iQ™5

Multicolor Real-Time PCR Detection System

Installation Guide
and System Manual

BIO-RAD

The iQ5 Real-Time PCR Detection System

Section 1

Safety Information

Important: Read this information carefully before using the iQ5 System.

Grounding

Always connect the iQ5 optics module to a three-prong, grounded AC outlet using the AC power cord provided with the system. Do not use an adaptor to a two-terminal outlet. Always ensure power switch is set to the off position when connecting or disconnecting power cords for the iQ5 system.

Handling

Handle all components of the iQ5 system with care and with clean, dry hands at all times. The optical system contains mirrors and lenses that may shatter if the unit is dropped or struck with great force. If the unit is damaged, such that internal components or wires are exposed, contact your local Bio-Rad office immediately, do not attempt to repair or power on the instrument.

Service

The only user-serviceable parts of the iQ5 optics module are the lamp and filters. Call your local Bio-Rad office for all other optics module and thermal cycler related service. When replacing the lamp or filters, open only the outer casing of the iQ5 optics module. The camera lamp may get extremely hot during system operation, do not attempt to remove the lamp without powering off the instrument and allowing the system to cool for at least 15 minutes. To prevent skin burns and fire hazards, do not attempt to operate the iQ5 system while the camera case is open. Do not open the casing of the iQ5 optics module when the instrument is in use.

Temperature

For normal operation, the maximum ambient temperature should not exceed 40°C (see Appendix A for specifications). To ensure adequate cooling of the system, maintain a clearance of at least 4 inches around the sides of the iQ5 optics module. Do not block the fan vents near the lamp, as this may lead to improper operation or cause physical damage to the iQ5 detector. Do not operate the iQ5 optics module in extreme humidity (>90%) or where condensation can short internal electrical circuits or fog optical elements.

Notice

This Bio-Rad instrument is designed and certified to meet EN-61010 safety standards.

EN-61010-certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified in any way. Alteration of this instrument will:

- Void the manufacturer's warranty
- Void the EN-61010 safety certification
- Create a potential safety hazard

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended, or by modifications to the instrument not performed by Bio-Rad or an authorized agent.

The iCycler® thermal cycler and iQ5 real-time PCR detection system are intended for laboratory research applications only.

Section 2

iQ5 System Installation

2.1 Preparing for the Installation Process

The iQ5 system should be installed on a clean, dry, level surface. Identify an appropriate work area for the installation process prior to unpacking any system components. The entire installation process should take approximately 15 minutes to complete.

System Checklist

- ❑ **iQ5 Optics Module (170-9751), 1 box**
  - ❑ Power cord
  - ❑ iQ5 optics module
  - ❑ iQ5 software installation disk (170-9753)
  - ❑ Amplification tech notes CD (version 1.0)
- ❑ **iCycler Chassis (170-8701), 1 box**
  - ❑ Power cord
  - ❑ iCycler chassis
  - ❑ iCycler thermal cycler operating instructions
- ❑ **iQ5 Optical Kit (170-9752), 1 box**
  - ❑ Optical reaction block
  - ❑ Modified sliding rear cover for iCycler thermal cycler
  - ❑ Serial cable
  - ❑ USB cable
  - ❑ Filter extraction tool
  - ❑ Optical tape applicator tools (3)
  - ❑ iQ5 optics support bracket
  - ❑ Support bracket screws
  - ❑ Hex driver
  - ❑ Hex screws
  - ❑ Spare part: halogen lamp
- ❑ **iQ Calibration Kit (170-8792), shipped on dry ice**
  - ❑ External well factor solution
  - ❑ 1x calibration dye solutions (9 dyes, 3 tubes of each dye)

Contact your local Bio-Rad office if any system components are missing or damaged.

In addition, the following accessories are required to complete the installation process:

- Scissors
- #2 Phillips screwdriver
- Optical-quality PCR plates or tubes
- Optical-quality sealing film or tube caps
- Calibrated micropipet(s)
- Aerosol barrier pipet tips

Handle the iQ5 optics module and iCycler thermal cycler with care and with clean, dry hands during unpacking and assembly. Do not handle the iQ5 system components with wet hands. Refer to Section 1 of this guide for additional safety information.

2.2 Installing the Optics Module on the iCycler Chassis

1. Remove the existing rear cover from the iCycler chassis by sliding the cover towards the front of the iCycler base (Figure 2.1).
2. Install the modified sliding rear cover provided with the optical reaction block, ensuring that the notch is oriented towards the rear of the iCycler thermal cycler.
3. Push the sliding rear cover on top of the chassis as far back as possible.
4. Rotate the green latches on the optical reaction block up towards the open lid.
5. Lift the optical reaction block by the handle and install it onto the chassis. Lower the front portion of the reaction block so that it engages with the chassis before the rear portion. The rear of the block lid should fit over the front lip of the sliding rear cover (Figure 2.2).
6. Secure the optical reaction block in place by rotating the green latches downward.
7. Close the optical lid.

Fig. 2.1. Slide the rear cover forward to remove it.

Fig. 2.2. Engage front portion of reaction block with chassis.

2.3 Installing the Support Bracket

A support bracket with roller is provided for the iQ5 optics module. It is mounted to the rear of the iCycler thermal cycler as shown in Figure 2.3.

Align the optics module support bracket with the two holes on the rear of the iCycler thermal cycler. Using a #2 Phillips screwdriver, adjust the height of the bracket with two of the appropriate screws provided with the system accessories. Both of the screws should be approximately in the center of the slots on the bracket.

Fig. 2.3. Secure the support bracket to the rear of the iCycler chassis.

2.4 Installing the iQ5 Optics Module

1. Remove the plastic sheath and protective label from the optics module and place the optical module on a flat surface, taking care not to touch the inside of the nose portion of the module.

Fig. 2.4. Slide module onto U-bracket.

2. Remove the protective label from the optical lid.
3. Slide the optics module onto the U-bracket as shown in Figure 2.4.
4. Secure the optics module to the U-bracket using the two long, thin hex screws and the hex driver provided.
5. After the optics module has been installed, confirm that the optics module and lid assembly can be opened and closed readily.

   If the lid is difficult to open, lower the support bracket slightly before tightening the bracket mounting screws.

   If the lid is difficult to close, try raising the support bracket slightly before tightening the rear screws.

2.5 Connecting Power and Communication Cables to the iQ5 System

Before connecting any communication or power cables to the system, confirm that the power switch for the iCycler thermal cycler and the iQ5 optics module are in the OFF position.

1. Close the optical reaction block by sliding the lid forward and pressing down on the lid handle. On the right side of the optics module are three connectors (Figure 2.5).

2. Using the cables provided in the iQ5 Optical Kit, establish power and communication with the computer as follows:

Fig. 2.5. Optics module connectors.

Recessed 3-pin power connector — Connect the supplied power cord between the optics module and a grounded power outlet. This connection provides power only to the optical module; a separate power cord must be connected to the iCycler thermal cycler.

Fig. 2.6. The serial connector.

Serial connector — A serial connector is located at the rear of the iCycler thermal cycler (Figure 2.6). Connect the serial cable to the rear of the iCycler chassis and to the serial port on the side of the iQ5 optics module. This connection enables communication between the iQ5 software and the iCycler thermal cycler.

USB port connector — Connect the supplied USB cable between the iQ5 optics module and a USB 2.0 high-speed enabled port on the computer. Data are transferred to the computer via this cable. This single connection directs the operation of both the iQ5 optics module and the iCycler thermal cycler by the iQ5 Optical System Software.

At the right rear corner of the optical reaction block is a single connector:

Positive docking connector — This self-aligning connector is secured into place when the iQ5 optics module is installed on the iCycler chassids. This connection senses when the lid handle is lifted.

2.6 Installing the iQ5 System Software

Locate the software installation disk provided with the iQ5 system. This installation disk is compatible with computers running Windows XP and Windows 2000 operating systems.

1. Insert the iQ5 Optical System Software installation CD in a CD-ROM drive.
2. If the installation program does not begin automatically, select Run… from the Start menu and then type "X:\Setup", where X is the drive letter of the CD-ROM drive. For example, if the CD-ROM is the E drive, type "E:\Setup".
3. Follow all screen prompts to finalize the installation.

Certain configurations of Windows 2000 and XP initialize new folders by assigning Read and Execute permission for the members of the Users group. If you have this type of operating system and this is a first-time installation, the administrator must change the protection on the Program Files/Bio-Rad folder or the Program Files/Bio-Rad/iQ5 folder so that you can save protocol, plate setup, and data files. If, after changing the protection on either of these folders, it is still not possible to write to the folders beneath Program Files/Bio-Rad/iQ5, check the properties of each individual folder to be sure that under the Securities tab, the box is checked that allows inheritable permissions to propagate to that folder.

2.7 Initializing the iQ5 Instrument

Before using the iQ5 system for the first time, five procedures must be performed on the new instrument: Camera Driver Installation, Mask Alignment, Background Calibration, Persistent Well Factor Collection, and Pure Dye Calibration.

1. Power on the iCycler thermal cycler and the iQ5 optics module.
2. Follow all screen prompts to finalize the camera driver installation.
3. Allow the instrument optics to warm up for 20 min prior to performing the Mask Alignment, Background Calibration, Persistent Well Factor Collection, or Pure Dye Calibration procedures.
4. Open the iQ5 system software by double-clicking on the shortcut icon located on the Windows desktop, or by selecting the iQ5 program icon from the Bio-Rad folder of the Windows Start menu.

Refer to the Help section of the iQ5 Optical System Software for detailed directions on the procedures and materials required to perform Mask Alignment, Background Calibration, Persistent Well Factor Collection, and Pure Dye Calibration.

Section 3

iQ5 Optical System Components

The optics module houses the excitation system and the detection system (Figure 3.1). The excitation system consists of a fan-cooled, 50 W tungsten-halogen lamp, a heat filter (infrared-absorbing glass), a six-position filter wheel fitted with five optical filters and one opaque filter "blank", and a dual mirror arrangement that allows simultaneous illumination of the entire sample plate. The excitation system is physically located on the right front corner of the optics module, with the lamp shining from right to left, perpendicular to the instrument axis. Light originates at the lamp, passes through the heat filter and a selected color filter, and is then reflected onto the 96-well plate in the thermal cycler by a set of mirrors. This light source excites the fluorescent molecules in the wells.

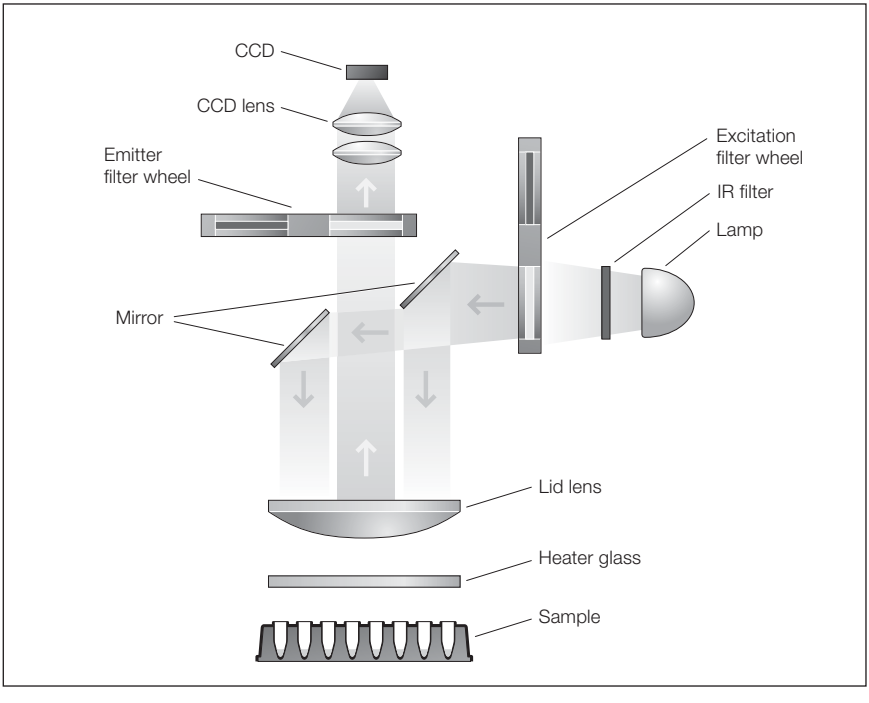

Fig. 3.1. Representation of the optical system layout. The detection system occupies the rear portion of the optics module housing. The primary detection components include a six-position emission filter wheel and a CCD detector. The emission filter wheel is identical to the wheel in the excitation system and is fitted with five colored-emission filters and one opaque filter "blank". Fluorescent light from the wells passes through the emission filter and is then detected by the CCD.

Appendix A

iQ5 System Specifications

Input power	100–250 VAC ± 10%, 50–60 Hz, 250 W max, 95 W typical
Operating temperature	5–40°C
Storage temperature	–20 to 40°C
Humidity	0–90%, noncondensing
Dimensions, optics module (W x D x H)	29.3 x 38.74 x 20.3 cm (11.5 x 15.25 x 8.75")
Weight, optics module	6.3 kg (13.9 lb)
Data communication	USB 2.0 high speed
Regulatory compliance	EN-61010, NRTL

Appendix B

Computer Specifications

A computer with the following system specifications is required to run the iQ5 real-time PCR detection system.

Minimum Computer Specifications

- Pentium or comparable processor, 1.0 GHz or faster
- Windows 2000 or Windows XP operating system
- Internet Explorer browser (version 5.0 or higher)
- 512 MB RAM
- 10 GB hard drive
- CD-ROM drive
- Mouse
- 1,024 x 768 x 256 colors minimum screen resolution
- USB 2.0 high-speed communications port

Recommended Computer Specifications

To ensure optimal performance of the iQ5 system and software, connect the iQ5 instrument to a computer with the following specifications:

- Pentium or comparable processor, 2.0 GHz or faster
- Windows XP operating system
- Internet Explorer browser (version 5.0 or higher)
- 512 MB RAM
- 20 GB hard drive
- CD-RW drive
- Mouse

- 1,024 x 768 x 256 colors minimum screen resolution
- At least 4 USB 2.0 high-speed communications ports

The following system accessories are also recommended:

- USB-compatible color printer
- Excel software
- High-speed modem with an Internet connection (suggested for downloading new releases)
- Uninterruptible power supply: at least 1,000 W for the base iCycler thermal cycler, 650 W for the computer, and 250 W for the iQ5 module

Appendix C

Ordering Information

Catalog #	Description
System Configuration	
170-8701	iCycler Chassis, includes iCycler base, power cord, quick reference cards, instruction manual
170-9750	iQ5 Optical System, includes optics module, software CD-ROM, 5 installed filter sets, 96-well reaction module, calibration solutions, optical-quality 96-well PCR plates, communication cables, power cord, instruction manual
Accessories	
223-9441	96-Well 0.2 ml Thin-Wall PCR Plates, 25
223-9443	96-Well 0.2 ml PCR Plate Caps, for 223-9441, 300
223-9444	Optical-Quality Sealing Tape, optimized for use with 223-9441, 100 sheets
170-8756	Replacement Halogen Lamp
170-9753	iQ5 Software Installation Disk
170-9761	iQ5 Filter Set, FAM/SYBR dyes
170-9762	iQ5 Filter Set, VIC/HEX/TET dyes
170-9763	iQ5 Filter Set, Cy3/TAMRA dyes
170-9764	iQ5 Filter Set, ROX/Texas Red dyes
170-9765	iQ5 Filter Set, Cy5 dyes
Calibration Solutions	
170-8792	iCycler iQ Calibration Kit, shipped on dry ice
170-8794	External Well Factor Solution
170-8793	FAM Calibrator Solution, 1.5 ml tubes, 5
170-8795	HEX Calibrator Solution, 1.5 ml tubes, 5
170-8797	Cy5 Calibrator Solution, 1.5 ml tubes, 5
170-8799	Texas Red Calibrator Solution, 1.5 ml tubes, 5

Appendix D

Care and Maintenance

Cleaning the Unit

Take care not to spill liquids onto or into the iCycler thermal cycler or the iQ5 optics module.

The outer casing of the instrument may be cleaned using a soft, lint-free cloth and water.

Replacing the Lamp

Before replacing the lamp, turn the instrument off and allow it to cool for at least 15 min.

The lamp is located on the right side of the optics module, (Figure D.1). To replace the lamp:

1. Turn off the power to the optics module.
2. Reach behind the iQ5 optics module and unscrew the short fastener that secures the lid of the module in place.
3. Using a gentle pressure with both hands, push inward on the rear vents located on the top half of the iQ5 casing. Lift upward to remove the cover of the optics module.
4. Push up on the lamp spring clip to release the lamp from the bracket.
5. Lift the lamp out of the socket.
6. Install the new lamp using the reverse of steps 1–5. Hold the new lamp by the outer reflector and do not touch the bulb. Be sure the spring clip is down before inserting the lamp into the socket. Push the lamp firmly into the bracket, then close the case and secure the lid.

Fig. D.1. Replacing the lamp.

iQ5 Filter Description and Installation Instructions

The filters designed for use in the iQ5 optics module are made of glass and mounted in plastic holders (see Figure D.2). The filter holders are held in either the excitation or emission filter wheel of the iQ5 optics module. Each filter wheel holds six filters. Every position in a filter wheel must have a filter or an opaque filter blank to avoid damage to the CCD detector. The first position in each filter wheel is designated as the "home" position and must always contain an opaque filter blank. Filters can be removed for cleaning or replacement. If a filter shatters or breaks during the installation process, contact your local Bio-Rad office immediately for service, do not attempt to remove the broken components from the interior of the camera housing.

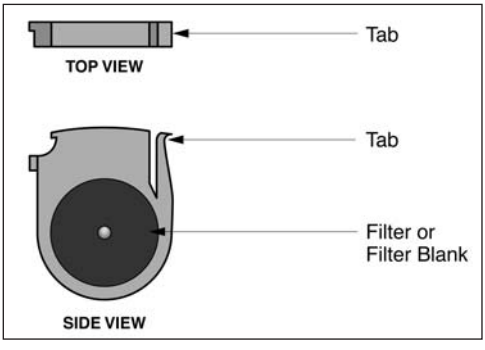

Fig. D.2. Filter in filter holder.

It is critically important that the excitation and emission filters are in the correct positions in the filter wheels, please confirm that the filters are in the proper location after cleaning or replacing filters in the iQ5 optics module.

The table below summarizes the positions of the filter pairs, their optical characteristics, and the recommended fluorophores with which they are compatible.

Position	Excitation	Emission	Recommended Fluorophores
2	490/20X	530/30M	Fluorescein (FAM),SYBR Green I
3	530/30X	575/20M	HEX, TET, VIC, JOE
4	545/30X	585/20M	TAMRA/Cy3
5	575/30X	620/30M	Texas Red, ROX
6	635/30X	680/30M	Cy5, LC640

Note: The filter designation 490/20X indicates that this filter will allow light between 480 and 500 nm to pass through. The first number, 490, indicates the center of the wavelength of light. The second number, 20 indicates the total breadth of wavelengths of light that can pass through it. The letter "X" indicates that the filter is specified for excitation only, and the letter "M" indicates emission only types of filters. Excitation and emission filters are not interchangeable.

To access the existing filters, proceed as follows:

1. Turn off the power to the optics module.
2. Reach behind the iQ5 optics module and unscrew the short fastener that secures the lid of the module in place.
3. Using gentle pressure and both hands, push inward on the rear vents located on the top half of the iQ5 casing. Lift upward to remove the cover of the optics module.
4. To access the excitation filter wheel, remove the black plug from the slot located near the lamp, at the right-hand side of the optics module. To access the emission filter wheel, remove the plug from the slot located at the top of the instrument (see Figure D.3).

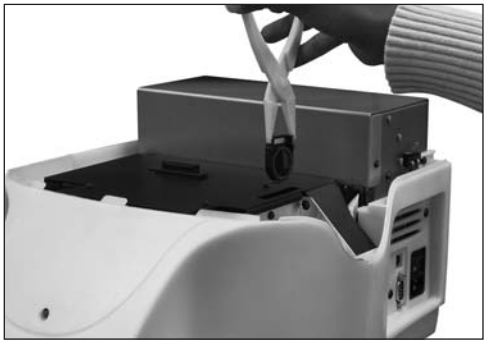

Fig. D.3. Installing the filter.

5. Turn the filter wheels to the desired positions using the supplied ball-end hex driver. As long as the power to the optics module is off, the filter wheels may be turned freely in either direction.
6. To remove a filter, grasp it on both sides with the filter removal pliers and squeeze the tab in; gently pull the filter up and out.
7. To insert a filter, grasp the filter with the pliers and insert it into a vacant slot. For the excitation filters, the tab on the filter should face toward the front of the instrument. For the emission filters, the tab on the filter should face the right of the instrument. Be sure that every position in the filter wheel has either an excitation or emission filter or a filter blank before powering on the system.
8. After the filters or filter blanks have been inserted, replace the rubber plugs over the slots of the filter wheels.
9. Realign the tabs on the front end of the iQ5 optics module cover with the tabs on the main housing. Lower the cover until the top half of the camera housing snaps into place.
10. Replace the screw in the rear of the optics module to secure the casing.

Appendix E

Installing New Versions of Firmware

Bio-Rad is committed to continuous improvement in iCycler features. Towards that end, the iCycler firmware will be upgraded to offer new features on a regular basis. We will make a firmware upgrade disk available at the time of each new release and announce the release on our web site, **discover.bio-rad.com**. The iCycler thermal cycler is upgraded via a serial port connection to a PC computer, or via a serial port pass-through with the iQ5 USB connection. The computer serial port must support a 57,600 baud rate. The connection may be made with a standard 9-pin serial cable. The iCycler firmware upgrade kit may be ordered from Bio-Rad using part number 170-8737.

Upgrading the iCycler Firmware

A recent version of the iCycler firmware and a related utility are contained on the iQ5 software installation CD provided with the iQ5 system. To complete a firmware upgrade of older iCycler instruments, follow the directions below to copy the iCycler upgrade files from installation CD to the computer connected to the iQ5 system.

1. Connect the 9-pin serial cable from the serial port located at the rear of the iCycler instrument to the iQ5 optics module, ensuring that the iQ5 optics module is connected to a computer via USB.
2. Close any other application that may have already established communication with the iCycler base unit (for example, **IQ5.EXE** or the iCycler report utility software).
3. Use Windows Explorer to create a new folder on the C: drive. Name this new folder “PC Firmware Upgrade”.
4. Open the CD directory \base unit\firmware upgrade and copy the files **UPGRADE.EXE** and **ICYCPROG.BIN** to the Firmware Upgrade folder created in step 3.
5. From Windows Explorer, open the Firmware Upgrade folder now located on the C: drive and double-click on **UPGRADE.EXE**. The utility will open and establish communication with the iCycler instrument through the serial port connection bridged by the iQ5 optics module.
6. Each new version of firmware is assigned a higher version number than the previous version. The **UPGRADE.EXE** utility will detect the version number of the installed firmware and display this information for comparison to the version ready for download. Download of a new firmware program will not proceed until the user enters “y” after reviewing the firmware version information.

The upgrade utility will display the following information:

Old program version: _____

New program version: _____

Proceed with download? y / n

Enter “y” to proceed with the download if the new program version has a HIGHER number than the old program version before proceeding with download.

Enter “n” if the new program version is the same or LOWER than the old program version. Entering “n” will exit and close the **UPGRADE.EXE** utility.

Once the download is complete, the **UPGRADE.EXE** utility will display a message confirming that the upgrade has been successfully completed. The **UPGRADE.EXE** utility will automatically shut down and restart the iCycler instrument in order to complete the installation of the new firmware.

Appendix F

System Warranty

The iQ5 real-time PCR detection system is warranted against defects in materials and workmanship. For specific warranty information, contact your local Bio-Rad office. If any defects should occur during the warranty period, Bio-Rad will replace the defective parts without charge. However, damage or defects resulting from any of the following causes are specifically excluded:

1. Improper operation.
2. Use of improper solvent or sample.
3. Use with tubes, plates, or sealing materials not specified by Bio-Rad Laboratories for use with the iQ5 real-time PCR detection system.
4. Deliberate or accidental misuse.
5. Repair or modifications done by anyone other than Bio-Rad Laboratories.
6. Natural disaster of any kind.

The warranty does not apply to fuses.

For inquiry or request for repair service, contact Bio-Rad Laboratories after confirming the model and serial number of your instrument. For technical support, call your local Bio-Rad office, or in the US, call 1-800-4BIORAD (1-800-424-6723), or visit us on the Web at **discover.bio-rad.com**

Appendix G

iCycler® Thermal Cycler Authorization Statement

This instrument, Serial No. _____, is an Authorized Thermal Cycler. Its purchase price includes the up-front fee component of a license under United States Patent Nos. 4,683,195, 4,683,202 and 4,965,188, owned by Roche Molecular Systems, Inc., and under corresponding claims in patents outside the United States, owned by F. Hoffmann-La Roche Ltd, covering the Polymerase Chain Reaction ("PCR") process, to practice the PCR process for internal research and development using this instrument. The running royalty component of that license may be purchased from Applied Biosystems or obtained by purchasing Authorized Reagents. This instrument is also an Authorized Thermal Cycler for use with applications licenses available from Applied Biosystems. Its use with Authorized Reagents also provides a limited PCR license in accordance with the label rights accompanying such reagents. Purchase of this product does not itself convey to the purchaser a complete license or right to perform the PCR process. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

No rights are conveyed expressly, by implication or estoppel to any patents on real-time PCR.

Applied Biosystems does not guarantee the performance of this instrument.

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Life Science Group

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