Installation

LightCycler® Software 4.05 controls the LightCycler® 2.0 Instrument using information you provide in an experiment protocol. The LightCycler® Software 4.05 includes the LightCycler® Software 4.05 application, a database, and the database object server (called "Exor 3"), which communicates with the database. The software needs to be installed in a local configuration.

In this configuration all the software components are installed on the LightCycler® computer connected to the LightCycler® 2.0 Instrument. Each instrument and computer function together as an independent system with its own database and its own set of user accounts. Computers not connected to an instrument, which can be used for the LightCycler® Software 4.05 application tools but not for running an experiment, are independent systems as well.

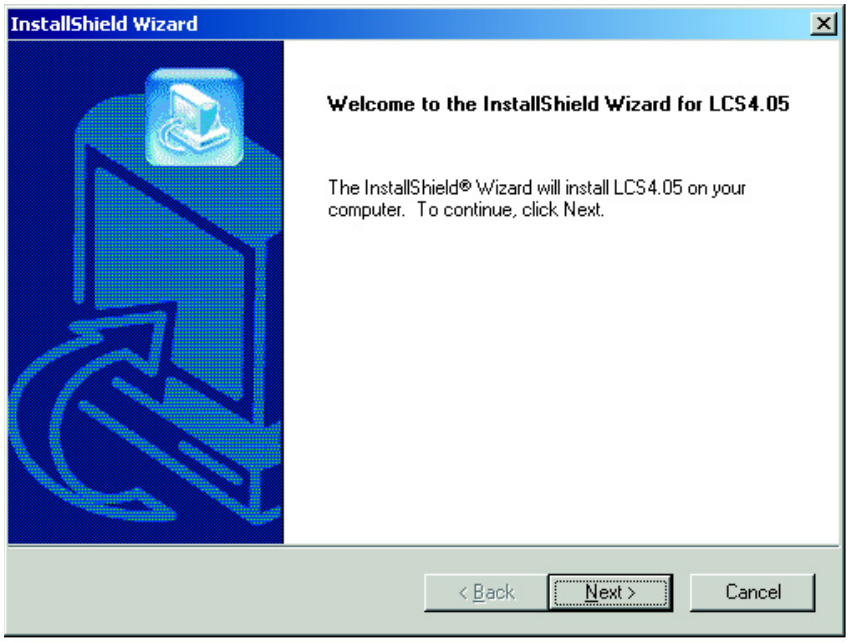
The LightCycler® 2.0 Instrument must not be connected to a network, although network hardware is present. Being connected to networks inherently carries a risk that the connected instrument may be infected by viruses and worms as well as targeted for attacks by malicious attackers. Roche is not responsible for any damages caused by connection of the LightCycler® 2.0 Instrument to a network by the customer.

12.2 Hardware and Software Requirements

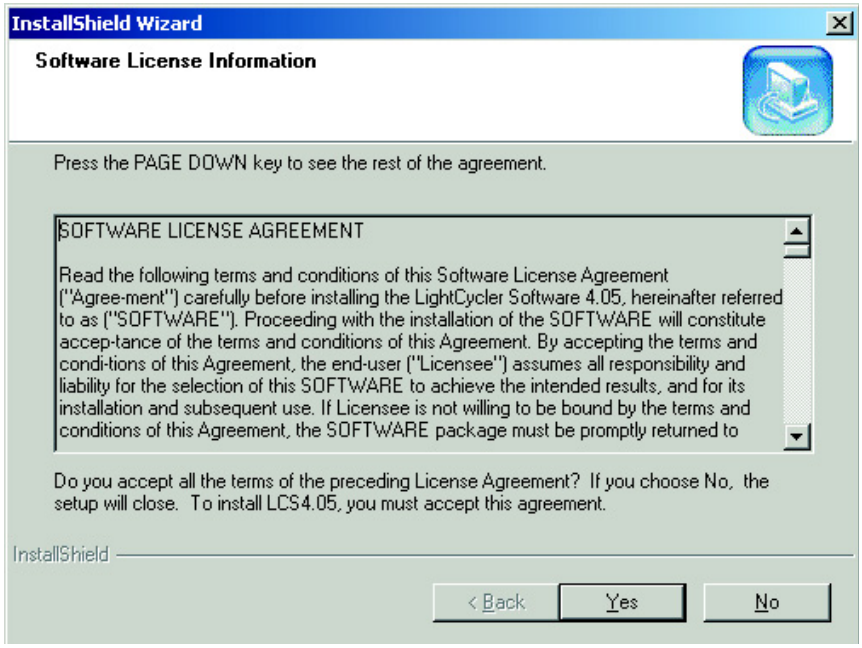
For hard- and software requirements of the computer used to connect to the LightCycler® 2.0 Instrument or to launch the LightCycler® Software 4.05 for data analysis, please refer to chapter B *System Description*.

12.3 Installing LightCycler® Software 4.05

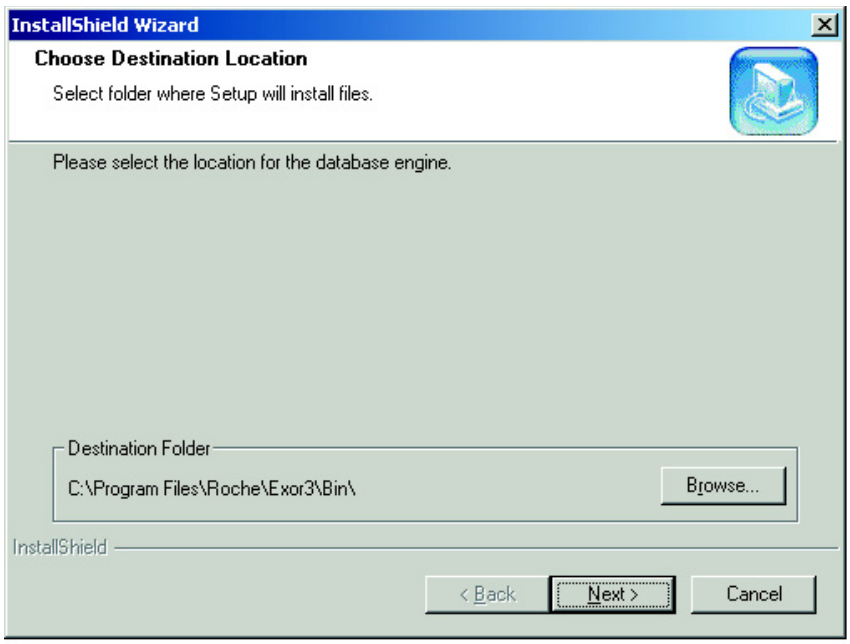
1. Insert the LightCycler® Software 4.05 CD. Double-click LightCycler_Software_405_Setup.exe, if installation doesn't start automatically. The installation process transfers files, extracts the files, and then prepares the installation wizard. The InstallShield Wizard Welcome window opens. Click *Next*.



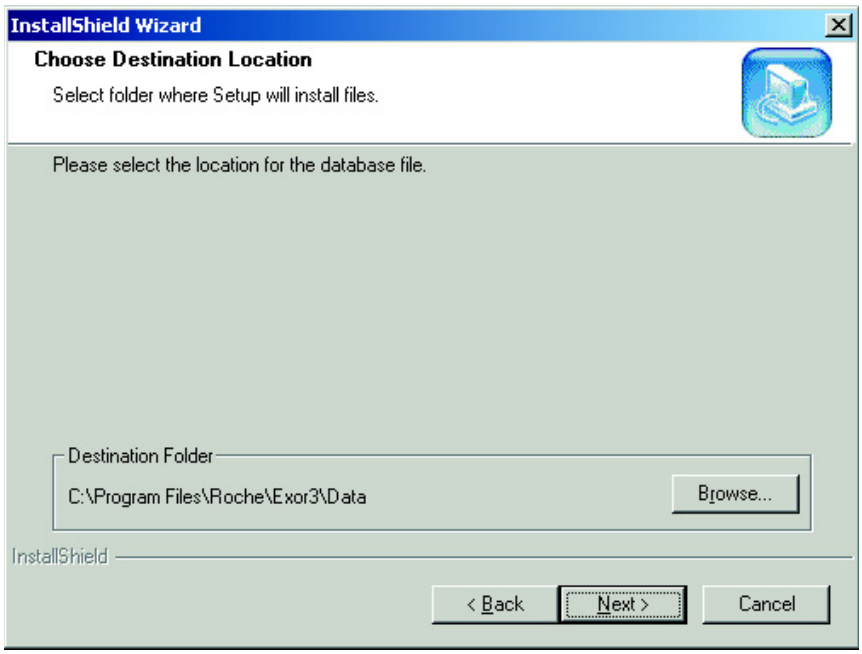
2. Your are prompted to agree to the license conditions. Click *Yes*.



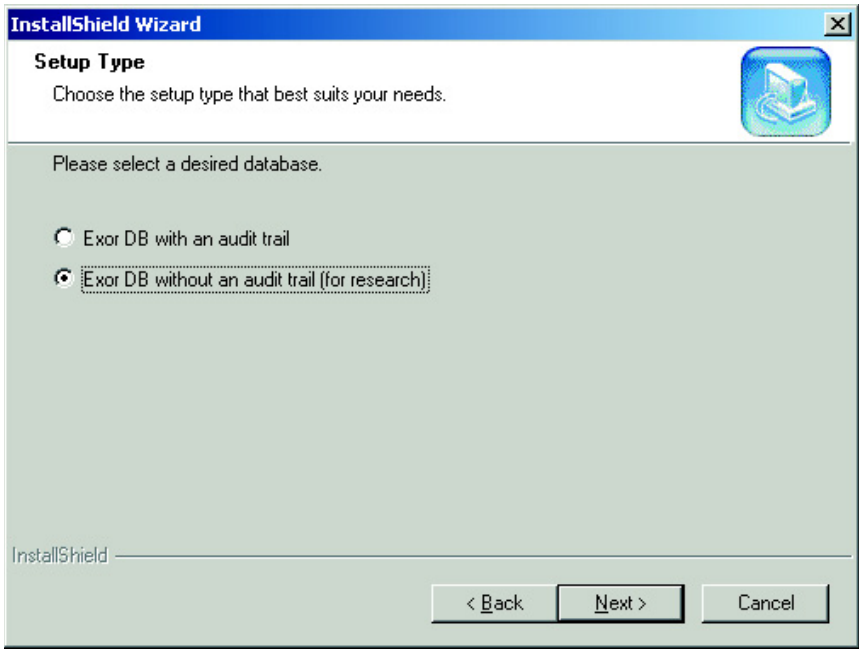
3 Select the default settings to install the database engine or browse to select a location. Click *Next*.



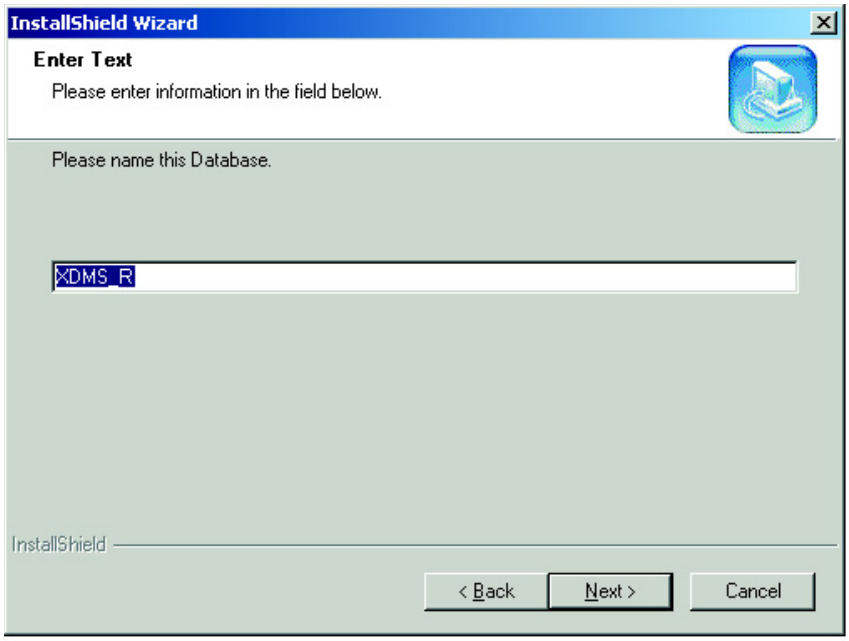
4 Select the default settings to install the database file or browse to select a location. Click *Next*.



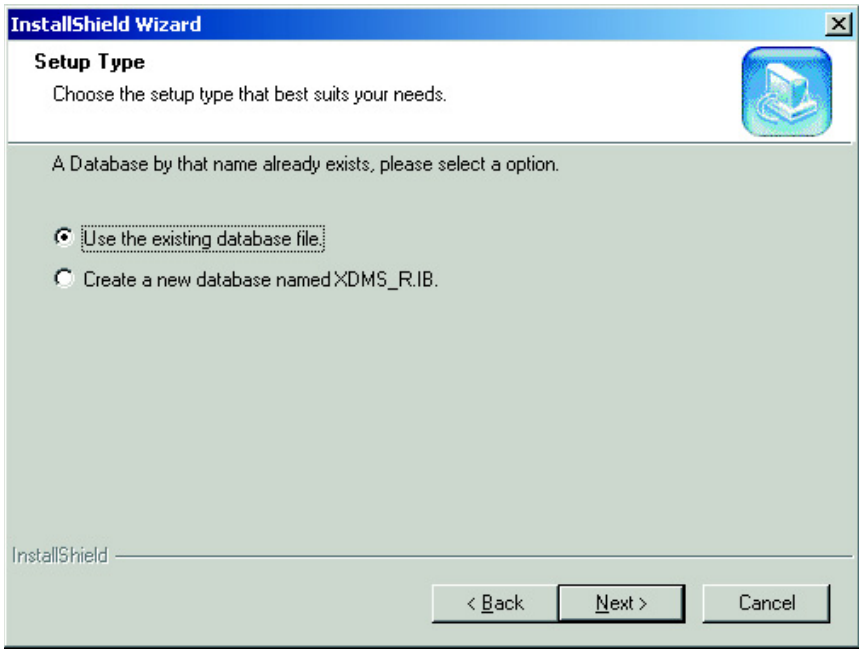
5 In the Setup Type window select *Exor DB without an audit trail (for research).* Click *Next.*



6 The software prompts you to name the new database. Enter a database name or leave the default, then click *Next.*

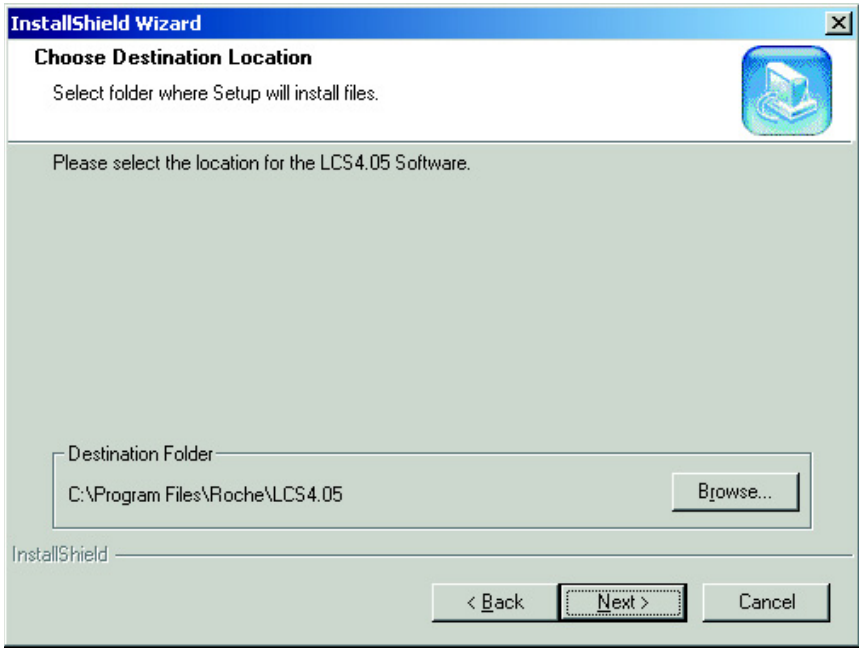


7 If a database with the same name already exists, you are prompted to use the existing database or create a new database with the same name. Click *Next*.

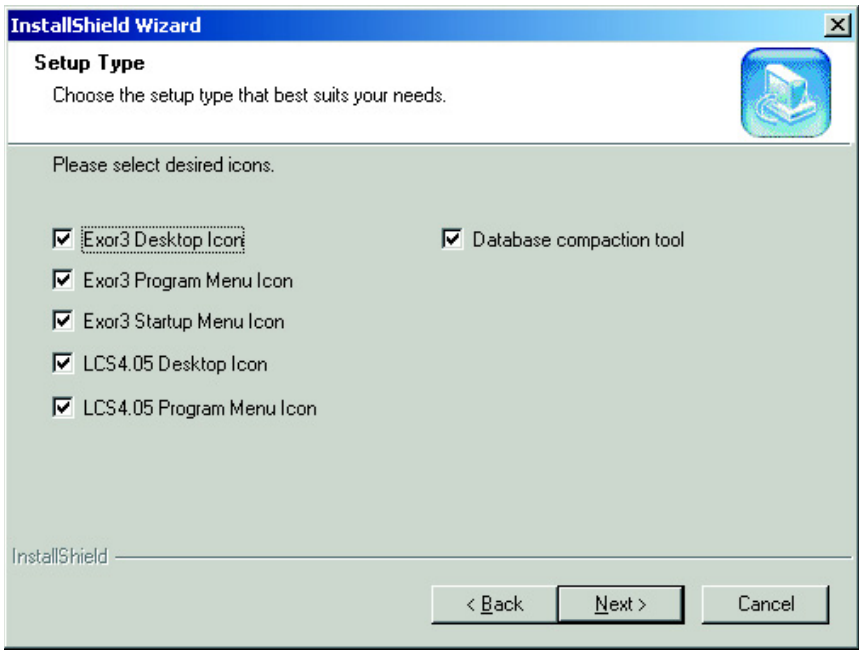


When you select Create a new database with the same name, the software automatically stores your old database with a time stamp in the database directory.

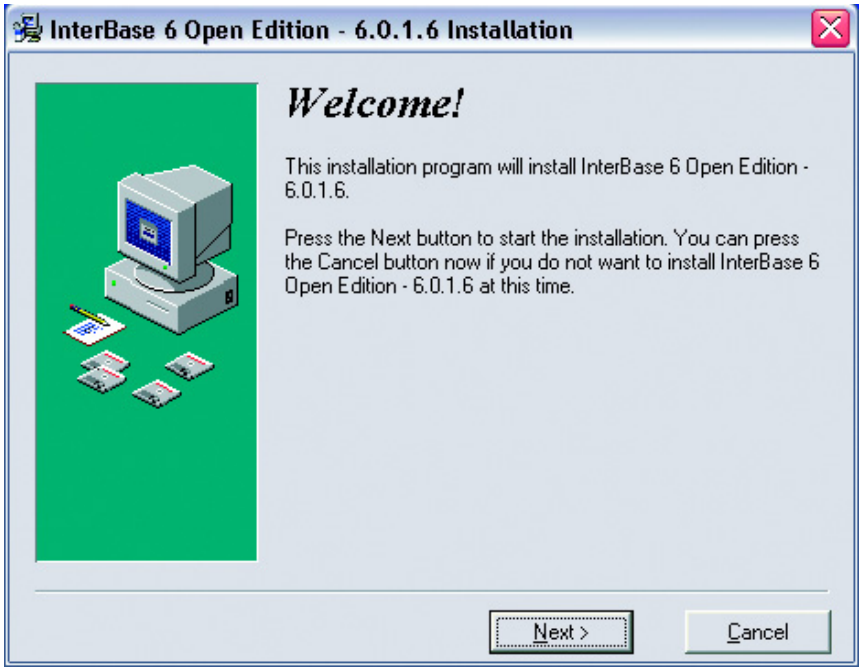
8 Select the default settings to install the LightCycler® Software 4.05 or browse to select a location. Click *Next*.



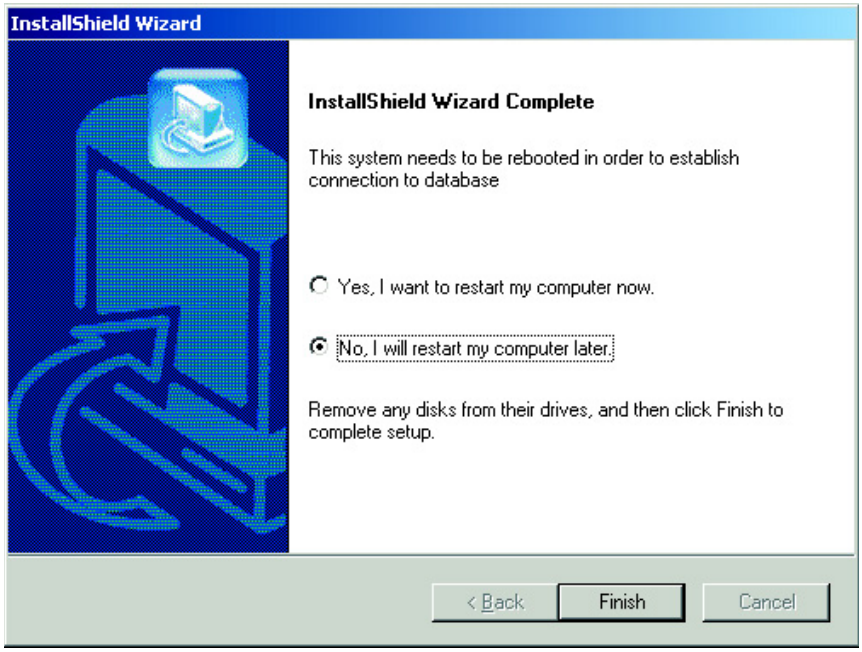
9 Select the location for program icons. These are locations to start the LightCycler® Software 4.05 from. Deselect the icon locations you do not want, and then click *Next*.



10 Installation of InterBase starts. In the Welcome screen click *Next* to continue. Accept the license conditions by clicking *I Agree*. When the Installation Completed window appears, click *Finish* to finish the InterBase installation.



11 When the *InstallShield Wizard Complete* window appears, the installation is complete. Click *Finish*. After a reboot of the computer you can launch the software by pressing the LightCycler® Software 4.05 icon.



The installation process installs the following icons on the desktop:

LightCycler® Software 4.05 icon , Exor 3 icon

12.3.1 Saving an existing database and installing additional databases

For backup purposes save a copy of your database routinely. 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 the Windows Explorer select C:\Program Files\Roche\Exor3\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 *Installing LightCycler® Software 4.05*, step 4).
2	Right-click on the database (*.IB) you want to check. From the menu select *Properties*; 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 Exor3 icon in the system tray and selecting *Shutdown*.
2	In the Windows Explorer select C:\Program Files\Roche\Exor3\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 *Installing LightCycler® Software 4.05*, step 4).
3	Right-click on the database you want to compress. From the menu select *Properties* and then 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  then navigate to a location, then click *Open*.
6	Click on *Compact* to start the process.

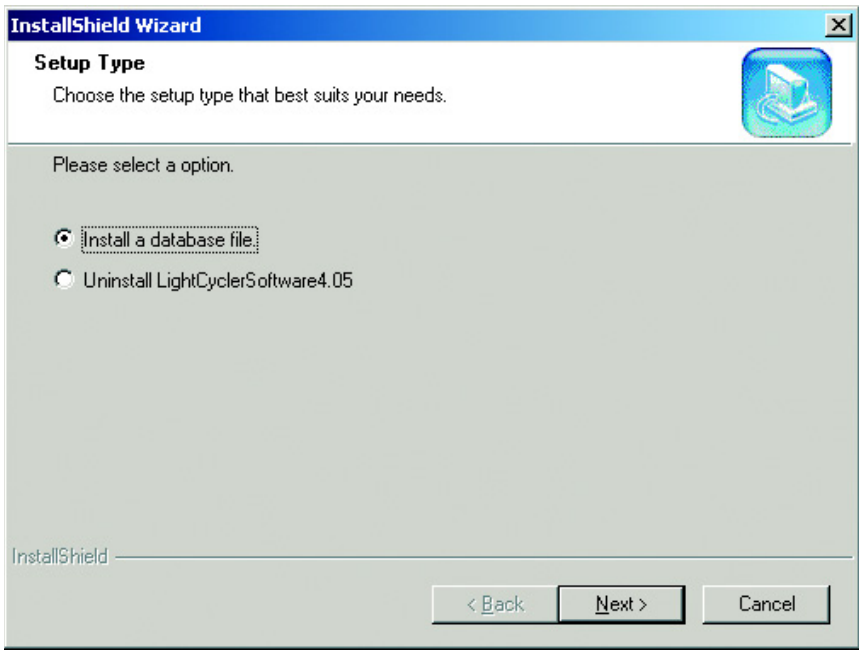
To save a database file:

1	Shutdown the Exor3 by right-clicking the Exor3 icon in the system tray and selecting *Shutdown*.
2	Save the database (*.IB) on a CD by following the procedure described in *Using the CD-RW drive*.

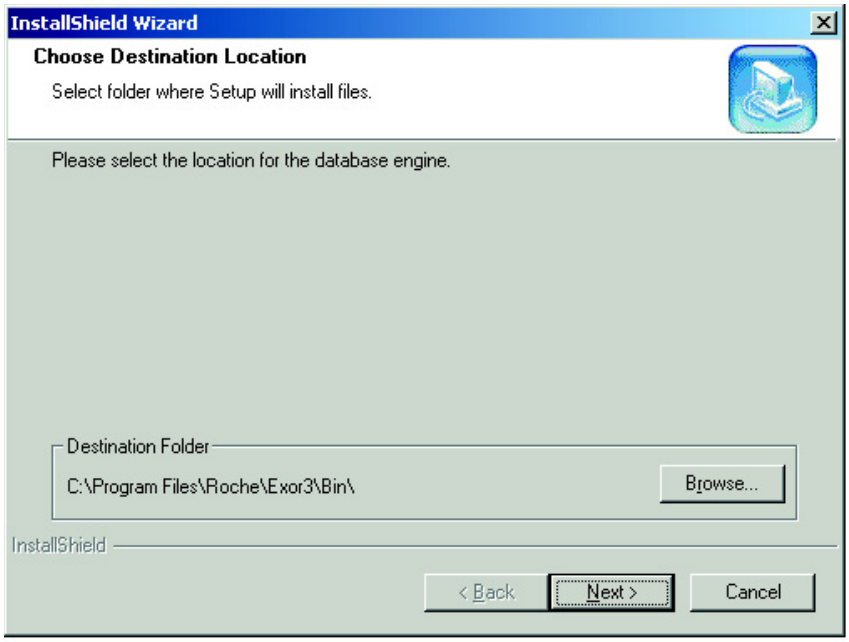
To install additional databases:

If the LightCycler® Software 4.05 is already installed on your computer, you can use the LightCycler® Software 4.05 installation utility to install additional databases:

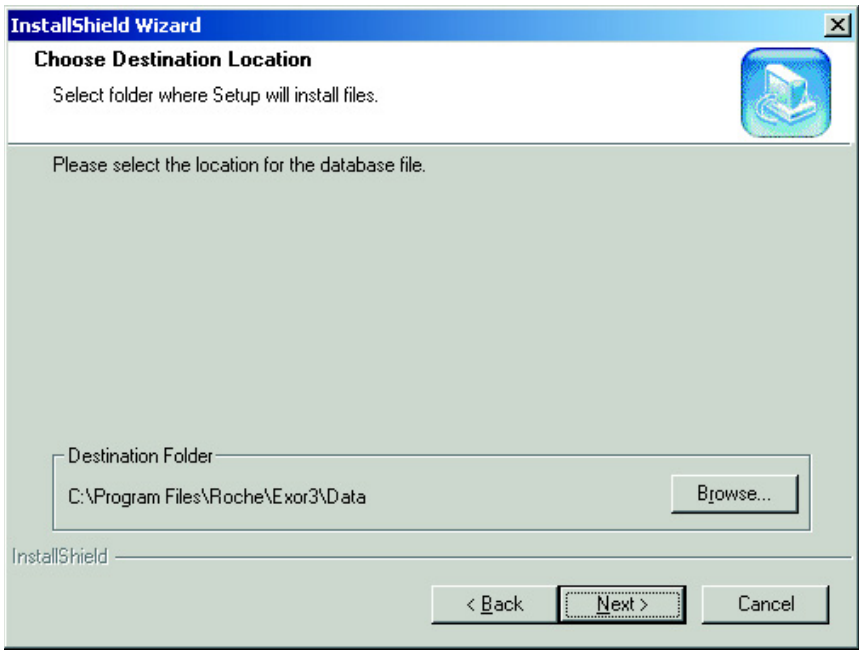
1. Shutdown all running database engines by right-clicking the Exor3 icon in the system tray and selecting *Shutdown*.
2. Insert the LightCycler® Software 4.05 CD. Double-click LightCycler_Software_405_Setup.exe, if installation doesn't start automatically.
3. The Setup Type window is displayed. Leave the default setting *Install a database file*, and then click *Next*.



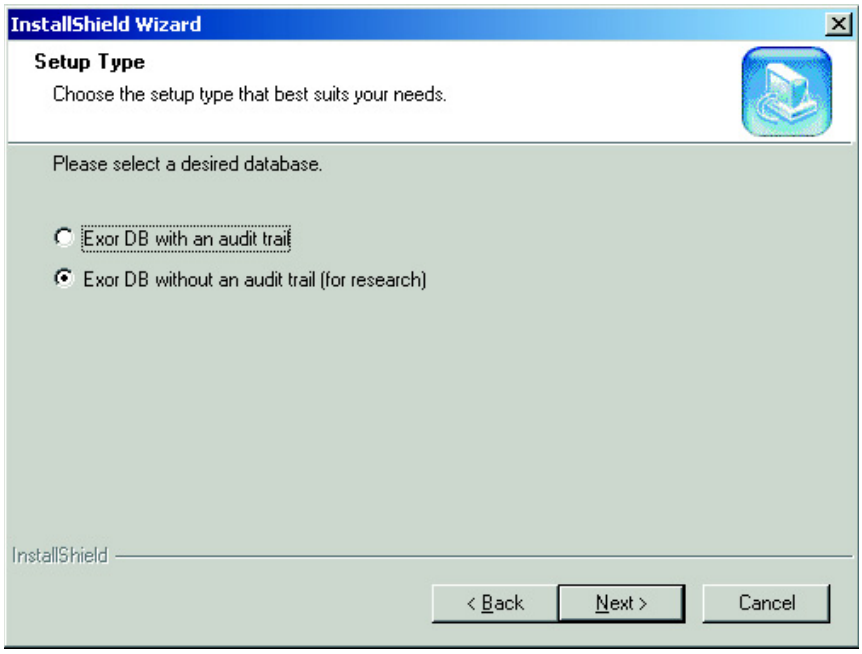
4 Select the default settings to install the database engine or browse to select a location. Click *Next*.



5 Select the default settings to install the database file or browse to select a location. Click *Next*.

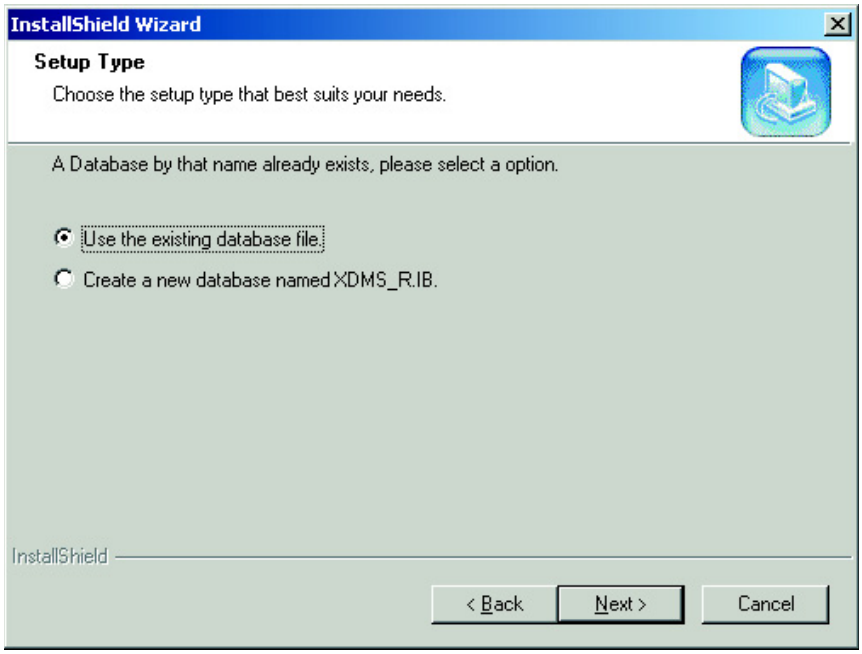


6 In the Setup Type window select *Exor DB without an audit trail (for research).* Click *Next.*



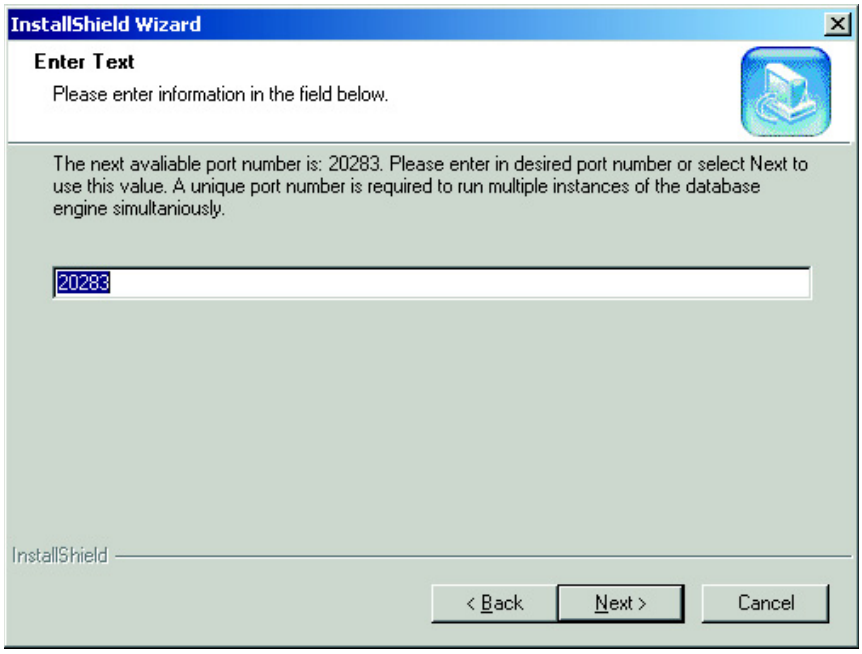
7 The software prompts you to name the new database. Enter a database name or leave the default, then click *Next.*

8 If a database with the same name already exists, you are prompted to use the existing database or create a new database with the same name. Click *Next*.

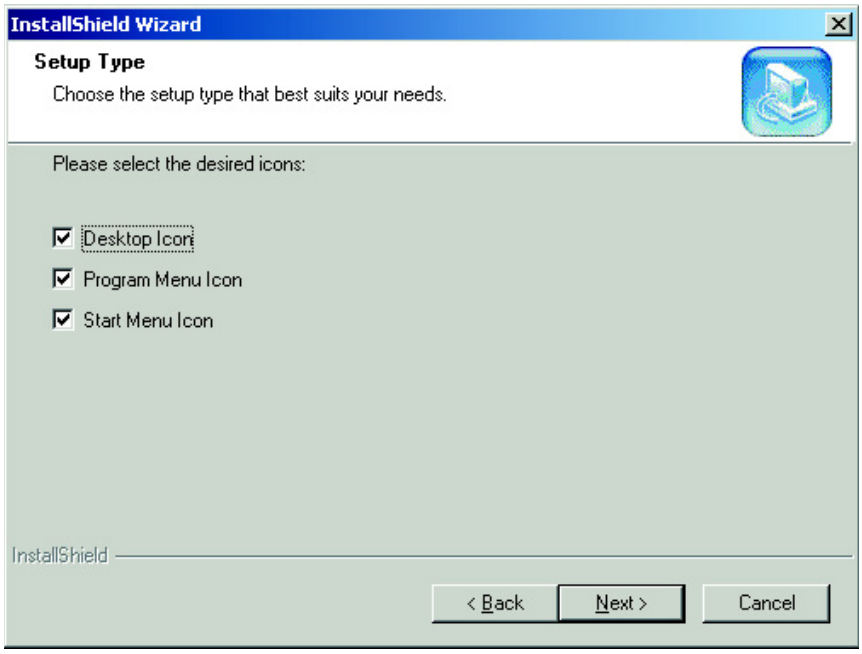


When you select Create a new database with the same name, the software automatically stores your old database with a time stamp in the database directory.

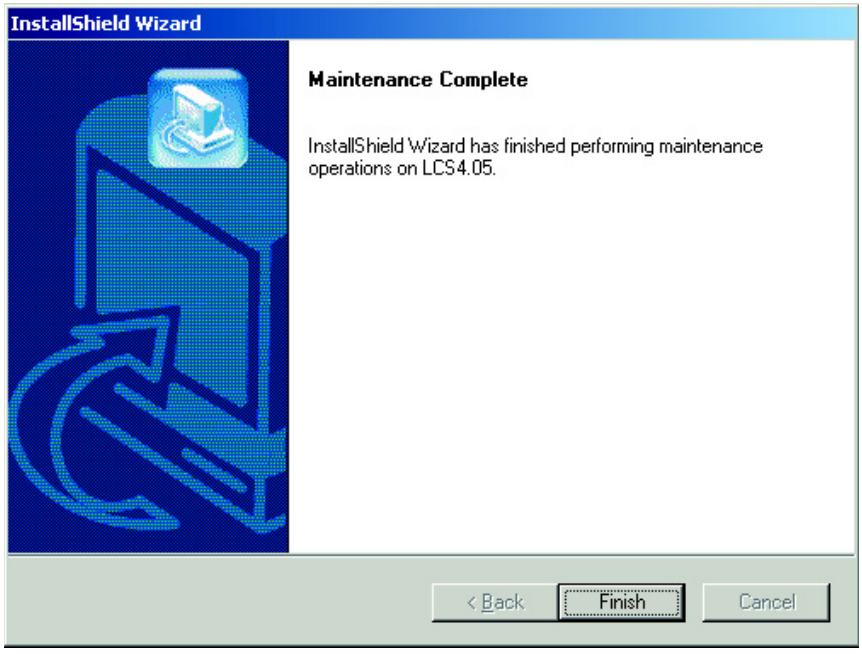
9 The software prompts you to enter a port number for the database. Leave the default value or enter a unique port number, then click *Next*.



10 Select the location for program icons. Deselect the icon locations you do not want, and then click *Next*.



11 A message states that the maintenance is complete. Click *Finish*.



The installation process installs another Exor3 icon on your desktop:

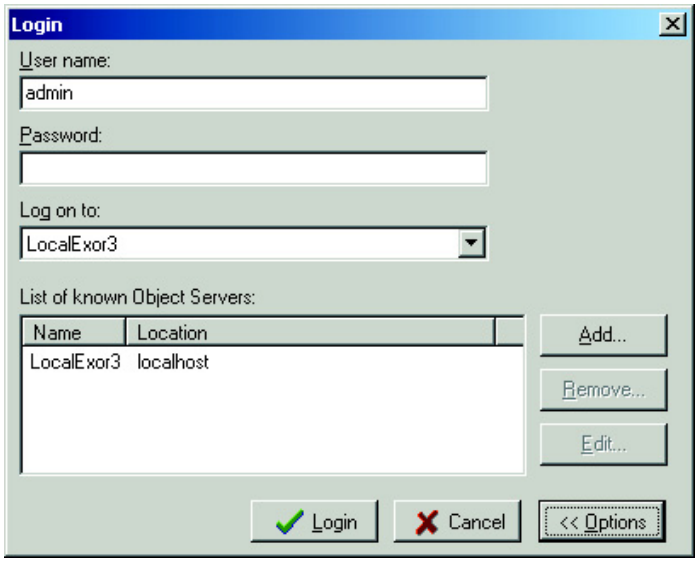
You must start the newly-installed Exor3 either by double-clicking on the icon on your desktop or by rebooting the system, before you can log on to the new database.

12.3.2 Logging on to different databases

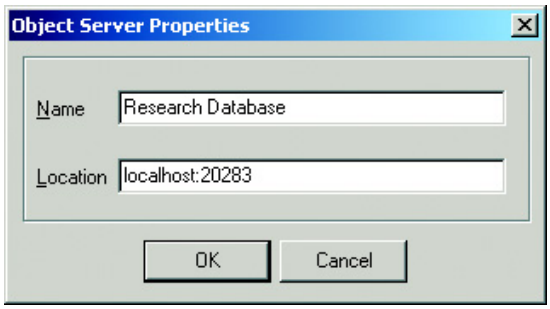
You can log on to an additionally installed database by selecting the database in the *Log on to* pull down menu.

To include an additionally installed database:

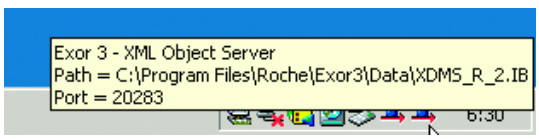
1. Double-click the LightCycler® Software 4.05 icon to launch the LightCycler® Software 4.05.
2. A login dialog box opens. Click on *Options* to display the list of known object servers.



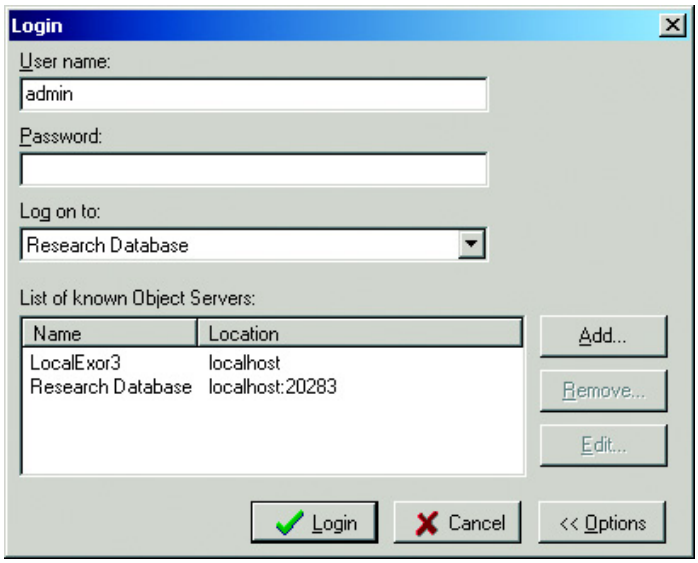
3. Click on *Add*. An *Objekt Server Properties* window opens.
4. Enter a name for the database and its location. The location is always composed of the word “localhost” and the port number of the database to be integrated, divided by a colon. Click *OK*.



To figure out the port number for a database, point at the Exor3 icon in the system tray, and then read the object server’s properties from the display.



5 The database is included in the *List of known Object Servers* and can be selected in the *Log on to* box.



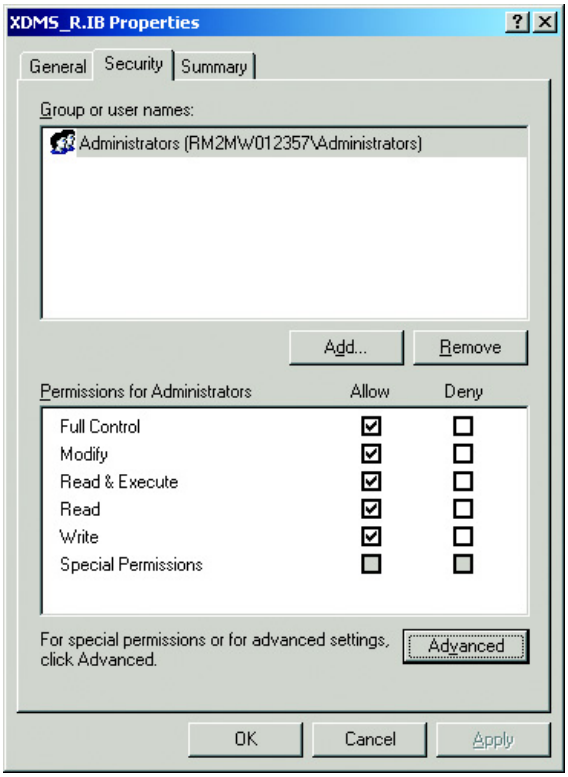
6 Select a database in the List of known Object Servers and click on *Remove* to remove it, or click on *Edit* to change the name or location of the database.

Replacing an existing database file by 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® Software 4.05 (no additional Exor3 service necessary).

To replace an existing database file through a database file of the same name:

1. Exit the LightCycler® Software 4.05.
2. Shutdown database engine corresponding to the database to be replaced by right-clicking the Exor3 icon in the system tray and selecting *Shutdown*.
3. In the Windows Explorer select C:\Program Files\Roche\Exor3\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 Installing LightCycler® Software 4.05*, step 4).
4. Right-click on the database you want to replace. From the menu select *Properties* and then select the *Security* tab. Check the *Allow* box for *Full Control* and *Modify*. You can now delete this database or store it under a different name.



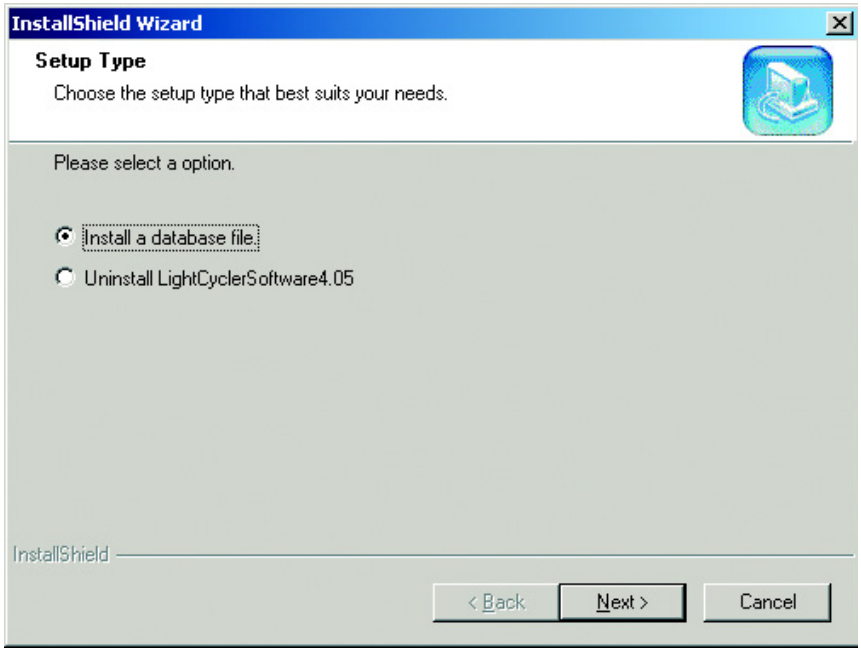
5. Copy the database file to be restored *e.g.*, from a CD, into the database directory.
   The name of the database must be identical with the name of the deleted database. Rename the database if necessary.
6. Disable the access rights for the database regarding *Full Control* and *Modify*. If you restored the database from a CD, right click on the database file and clear the *Read-only* box in the *Properties* menu on the *General* tab.
7. Start the Exor3 Service by double-clicking the icon on your desktop.
8. Start the LightCycler® Software 4.05.
   To log in on the restored database you must enter the User name and Password for this database.

Integrate a restored database file as an additional database in LightCycler® Software 4.05

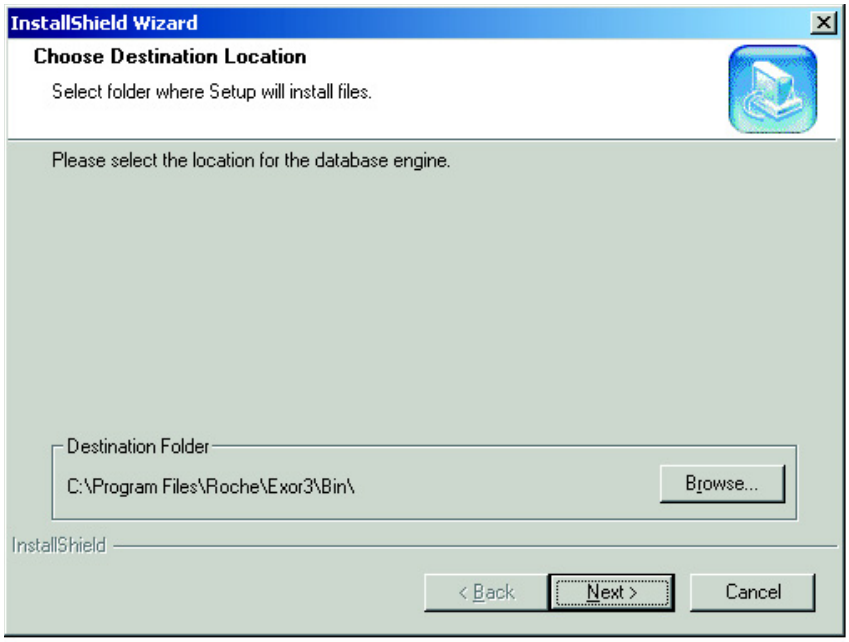
You can integrate an additional database *e.g.*, a restored database from a CD, in the LightCycler® Software 4.05 (additional Exor3 service necessary).

To integrate an additional restored database file in LightCycler Software 4.05

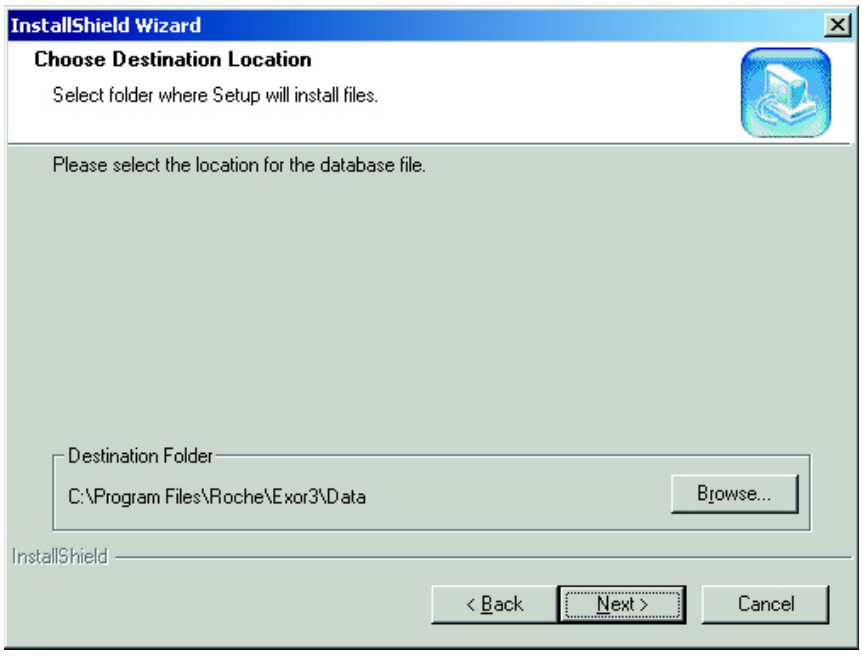
1. Copy the database file *e.g.*, from a CD, in the database directory. Assure that database names are unique.
2. Check if the access rights for the database regarding *Full Control* and *Modify* are disabled, and clear the *Read-only* box in the *Properties* menu on the *General* tab, as described above.
3. Shutdown all running database engines by right-clicking the Exor3 icon in the system tray and selecting *Shutdown*.
4. Insert the LightCycler® Software 4.05 CD. Double-click LightCycler_Software_405_Setup.exe, if installation doesn't start automatically.
5. In the *Setup Type* window select *Install a database file*. Click *Next*.



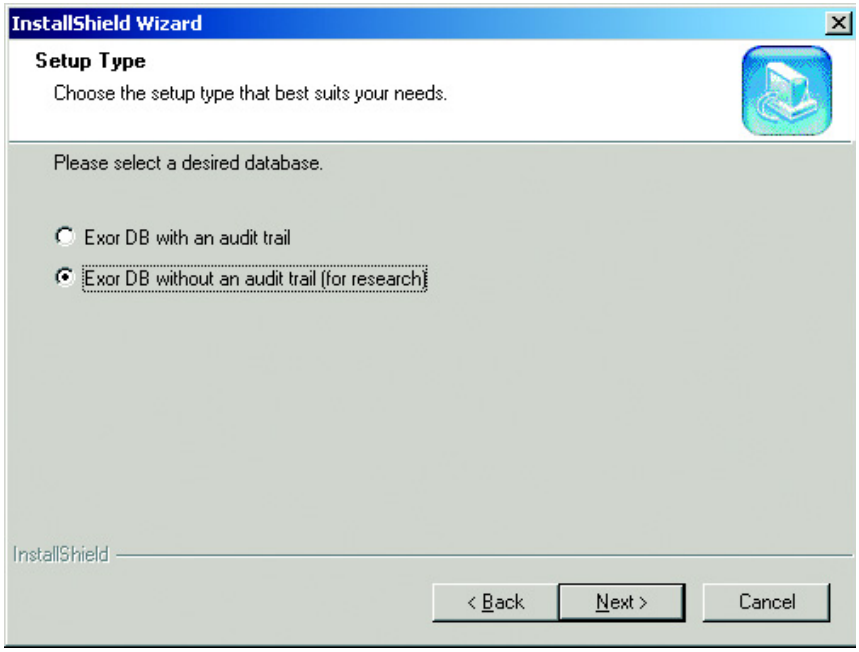
6 Select the default settings to install the database engine or browse to select a location. Click *Next*.



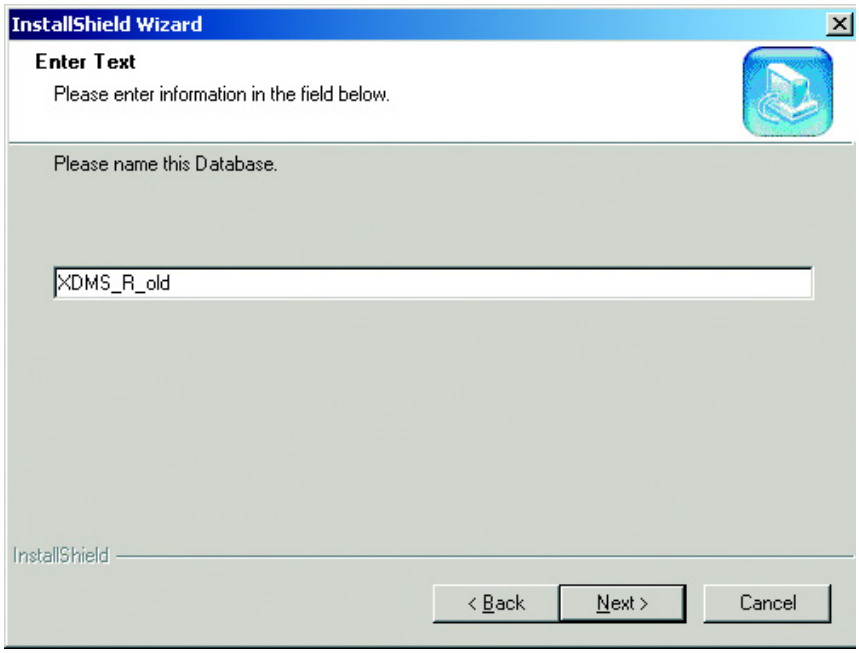
7 Select the default settings to install the database file or browse to select a location. Click *Next*.



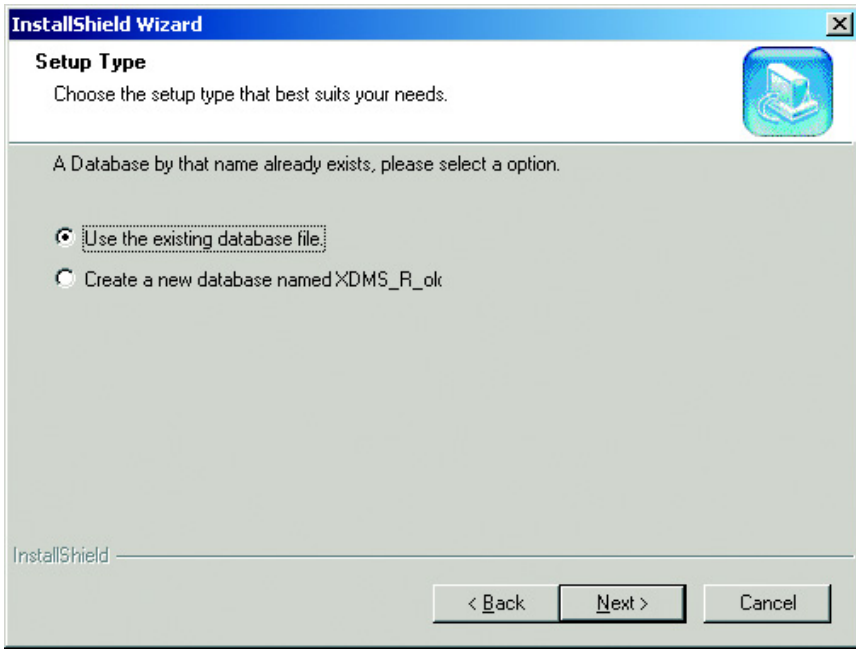
8 Select *Exor DB without an audit trail (for research).*



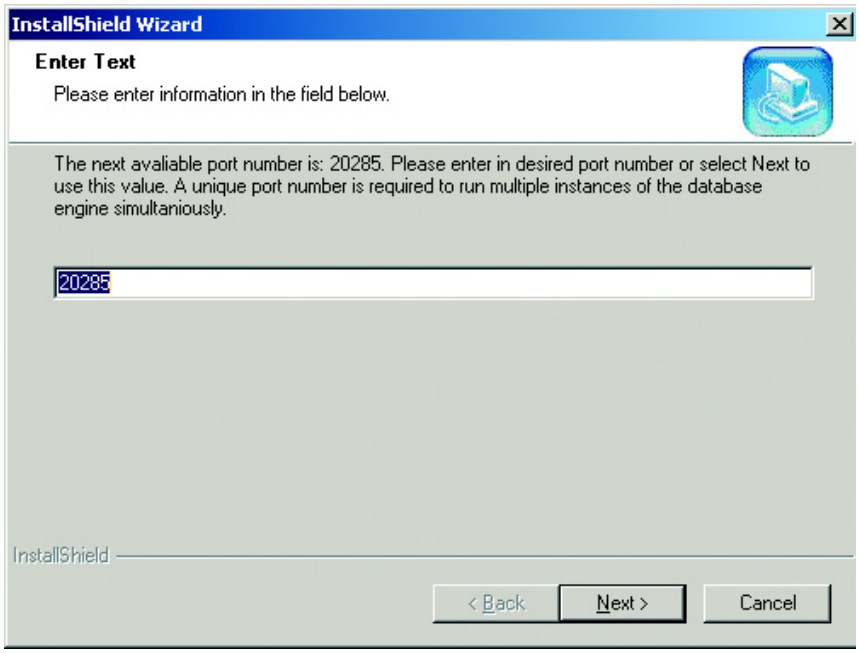
9 The software prompts you to name the database. Enter the name of the database you want to restore, and then click *Next*.



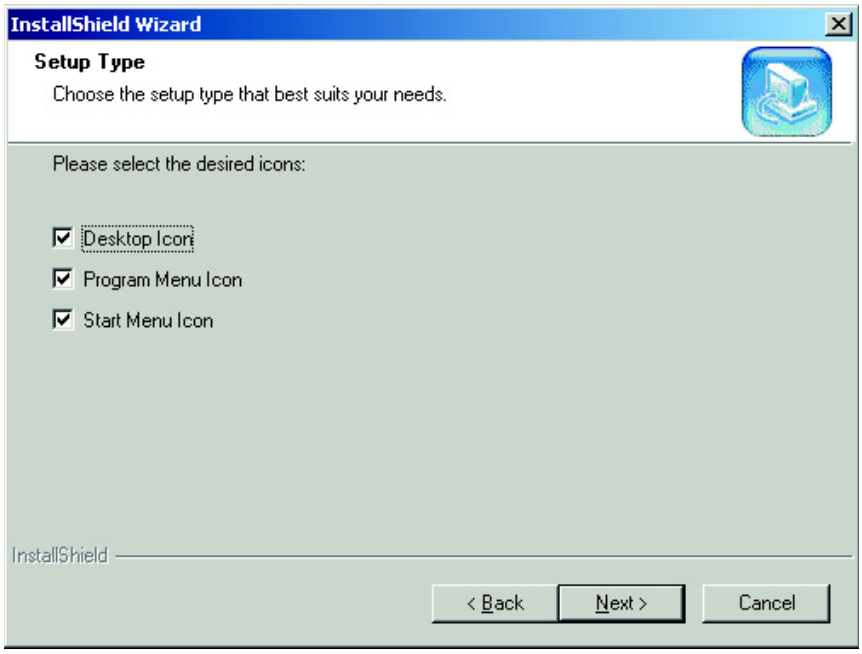
10 Select *Use the existing database file*. Click *Next*.



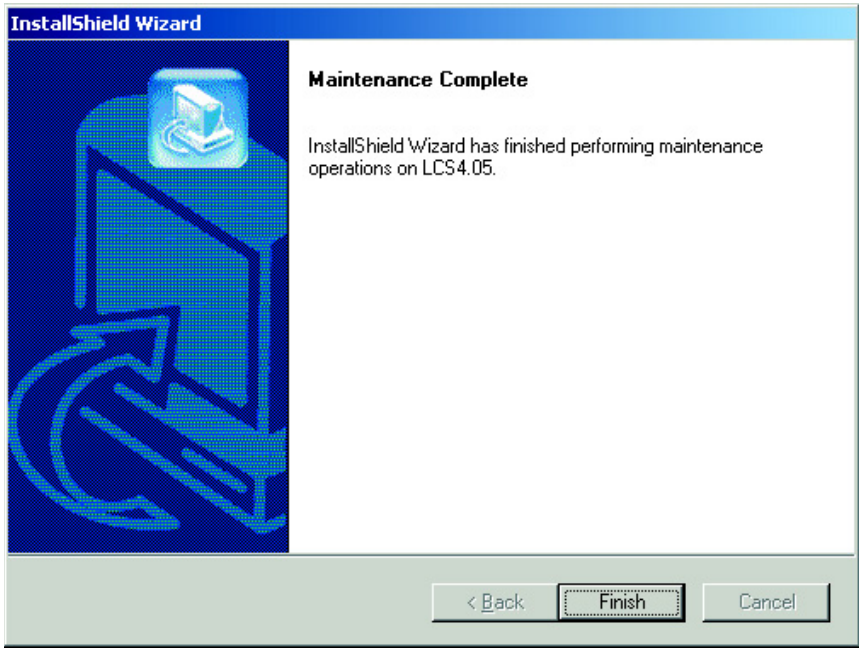
11 Note the new port number, and then click *Next*.



12 Select the location for program icons. Deselect the icon locations you do not want, and then click *Next*.



13 A message states that the maintenance is complete. Click *Finish*.



14 Start the newly installed Exor3 service by double-clicking the icon on your desktop, and then start the LightCycler® Software 4.05.

15 Integrate the restored database as described in *Logging on to different databases.*

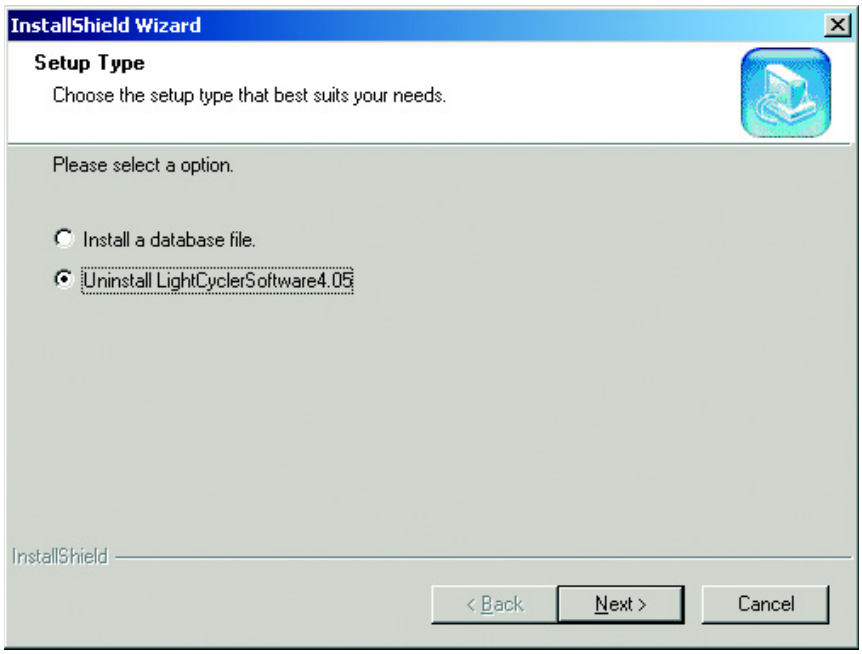
To log in on the restored database you must enter the User name and Password for this database.

12.4. Removing LightCycler® Software 4.05

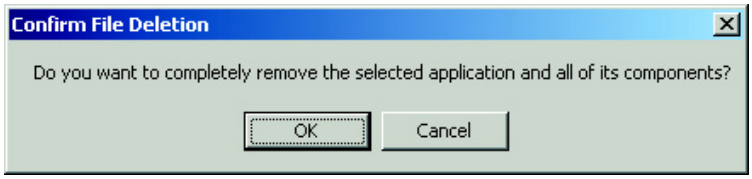
To uninstall LightCycler® Software 4.05:

1. Shutdown all running database engines by right-clicking the Exor3 icon in the system tray and selecting *Shutdown*.

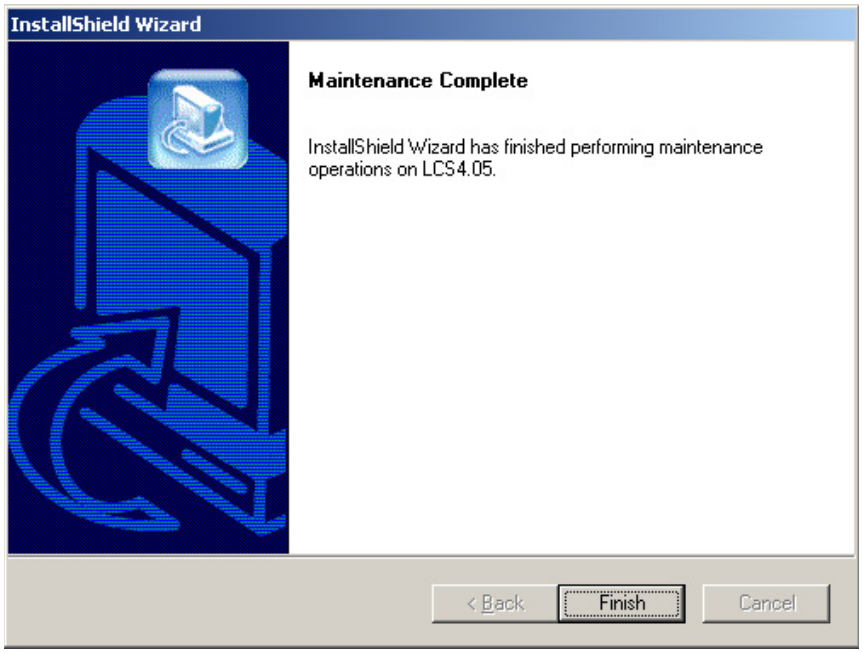
2. Insert the LightCycler® Software 4.05 CD.
Double-click LightCycler_Software_405_Setup.exe, if installation doesn't start automatically. The Setup Type window is displayed. Select *Uninstall LCS4.05 Software*, and then click *Next*.



3. You are prompted to confirm the deletion. Click *OK*.



4 After the software has been removed, a message states that the maintenance is complete. Click *Finish*.



12.5 Logging on to the Database as System Admin and Creating User Accounts

The LightCycler® Software 4.05 installation process creates a default user account with system administrator privileges. The user name is "admin" and the initial password is *LightCycler01*. You will be prompted to change your initial password upon your first login.

The default setting for the maximum login attempts on entering an invalid password is 3. To modify this setting or to disable this function see Determine conditions for access and modify a role's access rights.

After installing the software you will also need to create user accounts, so others can use the software. Each system (that is, each local computer) has its own set of user accounts. For each system you can create the following:

- Local Administrator accounts that have the same privileges as the System Admin account. Each person with a Local Administrator account can create other user accounts.
- Expert User and Standard User accounts, which have fewer privileges than Local Administrator accounts. For information about user accounts and their associated privileges see chapter *Managing User Access*.

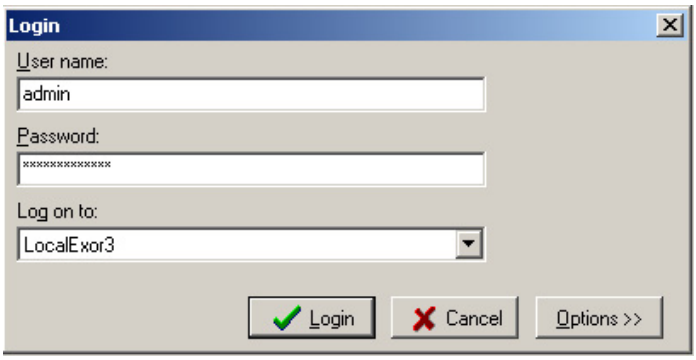
Before you begin determine the following:

- The new password for each System Admin account you want to change.
- The user names, login names, and passwords for the initial set of user accounts for the local computers.

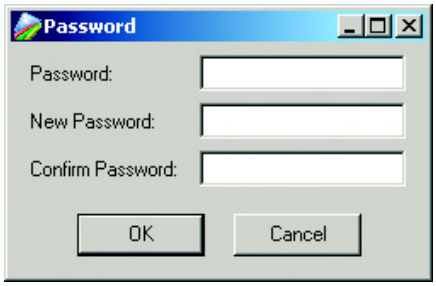
To log on to a local computer:

1. On a LightCycler® computer double-click the *LightCycler® Software 4.05* icon to launch the LightCycler® Software 4.05.

2. A login dialog box opens. Leave the default values for the user name *admin* and log on to location *LocalExor3*. Enter the password *LightCycler01*, and then click *Login*.



3. Upon your first login, you are prompted to change your password. Click *OK*. A Password dialog box is displayed.

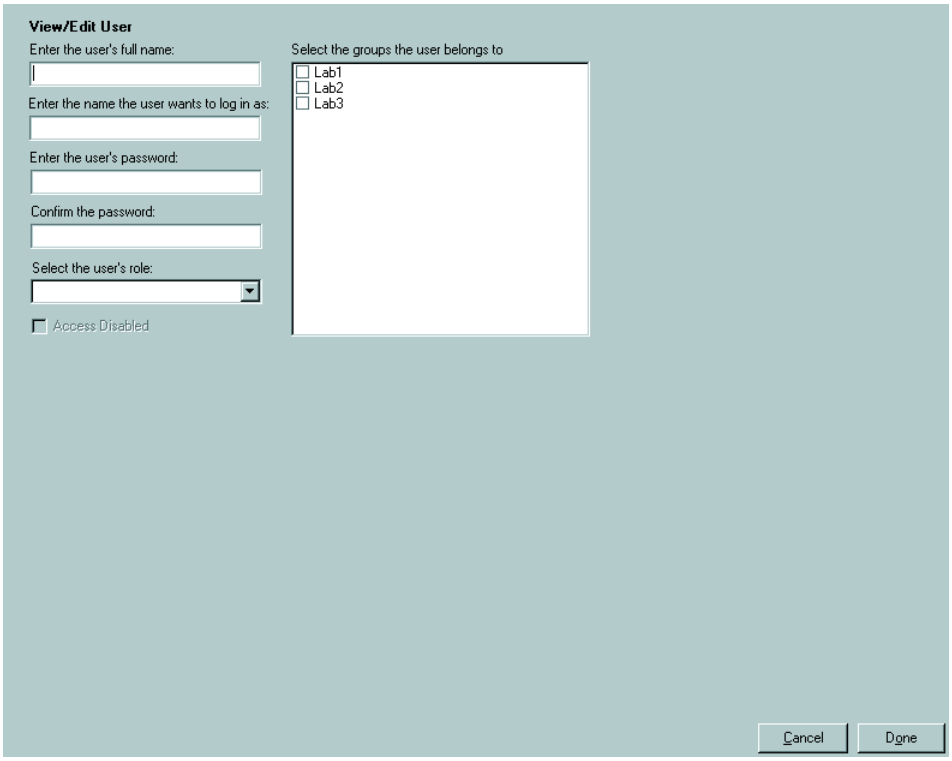


Enter the default password (LightCycler01) for the System Admin in the *Password* box. Enter the new password in the *New Password* box and again in the *Confirm Password* box. Click *OK*.

4. You are logged on to an Exor DB without an audit trail (research use). If you are logged on to the Exor DB without an audit trail, the word "Research" is displayed in the Statusbar.

To create user accounts:

To create new user accounts, from the *Tools* menu select *Manage Users*. The User Management tool opens. The *Work with Users* option is selected by default. To create a new user account, click *New User*. The *View/Edit User* dialog box opens.



In the first box, type the user's full name.
In the second box, type the user's "user name" he wants to use as the login name.
In the third box, type a password for the user.
In the fourth box, retype the password.
In the last box, select the user's role. The available roles are:

Local Administrator: Creates an administrator account that gives the user complete access to the software. A Local Administrator can create and edit other user accounts.

Expert User: Creates a general user account that gives the user extensive access to the software. The Expert User cannot create and edit user accounts.

Standard User: Creates a general user account that gives the user limited access to the software.

For detailed information about the privileges associated with each role see chapter *Managing User Access*.

When finished click *Done* and repeat the procedure to create other user accounts, as needed.

When finished, click *Close* to close the *Manage Users* window or *Exit* from the *File menu* to close the LightCycler® Software 4.05 application.

12.6 Connecting an Instrument

Before a LightCycler® Instrument can be used to run an experiment, the instrument information must be added to the database. Adding the instrument to the database also adds the instrument name to the list of available instruments in the Run module. This is typically done by Roche, but it might be useful to understand the procedure.

A new instrument will be automatically detected and added to the database during an experiment run, if no other instruments are connected to the local computer. Automatic instrument detection does not require Expert User or Local Administrator user privileges.

A user with Expert User or Local Administrator privileges can add an instrument to the database manually or by having the software search for instruments.

One PC must not be used with 2 LightCycler® Instruments simultaneously.

12.6.1 Adding a New Instrument Automatically

- You can add a new instrument to the database and to the list of available instruments by letting the software search for all instruments currently connected to the computer. This approach is useful when you do not know the instrument's COM port.
- The software will also detect a new instrument automatically when you start an experiment run, if no other instruments are currently in the list of available instruments. This is the easiest way, if you don't connect more than one LightCycler® Instrument to the computer.

Adding a new instrument automatically by letting the software search

This procedure detects all instruments currently connected to the computer, including those that are already in the database.

Prerequisites:
You must have Expert User or Local Administrator privileges. (For more information about user privileges, see chapter *Managing User Access*.)

To let the software detect a new instrument:

1. Make sure the instrument is physically connected to the computer and is powered on.
2. Turn on the computer, start the LightCycler® Software 4.05, then log in.
   Be sure the instrument is powered on before you start the software.
3. Click *Run* in the toolbar or *New Experiment* on the *Front* window to open the Run module.
4. Click *Options*, then select *Search for Instrument*. The *Options* button is located in the upper right corner of the Run window next to the instrument box.

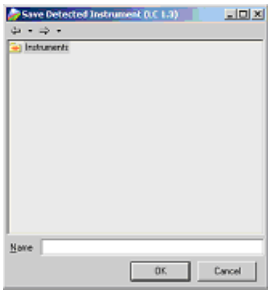
5. The *Discover Instruments* dialog box opens.
6. If you know the instrument's COM port, select *Find on selected com port*, then select the port from the pull-down list. If you do not know the port, select *Auto – Detect* and click *Next*.
7. When the software detects a new instrument that has no entry in the current LightCycler® Software 4.05 database, it prompts you to provide a name for the instrument. Enter a name, then click *OK*.
   If no instruments are found, the software displays "Unable to find any instruments". Make sure the instruments are connected and powered on, then click *Back* to restart the search.
8. After instruments are found, the dialog box displays a list of all the instruments connected to the computer.
9. Select the new instrument you want to add to the database, then click *Finish*.

The new instrument is added to the LightCycler® Software 4.05 database, and its name is added to the Instruments folder in the Navigator. The instrument name is now available from the instrument list in the Run module, so the instrument can be selected for experiments.

To add a new instrument during an experiment run:

1. Create a protocol and start the experiment run, as described in chapter *Creating and running an experiment*. (If your user account has the Standard User role, you must execute a macro to run an experiment. For more information see chapter *Using Templates and Macros*.)

2. The software automatically detects the new instrument when you start the run. The following window is displayed.



3. Enter a name for the instrument in the *Name* box, then click *OK*. The instrument is added to the LightCycler® Software 4.05 database, and its name is added to the Instruments folder in the Navigator. The instrument name is added to the list of available instruments.

   If the new instrument replaced an instrument that is no longer connected to the computer, you should remove the previous instrument from the instrument list. For more information see section Removing an instrument.

4. Continue the experiment using the detected instrument.

12.6.2 Adding a New Instrument Manually

Prerequisites:

You must have Expert User or Local Administrator privileges. (For more information about user privileges see chapter *Managing User Access*.)

You must know which COM port the instrument is attached to.

To add a new instrument manually:

1. Make sure the instrument is physically connected to the computer and is powered on.
2. Turn on the computer, start the LightCycler® Software 4.05, then log in.
   Be sure the instrument is powered on before you start the software.
3. Click *New* or select *New* from the *File* menu.
4. Select *LightCycler® Instrument*, then click *OK*.
5. The *New Instrument* window opens. The instrument fields are at the top of the work pane.

6. In the *Instrument Name* box, type a name of your choice for the instrument.
7. In the *I/O Port* box, select the COM port the instrument is attached to and click *Connect*.

The software finds the instrument connected to the designated COM port and then automatically fills in the Instrument Id, Instrument Version, and Last Connected Computer boxes.

The instrument is added to the current LightCycler® Software 4.05 database, and its name appears in the Instruments folder in the Navigator. The instrument name will now be available from the instrument pull-down list in the Run module, so the instrument can be selected for experiments.

12.6.3 Removing an Instrument

If an instrument is no longer connected to the local computer, its name should be removed from the list of instruments in the Run module. If the instrument is no longer available for use at all, it should also be removed from the LightCycler® Software 4.05 database.

Prerequisites:
You must have Local Administrator privileges to remove an instrument. (For more information about user privileges see chapter *Managing User Access*.)

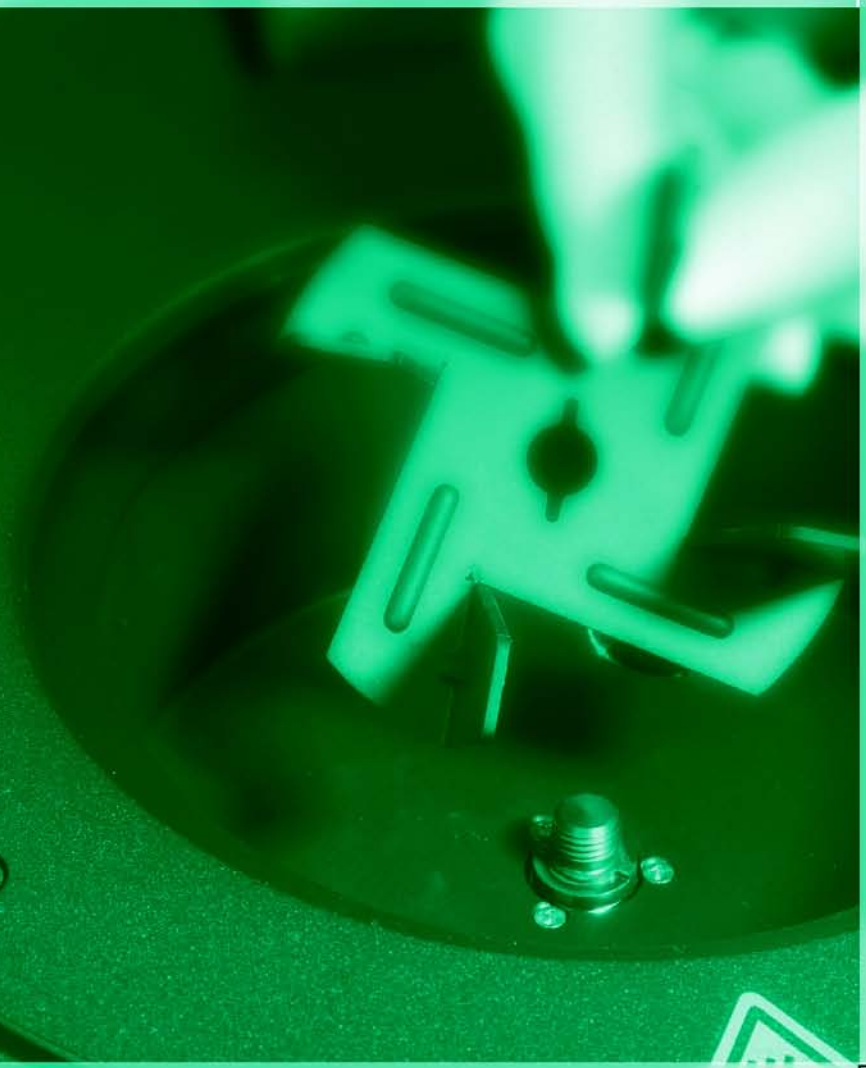
To remove an instrument from the instrument list:

1. To remove the instrument from the instrument list, select *Manage Known Instruments* from the *Tools* menu. The list of instruments is displayed.
2. Select the instrument you want to delete, then click *Delete*.

To remove an instrument from the database:

1. To remove the instrument from the current database, right-click the instrument name in the Navigator.
2. Click *Delete*.
3. You are prompted to confirm the deletion. Click *Yes*.

Maintenance



E

Chapter E • Maintenance
describes the maintenance procedures that are required for the LightCycler® 2.0 Instrument.

Maintenance

1. General Maintenance

The LightCycler® 2.0 Instrument is maintenance-free.

2. Cleaning Instructions

Never clean the LightCycler® 2.0 Instrument without turning the instrument power switch off and disconnecting the power cable.

Do not poor fluids into the thermal chamber.

As with all potentially biohazardous specimens, universal safety precautions shall be taken when handling and processing samples. Spills shall be immediately disinfected with an appropriate disinfectant solution to avoid spreading contamination to laboratory personnel or equipment.

Handling and disposal of infectious material shall be performed according to local safety guidelines.

2.1 General Cleaning

Clean the housing of the LightCycler® 2.0 Instrument with a mild commercial detergent. If necessary use 70% Ethanol for disinfecting the instrument housing.

To clean the LightCycler® Centrifuge Adapters, remove them from the cooling block before wiping them clean with a lab tissue soaked with decontamination solution. Use commercialized reagents such as License to kill (Biodelta) or DNA Zap (Ambion). Do not autoclave the cooling block.

If capillary breakage occurs, perform the following steps, as appropriate:

- Clean the LightCycler® 2.0 Sample Carousel, by removing capillary fragments using the brushes provided with the LightCycler® 2.0 Instrument.
- Refer to *Removable Fan* for details on cleaning the thermal chamber.

Contact your local Roche representative if capillary breakage occured.

The LightCycler® Sample Carousel can be autoclaved. In case the LightCycler® Sample Carousel is autoclaved regularly it is recommended to change the O-Ring after 50 autoclaving cycles.

2.2 Preventive Maintenance

The area around the instrument shall be checked regularly, to ensure that the air flow around the LightCycler® 2.0 Instrument is unrestricted and that books, papers, or other supplies are not interfering with the air flow.

2.3 Removable Fan

The fan is fixed in the thermal chamber with a knurled screw and can be released manually as indicated in the pictures. This allows easy cleaning of the thermal chamber.

Switch off and unplug the LightCycler® 2.0 Instrument before removing the fan.

1. To release the fan turn the knurled screw to the left.

2	Take out the fan.

To clean the thermal chamber proceed as follows:

1	Clean the chamber with a soft lint-free cloth. For cleaning purposes use 70% Mikrozid (Schülke & Mayr GmbH, Norderstedt, Germany) or 1:3 Clorox Regular (The Clorox Company, Oakland, USA) or 3% Kohrsolin (Bode Chemie GmbH, Hamburg, Germany) or 70% Ethanol according to manufacturers' instructions.
2	Clean the optical window with an optical polishing cloth.
3	Make sure that no liquid is left in the chamber before you put in the fan.
4	Insert the fan and fix it by following the steps, described to release it, in reverse order.

Only screw the fan into place, manually. Do not use any tools to screw the fan *e.g.*, an Allen key.

Do not poor fluids into the thermal chamber.

Make sure you don't touch or bend the thermal sensor in the chamber when cleaning it. In case the temperature sensor was bent by accident, this may cause faulty temperature measurements or even cause the capillaries to crash during a run. In case of doubt call your Roche representative.

3. Change of O-Ring

3.1 Removal of the O-Ring

1. To remove the O-Ring, use a suitable tool (*e.g.,* a blunt pair of tweezers or a small screwdriver as indicated in the picture), place it carefully under the O-Ring and raise it. The preferred position for doing this is one of the capillary cavities.

2. Remove the O-Ring completely from the carousel.

E

3.2 Insertion of the O-Ring

1. Put the new O-Ring on the carousel and press it down with your thumbs into the “furrow“. Always press opposite points.

2. Turn the carousel and proceed as described in step 1.

3. Proceed as described in steps 1 and 2 until the O-Ring fits in the “furrow“.
4. Finally smooth out the O-Ring over the whole carousel with your fingers.

4. Consumables and Spare Parts

The consumables and spare parts that are needed to operate the LightCycler® 2.0 Instrument are listed below. These materials may be obtained by contacting your Roche Diagnostics representative.

4.1 Consumables

LightCycler® Capillaries (20 µl), Cat.No.: 11 909 339 001

LightCycler® Capillaries (100 µl), Cat.No.: 03 337 090 001

4.2 Spare Parts

LightCycler® 2.0 Sample Carousel (20 µl), Cat.No.: 03 603 962 001
LightCycler® 2.0 Sample Carousel (100 µl), Cat.No.: 03 603 954 001
LightCycler® Centrifuge Adapters, Cat.No.: 11 909 312 001
LightCycler® Capillary Releaser, Cat.No.: 03 603 920 001
LightCycler® Capping Tool, Cat.No.: 03 357 317 001
LightCycler® Sample Carousel O-Ring, Cat.No.: 03 603 989 001

A printer and a barcode reader are provided locally upon request.

5. Disposal of Consumables and Reagents

- Discard the capillaries into a solid waste box after use.
- Discard reagents and waste material according to local safety guidelines.
- Contact your local Roche Diagnostics representative for disposal of the instrument or instrument parts.

6. Color Compensation

In a multicolor reaction, the wavelengths of light emitted by the dyes overlap, causing one channel to pick up signals from a dye measured by another channel. This bleed-over of fluorescence signal can result in uninformative data.To correct the bleed-over you can apply a color compensation object when you run the experiment or when you analyze the data. It is recommended to perform a color-compensation experiment once a year. Refer to chapter D *Software* for more details on performing a color-compensation experiment and applying it. Guide values are specified in the pack insert of the LightCycler® Color Compensation Kit.

Troubleshooting

F

Chapter F • Troubleshooting
lists all LightCycler® system messages, explains their meaning and indicates appropriate measures.

F

Troubleshooting

The monitoring of an experiment and the error history records are used to support the servicing of the LightCycler® 2.0 Instrument. The LightCycler® Software Version 4.05 offers the tools to collect all parameters related to the runs performed. Refer to Chapter D Software for more details on generating error log files.

System messages which may potentially occur are listed below. For each message the probable cause and corrective action typically required for solving the problem are shown. Call your Roche representative for troubleshooting assistance.

Data derived from a run where a system message appeared should be reviewed carefully. If the validity of the results is doubtful, repeat the run.

1. Instrument Errors

Error Code	System message	Possible Cause	Corrective Action
20	Temperature min error	Hardware problem concerning temperature control	Reboot system, call Roche Service if error persists
21	Temperature max error	Hardware problem concerning temperature control	Reboot system, call Roche Service if error persists
22	Temperature ADC timeout	Hardware problem concerning temperature control.	Call Roche Service
25	Cabinet fan will not switch off	Hardware error	Call Roche Service
26	Overheat from hardware sensor	Hardware problem concerning temperature control	Call Roche Service
27	Min fan error	Hardware error	Call Roche Service
28	Max fan error	Hardware error	Call Roche Service
29	Blue LED error	Hardware error	Call Roche Service
30	Fluorescence channel 1 min error	Hardware error	Call Roche Service
31	Fluorescence channel 1 max error	Hardware error	Call Roche Service
32	Fluorescence channel 2 min error	Hardware error	Call Roche Service
32	Fluorescence channel 2 max error	Hardware error	Call Roche Service
34	Fluorescence channel 3 min error	Hardware error	Call Roche Service
35	Fluorescence channel 3 max error	Hardware error	Call Roche Service
36	Rotor home position error	Hardware error	Call Roche Service
37	Photometer home position error	Hardware error	Call Roche Service
38	Photometer lost steps	Hardware error	Call Roche Service
39	Rotor lost steps	Hardware error	Reboot system, call Roche Service if error persists
40	Gain adjust error	Hardware error	Call Roche Service
41	Carousel setting out of scope	Hardware error	Call Roche Service
42	Carousel calculation out of scope	Hardware error	Call Roche Service
45	Parameter version mismatch	Hardware error	Call Roche Service

Error Code	System message	Possible Cause	Corrective Action
49	Checksum error	Temperature module error	Call Roche Service
50	EEPROM write error	Hardware module error	Reboot system, call Roche Service if error persists
51	EEPROM read error	Hardware module error	Reboot system, call Roche Service if error persists
52	EEPROM busy error	Hardware module error	Reboot system, call Roche Service if error persists
53	EEPROM page error	Hardware module error	Reboot system, call Roche Service if error persists
54	Internal bitbus error	Hardware module error	Reboot system, call Roche Service if error persists
55	Transmit buffer LC -> PC overflow	Communication error	Close all programs that were running on the computer at the same time as the LightCycler® software. If error still occurs, call Roche Service
56	Receive buffer LC <- PC overflow	Communication error	Reboot system, call Roche Service if error persists
57	Internal order buffer overflow	Communication error	Call Roche Service
58	Protocol address <> 'S' wrong processor address, software or PIC error	Communication error	Call Roche Service
59	Mathematics error for BASE-210	Communication error	Call Roche Service
60	Protocol data or order from PC not valid data exceed limits or unknown order, software error	Communication error	Call Roche Service
61	Protocol number of data not valid	Communication error	Call Roche Service
62	Internal protocol order not valid unknown order, firmware error	Communication error	Call Roche Service
70	Timeout transmit data to photometer	Photometer communication error	Reboot system, call Roche Service if error persists
71	Timeout receive data from photometer	Photometer communication error	Reboot system, call Roche Service if error persists
72	Timeout photometer measurement	Photometer error	Reboot system, call Roche Service if error persists
73	Checksum error in binary answer	Photometer error	Reboot system, call Roche Service if error persists
74	Timeout UART busy	Photometer error	Reboot system, call Roche Service if error persists
75	Error from photometer message register	Photometer error	Call Roche Service
80	Photometer bit 00: watch dog reset	Photometer error	Reboot system, call Roche Service if error persists
81	Photometer bit 01: brown out reset	Photometer error	Reboot system, call Roche Service if error persists

Error Code	System message	Possible Cause	Corrective Action
82	Photometer bit 02: communication error	Photometer error	Reboot system, call Roche Service if error persists
84	Photometer bit 04: ADC hardware error	Photometer error	Reboot system, call Roche Service if error persists
85	Photometer bit 05: LED hardware error	Photometer error	Reboot system, call Roche Service if error persists
86	Photometer bit 06: checksum error EEPROM	Photometer error	Reboot system, call Roche Service if error persists
87	Photometer bit 07: service mode enabled	Photometer error	Reboot system, call Roche Service if error persists
88	Photometer bit 08: write protection disabled	Photometer error	Reboot system, call Roche Service if error persists
89	Photometer bit 09: power supply error	Photometer error	Reboot system, call Roche Service if error persists
90	Photometer bit 10: self test active	Photometer error	Reboot system, call Roche Service if error persists
91	Photometer bit 11: not ready for commands	Photometer error	Reboot system, call Roche Service if error persists
92	Photometer bit 12: calculation error	Photometer error	Reboot system, call Roche Service if error persists
93	Photometer bit 13: checksum error Flash	Photometer error	Reboot system, call Roche Service if error persists
100	Fluorescence [1..12]=0 Min error	Fluorescence signal error	Reboot system, call Roche Service if error persists
101	Fluorescence 1 Max error	Fluorescence signal error	Check dye concentration, call Roche Service if error persists
102	Fluorescence 2 Max error	Fluorescence signal error	Check dye concentration, call Roche Service if error persists
103	Fluorescence 3 Max error	Fluorescence signal error	Check dye concentration, call Roche Service if error persists
104	Fluorescence 4 Max error	Fluorescence signal error	Check dye concentration, call Roche Service if error persists
105	Fluorescence 5 Max error	Fluorescence signal error	Check dye concentration, call Roche Service if error persists
106	Fluorescence 6 Max error	Fluorescence signal error	Check dye concentration, call Roche Service if error persists
107	Fluorescence 7 Max error	Fluorescence signal error	Check dye concentration, call Roche Service if error persists
108	Fluorescence 8 Max error	Fluorescence signal error	Check dye concentration, call Roche Service if error persists
109	Fluorescence 9 Max error	Fluorescence signal error	Check dye concentration, call Roche Service if error persists
110	Fluorescence 10 Max error	Fluorescence signal error	Check dye concentration, call Roche Service if error persists

Error Code	System message	Possible Cause	Corrective Action
111	Fluorescence 11 Max error	Fluorescence signal error	Check dye concentration, call Roche Service if error persists
112	Fluorescence 12 Max error	Fluorescence signal error	Check dye concentration, call Roche Service if error persists
127	Error in temp processor	Error from TEMP processor	Call Roche Service
148	Temperature min error	Hardware problem concerning temperature control	Reboot system, call Roche Service if error persists
149	Temperature max error	Hardware problem concerning temperature control	Reboot system, call Roche Service if error persists
150	Temperature heat up error	Hardware problem concerning temperature control	Call Roche Service
151	Temperature cool down error	Hardware problem concerning temperature control	Call Roche Service
152	Temperature heat up timeout	Hardware problem concerning temperature control	Call Roche Service
153	Temperature cool down timeout	Hardware problem concerning temperature control	Reboot system, call Roche Service if error persists
154	Overheat from hardware sensor	Hardware problem concerning temperature control	Call Roche Service
155	Temperature ADC timeout	Hardware problem concerning temperature control	Reboot system, call Roche Service if error persists
157	Fan Brake error	Fan error	Call Roche Service
158	Min fan error	Hardware problem concerning temperature control	Call Roche Service
159	Max fan error	Fan error	Call Roche Service
160	Fan alarm from hardware sensor	Fan error	Check if the fan is inserted correctly. If error occurs with correctly installed fan, call Roche Service
163	Lid unlock error	Lid lock error	Lid can be opened manually in urgent cases, refer to Chapter *Operation*. Call Roche Service
164	Lid lock error	Lid lock error	Reboot system, call Roche Service if error persists
173	Parameter version mismatch		Reboot system, call Roche Service if error persists
174	Checksum error on Main board EEPROM		Reboot system, call Roche Service if error persists
175	EEPOT invalid	Temperature module error	Reboot system, call Roche Service if error persists
176	Serial number T-Module invalid	Temperature module error	Reboot system, call Roche Service if error persists
177	Checksum error on T-Module EEPROM	Temperature module error	Call Roche Service

Error Code	System message	Possible Cause	Corrective Action
178	EEPROM write error	Hardware module error	Reboot system, call Roche Service if error persists
179	EEPROM read error	Hardware module error	Reboot system, call Roche Service if error persists
180	EEPROM busy error	Hardware module error	Reboot system, call Roche Service if error persists
181	EEPROM page error	Hardware module error	Reboot system, call Roche Service if error persists
182	Internal Bit-Bus Error	Hardware module error	Reboot system, call Roche Service if error persists
183	Transmit buffer LC -> PC overflow	Communication error	Close all programs that were running on the computer at the same time as the LightCycler® software. If error still occurs, call Roche Service
184	Receive buffer LC <- PC overflow	Communication error	Call Roche Service
185	Internal order buffer overflow	Communication error	Call Roche Service
186	Protocol address <> 'T'	Communication error	Call Roche Service
187	Mathematics error for BASE-210	Communication error	Call Roche Service
188	Protocol data or order from PC not valid	Communication error	Call Roche Service
189	Protocol number of data not valid	Communication error	Call Roche Service
190	Internal protocol order not valid	Communication error	Call Roche Service
255	Error in step processor	Error from STEP processor	Call Roche Service

2. Instrument-Related Errors

Instrument-related errors may occur during running an experiment, running the Real Time Fluorimeter or during operating the instrument screen of the software. In this list [...] is meant as a placeholder in certain error messages and may stand for an instrument name, a serial number etc.

Error Message	Possible Cause	Corrective Action
Are you sure the computer is connected to a LightCycler® instrument?	Communication error	Restart instrument and software.
Attempted to call the DetectOnPort method on a connected instrument [...] whose port [...] is different than the port to be used in the detection [...]!	Wrong port used	Check port connection.
Attempted to connect object [...] to a different instrument [...] on port [...]. Please choose the correct instrument object in the software, connect to a different instrument/port, or create a new instrument object if this is a new instrument (hasn't been connected before)!	Other instrument connected to port than expected	Choose the correct instrument object in the software and connect the associated instrument. Create a new instrument object if you want to connect a new instrument that has not been connected before.
Can only disconnect the instrument while it's not running!	User tried to disconnect an instrument in the instrument screen while it was running a selftest, run, etc.	Wait until current process is finished.
Cannot close window while [...] is running!	User tried to close the instrument screen while the instrument is running	Finish the run or abort it.
Can't apply templates to runs that have been started.	Handling error	Finish or abort current run. Apply new template to new run.
Can't change the I/O port while the instrument is connected!	User tried to change the I/O port in the instrument screen while connection was already established	Press 'Connect' button again to disconnect the instrument and to end communication.
Can't initialize the LightCycler® instrument. No I/O port has been assigned!	No I/O port chosen in the instrument screen.	Check I/O port chosen in the instrument screen.
Can't start the Run until the instrument lid is closed!	Handling error	Close the lid before starting a run.
Can't start the self-test until the instrument lid is closed!	Handling error	Close the lid before starting to operate the instrument.
Communication Error: Unable to load firmware!	Communication error	Restart instrument and software.
Disconnecting the instrument will terminate RTF. Are you sure you want to disconnect the instrument?	Depress 'Connect' in instrument screen while running RTF	Stop RTF first.

Error Message	Possible Cause	Corrective Action
Error reading firmware version. Error code = [...], Data = [...]!	Communication error	Restart instrument and software.
Information* Program 0, Segment 0: 30 is an invalid temperature target.	User imported a Nucleic Acid experiment containing a segment with 30°C as the temp target.	Correct the experiment settings.
Instrument [...] already exists.	User tried to create two instrument objects for the same instrument	Leave 2nd instrument object without saving. Select already existing instrument object for instrument.
Instrument [...] was detected. Do you want to start the run with this instrument?	User clicked "Start Run" on Run window when no instrument was connected	Answer the question. If it is the wrong instrument select an other instrument.
Instrument is already running!	Handling error	Finish or abort current run, start new run thereafter.
Internal Error: [...]. Firmware component was unable to return the firmware data!	Corrupted firmware in the firmware component.	Restart instrument and software. Call Roche Service if error persists.
Internal Error: [...]. Firmware property was unassigned!	Developer Error: The firmware component was not setup properly.	Restart instrument and software. Call Roche Service if error persists.
LightCycler® Error: [...]	Instrument error code	Restart instrument and software. Call Roche Service if error persists.
Please close the instrument lid before performing a self test.	Handling error	Close the instrument lid before starting a run.
Please close the instrument lid before starting a run	Handling error	Close the instrument lid before starting a run.
Please close the instrument lid before starting RTF.	Handling error	Close the lid before operating the instrument.
Port [...] is currently unavailable (possibly in use by another instrument or process). Details: [...]	Port already in use by another instrument or software.	Use other port. Wait until process is finalized. Close other software.
Real time fluorimeter aborted with an error	Instrument error	Check error log
Real time fluorimeter communication timed out! Please verify the instrument is properly connected.	Communication error	Check cable
Received a protocol with an error while performing a Step self test! Please verify the instrument is properly connected.	Communication error	Check cable
Received an error protocol while seeking samples! Please verify the instrument is properly connected.	Communication error	Check cable. Restart instrument and software.

Error Message	Possible Cause	Corrective Action
Received fatal error during run!	Instrument error	Check error log.
Run timed out after not receiving any data from the instrument!	Communication error	Check cable
Step self test timed out! Please verify the instrument is properly connected.	Communication error	Check cable
The firmware crc value is declared incorrectly!	Hardware error	Call Roche Service
The following samples were not found during the seek process. Do you want to continue the run?	Missing samples or missing fluorescent dye.	Check if all sample capillaries were inserted correctly. Check if fluorescent dye has been added. Confirm the message to continue the run or abort it by pressing “No”.
The instrument firmware failed to load. Please verify that the instrument is properly connected.	Communication error	Check cable, restart instrument and software.
The instrument is already running another experiment!	Handling error	Finish or abort current run before starting a new run.
The instrument is currently busy running Real Time Fluorimeter.	Handling error	Finish current process and restart action.
The instrument timed out while seeking samples! Please verify the instrument is properly connected.	Communication error	Check cable
The instrument timed out while waiting for sample search protocol! Please verify the instrument is properly connected.	Communication error	Check cable. Restart instrument and software.
The instrument’s lid was opened during the run!	Handling error	Repeat experiment.
The instrument's lid has been opened during the run. The run will now be terminated.	Handling error	Repeat run.
The instruments lid was opened during real time fluorimeter!	Handling error	Do not open the lid when operating the instrument.
The run aborted with an error. The instrument timed out while waiting for sample search protocol! Please verify the instrument is properly connected.	Communication error	Check cable. Restart instrument and software.
The run aborted with an error. Invalid checksum on returned protocol.	Communication error	Check cable. Restart instrument and software. Call Roche Service if error persists.
The seek process found the following samples which were not supposed to contain any data (their positions were initially flagged as empty): [...]!	Mismatch between SAM file and seek	Check loading; restart experiment.

Error Message	Possible Cause	Corrective Action
The selected instrument is of a different type ([…] Channel) than the instrument specified in the experiment ([…] Channel). Continuing could lead to a loss of data (e.g. loss of channel information in the sample editor). Do you want to continue?	Instrument is of different type (3 channel versus 6 channel) than selected in experiment	Chose correct instrument type for experiment or use other experiment.
The stored object reports the instrument subclass to be of type [...], but the instrument "says" the type is [...]!	Wrong instrument type connected.	Choose the correct instrument object in the software and connect the associated instrument. Create a new instrument object if you want to connect a new instrument that has not been connected before.
Timed out while waiting for instrument to adjust temperature! Please verify the instrument is properly connected.	Communication error	Check cable
Unable to backup run data!	Disk full; no access rights	Assure that the disk used has enough memory space and check the user rights.
Unable to communicate with the instrument. Please verify the instrument is properly connected.	Communication error	Check cable. Restart instrument and software.
Unable to find [...] on port [...]. Do you want to search for the instrument on other ports?	Instrument not connected to the specified port.	Choose the correct instrument object in the software and connect the adequate instrument. Create a new instrument object if you want to connect a new instrument that has not been connected before.
Unable to start self test with instrument [...]. Please make sure the instrument is properly connected.	Communication error	Check cable

3. Algorithm Errors and Messages

This list includes error messages that are possible within the module calculation sections of the LightCycler® Software 4.05.

Error Message	Possible Cause	Corrective Action
At least 11 cycles of data are required	Not enough data to compute Cp because user specified too few acquisitions.	Rerun experiment with at least 11 cycles of acquired data.
Coefs not correctly specified	.ccc file coefficients corrupted.	LCDA ABT file corrupted. Run experiment in SW 3.5 again.
Cycle and Fluorescence data matrices must be identically sized	Unequally sized fluorescence and cycle data. Data corrupted.	Rerun experiment.
Cycle and Fluorescence data matrices must be identically sized	Unequally sized fluorescence and cycle data. Data corrupted.	Rerun experiment.
Data dimensions are inconsistent	Inconsistent data, usually arising from importing SW 3.5 data.	Corrupted LCDA file. Reimport data.
Data in standards does not encompass sample temperature range.	Unknowns and standards incompatible, standards defined for temperatures outside sample temperature range.	Standards are incompatible with samples. Remelt samples at temperatures within the range of standards.
Different number of Channel and Position labels.	Internal error – can't find all data.	Rerun experiment; call Roche Service if error persists.
Different number of SampleID and Position labels.	Internal error – can't find all data.	Rerun experiment; call Roche Service if error persists.
Different number of Target and Position labels.	Internal error – can't find all data.	Rerun experiment; call Roche Service if error persists.
Different number of Type and Position labels.	Internal error – can't find all data.	Rerun experiment; call Roche Service if error persists.
Errors ocurred during analysis calculation. The report may display incorrect results!	User ran kit and aborted experiment during run.	Rerun experiment; call Roche Service if error persists.
Fluorescence & Time matrices of different size.	Data are inconsistent, SW 3.5 import problem or machine error.	Corrupted LCDA file. Reimport data; call Roche Service if error persists.
Gains not correctly specified	.ccc file gains corrupted.	LCDA ABT file corrupted. Run CC experiment in SW 3.5 again.
Invalid CCC data	CCC data do not match check sum.	Corrupted CCC data. Reload LCDA data if imported; redo CCC experiment.
Invalid format for number of Groups.	Too many standards for low-sensitivity mode.	Select different standards; use in-run standards; run in high sensitivity mode.
Invalid melt data	Data are constant, usually equal to zero.	Samples did not amplify. Redo experiment; call Roche Service if error persists
Maximum number of function evaluations exceeded	Data do not fit model, melt curves do not fit standard model.	Use manual Tm finder tool.
Maximum number of iterations exceeded'	Data do not fit model, melt curves do not fit standard model.	Use manual Tm finder tool.

Error Message	Possible Cause	Corrective Action
More measurements needed	Not enough data gathered in experiment.	Make sure run has acquisitions specified; decrease temperature ramp rate.
Negative Concentration not allowed	User specified negative conc. values.	Reset Cp limits in sample editor.
Negative CPRange limits not allowed	User specified negative Cp values.	Reset Cp limits in sample editor.
Negative Fluorescences not allowed	Negative values of fluorescence observed	Rerun experiment; call Roche Service if error persists.
No fluorescence data	Missing fluorescence information in raw data.	Make sure run has acquisitions specified.
Not enough data to color compensate	CCC experiment doesn't have enough data to compute compensation.	Rerun CC experiment, and gather enough data.
Qualitative Detection: Exception ECrossingPoint raised with message "At least 11 cycles of data are required."	Program did not have the necessary number of cycles for the analysis. Program may be inaccurate, or user may have aborted run before cycles complete.	Rerun experiment with at least 11 cycles of acquired data.
Resolution cutoff must be nonnegative and less than Score threshold	Invalid user input of resolution.	Decrease Resolution threshold below Score threshold.
Standard curve could not be calculated	Not enough standard data points.	Increase number or samples in experiment.
Temperature & Time matrices of different size.	Data are inconsistent, SW 3.5 import problem or machine error.	Corrupted LCDA file. Reimport data; call Roche Service if error persists.
Too much missing data, cycle numbers must be contiguous	Missing acquisitions in cycling. Machine didn't collect data. Data corrupted.	Rerun experiment; call Roche Service if error persists.
Unable to compute standard curve	Not enough standards for linear regression, regression requires 3 or more standards.	Increase the number of standards in experiment.
Upper limit of ConcRange greater than lower limit	User specifies incorrect conc. limits.	Reset concentration limits in sample editor.
Upper limit of CPRange greater than lower limit	User specifies incorrect Cp limits.	Reset Cp limits in sample editor.
[…] not a valid correction factor.	Non-positive correction factor.	Reset correction factor on paring tab.
[…] not a valid multiplication factor	Non-positive multiplication factor.	Reset multiplication factor on paring tab.

4. Miscellaneous messages

This section lists messages for modules and features not covered above.

Error Message	Possible Cause	Corrective Action
An error occurred while importing file […].FLO. Please verify that all ATF files exist and are valid.: ABT file not found.	User selected a .flo file to import that did not have a corresponding .abt file.	Check if the corresponding experiment data files are complete
Archive log entry was empty!!	User imported a file and saved it, then highlighted file in the Navigator and checked File > Revisions. Message appears when there are no previous revisions in the Revisions pane.	
Cannot stop Exor. There is <n> user connected.	The user tried to shut down Exor while one or more users were logged in.	Wait until all users are logged out
Please export modified objects before logging out or closing LCS4.	There was a failure in communication with Exor. The object cannot be saved in the database, but the user can export the object and reimport it when communication is reestablished.	Export the modified objects and reimport them after restart of the LightCycler® Software
The file does not have a checksum and will not be imported. Checksum failure!.	User tried to import an LightCycler® Software 4.0 .ixo file into LightCycler® Software 4.05	

5. Index

F

Index word **page**

F

Index word **page**

Index word **page**

Index word **page**

Index word **page**

F

Index word **page**

F

Index word **page**

F

Inspiring Discovery

Diagnostics

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany

www.roche-applied-science.com

0305-**04624505001**