# LightCycler® 2.0 Instrument Operator's Manual

## Software Version 4.1

## Manual A: for *in vitro* diagnostic use*


**www.roche-applied-science.com**

* in combination with Roche LightCycler® kits labeled for *in vitro* diagnostic use

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# Preface

## I. Revision History

| Version | Revision Date |
|---|---|
| 1.1 | October 2003 |
| 2.0 | April 2005 |
| 3.0 | April 2007 |



## II. Contact Addresses

| | |
|---|---|
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| Distribution in USA | Roche Diagnostics<br>9115 Hague Road<br>PO Box 50457<br>Indianapolis, IN 46250<br>USA |

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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.

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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.
The LightCycler® 2.0 Instrument is designed for *in vitro* diagnostic applications in combination with the 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). 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.

**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 the 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. 05 002 702 001; to obtain your local language version contact your Roche representative) while "Manual B, marked *for general laboratory use*" (id. no. 05 002 699 001) 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.

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# 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 using Roche Macros.

**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.<br>- 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 LightCycler® 2.0 Instrument or the Control Unit Components**

| | |
|---|---|
|  | Manufacturer of device.<br>On the instrument type plate. |
|  | Warning<br>On the instrument type plate (see *XII Handling Precautions*). |
|  | Hot surface<br>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<br>On the instrument type plate<br>(see Chapter *Overview, 2.2 General specifications*) |
|  | Electrical and electronic equipment marked with this symbol are covered by the European directive WEEE.<br>The symbol denotes that the equipment must not be disposed of in the municipal waste system. (WEEE Directive 2002/96/EC of the European Parliament and the Council of 27 January 2003 on waste electrical and electronic equipment.) |

# 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 informations required for installation and operation of the LightCycler® 2.0 Instrument are carefully read and observed. Please assure that these safety informations 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 is equipped with software, enabling the user of the Product to connect it with a network. Roche draws the attention of the user to the fact that such connection may have an adverse effect on the Product's integrity, *e.g.*, due to an infection of the Product with malicious code (viruses, Trojan horses, etc.) or access by unauthorized third parties (*e.g.*, intrusion by attackers). Roche therefore highly recommends to protect the Product against such risks by taking appropriate and state-of-the-art action. As the Product is not intended to be used within networks without an appropriate firewall and has not been designed for such use, Roche assumes no liability in that regard.
Roche offers the user the cobas IT firewall to be installed prior to the first connection of the Product to any network. For further information on this cobas IT firewall and/or the Roche network security concept please contact your local Roche representative.
In the event the user connects the Product with any network without using the cobas IT firewall, Roche cannot offer any Product support regarding any problem resulting from such network connection.
In case of stand-alone use of the software of the Product on or in connection with other IT components (*e.g.*, installation on other PCs) Roche assumes no liability with respect to any interference of the user's networks and/or other IT components such use might have. Roche's liability for the proper functioning of the software under the respective license and/or purchase agreements with the user shall remain unaffected.

Contact your local Roche representative for detailed information on the cobas IT firewall.

Additional software must not be installed on the LightCycler® 2.0 control units, except for Roche software specifically approved for installation on LightCycler® 2.0 control units. Installation of additional software contains the risk to interfere with LightCycler® Software 4.1 and may affect result security. For installation of additional Roche software, please refer to the Operator's Manual of this software or to the package insert of the corresponding test.

One PC must not be used with 2 LightCycler® 2.0 Instruments simultaneously.

Do not manipulate the instrument.

Exceeding the maximum number of 3 login attempts (default value) will block the access to your user account. Refer to Chapter *Software* for more information.

Do only use the supplied US keyboard. Usage of any other keyboard than the delivered US keyboard can cause wrong results.

### 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 115 V or 230 V line. Where an ungrounded receptacle is encountered, a qualified electrician must replace it with a properly (PE) grounded receptacle in accordance with the local electrical code. An extension must not be used. Any break in the electrical ground path, whether inside or outside the instrument, 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.

## XIII. Disposal of the Instrument and Control Unit Components

### Disposal Recommendations

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

#### Disposal of the LightCycler® 2.0 Instrument

The instrument must be treated as biologically contaminated-hazardous waste. Final disposal must be organized in a way that does not endanger waste handlers. As a rule, such equipment must be sterile before it is passed on for final disposal.

For more information contact your local Roche Support personnel.

#### Disposal of Control Unit Components

Components of your Control Unit such as the computer, monitor, keyboard, etc. which are marked with the crossed-out wheeled bin symbol are covered by the European Directive 2002/96/EC (WEEE).
These items must be disposed of via designated collection facilities appointed by government or local authorities.
For more information about disposal of your old product, please contact your city office, waste disposal service or your local Roche Support personnel.

#### Constraint

It is left to the responsible laboratory organization to determine whether control unit components are contaminated or not. If contaminated, treat in the same way as the instrument.

# 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


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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.

## 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.)<br>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;<br>relative humidity:<br>10% to 95% (no condensation) |
| **Temperatures allowed during operation** | +18°C to +30°C |
| **Relative Humidity** | 10% to 95%, no condensation |
| **Altitude/Pressure** | 0 to 2000 m above sea level<br>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 Control Unit

A fully equipped control unit is delivered by Roche with the LightCycler® 2.0 Instrument.

The control unit 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) | 20 µl: ≤ 15 sec<br>100 µl: ≤ 27.5 sec |
| Heating rate 50°C to 72°C (non-linear) | 20 µl: ≤ 8 sec<br>100 µl: ≤ 11 sec |
| Heating rate 72°C to 95°C (non-linear) | 20 µl: ≤ 8 sec<br>100 µl: ≤ 11.5 sec |

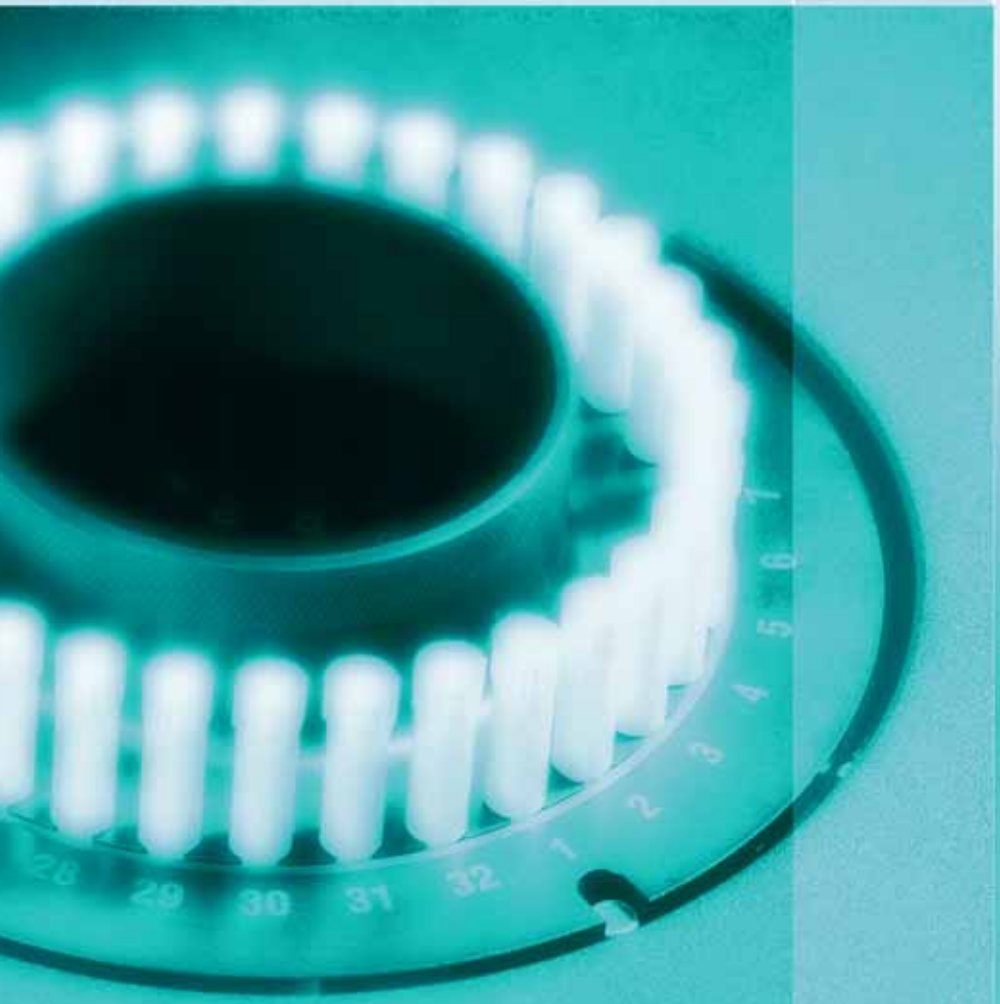
## 4.3 Capillary Cooling Rates

| | |
|---|---|
| Cooling rate 95°C to 50°C (non-linear) | 20 µl: ≤ 15 sec<br>100 µl: ≤ 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 |
| Precision of capillary temperature over all capillary positions when measured for 30 sec at 70°C | +/-0.15°C |
| Precision of capillary temperature over all capillary positions when measured for 30 sec at 50°C | +/-0.3°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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# 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)<br>  <br>▶ LightCycler® 2.0 Sample Carousel (20 µl) (Cat.No.: 03 603 962 001) mounted in LightCycler® 2.0 Instrument<br>  |

B

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

| Components | Description |
|---|---|
| System component 2 | ▶ LightCycler® 2.0 Instrument Operator's Manual<br>▶ LightCycler® Software 4.1 (Cat.No.: 04 898 915 001)<br>▶ LightCycler® Software 4.05 Tutorial<br>▶ Serial cable to connect the LightCycler® 2.0 Instrument to the Computer<br>  <br>▶ Power cord (one with German plug and one with US plug)<br>  |

A fully equipped control unit is delivered with the LightCycler® 2.0 Instrument.

Do only use the supplied US keyboard. Usage of any other keyboard than the delivered US keyboard can cause wrong results.

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<br>▶ 115 V (60 Hz)<br>▶ 230 V (50 Hz)<br>If the voltage in your country does not meet the voltage requirements, please contact your local Roche representative.<br>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.<br>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 10% and 95% (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.

B

## 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 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 Control Unit

## 3.1 Installation of the Computer

To install the PC, 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 the 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® 2.0 Instrument 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 Light-Cycler® control unit'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® 2.0 Instrument run.

B

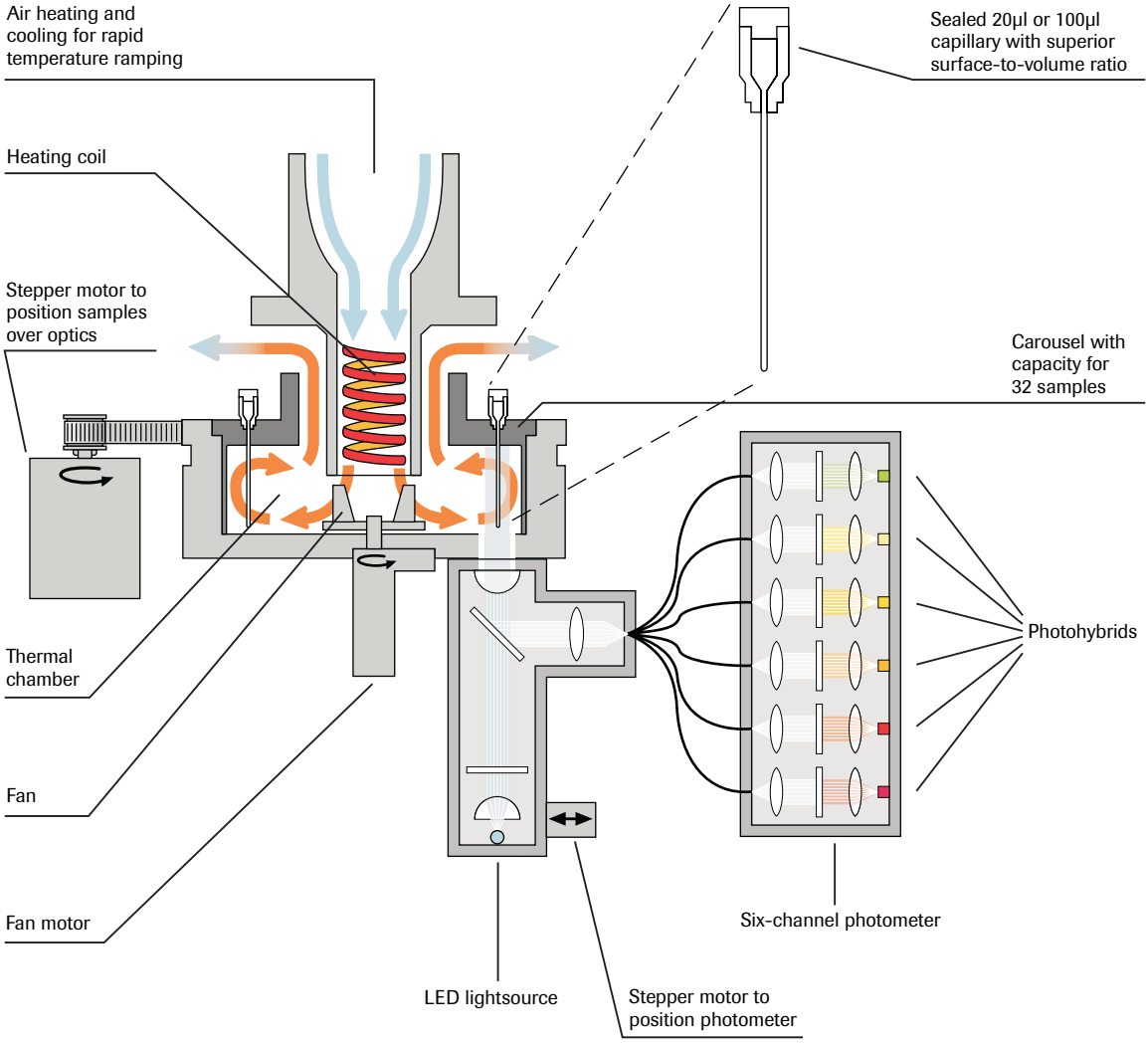
# 4. System Description

## 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.

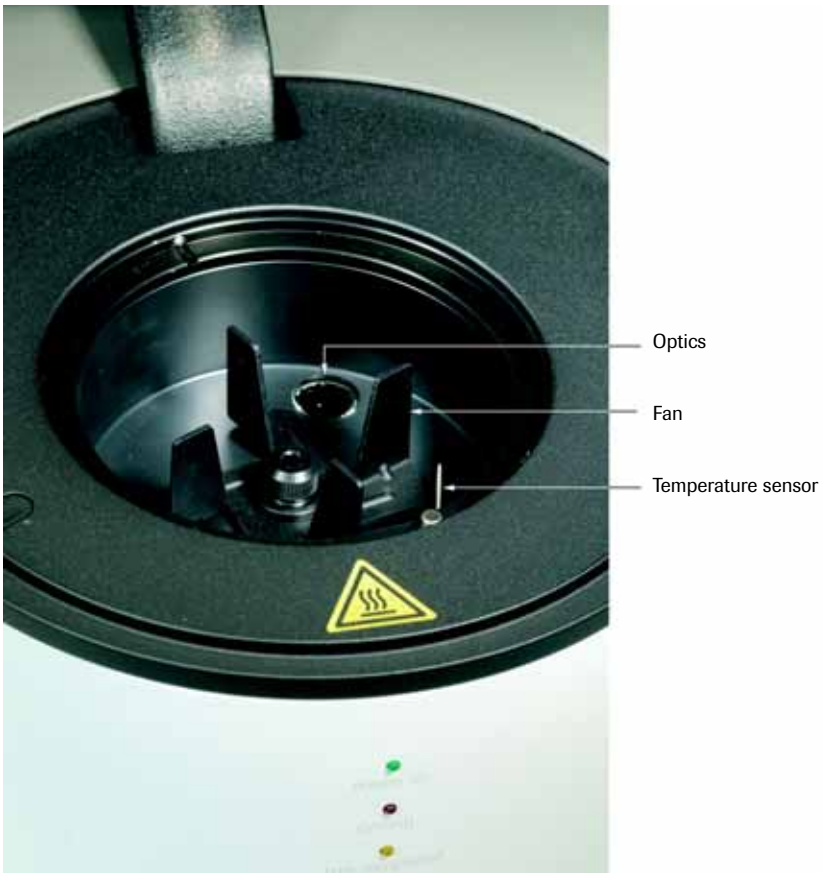
During measurements a stepper motor rotates the sample carousel within the thermal chamber to position the capillary tip precisely at the focal point of the photometer optics. The stepper motor of the sample carousel works with the horizontal photometer stepper motor to achieve optimal positioning of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

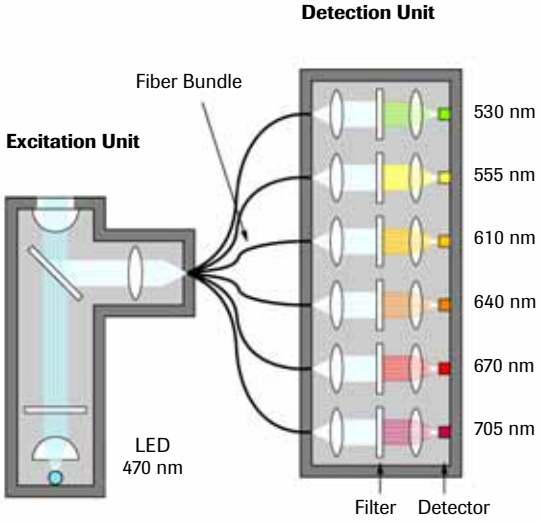
## 4.3 Photometer

### 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® 2.0 Instrument 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<br>530 nm | 2<br>555 nm | 3<br>610 nm | 4<br>640 nm | 5<br>670 nm | 6<br>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 a 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 E *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. It is used for the easy unloading of capillaries from the LightCycler® 2.0 Sample Carousel.

Once the loaded carousel is placed on the LightCycler® Capillary Releaser and pushed down using the flat of your hand, the capillaries are released from being firmly held by the rubber O-ring, and held at a slightly elevated position, allowing easy removal of the capillaries from the sample carousel.

B

When the capillaries are simply to be discarded, the loaded sample carousel may be put onto the table upside down. The LightCycler® Capillary Releaser is then inserted and pressed down to release the capillaries.

When the sample carousel is lifted, the LightCycler® Capillary Releaser will slide down into the carousel, to fully release 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.

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# 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 4.1. Details on working with the LightCycler® Software 4.1 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<br>▶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 upon starting a run or 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

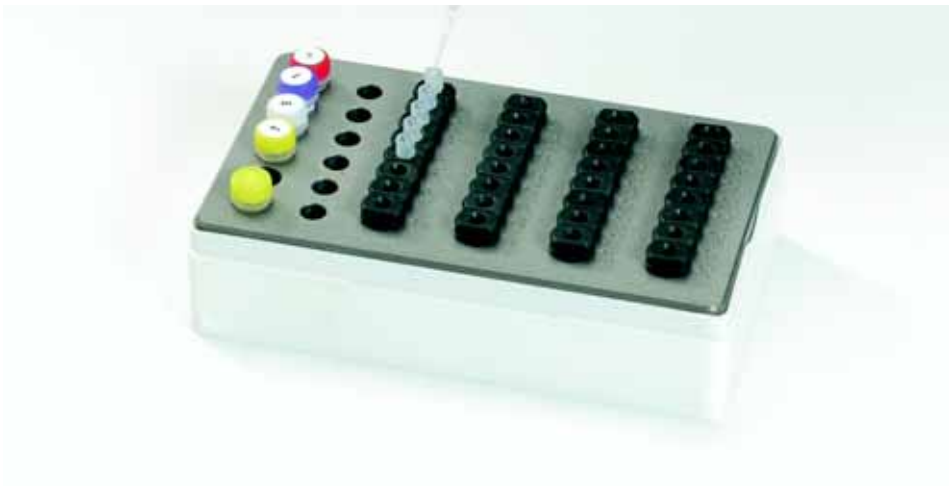
The lid lock is regulated electronically. For manual opening (*e.g.*, if no electricity is available), the lid can be unlocked manually by pressing the hidden button under the front left side of the instrument as indicated in the picture.

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

# 3. Preparing a LightCycler® 2.0 Instrument Run

| | |
|---|---|
| 1 | Install the Roche Macro. 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 during insertion into the LightCycler® 2.0 Sample Carousel. Do not use LightCycler® capillaries that fell down. LightCycler® capillaries with slight cracks, possibly invisible, might crack during a LightCycler® run.

When inserting 100 µl LightCycler® Capillaries in the LightCycler® 2.0 Sample Carousel, turn the capillaries slightly to unbend the O-Ring. A bent O-Ring may relax and push up the capillaries during a run and in consequence disturb the signal acquisition.

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, you can spin down the reaction mix within the capillaries with the help of the LightCycler® Centrifuge Adapters in a standard benchtop centrifuge, like the Biofuge 19 from Heraeus Instruments, 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 with 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 4.1 and shut-down the computer. |
| 2 | Put the LightCycler® 2.0 Instrument **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


**Chapter D • Software**
contains instructions for using Roche Macros.

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# Software

## 1. Overview of LightCycler® Software 4.1

This chapter provides a general introduction to the LightCycler® Software 4.1. It includes the following topics:

- Starting the LightCycler® Software 4.1
- Understanding the LightCycler® Software 4.1 window
- Opening, creating, and saving objects
- Renaming and deleting objects
- Importing and exporting objects

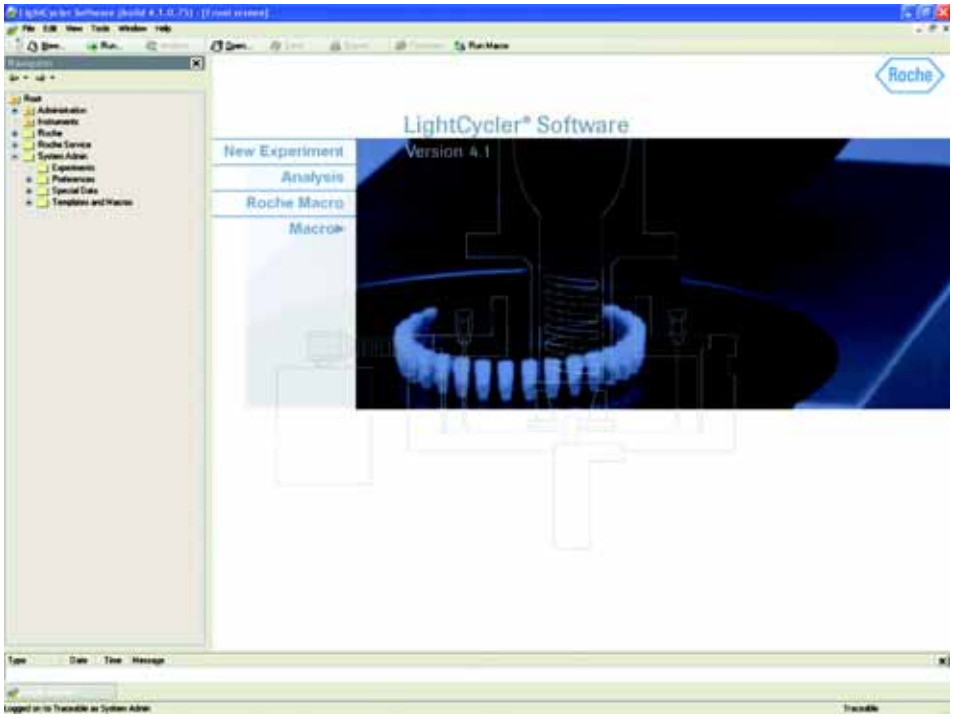
### 1.1 Starting the LightCycler® Software 4.1

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 4.1 described in the rest of this chapter.

**To start the LightCycler® Software 4.1 and view a demo experiment**

| | |
|---|---|
| 1 | Double-click the LightCycler® Software 4.1 icon on the desktop. |
| 2 | In the *Login* dialog box, type your user name and password. (The initial User name is admin and the initial password is LightCycler01. You will be prompted to change the initial password upon your first login. For more information see *Changing your Password*.)<br>*Exceeding the maximum number of 3 login attempts (default value) will block the access to your user account.* |
| 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 4.1 window containing the Navigator on the left and a picture of a LightCycler® 2.0 Instrument on the right. The activity of the buttons you see on the window depends on the role associated with your user account. For more information see chapter *Managing User Access*.




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.

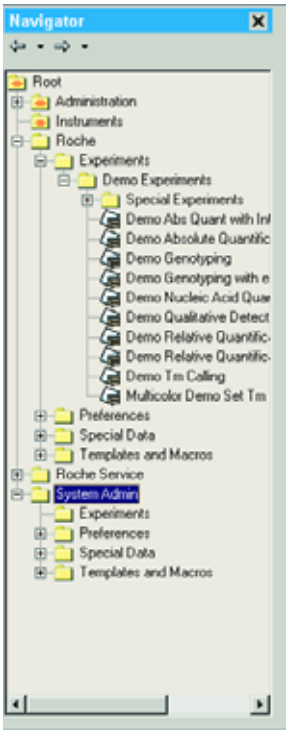
*New Experiment* launches the Run module. For more information about using the Run module to create experiments see chapter *Creating and Running an Experiment*, described in the LightCycler® 2.0 Operator's Manual, Manual B: for general laboratory use. Please note that this function is not part of the diagnostic workflow.

*Analysis* lets you select an existing experiment to analyze. For more information about analyzing experiments see chapter *Analyzing an experiment*, described in the LightCycler® 2.0 Operator's Manual, Manual B: for general laboratory use. Please note that this function is not part of the diagnostic workflow.

*Roche Macro* lets you select a previously installed Roche Macro. For more information about installing and executing Roche Macros see chapter *Using Roche Macros*.

*Macro* displays the protocol window, which contains custom buttons used to execute macros. For information about adding and deleting macro buttons on the front window see chapter *Using Roche 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 4.1 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 4.1 window, refer to the next section.

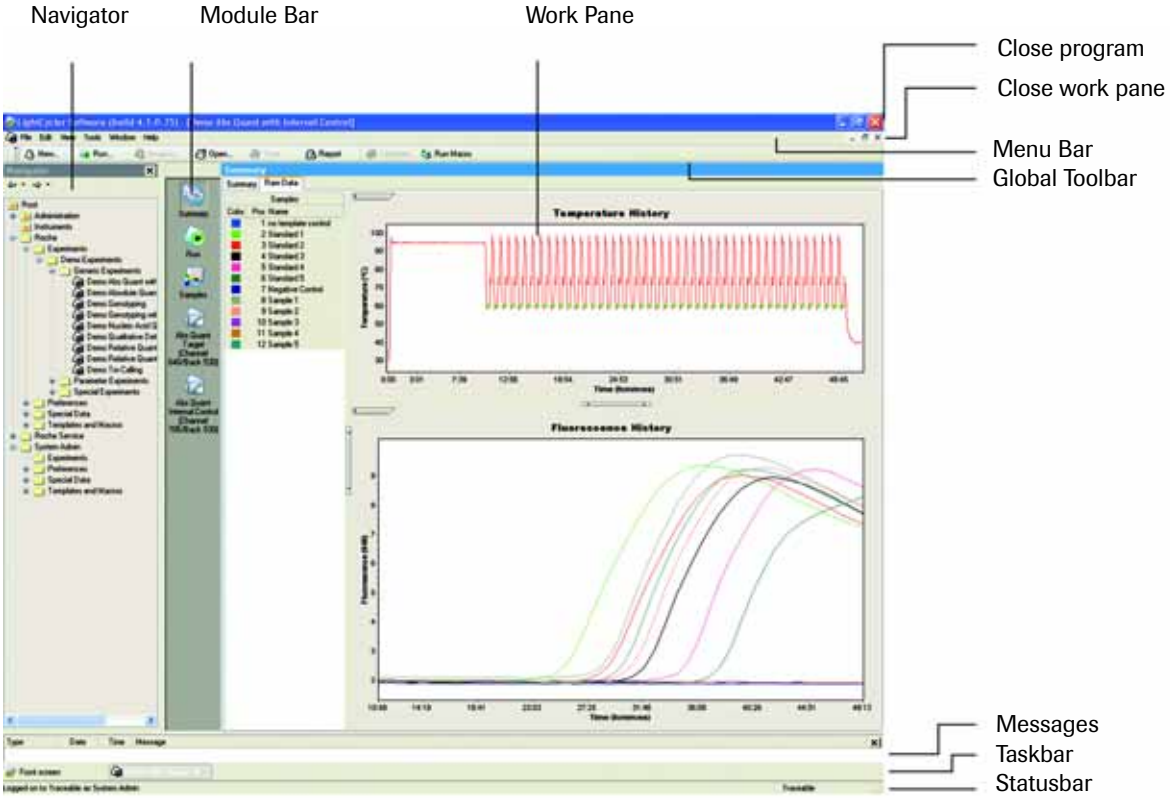
**9** To close an experiment select *Close* from the *File* menu or click the X in the upper right corner of the current experiment window.

**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

## 1.2 Understanding the LightCycler® Software 4.1 Window

The figure below illustrates a LightCycler® Software 4.1 window containing information from a demo experiment.




### The LightCycler® Software 4.1 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 *Taskbar* 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 4.1 window in more detail.

### 1.2.1 The Navigator

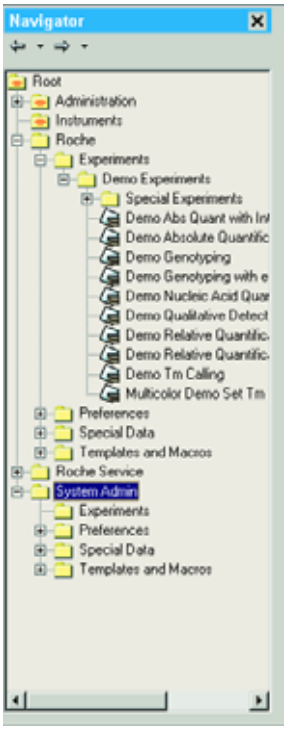
The Navigator on the left side of the LightCycler® Software 4.1 window provides access to items stored in the LightCycler® Software 4.1 database. Items include experiments, user accounts, instruments, macros, and so on. In the Navigator, items are organized in folders in a tree structure.

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 demo experiments and macros from Roche that can be used by anyone with access to the LightCycler® Software 4.1.
- An Instruments folder that is to contain an object for each LightCycler® 2.0 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.*

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 from the *View menu*, deselect *Navigator* 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 the LightCycler® 2.0 Instrument 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
- View the revision history for an object currently open in the LightCycler® Software 4.1 window
- 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 4.1 window: the Global Toolbar, Taskbar, Statusbar, Messages and Navigator
- Display the Front window (the window you see when you start LightCycler® Software 4.1) in the work pane

**Tools menu**

- Change your password
- Open the user management tool (used to create, delete, and edit users, groups, and roles)
- Run Roche Macros
- Manage instrument information
- View database status
- 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 others as *e.g.*, copyright information about the software

## Using toolbar buttons

Use toolbar buttons to perform key LightCycler® Software 4.1 tasks. 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. Please note that this function is not part of the diagnostic workflow.


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. Please note that this function is not part of the diagnostic workflow.


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.

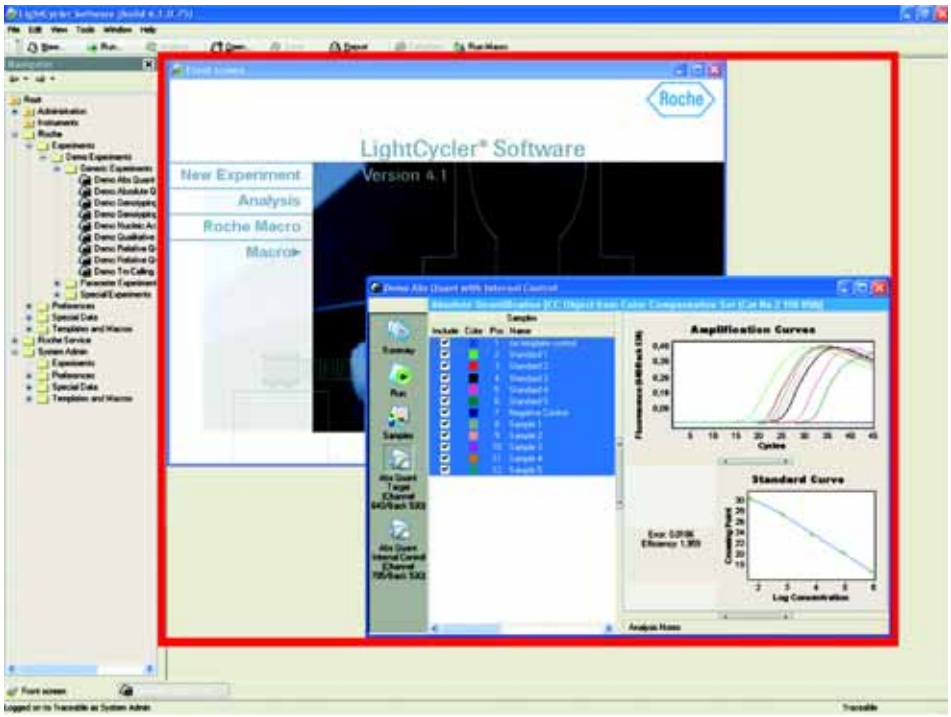

Click *Report* to open an experiment report window.


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 4.1 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 screen 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.


**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 the LightCycler® 2.0 Instrument 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 the LightCycler® 2.0 Instrument objects. Select the icon for the object you want, then click *OK*.
- 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 any item (except for experiment objects) 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).

### 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. You cannot delete an experiment object.

# 2. Using Reports, Charts, Queries and Instrument Tools

LightCycler® Software 4.1 includes the following tools and commands:

- A report generator used to generate reports that include experiment information and analysis results; for more information see *Viewing and Printing 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.
- Instrument and application diagnostic tools, including an instrument self test, error and operation logs. For more information see *Using diagnostic tools* below.

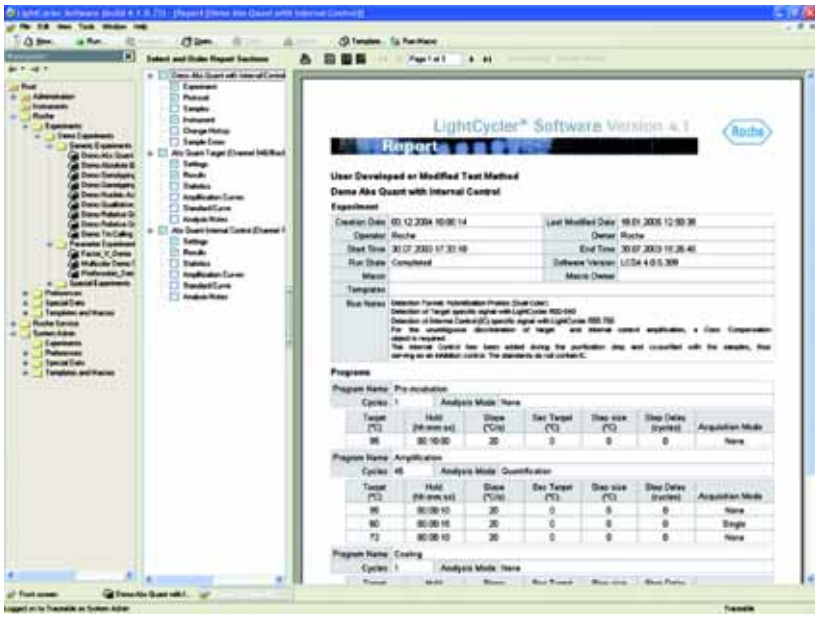
## 2.1 Viewing and Printing a Report

After the Roche Macro run is finished, LightCycler® Software 4.1 generates and displays a report containing experiment information and analysis results.

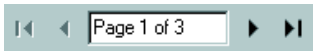
## To view and print a report:

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.




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




4. 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.

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

## 2.2 Working with Charts

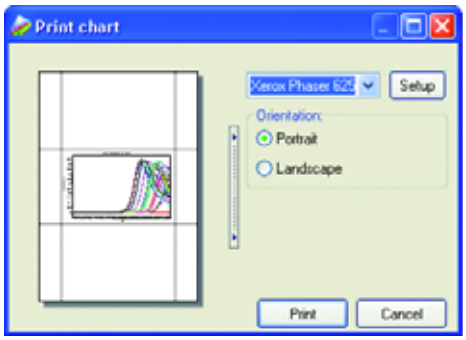
The LightCycler® Software 4.1 generates many kinds of charts during and after an experiment run and as part of an experiment analysis. You can print charts, export charts to various graphic formats, and copy and paste chart images and chart data. The charts include a zoom and a pan function, so you can enlarge details of a chart and move the chart left or right.

### 2.2.1 Printing, Exporting, and Copying Charts

You can print any chart displayed in the LightCycler® Software 4.1. 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:**

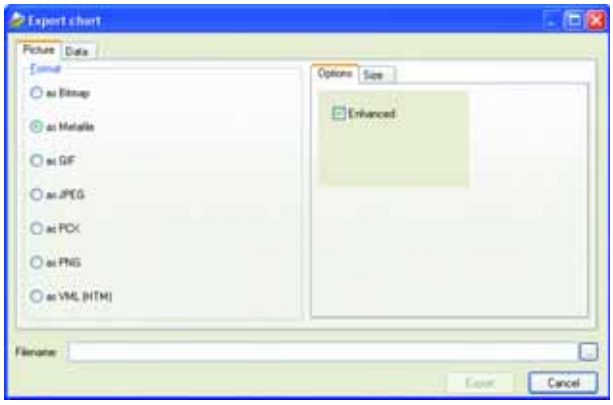
1. Display the chart you want to print.
2. Right-click within the chart boundaries, then select *Print*. A setup window is displayed.




3. To change the graph margins, and thereby 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. |

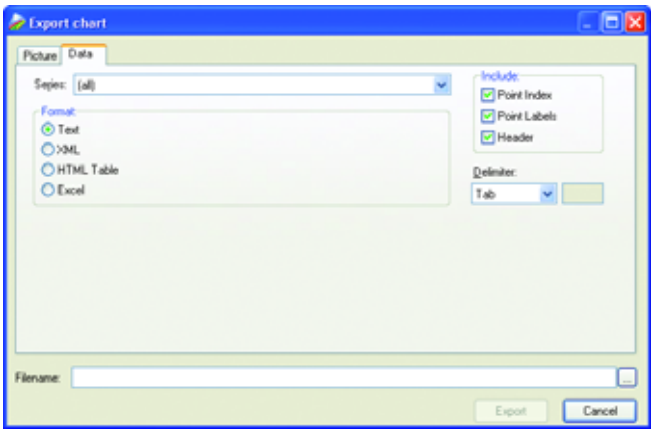



| | |
|---|---|
| 3 | On the *Picture* tab, under *Format*, select the graphic format in which you want to export the chart. |
| 4 | If an *Options* tab is displayed (on the right), select conversion options as needed. (The tab is not displayed for all graphic formats. If the tab is displayed, the options 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 *Save As* 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. |

D

## 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 *Save As* 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*.

### 2.2.2 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.

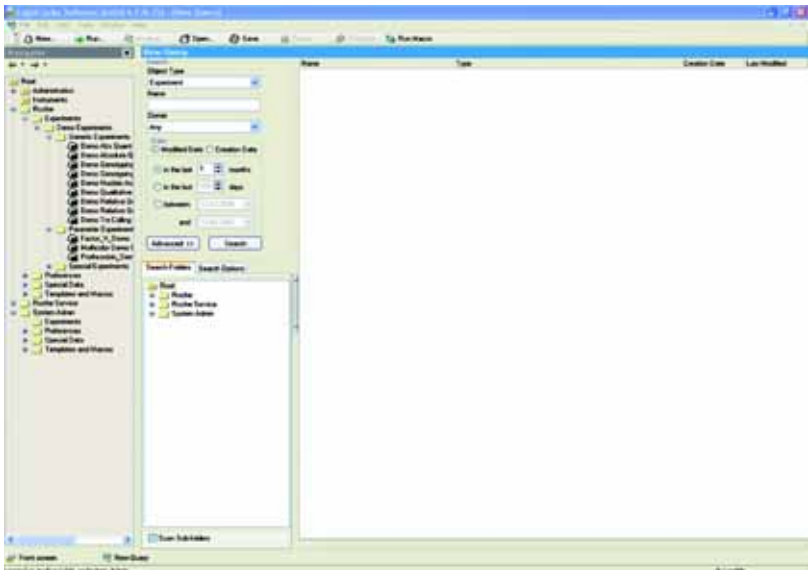
*You may be able to configure a 2-button mouse so that clicking both buttons at the same time equals the functionality of a middle button. See your system administrator or refer to the device driver instructions that came with your mouse.*

## 2.3 Using Queries to Find Information

The LightCycler® Software 4.1 includes a query tool you can use to retrieve experiments and other objects stored in the LightCycler® Software 4.1 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 4.1 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. 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 you 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

   *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. |

### 2.3.1 Updating the Database

Occasionally, the LightCycler® Software 4.1 database may need to be updated, for example if a LightCycler® Software 4.1 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, contact 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. |

## 2.4 Using Diagnostic Tools

The LightCycler® Software 4.1 includes the following diagnostic tools used to monitor the LightCycler® 2.0 Instrument's performance:

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

### 2.4.1 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® 2.0 Instrument runs. Always insert an empty carousel when performing an instrument self test.*

### 2.4.2 Viewing the Instrument Logs

The LightCycler® Software 4.1 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. The logs contain information that may be needed by support representatives if there is an instrument problem. In case of an instrument problem, it may be necessary to export a System Query Data object including both the Error Log and Operation Log, to forward to your support representative.

**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.

**To export a System Data Query object:**

1 In LightCycler® Software 4.1 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.


### 2.4.3 Viewing Application Errors

Error messages generated by the LightCycler® Software 4.1 are displayed in a message pane at the bottom of the LightCycler® Software 4.1 window. You can choose to display or hide the message 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.

# 3. Using Roche Macros

This chapter explains how to install and execute a Roche Macro, and how to add and delete macro buttons from the front window.

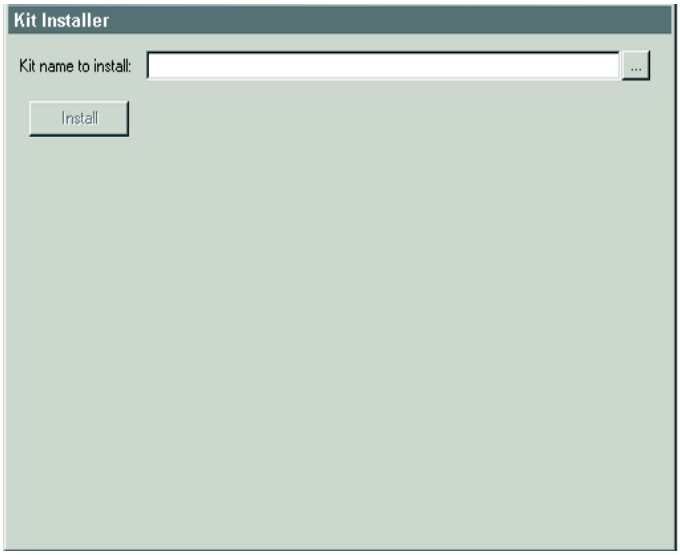
## 3.1 Installing and executing a Roche Macro

A Roche Macro is an experiment kit including run protocol, analysis modules and report. It includes templates for all the components of the experiment and a program called a wizard used to run the Roche Macro. When you execute a Roche Macro, the wizard guides you through the process of choosing an instrument, and then applies the experiment protocol, starts the experiment run, and after the run is finished, adds the analysis modules to the experiment.

To execute a Roche Macro you can use the Roche Macro button on the front screen. You can also link the Roche Macro with a macro button in the Macro menu on the LightCycler® Software 4.1 front screen. Clicking the button executes the Roche Macro.

### 3.1.1 Installing a Roche Macro

1. Assure to be logged on to the *Traceable* database. For details refer to *To log on to a local computer* in chapter *Changing the Default Administrator Password and Creating User Accounts*.
2. From the *Tools* menu select *Install Kit*.
3. The Kit Installer window opens.




4. Enter the path of the *.lckit or click ..., and then navigate to the location on your computer. Click *Install*.
5. The Roche Macro is automatically installed in the *Roche/Templates and Macros* folder.
6. After installation is finished, please assure to close the LightCycler® Software 4.1, remove the Roche Macro installation CD-ROM and restart the LightCycler® Software 4.1.

### 3.1.2 Executing a Roche Macro

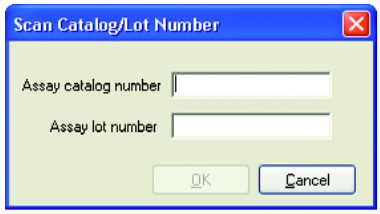
When you execute a Roche Macro, the LightCycler® Software 4.1 automatically launches a wizard to guide you through the process of using the Roche Macro to run the experiment. As you run the Roche Macro, you can edit the sample information.

**To execute a Roche Macro:**

1. Assure to be logged on to the *Traceable* database. For details refer to *To log on to a local computer* in chapter *Changing the Default Administrator Password and Creating User Accounts*.

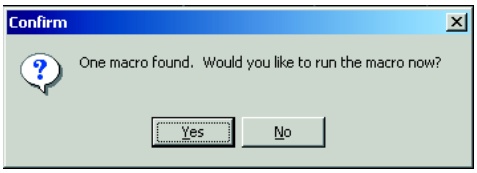
2. Click *Roche Macro* on the Front Screen.

3. The *Scan Catalog/Lot Number* window appears. Enter the Assay catalog number of the kit you use and enter the corresponding Assay lot number. You can also scan the catalog and lot number from the kit (printed on the back side of the respective package insert) with the barcode reader. Click *OK*.



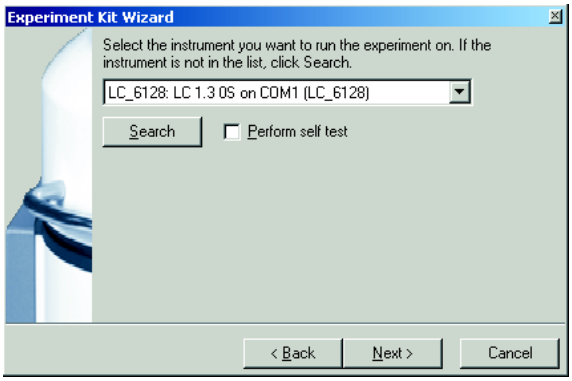

*In case the software finds more than one macro matching your entry, a list of the available macros is displayed. Select the appropriate macro from this list.*

4. The software confirms the availability of the macro. Click *Yes* to continue.

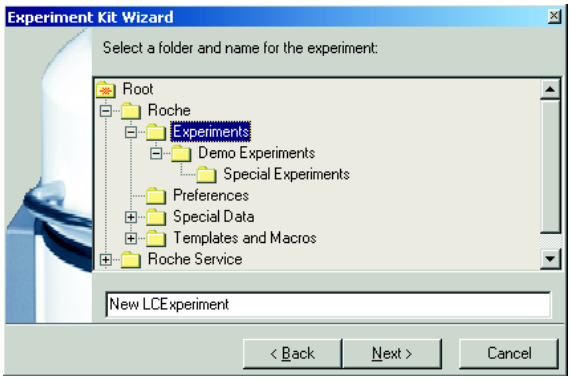



5. The Experiment Kit Wizard opens. Click *Next*.

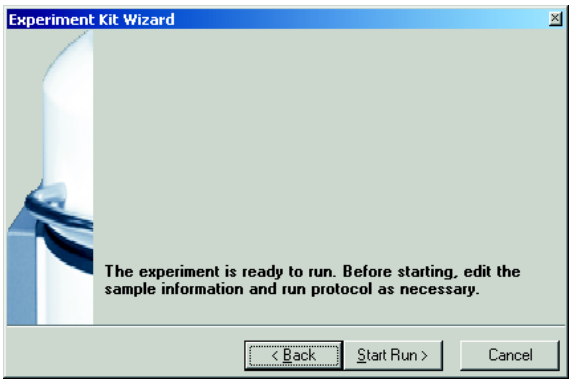
**6** The wizard prompts you to select an instrument. Check the *Perform self test* box if you want to perform an instrument self test before running the experiment. Click *Next*.




**7** Select a folder and enter a name for the experiment.




**8** The experiment is ready to run. Before starting the run, edit the sample information as necessary. Assure that the Windows screensaver function is turned off before starting a LightCycler® 2.0 Instrument run. Startup of the screensaver during a LightCycler® 2.0 Instrument run may lead to a loss of data. When finished, click *Start Run*.




*Do not operate the control unit while running a Roche Macro.*

9 After the run is finished, the report is displayed. You can switch to the analysis by clicking the corresponding icon in the taskbar.

LightCycler® Software Version 4.1

Report

Roche

**Demo Factor II**

**Experiment**

| | | | |
|---|---|---|---|
| Creation Date | 04.10.2004 13:20:19 | Last Modified Date | 07.11.2004 18:38:58 |
| Operator | System Admin | Owner | Roche |
| Start Time | 04.10.2004 13:26:54 | End Time | 04.10.2004 14:14:07 |
| Run State | Completed | Software Version | LCS4 4.0.5.314 |
| Macro | | Macro Owner | |
| Templates | | | |
| Run Notes | | | |

**Programs**

| | | | |
|---|---|---|---|
| Program Name | Denaturation | | |
| Cycles | 1 | Analysis Mode | None |

| Target (°C) | Hold (hh:mm:ss) | Slope (°C/s) | Sec Target (°C) | Step size (°C) | Step Delay (cycles) | Acquisition Mode |
|---|---|---|---|---|---|---|
| 95 | 00:00:30 | 20 | 0 | 0 | 0 | None |

| | | | |
|---|---|---|---|
| Program Name | Amplification | | |
| Cycles | 47 | Analysis Mode | Quantification |

It is possible to stop a running LightCycler® 2.0 Instrument experiment by clicking *Abort Run*.

*This will lead to a loss of data of the aborted experiment.*

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

*Additional software must not be installed on the LightCycler® 2.0 control units, except for Roche software specifically approved for installation on LightCycler® 2.0 control units. Installation of additional software contains the risk to interfere with LightCycler® Software 4.1 and may affect result security. For installation of additional Roche software, please refer to the Operator's Manual of this software or to the package insert of the corresponding test.*

*If you make modifications to an experiment after you have started the run you will be prompted to enter the reason for your changes in the Change Log window. This also applies to modifications of the experiment since the last save action. Previously saved versions of the experiment then become a read-only version and can be viewed in the File menu under Revisions.*

### 3.1.3 Import a SAM file

You can import a MPLC SAM file into a new experiment, using the Import SAM button in the LightCycler® Software 4.1 Sample Editor of an opened new experiment.

**To import a SAM file:**

| | |
|---|---|
| 1 | To import a MagNA Pure LC file containing the sample information of a MagNA Pure nucleic acid isolation open the Sample Editor by clicking *Samples* in the module bar. |
| 2 | Click *Import SAM* in the toolbar of the workpane, find and select the SAM file, then click *Open* or read the barcode of your SAM file generated by your MagNA Pure LC instrument with a barcode reader.<br>*Importing a SAM file is only possible before a run has started.* |
| 3 | 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 all sample information to the default values and resets any analysis-specific sample editor tabs to their default values. |
| 4 | When finished, click *Save* in the global toolbar to save the sample information with the experiment. |

*Do not change the SAM file manually.*

### 3.1.4 Modifying a Roche Macro

The report page header includes the seal status of the experiment *i.e.*, once the user changes any of the Roche Macro settings, the report seal is broken and the following line is displayed above the experiment name: ***User Developed or Modified Test Method.***


LightCycler® Software Version 4.1

Report

Roche

**User Developed or Modified Test Method**

**Factor_V_Demo**

**Experiment**

| | | | |
|---|---|---|---|
| Creation Date | 09.12.2004 08:15:35 | Last Modified Date | 18.01.2005 14:15:09 |
| Operator | System Admin | Owner | Roche |
| Start Time | 09.12.2004 09:50:43 | End Time | 09.12.2004 10:28:53 |
| Run State | Completed | Software Version | LCS4 4.0.5.389 |
| Macro | Factor_V_04618777001_00000000 | Macro Owner | System Admin |
| Templates | | | |
| Run Notes | | | |

**Samples**

| | | | | | |
|---|---|---|---|---|---|
| Sample Count | 8 | LC Carousel ID | | MPLC Batch ID | |
| Assay Cat. No. | 04618777001 | Assay Lot No. | 12345678 | Color Comp ID | |

**Changes that cause the report seal to be broken:**

**Run programming**

- Default channel
- Seek temperature
- Instrument type
- Capillary size
- Add or delete a program
- Program name
- Number of cycles
- Analysis mode
- Add or delete a temperature target
- Target temperature
- Hold time
- Ramp rate
- Secondary target temperature
- Step size
- Step delay
- Acquisition mode
- Automatically selected color compensation object

**Sample editor-general:**

- Selected analysis types
- Selected channels

**Capillary View sample editor:**

- Subsequent change of assay catalog number
- Subsequent change of assay lot number
- Color compensation ID
- Replicate information

**Absolute Quantification sample editor:**

- Enable controls selection status
- Unit entry
- Target name
- Sample type
- Standard concentration
- Cp low and Cp high
- Concentration low and concentration high

**Relative Quantification – Monocolor sample editor:**

- Target name
- Sample type
- Standard concentration
- Cp low and Cp high

**Relative Quantification – Dual Color sample editor:**

- Target name
- Sample type
- Standard concentration
- Cp low and Cp high

**Qualitative Detection sample editor:**

- Enable controls selection status
- Target name
- Sample type
- Cp low and cp high

**Tm Calling sample editor:**

- Target name
- Selected Tm calibrators
- Expected Tm

**Genotyping sample editor:**

- Target name
- Sample type
- Genotype

**Nucleic Acid Quantification sample editor:**

- Target name
- Sample type
- Standard concentration

**Color compensation sample editor:**

- Dominant channel

**Analyses – general:**

- Add or delete an analysis
- Analysis name

**Absolute Quantification analysis:**

- Channel nominator
- Channel denominator
- Automatically selected color compensation object, including selected channels
- Program used
- Standard curve used
- Method
- For Fit Points method:
  - Selected background correction
  - Selected noise band method and value
  - Number of fit points
  - Minimize error selection
  - Threshold value

**Relative Quantification – Monocolor analysis:**

- Add or delete a group
- Group name
- Channel nominator
- Channel denominator
- Automatically selected color compensation object, including selected channels
- Program used
- Efficiency correction method and standard curve used
- Selected reference experiment
- Add results sets
- Correction factor
- Multiplication factor

**Qualitative Detection analysis:**

- Channel nominator
- Channel denominator
- Automatically selected color compensation object including selected channels
- Program used

**Manual Tm Calling analysis:**

- Baseline
- Sample type
- Channel
- Run program
- Color compensation
- Changing to automated Tm calling analysis

**Automated Tm Calling analysis:**

- Channel nominator
- Channel denominator
- Automatically selected color compensation object including selected channels
- Program used
- Calculation mode (automatic, manual)
- For automated calculation:
  - Sensitivity mode
  - Maximum number of peaks
  - Show shoulders
  - Add or delete a melting peak
  - Change of automatically calculated melting temperature
- For manual determination:
  - Sample-specific background values

**Genotyping analysis:**

- Channel nominator
- Channel denominator
- Automatically selected color compensation object including selected channels
- Program used
- Grouping mode and used standard melting curve object
- Sensitivity mode
- Group name
- Score threshold
- Resolution threshold
- Minimum and maximum temperature

**Nucleic Acid Quantification analysis:**

- Channel nominator
- Channel denominator
- Program used

### Changes that do not break the report seal:

- Instrument used
- Run notes
- Maximum seek position/sample count
- LC carousel ID
- MPLC batch ID
- Assay lot number, if empty in the Roche Macro
- Sample name
- Sample notes
- Analysis notes
- Relative Quantification result set name
- Number of manually determined melting peaks
- Sample specific background values for manual peak height determination if not defined in the macro
- All display settings, such as:
  - Absolute Quantification, Fit Points method: show fit points selection
  - Relative Quantification – Monocolor: chart selection in pairing tab
  - Relative Quantification – Dual Color: chart selections in target/reference and pairing tab
  - Qualitative Detection: advanced result display
  - Tm Calling: Manual Tm display; result table columns
  - Color compensation: channel
- Manual Tm Calling analysis:
  - Tm values
  - Number of Tm values

## 3.2 Adding and Deleting Macro Buttons on the Front Window

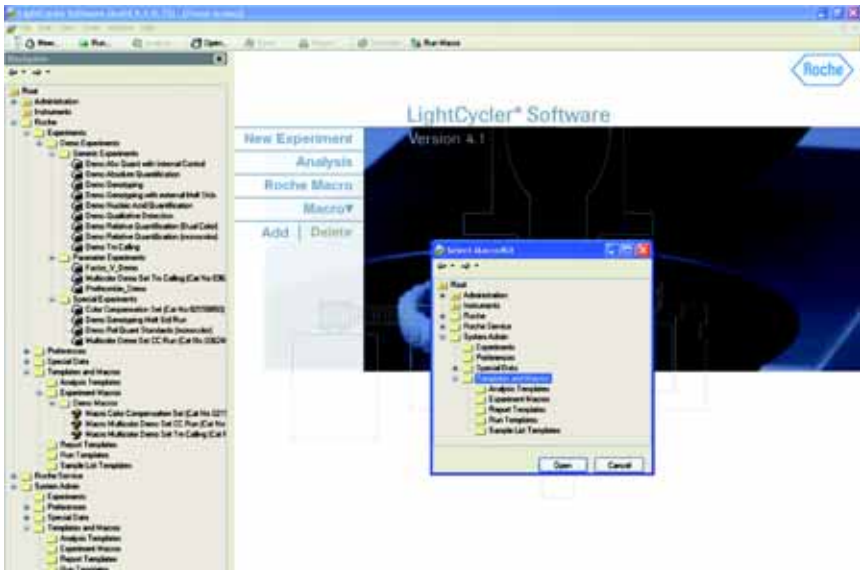
If you have installed Roche Macros, you can link each Roche Macro to a macro button on the LightCycler® Software 4.1 Front window. Clicking the button runs the experiment kit wizard.

Then follow the instructions below to assign the Roche 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 4.1 Front window is not currently displayed, click *Front* 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 4.1 *Front* window, click the arrow next to *Macro* and click *Delete*.<br>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.<br>*Only the macro button is deleted from the window, not the macro itself.* |

# 4. Managing User Access

To use the LightCycler® Software 4.1, you must have a user account in the LightCycler® Software 4.1 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 4.1 User Management tool. The chapter also explains how to change a user password.

Read this chapter if you are responsible for creating or modifying user accounts or if you want to understand the privileges associated with your account. Read the section on passwords if you need to change your password.

Your own user account must have the Local Administrator role to use the User Management tool.

## 4.1 Understanding User Accounts

A user account provides access to the LightCycler® Software 4.1. 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 4.1 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 4.1 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.

A user account once created cannot be edited or deleted, it can however be made inactive. An inactive user account cannot have a role and cannot be assigned to a group.

## 4.2 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, or move Bob's experiments. See the next section for more information about roles.

## 4.3 Understanding Roles

Each user account is assigned to 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.

### 4.3.1 Privileges of the Standard User Role

A Standard User has limited access to the LightCycler® Software 4.1. A Standard User can do the following:

- Install Roche macros to execute and analyze experiments.
- 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 and copy objects and execute macros owned by a Local Administrator or the Roche User.
- Open and 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.
- 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).
- Copy or rename any experiment object (including the user's own objects).
- Delete any object (including the user's own objects).

### 4.3.2 Privileges of the Expert User Role

An Expert User can do the following:

- Install Roche Macros to execute and analyze experiments.
- 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 (only possible if the folder does not contain any objects).
- Rename the user's own nondefault folders and objects.
- 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 objects 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 other Expert Users.
- See the folders or objects belonging to Standard and Expert Users who are not in the same group as the user.
- Copy, delete or rename experiment objects (including the user's own experiment objects).
- Delete, move, copy, or rename default folders (including the user's own folders).

### 4.3.3 Privileges of the Local Administrator Role

A Local Administrator can do the following:

- Use the Run programming module to create and execute experiments. Please note that this function is not part of the diagnostic workflow.
- Install Roche Macros to execute and analyze experiments.
- 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, execute and copy objects belonging to other Local Administrators.
- Open, execute, and delete objects 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.
- Modify, move or delete objects owned by other Local Administrators; for example one administrator cannot copy objects into another administrator's folder.
- Copy, delete or rename experiment objects (including the user's own experiment objects).
- Delete, move, copy, or rename default folders (including the user's own folders).



### 4.3.4 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's default folders.

## 4.4 Managing Users, Groups, and Roles

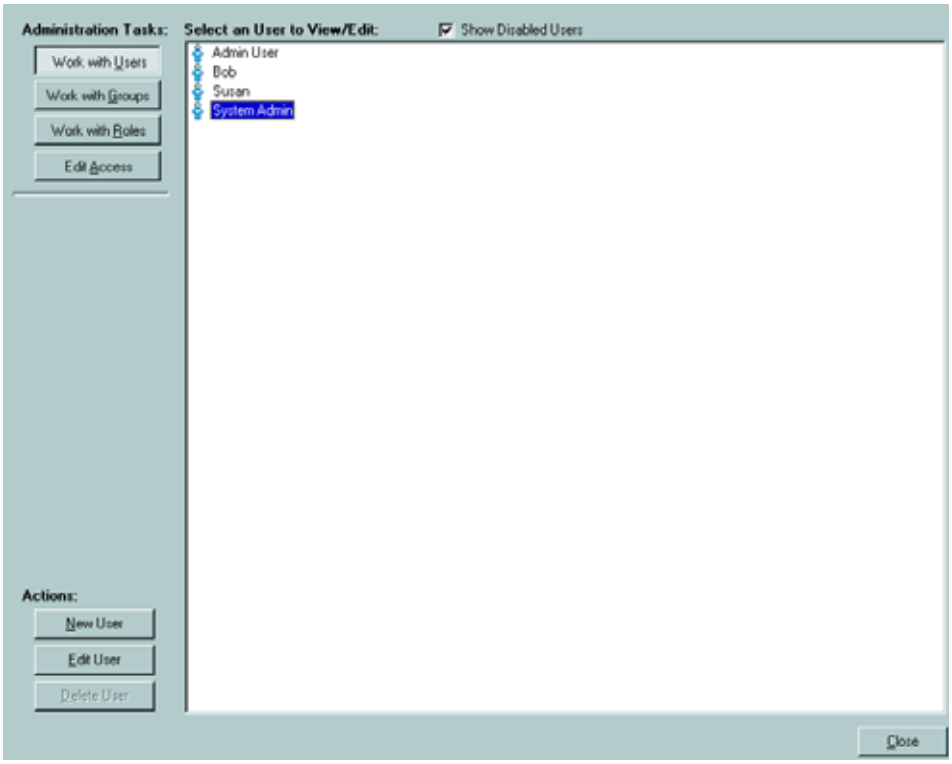
The User Management tool allows you to

- Create, modify, enable, or disable user accounts.
- Assign roles to user accounts (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.

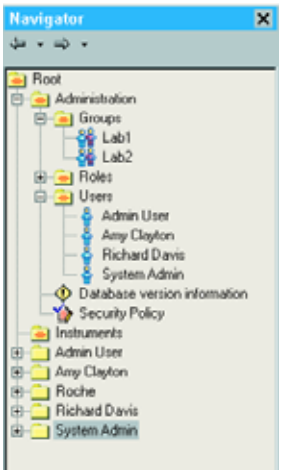


### 4.4.1 Working with Users

**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:

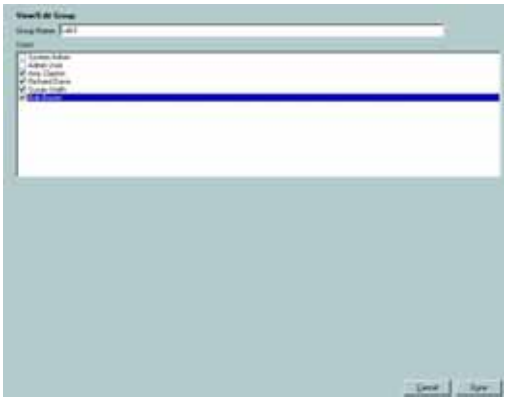
| | |
|---|---|
| 1 | In the *User Management Tool*, click *Work with Users* (if not already selected). |
| 2 | Select the user's name in the right pane, then click *Edit User*. |
| 3 | The only information you can change is the password and the belonging to a certain group. |
| 4 | To disable the user 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*. |

*You cannot delete a user account.*

### 4.4.2 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:**

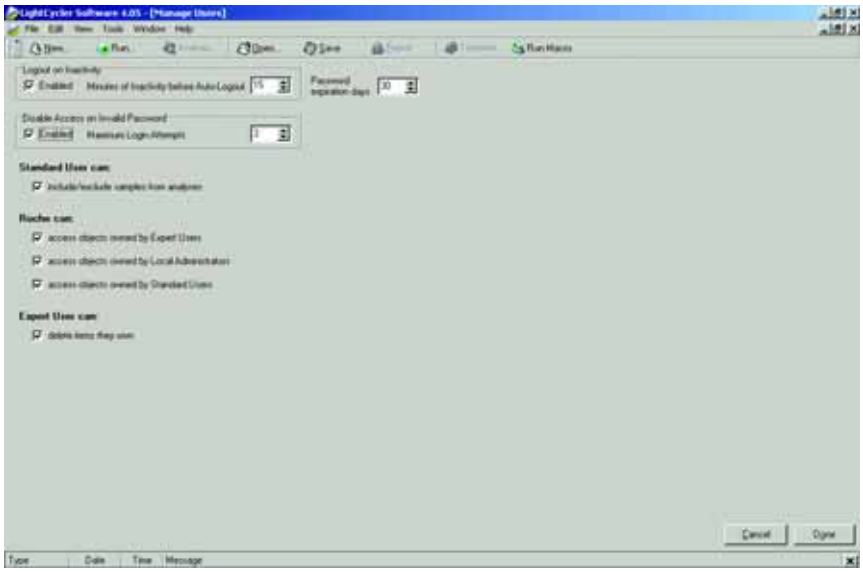
1. In the *User Management Tool*, click *Work with Groups*.
2. Select the group name in the right pane.
3. Click *Delete Group*. You are prompted to confirm the deletion.
4. Click *Yes* to delete the group.

### 4.4.3 Working with Roles

You cannot create or delete roles, and you cannot change a user's role assignment. You can modify certain access rights associated with the 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 an access is disabled on entering an invalid password.

**To determine conditions for access and modify a role's access rights:**

1. In the *User Management Tool*, click *Edit Access*. The access window displays the available options.



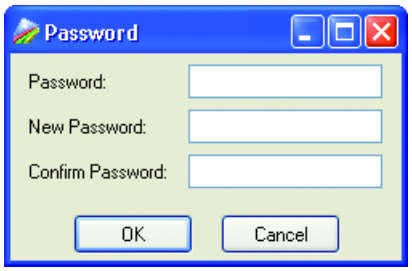
2. Select or deselect the available options.
3. When finished, click *Done*.

## 4.5 Changing your Password

When you are assigned an account on the LightCycler® Software 4.1 system, you receive an initial password you can use to log on to the LightCycler® Software 4.1 the first time (you will be prompted to change your initial password upon your first login). You can also change your password whenever you want.

**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.

# 5. Installation of LightCycler® Software 4.1

The complete LightCycler® software version 4.1 includes the LightCycler® Software 4.1 application, a database, and an object server. If you have received a license to install the LightCycler® Software 4.1 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 4.1. It includes the following topics:

- Overview of software installation
- Hardware and software requirements
- Installing LightCycler® Software 4.1
- Saving an existing database and installing additional databases
- Removing LightCycler® Software 4.1
- Changing the default administrator password and creating user accounts

## 5.1 Overview of Software Installation

LightCycler® Software 4.1 controls the LightCycler® 2.0 Instrument using information you provide in an experiment protocol. The LightCycler® Software 4.1 includes the LightCycler® Software 4.1 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.

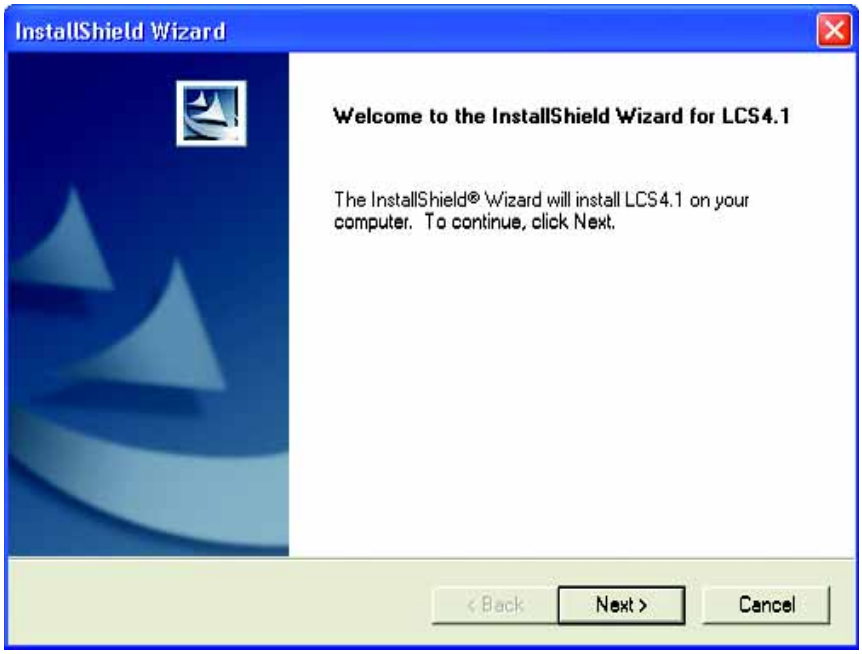
In this configuration all the software components are installed on the LightCycler® computer connected to the LightCycler® 2.0 Instrument. Each instrument and computer function together as an independent system with its own database and its own set of user accounts. Computers not connected to an instrument, which can be used for the LightCycler® Software 4.1 application tools but not for running an experiment, are independent systems as well.

## 5.2 Hardware and Software Requirements

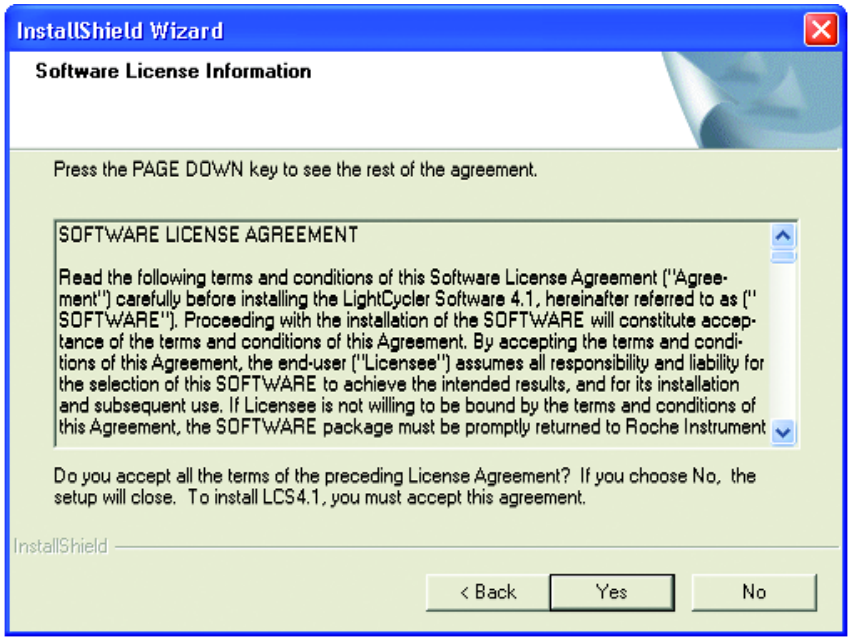
For hard- and software requirements of the computer used to connect to the LightCycler® 2.0 Instrument or to launch the LightCycler® Software 4.1 for data analysis, please refer to chapter B *System Description*.

## 5.3 Installing LightCycler® Software 4.1

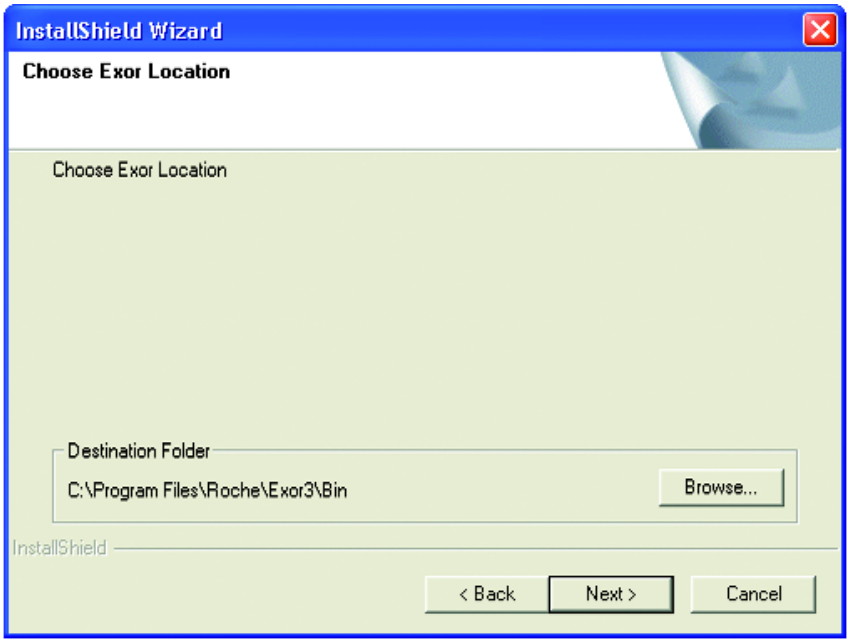
1. Insert the LightCycler® Software 4.1 CD. Double-click LightCycler_Software_41_Setup.exe, if installation doesn't start automatically. The installation process transfers files, extracts the files, and then prepares the installation wizard. The InstallShield Wizard opens. Click *Next.*



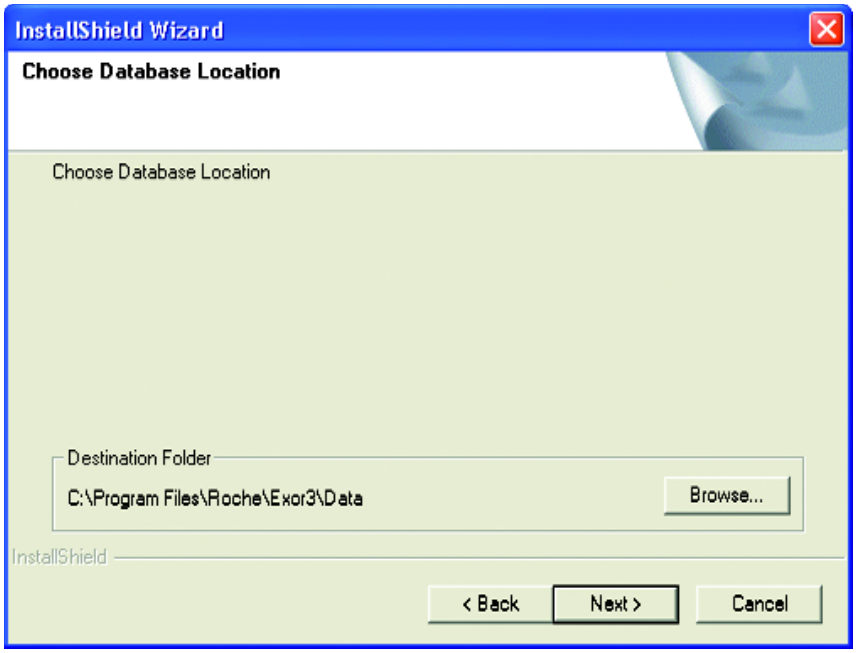

2. You are prompted to agree to the license conditions. Click *Yes.*



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*.

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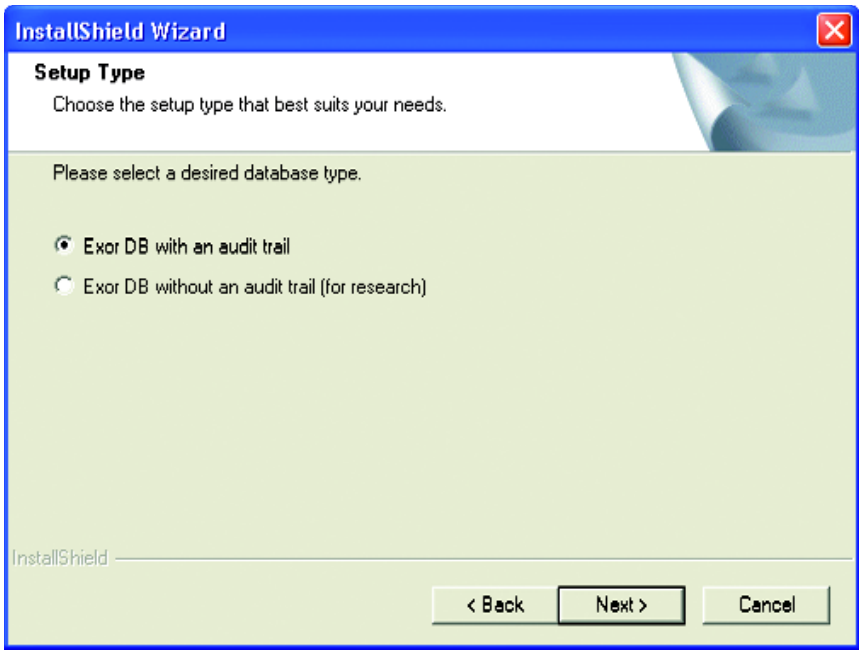
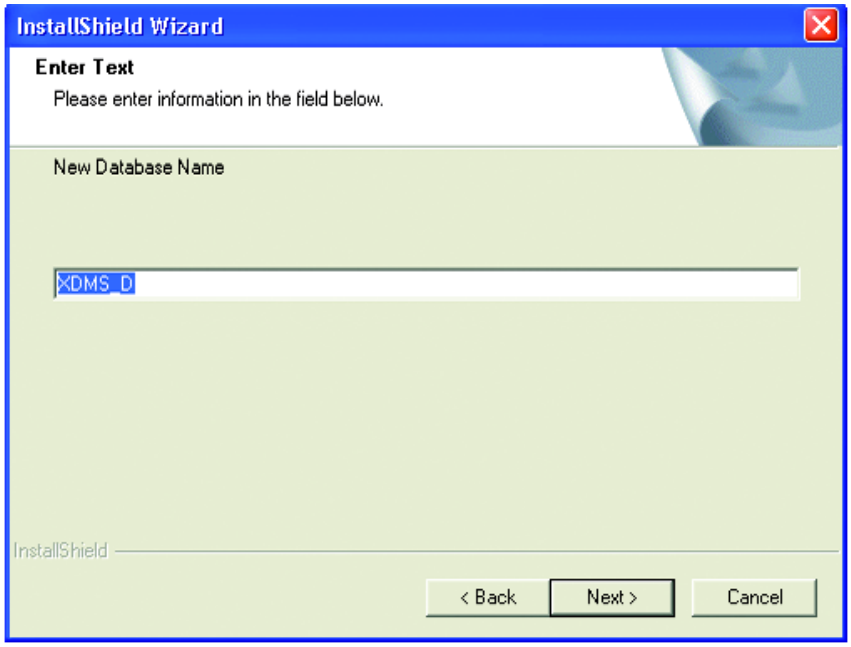
5 In the Setup Type window select *Exor DB with an audit trail*. This creates a database that generates an audit trail of modified experiments and provides other database controls. Click *Next*.

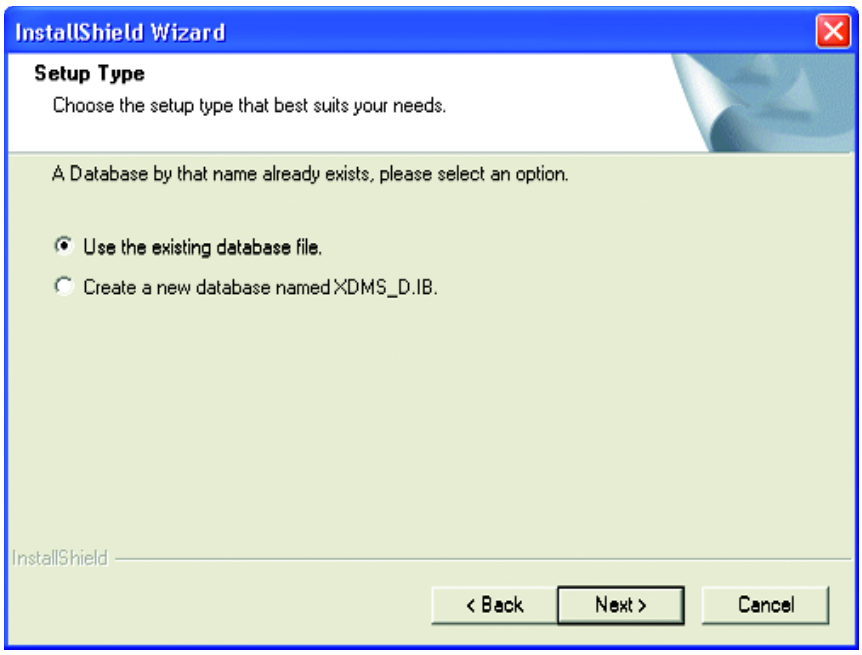



*An audit trail is a secure, computer-generated time-stamp which independently records the date, time and names of operator entries and actions that create, modify or delete electronic records. Record changes shall not obscure previously recorded information.*

6 The software prompts you to name the new database. Enter a database name or leave the default, then click *Next*.

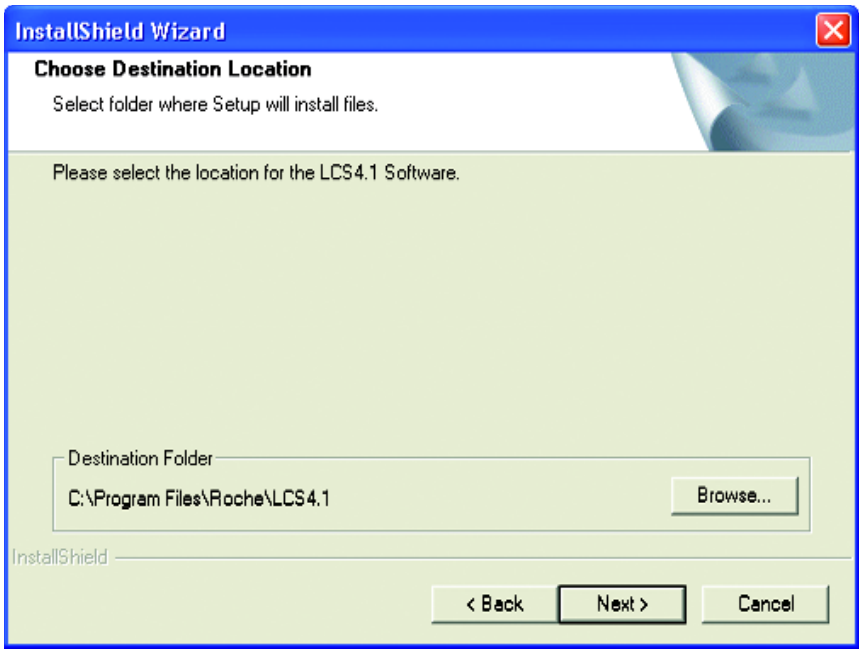


7 If a database with the same name already exists, you are prompted to use the existing database or create a new database with the same name. Click *Next*.

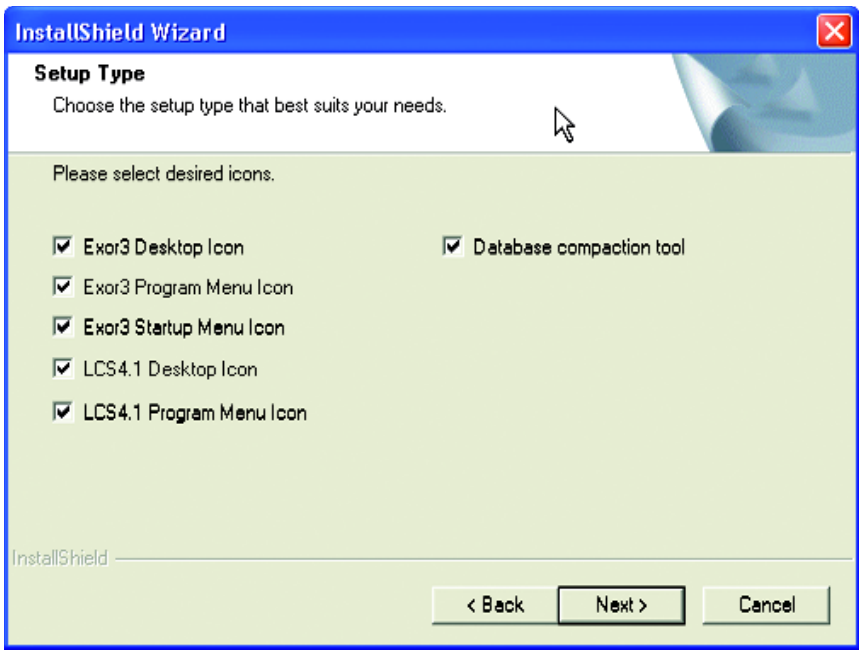



*When you select Create a new database with the same name, the software automatically stores your old database with a time stamp in the database directory.*

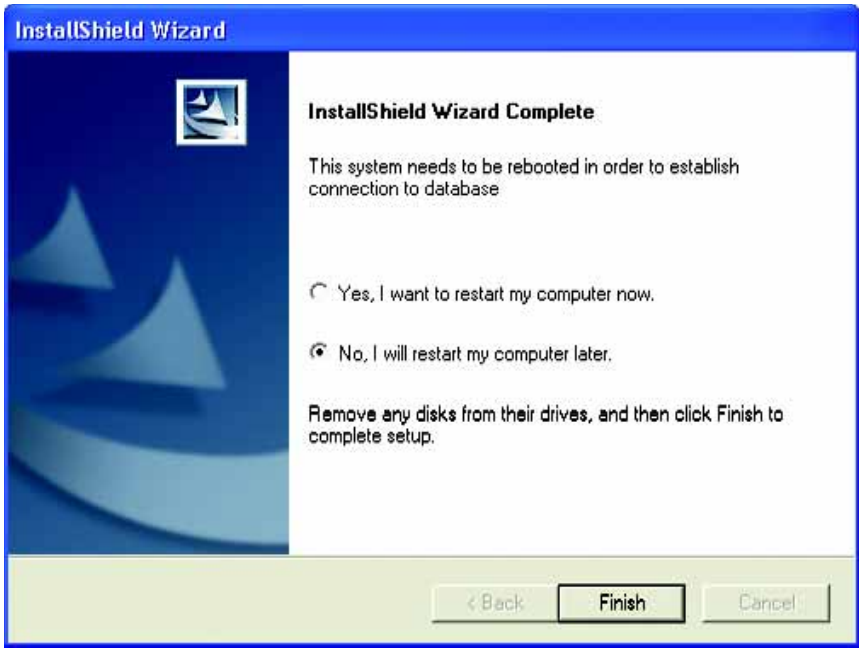
8 Select the default settings to install the LightCycler® Software 4.1 or browse to select a location. Click *Next*.



9 Select the location for program icons. These are locations to start the LightCycler® Software 4.1 from. Deselect the icon locations you do not want, and then click *Next*.




10 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 4.1 icon.




The installation process installs the following icons on the desktop:

LightCycler® Software 4.1 icon , Exor 3 icon

### 5.3.1 Saving an existing database and installing additional databases

For backup purposes it is recommended to save a copy of your database routinely. Limited by the capacity of a CD it is recommended to save your database at a size of maximally 700 MB. 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 4.1*, 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. |

In case 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 | In the Start menu select Programs/Roche/CompactIB |
| 2 | 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*. |
| 3 | Click on *Compact* to start the process. |

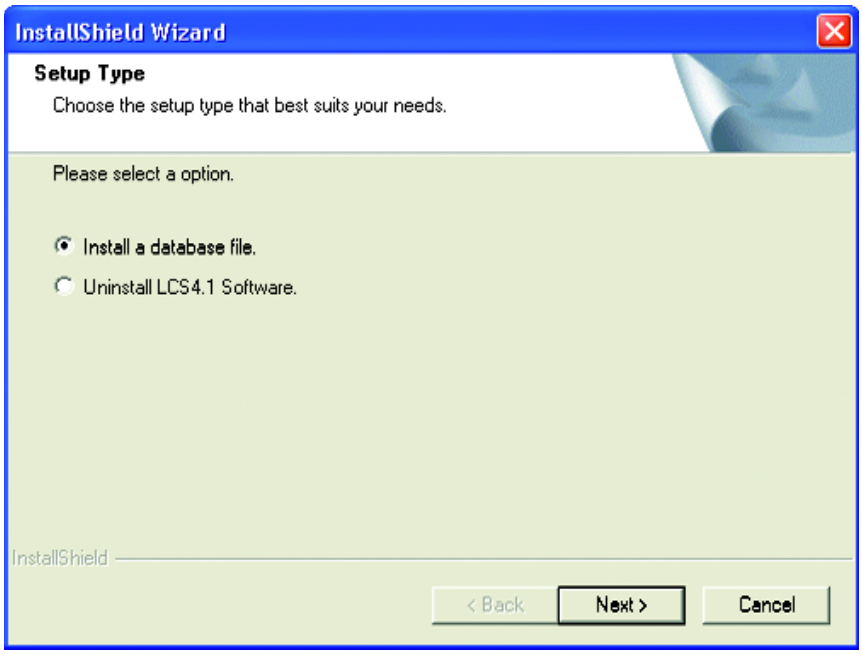
To save the database on a CD follow the procedure described in *Using the CD-RW Drive*.

### To install additional databases:

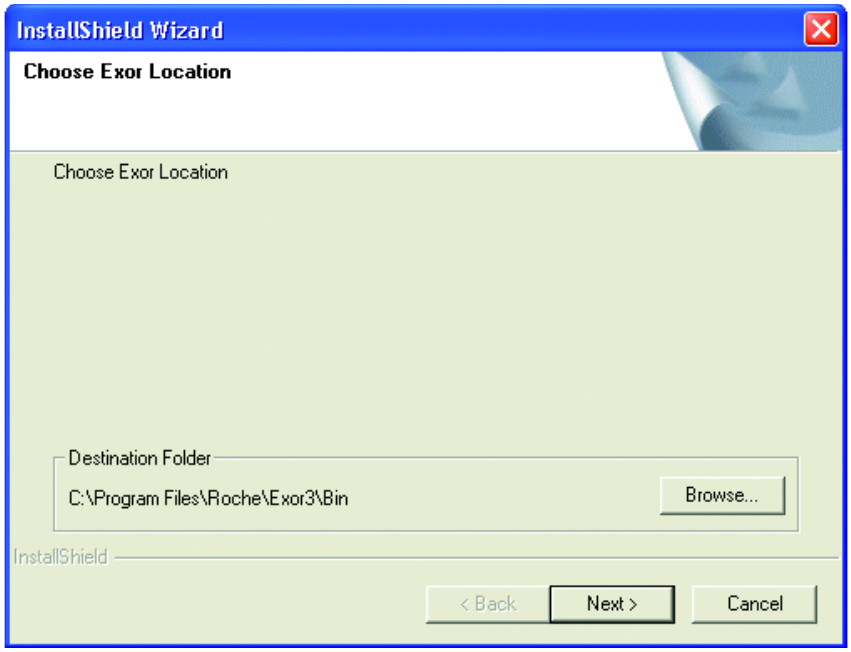
If the LightCycler® Software 4.1 is already installed on your computer, you can use the LightCycler® Software 4.1 installation utility to install additional databases:

1. Insert the LightCycler® Software 4.1 CD. Double-click LightCycler_Software_41_Setup.exe, if installation doesn't start automatically.

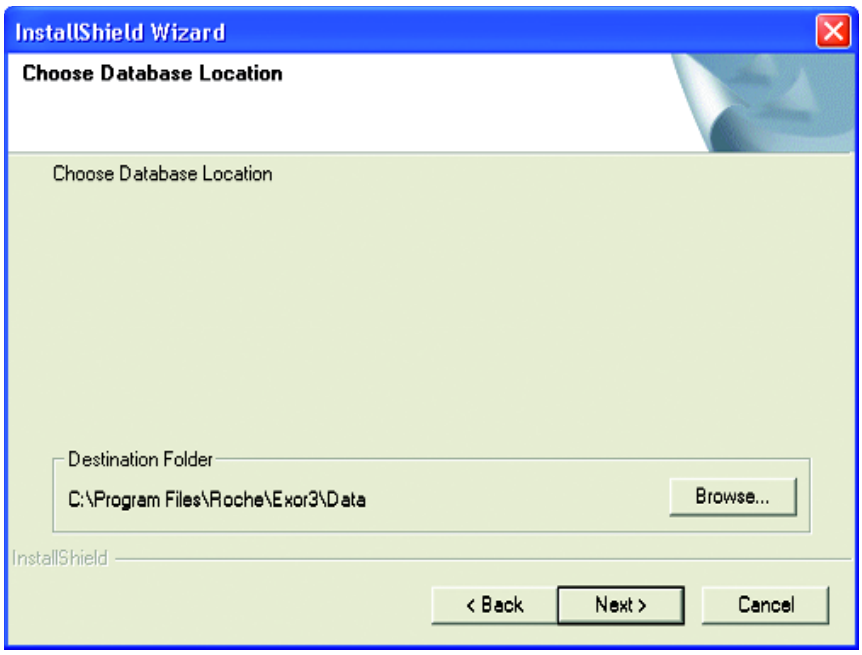
2. The Setup Type window is displayed. Leave the default setting *Install a database file*, and then click *Next*.



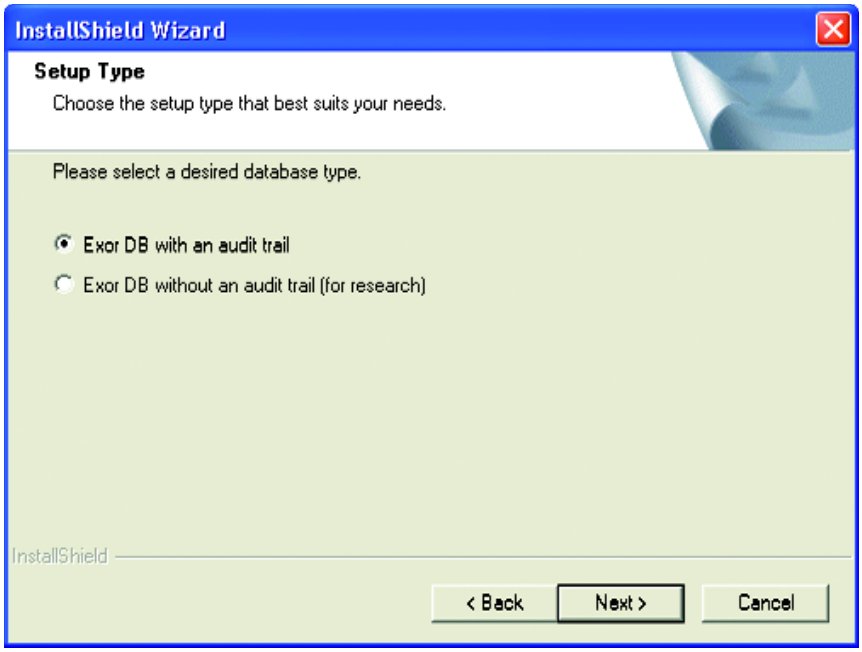

3. Select the default settings to install the database engine or browse to select a location. Click *Next*.



4 Select the default settings to install the database file or browse to select a location. Click *Next*.

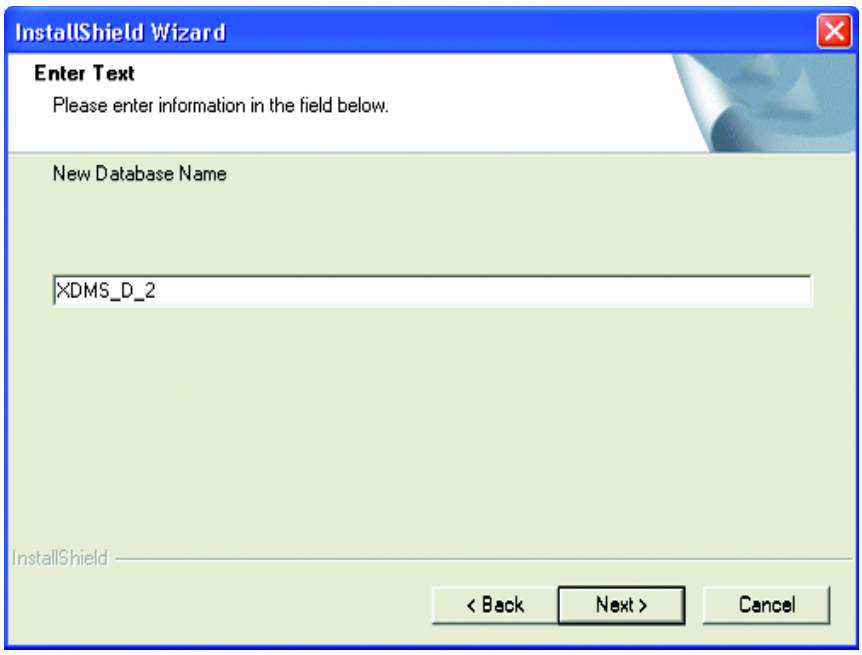



5 In the Setup Type window select *Exor DB with an audit trail*. This creates a database that generates an audit trail of modified experiments and provides other database controls. Click *Next*.

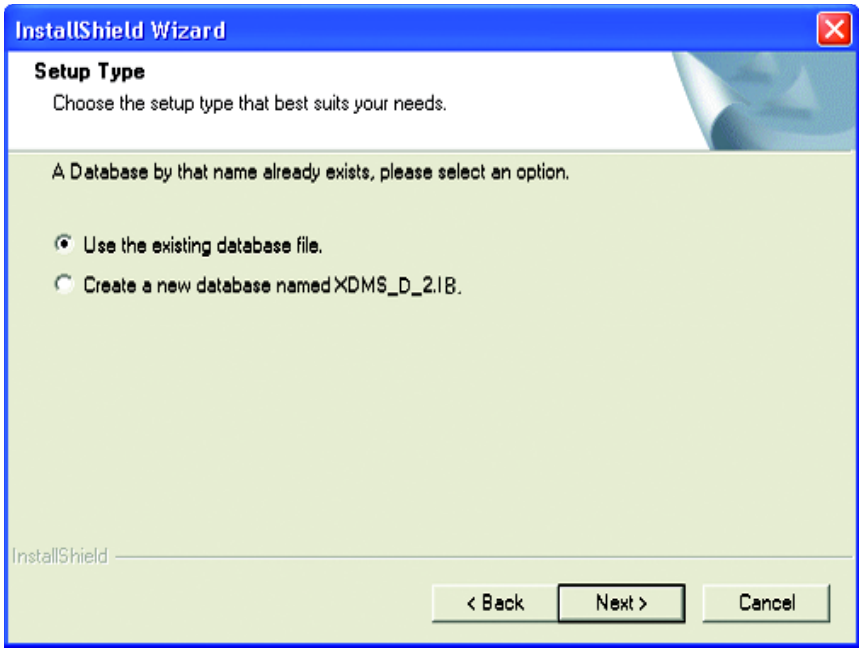



*An audit trail is a secure, computer-generated time-stamp which independently records the date, time and names of operator entries and actions that create, modify or delete electronic records. Record changes shall not obscure previously recorded information.*

6 The software prompts you to name the new database. Enter a database name or leave the default, then click *Next*.

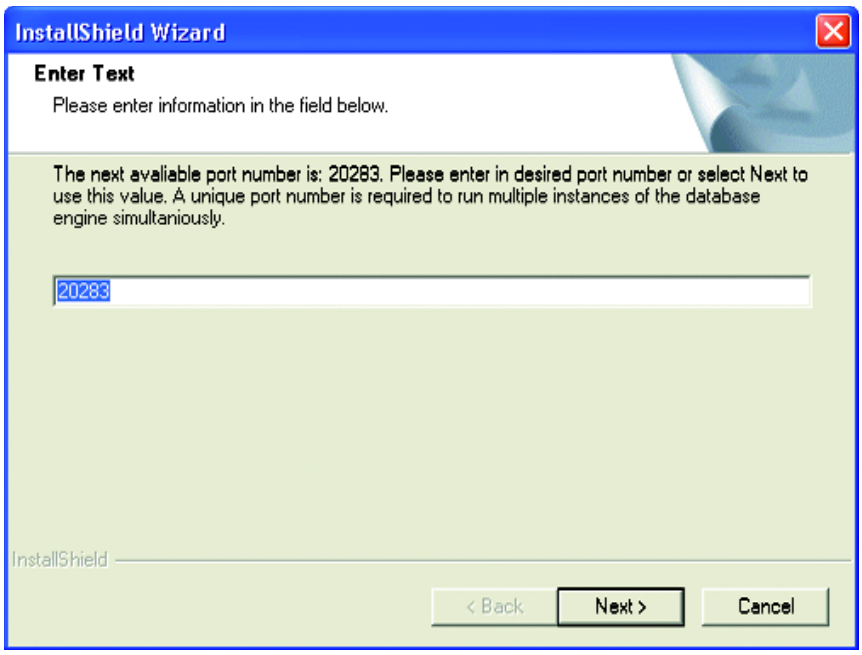



7 If a database with the same name already exists, you are prompted to use the existing database or create a new database with the same name. Click *Next*.

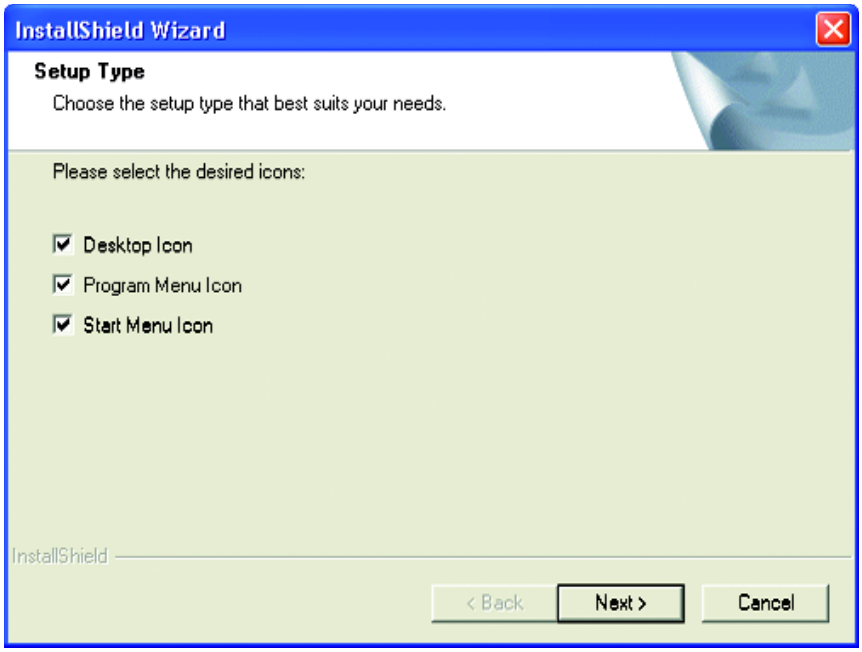



*When you select Create a new database with the same name, the software automatically stores your old database with a time stamp in the database directory.*

8 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*.




9 Select the location for program icons. Deselect the icon locations you do not want, and then click *Next*.

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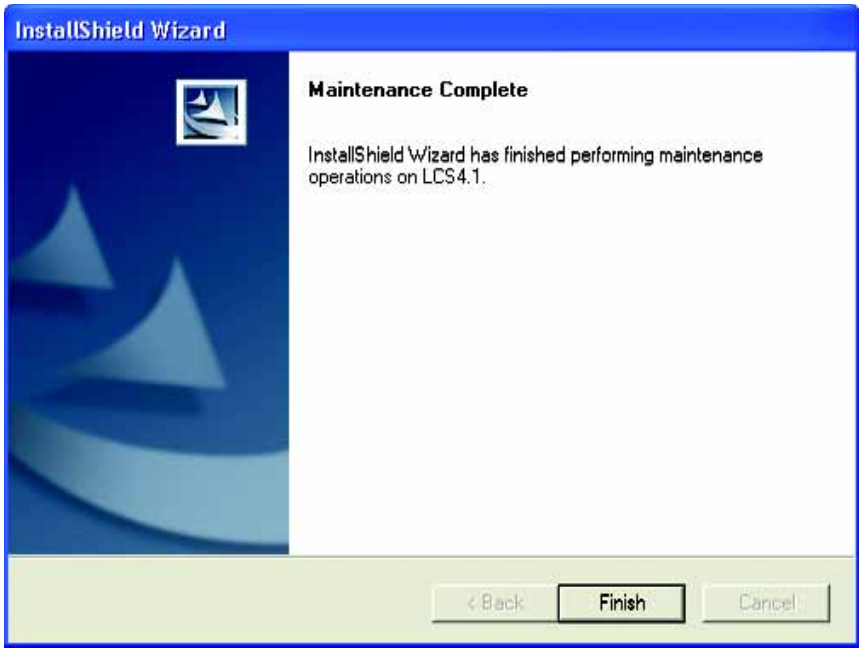
10 A message states that the maintenance is complete. Click *Finish*.




The installation process installs another Exor3 icon on your desktop: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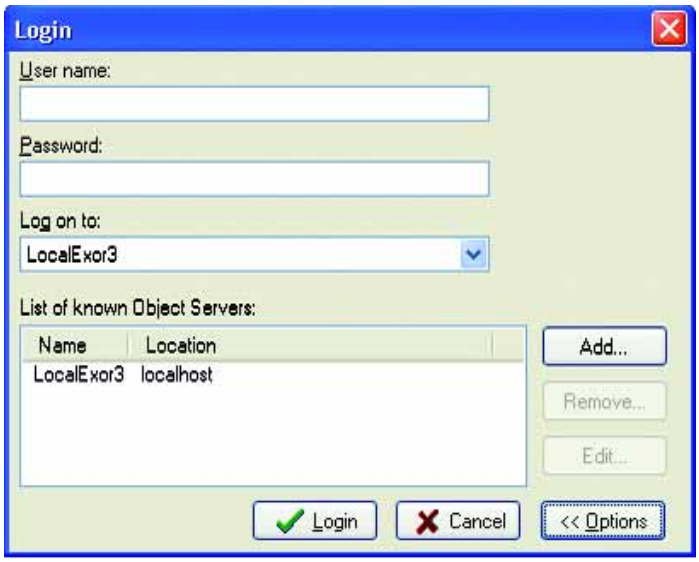
### 5.3.2 Logging on to different databases

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:**

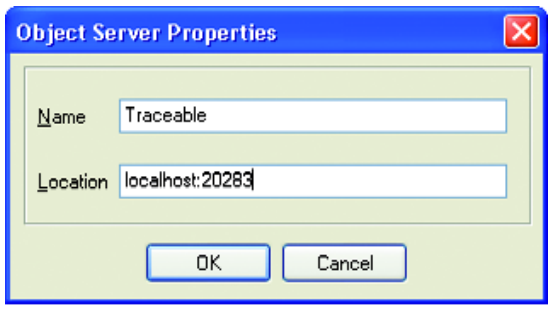
1. Double-click the LightCycler® Software 4.1 icon to launch the LightCycler® Software 4.1.

2. A login dialog box opens. Click on *Options* to display the list of known object servers.

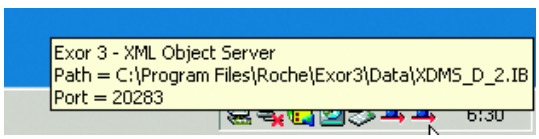



3. Click on *Add*. An *Object Server Properties* window opens.

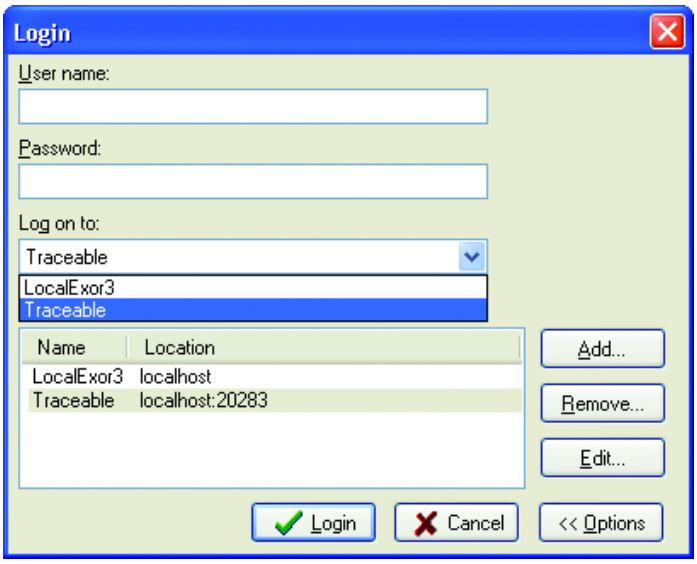
4. Enter a name for the database and its location. The location is always composed of the word “localhost” and the port number of the database to be integrated, divided by a colon. Click *OK*.




*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.

D

## 5.4 Setting up a Client/Server Network

LightCycler® Software 4.1 provides network functionality. This allows you to connect the application to a LightCycler® Software 4.1 database on a remote computer. It is even possible to host several connections from different users to a single remote database. Accordingly, a LightCycler® 2.0 Instrument client/server network can be set up that connects up to 5 LightCycler® 2.0 Instrument control units and data workstations (*i.e.*, PCs not connected to a LightCycler® 2.0 Instrument but having LightCycler® Software 4.1 installed for data analysis) to one LightCycler® 2.0 Instrument database server.

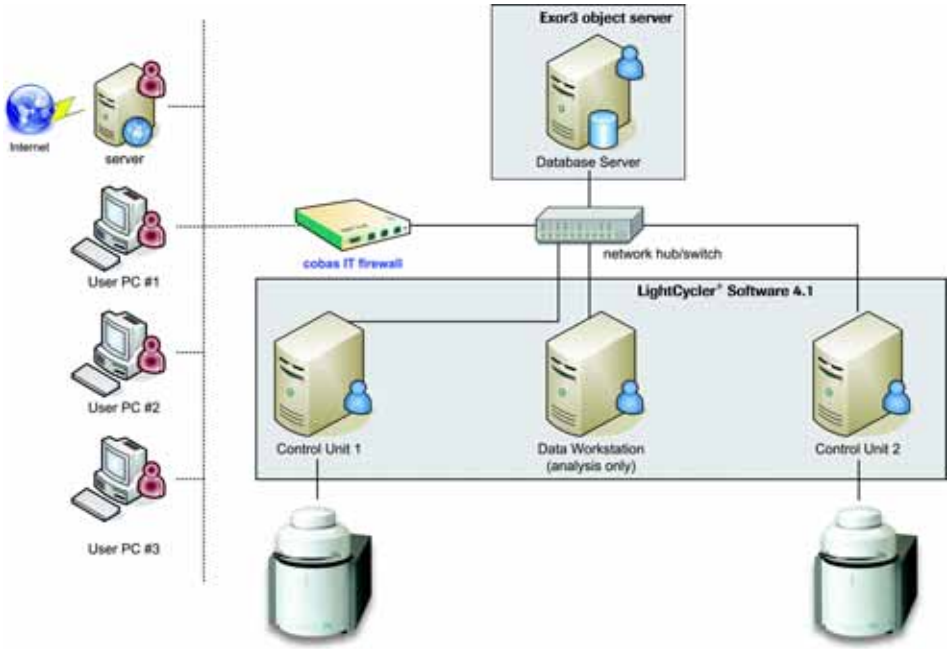
*You can also set-up a network of LightCycler® 2.0 Instrument applications without installing a shared remote database server simply to enable easy data exchange between several LightCycler® 2.0 Instrument control units and data workstations.*

*Before connecting LightCycler® 2.0 Instrument control units and data workstations to a remote database server via a network that is also connected to an unprotected, foreign network, you should carefully read and understand the disclaimer on connection of a LightCycler® 2.0 Instrument to a network under 'XII. General Precautions' on page 17.*

The following gives an overview of the principle options for setting up such a network solution. Note that also combinations of these sample configurations are possible.

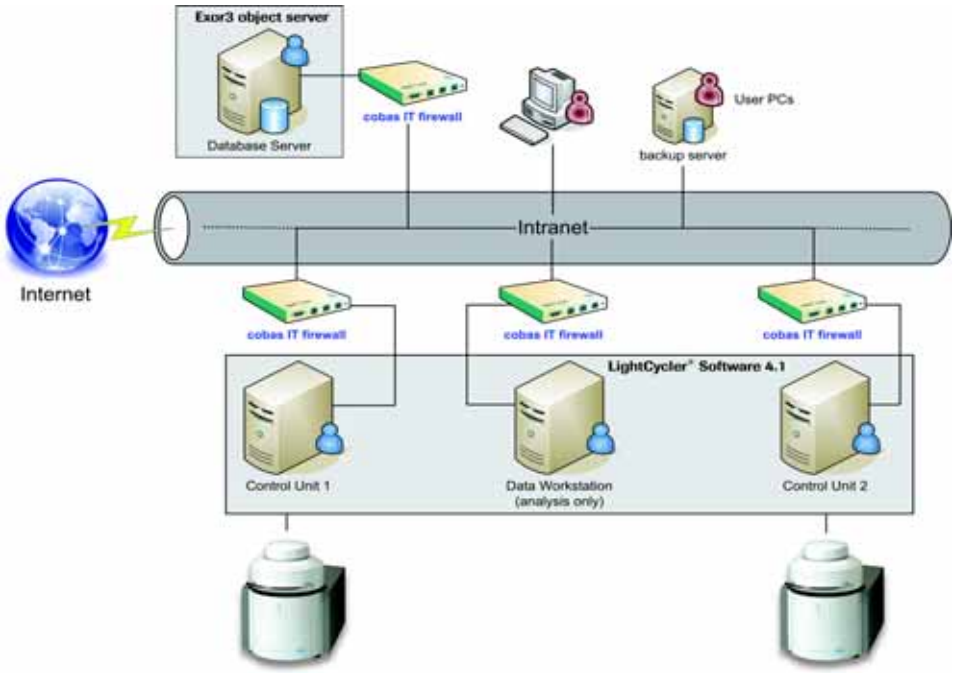
*The symbol denotes a Roche computer system, while the symbol denotes a non-Roche computer system provided by the user.*

1. A sub-network consisting of LightCycler® 2.0 Instrument applications on Roche computer systems only is connected to a foreign, unprotected network (*e.g.*, a laboratory network or Intranet). The LightCycler® 2.0 Instrument sub-network may consist of any combination of LightCycler® 2.0 Instruments with their control units, data workstations, and/or a database server. To secure the LightCycler® 2.0 Instrument sub-network from any potential threats (*e.g.*, viruses or network-borne attacks), network traffic to and from the LightCycler® 2.0 Instrument sub-network must be controlled by the cobas IT firewall.

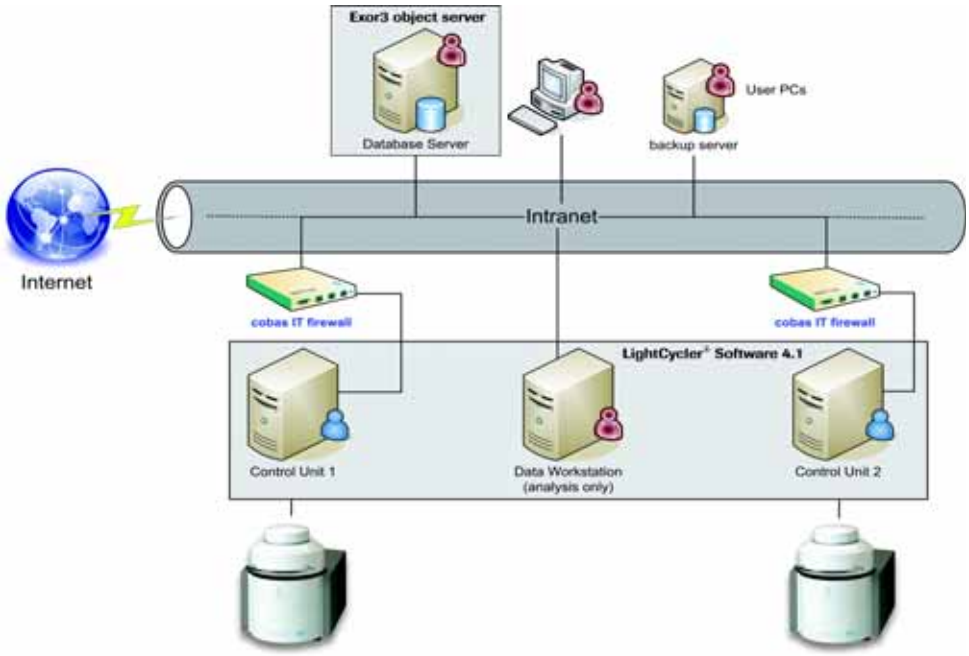


*The LightCycler® 2.0 Instrument sub-network may also be run as an isolated, local network with no connection to a foreign network. No router to another network or Internet connection needs to be installed in this case.*

2. All LightCycler® 2.0 Instrument applications (LightCycler® Software 4.1 on control units and data workstations, and Exor3 Object Server software on the remote database server) are installed on Roche computer systems, connected directly to a foreign, unprotected network (*e.g.*, a laboratory network or Intranet). In this case, each LightCycler® 2.0 Instrument application must be secured individually by a cobas IT firewall. Additionally, the security of the communication between the database server and the LightCycler® 2.0 Instrument applications is warranted by setting up Virtual Private Network connections (VPN) between them.



3. Some of the LightCycler® 2.0 Instrument applications (LightCycler® Software 4.1 on data workstations, and/or Exor3 Object Server software on the remote database server) are installed on non-Roche computer systems. These non-Roche systems and LightCycler® 2.0 Instrument control unit(s) are connected directly to a foreign, unprotected network (*e.g.*, a laboratory network or Intranet). In this case, each LightCycler® 2.0 Instrument control unit must be secured individually by a cobas IT firewall. Security of non-Roche systems has to be ensured by the user.

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When setting up a LightCycler® 2.0 Instrument client/server network solution, observe the following general conditions and restrictions:

*In case of doubt, contact your local Roche service engineer who will guide you through these rules.*

- Exor3 Object Server software, which must be installed together with the LightCycler® Software 4.1 database on the remote database server, is compatible with both Microsoft Windows XP Professional (service pack 1 or 2) and Microsoft Server 2003.

*Nevertheless, as Roche has not validated the functionality of Exor3 Object Server software in combination with Microsoft Server 2003, and since Microsoft Server 2003 can only be installed on a non-Roche computer system, Roche cannot support this configuration.*

*In addition to the remote database, it is also possible to use a local database on the control unit. When you start the LightCycler® Software 4.1 on the control unit, you can choose which database (local or remote) you want to log on to.*

- The same version of LightCycler® Software 4.1 must be installed on all control units and data workstations

*Only the Exor3 Object Server and the LightCycler® Software 4.1 database are installed on the database server.*

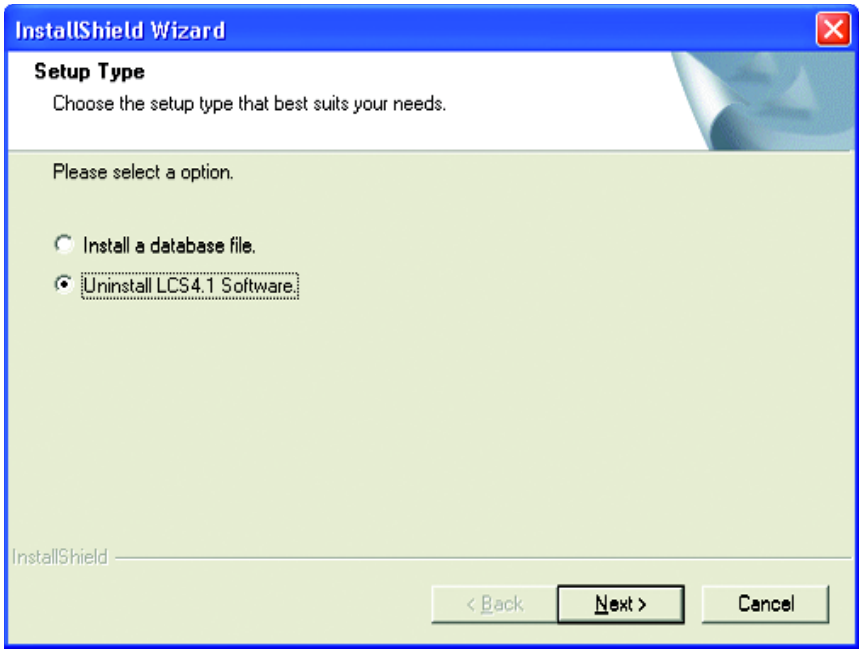
- Each LightCycler® 2.0 Instrument must be connected to a separate control unit.
- The LightCycler® 2.0 Instrument database server must run constantly while a user being is logged on to Windows. The Exor3 Object Server must be up and running.
- For safety reasons all users should log on to different accounts of the remote database.
- The number of active connections to the remote database should not exceed 5.
- Templates and macros must be defined either on LightCycler® 2.0 Instrument control units or in a separate local database on a LightCycler® 2.0 Instrument data workstation.
- Should the network connection between an instrument control unit and the database server be lost during a LightCycler® 2.0 Instrument run, the experimental data should be exported from the LightCycler® Software 4.1 on the control unit and imported into the remote database.
- Powering down of the database server during a LightCycler® 2.0 Instrument run may lead to an undefined status of the database and therefore to a possible loss of data.

## 5.5. Removing LightCycler® Software 4.1

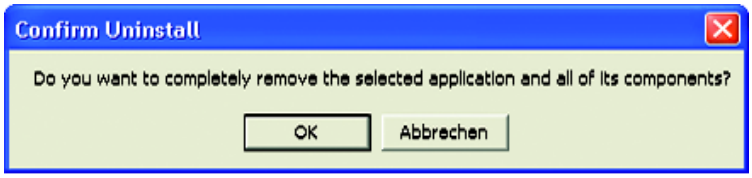
**To uninstall LightCycler® Software 4.1:**

1. Shutdown all running database engines by right-clicking the Exor3 icon in the system tray and selecting *Shutdown*.

2. Insert the LightCycler® Software 4.1 CD.
Double-click LightCycler_Software_41_Setup.exe, if installation doesn't start automatically. The Setup Type window is displayed. Select *Uninstall LCS4.1 Software*, and then click *Next*.




3. You are prompted to confirm the deletion. Click *OK*.

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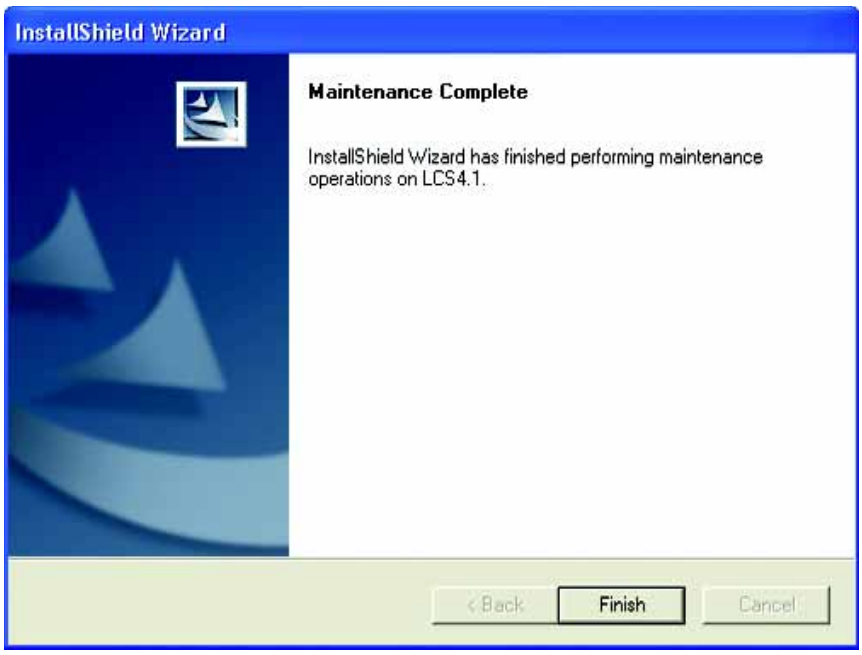
4 After the software has been removed, a message states that the maintenance is complete. Click *Finish*.

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## 5.6 Changing the Default Administrator Password and Creating User Accounts

The LightCycler® Software 4.1 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.

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*.

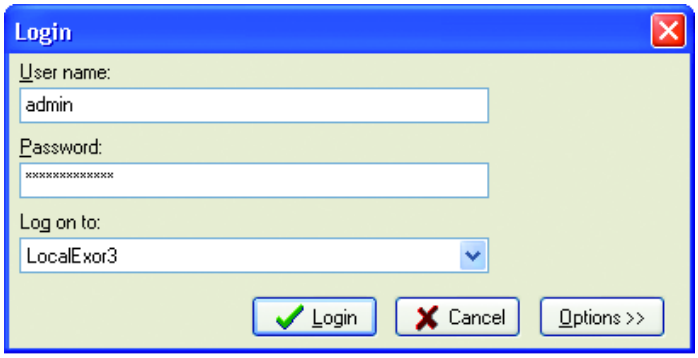
**Before you begin determine the following:**

- The new password for the System Admin account you need to change.
- The user names, login names, and passwords for the initial set of user accounts for the local computers.

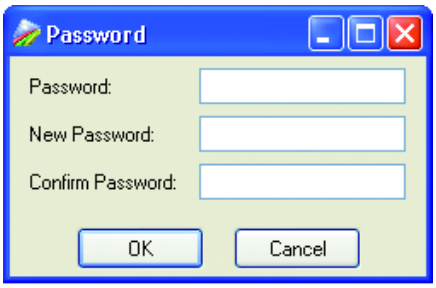
### To log on to a local computer:


On a LightCycler® computer double-click the *LightCycler® Software 4.1* icon to launch the LightCycler® Software 4.1.

A login dialog box opens. Enter admin as the User name and log on to location *LocalExor3*. Enter the password LightCycler01.




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*.

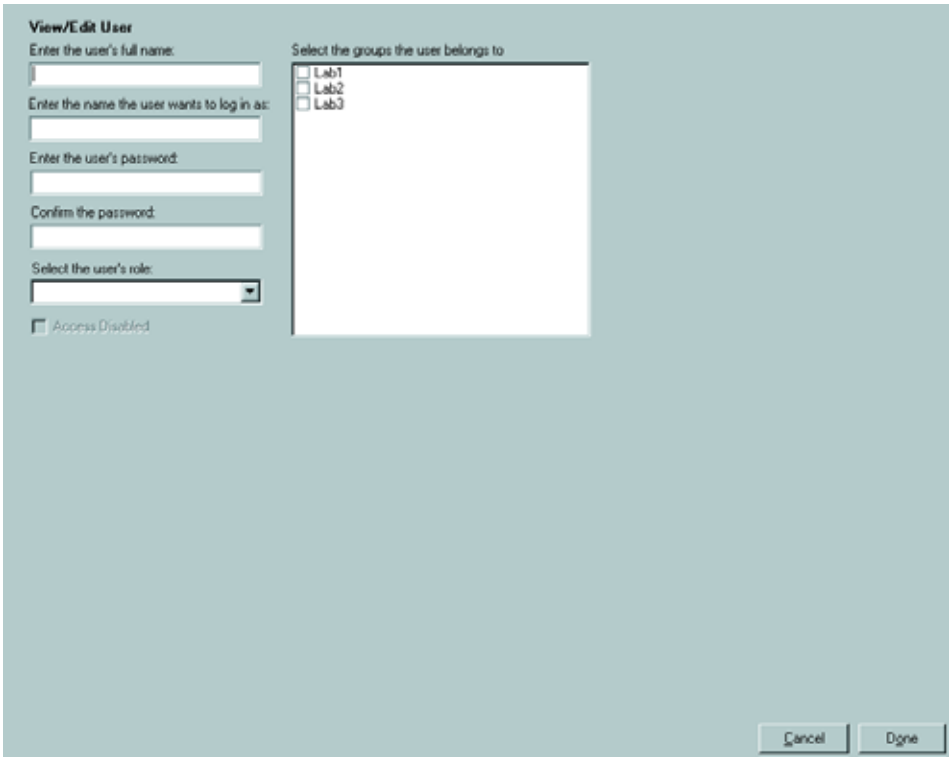
The password must contain at least six characters and contain one number and one upper case character.

*Passwords are case-sensitive. Remember the password or keep it in a safe place. Do not share your password with others.*

*Assure to be logged on to an Exor DB with an audit trail. If you are logged on to the Exor DB with an audit trail, the word "Traceable" 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 4.1 application.

## 5.7 Connecting an Instrument

Before a LightCycler® 2.0 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 when executing a Roche Macro. 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® 2.0 Instruments simultaneously.*

### 5.7.1 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.

### 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.

### 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 4.1, 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 instruments*. 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 4.1 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 4.1 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 LightCycler® 2.0 Instrument runs.

### 5.7.2 Adding a New Instrument Manually

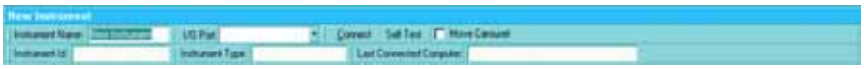
Prerequisites:

You must have Expert User or Local Administrator privileges.

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 4.1, 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 4.1 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 LightCycler® 2.0 Instrument runs.

### 5.7.3 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 4.1 database.

Prerequisites:
You must have Local Administrator privileges to remove an instrument.

**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*. |

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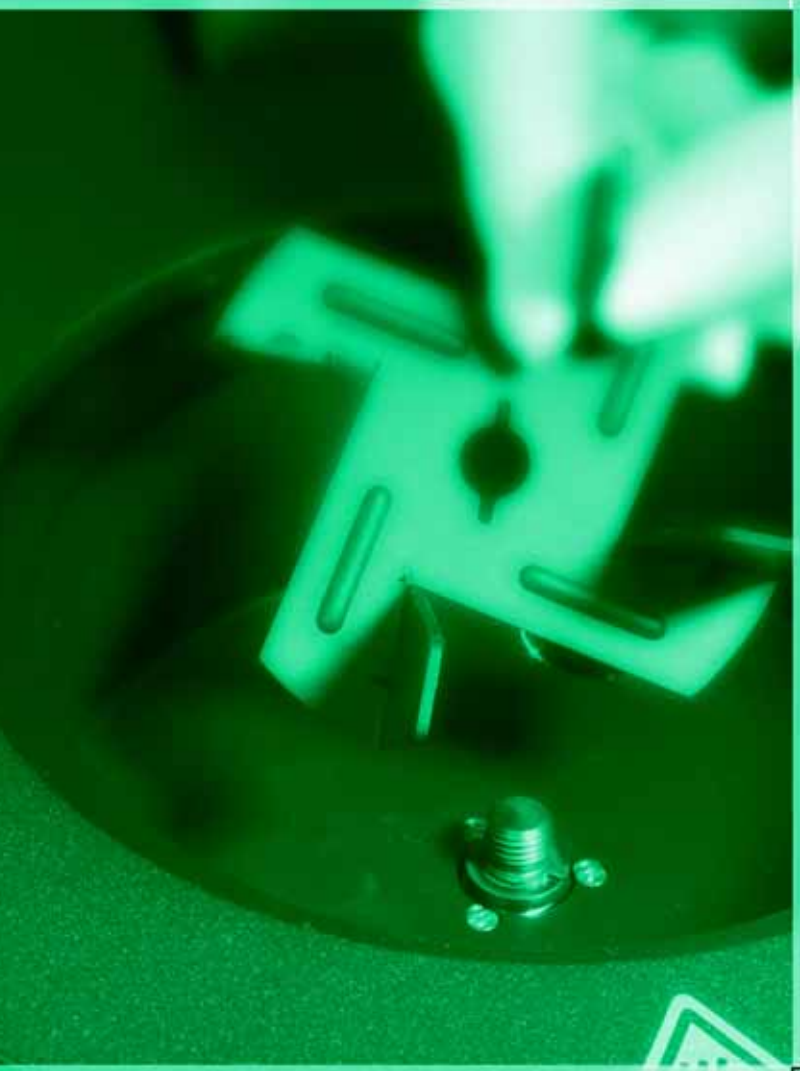
# Maintenance



E

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

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# 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.

E

| | |
|---|---|
| 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 Mikrozid AF Liquid (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 re-inserting of 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 thermal 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.


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## 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 (8 × 96 capillaries),
Cat.No.: 04 929 292 001 (5 × 96 capillaries)

LightCycler® Capillaries (100 µl), Cat.No.: 03 337 090 001

## 4.2 Spare Parts

LightCycler® 2.0 Sample Carousel (20 µl) marked with a brown tag,
Cat.No.: 03 603 962 001
LightCycler® 2.0 Sample Carousel (100 µl) marked with a purple tag,
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.

# Troubleshooting


F

**Chapter F • Troubleshooting**
lists all LightCycler® 2.0 Instrument system messages,
explains their meaning and indicates appropriate measures.

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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 4.1 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 considered critically. It is recommended to repeat the run where the validity of the results is doubtful.

## 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 running simultaneously to the LightCycler® software on the PC. 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 running simultaneously to the LightCycler® software on the PC. 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 adequate 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.<br>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 adequate 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 4.1.

| 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 over temperature 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 specifies negative conc. values. | Reset Cp limits in sample editor. |
| Negative CPRange limits not allowed | User specifies 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 4.1 | |

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