applied
biosystems®
by *life* technologies™

QuantStudio™ 6 and 7 Flex Real-Time PCR Systems

MAINTENANCE AND ADMINISTRATION

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About This Guide

Purpose

This user guide provides provides reference information for the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems and describes how to prepare, maintain, and troubleshoot the systems.

> ⚠ **WARNING!** The protection provided by the equipment may be impaired if the instrument is operated outside the environment and use specifications, the user provides inadequate maintenance, or the equipment is used in a manner not specified by the manufacturer (Life Technologies).

Note: For information and instructions on performing experiments on the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems, refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Getting Started Guide* (Pub. no. 4489822) and the *High Resolution Melt Module for QuantStudio™ 6 and 7 Flex Real-Time PCR System Software* (Pub. no. 4489823).

Audience

This user guide is written for laboratory staff who operate and maintain the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems.

Assumptions

This guide assumes that your QuantStudio™ 6 and/or 7 Flex System has been installed by a Life Technologies service representative.

This guide also assumes that you have:

- Familiarity with Microsoft® Windows® operating system.
- Knowledge of techniques for handling and preparing nucleic acid samples for PCR.
- A general understanding of data storage, file transfers, and copying and pasting.

User attention words

Four user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation or accurate chemistry kit use.

⚠ **CAUTION!** Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

⚠ **WARNING!** Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

⚠ **DANGER!** Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Except for IMPORTANTs, the safety alert words in user documentation appear with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instrument. See the "Safety" appendix for descriptions of the symbols.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the symbol is used along with the user attention words to highlight important safety information. The following table gives the meaning of these symbols.

Symbol	English	Français
⚠	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.

Contents of this manual

This guide is divided into the following chapters and appendixes:

Chapter/appendix	Content
Chapter 1, Getting Started	Introduces the QuantStudio™ 6 and 7 Flex Systems, including descriptions of all related hardware and software.
Chapter 2, Calibrate the Instrument	Describes how to maintain a QuantStudio™ 6 or 7 Flex System, including calibration and performance verification.
Appendix A, Manual Instrument Operation	Describes how to operate the QuantStudio™ 6 and 7 Flex Systems manually using the touchscreen interface.
Appendix B, Maintenance	Describes how to replace the user-serviceable parts of the QuantStudio™ 6 and 7 Flex Systems and how to resolve infrequent problems that can occur during normal use.
Appendix C, Networking	Describes how to install the QuantStudio™ 6 and 7 Flex Systems to a local area network for remote monitoring and control.
Appendix D, Power On or Off, Store, and Move	Describes how to store, move, and reinstall the components of the QuantStudio™ 6 and 7 Flex Systems.
Appendix E, Security, Audit, and Electronic Signature	Describes how to configure the security, audit, and electronic signature functions of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.
Appendix F, Calibration Consumable Preparation	Describes how to prepare consumables for custom dye calibration.
Appendix G, Parts and Materials	Describes how to order parts, accessories, and consumables for the QuantStudio™ 6 and 7 Flex Systems.
Appendix H, Instrument Specification and Layout	Describes the QuantStudio™ 6 and 7 Flex System specifications, requirements, layout, and connections.

1 Getting Started

Differences between the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems

The following table displays the differences between the QuantStudio™ 6 Flex System and the QuantStudio™ 7 Flex System.

QuantStudio™ 6 Flex System	QuantStudio™ 7 Flex System
Does not support the Twister® Robot	Supports the Twister® Robot
Does not support the Array Card block	Supports the Array Card block
Features a coupled five-color filter set	Features a decoupled six-by-six-color filter set

About the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems

The QuantStudio™ 6 and 7 Flex Systems use fluorescent-based polymerase chain reaction (PCR) reagents to provide:

- Quantitative research detection of target nucleic acid sequences (targets) using real-time analysis.
- Qualitative research detection of targets using post-PCR (endpoint) analysis.
- Qualitative analysis of the PCR product (achieved by melt curve analysis that occurs post-PCR).

Data collection

The QuantStudio™ 6 and 7 Flex Systems collect raw fluorescence data at different points during a PCR, depending on the type of run that the QuantStudio™ 6 and 7 Flex Systems perform:

	Run type	Data collection point
Real-time runs	Standard curve	The QuantStudio™ 6 and 7 Flex Systems collect data following each extension step of the PCR.
	Relative standard curve	
	Comparative C_T ($\Delta\Delta C_T$)	
	Melting curve	

Run type		Data collection point
Post-PCR (endpoint) runs	Genotyping	The QuantStudio™ 6 and 7 Flex Systems collect data: 1. Before the PCR. (For presence/absence experiments, data collection before the PCR is optional, but recommended.)
	Presence/absence	2. (Optional) During the PCR. The QuantStudio™ 6 and 7 Flex System can collect data during the run (real-time); collecting data during the run can be helpful for troubleshooting. 3. After the PCR.

The instrument collects raw fluorescence data following each extension step of the PCR. A data collection point, or read, on the QuantStudio™ 6 and 7 Flex System consists of three phases:

1. **Excitation:** The instrument illuminates all wells of the plate or array card, within the instrument, exciting the fluorophores in each reaction.

2. **Emission:** The instrument optics collect the residual fluorescence emitted from the wells of the plate or array card. The resulting image collected by the device consists only of light that corresponds to the range of emission wavelengths.

3. **Collection:** The instrument assembles a digital representation of the residual fluorescence collected over a fixed time interval. The software stores the raw fluorescent image for analysis.

After a run, the software uses calibration data (ROI, background, uniformity, dye, and normalization) to determine the location and intensity of the fluorescent signals in each read, the dye associated with each fluorescent signal, and the significance of the signal.

Instrument filters and supported dyes

System dyes

The QuantStudio™ 6 Flex System features a coupled five-color filter set while the QuantStudio™ 7 Flex System features a decoupled six-by-six-color filter set that supports the dyes shown in the following table. For more information about the spectral dye calibration kits available for the QuantStudio™ 6 and 7 Flex Systems, please contact Life Technologies.

Filter set	Color	Filter wavelength (nm)[†]		Supported dye
		Excitation	Emission	
x1-m1	Blue	470 ± 15	520 ± 15	FAM™ and SYBR® Green dyes
x2-m2	Green	520 ± 10	558 ± 12	JOE™, HEX™, TET™, and VIC® dyes[‡]
x3-m3	Yellow	549.5 ± 10	586.5 ± 10	Cy®3, NED™, and TAMRA™ dyes
x4-m4	Orange	580 ± 10	623 ± 14	ROX™ and Texas Red® dyes
x5-m5	Red	640 ± 10	682 ± 14	Cy®5 and LIZ® dyes[‡]
x6-m6[§]	Deep red	662 ± 10	711 ± 12	None[††]

[†] The central wavelengths are the optimized wavelengths.

‡ The HEX™, TET™, and LIZ® dyes from Life Technologies fall within the emission wavelength range of the system, therefore they can be added and adapted for use in experiments on the system. To add any of these dyes to the Dye Library, perform a custom dye calibration. For more information, see "Calibration Consumable Preparation" on page 149.

§ QuantStudio™ 7 Flex System only.

†† This filter set currently does not support any dyes supplied by Life Technologies.
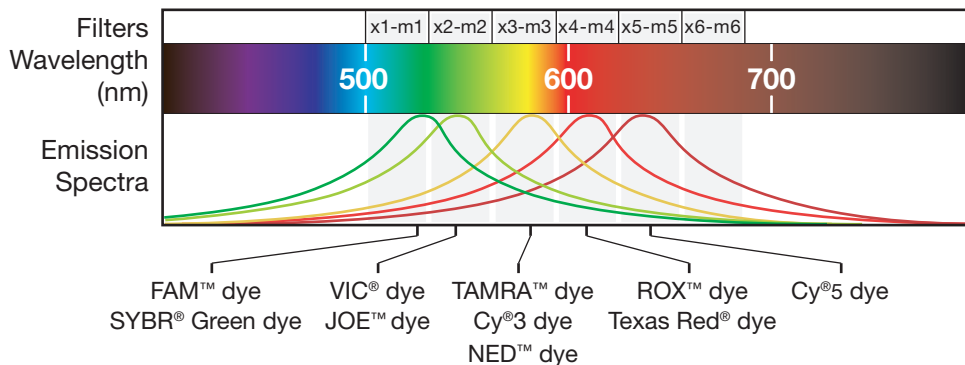
Custom dyes

The QuantStudio™ 6 and 7 Flex Systems can run assays designed with custom dyes (dyes not supplied by Life Technologies) that are excited between 455 to 672 nm and read between 505 to 723 nm. To add a custom dye to the Dye Library, perform a custom dye calibration. For more information, see "Calibration Consumable Preparation" on page 149.

Instrument hardware

The QuantStudio™ 6 and 7 Flex Systems consist of the components shown in the following figure and described in the subsequent table.

Front
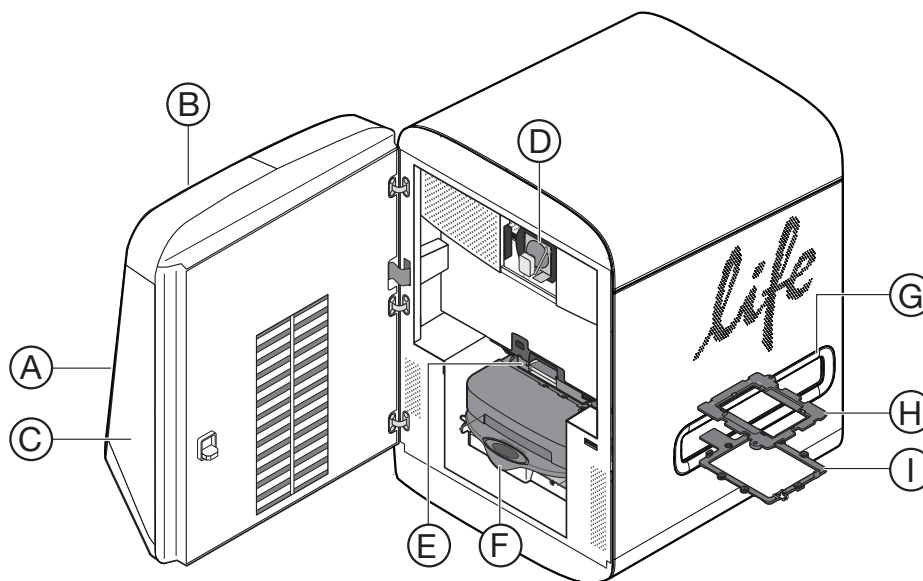
	Component	Description
A	USB ports	Provide USB communication with the instrument. Can be used to transfer data to and from the instrument. **Note:** If multiple USB drives are plugged into the instrument, the instrument mounts only the first drive that is installed, regardless of the USB port used.
B	Touchscreen	Provides access to the instrument functions. Can be used to run experiment, transfer data, and operate the instrument functions without the use of the computer.
C	Access door	Provides access to the instrument lamp, the heated cover, and the sample block.
D	Lamp	Illuminates the plate or array card during a run.
E	Heated cover	Covers the plate or array card during a run to prevent condensation and leakage through the cover.
F	Sample block	Heats the plate or array card during a run.
G	Side door	Opens to allow extension of the tray arm.
H	Plate adapter	Secures the plate or array card to the tray arm.
I	Tray arm	Conveys the plate or array card to and from the sample block in the interior of the instrument.

Rear
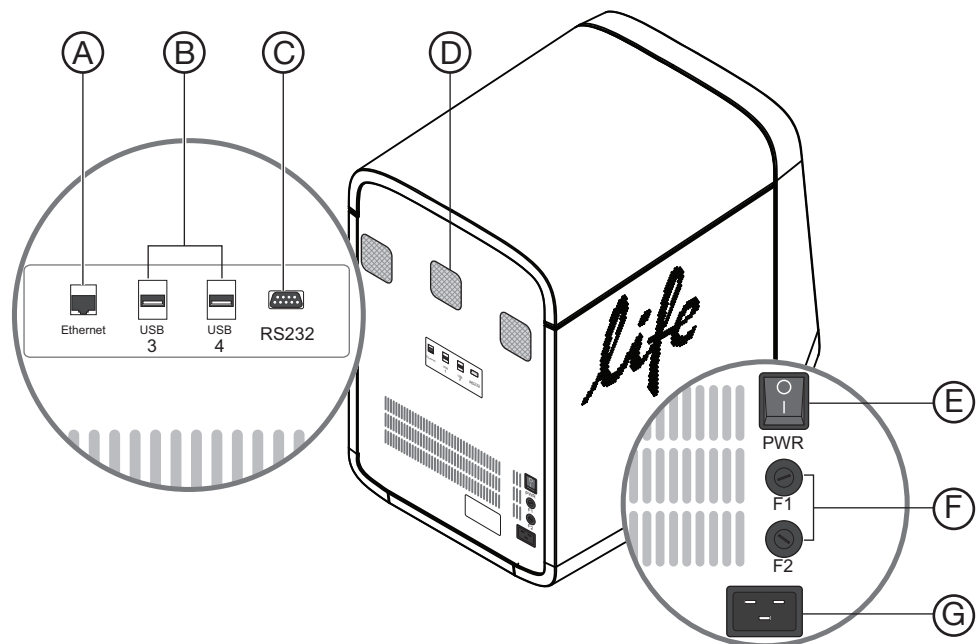
	Component	Description
A	Ethernet port	An RJ45 port that provides Ethernet (gigabit) communication with the instrument.[†]

	Component	Description
B	USB ports	Provide USB communication with the instrument. USB ports can be used to transfer data from the instrument.
		Note: If multiple USB drives are plugged into the instrument, the instrument mounts only the first drive that is installed, regardless of the USB port used.
C	RS232 port	Provides serial communication between the instrument and the computer.
		IMPORTANT! The serial port is for Life Technologies use only.
D	Instrument fans	Cool the interior of the instrument.
		IMPORTANT! The fans must be unobstructed to ensure adequate cooling and proper function of the instrument.
E	On/Off switch	Power switch for the instrument, where the states are on (**I**) or off (**O**).
F	Fuse cover	Dual 12.5A, Time-Lag T, 250 VAC, 5 × 20-mm electrical fuses that protect the instrument from excessive electrical current.
		For AU/NZ only - 6.3A, Time-Lag, 250VAC, 5 x 20-mm.
G	Power port	The 100–240 VAC port that provides power to the instrument.

† Use the Ethernet cable supplied with the instrument to connect the instrument (Ethernet port) to the network interface card in the computer.

Barcode scanner

The QuantStudio™ 6 and 7 Flex Systems include an optional Handheld Barcode Scanner (Part no. 4453271) that you can purchase to assist you with data entry. The barcode scanner uses a 670 nm Class II laser to scan plates or array cards (for only the QuantStudio™ 7 Flex System) and it is capable of reading Code 128 (alphanumeric), which supports 128 ASCII character barcodes.

⚠ **WARNING! LASER**. Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam.

The optional hand-held barcode scanner functions as an extension of the keyboard. You can use the scanner to scan barcodes into the instrument software.

To scan a barcode using the hand-held barcode scanner:

1. Select the field in the instrument software where you want to enter the barcode.

2. Hold the hand-held barcode scanner 20–30 cm away from a plate or array card and aim at the center of the barcode, then press the trigger. Slowly move the scanning beam across the barcode until the scanner emits a high-pitched tone.

When the scanner scans a barcode, it automatically:

• Transmits the alphanumeric equivalent of the barcode to the instrument software. The software enters the barcode text wherever the cursor is active.

• Transmits a carriage-return character (the equivalent of pressing the Enter key).

For more information on the hand-held barcode scanner, see the user documentation shipped with the barcode scanner.

Twister® Robot components

IMPORTANT! The Applied Biosystems Twister® Robot component is supported only by the QuantStudio™ 7 Flex System.

The QuantStudio™ 7 Flex System supports the use of the Applied Biosystems Twister® Robot, an optional QuantStudio™ 7 Flex System accessory that consists of the components shown below.

Note: See the *Applied Biosystems Twister® Robot Automation Accessory User Guide* (Pub. no. 4470693) for information on operating, calibrating, maintaining and integrating the Twister® Robot.

Front view
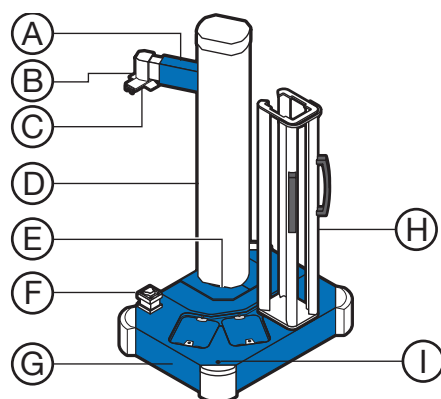
	Component	Description
A	Reach axis	Moves the grip horizontally 11.25 in (28.5 cm) to 19.75 in (50.1 cm) from the center of the robot post.
B	Wrist mechanism	Rotates materials to either the portrait or landscape positions, where the range of motion is ±135° (270° total).
C	Grip	Consists of two sets of fingers that grip the consumable. The fingers close to grasp a consumable and open to release it.
D	Robot tower/ vertical axis	Moves the arm up and down 21.5 in (54.6 cm), from 6.5 in (16.5 cm) to 28 in (71.1 cm) above the table.
E	Rotary axis	Rotates the arm 340° around the base of the Twister® Robot. Mechanical stops prevent continuous rotation.
F	Bar code reader	Scans the bar codes of consumables as they are loaded into the QuantStudio™ 7 Flex System.
G	Base cover	Removable cover that contains four access bolts, which secure the Twister® Robot to the Sciclone ALH 3000 base.
H	Racks	Provide storage for PCR consumables before and after they are run by the QuantStudio™ 7 Flex System (one of three shown).
I	Power LED	When lit, indicates the Twister® Robot is powered on.

Rear view
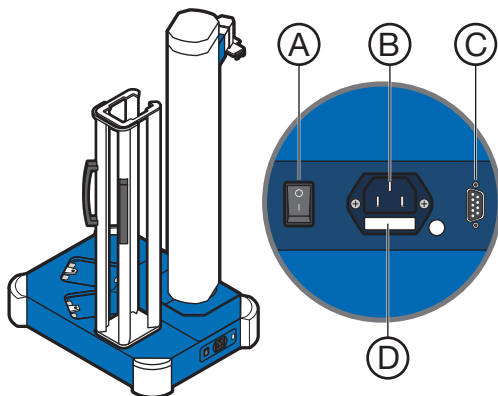
	Component	Description
A	On/Off switch	Power switch for the Twister® Robot, where the states are on (**I**) or off (**O**).
B	Power port	100–240V port that provides power to the Twister® Robot.
C	RS232 port	Provides serial communication with the computer.
D	Fuse cover	Two T1.6A 250VAC, 5 × 20-mm electrical fuses that protect the Twister® Robot from excessive electrical current.

Rack parts and functions

Racks are removable aluminum frames used as input and output locations for PCR consumables. Rack positions are numbered counter-clockwise, with position 1 closest to the front of the Twister® Robot (see below). Each rack is labeled for a specific position and cannot be exchanged with the other racks.
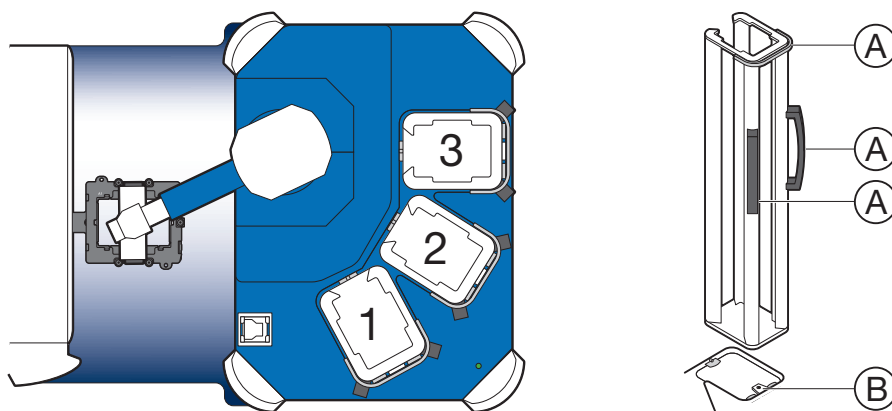
	Component	Description
A	Handles	For connecting or disconnecting racks from the pod.
B	Rack locator notch	Locks the rack onto the pod in the correct position.

Note: Do not drop the racks. If the rack is bent, the Twister® Robot cannot properly place the consumables.

**Electrical
protective devices**

We recommend several protective devices to protect the instrument in environments with large voltage and power fluctuations.

Power line regulator

We recommend the use of a 1.5-kVA power line regulator in areas where the supplied power fluctuates in excess of ± 10% of the normal voltage. Power fluctuations can adversely affect the function of the instrument.

Note: A power line regulator monitors the input current and adjusts the power supplied to the instrument or computer. It does not protect against a power surge or failure.

Uninterruptible power supply (UPS)

We recommend the use of a 1.5-kVA uninterruptible power supply (UPS), especially in areas prone to power failure. Power failures and other events that abruptly terminate the function of the QuantStudio™ 12K Flex System can corrupt data and possibly damage the computer or the instrument.

IMPORTANT! UPSs provide power for a limited time. They are meant to delay the effects of a power outage, not to serve as replacement power sources. In the event of a power loss, power off the instrument and the computer, unless you expect to regain power within the battery life of the UPS.

Surge protector

We recommend the use of a 10-kVA surge protector (line conditioner) in areas with frequent electrical storms or near devices that are electrically noisy, such as refrigerators, air conditioners, or centrifuges. Short-duration, high-voltage power fluctuations can abruptly terminate the function of, and thereby damage the components of, the computer and the instrument.

Note: A dedicated line and ground between the instrument/computer and the building's main electrical service can also prevent problems caused by power fluctuations.

Instrument software

The QuantStudio™ 6 and 7 Flex Systems include a suite of software applications that can be used to calibrate, run, and integrate the instrument into a laboratory workflow. The installation of the software contains the components described below.

The QuantStudio™ 6 and 7 Flex Systems include a suite of software applications that can be used to calibrate, run, automate, and integrate the instrument into a laboratory workflow. The installation of the software contains the components described below; however, additional software may be available for the QuantStudio™ 6 and 7 Flex Systems. Visit the QuantStudio™ 6 and 7 Flex Systems website for a complete list of compatible software, and for updates and patches for the QuantStudio™ 6 and 7 Flex System software and firmware:

www.lifetechnologies.com/us/en/home/technical-resources/software-downloads

Computer requirements

The requirements for the computer used to operate the QuantStudio™ 6 and 7 Flex Systems can vary depending on the version of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software that you are running. To determine the computer requirements for your QuantStudio™ 6 or 7 Flex System, check the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software release notes at the following location:

<drive>:\Program Files\Applied Biosystems\QuantStudio 6 and 7 Flex Software\QuantStudio 6 and 7 Software README.docx

Note: *<drive>* is the location where the software is installed.

IMPORTANT! Due to a known incompatibility, Life Technologies does not recommend installing the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to computers running the Windows Vista® operating system. If used with the operating system, the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software generates errors when saving experiment (.eds) files.

Software installation

The default installation of the instrument partitions the computer hard drive to create the logical drives as explained below.

Drive	Software	Description
C	Microsoft® Windows® OS†	Operating system files.
C or D drive	QuantStudio™ 6 and 7 Flex Real-Time PCR System Software	Used to calibrate and perform experiments on the QuantStudio™ 6 and 7 Flex System. Stores the installation files, application data files, and user files (experiments, exports, and imports).
	QuantStudio™ 6 and 7 Flex System Command Line Utility	Used to automate the creation of new experiments and the export of existing experiments.
	Twister® Robot Software‡	Controls the Twister® Robot, stores all of the taught positions for the robot, and includes the VBA code required to operate the Twister® Robot with the automation control software.

† We recommend that you do not install programs to the C drive.
‡ QuantStudio™ 7 Flex System only.

Third-party software

Before you install third-party software to the computer running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, confirm that the software will not:

- Restrict Ethernet communication
- Interfere with QuantStudio™ 6 and 7 Flex Real-Time PCR System Software operation (see below)

To confirm that third-party software does not interfere with the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software:

1. Install the software to the computer that contains the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

2. Perform several test experiments using "dummy" plates (plates that do not contain reagents).

   Note: The goal of the test experiments is to run plates under conditions that match normal instrument operation. Therefore, the characteristics of the test experiments (plate layout and run method) must closely resemble your actual experiments.

3. Confirm that the QuantStudio™ 6 and 7 Flex Systems perform each test experiment without producing errors.

   If the QuantStudio™ 6 and 7 Flex Systems perform the tests successfully, perform experiments normally. If the QuantStudio™ 6 and 7 Flex Systems encounter errors during the test runs, the software may not be compatible with the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.
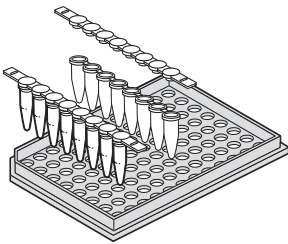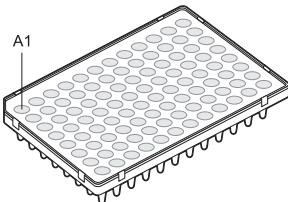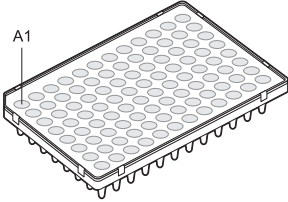
Instrument consumables
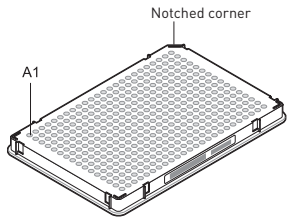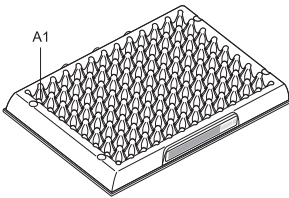
The QuantStudio™ 6 and 7 Flex Systems support a series of specialized consumables through interchangeable sample blocks. Use the consumables appropriate for the sample block of your QuantStudio™ 6 or 7 Flex Systems.

Note: The QuantStudio™ 6 Flex System does not support the Array Card block.

⚠ **WARNING!** Use the flat caps for 0.2 mL tubes and 0.1 mL tubes. Rounded caps can damage the heated cover.

Sample block	Consumable		Reaction volume
96-well plate, 0.2 mL		• MicroAmp® Optical 8-Cap Strip • MicroAmp® 8-Tube Strips (0.2-mL) • MicroAmp® Reaction Tubes without Caps (0.2-mL) • MicroAmp® 96-Well Tray/Retainer Set	100 µL
		• MicroAmp® Optical Adhesive Film • MicroAmp® Optical 96-Well Reaction Plate with Bar Code	100 µL
96-well plate, 0.1 mL		• MicroAmp® Optical Adhesive Film • MicroAmp® Optical 96-Well Fast Reaction Plate with Bar Code	50 µL

Sample block	Consumable		Reaction volume
384-well plate	Notched corner A1 	• MicroAmp® Optical Adhesive Film • MicroAmp® Optical 384-Well Reaction Plate with Bar Code	20 µL
Array card†	A1 	Life Technologies Array Card	1 µL

† QuantStudio™ 7 Flex System only.

Interchangeable sample blocks, heated covers, and plate retainers

The QuantStudio™ 6 and 7 Flex Systems feature a set of interchangeable sample blocks, heated covers, and plate retainers that allow the instruments to support multiple consumable formats. All of the hardware is user-installable. Replacement can be done without the need for recalibration of the instrument, provided that the calibrations for the new format have not expired. See Appendix B, "Maintenance" for more information about changing the sample block, heated cover, and plate retainer.

2 Calibrate the Instrument

IMPORTANT! Perform the procedures for preparing array cards only if you are verifying the performance of a QuantStudio™ 7 Flex System with an array card sample block. The QuantStudio™ 6 Flex System does not support the array card sample block.

Calibration overview

Calibration and maintenance schedule

The QuantStudio™ 6 and 7 Flex Systems require regular calibration and maintenance for proper operation. To ensure proper operation of your instrument, perform weekly, monthly, and semiannual maintenance as indicated in the following table.

IMPORTANT! Perform calibrations and run experiments under the environmental conditions specified in Appendix H, "Instrument Specification and Layout". Exposure to extreme temperatures can have adverse effects on the run results, as well as shortening the life span of the instrument components.

IMPORTANT! Do not use organic solvents to clean the QuantStudio™ 6 and 7 Flex Systems.

Frequency	User-performed maintenance task
Weekly	Check the computer disk space. If necessary, archive or back up your experiment files and instrument settings.
	Power off the computer that controls the instrument, then power on the computer after 30 seconds.
	Clean the surface of the instrument with a lint-free cloth.
Monthly	Perform a background calibration.[†]
	Run disk cleanup and disk defragmentation.
	Perform an instrument self test.
Semi-annually (every 6 months)	Perform an ROI calibration.
	Perform a background calibration.[†]
	Perform a uniformity calibration.
	Perform a dye calibration.
	Perform a normalization calibration.
As needed	Perform an RNase P instrument verification run.
	Replace the instrument lamp.[‡]

[†] You can perform a background calibration to check for contamination.

[‡] After replacing the instrument lamp, perform all calibrations and an RNAse P instrument verification run.

Planned maintenance

The instrument requires regular planned maintenance by Life Technologies service personnel to ensure that the instrument is operating within the installation specification. The planned maintenance service can help you maximize your productivity by minimizing downtime, lowering the cost of ownership by addressing potential problems before they manifest (lost data, samples, or reagents), and increasing the quality of your results by maintaining your instrument at optimal performance. For more information, contact your Life Technologies sales representative.

About maintenance reminders

The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software features an automated notification system that alerts you of required calibrations. The feature will also prevent you from operating an instrument that is out of calibration. See "Enable or change the calibration reminders" on page 31 for more information on setting the maintenance reminders.

Calibration workflow

The following figure shows the workflow for calibrating the QuantStudio™ 6 and 7 Flex Systems. Whether you are performing all calibrations or just a subset, perform them in the sequence shown below.

Start

▼

Perform an ROI calibration

▼

Perform a background calibration

▼

Perform a uniformity calibration

▼

Perform dye calibrations

▼

Perform a normalization calibration

▼

Perform an RNase P instrument verification test

▼

Finish
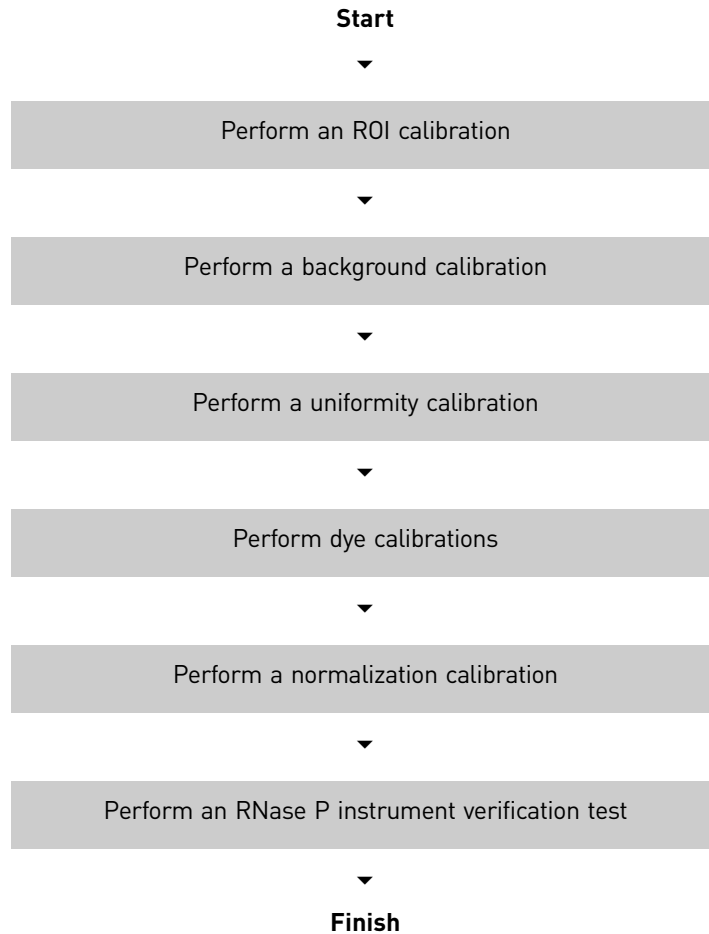
View the calibration status of an instrument

You can use the Instrument Console of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to view the calibration status of a QuantStudio™ 6 or 7 Flex System.

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click 🖥 **Instrument Console**.

2. From the Instrument Console open the Instrument Manager:

a. Select your instrument icon from the My Instruments group.

b. Click **Manage Instrument**.

Note: If the Manage Instrument option is inactive, connect your QuantStudio™ 6 or 7 Flex System to the My Instruments group.
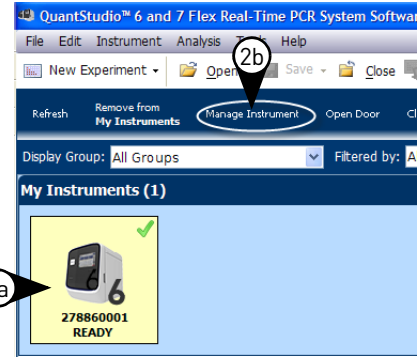
3. From the Instrument Manager, click **Monitor**, then click **Information** to open the instrument monitor.

The instrument monitor screen displays the:

a. The Instrument type (QuantStudio™ 6 Flex System or QuantStudio™ 7 Flex System).

b. Status of all calibrations, including the date and time of each calibration.

c. Maintenance status of the instrument.

d. Properties of the instrument.

278860001 (384-Well Block)
QuantStudio™ 6 Flex System
READY

Export Instrument Details | Monitor Running Experiment | Print Calibration Log | Print Maintenance Summary

Calibration Status

Type	Last Run	By	Status
ROI	06-20-2013 09:49:16 SGT	GUEST	Current
Background	06-20-2013 09:57:23 SGT	GUEST	Current
Uniformity	06-20-2013 10:04:14 SGT	GUEST	Current
⊟ Dye			
FAM	06-20-2013 10:11:09 SGT	GUEST	Current
VIC	06-20-2013 10:18:06 SGT	GUEST	Current
ROX	06-20-2013 10:27:22 SGT	GUEST	Current
SYBR	06-20-2013 10:37:27 SGT	GUEST	Current
NED	06-20-2013 10:53:57 SGT	GUEST	Current
TAMRA	06-20-2013 10:44:41 SGT	GUEST	Current
MELTDOCTOR	06-21-2013 14:40:54 SGT	GUEST	Current
⊟ Normalization			
FAM/ROX	06-20-2013 11:01:35 SGT	GUEST	Current
VIC/ROX	06-20-2013 11:08:56 SGT	GUEST	Current
⊞ HRM			
RNase P	06-21-2013 09:26:48 SGT	GUEST	Current

Maintenance Info

Property	Value
Lamp Life	46 hours
Total Cycles for Block	3,856
Total Degrees for Block	121,250

Instrument Properties

Property	Value
Instrument Label	ruo
Serial Number	278860001
Instrument Firmware Version	1.0.3
IP Address	172.28.1.105
Block Type	384-Well Block
Controller Firmware Version	1.0.2
Optics Firmware Version	24
Thermal Block Firmware Version	20
Heated Cover Firmware Version	10

Note: While viewing the Monitor screen, you can generate reports of the calibration history and status for the instrument. The following options are available from the Instrument Manager:

· **Print Calibration Log** – Prints the calibration history of the instrument.

· **Print Maintenance Summary** – Prints a summary of the current instrument calibrations.

Note: The details for an instrument in the My Preferred list can be exported even if the network connection has been interrupted. The exported details from the disconnected instrument would contain the data most recently downloaded from the instrument before the interruption.

Enable or change the calibration reminders

The calibration reminder settings allow you to configure the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to alert you by email when the QuantStudio™ 6 or 7 Flex System requires calibration. The notifications settings feature is optional, and it does not affect instrument performance.

IMPORTANT! The calibration reminders feature requires a connection between the QuantStudio™ 6 or 7 Flex System computer and a computer network. If you choose to set up the feature as described below, ensure that your network has appropriate security measures in place to protect data from threats such as computer viruses.

IMPORTANT! The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software transmits emails only while the QuantStudio™ 6 or 7 Flex System is monitored. If the network connection is interrupted, the software will stop transmitting updates.

Collect the required information

The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software requires access to a Simple Mail Transfer Protocol (SMTP) server to email calibration reminders. Contact your systems administrator or information technology department for the following information:

• Network address of an SMTP server.

• A user name and password for the server, if required for access.

• The Secure Sockets Layer (SSL) setting of the server (on or off).

Define the mail server settings

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ▸ Preferences**.

2. From the Preferences dialog box, select the **SMTP Settings** tab.

3. Define the settings for the SMTP server:

   a. From the **Outgoing Mail Server (SMTP)** field, enter the network address of a Simple Mail Transfer Protocol (SMTP) server.

   Optionally, you can specify the transmission control protocol (TCP) port for the server by appending the port number to the server name, separating the two using a colon (:).

For example: smtp.mycompany.com:2023

Note: If a TCP port is not specified, the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software uses the default port number (25).

b. Select **Encryption Required** if the mail server has SSL enabled.

c. Select **Authentication Required** if the mail server requires a user name and password.

d. If the mail server requires authentication, enter the user name and password provided by your systems administrator.
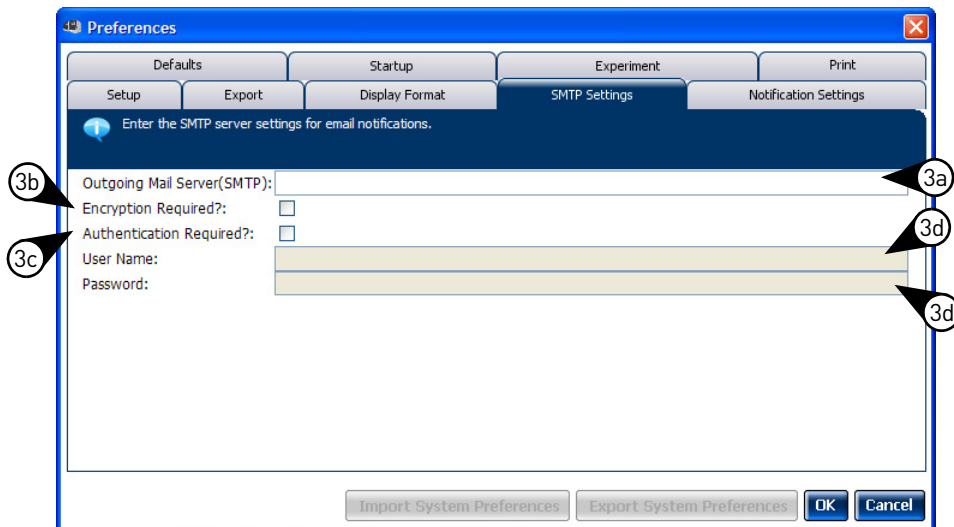
4. Click **OK**.

5. Test the mail server settings:

a. From the Home tab of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select any experiment in the Set Up panel, then click **Set Up** to create a new experiment.

b. From the experiment document, click ⚙ **Run** in the Experiment Menu, then click **Notification Setting** to view the notification settings for the experiment.

c. From the Notification Settings of the experiment document, select **Yes** to enable the Enable Notifications setting.

d. From the Enter e-mail addresses for notifications field, enter the email address that you want to receive the test emails.

e. Click **Test Configuration** to test the mail server settings.

Confirm that the email account you specified in step 5d received the test messages from the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software. If not, consult your network administrator to confirm that the mail server settings are defined correctly.
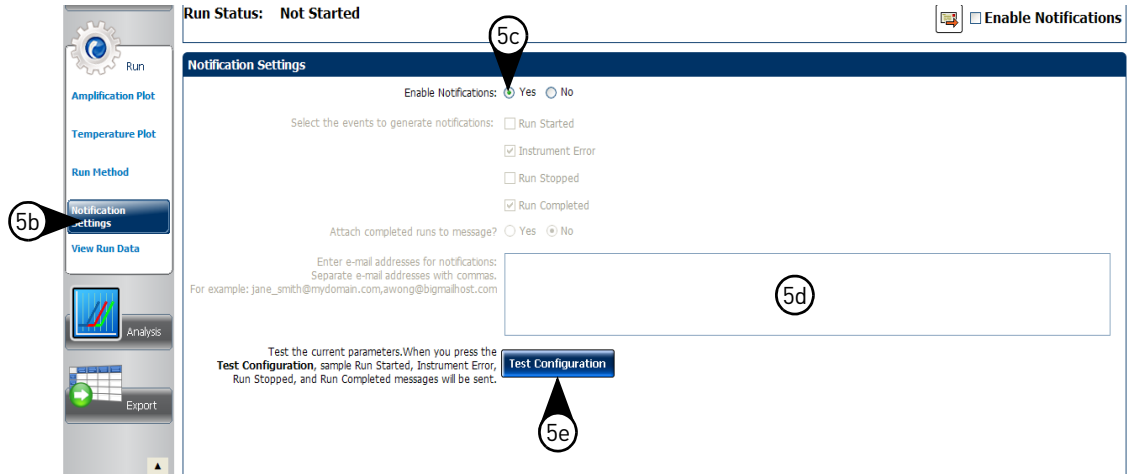
Modify the notification settings for a monitored instrument

1. Open the Calibration Reminders screen for the QuantStudio™ 6 or 7 Flex System:

    a. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

    b. From the Instrument Console, select the icon for your instrument, then click **Manage Instrument**.

        Note: If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.

    c. From the Instrument Manager, click 🛠 **Maintenance**, then click **Calibration Reminders**.

2. From the Calibration Reminders Setting table, configure the notification settings for the calibrations in interest. For each calibration that you want to monitor:

    a. From the Send a Reminder column, select the check box to configure the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to email a reminder to perform the calibration.

    b. From the Reminder Interval column, enter the number of days before the calibration expires that the software should send a reminder email.

3. From the Enter e-mail addresses for notifications field, enter the email address(es) that you want to receive notifications. Separate multiple email addresses with commas (,).

2

4. Click **Apply** to change the notification settings.

2a   2b

Calibration Reminders Settings

287880008

Calibration	Date Last Calibrated	Expiry Interval	Date Calibration Ex...	Send an Email Remi...	Reminder Interval	Reminder Date
ROI	13-07-2012 11:12:58 PDT	180	09-01-2013 11:12:58 PST	☐	7	02-01-2013 11:12:58 PST
Background	13-07-2012 11:27:13 PDT	30	12-08-2012 11:27:13 PDT	☐	7	05-08-2012 11:27:13 P...
Uniformity	13-07-2012 11:39:39 PDT	180	09-01-2013 11:39:39 PST	☐	7	02-01-2013 11:39:39 PST
⊟ Dye		180		☐	7	
Dye-FAM	13-07-2012 11:50:46 PDT		09-01-2013 11:50:46 PST	☐		02-01-2013 11:50:46 PST
Dye-VIC	13-07-2012 12:05:52 PDT		09-01-2013 12:05:52 PST	☐		02-01-2013 12:05:52 PST
Dye-ROX	13-07-2012 12:17:14 PDT		09-01-2013 12:17:14 PST	☐		02-01-2013 12:17:14 PST
Dye-CY5	13-07-2012 13:51:56 PDT		09-01-2013 13:51:56 PST	☐		02-01-2013 13:51:56 PST
⊟ Normalization		180		☐	7	
Normalization-FAM/ROX	13-07-2012 12:28:14 PDT		09-01-2013 12:28:14 PST	☐		02-01-2013 12:28:14 PST
Normalization-VIC/ROX	13-07-2012 12:38:04 PDT		09-01-2013 12:38:04 PST	☐		02-01-2013 12:38:04 PST
RNase P	13-07-2012 14:27:41 PDT	180	09-01-2013 14:27:41 PST	☐	7	02-01-2013 14:27:41 PST
⊟ HRM		180		☐	7	

Monitor

Maintenance

ROI

Background

Uniformity

Dye

Normalization

HRM

RNase P Run

1c → **Calibration Reminders**

Manage Files

▲

Email Addresses Settings

Enter e-mail addresses for notifications:
Separate e-mail addresses with ","   3

For example, "john@email.com, Kevin@email.com"

Apply ← 4

🏠 Home | 📋 Instrument Con... | 📄 287880008 ✕

Guidelines for preparing calibration plates and array cards

Observe the following guidelines when preparing calibration plates and array cards:

- Wear appropriate protective eyewear, clothing, and gloves.
- Prepare and run calibration plates and array cards within the recommended time limits listed below.

Consumable	Time to thaw	After thawed, run within...
All 96-/384-well calibration consumables[†]	30 minutes	120 minutes
All array card calibration consumables[‡]	30 minutes	120 minutes
RNase P plate (96-/384-well)	5 minutes	30 minutes
RNase P array card[§]	15 minutes	60 minutes

[†] All 96-well plates and 384-well plates used in the calibration of the instrument (ROI, background, uniformity, dye, and normalization).
[‡] All array cards used in the calibration of the QuantStudio™ 7 Flex System only (ROI, background, uniformity, dye, and normalization).
[§] For use with QuantStudio™ 7 Flex System only.

- *(Plates only)* Do not discard the packaging for the calibration plates. Each plate can be used to calibrate the QuantStudio™ 6 and 7 Flex Systems 3 times for up to 6 months if it is stored in its packing sleeve.
- Store calibration plates or array cards in a dark place until you are ready to use them. The fluorescent dyes in the wells of calibration consumables are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dyes.
- Do not allow the bottoms of the plates or array cards to become dirty. Fluids and other contaminants that adhere to the bottoms of the consumables can contaminate the sample block and cause an abnormally high background signal.
- Confirm that your centrifuge is clean. Before centrifugation, wipe down the bucket(s) using a tissue.
- Vortex and centrifuge all calibration plates to ensure complete mixing and that all reagents are contained at the bottom of the wells. The calibration plates must be well mixed and centrifuged before use.

Preparing array cards for instrument calibration

IMPORTANT! Perform the following procedure only if you are verifying the performance of a QuantStudio™ 7 Flex System with an array card sample block. The QuantStudio™ 6 Flex System does not support the array card sample block.

Required materials
- QuantStudio™ 7 Flex System Array Card Spectral Calibration Dye Kit:
  - Array Cards, empty
  - Array Card Spectral Calibration Dye Kit, including: FAM™ dye mix, VIC® dye mix, ROX™ dye mix, ROI dye mix, Background Buffer, FAM™/ROX™ dye mix, and VIC®/ROX™ dye mix
- Array Card Staker/Sealer
- Centrifuge with array card buckets and array card carrier clips
- Permanent marker or pen
- Pipettor, 200-μL (with pipette tips)
- Powder-free gloves
- Safety glasses

Filling the calibration array cards

IMPORTANT! Wear powder-free gloves while creating the calibration array cards.

This procedure explains how to create *all* of the array cards required to calibrate the QuantStudio™ 7 Flex System, but not all of them are required for a monthly maintenance. Before preparing array cards for calibration, see "Calibration and maintenance schedule" on page 28 to determine which calibrations are required.

Note: For more information on the array card loading procedure, contact Life Technologies.

1. Remove the tubes of calibration solutions from the freezer, allow them to thaw, then vortex the tubes to mix the contents well.

2. Remove the array cards from their box and place them on a clean, dry surface.

3. Mark the side of the empty array cards with: Background, FAM, ROI, ROX, VIC, FAM/ROX, and VIC/ROX.

Mark here
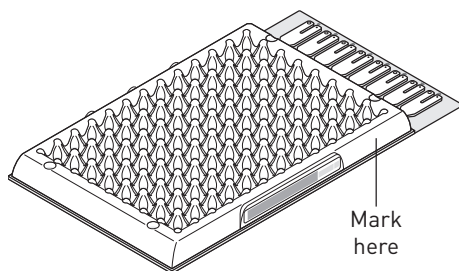
4. For each array card, pipet 100 μL of the appropriate calibration solution into each of the eight reservoirs in the array card:

   a. Place the array card on a lab bench, with the foil side down.

   b. Load 100 μL of the calibration solution into a pipette.

c. Hold the pipette in an angled position (~45 degrees) and place the tip into the fill port. The fill port is the larger of the two holes on the left side of the fill reservoir.
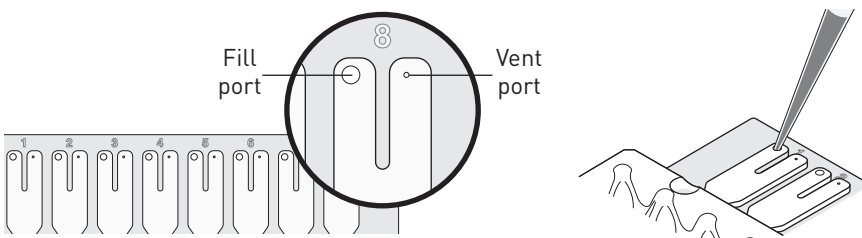
d. Dispense the fluid so that it sweeps in and around the fill reservoir toward the vent port.

Pipet the entire 100 μL into the fill reservoir, but *do not* go past the first stop of pipettor plunger or you may blow the solution out of the port.
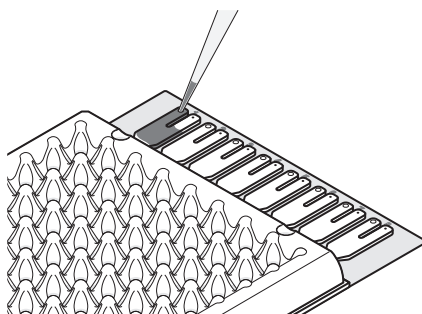
IMPORTANT! Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.

5. Repeat step 4 to fill the remaining array card with the appropriate calibration reagents.

6. Centrifuge the array card(s):

   a. Place the filled array card(s) into a centrifuge array card carrier clip and place empty array cards in the remaining slots. Confirm that the labels on the buckets and clips are oriented in the same direction.

   b. Place the filled carrier clips into the centrifuge buckets. Make sure that the array card fill reservoirs and bucket and clip labels face outward when loaded into the centrifuge.

   IMPORTANT! You must run the centrifuge with all four buckets in place and each of the two carriers filled with array cards. Place empty array cards into unfilled slots.

   IMPORTANT! Balance the loads in opposite buckets in the centrifuge.

Filled array
Empty array
This Side Out
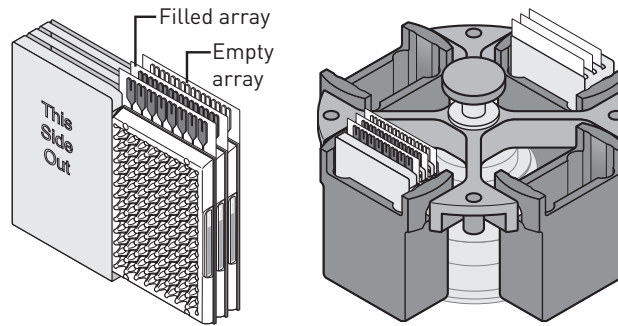
c. Close the centrifuge cover, then spin the array card(s) for 1 minute at 1200 rpm.

d. When the run is finished, stop the centrifuge, then spin the array card(s) again for 1 minute at 1200 rpm.

> **IMPORTANT!** Do not try to save time by doing one spin for 2 minutes. The two sets of ramps are important for a good fill into the array card.

7. When the second run is finished, open the centrifuge and check that the fluid levels in the reservoirs of each array card have decreased by the same amount. Also, check all wells for bubbles and note the locations as possible problems.

Correct fill	Incorrect/partial fill
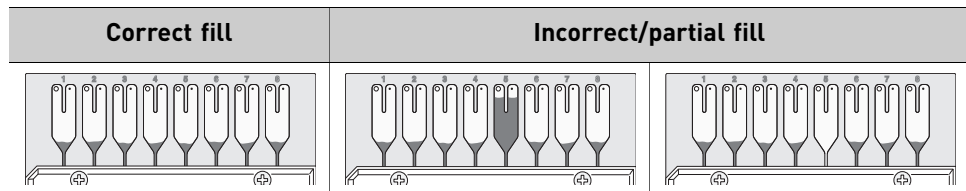
If necessary, centrifuge the array cards for an additional minute to fill any unfilled wells. Do not exceed three 1-minute runs or centrifuge the array card for longer than 1 minute at a time.

8. Seal the array card(s):

a. With the carriage (roller assembly) of the Array Card Staker/Sealer in the Start position, place a filled array card into the fixture with the foil side up so that the fill reservoirs are the farthest away from the carriage.

b. Press down on all four corners of the array card to ensure that it is fully seated within the fixture.

8a   8b   Pins
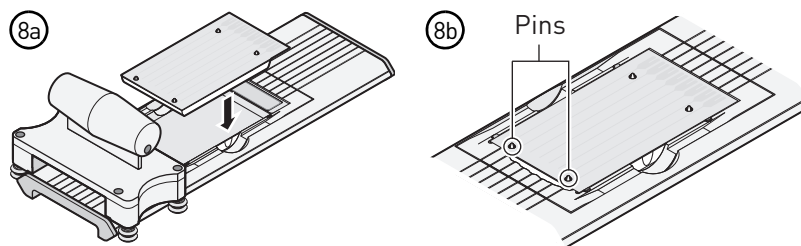
    c. Use the two alignment pins in the fixture to position the array card correctly.

    d. Seal the array card by running the carriage slowly over it. Run the carriage over the array card in one direction only. Do not apply downward force on the carriage as you move it forward over the card.
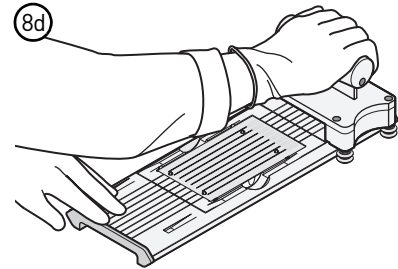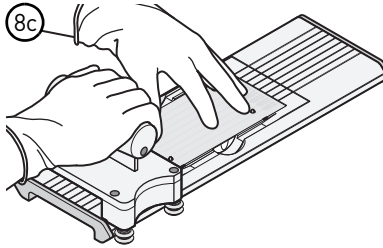
    e. Remove the sealed array card from the fixture and trim the fill reservoirs from the array card assembly using scissors. Trim the foil array card so that the edge is even with the plastic carrier.
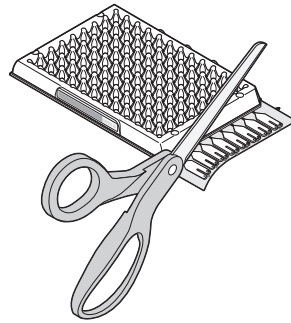
IMPORTANT! Completely remove the fill reservoirs from the array card so that the edge is free of residual plastic. The plastic from the fill reservoirs that extends beyond the edge of the card can prevent the array card from seating properly on the sample block and can affect amplification.

Correct trim	**Incorrect trim**
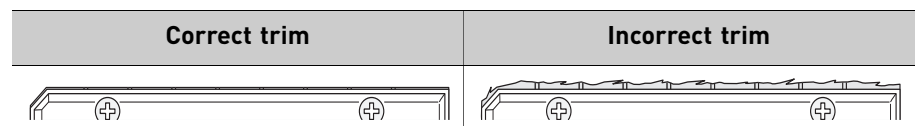
  9. Repeat step 8 to seal the remaining array cards.

IMPORTANT! If an array card is sealed improperly, the it can leak and contaminate the sample block and/or it can cause the associated calibration or RNase P test to fail.

IMPORTANT! As you seal the remaining filled array cards, store them in a dark place. Do not expose the array cards to light until you are ready to use them. The dyes in the array cards are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dye.

ROI calibration

A region of interest (ROI) calibration maps the positions of the wells on the sample block of the instrument. The software uses the ROI calibration data to associate increases in fluorescence during a run with the wells on the plate or array card. The instrument uses a set of optical filters to distinguish the fluorescence emissions gathered during runs. The software generates a calibration image for each filter to account for minor differences in the optical path.

When to perform the calibration

Perform the ROI calibration once every 6 months.
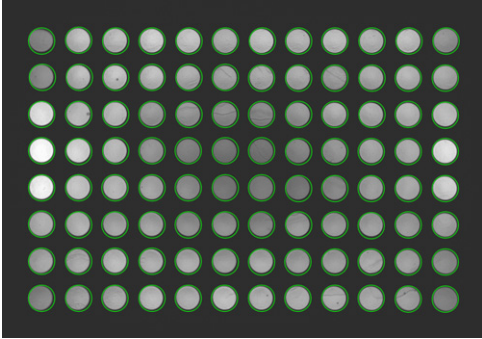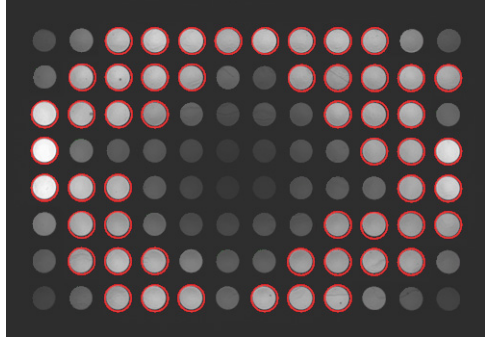
IMPORTANT! After every ROI calibration, you must perform (in order): a background calibration, uniformity calibration, dye calibration, and a normalization calibration.

About the calibration data

During the ROI calibration, the instrument software captures images of the ROI calibration plate or array card at each instrument filter. An ROI calibration passes if the collected image for each filter distinguishes all wells of the ROI plate or array card. Each well in the image must be distinct and visible at the same luminosity relative to the other wells in the image.

Note: You can review the ROI calibration image for each filter set by selecting the desired filter combination from the Filter Set menu of the ROI tab in the Instrument Manager.

Passing image	Failing image
Green circles around *all* wells indicate that the wells calibrated successfully. Each green circle indicates that the region of interest for the well position is sufficiently bright.	Red circles around some of the wells indicate that the wells did not calibrate. The absence of a circle indicates that the region of interest for the well position is not sufficiently bright.

Preparing the calibration plate or array card

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare plates or array cards.

Prepare the calibration consumable appropriate for your QuantStudio™ 6 or 7 Flex System:

Prepare the ROI calibration plate

Materials required

- Region of Interest (ROI) Plate
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Prepare the plate

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare the plate.

1. Remove the ROI calibration plate from the freezer, then thaw it at room temperature for approximately 30 minutes.

   IMPORTANT! Use the calibration plate within 2 hours of defrosting it. Until you are ready to run the plate, store it in the dark and at ambient temperature (15–30°C). Do not remove the calibration plate from its packaging until you are ready to run it. The fluorescent dyes in the wells of the plate are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dyes.

2. Remove the ROI plate from its packaging. Do not remove the optical film.

3. Vortex and centrifuge the plate:

   a. Vortex the ROI calibration plate for 5 seconds.

   b. Centrifuge the plate for 2 minutes at < 1500 rpm.

      IMPORTANT! The ROI calibration plate must be well mixed and centrifuged.

   c. Confirm that the liquid in each well of the ROI calibration plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.
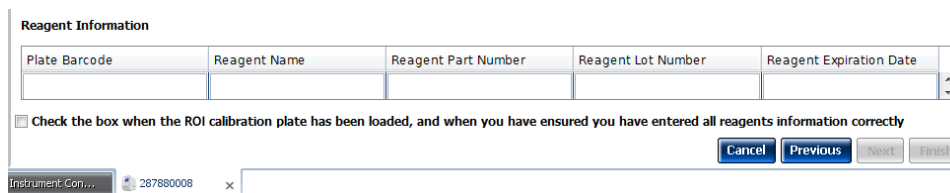
Correct	Incorrect	
Liquid is at bottom of well.	• Not centrifuged with enough force, *or* • Not centrifuged for enough time.	
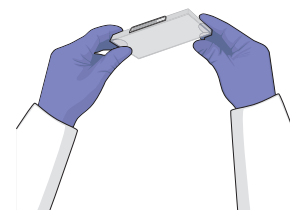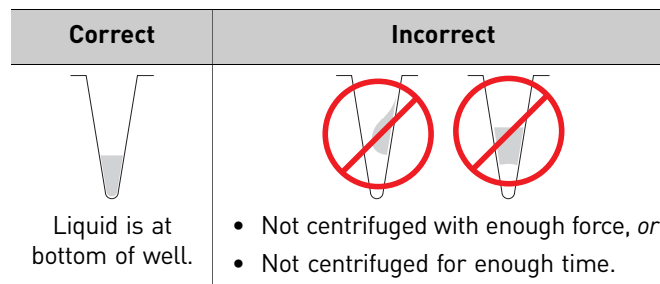
Perform the ROI calibration

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

2. From the Instrument Console, select the icon for your instrument, then click **Manage Instrument**.

   Note: If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.

3. From the Instrument Manager, start the calibration:

   a. Click 🛠 **Maintenance**, then click **ROI**.

   b. From the ROI Calibration screen, click **Start Calibration**.

4. Click **Next**, then prepare for the calibration as instructed.

5. From the bottom of the Setup tab, enter the reagent information for the ROI plate or array card that you are using.

6. Load the calibration plate or array card into the instrument:

   a. From the instrument touchscreen, touch ⏏ to eject the instrument tray.

   b. Load the plate or array card into the plate holder so that:

      • Well A1 of the plate or array card is in the top-left corner of the plate adapter.

      • The barcode faces the front of the instrument.

   IMPORTANT! Plates and array cards should be loaded and unloaded by operators who have been warned of the moving parts hazard and have been adequately trained.

   c. From the instrument touchscreen, touch ⏏ to close the instrument tray.

7. After loading the plate or array card, start the calibration:

   a. From the bottom of the Setup tab, select **Check the box when the ROI calibration plate has been loaded**, then click **Next**.

      b. From the Run screen, click **START RUN**.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the instrument is in operation.

Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach the required temperature.

8. When the run is complete and the software displays the Analysis screen, select each filter from the Filter Set drop-down list, then confirm that the corresponding ROI Image displays a green circle around each well area.

9. After you inspect all ROI images, confirm the status of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or that the data it collected is unusable.

Analysis status	Action
Passed	Click **Next**.
Failed	Troubleshoot the failed calibration as described in "ROI calibration troubleshooting" on page 70.

10. Unload the calibration plate or array card:

    a. After the calibration, touch ⏏ on the touchscreen to eject the plate or array card.

    Note: If the instrument does not eject the plate or array card, remove it as explained in "ROI calibration troubleshooting" on page 70.

    b. Remove the ROI calibration plate or array card from the instrument tray and store it in the dark at room temperature until you are ready to perform the uniformity calibration.

⚠ **WARNING! PHYSICAL INJURY HAZARD**. During instrument operation, the plate or array card temperature can reach 100°C. Allow the consumable to cool to room temperature before removing.

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    c. Touch ⏏ on the instrument touchscreen to close the instrument tray.

11. From the ROI Calibration screen, click **Finish** to complete the calibration, then click **Yes** when prompted to save the results.

Note: If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays the warning, "Saving ROI calibration will invalidate other existing calibrations except HRM Calibrations", click **OK** to proceed.

Note: HRM (High Resolution Melt) is a separately licensed module. If your instrument requires this module, contact Life Technologies.

12. (Optional) Click **Print Report** in the upper right corner of the screen to print a summary of the calibration results for your records.

Background calibration

The software uses the background calibration data during subsequent experiments to remove background fluorescence from the experiment data.

When to perform the calibration

Perform the background calibration monthly.

About the calibration data

During the background calibration, the software captures a series of images of the background plate or array card using each instrument filter. The software compares the fluorescence from each well to the average for the plate or array card. A background calibration passes if the collected images for all filters are free of abnormal fluorescence.

Background fluorescence

Fluorescence data collected by the instrument includes a fluorescence signal inherent to the instrument, referred to as "background fluorescence." Background fluorescence is a composite signal found in all spectral data that consists of fluorescence from several sources, including:

- Background electronic signal
- Contaminants in the sample block
- The plastic consumable (plate or array card)

Preparing the calibration plate or array card

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare plates or array cards.

Prepare the calibration consumable appropriate for your QuantStudio™ 6 or 7 Flex System:

Prepare the background calibration plate	**Materials required** • Background Plate • Centrifuge with plate adapter • Powder-free gloves • Safety goggles

Prepare the plate

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare the plate.

1. Remove the background calibration plate from the freezer, then thaw it at room temperature for approximately 30 minutes.

    IMPORTANT! Use the calibration plate within 2 hours of defrosting it. Until you are ready to run the plate, store it in the dark and at ambient temperature (15–30°C). Do not remove the plate from its packaging until you are ready to run it.

2. Remove the background calibration plate from its packaging. Do not remove the optical film.

3. Vortex and centrifuge the plate:

    a. Vortex the background calibration plate for 5 seconds.

    b. Centrifuge the plate for 2 minutes at < 1500 rpm.

    IMPORTANT! The background calibration plate must be well mixed and centrifuged.

    c. Confirm that the liquid in each well of the background calibration plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

    IMPORTANT! Do not allow the bottom of the plate to become dirty. Fluids and other contaminants that adhere to the plate bottom can contaminate the sample block and cause an abnormally high background signal.

Correct	**Incorrect**
Liquid is at bottom of well.	• Not centrifuged with enough force, *or* • Not centrifuged for enough time.
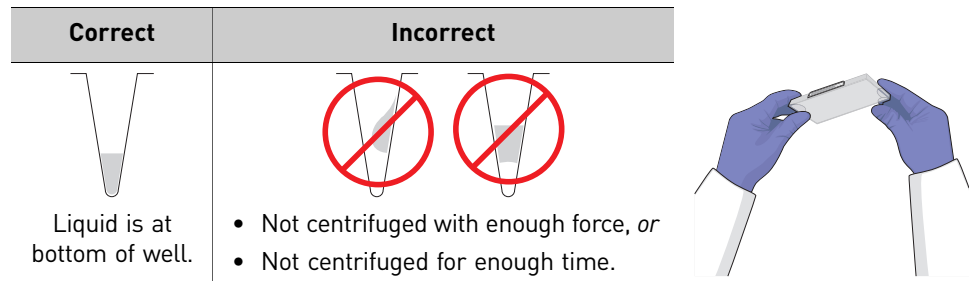
Perform the background calibration

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

2. From the Instrument Console, select the icon for your instrument, then click **Manage Instrument**.

   Note: If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.

3. From the Instrument Manager, start the calibration:

   a. Click ⚒ **Maintenance**, then click **Background**.

   b. From the Background Calibration screen, click **Start Calibration**.

4. Click **Next**, then prepare for the calibration as instructed.

5. From the bottom of the Setup tab, enter the reagent information for the background plate or array card that you are using.

6. Load the calibration plate or array card into the instrument:

   a. From the instrument touchscreen, touch ⏏ to eject the instrument tray.

   b. Load the calibration plate or array card into the plate holder so that:

      • Well A1 of the plate or array card is in the top-left corner of the plate adapter.

      • The barcode faces the front of the instrument.

   IMPORTANT! Plates or array cards should be loaded and unloaded by operators who have been warned of the moving parts hazard and have been adequately trained.

   c. From the instrument touchscreen, touch ⏏ to close the instrument tray.

7. After loading the plate or array card, start the calibration:

   a. From the Setup tab, select **Check the box when the background calibration plate has been loaded**, then click **Next**.

   b. From the Run screen, click **START RUN**.

   IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the instrument is in operation.

   Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach the required temperature.

8. When the run is complete and the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays the Analysis screen, confirm the status of the calibration. The analysis status indicates the success of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or that the data it collected is unusable.

Analysis status	Action
Passed	Click **Next**.
Failed	Troubleshoot the failed calibration as described in "Background calibration troubleshooting" on page 71.

   Note: Abnormal spectra or abnormally high background fluorescence can indicate the presence of contamination on the plate or array card or sample block, which can cause the calibration to fail.

9. Select the QC tab to review the quality check summary. The QC status indicates the quality of the calibration data, where "Passed" indicates that all wells produced data that passed the quality check, and "Failed" indicates that one or more wells produced spectra that deviate significantly from the other wells on the plate or array card.

   Note: If the calibration failed, the QC tab can provide information regarding the cause of the failure.

10. Unload the calibration plate or array card:

   a. After the calibration, touch 🔺 on the touchscreen to eject the plate or array card.

   Note: If the instrument does not eject the plate or array card, remove it as explained in "Background calibration troubleshooting" on page 71.

   b. Remove the plate or array card from the instrument tray and discard it.

   ⚠ **WARNING! PHYSICAL INJURY HAZARD**. During instrument operation, the plate or array card temperature can reach 100°C. Allow the consumable to cool to room temperature before removing.

---

   c. Touch 🔺 on the instrument touchscreen to close the instrument tray.

11. From the Background Calibration screen, click **Finish** to complete the calibration, then click **Yes** when prompted to save the results.

12. (Optional) Click **Print Report** in the upper right corner of the screen to print a summary of the calibration results for your records.

Uniformity calibration

The uniformity calibration generates data that allows the software to compensate for the physical effects of the instrument filters.

When to perform the calibration

Perform a uniformity calibration once every 6 months.

Preparing the calibration plate or array card

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare plates or array cards.

Prepare the calibration consumable appropriate for your QuantStudio™ 6 or 7 Flex System:

Prepare the ROI calibration plate

Materials required

Note: The uniformity calibration requires the same materials used to perform the ROI calibration.

- Region of Interest (ROI) Plate
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Prepare the plate

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare the plate.

If you have a prepared ROI calibration plate from a recent ROI calibration, go to step 3b below; otherwise, prepare the ROI calibration plate as follows:

1. Remove the ROI calibration plate from the freezer, then thaw it at room temperature for approximately 30 minutes.

   IMPORTANT! Use the calibration plate within 2 hours of defrosting it. Until you are ready to run the plate, store it in the dark and at ambient temperature (15–30°C). Do not remove the plate from its packaging until you are ready to run it.

2. Remove the ROI plate from its packaging. Do not remove the optical film.

3. Vortex and centrifuge the plate:
   a. Vortex the ROI calibration plate for 5 seconds.
   b. Centrifuge the plate for 2 minutes at < 1500 rpm.

      IMPORTANT! The ROI calibration plate must be well mixed and centrifuged.

c. Confirm that the liquid in each well of the ROI calibration plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.
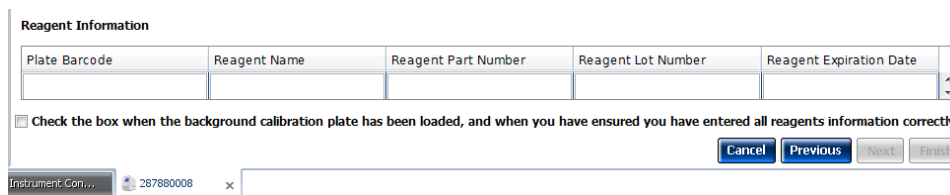
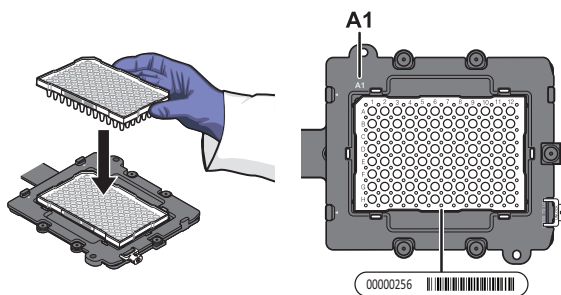| Correct	Incorrect
Liquid is at bottom of well.	• Not centrifuged with enough force, *or* • Not centrifuged for enough time.

Perform the uniformity calibration

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

2. From the Instrument Console, select the icon for your instrument, then click **Manage Instrument**.

   Note: If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.

3. From the Instrument Manager, start the calibration:

   a. Click ⚒ **Maintenance**, then click **Uniformity**.

   b. From the Uniformity Calibration screen, click **Start Calibration**.

4. Click **Next**, then prepare for the calibration as instructed.

5. From the bottom of the Setup tab, enter the reagent information for the ROI plate or array card that you are using.

Reagent Information

Plate Barcode	Reagent Name	Reagent Part Number	Reagent Lot Number	Reagent Expiration Date	

☐ Check the box when the ROI calibration plate has been loaded, and when you have ensured you have entered all reagents information correctly

Cancel   Previous   Next   Finish

Instrument Con...   287880008   ×

6. Load the calibration plate or array card into the instrument:

   a. From the instrument touchscreen, touch ⏏ to eject the instrument tray.

   b. Load the calibration plate or array card into the plate holder so that:

      • Well A1 of the plate or array card is in the top-left corner of the plate adapter.

      • The barcode faces the front of the instrument.

IMPORTANT! Plates or array cards should be loaded and unloaded by operators who have been warned of the moving parts hazard and have been adequately trained.

---

   c. From the instrument touchscreen, touch ⏏ to close the instrument tray.

7. After loading the plate or array card, start the calibration:

   a. From the Setup tab, select **Check the box when the ROI calibration plate has been loaded**, then click **Next**.

   b. From the Run screen, click **START RUN**.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the instrument is in operation.

Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach the required temperature.

8. When the run is complete and the Analysis screen displays, confirm the status of the calibration. The analysis status indicates the success of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or that the data it collected is unusable.

Analysis status	Action
Passed	Click **Next**.
Failed	Troubleshoot the failed calibration as described in "Uniformity calibration troubleshooting" on page 72.

9. Select the QC tab to review the quality check summary.

   Note: The QC tab can provide information on the cause of a failed calibration.

10. Unload the calibration plate or array card:

    a. After the calibration, touch 🔺 on the touchscreen to eject the plate or array card.

      Note: If the instrument does not eject the plate or array card, remove it as explained in "Uniformity calibration troubleshooting" on page 72.

    b. Remove the calibration plate or array card from the instrument tray and discard it.

> ⚠ **WARNING! PHYSICAL INJURY HAZARD**. During instrument operation, the plate or array card temperature can reach 100°C. Allow the consumable to cool to room temperature before removing.

    c. Touch 🔺 on the instrument touchscreen to close the instrument tray.

11. From the Uniformity Calibration screen, click **Finish** to complete the calibration, then click **Yes** when prompted to save the results.

12. (Optional) Click **Print Report** in the upper right corner of the screen to print a summary of the calibration results for your records.

Dye calibration

The software uses the calibration data collected from the dye standards to characterize and distinguish the individual contribution of each dye in the total fluorescence collected by the instrument. After each run, the instrument software receives data in the form of a raw spectra signal for each reading. The software determines the contribution of the fluorescent dyes used in each reaction by comparing the raw spectra to the pure spectra calibration data. When you save an experiment after analysis, the instrument software stores the pure spectra with the collected fluorescence data for that experiment.

When to perform the calibration

Perform a dye calibration once every 6 months.

IMPORTANT! Perform a background calibration before every series of dye calibrations. Because the age and use of instrument components can affect spectra readings, you must perform a dye calibration at least every 6 months.

About the dye calibration data

The product of a dye calibration is a collection of spectral profiles that represent the fluorescence signature of each dye standard. Each profile consists of a set of spectra that correspond to the fluorescence collected from the wells of the calibration plate or array card. Following the calibration of each dye, the software "extracts" a spectral profile for each dye. The software plots the resulting data for each profile in a graph of fluorescence versus filter.

When the software extracts the dye calibration data, it evaluates the fluorescence signal generated by each well in terms of the collective spectra for the entire calibration plate or array card. Dye spectra are generally acceptable if they peak within the same filter as their group, but diverge slightly at other wavelengths (see below).

Acceptable spectra	Unacceptable spectra
Spectra peak at the same wavelength and do not diverge significantly	Spectra peak at the different wavelengths
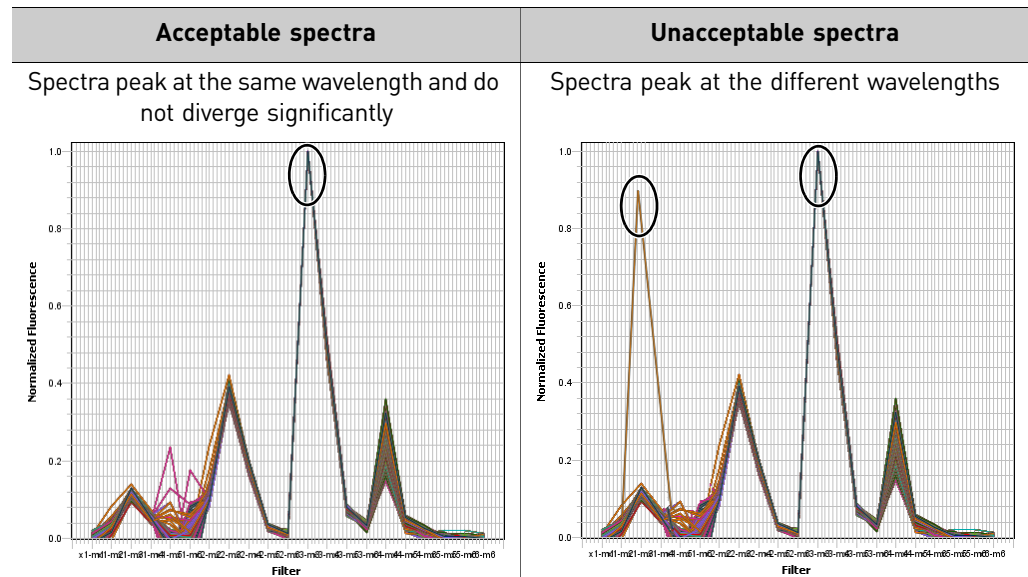
Note: Because the wells of a calibration plate or array card contain identical concentrations of a dye, the resulting signals for the wells should be similar. Variations in spectral position and peak position are caused by minor differences in the optical properties and excitation energy between the individual wells.

Preparing the calibration plate or array card

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare plates or array cards.

Prepare the calibration consumable appropriate for your QuantStudio™ 6 or 7 Flex System:

Prepare the dye calibration plates

Materials required

- Spectral calibration plates
  – Spectral Calibration Plate with FAM™ Dye
  – Spectral Calibration Plate with VIC® Dye
  – Spectral Calibration Plate with ROX™ Dye
  – Spectral Calibration Plate with NED™ Dye
  – Spectral Calibration Plate with TAMRA™ Dye
  – Spectral Calibration Plates as required by the experiment that you intend to run
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Prepare the plate

IMPORTANT! Before performing a dye calibration, you must perform an ROI calibration, a background calibration, and a uniformity calibration.

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare the plate.

1. Remove the dye calibration plate from the freezer, then thaw it at room temperature for approximately 30 minutes.

   IMPORTANT! Use the calibration plates within 2 hours of defrosting them. Until you are ready to run the plates, store them in the dark and at ambient temperature (15–30°C). Do not remove the plates from their packaging until you are ready to run them.

2. Before using each dye plate, vortex the plate for 5 seconds, centrifuge it for 2 minutes at < 1500 rpm, then confirm that the liquid in each dye plate is at the bottom of the wells. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

Correct	Incorrect	
Liquid is at bottom of well.	• Not centrifuged with enough force, *or* • Not centrifuged for enough time.	
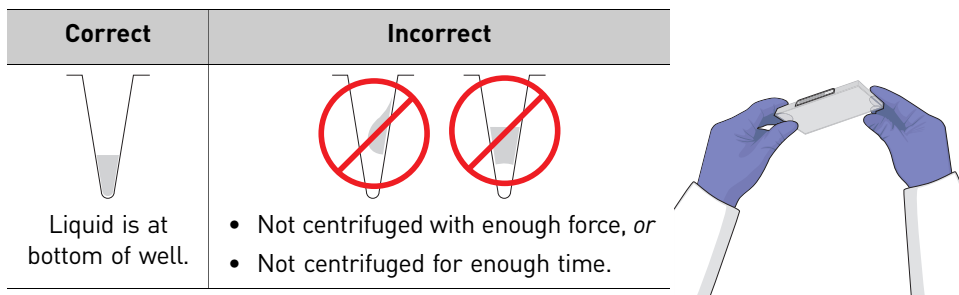
Perform the dye calibration

IMPORTANT! The software guides you through the calibration of each dye separately. You must set up, run, and analyze each dye independently.

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

2. From the Instrument Console, select the icon for your instrument, then click **Manage Instrument**.

   Note: If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.

3. From the Instrument Manager, start the calibration:

   a. Click ⚒ **Maintenance**, then click **Dye**.

   b. From the Dye Calibration screen, select **System Dye Calibration**, then click **Start Calibration**.

4. From the Dye Calibration screen, select the dye to calibrate from the Dye Name drop-down list, then perform the calibration as instructed.

5. From the bottom of the Setup tab, enter the reagent information for the dye plate or array card that you are using.

   Reagent Information

Plate Barcode	Reagent Name	Reagent Part Number	Reagent Lot Number	Reagent Expiration Date	

   ☐ Check the box when the dye calibration plate has been loaded, and when you have ensured you have entered all reagents information correctly

   Cancel   Previous   Next   Finish

   Instrument Con...   287880008   ✕

6. Load the dye plate or array card into the instrument:

   a. From the instrument touchscreen, touch ⏏ to eject the instrument tray.

   b. Confirm that the dye plate or array card matches the dye selected in the software, then load it into the plate holder.

      Load the plate or array card so that:

      • Well A1 of the plate or array card is in the top-left corner of the plate adapter.

      • The barcode faces the front of the instrument.
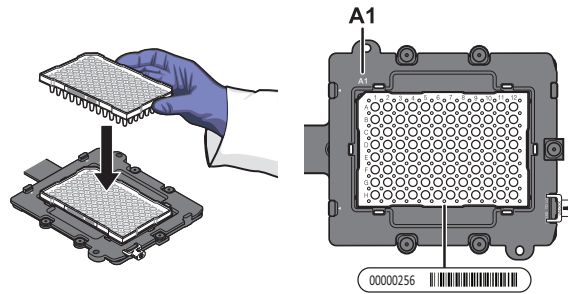
Note: The name of the dye contained by the plate or array card is next to the barcode on the front of it.

IMPORTANT! Plates or array cards should be loaded and unloaded by operators who have been warned of the moving parts hazard and have been adequately trained.

---

    c. From the instrument touchscreen, touch ▲ to close the instrument tray.

7. After loading the plate or array card, start the calibration:

    a. From the Dye Calibration screen, select **Check the box when the dye calibration plate has been loaded**, then click **Next**.

    b. From the Run screen, click **START RUN**.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the instrument is in operation.

Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach the required temperature.

8. When the run is complete and the software displays the Analysis screen, confirm the grouping of the dye spectra:

    a. Select the **Plate Layout** tab, then review the raw data. For each spectrum, confirm that the peak is:

      • Within the detectable range for the QuantStudio™ 6 and 7 Flex Systems.

      • Free of irregular spectral peaks.

      • Present at the correct filter for the dye (see "Instrument filters and supported dyes" on page 17).

    Note: Among wells containing the same dye, variations in spectral position and peak position are caused by minor differences in the optical properties and excitation energy between the individual wells.

    b. Select the **QC** tab, then review the summary of wells that failed the quality check (QC).

9. After you inspect the dye spectra, confirm the analysis status of the calibration. The analysis status indicates the success of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or that the data it collected is unusable.

Analysis status	Action
Passed	1. Click **Next**. 2. Enter comments in the Comments field, click **Finish**, then click **Yes** when prompted to save the results.
Failed	Troubleshoot the failed calibration as described in "Dye calibration troubleshooting" on page 73.

10. Select the QC tab to review the quality check summary. The QC status indicates the quality of the calibration data, where *passed* indicates that all wells produced data that passed the quality check, and *failed* indicates that one or more wells produced dye spectra that differ significantly from the other wells on the plate or array card.

    Note: If the calibration failed, the QC tab can provide information regarding the cause of the failure.

11. Unload the calibration plate or array card:

    a. After the calibration, touch on the touchscreen to eject the plate or array card.

        Note: If the instrument does not eject the plate or array card, remove it as explained in "Dye calibration troubleshooting" on page 73.

    b. Remove the calibration plate or array card from the instrument tray and discard it.

        ⚠ **WARNING! PHYSICAL INJURY HAZARD**. During instrument operation, the plate or array card temperature can reach 100°C. Allow the consumable to cool to room temperature before removing.

    c. Touch on the instrument touchscreen to close the instrument tray.

12. Repeat the calibration to calibrate the instrument for the remaining dyes that are present in the experiments that you will be running.

13. (Optional) Click **Print Report** in the upper right corner of the screen to print a summary of the calibration results for your records.

Normalization calibration

During the normalization calibration, the instrument collects and stores the data from the normalization standards.

When to perform the calibration

Perform a normalization calibration once every 6 months.

About the calibration data

During the normalization calibration, the software captures a series of images of each normalization plate or array card using each instrument filter. The normalization calibration yields a "Pass" or "Fail" result for each normalization consumable used.

Preparing the calibration plate or array card

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare plates or array cards.

Prepare the calibration consumable appropriate for your QuantStudio™ 6 or 7 Flex System:

Prepare the normalization calibration plates

Materials required
- Normalization Plate with FAM™/ROX™ dyes
- Normalization Plate with VIC™/ROX™ dyes
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Prepare the plate

IMPORTANT! Before performing a normalization calibration, you must perform an ROI calibration, a background calibration, a uniformity calibration, and a dye calibration.

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare the plate.

1. Remove the normalization calibration plates from the freezer, then allow them to warm to room temperature (approximately 30 minutes).

   IMPORTANT! Use the calibration plates within 2 hours of defrosting them. Until you are ready to run the plates, store them in the dark and at ambient temperature (15–30°C). Do not remove the plates from their packaging until you are ready to run them.

2. Go to "Perform the normalization calibration" on page 58.

   Before using each normalization plate, vortex the plate for 5 seconds, centrifuge it for 2 minutes at < 1500 rpm, then confirm that the liquid in each dye plate is at the bottom of the wells. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

Correct	Incorrect
Liquid is at bottom of well.	• Not centrifuged with enough force, *or* • Not centrifuged for enough time.

Perform the normalization calibration

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

2. From the Instrument Console, select the icon for your instrument, then click **Manage Instrument**.

   Note: If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.

3. From the Instrument Manager, start the calibration:

   a. Click 🔧 **Maintenance**, then click **Normalization**.

   b. From the Normalization Calibration screen, click **Start Calibration**.

4. From the Normalization Calibration screen, select the reporter/passive dye combination that you want to calibrate, then perform the calibration as instructed.

5. From the bottom of the Setup tab, enter the reagent information for the normalization plate or array card that you are using.

6. Load the calibration plate or array card into the instrument:

    a. From the instrument touchscreen, touch ⏏ to eject the instrument tray.

    b. Confirm that the normalization plate or array card matches the selection in the software, then load it into the plate holder so that:

        • Well A1 of the plate or array card is in the top-left corner of the plate adapter.

        • The barcode faces the front of the instrument.

A1

00000256
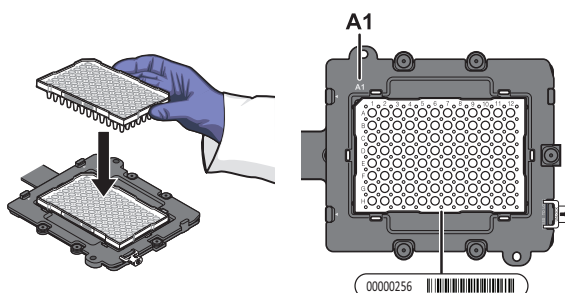
        Note: The dyes contained in each plate or array card appear next to the barcode on the front of it.

        IMPORTANT! Plates or array cards should be loaded and unloaded by operators who have been warned of the moving parts hazard and have been adequately trained.

    c. From the instrument touchscreen, touch ⏏ to close the instrument tray.

7. After loading the plate or array card, start the calibration:

    a. From the Normalization Calibration screen tab, select **Check the box when the normalization calibration plate has been loaded**, then click **Next**.

    b. From the Run screen, click **START RUN**.

    IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the instrument is in operation.

    Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach the required temperature.

8. When the run is complete and the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays the Analysis screen, confirm the status of the calibration. The analysis status indicates the success of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or that the data it collected is unusable.

Analysis status	Action
Passed	Enter comments in the Comments field, click **Next**.
Failed	Troubleshoot the failed calibration as described in the "Normalization calibration troubleshooting" on page 74.

9. Select the QC tab to review the quality check summary. The QC status indicates the quality of the calibration data, where *passed* indicates that all wells produced data that passed the quality check, and *failed* indicates that one or more wells produced unusable data.

   Note: If the calibration failed, the QC tab can provide information regarding the cause of the failure.

10. Unload the calibration plate or array card:

    a. After the calibration, touch ⏏ on the touchscreen to eject the plate or array card.

       Note: If the instrument does not eject the plate or array card, remove it as explained in "Normalization calibration troubleshooting" on page 74.

    b. Remove the calibration plate or array card from the instrument tray and discard it.

       ⚠ **WARNING! PHYSICAL INJURY HAZARD**. During instrument operation, the plate or array card temperature can reach 100°C. Allow the consumable to cool to room temperature before removing.

    c. Touch ⏏ on the instrument touchscreen to close the instrument tray.

11. From the Normalization Calibration screen, click **Finish** to complete the calibration, then click **Yes** when prompted to save the results.

12. Repeat the calibration procedure to perform the remaining normalization calibration.

13. (Optional) Click **Print Report** in the upper right corner of the screen to print a summary of the calibration results for your records.

Verify the instrument performance

When necessary, you can perform the RNase P instrument verification test to confirm the performance of the QuantStudio™ 6 and 7 Flex Systems.

When to perform the test

We recommend performing an RNase P instrument verification test:

- After moving the instrument to another location.
- As needed to confirm the function of the instrument.

About the RNase P kits

The RNase P instrument verification test uses instrument verification kits available from Life Technologies. The kits differ only in the consumable format for which they are designed: an RNase P Instrument Verification Plate for QuantStudio™ 6 and 7 Flex Systems with 96/384-well sample blocks and an Array Card RNase P Kit for the QuantStudio™ 7 Flex System with array card sample blocks.

RNase P Instrument Verification Plates

The RNase P plate is preloaded with the reagents necessary for the detection and quantitation of genomic copies of the human RNase P gene (a single-copy gene encoding the RNase moiety of the RNase P enzyme). Each well contains: PCR master mix, RNase P primers, FAM™ dye-labeled probe, and a known concentration of human genomic DNA template.

The figure to the right illustrates the arrangement of the standard and unknown populations on a 96-well and Fast 96-well RNase P plate. The RNase P plate contains five replicate groups of standards (1250, 2500, 5000, 10000, and 20000 copies), two unknown populations (5,000 and 10,000 copies), and a no template control (NTC).
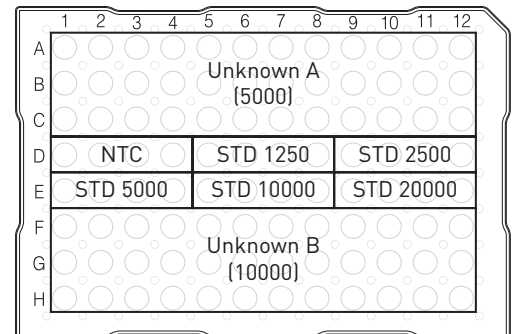
The figure to the right illustrates the arrangement of the standard and unknown populations on a 384-well RNase P plate. The RNase P plate contains five replicate groups of standards (1250, 2500, 5000, 10000, and 20000 copies), two unknown populations (5,000 and 10,000 copies), and a no template control (NTC).
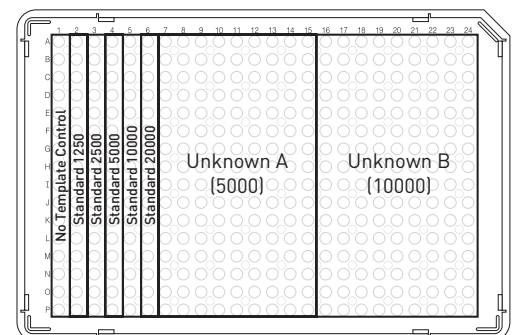
Array Card RNase P Kits

The RNase P Kits include one empty array card and eight tubes of solution. Each tube contains reaction mix (PCR master mix, RNase P primers, and FAM™-MGB dye-labeled probe) and a known concentration of human genomic DNA template.

To perform an instrument verification run, each solution is loaded into the empty array card in the arrangement shown right. When complete, the array card contains five replicate groups of standards (200, 400, 800, 1600, and 3200 copies), two of unknown populations (800 and 1600 copies), and one that serves as a no template control (NTC).

	PORT
No Template Control	1
Unknown A (800)	2
Unknown B (1600)	3
Standard 200	4
Standard 400	5
Standard 800	6
Standard 1600	7
Standard 3200	8

Analytical performance

The software performs the same analysis of data from an instrument verification run regardless of the type of consumable used.

After the run, the software:

1. Generates a standard curve from the averaged threshold cycle (C_T) values of the replicate groups of standards.

2. Calculates the concentration of the two unknown populations using the standard curve.

3. Calculates the following to assess the QuantStudio™ 6 or 7 Flex System performance:

$$[(CopyUnk_2) - 3(\sigma_{CopyUnk2})] > [(CopyUnk_1) + 3(\sigma_{CopyUnk1})]$$

where:

- $CopyUnk_1$ = Average copy number of unknown population A
- $\sigma_{CopyUnk1}$ = Standard deviation of unknown population A
- $CopyUnk_2$ = Average copy number of unknown population B
- $\sigma_{CopyUnk2}$ = Standard deviation of unknown population B

Note: Unknown population A refers to the 5,000-copy population and unknown population B refers to the 10,000-copy population in the RNase P plate.

Installation specification

The QuantStudio™ 6 or 7 Flex System passes the installation specification if the inequality holds and the instrument successfully distinguishes between unknown populations A and B with a statistical confidence level of 99.7%.

As shown in the following table, you can omit a limited number of outlier wells from the unknown populations to meet the installation specification.

Sample block	Maximum number of outlier wells that can be removed			
	Unknown population A[†]	Unknown population B[‡]	Standards (STD)[§]	No template controls (NTC)
96-well plate[††]	6	6	1	0
384-well plate	10	10	2	0
Array card[‡‡]	4	4	4	0

† 5,000-copy population for 384-well plates; 800-copy population for array cards.
‡ 10,000-copy population for 384-well plates; 1,600-copy population for array cards.
§ Maximum number of wells that can be removed from *each* standard population.
†† Standard 96-well plates or Fast 96-well plates
‡‡ QuantStudio™ 7 Flex System only.

Prepare the RNase P plate

Materials required

- RNase P Instrument Verification Plate
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Prepare the plate

IMPORTANT! When performing the RNase P instrument verification test:
- Perform all calibrations beforehand.
- Run the RNase P plate soon after you allow the plate to thaw. Minimizing the time that the prepared plate or array card sits on the bench ensures optimal performance.
- Wear powder-free gloves and safety glasses when you prepare plates.

1. Obtain the RNase P Instrument Verification Plate from the freezer, then allow the plate to warm to room temperature (for approximately 5 minutes).

   IMPORTANT! Use the RNase P plate within 30 minutes of defrosting it. Until you are ready to run the plate, store it in the dark and at ambient temperature (15–30°C). Do not remove the plate from its packaging until you are ready to run it.

2. Remove the RNase P plate from its packaging.

3. Briefly vortex and centrifuge the RNase P plate:

   a. Vortex the plate for 5 seconds.

    b. Centrifuge the reaction plate for 2 minutes at < 1500 rpm.

> **IMPORTANT!** The reaction plate must be well mixed and centrifuged.

    c. Confirm that the liquid is at the bottom of each well of the reaction plate. If not, centrifuge the reaction plate again at a greater rpm and for a longer period of time.

> **IMPORTANT!** Do not allow the bottom of the plate to become dirty. Fluids and other contaminants that adhere to the bottom of the reaction plate can contaminate the sample block and cause an abnormally high background.

Correct	Incorrect	
Liquid is at bottom of well.	• Not centrifuged with enough force, *or* • Not centrifuged for enough time.	
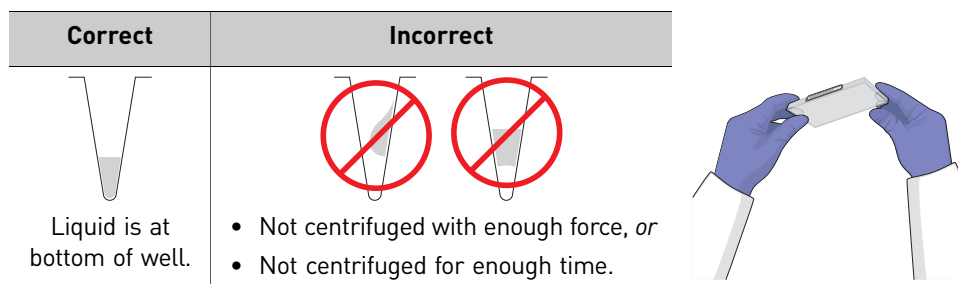
Preparing an array card for instrument verification

> **IMPORTANT!** Perform the following procedure only if you are verifying the performance of a QuantStudio™ 7 Flex System with an array card sample block.

Required materials

- Array Card Staker/Sealer
- Centrifuge with centrifuge array card carrier clips and buckets
- Powder-free gloves
- Safety goggles
- Pipettor, 200-µL (with pipette tips)
- RNase P Array Card Instrument Verification Reagents Kit:
    - Array Card
    - RNase P Array Card Instrument Verification Reagents Kit, including tubes with reagent mix for each port (8 tubes total)

Preparing the RNase P Array Card

> **IMPORTANT!** Wear powder-free gloves while preparing the array card.

1. Remove the Array Card RNase P Kit from the freezer, then allow it to warm to room temperature (for approximately 15 minutes).

> **IMPORTANT!** You must fill and run the RNase P array card within 60 minutes of thawing the Array Card RNase P Kit.

2. Remove an array card from its box and place it on a clean, dry surface.

3. Using a permanent marker, mark the side of the empty array card with RNase P.

Mark here

4. Transfer 100 µL of each solution into the appropriate port of the array card:

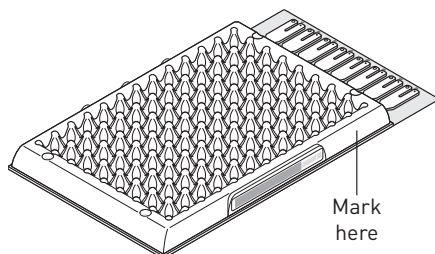|	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	PORT
A B								No Template Control																	1
C D								Unknown A (800)																	2
E F								Unknown B (1600)																	3
G H								Standard 200																	4
I J								Standard 400																	5
K L								Standard 800																	6
M N								Standard 1600																	7
O P								Standard 3200																	8

For each transfer:

a. Place the array card on a lab bench, with the foil side down.

b. Load 100 µL of fluid into a pipette.

c. Hold the pipette in an angled position (~45 degrees) and place the tip into the fill port. The fill port is the larger of the two holes on the left side of the fill reservoir.

Fill port   Vent port

d. Dispense the fluid so that it sweeps in and around the fill reservoir toward the vent port. Pipet the entire 100 µL into the reservoir, but *do not* go past the first stop of pipettor plunger or you may blow the solution out of the port.

IMPORTANT! Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.

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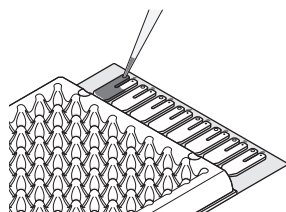
5. Centrifuge and seal the array card as explained in steps a through 8 on page 37.

6. Run the prepared array card as soon as possible after filling it.
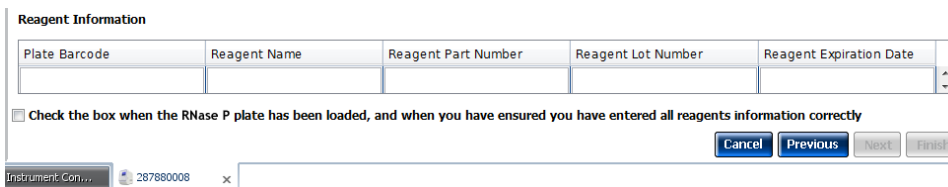
IMPORTANT! Store the array card in a dark place until you are ready to run it. The fluorescent dyes in the array card are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dye.

Run the RNase P plate or array card

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

2. From the Instrument Console, select your instrument icon, then click **Manage Instrument**.

   Note: If the Manage Instrument button is inactive, add your QuantStudio™ 6 and 7 Flex Instrument to the My Instruments group.

3. From the Instrument Manager, start the RNase P run:

   a. Click ⚒ **Maintenance**, then click **RNase P Run**.

   b. From the RNase P Run screen, click **Start RNase P Run**.

4. Click **Next**, then prepare for the instrument verification test as instructed.

5. From the bottom of the Setup tab, enter the reagent information for the RNase P plate or array card that you are using.

6. Load the RNase P plate or array card into the instrument:

   a. From the instrument touchscreen, touch 🔺 to eject the instrument tray.

   b. Load the plate or array card into the plate holder so that:

      • Well A1 of the plate or array card is in the top-left corner of the adapter.

      • The barcode faces the front of the instrument.
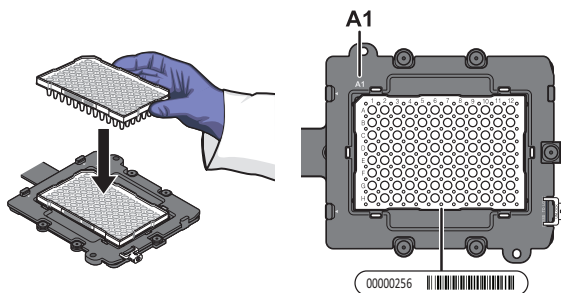
IMPORTANT! Plates and array cards should be loaded and unloaded by operators who have been warned of the moving parts hazard and have been adequately trained.

---

   c. From the instrument touchscreen, touch 🔺 to close the instrument tray.

7. After loading the plate or array card, start the instrument verification test:

   a. From the Overview screen, select **Check the box when the RNase P plate has been loaded**, then click **Next**.

   b. From the Run screen, click **START RUN** to start the test.

   IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the instrument is in operation.

   Note: Before starting the instrument verification test, the instrument may pause (up to 10 minutes) to allow the heated cover to reach the required temperature.

8. When the run is complete and the software displays the Analysis screen, confirm the status of the run.

Analysis status	Action
Passed	The QuantStudio™ 6 or 7 Flex System passed the RNase P run.
Failed	The QuantStudio™ 6 or 7 Flex System failed the RNase P run. Review the data for outliers.
	If the run fails, the software may have included outliers that caused the initial analysis to fail. Error may cause some wells to be amplified insufficiently or not at all. These wells typically produce C_T values that differ significantly from the average for the associated replicate wells. If included in the calculations, these outlying data (outliers) can result in erroneous measurements.

9. From the Amplification Plot, select **Ct vs. Well** from the Plot Type menu.

10. Confirm the uniformity of each replicate population (controls, standards, and unknowns) on the plate or array card by comparing the groupings of C_T values:

    a. From the Plate Layout, select the wells containing Unknown Population A:

    b. From the plot, confirm that the C_Ts of the replicate population are equivalent.

       Note: The numbers on the X-axis of the plot correspond to the wells of the plate or array card. Beginning with well A1, the wells are numbered from left-to-right, and top-to-bottom.

c. If an outlier is present in the selected population, select the corresponding well of the plate layout, then click **Omit** to remove the well from the analysis. If the total number of outliers for the replicate population exceeds the limit in the table below, repeat the test using another RNase P plate or array card.

Sample block	Maximum number of outlier wells that can be removed			
	Unknown population A[†]	Unknown population B[‡]	Standards (STD)[§]	No template controls (NTC)
96-well plate[††]	6	6	1	0
384-well plate	10	10	2	0
Array card[‡‡]	4	4	4	0

† 5,000-copy population for 384-well plates; 800-copy population for array cards.
‡ 10,000-copy population for 384-well plates; 1,600-copy population for array cards.
§ Maximum number of wells that can be removed from *each* standard population.
†† Standard 96-well plates or Fast 96-well plates
‡‡ QuantStudio™ 7 Flex System only.

11. Repeat step 10 for each replicate population (unknowns, standards, and no template controls) on the plate or array card.

12. Review the Results Table for quality flags generated by the test:

    a. Select the **Results Table** tab.

    b. Review the Flag column for wells that generated quality flags.

    c. Troubleshoot each well that generated a flag as explained in "RNase P test troubleshooting" on page 75.

       • AMPNC – Amplification in negative control
       • BADROX – Bad passive reference signal
       • BLFAIL – Baseline algorithm failed
       • CTFAIL – C_T algorithm failed
       • EXPFAIL – Exponential algorithm failed
       • HIGHSD – High standard deviation in replicate group
       • NOAMP – No amplification
       • NOISE – Noise higher than others on the consumable
       • NOSIGNAL – No signal in well
       • OFFSCALE – Fluorescence is offscale
       • OUTLIERRG – Outlier in replicate group
       • SPIKE – Noise spikes
       • THOLDFAIL – Thresholding algorithm failed

13. If you omitted outliers, click **Reanalyze** to analyze the run.

    If the status of the RNase P Run is "Failed" after performing step 8–12, repeat the RNase P test using a different RNase P plate or array card. If the problem persists, contact Life Technologies.

14. Review the standard curve:

   a. Select the **Standard Curve** tab.

   b. Click the upper-left corner of the Plate Layout to select all wells.

   c. Confirm that the R^2 value is ≥ 0.990.

   If the R^2 value is < 0.990, repeat the RNase P test using a different RNase P plate or array card. If the problem persists, contact Life Technologies.

15. In the Analysis screen, click **Next**, remove the plate or array card when the instrument ejects the tray arm, then discard the consumable.

   > ⚠ **WARNING! PHYSICAL INJURY HAZARD**. During instrument operation, the plate or array card can reach 100°C. Before removing the consumable, be sure to wait until it reaches room temperature.

   Note: If the instrument does not eject the plate or array card, remove it as explained in "RNase P test troubleshooting" on page 75.

16. Click **Finish**, then click **Yes** when prompted to save the test.

17. (Optional) Click **Print Report** in the upper right corner of the screen to print a summary of the RNase P test results for your records.

Troubleshooting

The tables in this section describe how to troubleshoot failed calibrations and RNase P instrument verification tests:

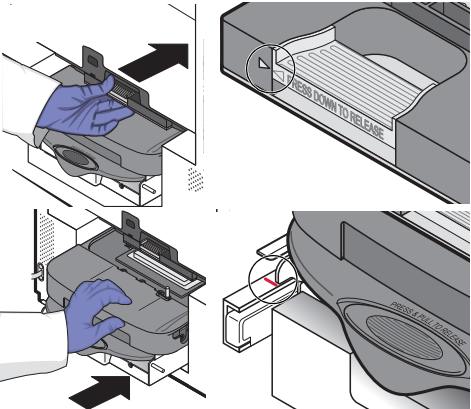**Table 1** ROI calibration troubleshooting

Problem/symptom	Possible cause	Action

ROI calibration failed.
• ROI image is blurry
• ROI image is faint
	• Plate or array card was centrifuged insufficiently.
	• Plate or array card contains old or insufficient reagents.
	• The sample block or heated cover may not be seated correctly.
		⚠ **CAUTION! PHYSICAL INJURY HAZARD**. During operation, the sample block can reach 100°C. Before removing the plate or array card, wait until the block reaches room temperature.
		
		1. Unload the instrument and confirm that you loaded the correct plate or array card. If not, perform the calibration again using the correct type of consumable.
		
		2. View the wells of the plate or array card. If the liquid in the wells is not:
		  • At the bottom of the wells, centrifuge the plate or array card for a longer time, then repeat the calibration.
		  • Equivalent in volume, the plate or array card is not sealed properly and the reagents have evaporated. Discard the consumable and run another.
		
		3. If the calibration plate or array card appears to be normal, discard it and run another.
		
		4. If the calibration fails again, check the sample block and heated cover positions:
		  a. Power off and unplug the instrument. Wait 15 minutes, then open the access door.
		  b. Firmly push the sample block and the heated cover toward the back of the instrument to confirm that they are seated correctly.
		
		    IMPORTANT! Align the arrows on the front handle of the heated cover as shown. If the arrows are not aligned, push the heated cover into the instrument until the handle locks into place.
		
		    IMPORTANT! Confirm that the indicator on the left side of the sample block is positioned behind the red line on the instrument rail. If not, push the sample block into the instrument until it is seated correctly.
		
		  c. Repeat the calibration.
		
		5. If the calibration fails again, contact Life Technologies.

Instrument does not eject the ROI plate or array card (it is stuck within the instrument).	The adhesive cover may have adhered the plate or array card to the heated cover.	Remove the plate or array card from the instrument as explained in "Remove a plate stuck within the instrument" on page 78.
Instrument malfunction.	Multiple possible causes.	Contact your local Life Technologies Field Service Office.

Table 2 Background calibration troubleshooting

Problem/symptom	Possible cause	Action

Background calibration failed.	• Plate or array card was centrifuged insufficiently.
	• Plate or array card contains old or insufficient reagents.
	• One or more wells of the background plate or array card produced spectra that exceed the maximum limit for the instrument.
		⚠ **CAUTION! PHYSICAL INJURY HAZARD**. During operation, the sample block can reach 100°C. Before removing the plate or array card, wait until the block reaches room temperature.
		
		1. Unload the QuantStudio™ 6 or 7 Flex System and confirm that you loaded the correct plate or array card into the instrument. If not, perform the calibration again using the correct type of consumable.
		2. View the wells of the plate or array card. If the liquid in the wells of the consumable is not:
		  • At the bottom of the wells, centrifuge the plate or array card for a longer time, then repeat the calibration.
		  • Equivalent in volume, the plate or array card is not sealed and the reagents have evaporated. Discard the consumable and run another.
		3. If the calibration plate or array card appears to be normal, discard it and run another.
		4. If the calibration fails again, determine the source of the contamination, as explained in "How to identify contamination" on page 78.

Instrument does not eject the background plate or array card (it is stuck within the instrument).	The adhesive cover may have adhered the plate or array card to the heated cover within the instrument.	Remove the plate or array card from the instrument as explained in "Remove a plate stuck within the instrument" on page 78.
Instrument malfunction.	Multiple possible causes.	Contact your local Life Technologies Field Service Office.

Table 3 Uniformity calibration troubleshooting

Problem/symptom	Possible cause	Action
Uniformity calibration failed.	• Plate or array card was centrifuged insufficiently. • Plate or array card contains old or insufficient reagents. • Abnormally low spectra across all wells of the plate or array card.	⚠ **CAUTION! PHYSICAL INJURY HAZARD**. During operation, the sample block can reach 100°C. Before removing the plate or array card, wait until the block reaches room temperature. 1. Unload the QuantStudio™ 6 or 7 Flex System and confirm that you loaded the correct plate or array card into the instrument. If not, perform the calibration again using the correct type of consumable. 2. Unload the QuantStudio™ 6 or 7 Flex System and view the wells of the plate or array card. If the liquid in the wells of the consumable is not: • At the bottom of the wells, centrifuge the plate or array card for a longer time, then repeat the calibration. • Equivalent in volume, the plate or array card is not sealed and the reagents have evaporated. Discard the consumable and run another. 3. If the calibration plate or array card appears to be normal, discard it and run another. 4. If the calibration fails again, contact Life Technologies.
	One or more wells produced spectra that deviate significantly from the rest of the plate or array card.	1. While viewing the calibration data in the Analysis screen, locate the well(s) with abnormal signal in the Plate Layout tab. 2. Rotate the plate or array card 180°, then perform the calibration again. 3. Determine the location of the contaminated wells again. If the position(s) of the well(s) identified in the steps above are: • Identical – The sample block is contaminated. Decontaminate the sample block. • Reversed – The ROI plate or array card is contaminated. Discard the consumable, then perform the uniformity calibration using a new ROI plate or array card. 4. If the calibration fails again, contact Life Technologies.
Instrument does not eject the ROI plate or array card (the consumable is stuck within the instrument).	The adhesive cover may have adhered the plate or array card to the heated cover within the instrument.	Remove the plate or array card from the instrument as explained in "Remove a plate stuck within the instrument" on page 78.
Instrument malfunction.	Multiple possible causes.	Contact your local Life Technologies Field Service Office.

Table 4 Dye calibration troubleshooting

Problem/symptom	Possible cause	Action

Dye calibration failed.
 	
One or more raw spectra are at or below the detectable threshold for the calibration.
	• Dye plate or array card was centrifuged insufficiently.
	• Dye plate or array card contains old or insufficient reagents.
		⚠ **CAUTION! PHYSICAL INJURY HAZARD**. During operation, the sample block can reach 100°C. Before removing the plate or array card, wait until the block reaches room temperature.
		
		1. Unload the QuantStudio™ 6 or 7 Flex System and view the wells of the plate or array card. If the liquid in the wells of the consumable is not:
		  • At the bottom of the wells, centrifuge the plate or array card for a longer time, then repeat the calibration.
		  • Equivalent in volume, the plate or array card is not sealed and the reagents have evaporated. Discard the consumable and run another.
		
		2. If the dye calibration plate or array card appears to be normal, discard it and run another.
		
		3. If the problem persists, contact Life Technologies.

• Spectra contain peaks in more than one filters. • One or more raw spectra exceed the maximum limit for the instrument.	Fluorescent contaminants are present on the sample block or dye plate or array card.	Confirm that contaminants are not present by performing a background calibration. If the background calibration does not show sample block contamination, the dye plate or array card may be contaminated.
Instrument does not eject the dye plate or array card (the consumable is stuck within the instrument).	The adhesive cover may have adhered the plate or array card to the heated cover within the instrument.	Remove the plate or array card from the instrument as explained in "Remove a plate stuck within the instrument" on page 78.
Instrument malfunction.	Multiple possible causes.	Contact your local Life Technologies Field Service Office.

Table 5 Normalization calibration troubleshooting

Problem/symptom	Possible cause	Action
Normalization calibration failed.	Abnormally low spectra across all wells of the plate or array card.	⚠ **CAUTION! PHYSICAL INJURY HAZARD**. During operation, the sample block can reach 100°C. Before removing the plate or array card, wait until the block reaches room temperature. 1. Unload the QuantStudio™ 6 or 7 Flex System and confirm that you loaded the correct plate or array card into the instrument. If not, perform the calibration again using the correct consumable. 2. If you are using the correct plate or array card, perform the calibration again using a different normalization consumable. 3. If the calibration fails again, contact Life Technologies Field Service Office.

	One or more wells produced spectra that deviate significantly from the rest of the plate or array card.	1. While viewing the calibration data in the Analysis screen, locate the well(s) with abnormal signal in the Plate Layout tab.
		2. Rotate the plate or array card 180°, then perform the calibration again.
		3. Determine the location of the contaminated wells again. If the position(s) of the well(s) identified in the steps above are:
		  • **Identical**: The sample block is contaminated. Decontaminate the sample block.
		  • **Reversed**: The normalization plate or array card is contaminated. Discard the consumable, then perform the normalization calibration using a new normalization plate or array card.
		4. If the calibration fails again, contact your local Life Technologies Field Service Office.

Instrument does not eject the normalization plate or array card (it is stuck within the instrument).	The adhesive cover may have adhered the plate or array card to the heated cover within the instrument.	Remove the plate or array card from the instrument as explained in "Remove a plate stuck within the instrument" on page 78.
Instrument malfunction.	Multiple possible causes.	Contact your local Life Technologies Field Service Office.

Table 6 RNase P test troubleshooting

Problem/symptom	Possible cause	Action

RNase P plate or array card verification run failed.	• Contamination
	• Defective seal
	• Inaccurate fluid transfer during plate or array card manufacturing
	• Insufficient centrifugation
		⚠ **CAUTION! PHYSICAL INJURY HAZARD**. During operation, the sample block can reach 100°C. Before removing the plate or array card, wait until the block reaches room temperature.
		
		1. Unload the RNase P plate or array card from the instrument.
		
		2. Hold the plate or array card up to a light source, and confirm that all wells contain the same volume of fluid.
		
		   If there are differences in fluid volumes, check the heat seal of the wells with lower volumes for signs of damage or evaporation.
		
		   Compare the position of the wells that have lower volumes with the outliers that you have removed from the plate or array card. If the well positions coincide, the heat seal on the consumable may be defective, resulting in the evaporation of the associated samples.
		
		3. Contact Life Technologies to order a replacement RNase P plate or array card. If the replacement consumable fails, contact Life Technologies for assistance.

Instrument does not eject the RNase P plate or array card (the consumable is stuck within the instrument).	Adhesive cover may have adhered the plate or array card to the heated cover within the instrument.	Remove the plate or array card from the instrument as explained in "Remove a plate stuck within the instrument" on page 78.
Negative control well displays the AMPNC flag, indicating that the well amplified.	Contamination in one or more PCR reaction components contained in the negative control well.	Contact Life Technologies to order a replacement RNase P plate or array card kit. If the replacement RNase P consumable fails, contact Life Technologies for assistance.
Well displays the BADROX flag, indicating that the passive reference signal is unacceptable for the normalization of the reporter dye signal.	• Droplets on the sides of the wells • Improper sealing or seal leaks • Condensation on the plate or array card	If a well is flagged, confirm the results: 1. Select the flagged well(s) in the plate layout or well table. 2. View the amplification plot (R_n vs. Cycle), and review the data in the C_T region for abnormalities. 3. Examine the plate or array card to check for condensation and/or inconsistent reaction volumes. 4. Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test and make sure to properly centrifuge the RNase P consumable. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance.

Problem/symptom	Possible cause	Action
Well displays the BLFAIL flag, indicating that the software cannot calculate the best fit baseline for the data.	• Amplification too late • No amplification	If a well is flagged, confirm the results: 1. Select the flagged well(s) in the plate layout or well table. 2. View the amplification plot (R_n vs. Cycle and DR_n vs. Cycle) and check for early, late, low, or no amplification. 3. Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test, making sure to properly seal and centrifuge the RNase P plate or array card. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance.
• Well displays the CTFAIL flag, indicating that the software cannot calculate the threshold cycle (C_T). • Well displays the EXPFAIL flag, indicating that the software cannot identify the exponential region of the amplification plot.	• Amplification too early • Amplification too late • Low amplification • No amplification	
Well displays the HIGHSD flag, indicating that the C_T standard deviation for the replicate group exceeds the current flag setting.	• Droplets on the sides of the wells • Improper sealing or seal leaks • Condensation on the plate or array card • Inconsistent volumes across the plate or array card	If a well is flagged, confirm the results: 1. Select the flagged well(s) and the associated replication group(s) in the plate layout or well table. 2. View the amplification plot (R_n vs. Cycle), and review the data for abnormalities. 3. Hold the plate or array card up to a light source, and check for condensation or evaporation. If there are differences in fluid volumes, check the heat seal of the wells with lower volumes for signs of damage or evaporation. 4. Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test, and make sure to properly centrifuge the RNase P consumable. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance.
Well displays the NOAMP flag, indicating that the sample did not amplify.	• Missing template • Excitation source in the instrument stopped functioning	
Well displays the NOISE flag, indicating that the well produced more noise in the amplification plot than other wells on the plate or array card.	• Droplets on the sides of the wells • Improper sealing or seal leaks • Condensation on the plate or array card	

Problem/symptom	Possible cause	Action
Well displays the NOSIGNAL flag, indicating that the well produced very low or no fluorescence signal.	Missing reaction mix resulting from pipetting error.	If a well is flagged, confirm the results: 1. Consider omitting the well from the analysis. 2. Note the location for each flagged well, and check each corresponding well in the plate or array card for evaporation or low reaction volume. 3. Contact Life Technologies to order a replacement RNase P plate or array card. If the replacement RNase P consumable fails, contact Life Technologies for assistance
Well displays the OFFSCALE flag, indicating that the fluorescence signal for one or more dyes in the well exceeds the instrument's maximum detectable range for one or more cycles.	• Fluorescent contaminant on the plate or array card or sample block • Fluorescent contaminant in the reaction	1. Perform a background calibration. If you detect fluorescent contamination, decontaminate the sample block. 2. Contact Life Technologies to order a replacement RNase P plate or array card. If the replacement consumable fails, contact Life Technologies for assistance
Well displays the OUTLIERRG flag, indicating that the C_T of the well deviates significantly from C_T values in the associated replicate group (only the outlier is flagged).	• Contamination • Improper sealing or seal leaks	1. Decontaminate the work area and pipettors. 2. Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test and make sure that the RNase P consumable is properly sealed. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance.
Well displays the SPIKE flag, indicating that the amplification curve contains one or more data points inconsistent with the other points in the curve.	• Bubbles in the reaction • Evaporation during the denaturation step because of improper sealing or seal leaks	Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test, and make sure to properly centrifuge the RNase P consumable. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance.
Well displays the THOLDFAIL flag, indicating that the software cannot calculate the threshold.	• Amplification too early • Amplification too late • Low amplification • No amplification	If a well is flagged, confirm the results: 1. Select the flagged well(s) in the plate layout or well table. 2. View the amplification plot (R_n vs. Cycle and DR_n vs. Cycle), and check for early, late, low, or no amplification. 3. Contact Life Technologies to order a replacement RNase P plate or array card. If the replacement RNase P consumable fails, contact Life Technologies for assistance.
Instrument malfunction.	Multiple possible causes	Contact your local Life Technologies Field Service Office.

How to identify contamination

Signals that exceed the limit of normal background fluorescence may indicate fluorescent contaminants on the calibration plate or array card or the sample block. Common contaminants include ink residue from permanent pens, powder from disposable gloves, and dust.

To identify and resolve a possible contamination problem:

1. While viewing the background calibration data in the Analysis screen, select the QC tab to review the list of wells that failed the quality check.

2. Eject the background plate or array card from the instrument, rotate the plate 180°, then perform the background calibration again.

3. Determine the location of the contaminated wells again as in step 1.

   If the position(s) of the contaminated well(s) in the steps above are:

   • **Identical** – The sample block is contaminated. Decontaminate the sample block as explained in "Decontaminate the sample block" on page 96.

   • **Reversed** – The background plate or array card is contaminated. Discard it, then perform the background calibration using a new background consumable.

4. If the calibration fails after you replace the background plate or array card and decontaminate the sample block, contact Life Technologies for further support.

Remove a plate stuck within the instrument

If the QuantStudio™ 6 or 7 Flex System does not eject a plate after you touch ⏏ on the touchscreen, the adhesive cover of the plate may have adhered it to the heated cover within the instrument. If so, you can remove the plate from the instrument as follows.

1. Power off the instrument. Wait 15 minutes, then power on the instrument and eject the plate.

2. If the plate does not eject, power off and unplug the instrument, then open the access door.

3. While wearing powder-free gloves, reach into the instrument and remove the plate from the heated cover, then close the access door.

4. Perform a background calibration to confirm that the sample block has not been contaminated.

A

Manual Instrument Operation

Note: This appendix describes how to operate the QuantStudio™ 6 and 7 Flex Systems manually using the touchscreen interface. Although the QuantStudio™ 6 and 7 Flex Systems can be used without an Ethernet connection to a computer, the touchscreen allows you to perform only a subset of the total instrument functions.

Overview of instrument touchscreen functions

The QuantStudio™ 6 and 7 Flex Systems feature a touchscreen interface that you can use to transfer data, manage instrument settings, and configure the instrument for network use. The touchscreen does not provide access to all instrument functions. Features such as analyzing experiments, instrument calibration, and remote notification are available only through the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

The following table summarizes the functions that are available from the QuantStudio™ 6 or 7 Flex System touchscreen.

Category	Function	Available in secure mode?[†]
Operational[‡]	Create experiments from templates	No
	Run experiments	
	Transfer and delete experiments, templates, and results to/from a USB drive	
Maintenance[§]	Back up and restore the instrument settings	Yes
	Perform an instrument self test	
	Install and record a new lamp	
	Update the instrument firmware	
Administration[††]	Define the date and time	
	Define the instrument settings	
	Define the network settings	
	Define the maintenance reminders	
	Define the system shortcuts	
	Enable or disable instrument security	
	Review the instrument statistics	
	Review the instrument version information	
	View the QuantStudio™ 6 and 7 Flex Systems log	

[†] See "Enable/disable instrument security" on page 92.
[‡] Tasks for users responsible for day-to-day use of the instrument.
[§] Tasks for users who maintain the instrument.
[††] Tasks for systems administrators or for information technology personnel.

Operate the instrument from the touchscreen

The touchscreen provides limited control of the QuantStudio™ 6 and 7 Flex Systems to run experiments and transfer data. You can perform the following functions from the touchscreen to operate the QuantStudio™ 6 and 7 Flex Systems without using the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software:

Note: If the QuantStudio™ 6 or 7 Flex System is operating in secure mode (see "Enable/disable instrument security" on page 92), users can only open and close the side door.

Create an experiment from a template

1. If necessary, download the experiment template to the QuantStudio™ 6 or 7 Flex System as described in "Transfer experiments from a USB drive" on page 83.

2. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press 🔘 .

3. In the Main Menu, touch 👁 **View Templates**.

4. In the View Templates screen, touch a template, then touch **+ New**:

   To view the parameters of a template, select the desired template, then touch 👁 **View**. When finished, touch ◀ to return to the View Templates screen.

   Note: You cannot modify the experiment parameters of a template.

5. In the Create New Experiment screen, touch each field to set the:
   - Touch the **New Experiment Name** field and use the keypad to enter a name (up to 100 characters) for the experiment.
   - Touch the **Save to Folder** field to open the Select Folder Screen.
   - Touch the **Reaction Volume** field to enter a reaction volume in µL.
   - (Optional) Touch the **Barcode Number** field to enter a barcode and touch the **Notes** field to enter notes (up to 200 characters) about the experiment.

6. When finished, either:
   - Touch 🗄 **Save & Exit**.
     or
   - Touch ⚡ **Save & Start Run** to proceed to the Start Run screen.
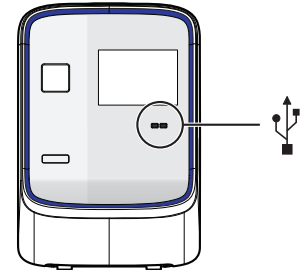
Run an experiment

1. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press ⏻ .

2. In the Main Menu screen, then touch ⏏.

3. When the side door opens, load the appropriate plate or array card. Ensure that the consumable is properly aligned in the holder.

   • (**A**) Load 96/384-well plates with the A1 position at the top-left corner of the plate adapter.

   • (**B**) Load both plates and array cards with the barcode facing the front of the instrument.

4. In the Main Menu, touch **Browse Experiments**.

5. In the Experiments screen, touch the desired experiment, then touch either:

   • ⚡ **Start Run** to start the run immediately, then go to step 10.

     or

   • 👁 **View/Edit** to view or edit the experiment before starting the run.

6. Modify the experiment parameters as needed. To:

   • Add a stage or step to the thermal profile, touch the stage or step to the left of where you want to add the stage or step, then touch 🔲 **Add**.

   • Add a melt curve to the end of the thermal profile, touch 🔲 **Add Melt Curve**.

   • Change the time or temperature of a stage or step, touch the time/ temperature field of the stage or step, modify the settings as desired, then touch **Close**.

   • Change the cycle parameter of a stage, touch the cycle field, modify the setting as desired, then touch **Close**.

   • Delete a stage or step from the thermal profile, touch the stage or step you want to remove, then touch 🗑 **Delete**.

7. When finished modifying the parameters, touch 🔲 **Save**.

8. In the Save Experiment screen, touch each field to set the experiment, name, reaction volume, barcode, and any additional information to save to the experiment

9. When finished, touch ⚡ **Save & Start Run** to start the experiment.

10. In the Start Run screen, touch each field as needed to modify the associated parameter, then touch ⚡ **Start Run Now** to start the experiment.
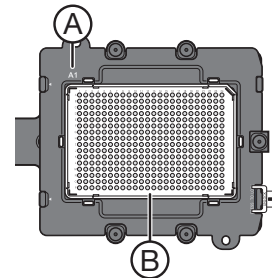
    Note: When the run is complete, touch ⏏ to unload the plate. You can download the experiment results to a computer if the QuantStudio™ 6 or 7 Flex System is connected to a network, or you can copy the data to a USB device (see "Transfer experiments, templates, and results data" on page 83).

Transfer experiments, templates, and results data

You can transfer experiments, templates, and results data to/from the QuantStudio™ 6 and 7 Flex Systems using a USB flash drive. Before transferring data, you must plug the drive into the USB port below the touchscreen.

IMPORTANT! Do not use the USB ports on the rear panel of the QuantStudio™ 6 and 7 Flex Systems. The rear USB ports are only for use by Life Technologies personnel to service the instrument.

Transfer templates from a USB drive

1. Plug a USB drive into the USB port below the touchscreen.

2. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press ⏻ .

3. In the Main Menu, touch 👁 **View Templates**.

4. In the Browse Experiments screen, select the template:

    a. Touch 🗀 , then touch **USB**.

    b. Touch the desired template, then touch 🖫 **Save**.

5. In the Save Experiment As screen, set the name for the file.

    a. Touch the New Template Name field, then enter a name for the copied file.

    b. Touch the Save to Folder field, then select the folder to receive the file.

    c. Touch **Save**.

6. Touch 🏠 to return to the Main Menu.

7. Unplug the USB drive.

Transfer experiments from a USB drive

1. Plug a USB drive into the USB port below the touchscreen.

2. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press ⏻ .

3. In the Main Menu, touch **Browse Experiments**.

4. In the Browse Experiments screen, select the experiment:

    a. Touch 🗀 , then touch **USB**.

    b. Touch the desired experiment, then touch 🖫 **Save**.

5. In the Save Experiment As screen, touch the experiment that you want to transfer to the USB drive, then touch **Save**.

6. Touch 🏠 to return to the Main Menu.

7. Unplug the USB drive.

Copy experiment results to a USB drive

1. Plug a USB drive into the USB port below the touchscreen.

2. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press ⏻ .

3. From the Main Menu of the instrument touchscreen, touch **Collect Results**.

4. From the list of experiments, touch the experiment(s) of interest or touch 🔾 **Select All**.

5. Touch 🖉 **Save to USB**.

6. From the Copy Results To USB screen, check that the name of the USB drive is correct to ensure that it is mounted, then touch 🖉 **Save to USB**.

7. Touch 🏠 to return to the Main Menu.

8. Unplug the USB drive.

Note: After the results from a completed run have been collected, the corresponding experiment displays "Collected" and it can be deleted.

Delete results

Once you have collected results from the QuantStudio™ 6 or 7 Flex System, you can use the touchscreen to delete the files from the instrument hard drive.

1. From the Main Menu of the instrument touchscreen, touch **Collect Results**.

2. From the list of experiments, touch the experiment(s) of interest, then touch 🗑 **Delete**.

3. When asked to confirm the deletion, touch **OK**.

4. Touch 🏠 to return to the Main Menu.

Maintain the instrument from the touchscreen

The QuantStudio™ 6 and 7 Flex Systems touchscreen provides access to a few maintenance functions that cannot be accessed remotely from the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software. Unlike the maintenance performed using the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, some of the touchscreen functions are not part of the recommended maintenance schedule. The frequency that you perform them depends on your laboratory policies.

Note: The touchscreen does not provide access to all instrument functions. Features such as instrument calibration and remote notification are available only through the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

Back up and restore the instrument settings

You can use the QuantStudio™ 6 or 7 Flex System touchscreen to back up the instrument settings (icon, standby time-out, and cover idle temperature), and some network settings (the Autodiscovery and Smart Monitoring options). If the QuantStudio™ 6 or 7 Flex Instrument settings are reset, you can restore the settings using the backup.

Note: The backup feature can be used as an administrative tool to manage QuantStudio™ 6 and 7 Flex Systems. You can use the feature to create a standard "image" for a QuantStudio™ 6 or 7 Flex Instrument that can then be restored on other instruments to bypass the manual setup process.

The QuantStudio™ 6 and 7 Flex Systems back up to and restores instrument settings from a USB flash drive. Before backing up or restoring settings, you must plug the drive into one of the USB ports below the touchscreen.
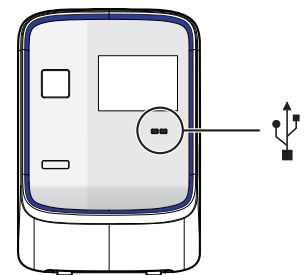
IMPORTANT! Do not use the USB ports on the rear panel of the QuantStudio™ 6 and 7 Flex Systems. The rear USB ports are for use by Life Technologies personnel to service the instrument.

Back up the instrument settings

1. Plug a USB drive into the USB port below the touchscreen.

2. From the Main Menu of the instrument touchscreen, touch **Tools**, then touch **Backup Settings**.

3. From the Backup Settings screen, touch ⚙ **Backup**.

4. Wait for the backup to complete, then touch 🏠 to return to the Main Menu.

5. Unplug the USB drive.

Note: For administrative purposes, you can reuse the instrument settings saved to the USB drive to configure more than one QuantStudio™ 6 or 7 Flex System. Note that you must configure the network settings for each instrument individually.

Restore the instrument settings

1. Plug the USB drive that contains the instrument settings into the USB port below the touchscreen.

2. From the Main Menu of the instrument touchscreen, touch **Tools**, then touch **Restore Settings**.

3. From the Restore Settings screen, select the settings to restore:

   a. Touch the settings that you want to restore from the list.

   b. Touch 🔧 **Restore** to upload the instrument settings from the USB drive.

   IMPORTANT! Do not remove the USB drive from the QuantStudio™ 6 or 7 Flex System until instructed.

   Note: Alternatively, touch **Restore Default Settings** to restore the QuantStudio™ 6 or 7 Flex System to the factory settings.

4. After the QuantStudio™ 6 or 7 Flex System restarts, unplug the USB drive.

Perform an instrument self test

You can use the QuantStudio™ 6 or 7 Flex System touchscreen to perform a comprehensive self test of the QuantStudio™ 6 or 7 Flex System subsystems. After the self test is complete, the QuantStudio™ 6 or 7 Flex System generates two files that provide a detailed summary of the instrument condition and function. In the event of a problem, you can save the results files to a USB drive and email them to Life Technologies technical support for a diagnosis.

Note: We recommend running the self test as part of regular maintenance to ensure optimal performance of the QuantStudio™ 6 or 7 Flex System.

1. From the Main Menu of the instrument touchscreen, touch **Tools**, then touch **Run Self Test**.

2. From the Self Test screen, touch ☑ **Start Self Test**, then wait for the test to complete.

3. (Optional) When the QuantStudio™ 6 or 7 Flex System completes the self test, save the results to a USB drive:

   a. Plug a USB drive into the USB port below the touchscreen.

      IMPORTANT! Do not use the USB ports on the rear panel of the QuantStudio™ 6 and 7 Flex Systems. The rear USB ports are for use by Life Technologies personnel to service the instrument.

      ---
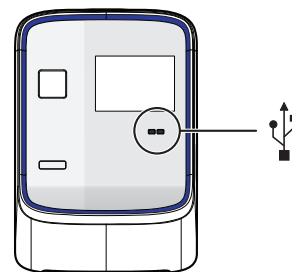
    b. Touch 🗄 **Save**.

> **IMPORTANT!** Do not remove the USB drive from the QuantStudio™ 6 or 7 Flex System until instructed.

    c. When the QuantStudio™ 6 or 7 Flex System finishes writing the results to the USB drive, touch **OK**, then remove the USB drive.

4. Touch 🏠 to return to the Main Menu.

Update the instrument firmware

You can download QuantStudio™ 6 and 7 Flex Systems firmware updates directly from the service section of the Life Technologies website. After obtaining a firmware update, transfer the update to the QuantStudio™ 6 or 7 Flex System using a USB drive.

1. Download the firmware update:

    a. Go to **www.lifetechnologies.com/us/en/home/technical-resources/ software-downloads**

    b. In the Software Downloads page, select **Applied Biosystems QuantStudio™ 6 and 7 Flex Real-Time PCR Systems** from the menu.

    c. In the Software Downloads page for your QuantStudio™ 6 or 7 Flex System, click **Updates - Patches**.

    d. Download the QuantStudio™ 6 and 7 Flex Systems firmware to a USB drive.

2. Plug a USB drive into the USB port below the touchscreen.

> **IMPORTANT!** Do not use the USB ports on the rear panel of the QuantStudio™ 6 and 7 Flex Systems. The rear USB ports are for use by Life Technologies personnel to service the instrument.
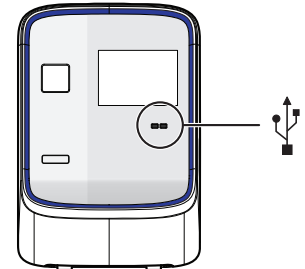
3. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press ⏻ .

4. In the Main Menu, touch **Tools**, then touch ⬆ **Upgrade Firmware**.

5. In the Upgrade Firmware screen, select the update package, then touch ⬆ **Upgrade Firmware**. Allow the QuantStudio™ 6 or 7 Flex System to complete the upgrade.

> **IMPORTANT!** Do not remove the USB drive from the QuantStudio™ 6 or 7 Flex System until you are instructed to do so.

6. After the upgrade is complete and the QuantStudio™ 6 or 7 Flex System reboots, confirm the upgrade success:

    a. Unplug the USB drive.

    b. Touch **Settings**, then touch **About this instrument** to view the software version number to confirm that the firmware has been upgraded.

Administrate the instrument from the touchscreen

The touchscreen provides access to several administrative functions that you can use to integrate the QuantStudio™ 6 and 7 Flex Systems into a laboratory workflow. The following functions are available from the touchscreen and can be used after installation to customize the instrument settings and configure it for network use.

Note: The touchscreen does not provide access to all instrument functions. Features such as instrument calibration and remote notification are available only through the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

Define the date and time

IMPORTANT! If your laboratory is subject to daylight savings time changes, you must manually adjust the time settings during daylight savings to ensure that the internal clocks of your computer and instrument are synchronized.

1. From the Main Menu of the instrument touchscreen, touch **Settings**, then touch **Set Date & Time**.

2. From the Set Date & Time screen:

    a. Touch the **Time zone** field, then touch the correct time zone from the list.

    b. Touch the **Date** field, enter the current date, then touch **Done**.

    c. Touch the **Date Format** drop-down list, then select the format for your region.

    d. Touch each Time field, enter the appropriate time units, then touch **Done**.

    e. Touch **12 Hour** or **24 Hour** to select the appropriate time format.

    f. Touch **Save** to save the settings, then touch **OK** when prompted.

3. Touch to return to the Main Menu.

Define the instrument settings

1. From the Main Menu of the instrument touchscreen, touch **Settings**, then touch **Configure the Instrument**.

2. Touch the **Instrument Name** field, enter up to a 25-character name for the QuantStudio™ 6 or 7 Flex System, then touch **Done**.

The instrument name is the alphanumeric string used to identify the QuantStudio™ 6 or 7 Flex Instrument on the network.

IMPORTANT! To connect the QuantStudio™ 6 or 7 Flex System to a network, the name must be unique.

IMPORTANT! The instrument name cannot include spaces or special characters (such as; : " < > * + = \ | ? ,).

3. Upload the instrument icon:

   The instrument icon is the graphic used to represent the QuantStudio™ 6 or 7 Flex System in the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Instrument Console:

   a. Save the replacement graphic to a USB drive, then plug the drive into the USB port below the touchscreen.

      IMPORTANT! Do not use the USB ports on the rear panel of the QuantStudio™ 6 and 7 Flex Systems. The rear USB ports are for use by Life Technologies personnel to service the instrument.
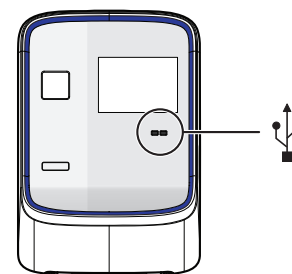
   b. Touch **Upload Icon**, select the desired graphic file, then touch **Done**.

      Note: The replacement graphic must be a maximum of 48 × 48 pixels and be stored in the portable net graphic (PNG) format.

4. Define the standby time-out setting:

   a. Select **Standby Time-out** to activate the feature.

   b. Touch the **Standby Time-out** field.

   c. Enter the number of minutes (1–300) that the QuantStudio™ 6 or 7 Flex System should remain idle until it enters standby mode, then touch **Done**.

   Note: When in standby mode, the QuantStudio™ 6 or 7 Flex System powers off the LCD screen backlight and enters low-power mode.

5. Define the heated cover temperature setting:

   a. Select **Cover Idle Temperature** to activate the feature.

   b. Touch the **Cover Idle Temperature** field.

   c. Enter the temperature (50.0–110.0°C) that the heated cover should maintain when the QuantStudio™ 6 or 7 Flex System is idle, then touch **Done**.

      Note: The recommended Cover Idle Temperature is 60.0°C for reaction plate formats and 85.0°C for the array card format.

6. Touch **Save** to save the settings, then touch **OK** when prompted.

7. Unplug the USB drive.

8. Touch 🏠 to return to the Main Menu.

Define the maintenance reminders

You can use the QuantStudio™ 6 or 7 Flex System touchscreen screen to:

- Set the expiration period for instrument calibrations and lamp replacement.
- Activate, deactivate, or change the frequency of the maintenance reminders displayed by the QuantStudio™ 6 or 7 Flex System.

To set the reminder:

1. From the Main Menu of the instrument touchscreen, touch **Settings**, then touch **Set Maintenance Reminders**.

2. For each reminder:

   a. Touch the **Calibration expires after** field.

   b. Use the keypad to enter the number of days (or, for the lamp, the number of hours) that should elapse before expiration, then touch **Done**.

   c. (Optional) Touch the checkbox to activate or deactivate an expiration reminder, then go to step 3.

3. (Optional) Configure the reminder:

   a. Touch the **Display reminders before** field.

   b. Enter the number of days (or, for the lamp, the number of hours from 1-2000) before which the instrument should display warnings of the impending expiration, then touch **Done**.

4. Touch **Save** to save the settings, then touch **OK** when prompted.

5. Touch 🏠 to return to the Main Menu.

Define the network settings

1. From the Main Menu of the instrument touchscreen, touch **Settings**, then touch **Set Network Information**.

   Note: The Set Network Information screen displays the Media Access Control (MAC) address of the QuantStudio™ 6 or 7 Flex System below the Autodiscovery and Smart Monitoring check boxes. The MAC address can be used to uniquely identify the QuantStudio™ 6 or 7 Flex System on the network.

2. Touch **Autodiscovery** to make the QuantStudio™ 6 or 7 Flex System discoverable by computers that are running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

3. Touch **Smart Monitoring** to enable the feature on the QuantStudio™ 6 or 7 Flex System.

   The Smart Monitoring feature allows Life Technologies service personnel to monitor the status of the QuantStudio™ 6 or 7 Flex System remotely through an internet connection. Smart Monitoring employs multiple layers of security, including a Secure Sockets Layer (SSL) and Lightweight Directory Access Protocol (LDAP) authentication, to provide real-time troubleshooting and problem resolution for the QuantStudio™ 6 or 7 Flex System. For a detailed description of the Smart Monitoring Service, see the *Smart Monitoring Service Product Bulletin: Leveraging the power of the Internet while maintaining system security* (Part no. 121PB07-03).

4. Set the Internet Protocol (TCP/IP) Properties for either DHCP or Static IP communication.

Network service	Action
DHCP	Touch **Obtain an IP address automatically**.
Static IP	1. Touch **Use the following IP address**. 2. Touch the **IP Address** field, enter the IP address using the keypad, then touch **Done**. 3. Assign the: • IP addresses for the DNS Servers (primary and secondary) • Subnet Mask setting • Default Gateway setting

5. Touch ☰ **Save** to save the settings, then touch **OK** when prompted.

6. Touch 🏠 to return to the Main Menu.

Define the system shortcuts

You can use the QuantStudio™ 6 or 7 Flex System touchscreen to map the shortcut buttons that appear in the Main Menu. You can configure shortcuts to automatically open specific files and folders so that you can access data quickly and easily without having to navigate to it.

1. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press 🔘 .

2. In the Main Menu, configure the shortcuts as desired:

   To *add* a shortcut:

   a. Touch the shortcut of interest, then touch 🔗 **Set Shortcut**.

   b. Touch 📁 **From Templates** to link to a specific template file or touch 📁 **From Folders** to link to a folder.

   c. Touch the desired template file or folder to configure the shortcut.

   To *delete* a shortcut, touch the shortcut of interest, then touch 🗑 **Remove Shortcut**, or touch **Remove All** to delete all shortcuts.

3. When you are finished configuring the shortcuts, touch 🏠 to return to the Main Menu.

Review the instrument statistics

You can use the QuantStudio™ 6 or 7 Flex System touchscreen to view usage statistics on the heated cover, lamp, and other instrument components.

1. From the Main Menu of the instrument touchscreen, touch **Tools**, then touch **Show Statistics**.

2. When you are finished, touch 🏠 to return to the Main Menu.

Review the instrument version information

You can use the QuantStudio™ 6 or 7 Flex System touchscreen to view instrument version data, such as instrument serial number and version numbers for most instrument components and firmware.

1. From the Main Menu of the instrument touchscreen, touch **Tools**, then touch **About the Instrument**.

2. When you are finished, touch 🏠 to return to the Main Menu.

A

Enable/disable instrument security

The QuantStudio™ 6 and 7 Flex Systems feature a secure mode that can be enabled to restrict local instrument functionality. When security is enabled, use of the touchscreen is restricted to administrative functions that change the instrument settings. After the QuantStudio™ 6 or 7 Flex System is secured, you must enter an administrator password to modify the instrument settings, use the firmware tools, or deactivate the secure mode.

IMPORTANT! If you enable or disable the QuantStudio™ 6 and 7 Flex Systems security, auditing, and electronic signature feature, you must similarly enable or disable the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software security (see page 131). The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software cannot connect to QuantStudio™ 6 and 7 Flex Systems that do not match security settings.

Note: Secure mode limits the number of features that are available from the QuantStudio™ 6 or 7 Flex System touchscreen; it does not provide user authentication functionality through the instrument touchscreen.

1. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press 🔘 .

2. In the Main Menu, touch **Settings**, then touch **Set Administrator Options**.

3. In the Set Administrator Options screen, touch **Secure Environment** to enable (checked) or disable (unchecked) system security.

4. (Optional) To change the administrator password:

   a. Touch **Change Password**.

   b. Enter the current password, then touch **Done**.

   c. Enter the new password, then touch **Done**.

   d. Reenter the password when prompted.

   e. Touch **OK** when prompted.

   Note: The default password for the QuantStudio™ 6 or 7 Flex System touchscreen is *password*; however, the password can be changed during installation.

5. Touch 📁 **Save**.

6. Touch the Administrator Password field, enter the administrator password, then touch **Done**.

7. Touch 🏠 to return to the Main Menu.

View the instrument log

You can use the QuantStudio™ 6 or 7 Flex System touchscreen to view a log that summarizes instrument activity from the last 6 months. For each recorded activity, the activity log provides a description of the activity and the date/time when it occurred.

1. From the Main Menu of the instrument touchscreen, touch **Tools**, then touch **View Log**.

2. From the View Log screen, configure the settings to display the records of interest:

   • Select an option from the drop-down menu to filter the log.

   • Select **Earliest First** or **Latest First** to determine the order to sort the records.

3. (Optional) Plug the USB drive into the port below the touchscreen, then touch **Export to USB** to save the log to the drive.

IMPORTANT! Do not use the USB ports on the rear panel of the QuantStudio™ 6 and 7 Flex Systems. The rear USB ports are for use by Life Technologies personnel to service the instrument.

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4. Touch 🏠 to return to the Main Menu.

B   Maintenance

IMPORTANT! This chapter contains all user service procedures for the QuantStudio™ 6 and 7 Flex Systems. Procedures other than those described in this document must be performed by a qualified Life Technologies Service Engineer.

Decontaminate the sample block

Perform this procedure to eliminate fluorescent contaminants from the QuantStudio™ 6 or 7 Flex System sample block. Contamination is generally evident in failed background calibrations where one or more wells consistently exhibit abnormally high signals.

> ⚠ **CAUTION! PHYSICAL INJURY HAZARD**. Do not remove the QuantStudio™ 6 or 7 Flex System cover. There are no components inside the instrument that you can safely service yourself. If you suspect a problem, contact a Life Technologies Service Representative.

> ⚠ **CAUTION! PHYSICAL INJURY HAZARD**. During instrument operation, the sample block can reach 100°C. Allow the sample block to cool to room temperature before performing the procedure.

> ⚠ **CAUTION!** Before using a cleaning or decontamination method other than those recommended by the Life Technologies, confirm with Life Technologies that the proposed method will not damage the equipment.

Materials required

- Bleach, 10% solution
- Tissue, lint-free
- Cotton or nylon swabs and lint-free cloths
- Ethanol, 95% solution
- Safety glasses
- Pipette (100-μL) with pipette tips
- Powder-free gloves
- Screwdriver
- Deionized water

How to handle the sample block

To prevent damaging or contaminating the sample block, handle the assembly as shown. Also, when the assembly has been removed from the QuantStudio™ 6 or 7 Flex System, place the sample block on a clean, dry surface or in its shipping container.
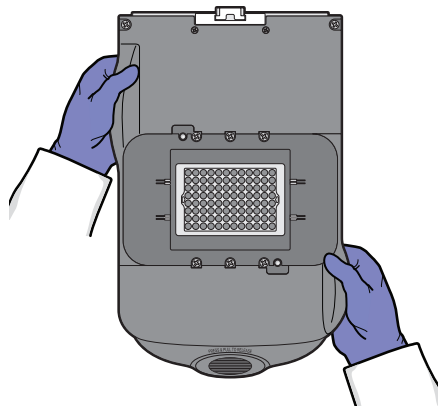
Clean the sample block

⚠ **WARNING! PHYSICAL INJURY HAZARD**. During instrument operation, the sample block can reach 100°C. Allow the sample block to cool to room temperature before performing the procedure.

IMPORTANT! Wear powder-free gloves when you perform this procedure.

1. Identify the contaminated wells of the sample block (see "How to identify contamination" on page 78).

2. Power off and unplug the QuantStudio™ 6 or 7 Flex System, then allow it to cool for 15 minutes.

3. Open the access door.

4. Firmly press down on the handle of the sample block, then remove it from the QuantStudio™ 6 or 7 Flex System. Place the sample block on a clean, dry surface.
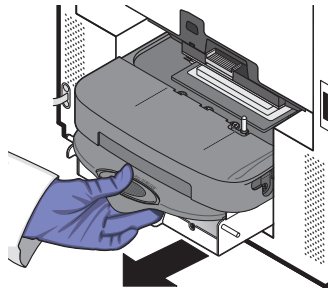
5. Clean the contaminated wells of the sample block using deionized water:

    a. Pipet a small volume of deionized water into each contaminated well.

    b. In each well, pipet the water up and down several times to rinse the well.

    c. Pipet the water to a waste beaker.

    d. Use a cotton swab to scrub inside of each contaminated well.

    e. Use a lint-free cloth to absorb the excess deionized water.
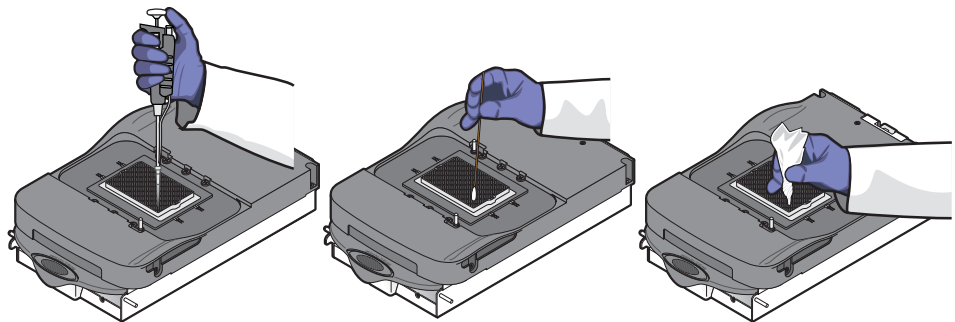
6. Load the sample block into the QuantStudio™ 6 or 7 Flex System, then close the access door.

> **IMPORTANT!** After installing the sample block, confirm that the indicator on the left side of the sample block is positioned behind the red line on the instrument rail. If the indicator is forward of the red line, push the sample block into the QuantStudio™ 6 or 7 Flex Instrument until it is seated correctly.
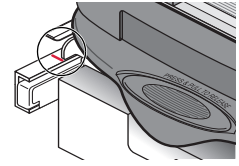
7. Close the access door.

> **IMPORTANT!** Confirm that the access door is completely closed. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays an error message if the door is not completely closed and latched, or if the sample block is not seated correctly.

8. Plug in, then power on the QuantStudio™ 6 or 7 Flex System.

9. Perform a background calibration to confirm that you have eliminated the contamination.

10. If the contamination remains, repeat steps 2–5, then clean the contaminated wells of the sample block using a 95% ethanol solution:

    a. Pipet a small volume of 95% ethanol solution into each contaminated well.

    b. In each contaminated well, pipet the solution up and down several times to rinse the well.

    c. Pipet the ethanol solution to a waste beaker.

11. Repeat steps 5–9 to rinse the wells of the sample block and to ensure that you have eliminated the contamination.

> **IMPORTANT!** Always use deionized water to rinse wells after cleaning with bleach or ethanol solution.

12. If the contamination remains, repeat steps 2–5, then clean the contaminated wells of the sample block using 10% bleach solution:

    a. Pipet a small volume of 10% bleach solution into each contaminated well.

    b. In each contaminated well, pipet the solution up and down several times to rinse the well.

    c. Pipet the bleach solution to a waste beaker.

13. Repeat steps 5–9 to rinse the wells of the sample block and to confirm that you have eliminated the contamination.

> **IMPORTANT!** Always use deionized water to rinse wells after cleaning with bleach or ethanol solution.

14. If the contamination remains, contact Life Technologies.

Replace the halogen lamp

Replace the halogen lamp at or before 2000 hours of life.

> ⚠ **WARNING! PHYSICAL INJURY HAZARD**. The QuantStudio™ 6 or 7 Flex System and lamp are hot! The lamp can become very hot while in use. Before handling the lamp, allow it to cool for 15 minutes and put on protective, powder-free gloves.

> ⚠ **CAUTION! PHYSICAL INJURY HAZARD**. Wear disposable, powder-free gloves when handling the lamp to prevent burns and to prevent shortening the life of the replacement lamp.

> ⚠ **WARNING!** This QuantStudio™ 6 or 7 Flex System is designed for 12 V, 75 W halogen lamps only. Replace with halogen bulbs supplied by Life Technologies.

Materials required

- Safety glasses
- Powder-free gloves
- Halogen bulb (12 V, 75 W)

Halogen lamp warnings

The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can display the following warnings before or during an experiment:

- The lamp current is below the acceptable level at the start of the run.

  Replace the halogen bulb as explained in "Replace the lamp" on page 100.

- The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software stopped the run because the lamp current decreased below the acceptable level during the run.

  Replace the halogen bulb as explained in "Replace the lamp" on page 100. Click **OK** in the message box, then replace the lamp bulb.

- The lamp usage exceeds 2000 hours at the start of a run.

  Click **Cancel Run**, then replace the lamp before you perform another experiment.

View the lamp status

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

2. From the Instrument Console, select your QuantStudio™ 6 or 7 Flex System from the list of instruments, then review the Lamp Life and Last Serviced readings in the Maintenance Info pane.

   If the lamp usage has reached 2000 hours, replace the lamp (see "Replace the lamp" below).

   Note: The Lamp Life report displays the total number of hours that the halogen lamp has been illuminated. The Last Serviced report displays the date that the lamp was installed.

Replace the lamp

IMPORTANT! Always wear powder-free gloves when you handle the lamp. Finger prints shorten the lamp life.

1. Record the lamp installation:

   a. From the Main Menu of the touchscreen, touch **Tools**, then touch **Record Lamp Installation**.

   b. Touch the **Name** field, enter any relevant information (such as the make, model, or serial number of the lamp), then touch **Done**.

   c. Touch the **Comments** field, enter any additional information that you want to record, then touch **Done**.

   d. Touch **Save**, then touch **OK**.

   e. When asked to confirm lamp replacement, touch **OK**.

   Note: The data that you enter for the new lamp appears in the instrument log.

2. Power off and unplug the QuantStudio™ 6 or 7 Flex System, then allow it to cool for 15 minutes.

3. Open the access door.

4. Remove the lamp from the QuantStudio™ 6 or 7 Flex System:

   a. Slide the lamp release lever downward.

   b. Firmly grasp the lamp and lift it up and out of the slotted mount.
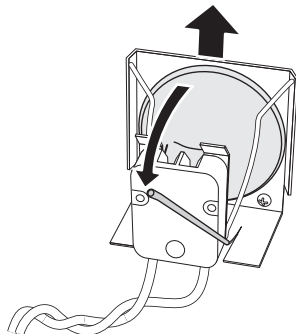
5. Inspect the lamp for damage (carbon usually coats the inside of a failed lamp). If necessary, dispose of the lamp and obtain a replacement.

   IMPORTANT! Dispose of the lamp in accordance with your local municipal waste ordinances. Do not dispose of the lamp as unsorted municipal waste.

6. Install the new lamp into the QuantStudio™ 6 or 7 Flex System:

   a. Slide the lamp release lever upward.

b. Firmly grasp the lamp, place it into the slotted mount, then carefully slide the lamp downward into place.
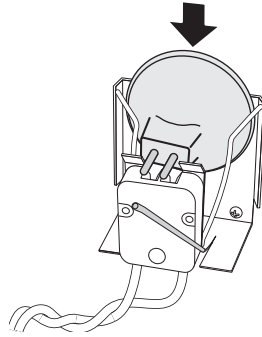
7. Close the access door.

8. Plug in and power on the QuantStudio™ 6 or 7 Flex System.

IMPORTANT! Confirm that the access door is completely closed. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays an error message if the door is not completely closed and latched, or if the lamp is not seated correctly.

---

9. Start the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, then perform an ROI calibration.

   While the QuantStudio™ 6 or 7 Flex System is running, look through grating of the access door and confirm that the lamp is illuminated.

   • If the lamp is illuminated, the lamp has been installed successfully.

   • If the lamp is not illuminated, the replacement halogen lamp may be defective. Replace the lamp again. If the second lamp does not illuminate, check the QuantStudio™ 6 or 7 Flex System fuses for failure (see page 102).

10. After replacing the lamp, perform the following calibrations in the specified order:

   a. ROI calibration

   b. Background calibration

   c. Uniformity calibration

   d. Dye calibration

   e. Normalization calibration

   f. RNase P test

Replace the instrument fuses

Replace the QuantStudio™ 6 or 7 Flex System fuses when the fuses fail.

> ⚠ **CAUTION! FIRE HAZARD**. For continued protection against the risk of fire, replace fuses only with listed and certified fuses of the same type and rating as those currently in the QuantStudio™ 6 or 7 Flex System.

Materials required

- Fuses, 12.5A, Time-Lag T, 250VAC, 5 × 20-mm (2)
- Fuses for AU/NZ only, 6.3A, Time-Lag T, 250VAC, 5 x 20-mm (2)
- Safety glasses
- Powder-free gloves
- Screwdriver, flathead

Replace the fuses

1. Power off, then unplug the QuantStudio™ 6 or 7 Flex System. Allow it to cool for 15 minutes.

2. Using a flat-head screwdriver, unscrew and remove the fuse holder.
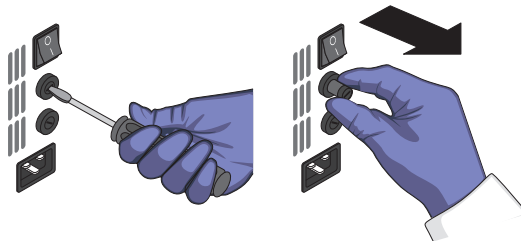
3. Remove each fuse from its fuse holder and inspect it for damage. Carbon typically coats the inside of failed fuses.

Good	Failed
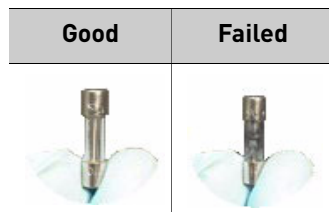
4. Replace each failed fuse with a 12.5A, Time-Lag T, 250VAC, 5 × 20-mm Fuse.

   Note: The voltage and amperage ratings are on the fuse holder.

   Note: For AU/NZ only, replace each failed fuse with a 6.3A, Time-Lag T, 250VAC, 5 x 20-mm Fuse.

5. Install the fuse holder.

6. Plug in, then power on the QuantStudio™ 6 or 7 Flex System. The installation is successful if the instrument powers on.

Note: Fuse failure can result from fluctuations in the supplied power to the QuantStudio™ 6 or 7 Flex System. To prevent further failures, consider installing an electrical protective device, such as a UPS or a surge protector.

Install the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software

If you have extra software licenses, you can install the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to computers that meet the operating requirements (not recommend).

Computer requirements

See "Computer requirements" on page 24 for a list of hardware and operating system requirements for the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

Install the software

1. Load the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software CD into the computer.

2. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Installer, click **Install QuantStudio™ 6 and 7 Flex Real-Time PCR System Software**.

3. Complete the installation as instructed using the default settings:

   • From the Welcome window, enter your customer information.

   • From the License Agreement screen, accept the terms of the agreement.

   • From the Installation Location screen, accept the default location.

   • From the License screen, install the license key/file that you obtained from Life Technologies.

   The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software installation requires several minutes.

4. Eject the CD when the installation is complete.

Manage QuantStudio™ 6 and 7 Flex Real-Time PCR System Software licenses

You can use the License Central feature to activate, or install the licenses that control access to the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software base application and associated modules.

QuantStudio™ 6 and 7 Flex Real-Time PCR System Software license keys and files

The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software and associated modules require the installation and maintenance of valid license files for continued operation. The license files are generated by the Life Technologies website when a license key is activated. Each file pairs a software license key with the computer from which the key was activated. After a key is activated and a license file is generated, the file cannot be transferred to another computer. To transfer a license between computers, you must reactivate the license key using the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software on the target computer.

Note: License keys are found on the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software CD packaging, or they can be supplied by Life Technologies support.

Activate a license

If you have a valid license key for the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software or an associated module, you can activate the license as explained below.

IMPORTANT! An internet connection, a web browser, and a valid email account are required to activate a QuantStudio™ 6 and 7 Flex Real-Time PCR System Software license. If the computer that contains the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is not connected to the internet or it lacks a web browser application, contact Life Technologies support to request the license file.

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, select **Tools ▸ License Central**.

2. From the License Central dialog box, select the license of interest from the table, click **Activate License**, then wait for the default web browser application to connect to the Life Technologies website.

3. From the Life Technologies Software License Activation website, click **QuantStudio™ 6 and 7 Flex Real-Time PCR System Software** from the list of products, then activate the license as instructed.

   After you successfully activate the license, the Life Technologies website emails you the activated license file (.lic) to install on your computer.

Install a license file

After you activate a license and receive an activated license file (.lic), install the file as explained below to unlock the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software or module.

Note: Each license file is generated specifically for the computer that was used to activate the license key.

1. Save the license (.lic) file to the computer that contains the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

2. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, select **Tools ▸ License Central**.

3. From the License Central dialog box, click **Install License**.

4. From the Open dialog box, navigate to and select the license file, then click **Open**.

5. Click **OK** to close the License Central dialog box.

Note: Contact Life Technologies support if the software fails to install the license.

Update the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software

Life Technologies may release updates to the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software that you can install without the aid of Life Technologies service personnel. You can obtain updates directly from the service section of the Life Technologies website.

For the latest services and support information for the QuantStudio™ 6 or 7 Flex System:

1. Go to **www.lifetechnologies.com/us/en/home/technical-resources/software-downloads**

2. From the Software Downloads page, select **Applied Biosystems QuantStudio™ 6 and 7 Flex Systems** from the menu.

3. From the Applied Biosystems QuantStudio™ 6 and 7 Flex Systems Software Downloads page, click **Updates - Patches**.

The website opens the page describing the latest software updates for the QuantStudio™ 6 or 7 Flex System.

Update the software

Prepare for the upgrade

To prepare for the software update, back up all data files and experiments by creating a copy of the directory that you are using to store files. The default directories are:

- **Installation directory** – *<drive>*`:\Program Files\Applied Biosystems\QuantStudio 6 and 7 Flex Software`
- **Data directory** – *<drive>*`:\Applied Biosystems\AppData\QuantStudio 6 and 7 Flex Software`
- **User files directory** – *<drive>*`:\Applied Biosystems\QuantStudio 6 and 7 Flex Software\User Files\experiments`

Note: *<drive>* is the location where the software is installed.

Install the software

Install the software update according to the instructions that download with the software. If you are installing the update to a computer that already contains the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, the update automatically acquires the software license from the existing installation. If you are installing the

QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to a computer that does not contain a previous installation, you must have a license file supplied by Life Technologies. If you do not have a license file, obtain one as explained in "Manage QuantStudio™ 6 and 7 Flex Real-Time PCR System Software licenses" on page 104.

Update the Windows® operating system

Do not upgrade or update the Microsoft® Windows® operating system of the computer running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software without first consulting the software release notes or the Life Technologies website. Future versions and updates to the Windows® operating system may conflict with the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

To determine compatibility of an upgrade or update:

1. Open ***<drive>*:\Program Files\Applied Biosystems\QuantStudio 6 and 7 Flex Software**, double-click **QuantStudio 6 and 7 Software README.docx**, then read the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Release Notes for the compatibility of interest.

   Note: *<drive>* is the location where the software is installed.

2. If the website does not contain the information of interest, contact Life Technologies.

Change the sample block

⚠️ **WARNING! PHYSICAL INJURY HAZARD**. During instrument operation, the sample block and heated cover can reach 100°C. Allow the sample block to cool to room temperature before removing.

When to change the sample block

You can change the sample block:

- To change the consumable format of the QuantStudio™ 6 or 7 Flex System.
- In the event of a hardware failure.

Materials required

- Safety glasses
- Powder-free gloves
- Sample block

How to handle the sample block

To prevent damaging or contaminating the sample block, handle the assembly as shown below. After the assembly has been removed from the QuantStudio™ 6 or 7 Flex System, place the sample block on a clean, dry surface or in its shipping container.
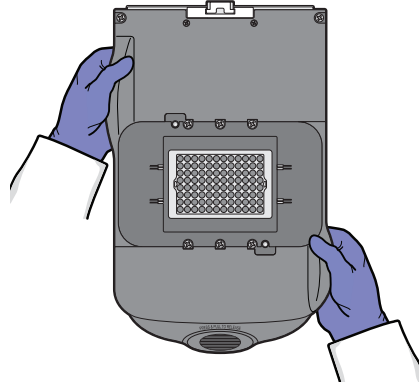
Change the sample block

1. Power off and unplug the QuantStudio™ 6 or 7 Flex System, then allow it to cool for 15 minutes.

2. Open the access door.
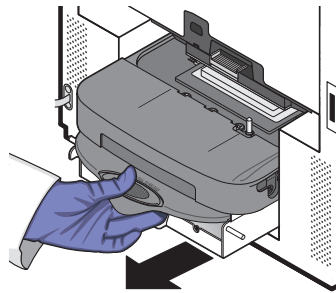
3. Firmly press down on the handle of the sample block, then remove it from the QuantStudio™ 6 or 7 Flex System. Place the sample block on a clean, dry surface.

4. Install the new sample block into the QuantStudio™ 6 or 7 Flex System.

   IMPORTANT! After installing the sample block, confirm that the indicator on the left side of the sample block is positioned behind the red line on the instrument rail. If not, push the sample block into the QuantStudio™ 6 or 7 Flex System until it is seated correctly.
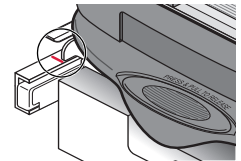
5. If you are installing a sample block of a different consumable format, replace the heated cover and plate adapter if necessary.

6. Close the access door.

   IMPORTANT! Confirm that the access door is completely closed. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays an error message if the door is not completely closed and latched, or if the sample block is not seated correctly.

7. Plug in and power on the QuantStudio™ 6 or 7 Flex System.

8. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

9. From the Instrument Console, select your QuantStudio™ 6 or 7 Flex System from the list of instruments, then review the Block Type field in the Instrument Properties pane.

   The installation is successful if the QuantStudio™ 6 or 7 Flex System powers on and if the Block Type field displays the correct type of sample block.

   Note: The Block Type field displays the type of sample block installed to the QuantStudio™ 6 or 7 Flex System.

10. Check the calibration status of the QuantStudio™ 6 or 7 Flex System (see "View the calibration status of an instrument" on page 29).

    If the calibrations for the new sample block/heated cover have expired, perform the following calibrations in the specified order:

    a. ROI calibration

    b. Background calibration

    c. Uniformity calibration

    d. Dye calibration

    e. Normalization calibration

    IMPORTANT! If the calibrations for the new sample block/heated cover are valid, you do not need to recalibrate the QuantStudio™ 6 or 7 Flex System.

Change the heated cover

⚠ **WARNING! PHYSICAL INJURY HAZARD**. During instrument operation, the sample block and heated cover can reach 100°C. Allow the heated cover to cool to room temperature before removing.

When to change the heated cover

You can change the heated cover:

- To change the consumable format of the QuantStudio™ 6 or 7 Flex System.
- In the event of a hardware failure

Materials required

- Safety glasses
- Powder-free gloves
- Heated cover

How to handle the heated cover

To prevent damaging or contaminating the heated cover, handle the assembly as shown below. After the assembly has been removed from the QuantStudio™ 6 or 7 Flex System, place the heated cover on a clean, dry surface or in its shipping container.
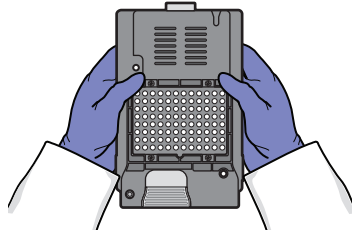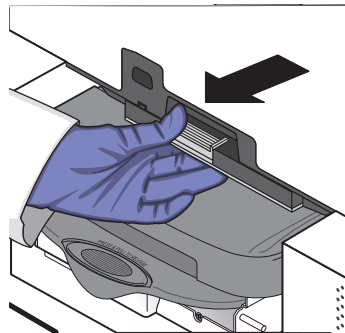
Change the heated cover

Note: Confirm that the replacement heated cover supports the plate or array card format that you want to use. Some heated covers support more than one consumable type.

1. Power off and unplug the QuantStudio™ 6 or 7 Flex System, then allow it to cool for 15 minutes.

2. Open the access door.

3. Unlock the heated cover by pinching the handle together, then pull the assembly from the QuantStudio™ 6 or 7 Flex System and place it on a clean, dry surface.

4. Install the new heated cover into the QuantStudio™ 6 or 7 Flex System.

IMPORTANT! Align the arrows on the front handle as shown below. If the arrows are not aligned, push the heated cover into the QuantStudio™ 6 or 7 Flex System until the handle locks into place.

Correct	**Incorrect**
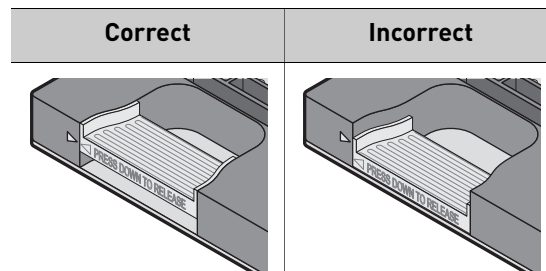
5. If you are installing a heated cover of a different consumable format, replace the sample block and plate adapter if necessary.

6. Close the access door and ensure it is completely closed.

   Note: The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays an error message if the door is not completely closed and latched, or if the sample block is not seated correctly.

7. Plug in and power on the QuantStudio™ 6 or 7 Flex System.

8. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

9. From the Instrument Console, select your QuantStudio™ 6 or 7 Flex System from the list of instruments, then review the Heated Cover Firmware Version field in the Instrument Properties pane.

   The installation is successful if the QuantStudio™ 6 or 7 Flex System powers on and if the Heated Cover Firmware Version field displays a version number.

10. Check the calibration status of the QuantStudio™ 6 or 7 Flex System (see "View the calibration status of an instrument" on page 29).

    If the calibrations for the new sample block/heated cover have expired, perform the following calibrations in the specified order:

    a. ROI calibration

    b. Background calibration

    c. Uniformity calibration

    d. Dye calibration

    e. Normalization calibration

    IMPORTANT! If the calibrations for the new sample block/heated cover are valid, you do not need to recalibrate the QuantStudio™ 6 or 7 Flex System.

Change the plate adapter

Change the plate adapter in the event of a hardware failure or if you want to change the consumable format of the QuantStudio™ 6 or 7 Flex System.

> ⚠ **WARNING! PHYSICAL INJURY HAZARD**. During instrument operation, the plate adapter can reach 100°C. Allow the plate adapter to cool to room temperature before removing.

When to change the plate adapter

You can change the plate adapter:

- To change the consumable format of the QuantStudio™ 6 or 7 Flex System.
- In the event of a hardware failure

Materials required

- Safety glasses
- Powder-free gloves
- Plate adapter

Change the plate adapter

1. Touch the instrument touchscreen to activate it, then press ⏻.

2. From the Main Menu, touch ⏏.

3. When the tray arm opens, pull the latch, then lift and remove the plate adapter.
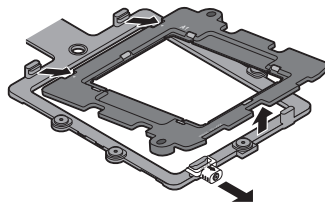
4. Attach the new adapter to the tray arm, then pull the latch to allow the adapter to lower into place. If necessary, apply pressure as indicated until the adapter snaps into place.
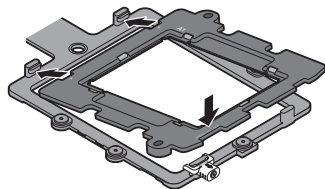
5. From the Main Menu, touch ⏏.

6. If you are installing a tray adapter of a different consumable format, replace the sample block and heated cover if necessary.

B

C Networking

IMPORTANT! This chapter *does not* provide adequate detail to integrate the QuantStudio™ 6 or 7 Flex Systems into all possible network architectures. Because your network may contain advanced features (such as a firewall or network domains), Life Technologies recommends that you consult a network administrator before connecting the QuantStudio™ 6 or 7 Flex Systems to your laboratory network.

Networking overview

After installing the QuantStudio™ 6 or 7 Flex System, you can connect the QuantStudio™ 6 or 7 Flex System to a local area network to enhance its functionality.

This appendix describes how to:

- Set up the QuantStudio™ 6 or 7 Flex System for use on a network.
- Set up a computer for remote monitoring.
- Test the network connection by engaging the remote monitoring feature.

Controlling and monitoring networked QuantStudio™ 6 and 7 Flex Systems

When the QuantStudio™ 6 or 7 Flex System is connected to a network, computers on the network that are running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can control or monitor it. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can control up to 4 instruments and monitor up to 10 instruments simultaneously. A networked QuantStudio™ 6 or 7 Flex System can be controlled by only one computer at a time. A networked computer running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can transfer experiments to and from an instrument, begin or stop a run, and perform some maintenance functions. During a run, the Remote Monitoring feature of the software can be used to view the run status, temperature, and amplification data in real-time.

Note: Remote monitoring does not allow you to control the QuantStudio™ 6 or 7 Flex System.

About the Ethernet port

The QuantStudio™ 6 or 7 Flex System features a Gigabit Ethernet port for direct communication with the QuantStudio™ 6 or 7 Flex System computer and for network communication. When the QuantStudio™ 6 or 7 Flex System is connected to a network, computers on the network that run the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can:

- Send and download experiments to and from the QuantStudio™ 6 or 7 Flex System.
- Run experiments on the QuantStudio™ 6 or 7 Flex System.
- Remote monitor the QuantStudio™ 6 or 7 Flex System as it performs runs.

The Ethernet port of the QuantStudio™ 6 or 7 Flex System supports:

- Static IP network service with subnet mask, primary and secondary data network service (DNS), and default gateway settings, or dynamic host configuration protocol (DHCP) network service
- mDNS/DNS for local domains

   Note: Because mDNS is limited to direct network connections, a QuantStudio™ 6 or 7 Flex System set for mDNS may not be visible to other nodes that are separated by a router, hub, or another network device.

- IPv4 link-local (IPV4LL) in the RFC (also known as Automatic Private IP Addressing [APIPA] or Internet Protocol Automatic Configuration [IPAC])
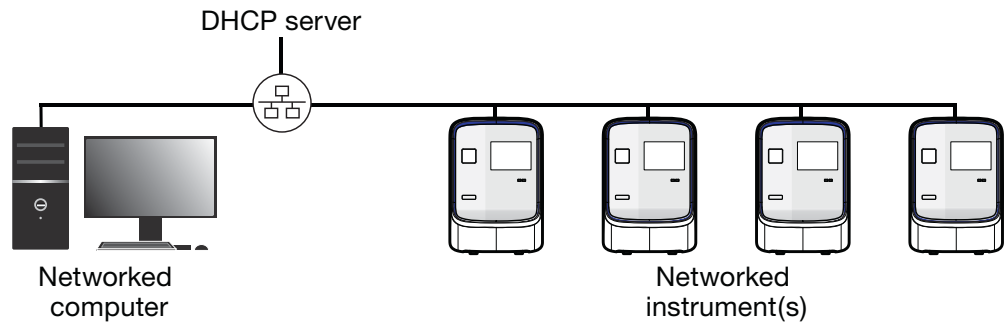
   Note: When the QuantStudio™ 6 or 7 Flex System is set for DHCP, APIPA is automatically enabled, and the QuantStudio™ 6 or 7 Flex System provides an IP address when no address is supplied by the DHCP server.

Example network layouts

Example 1

In the following example, one or more QuantStudio™ 6 or 7 Flex Systems, which have been configured for dynamic host configuration protocol (DHCP) operation, are connected to a network by their Ethernet ports. In this layout, any computer on the network can monitor and control the QuantStudio™ 6 or 7 Flex Systems.

Note: A networked computer running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can simultaneously control up to 4 instruments and monitor up to 10 instruments that have been connected to the network.

DHCP server

Networked computer

Networked instrument(s)

Example 2

In the example shown below, the QuantStudio™ 6 or 7 Flex System is connected directly to the QuantStudio™ 6 or 7 Flex System computer, which is also connected to the local area network. In this configuration, the computers on the network can exchange experiments with the QuantStudio™ 6 or 7 Flex System computer; however, the QuantStudio™ 6 or 7 Flex System can be neither monitored nor controlled remotely because it is physically isolated from the network.

DHCP server

Networked computer

Networked computer

Instrument
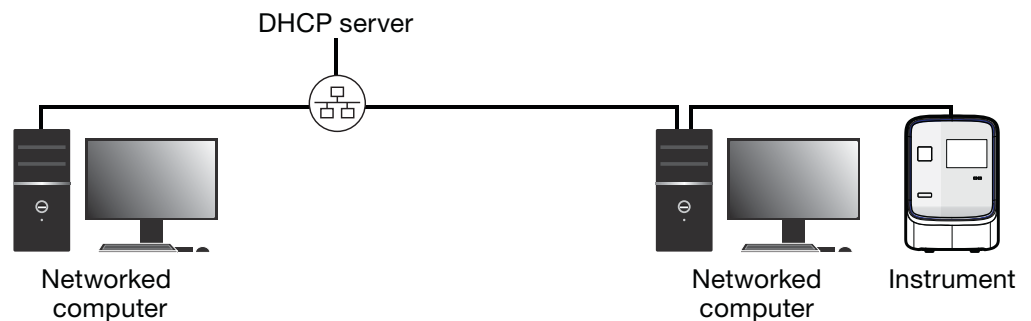
Networking guidelines and best practices	• Consult a network administrator.

- – We recommend that you consult a network administrator before connecting the QuantStudio™ 6 or 7 Flex System to your laboratory network.
- – To enable the full functionality of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, the computer requires a network connection.

- Limit remote monitoring to 10 computers.

  Avoid using more than 10 computers to simultaneously monitor the QuantStudio™ 6 or 7 Flex System remotely. Although the QuantStudio™ 6 or 7 Flex System supports remote monitoring from multiple computers, each connection taxes the instrument microprocessor. Too many connections can overburden the QuantStudio™ 6 or 7 Flex System and result in instrument errors.

  Note: The effects of an overburdened QuantStudio™ 6 or 7 Flex System are evident in the Temperature Plot during a run. Symptoms can include extended hold times or brief, unexpected plateaus in the instrument Temperature Plot.

- Observe the restrictions to mDNS and Autodiscovery.

  The QuantStudio™ 6 or 7 Flex System supports mDNS but only when the QuantStudio™ 6 or 7 Flex System and computer share a direct network connection and are within the same subnet. Consequently, network computers that are separated from the QuantStudio™ 6 or 7 Flex System by a router, hub, or another network device may not be able to access the QuantStudio™ 6 or 7 Flex System by its host name.

- Confirm the uniqueness of the instrument name.

  The QuantStudio™ 6 or 7 Flex System does support name resolution but the instrument name must be unique within the subnet. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can automatically discover QuantStudio™ 6 or 7 Flex Systems on the link-local network that are configured for Autodiscovery.
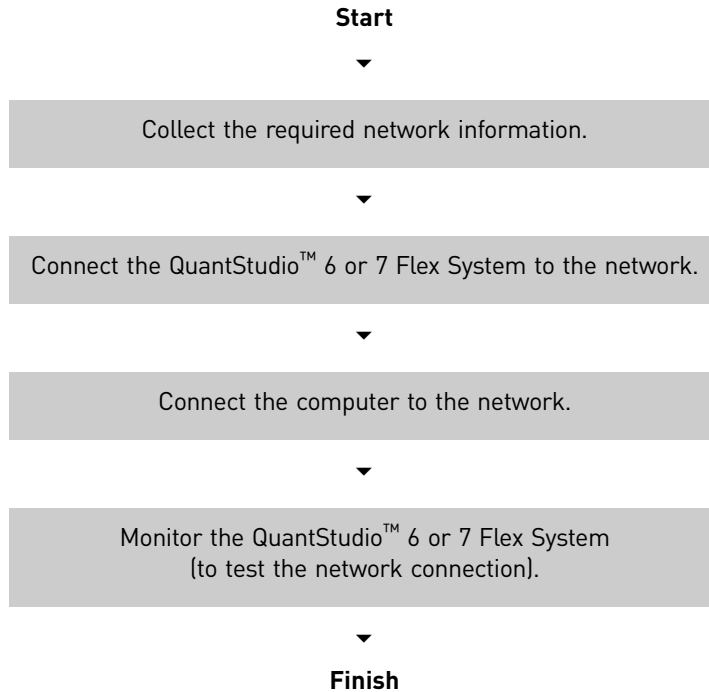
  Note: The QuantStudio™ 6 or 7 Flex System does not test the uniqueness of the instrument name when it is set.

- Name QuantStudio™ 6 and 7 Flex Systems using lowercase letters.

  When you define the QuantStudio™ 6 and 7 Flex Systems settings, enter the instrument name using lowercase letters only.

Network setup workflow

The following figure illustrates the workflow for installing the QuantStudio™ 6 or 7 Flex System to a local area network.

Start

▼

Collect the required network information.

▼

Connect the QuantStudio™ 6 or 7 Flex System to the network.

▼

Connect the computer to the network.

▼

Monitor the QuantStudio™ 6 or 7 Flex System
(to test the network connection).

▼

Finish

Collect the required network information

Obtain the following information from your network administrator:

- Network policy for obtaining IP addresses (DHCP or static IP).

  IMPORTANT! When the QuantStudio™ 6 or 7 Flex System is set for DHCP, APIPA is automatically enabled and the QuantStudio™ 6 or 7 Flex System self assigns an IP address when no address is supplied by a DHCP server.

- If the network requires static IP addresses, obtain the IP address, subnet mask, and gateway address for the QuantStudio™ 6 or 7 Flex System.

Connect the QuantStudio™ 6 or 7 Flex System to the network

After deciding how to connect the QuantStudio™ 6 or 7 Flex System to a network, set up the QuantStudio™ 6 or 7 Flex System according to your network policies.

Materials required Ethernet cable with RJ45 connectors (a CAT6 Ethernet cable for a 1000Mbit/s network connection or a CAT5 for 100Mbit/s connection)

Define the instrument internet protocol settings

1. Use the Ethernet cable to connect the Ethernet port of the QuantStudio™ 6 or 7 Flex System to the nearest network port.

2. Power on the QuantStudio™ 6 or 7 Flex System.

3. From the Main Menu of the touchscreen, touch **Settings**, then touch **Set Network Information**.

   Note: The Set Network Information screen displays the Media Access Control (MAC) address of the QuantStudio™ 6 or 7 Flex System below the Autodiscovery and Smart Monitoring check boxes. The MAC address can be used to uniquely identify the QuantStudio™ 6 or 7 Flex System on the network.

4. Touch **Autodiscovery** to make the QuantStudio™ 6 or 7 Flex System discoverable by computers that are running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

5. Touch **Smart Monitoring** to enable the feature on the QuantStudio™ 6 or 7 Flex System.

   The Smart Monitoring feature allows Life Technologies service personnel to monitor the status of the QuantStudio™ 6 or 7 Flex System remotely through an internet connection. Smart Monitoring employs multiple layers of security, including a Secure Sockets Layer (SSL) and Lightweight Directory Access Protocol (LDAP) authentication, to provide real-time troubleshooting and problem resolution for the instrument. For a detailed description of the Smart Monitoring Service, see the *Smart Monitoring Service Product Bulletin: Leveraging the power of the Internet while maintaining system security* (Part no. 121PB07-03).

6. Set the Internet Protocol (TCP/IP) Properties for either DHCP or Static IP communication.

Network service	Action
DHCP	Touch **Obtain an IP address automatically**.

Static IP	1. Touch **Use the following IP address**.
	2. Touch the **IP Address** field, enter the IP address using the keypad, then touch **Done**.
	3. Assign the:
	  • IP addresses for the DNS Servers (primary and secondary)
	  • Subnet Mask setting
	  • Default Gateway setting

7. Touch **Save** to save the settings, then touch **OK** when prompted.

8. Touch 🏠 to return to the Main Menu.

Connect the computer to the network

Materials required   Ethernet cable with RJ45 connectors

Computer requirement   If you are connecting a computer that you provided to a network, confirm that the computer contains a free network port.

Collect required information   Obtain the following information from your network administrator:

- Network policy for obtaining IP addresses (DHCP or static IP)
- If the network requires static IP addresses, obtain the IP address, subnet mask, and gateway address for the computer

Set up the computer

IMPORTANT! We recommend that you arrange for a network administrator to connect your computer to the network. The following procedure does not provide adequate detail for all network architectures.

Note: The following procedure is valid for the Microsoft® Windows® 7 operating system.

1. Use the Ethernet cable to connect the computer to the nearest network port.

2. Power on the computer, then log in using a user account that belongs to the Administrators user group.

3. From the desktop, right-click **My Network Places**, then select **Properties**.

4. Right-click **Local Area Connection**, then select **Properties**.

5. Select **Internet Protocol (TCP/IP)**, then click **Properties**.

6. Set the Internet Protocol (TCP/IP) Properties for either DHCP or Static IP communication:

Network configuration	Action

DHCP	1. Select **Obtain an IP address automatically**.
	 2. Set the DNS address. If the computer obtains DNS addresses:
	    • Automatically – Select **Obtain DNS server address automatically**.
	    • Statically – Select **Use the following DNS address**, enter the address of the preferred and alternate DNS servers if available.

Static IP	1. Select **Use the following IP address**. 2. In the IP Address field, enter the static IP address. 3. If necessary, enter a subnet mask. 4. If necessary, enter a static gateway address in the Default Gateway field.

7. If your network requires advanced TCP/IP setup (such as WINS), define the settings:

   a. Click **Advanced** in the Internet Protocol (TCP/IP) Properties dialog box.

   b. Define the IP Settings, DNS, and WINS tabs as instructed by your systems administrator, then click **OK**.

8. Close all dialog boxes by clicking **OK**.

9. Restart the computer.

   The computer is now visible to other computers on the network.

Install the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software

If you are using a computer that you have provided, install the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software using the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software CD.

Note: You must install the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to monitor the QuantStudio™ 6 or 7 Flex System over the network.

Monitor the QuantStudio™ 6 or 7 Flex System

After connecting theQuantStudio™ 6 or 7 Flex System and a computer to the network, you can enable remote monitoring in the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to observe the instrument status remotely.

About remote monitoring

When the QuantStudio™ 6 or 7 Flex System is connected to the network, any computer on the network that is running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can:

- Monitor the status of a run . . . . see below
- Download experiment files 121
- Enable or change the calibration reminders 121

Guidelines for remote monitoring

To ensure optimal performance of the remote monitoring feature, observe the following guidelines:

- The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can monitor up to 10 instruments.
- We do not recommend that a QuantStudio™ 6 or 7 Flex System be monitored by more than 10 computers simultaneously.
- Unless you are sure that your QuantStudio™ 6 or 7 Flex System and computer exist on the same subnet, we recommend that you use the IP address of the QuantStudio™ 6 or 7 Flex System to add it for remote monitoring.

Monitor the status of a run

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

2. From the Instrument Console, select the icon for your instrument, then click **Manage Instrument**.

   Note: If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.

3. From the Instrument Manager, click **Monitor**, then click **Information**.

4. From the Monitor Instrument screen, click **Monitor Running Instrument**.

   The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays the status, attributes, calibration status, and plot data for the selected QuantStudio™ 6 or 7 Flex System. If a communications warning appears, contact your network administrator to troubleshoot the problem.

You can lose the software connection to the QuantStudio™ 6 or 7 Flex System if you:

- Change the QuantStudio™ 6 or 7 Flex System that is connected directly to your computer
- Use the touchscreen to change the instrument name or IP address

Note: To reestablish the connection, restart the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

Download experiment files

See "Transfer experiments, templates, and results data" on page 83 for more information on using QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to transfer experiment results from the QuantStudio™ 6 or 7 Flex System.

Enable or change the calibration reminders

The calibration reminder settings allow you to configure the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to alert you by email when the QuantStudio™ 6 or 7 Flex System requires calibration. The notifications settings feature is optional, and it does not affect performance.

IMPORTANT! The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software transmits email only while the QuantStudio™ 6 or 7 Flex System is monitored. If the network connection is interrupted, the software stops transmitting updates.

Collect the required information

The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software requires access to a Simple Mail Transfer Protocol (SMTP) server to email calibration reminders. Contact your systems administrator or information technology department for the following information:

- Network address of a SMTP server.
- A user name and password for the server, if required for access.
- The Secure Sockets Layer (SSL) setting of the server (on or off).

Define the mail server settings

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ▸ Preferences**.

2. From the Preferences dialog box, select the **SMTP Settings** tab.

3. From the SMTP Settings tab, define the settings for the SMTP server:

  - **Outgoing Mail Server (SMTP) field** – Enter the network address of a Simple Mail Transfer Protocol (SMTP) server. Optionally, you can specify the transmission control protocol (TCP) port for the server by appending the port number to the server name, separating the two using a colon (:).

    For example: smtp.mycompany.com:2023

    Note: If a TCP port is not specified, the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software uses the default port number (25).

  - **Encryption Required** – Select if the mail server has SSL enabled.

  - **Authentication Required** – Select if the mail server requires a user name and password.

  - **User Name and Password fields** – If the mail server requires authentication, enter the user name provided by your systems administrator.

4. Click **OK**.

Modify the notification settings for a monitored QuantStudio™ 6 or 7 Flex System

1. Open the Calibration Reminders screen for the QuantStudio™ 6 or 7 Flex System:

  a. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

  b. From the Instrument Console, select your QuantStudio™ 6 or 7 Flex System from the list of instruments on the network, then click **Add to My Instruments**.

    Note: You must add a QuantStudio™ 6 or 7 Flex System to your list before you can manage it.

  c. After the QuantStudio™ 6 or 7 Flex System is added to your list, select it, then click **Manage Instrument**.

  d. From the Instrument Manager, click 🛠 **Maintenance**, then click **Calibration Reminders**.

2. From the Calibration Reminders Setting table, configure the notification settings for the calibrations in interest. For each calibration that you want to monitor:

  a. In the Expiry Interval column, enter the number of days that elapse before the type of calibration expires on the QuantStudio™ 6 or 7 Flex System.

  b. In the Send a Reminder column, select the check box to configure the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to email a reminder to perform the calibration.

  c. In the Reminder Interval column, enter the number of days that elapse before the software emails recipients a reminder to perform the calibration.

3. From the Enter e-mail addresses for notifications field, enter the email address(es) that you want to receive email notifications. Separate multiple email addresses with commas (,).

4. Click **Apply** to change the notification settings.

D

Power On or Off, Store, and Move

Place the QuantStudio™ 6 or 7 Flex System on standby

If left unattended, the QuantStudio™ 6 or 7 Flex System automatically enters standby mode to conserve power. To enter standby mode manually, touch 🔘 on the QuantStudio™ 6 or 7 Flex System touchscreen.

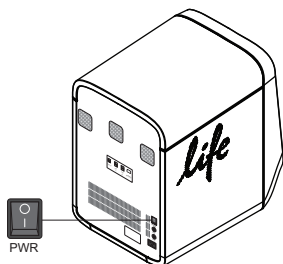# Power on the QuantStudio™ 6 or 7 Flex System

To power on the QuantStudio™ 6 or 7 Flex System from a powered-off state:

1. Touch anywhere on the touchscreen to determine if the QuantStudio™ 6 or 7 Flex System is in standby mode.

   Does the touchscreen display the Standby screen after you touch it?

   - **Yes** – The instrument is ready for use. Go to step 4.
   - **No** – Go to step 2 to power on the instrument.

2. Power on the QuantStudio™ 6 or 7 Flex System. Toggle the power button on the rear of the QuantStudio™ 6 or 7 Flex System, then wait for it to start.

   The QuantStudio™ 6 or 7 Flex System is ready to use when the touchscreen displays the Main Menu.

3. *(QuantStudio™ 7 Flex System only)* If you have an Applied Biosystems® Twister® Robot, toggle the power button on the rear of the Twister® Robot.

   Note: The Twister® Robot is ready to use when the power LED illuminates.

4. Power on the monitor.

5. Power on the computer:

   a. Press the computer power button, then wait for it to start.

   b. When the Login screen appears, enter your user name and password, then click **OK**.

6. Start the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software:

   a. From the desktop, double-click **QuantStudio 6 and 7 Flex System Software**.

      Note: If the shortcut is not present on the desktop, select **Start ▸ All Programs ▸ Applied Biosystems ▸ QuantStudio 6 and 7 Flex Software ▸ QuantStudio 6 and 7 Flex Software** to start the software.

> **IMPORTANT!** If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software does not start, confirm that no other instances of the instrument control software are open. If any instance of the software is open, close it before starting the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

b. If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Login appears, enter your user name and password, then click **Log In**.
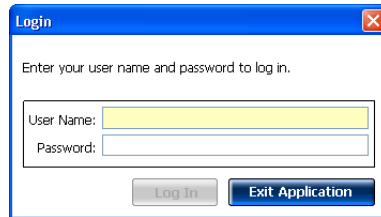
Note: Security is a separately licensed module. If your system requires this module, contact Life Technologies.

Note: If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays the License Central screen after you log into the software, your license file may have become corrupt. Contact Life Technologies support to obtain a replacement license file.

Power off the QuantStudio™ 6 or 7 Flex System

The QuantStudio™ 6 or 7 Flex Systems operate in low-power mode when not in use; however, the instrument can be powered off completely so that the components draw no power.

Note: If the QuantStudio™ 6 and 7 Flex Systems are inactive for extended periods of time, prepare them for storage as explained in "Store the QuantStudio™ 6 or 7 Flex System" on page 126.

1. Power off the QuantStudio™ 6 or 7 Flex System:

   a. If the touchscreen is not blank, touch 🔵 to place the QuantStudio™ 6 or 7 Flex System into stand-by mode.

   b. Toggle the power button on the rear of the QuantStudio™ 6 or 7 Flex System.
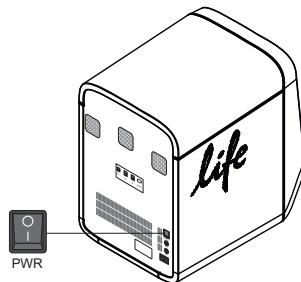
2. Power off the QuantStudio™ 6 or 7 Flex System computer:

   a. From the desktop, select **Start ▸ Shut Down**.

   b. From the Shut Down Windows dialog box, select **Shut Down**, then click **OK**.

3. Power off the monitor.

4. *(QuantStudio™ 7 Flex System only)* If you have an Applied Biosystems®
Twister® Robot, toggle the power button on the rear of the Twister® Robot.

Store the QuantStudio™ 6 or 7 Flex System

The QuantStudio™ 6 or 7 Flex System can be powered off and stored for extended
periods of time. The length of the period of inactivity determines the method you use
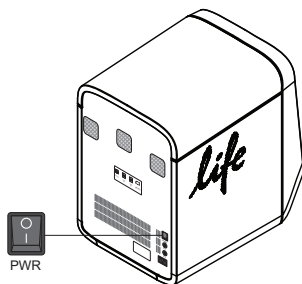to power off the QuantStudio™ 6 or 7 Flex System.

Materials required   Reaction plate or array card (unused)

**Prepare the
QuantStudio™ 6 or
7 Flex System**

1. If you plan to store the QuantStudio™ 6 or 7 Flex System for more than a week or
you plan to move it, load an unused plate or array card into the QuantStudio™ 6
or 7 Flex System:

Note: The empty plate or array card protects the internal components of the
QuantStudio™ 6 or 7 Flex System during transport or during periods of inactivity
lasting more than a week.

    a. Touch 🔘 in the QuantStudio™ 6 or 7 Flex System touchscreen.

    b. Touch ⏏ to eject the tray arm, place a plate or array card onto the plate
adapter, then press ⏏ again to load the plate.

    c. Touch 🔘 to place the QuantStudio™ 6 or 7 Flex System into stand-by mode.

2. Toggle the power button on the rear of the QuantStudio™ 6 or 7 Flex System.

3. Power off the computer:

    a. Select **Start ▸ Shut Down**.

    b. From the Shut Down Windows dialog box, select **Shut Down,** then click **OK**.

4. Power off the monitor.

5. *(QuantStudio™ 7 Flex System only)* If you have an Applied Biosystems®
Twister® Robot, toggle the power button on the rear of the Twister® Robot.

Move the QuantStudio™ 6 or 7 Flex System

Perform this procedure to safely move the QuantStudio™ 6 or 7 Flex System short distances (for example, between laboratories of the same building).

⚠ **CAUTION! PHYSICAL INJURY HAZARD**. Do not attempt to lift the QuantStudio™ 6 or 7 Flex System or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the QuantStudio™ 6 or 7 Flex System. At least two people are required to lift it.

IMPORTANT! Moving your QuantStudio™ 6 or 7 Flex System can create subtle changes in the alignment of the instrument optics. Recalibrate the instrument if necessary.

How to handle the sample block and heated cover

To prevent damaging or contaminating the sample block or the heated cover, handle the assemblies as shown below. After you remove each assembly from the QuantStudio™ 6 or 7 Flex System, place them on a clean, dry surface or in its shipping container.
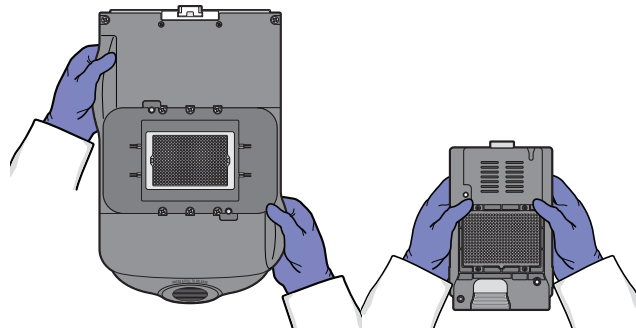
Prepare the QuantStudio™ 6 or 7 Flex System components

1. Power off the QuantStudio™ 6 or 7 Flex System and computer.

2. When the QuantStudio™ 6 or 7 Flex System and computer are powered off, disconnect all QuantStudio™ 6 or 7 Flex System components and package the cabling for the move.

3. Prepare the QuantStudio™ 6 or 7 Flex System for the move:

   a. Open the QuantStudio™ 6 or 7 Flex System access door.

   b. Firmly press down on the sample block handle, pull the sample block from the QuantStudio™ 6 or 7 Flex System, then place it on a clean, dry surface.

   c. Pinch the handle of the heated cover together, then pull the assembly from the QuantStudio™ 6 or 7 Flex System and place it on a clean, dry surface.

D

   d. Package the sample block and heated cover assemblies in a clean, dust-free container.
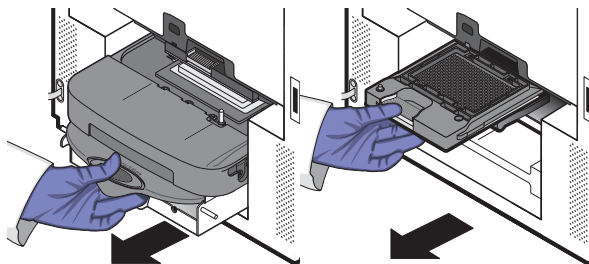
Move the QuantStudio™ 6 or 7 Flex System

Move the QuantStudio™ 6 or 7 Flex System according to the following guidelines:

- Ensure that the surface on which you will place the QuantStudio™ 6 or 7 Flex System can support at least 77.9 ± 0.6 kg (171.5 ± 0.13 lbs).
- Ensure that the path to transport the QuantStudio™ 6 or 7 Flex System is clear of obstructions.
- At least two people are needed to lift and carry the QuantStudio™ 6 or 7 Flex System.
- Keep your spine in a good neutral position.
- Bend at the knees and lift with your legs.
- Do not lift an object and twist your torso at the same time.
- Coordinate your intentions with your assistant before lifting and carrying.

Reinstall the QuantStudio™ 6 or 7 Flex System

1. Reconnect the components of the QuantStudio™ 6 or 7 Flex System. Use the Ethernet cable supplied with the instrument to connect the QuantStudio™ 6 or 7 Flex System to the network interface card in the computer.

   IMPORTANT! Do not use a standard Ethernet cable to connect the QuantStudio™ 6 or 7 Flex System to the computer.
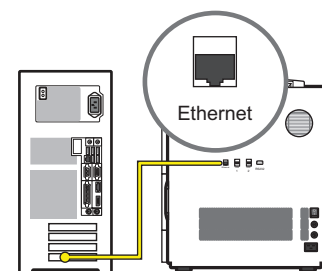
2. Install the sample block and heated cover assemblies.

3. Perform a RNase P instrument verification run. If the run:

   Passes – Do not recalibrate the QuantStudio™ 6 or 7 Flex System. No further action is necessary.

   Fails – Perform the following calibrations in the specified order:

   a. ROI

   b. Background

   c. Uniformity

   d. Dye

   e. Normalization

E

Security, Audit, and Electronic Signature

Administrator overview

IMPORTANT! Security, Audit, and Electronic Signature (SAE) is a separately licensed module available only on purchase. If your system requires this module, contact Life Technologies. The feature is disabled by default even after the installation of the license and needs to be enabled.

The Security, Audit, and Electronic Signature (SAE) module is a component of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software that allows you to configure the QuantStudio™ 6 and 7 Flex Systems to meet specific requirements. The module provides the following functionality:

- **Security** – Controls user access to the software. A default Administrator user account is provided, and additional user accounts and permissions can be user-defined.

  Note: You can enable or disable system security globally.

  Note: The default password for the Administrator user account is *Administrator*; however, the password can be changed during installation.

- **Auditing** – Tracks changes made to library items, actions performed by users, and changes to the Security, Audit, and Electronic Signature settings. The software automatically audits some actions silently. You can select other items for auditing and specify the audit mode. The Auditing function provides reports for audited library items, Security, Audit, and Electronic Signature changes, and actions.

  Note: You can enable or disable auditing globally and by record type.

- **Electronic signature (e-sig)** – Determines if users are required to provide a user name and password when performing certain functions. You can configure e-sig so that a user can print a report or start a run only if the associated data are signed. You can also configure each e-sig event to require multiple signatures and to require users with specific permissions to sign.

  Note: You can enable or disable electronic signature globally.

Example applications

You can configure the Security, Audit, and Electronic Signature module in a variety of ways. For example, you can:

- Require users to log in, and leave audit disabled.
- Allow only certain users to create or modify protocols.
- Allow only certain users to approve reviewed samples.
- Require experiments to be signed before users can run or print them.

Configure the security system

Access the Security screen and enable or disable security

Use the Security screen to disable and enable security, control restrictions and security policies for all user accounts, and set up notifications when certain security events occur.

Note: Security is disabled by default.

To enable or disable security:

1. In the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ▸ Security ▸ Settings**.

2. In the Security Settings dialog box, select the **System** tab.

3. Select or deselect **Enable Security**. Note the following:
   - The software requires you to enter your user name and password when you enable security.
   - The enable commands are grayed when a run is in process.
   - When security is disabled, the 🔵 is not active in lower parts of the screen.
   - Disabling Security inactivates Auditing and E-Signature.

   IMPORTANT! If you disable security, you inactivate audit and electronic signature functions; however, no audit record is generated to indicate that audit and electronic signature functions are disabled.

   IMPORTANT! If you enable or disable the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software security, auditing, and electronic signature feature, you must similarly enable or disable the QuantStudio™ 6 and 7 Flex Systems security (see "Enable/disable instrument security" on page 92). The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software cannot connect to QuantStudio™ 6 and 7 Flex Systems that do not match security settings.

4. Click **Apply Settings**.

Set account setup and security policies

Note: Security policies apply to all user accounts.

1. In the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ▸ Security ▸ Settings**.

2. In Account Setup, specify User Name limits.

   IMPORTANT! The software allows spaces in user names. Use spaces in user names with caution. For information, see "Spaces in user names and/or passwords" on page 132.

3. Specify User Password limits:
   a. Specify the passwords length limits.
   b. Specify password reuse. You cannot disable the password reuse restriction.

    c. Specify the allowed characters in user passwords: spaces and alphabetical, numeric, uppercase, lowercase, and special characters (commas, periods, semicolons, dashes, underscores, and tildes).

4. In Security Policies, specify Password Expiration, Account Suspension, and Session Timeout settings.

   Note: A session times out while a run is in progress if the time-out period is exceeded and there is no other user activity.

5. In the Open Non-Secure Data option, select **Yes** or **No** to determine whether users can open experiments and templates that were created without security settings.

6. Click **Set Up Messaging Notification Settings** to specify when and how the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software notifies the administrator of certain security events. For information, see "Set up messaging notifications" on page 132.

7. Click **Apply Settings**.

The new settings are applied to the user account the next time that the user logs in.

Spaces in user names and/or passwords

If you allow spaces in user names and/or passwords, be aware of the following issues:

- Leading and trailing spaces in user names are difficult to detect on the screen or in printed reports.
- The number of consecutive spaces in a user name is difficult to determine on the screen or in printed reports.

Spaces in user names may cause confusion when a user searches for an audit record associated with a user name. To find a record associated with a user name, specify the user name exactly, including leading, consecutive, and trailing spaces.

Set up messaging notifications

1. In the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ▸ Security ▸ Settings**.

2. In the Security screen, click **Set Up Messaging Notifications** to display the Setup Notifications dialog box.

3. Select the events for notification:

   - **System security enabled or disabled** – Security has been enabled or disabled.
   - **User did not enter correct password** – A user attempts to log in with an incorrect password. The message indicates the number of failed authentications.
   - **User account suspended** – The user exceeds maximum number of allowed failed authentications (login attempts with an incorrect password).
   - **User session timed out** – No activity occurred in a user account for the specified period of inactivity.

4. Select the notification method:

- **Notify Admin at Login** – If an event triggers notification, the next time any user with an Administrator role logs in, the software lists those events, indicating the time each event occurred and the user who triggered the event.

  The Administrator has the option of acknowledging the event, which removes it from the notification list.

- **Email Notification** – If an event triggers notification, the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software sends an email to the addresses in the adjoining Email Address column of the table. The email notification displays the triggered event and displays the time that the event occurred and the user who triggered the event.

5. Click **OK**.

User account management

Create or edit user accounts

The software includes a default Administrator user account with permissions (defined by the account user role) to perform all functions in the software. You cannot modify this account.

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ▸ Security ▸ Settings**.

2. From the Security Settings dialog box, select the **Users** tab.

From the Users tab, you can perform the actions in the following table:

To...	Action
Create a user account	1. Click **Create** to display the New User dialog box.
	2. Enter user name, password, first name, middle initial (optional), and last name. Click a field to display the field limits, which are specified in Security settings.
	Note: First name, MI (middle initial), and last name are used to create User Full Name, which is displayed as the name of the logged-in user.
	Note: You cannot change the user name after you save the user account.
	3. Select **Password Expires at First Login** to require the user account to specify a new password at first log in. The Password Expires On date is specified in Security settings.
	4. Select the user role (described in "Create or edit a user role" on page 134) and the electronic signature state (determines if a user account has permission to electronically sign objects). Leave the status set to **ACTIVE**.
	5. (Optional) Enter email (for information only), phone, and comments.
	6. Click **Save**. A shaded Save button indicates an invalid entry in a field. Click a field to display the limits for the field, then enter a valid entry.

To...	Action
Edit a user account	1. From the Users screen, select a user account, then click **Edit**. **Note:** If you select multiple users, only Status and Role will be changed. 2. Edit settings as needed. You cannot edit the user name of an existing user. 3. Click **Save**.
Activate a suspended user account	1. From the Users screen, select the user. 2. Click **Edit**. 3. Change the Status from **SUSPENDED** to **ACTIVE**, then click **Save**.
Disable (inactivate) a user account	**IMPORTANT!** You cannot delete a user, because user records are required for auditing. To disable a user account, inactivate it as follows. 1. From the Users screen, select the user. 2. Click **Edit**. 3. Change the Status from **ACTIVE** to **INACTIVE**, then click **Save**.
Reset a forgotten password	1. From the Users screen, select the affected user account, then click **Edit**. 2. Enter a replacement password for the user, then re-enter the password for confirmation. 3. If you assigned the user a temporary password, then select **Password Expires at First Login** to require the user to specify a new password at log in. 4. Click **Save**.

Determine the name of the logged-in user

The title bar of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software window displays the name of the user.

Create or edit a user role

User roles determine the permissions associated with a user account. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software includes three default user roles:

- Administrator (cannot be edited or deleted)
- Lab Director
- Technologist

You can modify the Lab Director and Technologist roles, and you can create your own roles with customized settings as needed.

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ▸ Security ▸ Settings**.

2. From the Security Settings dialog box, select the **Roles** tab.

From the Roles tab, you can perform the actions in the following table:

To...	Action
Create a user role	1. Click **Create**. 2. Enter a role name and (optional) description. 3. Select permissions (see "Permissions and default user roles" on page 135). To select all permissions in a category, select the check box next to the category. **Note:** Operations not shown in the following table are available to all user roles. 4. Click **Save Role**.
Edit a user role	1. From the Roles screen, select a user role, then click **Edit**. 2. Edit settings as needed. You cannot edit the Administrator user role. 3. Click **Save Role**.

Permissions and default user roles

Note: To determine the permissions for a default role or to edit it, select the role, then click **Edit**.

The following table shows all user-configurable permissions and the settings for the default user accounts.

Permissions		Default user roles		
Category	Function	Lab Director	Technologist	Administrator
Setup	Create and edit experiments or experiment templates (includes running experiments)	Yes	No	Yes
	Create, edit, and import sample attributes	Yes	No	Yes
Run	Perform a run using the Quickstart function	Yes	No	Yes
	Start a run	Yes	No	Yes
	Stop a run	Yes	No	Yes
Targets (Library)	Create targets	Yes	No	Yes
	Edit targets	Yes	No	Yes
	Delete targets	Yes	No	Yes
Analysis Settings (Library)	Create analysis settings (includes default settings)	Yes	No	Yes
	Edit analysis settings (includes default settings)	Yes	No	Yes
	Delete analysis settings	Yes	No	Yes
Run Methods (Library)	Create a run method	Yes	No	Yes
	Delete a run method	Yes	No	Yes
Dye (Library)	Create a custom dye	Yes	No	Yes
	Delete a dye	Yes	No	Yes

Permissions		Default user roles		
Category	**Function**	**Lab Director**	**Technologist**	**Administrator**
Study	Create or edit a study (for example, edit a plate or analysis settings)	Yes	No	Yes
	Add (transfer) experiments to a study	Yes	No	Yes
	Remove experiments from study	Yes	No	Yes
Preferences	Edit the system preferences	Yes	No	Yes
	Export the system preferences	Yes	No	Yes
	Import the system preferences	Yes	No	Yes
Calibrations	Perform calibrations	Yes	Yes	Yes
RNaseP	Perform an RNase P run	Yes	Yes	Yes
Instrument Configuration	Add and remove instruments from monitoring	Yes	No	Yes
	Upgrade and downgrade instrument firmware	Yes	No	Yes
Security Configuration	Configure the security and audit feature	No	No	Yes
	Log in to user sessions that have timed out	No	No	Yes
	Perform E-Signing	Yes	No	Yes
Export	Export primary and secondary analysis results	Yes	Yes	Yes

View or print a user report

Note: The landscape and portrait functions are not available for the User or Roles reports. To print either report in landscape, save the report electronically (.pdf), then print the file in the desired orientation.

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ▸ Security ▸ Settings**.

2. From the Security Settings dialog box, select the **Users** or **Roles** tab.

3. Click **View Report**.

4. From the Report screen, click tool bar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.

5. Click 🖨 (Print) to print the report, or click 💾 (Save) to save the report electronically (.pdf). Close the report.

Audit trail management

Access the Audit screen and enable or disable auditing

Use the Audit screen to control the auditing state (disabled or enabled), the events that are audited, and the reasons available to users when audit mode is set to Prompt or Required.

Note: Auditing is disabled by default.

IMPORTANT! If you disable security, you inactivate audit and electronic signature functions; however, no audit record is generated to indicate that audit and electronic signature functions are disabled.

To enable auditing:

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ▸ Security ▸ Settings**.

2. From the Security Settings dialog box, select the **Audit** tab.

3. Select or deselect **Enable Audit**.

4. Click **Apply Settings**.

Modify the auditing settings

To...	Action
Select objects to audit	1. In the Audit Settings group of the Audit tab, select the objects to audit and the mode for each enabled item (Experiments, Experiment Templates, or Study). 2. Set the Audit Mode for each item you enable for auditing: • **Optional** – The event is audited, a reason prompt is displayed, but the user can cancel and continue without entering a reason. • **Required** – The event is audited, a reason prompt is displayed, and the user must specify a reason. • **Silent** – The event is audited, no reason prompt is displayed. 3. Click **Apply Settings**.
Create audit reason settings	You can create, modify and delete the reasons that are available for selection in the Audit Reason dialog box (displayed when a user performs an audited action). 1. To require users to select a pre-defined reason in the Audit Reason dialog box (displayed when a user performs an audited action), enable **Require users to select a reason to change from the list**. Users are not permitted to enter a reason. 2. As needed, click **Create**, or select a reason from the list, then click **Edit** or **Delete**.

Audit report generation

You can use the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to generate reports of audit history from both the Security Settings dialog box and from open experiments, templates, or studies.

Viewing audit histories from the Security Settings dialog box

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ▸ Security ▸ Settings**.

2. From the Security Settings dialog box, select the **Audit** tab, then click **View Reports**.

   Note: To access the Audit Reports screen, the user role for an account must have the "Configure the security and audit feature" permission. Users without the permission can view object audit histories for individual entries in the libraries by selecting entries, then clicking **View Audit History**.

From the Audit Reports dialog box, you can perform the actions in the following table:

To...	Action
Display audit histories	1. Select a tab to display: • **System Configuration History** – Security, audit, and electronic signature configuration records, including audit history for each user account. • **Action Record** – System-specified audit events. 2. (Optional) Select **Filter by**, then filter the table: • Sort the table. • Specify filters (date range, user name, action, object or record type, object or record name, reason), then click **Refresh**. **Note:** The Reason field in System Configuration History is not used. • Select one or more records, then click **View Report**.
Review the action log	The Action Record log lists system-specified audit events. All items in the action log are audited silently, except for the items noted as configurable. Configurable items may include comments in the action log. • Audit Settings (Update) • Auditing Event (Archive, Restore, Purge) • Configuration (Import, Export) • Data Audit (Archive, Restore, Purge) • Login (Success, Failure) • Logout (Success) • Run (Start, Stop, Completed, Failed, Aborted, Error) • User Account (Create, Update)

To...	Action

View and print audit reports	1. Select the **System Configuration History** tab.
	
	2. Display the records of interest.
	
	3. Filter the list to decrease the time required to generate reports.
	
	    IMPORTANT! You cannot cancel a report after you click a view button.
	
	4. Click **View Report**.
	
	    Note: From the audit report, the "Create" action value is listed as "Added", "Delete" action value is displayed as "Removed", and "Update" action value is displayed as "Updated".
	
	5. From the Report screen, click tool bar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.
	    • To print the report, click 🖨 (Print).
	    • To save the report electronically (PDF), click 💾 (Save).
	
	6. Close the report.

Archive, purge, and restore audit records	The audit archive function makes a copy of audit records. Purge makes a copy of audit records, and then deletes them. You can use the Restore function to restore purged audit records. **Archive and purge** To selectively archive or purge (delete) system configuration or action audit records: 1. Select the **System Configuration History** tab. 2. Select records in the appropriate screen. 3. Click **Archive** or **Purge**. 4. If you select Archive, specify a location and name for the archive file (.asz). **Restore** To restore system configuration or action audit records, click **Restore**, then select the .asz file to restore.
Export audit records	You can export audit records to a .txt file for additional manipulation and reporting outside the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software. 1. Display the records of interest as described above. 2. Click **Export**. 3. Specify a name and location for the export .txt file, then click **Save**. Note: If you export audit records for samples that have been deleted or moved, an error message is displayed. Return sample data files to their original location, then export again.
Review the system configuration history	The System Configuration History lists security, audit, and electronic signature configuration records.

Review the system configuration history

The following table summarizes the actions that can be audited using the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software. The System Configuration History lists security, audit, and electronic signature configuration records.

Note: If you have installed additional modules to the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, then the list can contain additional auditable records.

Record type	Action	Corresponds to...
Security Settings	Update	Disable, enable, or modify security policies and session time-out settings
Account Settings	Update	Modify password settings, security policies (password expiration and account suspension), or user name settings
User Group Manager	Update	Create, delete, or modify reason for change
User Role	Create	Create user role
	Delete	Delete user role
	Update	Modify user role
User Account	Create	Create new user account
	Update	Edit or suspend a user account
Role Assignment	Delete	Assign a different user role to an existing user account
	Update	Create a user account, or assign a different user role to an existing account
Audit Settings	Update	Enable or disable auditing
Audit Type	Update	Modify audit settings
Function Management Settings	Update	Update function management
Function Access Manager	Update	Update function access management
Function	Create	Create function
	Delete	Delete function
	Update	Update function
Role Permissions	Create	Create a user role[†]
	Delete	Delete a user role
	Update	Modify user role permissions
Audit Reason for Change	Delete	Create reason for change
	Update	Delete or modify reason for change
Event Manager	Update	Update the event manager
E-signature Manager	Update	Enable or disable e-signature
E-signature Type	Create	Create an e-signature meaning
	Delete	Delete an e-signature meaning
	Update	Edit an e-signature meaning or an e-signature action
E-signature Function	Update	Edit an action requiring e-signature
File Name	Update	Update the file name
File Version	Update	Update the file version

† Creates one role assignment record for each permission in a role.

Viewing audit histories from an experiment, template, or study

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, open an experiment (.eds), template (.edt), or study (.edm) file.

2. From the open file, click 🔍 **Audit**, then click **Audit Records**.

3. *(Studies only)* Select the audit records of interest:
   - **Study** to view the audit records for the study.
   - The name of an experiment to view the audit records for the experiment.

From the Audit Records screen of the open file, you can perform the actions in the following table:

To...	Action
Display the audit history	(Optional) Filter the table. 1. Check the **Filter by** check box. 2. Enter criteria for the records of interest, such as a date range, a user name, or a type of action. 3. Click **Refresh**. To view details for a specific record: 1. Click the row in the list on the left to view the details of the record in the table on the upper right. 2. Click any row to view details for individual records in the table on the bottom right.

Export audit records	1. From the table on the left, select the records to be exported:
	  • Click in the table, then press **Ctrl**-**A** to select all the records in the table.
	  • Click and drag or press **Shift** to select continuous rows.
	  • Press **Ctrl** to select discontinuous rows.
	2. Export the records:
	  • Click **Export Summary** to export only the records in the left table.
	  • Click **Export Details** to export the records in the left table and the associated details.
	3. Select a location for the export file, enter a name for the file, then click **Save**.
	4. Click **OK** in the confirmation message.

Print audit records	1. Click **View Report** to open the Print Preview dialog box.
	2. Preview, save or print the report:
	  • Click 💾 (Save) to save the report as a .pdf or .html file. Enter the file name, select a location, select the file type, then click **Save**.
	  • Click 🖨 (Print) to send the report to the printer. From the Print dialog box, select the printer and print options, then click **OK**.
	3. Click ❌ to close the Print Preview dialog box.

Manage electronic signature

Access the e-signature settings screen and enable or disable e-sig

Use the Security screen to disable and enable electronic signature.

Note: Electronic signature is disabled by default.

To enable or disable electronic signature:

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ▸ Security ▸ Settings**.

2. From the Security Settings dialog box, select the **e-Signature** tab.

3. Select or deselect **Enable e-Signature**.

   IMPORTANT! Enabling the electronic signature feature can substantially increase the size of experiment, template, and study files.

   IMPORTANT! If you disable security, you inactivate audit and electronic signature functions. No audit record is generated for the disabling of audit and electronic signature functions when you disable security.

4. Click **Apply Settings**.

Configure the meanings of the electronic signatures

The e-signature meanings are the text that a user can select to describe a reason for an electronic signature. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is installed with the following default meanings.

E-Signature definition	Default data types			
	Plate setup	**Thermocylcer protocol**	**Analysis protocol**	**Analysis results**
Reviewed and Approved Plate Set Up	Yes	Yes	No	No
Reviewed and Approved Results	No	No	Yes	Yes
Reviewed and Approved Template	No	No	Yes	Yes

Use the Security Settings dialog box to add or remove electronic signature meanings and to determine the data types to which they apply.

To...	Action
Add a meaning	1. From the e-Signature tab of the Security Settings dialog box, click **Add** in the e-Signature Meanings settings. 2. From the Create Meaning dialog box, enter a description of the e-Signature meaning, then click **OK**. 3. Select what data is signed for the selected meaning. 4. Click **Apply Settings**.
Delete a meaning	1. Select the meaning from the e-Signature Meanings list, then click **Remove**. 2. Click **Apply Settings**.

Configure the e-signature rights for user roles

To determine the user roles that can perform an electronic signature:

1. From the e-Signature tab of the Security Settings dialog box, select the check box next to the appropriate user roles in the User Role signature rights table.

2. Click **Apply Settings**.

Select the actions that require signature

IMPORTANT! Do not change electronic signature settings during calibration.

1. In the Signature Required column, select the check box next to each action for which you want to require electronic signatures (see below). This selection causes the software to present an e-sig prompt if a user performs the action on a data file that does not have the required signatures. The data must be signed before the user can perform the action.

Action	The software requires e-signatures when a user…
Print Report	Prints a report from an experiment or study.
Start Run	Initiates a run from the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

2. For each meaning of each selected action, enter the number of e-signatures from each user role that are required before the software can execute the associated action. For example, in the following figure, at least two users from the Administrator user role must sign an experiment using the "Reviewed and Approved Plate Set Up" meaning before a user can start the associated run.

Actions requiring signatures

Signature Required	Action	Number of signatures required for the selected action			
	Print Report	Meaning	Administrator	Technician	Scientist
✓	Start Run	Reviewed and Approved Plate Set Up	2	0	0
		Reviewed and Approved Results	0	0	0
		Reviewed and Approved Template	0	0	0

3. Click **Apply Settings**.

How the software prompts electronic signature

If the system is configured to check for a signature before starting a run or printing a report and the data are not signed, the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays a message when the user clicks **Start Run** or **Print Report**.

Example

The e-signature system is configured to require signatures from two users from the user account named Administrator before a user can start a run. The experiment has not been signed.

A user attempts to begin the run. The following message is displayed:

Missing Required Signatures

The requested operation cannot be performed because some required signatures are missing. The following e-Signatures must be collected before this operation can be performed:

Reviewed and Approved Plate Set Up by 2 Administrator(s)

OK
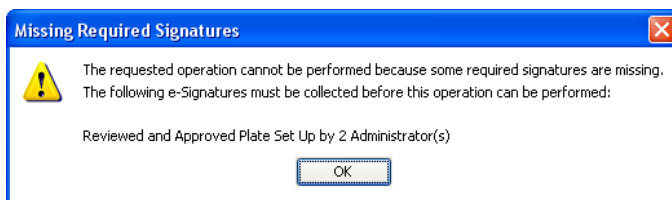
Before the run can start, two administrators must sign. If a user with an incorrect user role signs, the message is displayed again.

Generate e-signature reports

You can use the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to generate reports of e-signature history from open experiment (.eds) or template (.edt) files.

Task	Description
Display the e-sig records	1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, open an experiment or template file. 2. From the open experiment, click **Audit**, then click **E-Signatures**. 3. (Optional) Click any row to view details for individual signatures.
Save or print e-sig records	1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, open an experiment or template file. 2. From the open experiment, click **Audit**, then click **E-Signatures**. 3. From the table, select the record to be saved or printed. 4. Save or print the record: • Click (Save), select a location for the export file, enter a name for the file, then click **Save**. or • Click (Print). 5. Click **OK** in the confirmation message.
Save or print the table of e-signature events	1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, open an experiment or template file. 2. From the open file, click **Audit**, then click **Print E-Signatures**. 3. Save or print the record: • Click (Save), select a location for the export file, enter a name for the file, then click **Save**. or • Click (Print). 4. Click **OK** in the confirmation message.

Appendix E Security, Audit, and Electronic Signature
Export and import user, security, audit, and e-signature settings

E

Export and import user, security, audit, and e-signature settings

Note: The export/import feature can be used to replicate identical security, audit, and e-signature settings across multiple computers. The feature allows you to create a standard security, audit, and e-signature settings "image" for the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software that can then be imported by other copies of the software to bypass manual setup.

Task	Description
Export	1. In any screen of the Security Settings dialog box, click **Export**. 2. Select the items to export: **All** or **Custom**. Custom contains select settings: • **Users & Roles** – All user accounts with "Active" status and all user roles and associated permissions (in case a user account specifies a user role that does not exist on the system into which you import the profiles). • **System & Roles** – All system settings and all user roles and associated permissions. 3. Click **Export** or **OK**. 4. When prompted, specify the name and location for the exported file (.dat), then click **Save**. A message is displayed when the export completes.
Import	1. In any screen in the Security Settings dialog box, click **Import** in the Experiment Menu. 2. Select the .dat file to import, then click **Open**. A message is displayed asking if you want to overwrite the current system configuration. Click **Yes**. If any imported user accounts already exist on the system, you are prompted to overwrite or skip each account.

User overview

The Security, Audit, and Electronic Signature (SAE) module is a component of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software that provides the following functionality:

- **System security** – Controls user access to the software.
- **Auditing** – Tracks changes made to library items, actions performed by users, and changes to the Security, Audit, and Electronic Signature settings.
- **Electronic signature** – Requires users to provide a user name and password when performing certain functions.

Depending on the way that your administrator configures these features, you may see the following dialog boxes and prompts when you use the software.

User account permissions

If your user account does not have permission to perform any function in the software, menu commands are shaded.

Account suspension

If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is configured to suspend a user account for failed logins, and if you repeatedly enter an incorrect user name and/or password, your user account is suspended, and the Log In dialog box indicates that your account is inactive.

There are two ways to activate a suspended account:

- You can wait until the suspension period ends.
- An administrator can change the account status from Suspended to Active.

Note: While a user is suspended, another user can click Reset, then log in and replace the suspended user.

Session time-out

If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is configured to time-out and there is no user activity for the specified time, the Log In dialog box indicates that your user session has timed out. You must enter your user name and password to access the software.

The administrator or another user with permission to log in to timed-out sessions can click **Reset**, then log in.

User security tasks

Task	Description
Log in	Your access to functions in the software is based on the permissions associated with your user account. Functions for which you do not have permissions are shaded. **Note:** If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is configured for password expiration, you are periodically prompted to change your password. **Note:** If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is configured to monitor failed log in attempts, you will be locked out of the software if you repeatedly enter an incorrect user name and/or password.
Change your password when it expires	When your password is about to expire, a message is displayed when you log in. To change your password, select **Tools ▸ Change Password**. Enter your current password, then enter the new password two times, then click **OK**.
Enter a reason for change	If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is configured for auditing, you may be prompted to specify a reason when you make certain changes in the software. Depending on your QuantStudio™ 6 and 7 Flex Real-Time PCR System Software configuration, you can either select a reason from the list or enter a reason for change.
Perform an electronic signature	If your system is configured for electronic signature, you may be required to have the experiment signed by other users before you can print a report or start a run. If an item is set to require multiple signatures, all approvers must sign the associated data before the action can be completed. If electronic signature is enabled for experiments, any of the following may apply: • The **Tools ▸ Security ▸ Sign Data** menu option is enabled. • You are prompted to sign.

F Calibration Consumable Preparation

Create a background plate or array card

Whenever possible, use a Background Plate or the TaqMan® Array Background Buffer that is included with the spectral calibration kit. The plates/array cards supplied in the kit contain a buffer that accurately simulates the reagents used for PCR, and, therefore, produces high-quality calibration data. If a background plate or array card from a spectral calibration kit is not available, you can create one as described below.

Required materials

96/384-Well Plate Sample Block

- MicroAmp® Optical 96/384-Well Reaction Plate
- Safety glasses
- Optical Adhesive Cover or Optical Flat Caps
- Pipettor, 200-μL (with pipette tips)
- Powder-free gloves
- Deionized water

Array Card Sample Block *(QuantStudio™ 7 Flex System only)*

- Array Cards
- Applied Biosystems® Array Card Staker/Sealer
- Centrifuge with array card buckets and array card carrier clips
- Permanent marker or pen
- Pipettor, 200-μL (with pipette tips)
- Powder-free gloves
- Safety glasses
- Deionized water

Create a background plate

IMPORTANT! Wear powder-free gloves while creating the background plate.

1. Remove a MicroAmp®96/384-Well Optical Reaction Plate from its box and place it on a clean, dry surface.

2. Aliquot 20 μL deionized water to each well of the reaction plate.

3. Seal the plate using an optical adhesive cover or optical flat caps.

4. Use the plate for background calibration as you would a background plate from the spectral calibration kit.

Create a background array card *(QuantStudio™ 7 Flex System only)*

1. Remove an array card from its box and place it on a clean, dry surface.

2. Using a permanent marker, write "Background" on the side of the empty card.

Mark here
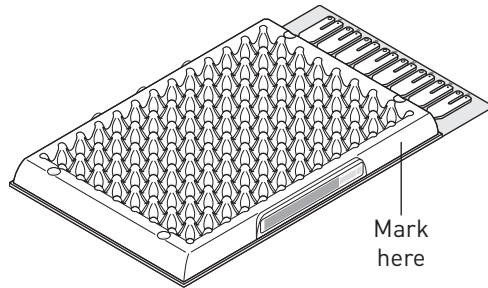
3. Pipet 100 µL of deionized water into each of the eight reservoirs in the card:

    a. Place the array card on a lab bench, with the foil side down.

    b. Load 100 µL of the solution into a pipette.

    c. Hold the pipette in an angled position (~45°) and place the tip into the fill port.

    There is a fill port on the left arm of each fill reservoir – the larger of the two holes.

Fill port   Vent port
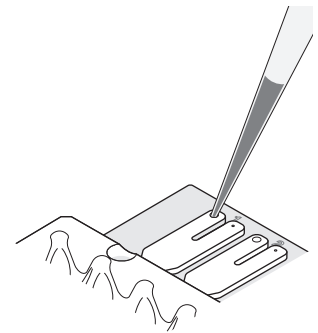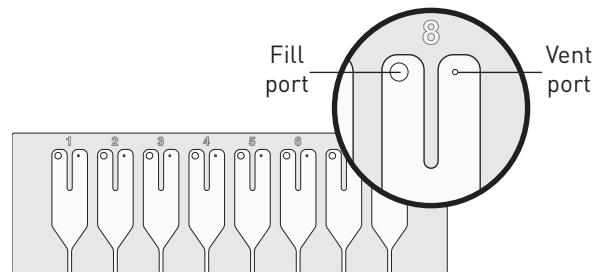
    d. Dispense the fluid so that it sweeps in and around the fill reservoir toward the vent port.

    When pipetting the reagents into the array card, pipet the entire 100-µL volume into the fill reservoir, but *do not* go past the first stop of pipettor plunger or you may blow the solution out of the port.
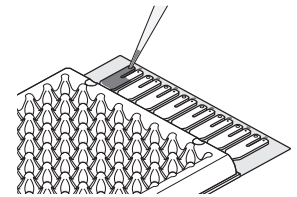
IMPORTANT! Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.

4. Centrifuge and seal the array card as explained in "Filling the calibration array cards" on page 36.

Create a custom dye plate for calibration

The QuantStudio™ 6 or 7 Flex System can be used to run assays designed with custom dyes (dyes not manufactured by Life Technologies). Custom dyes must excite between 455 and 672 nm and read between 505 and 723 nm.

Before you use custom dyes

Before using custom dyes with the QuantStudio™ 6 or 7 Flex System, you must:

- Determine optimum dye concentration.
- Create a custom dye plate.
- Add the custom dye to the software.
- Perform a dye calibration.

Required materials

- Centrifuge with plate adapter
- Custom dye(s)
- Safety glasses
- Powder-free gloves
- MicroAmp® Optical 96/384-Well Reaction Plate
- Optical Adhesive Cover
- Pipettors and pipette tips (200-μL and 1000-μL)
- Tubes (2-mL and 10-mL)
- Deionized water

Determine optimum dye concentration

Note: Wear powder-free gloves while creating the dye plate.

1. Prepare and load the custom dye plate:

   a. In the center of a 96/384-well plate, prepare a dilution series of the custom dye (for example, 25, 50, 100, 200, 400, 800, 1600, and 3200 nM) using 20 μL volumes for a 96/384-well plate.

   b. Seal the reaction plate using an optical adhesive cover.

   c. Load the prepared reaction plate.

2. Start the calibration wizard:

   a. In the Home screen of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, click **Instrument Console**.

   b. In the Instrument Console, select your QuantStudio™ 6 or 7 Flex System, then click **Add to My Instruments**.

   c. Select the QuantStudio™ 6 or 7 Flex System, then click **Manage Instrument**.

   d. In the Instrument Manager, click **Maintenance**, then click **ROI**.

   e. In the ROI Calibration screen, click **Start Calibration**.

   f. In the ROI dialog box, click **Next** until prompted to load the QuantStudio™ 6 or 7 Flex System. When the side door opens, load the sealed plate. Ensure that the plate/array card is properly aligned in the holder.

    g. In the ROI dialog box, select **Check the box when the ROI calibration plate has been loaded**, click **Next** twice, then click **START RUN** to start the calibration.

3. When the run is complete, inspect the ROI images:

    a. Select the first filter from the Filter drop-down list.

    b. Record the coordinate of the well that contains the lowest concentration of dye and that is encircled by a ring. This well contains the optimal concentration of the custom dye at the given filter.

    c. Repeat steps a and b for the remaining filters.

    d. After you determine the optimum concentration for each filter, determine the optimum concentration for the custom dye. Compare the results from all filters, then select the concentration that yields the highest possible signal in all filters.

4. Discard the plate.

    ⚠ **WARNING! PHYSICAL INJURY HAZARD**. During instrument operation, the plate can reach 100°C. Allow the plate to reach room temperature before removing.

5. In the ROI dialog box, click **Finish** to complete the calibration, then click **No** when prompted to save the results.

Create a custom dye plate

IMPORTANT! Wear powder-free gloves while creating the dye plate.

1. Prepare 2 mL of the custom dye at the concentration determined in "Determine optimum dye concentration" on page 152.

2. Pipet 20 µL of the diluted custom dye to all wells of an optical reaction plate.

3. Seal the wells of the reaction plate using an optical adhesive cover.

4. Centrifuge the plate for 2 minutes at < 1500 rpm.

    Note: The custom dye calibration plate must be well mixed and centrifuged.

5. Verify that the liquid in each well of the plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

Correct	Incorrect	
Liquid is at bottom of well.	• Not centrifuged with enough force, *or* • Not centrifuged for enough time	
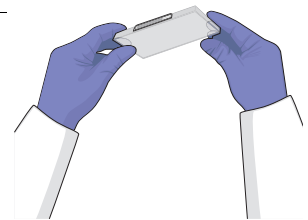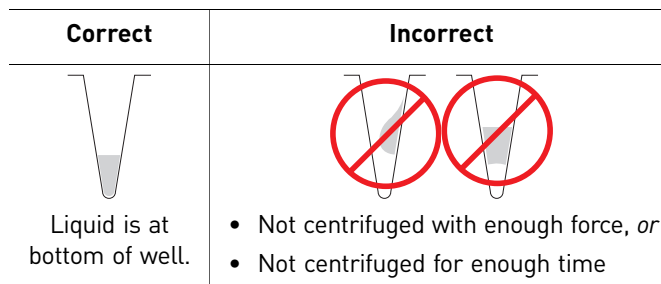
Add the custom dye to the software

1. Start the dye calibration:

   a. In the Home screen of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, click **Instrument Console**.

   b. In the Instrument Console, select your QuantStudio™ 6 or 7 Flex System from the list of instruments on the network, then click **Add to My Instruments**.

   c. Select the QuantStudio™ 6 or 7 Flex System, then click **Manage Instrument**.

   d. In the Instrument Manager, click **Maintenance**, then click **Dye**.

   e. In the Background Calibration screen, click **Start Calibration**.

2. In the Dye window, select a custom dye from the list or create the custom dye:

   a. Click **New Dye**.

   b. In the Dye Library dialog box, click **New**.

   c. Complete the New Dye dialog box, then click **OK**.

Field/option	Action
Name	Enter a name for the custom dye.
Wavelength	Enter the wavelength at which the dye fluoresces.
Type	Select: • **Reporter** if the dye works in conjunction with a quencher dye to report an increase of PCR product. • **Quencher** if the dye suppresses the fluorescence of a reporter dye until amplification of PCR product. • **Both** if the dye reports an increase of PCR product without the aid of a quencher dye.

   d. Click **Close**.

3. In the Dye window, enter a temperature setting for the calibration. Set the temperature to match the temperature at which you intend to collect data. For example, the temperature for all Life Technologies system dyes is 60°C because data collection for TaqMan® reagents occurs during the 60°C extension step of the PCR.

4. Load the appropriate dye plate into the plate adapter, select **Please check the box when the dye calibration plate has been loaded**, click **Next** twice, then click **START RUN** to start the calibration.

5. When the run is complete and the QuantStudio™ 6 or 7 Flex System ejects the plate, remove and discard the plate or array card.

   ⚠ **WARNING! PHYSICAL INJURY HAZARD**. During instrument operation, the plate can reach 100°C. Allow the plate to reach room temperature before removing.

6. In the Dye dialog box of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, click **Next**.

7. Verify the grouping of the dye spectra:

a. In the plate layout, select the wells of the plate.

b. Inspect the raw data. For each spectrum, verify that the peak is:

- Within the detectable range for the QuantStudio™ 6 and 7 Flex Systems.
- Free of irregular spectral peaks.
- Present at the correct filter for the dye.

Note: Among wells containing the same dye, variations in spectral position and peak position are caused by minor differences in the optical properties and excitation energy between the individual wells.

8. Verify the status of the calibration. If the calibration:

Passed – If all spectra are acceptable, finish the calibration:

a. Click **Next**.

b. Enter any comments you have in the Comments field, click **Finish**, then click **Yes** when prompted to save the calibration results.

Failed – Create another custom dye plate using the next dye concentration greater than the concentration determined in "Determine optimum dye concentration" on page 152, then perform the calibration again.

G

Parts and Materials

How to order

You can order materials and accessories directly from the Life Technologies store over the internet.

Note: Product availability and pricing may vary according to your region or country. Online ordering through the Life Technologies Store is not available in all countries. Contact your local Life Technologies representative for help.

To order through the website or the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software:

- Confirm that your computer has an Internet connection.
- We recommend the following browsers and Adobe® Acrobat® Reader versions to use the Life Technologies website:

Operating system	Microsoft® Internet Explorer®	Apple® Safari®	Mozilla® Firefox®	Adobe® Acrobat® Reader
Microsoft® Windows®	v6.x or later	None†	v2.x or later	v4.0 or later
Macintosh®	None†	v2.0.4 or later		

† Browser not available for this platform.

Note: Confirm that cookies and Javascript are turned on for the website to function correctly.

How to order from the Life Technologies website

To order...	Procedure
Assays and reagents	1. Go to **www.lifetechnologies.com** 2. Under "I Want to Buy," select the product of interest.
Instrument parts and accessories Calibration kits	1. Go to **www.lifetechnologies.com/quantstudio6flex** or **www.lifetechnologies.com/quantstudio7flex** 2. Click **Parts and accessories**. 3. Select the desired components, the complete the order as instructed. See "Kits, consumables, and accessories" on page 160 for a complete list of compatible instrument parts, accessories, and kits.

How to order from the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software

1. To find your assay on the Life Technologies Store, complete the Find Assay pane in the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software:

   a. Enter a gene name in the Enter Gene Name field, then click **Find Assay**.

   b. In the Find Assay Results dialog box, select your assay.

   c. Click **Apply Assay Selection**. The selected assay gets added to your shopping list.

2. Check that the Experiment Shopping List contains the desired materials, other than the assay selected in the previous step, and that the quantities are correct, then click **Order Materials in List**.

3. In the Order Materials - Login dialog box, enter your user name and password for the Life Technologies Store, then click **Log In and Submit**.

Note: If you do not have an account with the Life Technologies Store, click **Register Now** to create an account.

When you are connected to the Life Technologies Store, follow the prompts to complete your order.

Kits, consumables, and accessories

The following kits, consumables, and reagents are to be used with the QuantStudio™ 6 and 7 Flex Systems.

Note: For reagent or consumable shelf-life expiration date, see the package label.

Note: The QuantStudio™ 6 Flex System does not support the Array Card block.

Fast 96-well consumables

Consumable	Part no.	Storage (°C)	Shelf life at storage temperature
Fast 96-Well Spectral Calibration Plate with FAM™ Dye	4432600	≤–20	Use the consumable by the expiration date on the packaging
Fast 96-Well Spectral Calibration Plate with VIC® Dye	4432607		
Fast 96-Well Spectral Calibration Plate with ROX™ Dye	4432614		
Fast 96-Well Spectral Calibration Plate with TAMRA™ Dye	4432632		
Fast 96-Well Region of Interest (ROI) and Background Plates • Fast 96-Well Region of Interest (ROI) Calibration Plate • Fast 96-Well Background Plate	4432638		
Fast 96-Well Normalization Plates with FAM™/ROX™ and VIC®/ROX™ Dyes • Fast 96-Well Normalization Plate with FAM™/ROX™ Dye • Fast 96-Well Normalization Plate with VIC®/ROX™ Dye	4443774		
RNase P Fast 96-Well Instrument Verification Plate	4432656		
Fast 96-Well Background Plate	4481308		

96-well consumables

Consumable	Part no.	Storage (°C)	Shelf life at storage temperature
96-Well Spectral Calibration Plate with FAM™ Dye	4432538	≤–20	Use the consumable by the expiration date on the packaging.
96-Well Spectral Calibration Plate with VIC® Dye	4432545		
96-Well Spectral Calibration Plate with ROX™ Dye	4432551		
96-Well Spectral Calibration Plate with TAMRA™ Dye	4442918		
96-Well Region of Interest (ROI) and Background Plates • 96-Well Region of Interest (ROI) Calibration Plate • 96-Well Background Plate	4432626		
96-Well Normalization Plates with FAM™/ROX™ and VIC®/ROX™ Dyes • 96-Well Normalization Plate with FAM™/ROX™ Dye • 96-Well Normalization Plate with VIC®/ROX™ Dye	4443661		
RNase P 96-Well Instrument Verification Plate	4432593		
96-Well Background Plate	4481309		

384-well consumables

Consumable	Part no.	Storage (°C)	Shelf life at storage temperature
384-Well Spectral Calibration Plate with FAM™ Dye	4432445	≤–20	Use the consumable by the expiration date on the packaging.
384-Well Spectral Calibration Plate with VIC® Dye	4432452		
384-Well Spectral Calibration Plate with ROX™ Dye	4432458		
384-Well Spectral Calibration Plate with TAMRA™ Dye	4443619		
384-Well Region of Interest (ROI) and Background Plates • 384-Well Region of Interest (ROI) Calibration Plate • 384-Well Background Plate	4432482		
384-Well Normalization Plates with FAM™/ROX™ and VIC®/ROX™ Dyes • 384-Well Normalization Plate with FAM™/ROX™ Dye • 384-Well Normalization Plate with VIC®/ROX™ Dye	4432620		
RNase P 384-Well Instrument Verification Plate	4432500		
384-Well Background Plate	4481310		

Array card consumables

Consumable	Part no.	Storage (°C)	Shelf life at storage temperature
Array Card Spectral Calibration Dye Kit • Array Calibration with FAM™ Dye • Array Calibration with VIC® Dye • Array Calibration with ROX™ Dye • Array Calibration with ROI Dye • Array Background Buffer • Array Calibration with FAM™/ROX™ Dye • Array Calibration with VIC®/ROX™ Dye	4432644	≤–20	Use the consumable by the expiration date on the packaging.
Kit, RNase P Array Card Instrument Verification Reagents • Port 1 NTC • Port 2 Unknown A • Port 3 Unknown B • Port 4 Standard 200 Copies • Port 5 Standard 400 Copies • Port 6 Standard 800 Copies • Port 7 Standard 1600 Copies • Port 8 Standard 3200 Copies	4432650		
Array Card Background Buffer Kit • Background Buffer, 6 tubes • Array Card Kit (empty), 6-pack	4481307		

Note: The Array Card block consumables are applicable only to the QuantStudio™ 7 Flex System.

QuantStudio™ 6 and 7 Flex Systems accessories

Accessory	Part no.
384-Well Plate/Array Card Heated Cover	4454163
384-Well Plate Sample Block	4454165
96-Well Plate Heated Cover	4459841
96-Well Plate Sample Block	4459839
Array Card Bucket/Clip Set	4452205
Array Card Sample Block	4454164
Fast 96-Well Plate Heated Cover	4459842
Fast 96-Well Plate Sample Block	4459840
Halogen lamp (12 V, 75 W)	4480914
Handheld Barcode Scanner	4453271
Life Technologies Array Card Staker/Sealer	4456102

QuantStudio™ 6 and 7 Flex Systems empty plates and array cards

Consumable	Part no.
96-Well Fast Plate (empty), 10-pack	4346907
96-Well Fast Plate with Barcode (empty), 10-pack	4346906
96-Well Plate (empty), 10-pack	N8010560
96-Well Plate with Barcode (empty), 10-pack	4306737
Adhesive Seal Applicator Kit, 5-pack	4481196
Array Card Kit (empty), 1-pack[†]	4445702
Array Card Kit (empty), 4-pack[†]	4445701
Optical Adhesive Covers, 25-pack	4481193

† QuantStudio™ 7 Flex System only.

General-use materials and consumables

The following general-use materials and consumables are required to calibrate, maintain, and operate the QuantStudio™ 6 and 7 Flex Systems. Unless indicated otherwise, all materials shown below are available from major laboratory suppliers (MLS).

Material/consumable	Source
Bleach, 10% solution	MLS
Centrifuge with 96-well plate buckets	MLS
Cotton or nylon swabs and lint-free cloths	MLS
Ethanol, 95% solution	MLS
Optical clear adhesive film for PCR	MLS
Pipettors, 100-µL and 200-µL (with pipette tips)	MLS
Powder-free gloves	MLS
Safety glasses	MLS
Screwdriver, flathead	MLS
Tissue, lint-free	MLS
Deionized water	MLS

Instrument Specification and Layout

QuantStudio™ 6 and 7 Flex Systems specifications

The figure below summarizes the specifications and requirements for the QuantStudio™ 6 and 7 Flex Systems. For more information, refer to the *QuantStudio™ 6 and 7 Flex Systems Site Preparation Guide*.

Component	Height	Depth	Width	Weight
Instrument†	75.0 cm (29.5 in.)	70.0 cm (27.6 in.)	53.0 cm (20.9 in.)	70 kg (154.3 lbs)
Computer	56.5 cm (22.3 in.)	54.7 cm (22.4 in.)	21.6 cm (8.5 in.)	10 kg (22.0 lbs)
Monitor	38.0 cm (15.0 in.)	13.7 cm (5.4 in.)	37.4 cm (14.7 in.)	3.0 kg (6.7 lbs)
Keyboard	2.75 cm (1.1 in.)	44.5 cm (17.8 in.)	13.75 cm (5.5 in.)	0.75 kg (1.65 lbs)

† Weight varies depending on the sample block installed.
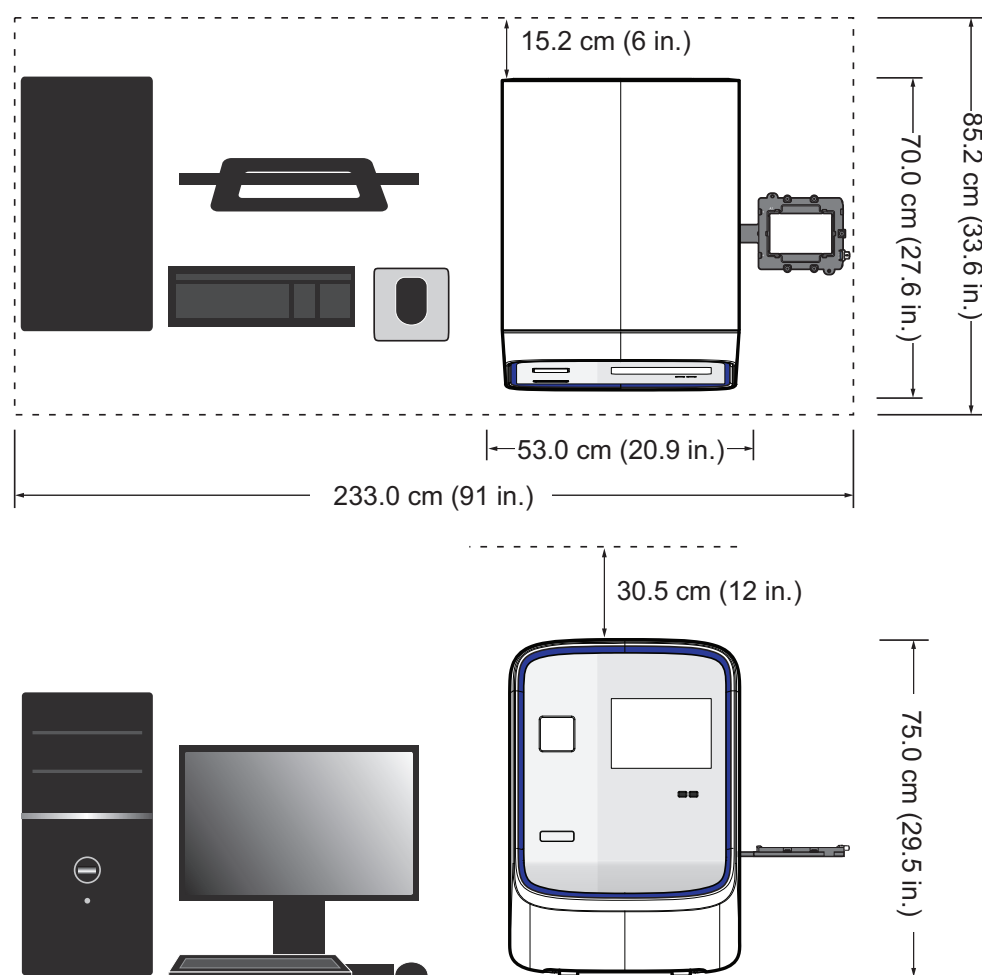
Required clearances

The QuantStudio™ 6 and 7 Flex Systems require the following additional clearances:

Component	Top	Front	Sides	Back
Instrument	30.5 cm (12.0 in.)	122 cm (48.0 in.)	51.0 cm (20.0 in.)	15.2 cm (6.0 in.)
Computer	—	30.5 cm (12.0 in.)	—	15.2 cm (6.0 in.)

Electrical requirements

⚠️ **WARNING!** For safety, the power outlet used for powering the instrument must be accessible at all times. See "Required clearances" on page 168 for information about the space needed between the wall and the instrument. In case of emergency, you must be able to immediately disconnect the main power supply to all the equipment. Allow adequate space between the wall and the equipment so that the power cords can be disconnected in case of emergency.

Note: Place the QuantStudio™ 6 or 7 Flex System and computer power receptacle on an electrical circuit that is not shared with electrically noisy devices or devices that can cause power surges, such as refrigeration units.

The following table provides electrical specifications for the instrument and associated devices. For all indicated input voltages, a 15 A circuit is required.

Device	Rated current	Rated power	Rated voltage	Rated frequency
Instrument	12.5 A	950 VA	100–240 ± 10% VAC	50/60 Hz
Computer	2.1 A	125 VA		
Monitor	1.5 A	65 VA		

Note: The instrument, monitor, and computer self-adjust for 100–240 V input voltages of 50/60 Hz.

Environmental requirements

Condition	Description
Altitude	Located between sea level and 6000 ft (~1800 m) above sea level
Electromagnetic interference	Do not use this device in close proximity to sources of strong electromagnetic radiation (for example, unshielded intentional RF sources). Strong electromagnetic radiation may interfere with the proper operation of the device.
Humidity	15–80% (noncondensing)
Installation site	For indoor use only
Pollution	The instrument has a Pollution Degree rating of II. The instrument may only be installed in an environment that has nonconductive pollutants such as dust particles or wood chips. Typical environments with a Pollution Degree II rating are laboratories and sales and commercial areas. The noise output of the instrument is <60 dB at idle.
Temperature	15–30°C (60–85°F)
Thermal output	During operation the net thermal output, based on the actual current draw of the QuantStudio™ 6 and 7 Flex Instrument and computer, is expected to be approximately 2731 Btu/h (800 W).
Vibration	Ensure that the instrument is not adjacent to strong vibration sources, such as a centrifuge, pump, or compressor. Excessive vibration will affect instrument performance.
Other conditions	Ensure the room is away from any vents that could expel particulate material on the instrument components. Avoid placing the instrument and computer adjacent to heaters, cooling ducts, or in direct sunlight.

Network requirements

The computer is factory-configured for the TCP/IP protocol. The product includes a fast Ethernet adapter (10/100 Mbps) with an RJ45-type connector and one 3-m (9.8-ft) crossover Ethernet cable that connects the computer and the instrument.

If the instrument will be connected to a LAN, an active, tested LAN connection must be in place before the scheduled installation date. Due to differences in network connections, the Life Technologies service representative cannot configure the instrument to access a specific network.

You must supply a standard Category 5 Ethernet cable of the required length to connect the computer to your LAN.

QuantStudio™ 6 and 7 Flex Systems layout and connections

The QuantStudio™ 6 and 7 Flex Systems consist of the components in the following figure.
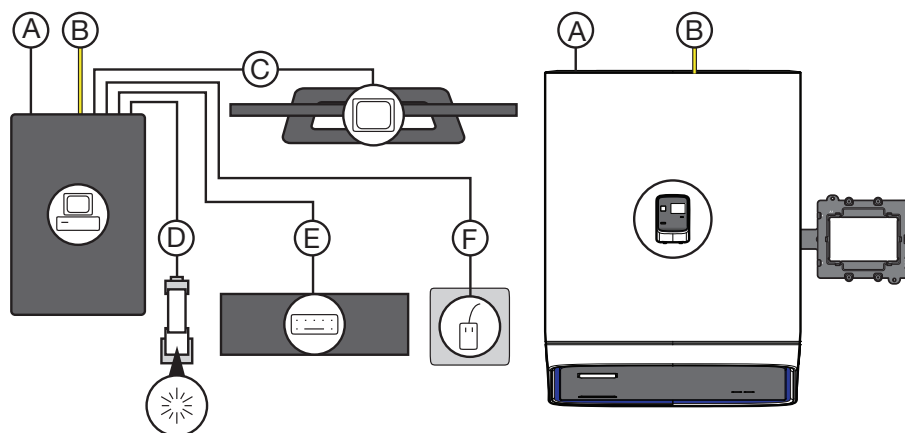
	Component	Description
	QuantStudio™ 6 or 7 Flex System	Performs fluorescence detection and data collection of experiment and calibration plates or array cards.
	Computer	Run the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software that is used to:
	Monitor	• Calibrate the QuantStudio™ 6 and 7 Flex Systems.
	Keyboard	• Set up experiments.
		• (Optional) Run experiments.
	Mouse	• Analyze experiments.
	Barcode scanner	Scans the barcodes of plates and array cards.

	Connection	Description
A	Power cables	Supply power to the computer and the QuantStudio™ 6 and 7 Flex Systems.†
B	LAN connection or Ethernet cable‡	Connects the QuantStudio™ 6 and 7 Flex Systems (Ethernet port) to the Ethernet port on the network interface card in the computer. The components can be connected over a local area network or a via a direct physical connection.
C	DVI cable	Connects the monitor to the computer (DVI port).
D	Barcode scanner cable	Connects the optional barcode scanner to the computer (USB port).
E	Keyboard cable	Connects the keyboard to the computer (USB port).
F	Mouse cable	Connects the mouse to the computer (USB port).

† Supplies 115/230 V depending on the geographic location of the installation.

‡ Supplied with the QuantStudio™ 6 and 7 Flex Systems.

I

Safety

⚠ **WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.

- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the symbol is used along with user attention words described in the "About This Guide" section to highlight important safety information. The following table gives the meaning of these symbols.

Symbol	English	Français
	Caution, risk of danger Consult the manual for further safety information.	Attention, risque de danger Consulter le manuel pour d'autres renseignements de sécurité.
	Caution, hot surface	Attention, surface chaude
	Caution, risk of electrical shock	Attention, risque de choc électrique
	Laser radiation	Rayonnement laser
	Moving parts	Parties mobiles
	Potential biohazard	Danger biologique potentiel
	Ultraviolet light	Rayonnement ultraviolet
	Potential slipping hazard	Danger de glisser potentiel
	On	On (marche)
	Off	Off (arrêt)
	On/Off	On/Off (marche/arrêt)
	Standby	En attente
	Earth (ground) terminal	Borne de (mise à la) terre
	Protective conductor terminal (main ground)	Borne de conducteur de protection (mise à la terre principale)
~	Terminal that can receive or supply alternating current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant de type alternatif

Symbol	English	Français
≈	Terminal that can receive or supply alternating or direct current or voltage	Borne pouvant recevoir ou envoyer une tension ou un courant continu ou alternatif
	Do not dispose of this product in unsorted municipal waste **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.	Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif. **CAUTION!** Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable.

Conformity mark	Description
CE	Indicates conformity with European Union Directives (see "Safety and electromagnetic compatibility (EMC) standards" on page 181).
C (UL) US	Indicates conformity with safety requirements for Canada and U.S.A.
(C-tick)	Indicates conformity with Australian standards for electromagnetic compatibility.

Conforming symbols

The following table describes symbols that may be displayed on the Life Technologies QuantStudio™ 6 and 7 Flex Systems, consumables, or reagents.

Symbol	Description
LOT	BATCH CODE This symbol is accompanied by the manufacturer's batch code. It indicates the lot number.
REF	CATALOGUE NUMBER The manufacturer's catalogue number will appear after or below this symbol.
[i]	CONSULT INSTRUCTIONS FOR USE This symbol indicates that you should consult the product insert for further information. If a product insert does not exist, or if the product insert does not contain the symbol or the required information, consult the user manual.
CONT	CONTAINS
⊘	DO NOT REUSE For one time use only.
	LOWER LIMIT OF TEMPERATURE The lower limit of temperature is indicated adjacent to the symbol.
	MANUFACTURER The name and the address of the manufacturer will appear next to this symbol.
SN	SERIAL NUMBER The serial number will appear after or below this symbol.
	TEMPERATURE LIMITATION The upper and lower limits of temperature are indicated adjacent to the upper and lower horizontal lines.
	UPPER LIMIT OF TEMPERATURE The upper limit of temperature is indicated adjacent to the symbol.
	USE BY This symbol is accompanied by a date to indicate that the device should not be used after the end of the date shown.

Locations of safety labels on instrument

The QuantStudio™ 6 and 7 Flex Systems contain warnings at the following locations:
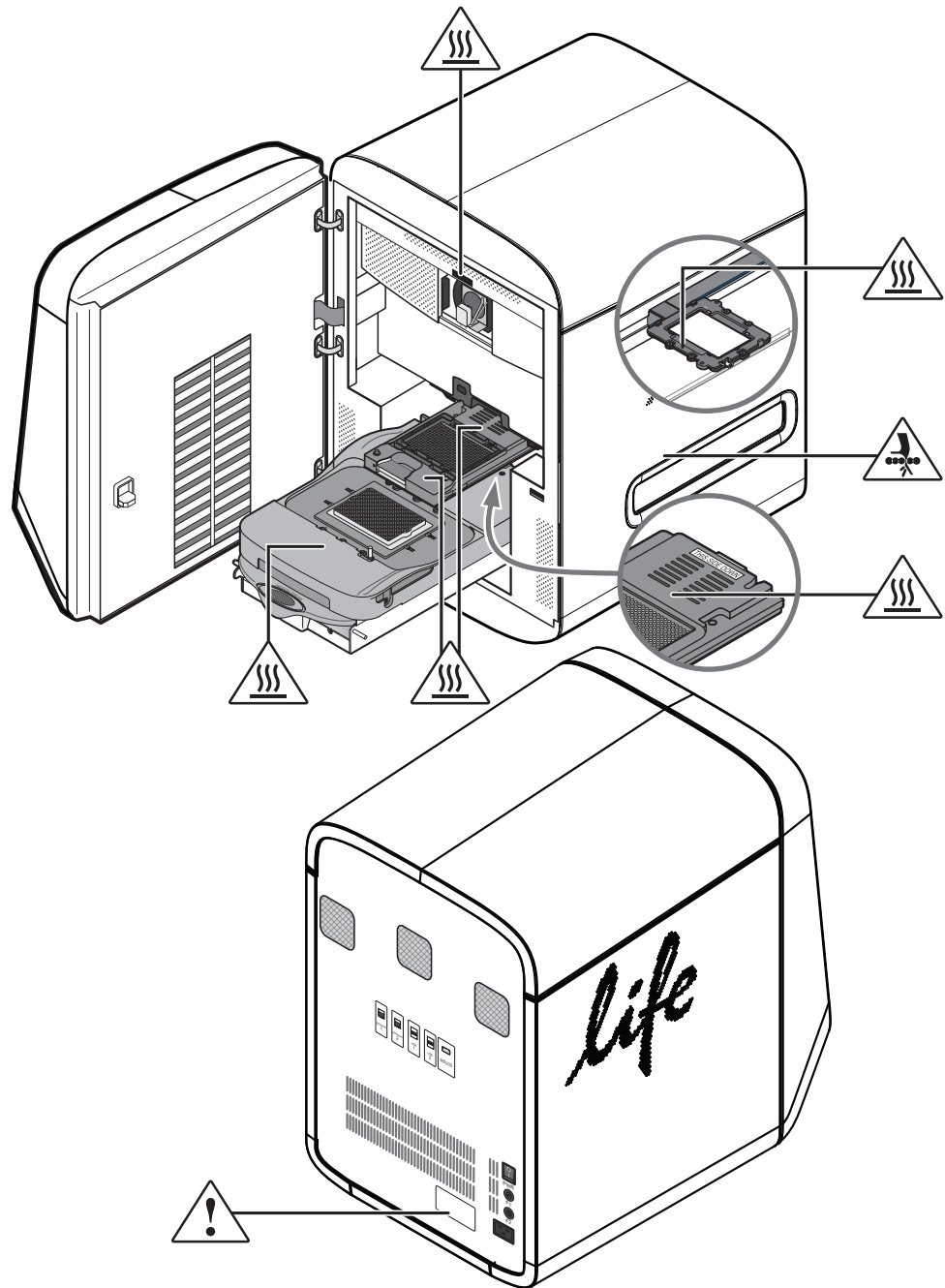
Safety alerts on this instrument

Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

English	French translation
CAUTION! **Hazardous chemicals.** Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION! Produits chimiques dangereux.** Lire les fiches signalétiques (FS) avant de manipuler les produits.
CAUTION! **Hazardous waste.** Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION! Déchets dangereux.** Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l'élimination des déchets.
CAUTION! Class 2(II) visible and/or invisible laser radiation present when open Do not stare directly into the beam or view directly with optical instruments.	**ATTENTION!** Rayonnement laser visible ou invisible de classe 2 (II) présent en position ouverte. Ne pas regarder directement dans le faisceau ou avec des instruments optiques.

Instrument safety

General

CAUTION! **Do not remove instrument protective covers.** If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Physical injury

CAUTION! **Moving and Lifting Injury.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide.

Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Ensure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

CAUTION! **Moving Parts.** Moving parts can crush, pinch, and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical

WARNING! **Fuse Installation.** Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

WARNING! **Ensure appropriate electrical supply.** For safe operation of the instrument:
- Plug the instrument into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! **Power Supply Line Cords.** Use properly configured and approved line cords for the power supply in your facility.

WARNING! **Disconnecting Power.** To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Cleaning and decontamination

CAUTION! **Cleaning and decontamination.**
- If hazardous materials are spilled onto the instrument, the instrument should be appropriately decontaminated.
- Using cleaning or decontamination methods other than those recommended by the manufacturer may compromise the safety or quality of the instrument.
- Care should be taken to not use decontamination or cleaning agents which would cause a hazard as a result of a reaction with parts of the equipment or with material contained in it.
- For the protection of others, ensure the instrument is properly decontaminated prior to having the instrument serviced at your facility or before sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan. Decontamination forms may be requested from customer service.

Instrument, component, and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do no dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Barcode scanner laser safety

Laser classification   The optional barcode scanners included with the QuantStudio™ 6 and 7 Flex Systems are categorized as Class 2 (II) lasers.

Laser safety requirements   Class 2 (II) lasers are low-power, visible-light lasers that can damage the eyes. Never look directly into the laser beam. The scanner is designed to prevent human access to harmful levels of laser light during normal operation, user maintenance, or during prescribed service operations.

> **WARNING! LASER HAZARD.** Class 2 (II) lasers can cause damage to eyes. Avoid looking into a Class 2 (II) laser beam or pointing a Class 2 (II) laser beam into another person's eyes.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

> **CAUTION! MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD.** These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

Safety

Reference	Description
EU Directive 2006/95/EC	European Union "Low Voltage Directive"
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1:* *General requirements*
IEC 61010-2-010 EN 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010:* *Particular requirements for laboratory equipment for the heating of materials*
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081:* *Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*

EMC

Reference	Description
Directive 2004/108/EC	European Union "EMC Directive"
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 18 (47 CFR)	U.S. Standard "Industrial, Scientific, and Medical Equipment"
AS/NZS 2064	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 3	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Environmental design

Reference	Description
Directive 2002/96/EC	European Union "WEEE Directive" – Waste electrical and electronic equipment
Directive 2002/95/EC	European Union "RoHS Directive" – Restriction of hazardous substances in electrical and electronic equipment

Chemical safety

⚠ **WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

· Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.

· Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).

· Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).

· Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.

· Handle chemical wastes in a fume hood.

· Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)

· After emptying a waste container, seal it with the cap provided.

· Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.

· Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

· **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

⚠ **WARNING! HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

⚠ **WARNING! 4L Reagent and Waste Bottle Safety.** Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

⚠ **WARNING! Potential Biohazard.** Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

⚠ **WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**

- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**

- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

⚠

Documentation and Support

Obtaining information from the Help system

The QuantStudio™ 6 and 7 Flex Systems have a Help system that describes how to use each feature of the user interface. Access the Help system by doing one of the following:

- Click 🔵 in the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software window.
- Select **Help ▸ QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Help**.
- Press **F1**.

You can use the Help system to find topics of interest by:

- Reviewing the table of contents.
- Searching for a specific topic.
- Searching an alphabetized index.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com

At the Life Technologies website, you can:

- Access worldwide telephone and fax numbers to contact Life Technologies Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Life Technologies user documents, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
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- Download software updates and patches.

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Documentation and Support
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Glossary

aligned melt curve plot	Plot of the re-scaled melt curve.
allele	In a diploid organism, one of two DNA sequences found at the same locus (for example, a particular gene), but located on homologous chromosomes. Two corresponding alleles may have the identical sequence, or they may differ somewhat, often at one or more single-base sites (SNPs).
amplicon	A segment of DNA amplified during PCR.
amplification	Part of the instrument run in which PCR amplifies the target. Fluorescence data collected during amplification are displayed in an Amplification Plot, and the data are used to calculate results. **Note:** Only quantitative real-time PCR experiments, not end-point experiments, take amplification data into account.
amplification efficiency (EFF%)	Calculation of the efficiency of the PCR amplification in an experiment. EFF% is calculated using the slope of the regression line in the standard curve. A slope close to –3.32 indicates optimal, 100% PCR amplification efficiency.
Amplification Plot	Display of data collected during the cycling stage of PCR amplification. The Amplification Plot can be viewed as: • Baseline-corrected normalized reporter (ΔR_n) vs. cycle • Normalized reporter (R_n) vs. cycle • Threshold cycle (C_T) vs. well
amplification stage	Part of the instrument run in which PCR amplifies the target. The amplification stage, called a cycling stage in the thermal profile, consists of denaturing, primer annealing, and extension steps that are repeated. Fluorescence data collected during the extension stage are displayed in an amplification plot, and the data are used to calculate results. With chemistry, the last two steps of a PCR stage are typically combined. See also cycling stage.
assay	In a PCR reaction mix, two target-specific primers or two primers and a probe used to amplify a target.
assay mix	PCR reaction component in assays. The assay mix contains primers designed to amplify a target and a probe designed to detect amplification of the target.

automatic baseline	An analysis setting in which the software calculates the baseline start and end cycles for the amplification plot. See also baseline.
automatic threshold	An analysis setting in which the software calculates the baseline start and end cycles and the threshold in the amplification plot. The software uses the baseline and threshold to calculate the threshold cycle (C_T). See also threshold cycle (CT).
background calibration	Type of calibration in which the instrument performs reads of a background plate or array card, averages the spectra recorded during the run, and extracts the resulting spectral component to a calibration file. The software then uses the calibration file during subsequent runs to remove the background fluorescence from the run data.
baseline	From the Amplification Plot, a cycle-to-cycle range that defines background fluorescence.
baseline-corrected normalized reporter (ΔR_n)	The magnitude of normalized fluorescence signal generated by the reporter. In experiments that contain data from real-time PCR, the magnitude of normalized fluorescence signal generated by the reporter at each cycle during the PCR amplification. From the ΔR_n vs. Cycle Amplification Plot, ΔR_n is calculated at each cycle as: $\Delta R_{n\ (cycle)} = R_{n\ (cycle)} - R_{n\ (baseline)}$, where R_n = normalized reporter See also normalized reporter (Rn).
blocked IPC	In presence/absence experiments, a reaction that contains IPC blocking agent, which blocks amplification of the internal positive control (IPC). In QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, also the name of the task for the IPC target in wells that contain IPC blocking agent. See also negative control-blocked IPC wells.
CE mark $C\epsilon$	The CE Mark (an acronym for the French "Conformite Europeene") of conformity certifies that a product has met the essential requirements of the applicable European Directive.
chemistry	See reagents.
control	Sample of known genotype or melt-curve shape.
C_T	See threshold cycle (CT).
C_T algorithm	The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software uses the Baseline Threshold algorithm.
cycle threshold	See threshold cycle (CT).
cycling stage	In the thermal profile, a stage that is repeated. A cycling stage is also called an amplification stage. See also amplification stage.

data collection	During the instrument run, a process in which an instrument detects fluorescence data from each well of the plate or array card. The instrument transforms the signal to electronic data and saves the data in the experiment file.
delta R$_n$ (ΔR$_n$)	See baseline-corrected normalized reporter (DRn).
derivative melt curve plot	The default view of the melting curve. It plots the negative derivative data (–R$_n'$) vs. temperature.
	Note: An alternative view is the raw data view, which plots the normalized fluorescence from reporter dye vs. temperature.
derivative reporter (–R$_n'$)	The rate of change in fluorescence as a function of temperature. R$_n'$ is used to plot a melting curve. Significant decrease of the fluorescent signal generates a positive peak on the derivative view of the melting curve.
dissociation curve	See melt curve.
dye calibration	Type of calibration in which the software collects spectral data from a series of dye standards and stores the spectral information for the dye standards in a pure spectra calibration file. This file is used during experiment runs to characterize and distinguish the individual contribution of each dye in the total fluorescence collected by the instrument.
EFF%	See amplification efficiency (EFF%).
error	The standard error of the slope of the regression line in the standard curve.
	The error can be used to calculate a confidence interval (CI) for the slope. Because the amplification efficiency (EFF%) is calculated from the slope, knowing the error allows a CI for the amplification efficiency to be calculated.
filter	Dye excitation and emission filter combination that is set for a experiment. The QuantStudio™ 7 Flex System includes a decoupled, six by six-color filter set. The QuantStudio™ 6 Flex System includes a coupled five-color filter set. The excitation and emission filter combinations are preset by the experiment document (.eds) file for your experiment.
flag	A quality control (QC) indicator which, when applied by the software to a well during analysis, indicates a possible issue with that reaction. For example, a flag may be issued if no amplification is detected in a well. Flags indicating potential problems are displayed in the Quality Control tab of the Plate Layout, Well Table, and QC Summary screens.
forward primer	Oligonucleotide that flanks the 5′ end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.
heterozygote	Samples having both allele 1 and allele 2.
holding stage	In the thermal profile, the stage that holds the temperature constant for a defined period of time.

homozygote	Samples having only allele 1 or only allele 2.
Instrument Console	A software feature that allows you to monitor the status of any instrument on the network; view calibration, maintenance, and instrument properties for a selected instrument; and open and close the instrument drawer.
Instrument Manager	A software feature that allows you to monitor the status of an instrument; monitor Amplification Plots and temperature plots in real time; view the calibration status, perform calibrations and manage files on the instrument, including downloading completed experiments to your computer.
internal positive control (IPC)	In presence/absence experiments, a short synthetic DNA template that is added to PCR reactions. The IPC can be used to distinguish between true negative results (the target is absent in the samples) and negative results caused by PCR inhibitors, incorrect assay setup, or reagent or instrument failure.
IPC	See internal positive control (IPC).
IPC+	See negative control-IPC wells.
melt curve	A plot of data collected during the melt curve stage. Peaks in the melt curve can indicate the melting temperature (T_m) of the target, or they can identify nonspecific PCR amplification. In the software, you can view the melt curve as normalized reporter (R_n) vs. temperature or as derivative reporter ($-R_n'$) vs. temperature.
melt curve characteristics	The melt curve shape and the difference in melting temperature (T_m) values.
melt curve stage	In the thermal profile, a stage with a temperature increment to generate a melt curve.
melting temperature (T_m)	The temperature at which 50% of the DNA is double-stranded and 50% of the DNA is dissociated into single-stranded DNA. In a melt curve experiment, the melt curve plot displays the melting temperature.
melting transition region	In Melt Curve experiments, the region before and after the melting temperature (Tm).
negative control (NC)	The task for targets or SNP assays in wells that contain water or buffer instead of sample. No amplification of the target should occur in negative control wells. Previously called no template control (NTC).
negative control-blocked IPC wells	In presence/absence experiments, wells that contain IPC blocking agent instead of sample in the PCR reaction. No amplification should occur in negative control-blocked IPC wells because the reaction contains no sample and amplification of the IPC is blocked. Previously called no amplification control (NAC).
negative control-IPC wells	In presence/absence experiments, wells that contain IPC template and buffer or water instead of sample. Only the IPC template should amplify in negative control-IPC wells because the reaction contains no sample. Previously called IPC+.

no template control (NTC)	See negative control (NC).
nonfluorescent quencher-minor groove binder (NFQ-MGB)	Molecules that are attached to the 3′ end of probes. When the probe is intact, the nonfluorescent quencher (NFQ) prevents the reporter dye from emitting fluorescence signal. Because the NFQ does not fluoresce, it produces lower background signals, resulting in improved precision in quantification. The minor groove binder (MGB) increases the melting temperature (T_m) of the probe without increasing its length, allowing for the design of shorter probes.
normalization calibration	Type of calibration in which the software collects data from the normalization standards, then stores it in a normalization calibration file.
normalized quantity	Either the C_T Avg. of the target gene minus the C_T Avg. of the endogenous control (Comparative C_T experiments), or the Q Avg. of the target divided by the Q Avg. of the endogenous control (Relative Standard Curve experiments).
normalized quantity mean	The relative standard curve equivalent of the ΔC_T mean value found in Comparative C_T experiments (computed as the geometric mean).
normalized quantity SE	The relative standard curve equivalent of the ΔC_T SE value found in Comparative C_T experiments (computed as the geometric standard error of the mean).
normalized reporter (R_n)	Fluorescence signal from the reporter dye normalized to the fluorescence signal of the passive reference dye (usually ROX™ dye on Life Technologies instruments).
outlier	A measurement (such as a C_T) that deviates significantly from the measurement of the other replicates for that same sample.
passive reference	A dye that produces fluorescence signal independent of PCR amplification, and that is added to each reaction at a constant concentration. Because the passive reference signal should be consistent across all wells, it is used to normalize the reporter dye signal to account for non-PCR related fluorescence fluctuations caused by minor well-to-well differences in volume. Normalization to the passive reference signal generally results in data with noticeably high precision among technical replicates.
plate layout	An illustration of the grid of wells and assigned content in the plate or array card. The number of rows and columns in the grid depends on the sample block that you use. From the software, you can use the plate layout as a selection tool to assign well contents, to view well assignments, and to view results. The plate layout can be printed, included in a report, exported, and saved as a slide for a presentation.
point	One standard in a standard curve. The standard quantity for each point in a standard curve is calculated based on the starting quantity and serial factor.
positive control	In genotyping and presence/absence experiments, a DNA sample with a known genotype, homozygous or heterozygous. In the software, the task for the SNP assay in wells that contain a sample with a known genotype.

pure dye	Fluorescent compound used to calibrate the instrument. See system dye.
quantity	In quantification experiments, the amount of target in the samples. Absolute quantity can refer to copy number, mass, molarity, or viral load. Relative quantity refers to the fold-difference between normalized quantity of target in the sample and normalized quantity of target in the reference sample.
quencher	A molecule attached to the 3′ end of probes to prevent the reporter from emitting fluorescence signal while the probe is intact. With reagents, a nonfluorescent quencher-minor groove binder (NFQ-MGB) can be used as the quencher.
R^2 value	Regression coefficient calculated from the regression line in the standard curve. An important quality value, the R^2 value indicates the closeness of fit between the standard curve regression line and the individual C_T data points from the standard reactions. A value of 1.00 indicates a perfect fit between the regression line and the data points.
Raw Data Plot	A plot of raw fluorescent signal as detected through each emission filter, used to view raw data for individual wells and at individual cycles.
reaction mix	A solution that contains all components to run the PCR reaction, except for the template (sample, standard, or control). Also called a "PCR cocktail".
reagents	The PCR reaction components used to amplify the target and to detect amplification.
real-time PCR	Process of collecting fluorescence data during PCR. Data from the real-time PCR are used to calculate results for quantification experiments or to troubleshoot results for genotyping or presence/absence experiments.
reference sample	In relative standard curve and Comparative C_T ($\Delta\Delta C_T$) experiments, the sample used as the basis for relative quantification results. Also called the calibrator.
region of interest (ROI) calibration	Type of calibration in which the software maps the positions of the wells on the sample block of the instrument. The software uses the ROI calibration data to associate increases in fluorescence during a run with specific wells of the plate or array card. A calibration image for each individual filter must be generated to account for minor differences in the optical path.
regression coefficients	Values calculated from the regression line in standard curves, including the R^2 value, slope, and y-intercept. You can use the regression coefficients to evaluate the quality of results from the standards. See also standard curve.
regression line	In standard curve and relative standard curve experiments, the best-fit line from the standard curve. Regression line formula: $C_T = m \, [\log (Qty)] + b$ where m is the slope, b is the y-intercept, and Qty is the standard quantity. See also regression coefficients.

reject well	An action that the software performs during analysis to remove one or more wells from further analysis if a specific flag is applied to the well.
relative standard curve method	A method to determine relative quantities. This method compensates for target and endogenous control efficiency differences within each run. In all experiments, unknown samples and dilution series of template (such as cDNA) are amplified. Following a run, the instrument software interpolates relative quantities for each unknown sample from the appropriate dilution curve, then normalizes the data for each sample (or set of replicates) as follows: target QAvg. ÷ endogenous control QAvg.
replicate group	A user-defined biological grouping. A replicate group may be a set of identical reactions in a experiment.
replicates	Total number of identical reactions containing identical components and identical volumes.
reporter	A fluorescent dye used to detect amplification. With reagents, the reporter dye is attached to the 5′ end.
reverse primer	An oligonucleotide that flanks the 3′ end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.
reverse transcriptase	An enzyme that converts RNA to cDNA.
R_n	See normalized reporter (Rn).
ROX™ dye	A dye supplied by Life Technologies, which is used as a passive reference in some experiments.
run method	Definition of the reaction volume and the thermal profile for the instrument run. The run method specifies the temperature, time, ramp, and data collection points for all steps and stages of the instrument run.
sample	The biological tissue or specimen that you are testing for a target gene.
sample definition file	A tab-delimited file (.txt) or Microsoft® Excel® file (.xls or .xlsx) that contains the sample name and custom sample properties for a single experiment.

security, auditing and e-signature	A separately licensed software module that provides:

- **System Security** – Controls user access to the software. Provides a default Administrator user account. You can define additional user accounts and permissions.

- **Auditing** – Tracks changes made to library items, actions performed by users, and changes to the Security and Audit settings. The software automatically audits some actions silently. You can select other items for auditing and specify the audit mode. Provides reports for audited library items, Security and Audit changes, and actions.

- **Electronic Signature (e-signature)** – Controls whether users are permitted, prompted, or required to provide a user name and password when accessing certain software features. You can select which features are controlled and the number of signatures required for access. When authorized persons use this feature, they are creating a legally binding signature.

serial factor	In the software, a numeric value that defines the sequence of quantities in the standard curve. The serial factor and the starting quantity are used to calculate the standard quantity for each point in the standard curve. For example, if the standard curve is defined with a serial factor of 1:10 or 10X, the difference between any two adjacent points in the curve is 10-fold.
slope	Regression coefficient calculated from the regression line in the standard curve. The slope indicates the PCR amplification efficiency for the assay. A slope of -3.32 indicates 100% amplification efficiency.
	See also amplification efficiency (EFF%) and regression line.
stage	In the thermal profile, a group of one or more steps. Examples: PCR stage, cycling stage (also called amplification stage), and hold stage.
standard	A sample that you dilute and amplify along with unknown samples. This dilution series can contain known starting quantities of the target of interest (absolute standard curve) or it can be of known dilution factor (relative standard curve). Following the run, the software interpolates the C_T values of the unknowns to this curve, yielding either specific quantities of the target (for absolute curves) or relative quantities (for relative dilution curves).
	See also standard curve.
standard curve	In standard curve and relative standard curve experiments:

- The best-fit line in a plot of the C_T values from the standard reactions plotted against standard quantities. See also regression line.

- A set of standards containing a range of known quantities. Results from the standard curve reactions are used to generate the standard curve. The standard curve is defined by the number of points in the dilution series, the number of standard replicates, the starting quantity, and the serial factor.

standard curve method	Method for determining absolute target quantity in samples. With the standard curve method, the software measures amplification of the target in samples and in a standard dilution series. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates the absolute quantity of target in the samples.

See also standard and standard curve.

standard dilution series	In standard curve and relative standard curve experiments, a set of standards containing a range of known quantities. The standard dilution series is prepared by serially diluting standards. For example, the standard stock is used to prepare the first dilution point, the first dilution point is used to prepare the second dilution point, and so on. In the software, the volumes needed to prepare a standard dilution series are calculated by the number of dilution points, the number of standard replicates, the starting quantity, the serial factor, and the standard concentration in the stock. See also standard curve.
standard quantity	In the PCR reaction, a known quantity. In standard curve experiments, the quantity of target in the standard. From the software, the units for standard quantity can be for mass, copy number, viral load, or other units for measuring the quantity of target. Standard quantity can also refer to dilution factor.
starting quantity	When defining a standard curve in the software, the highest quantity.
step	A component of the thermal profile. For each step in the thermal profile, you can set the ramp rate (ramp increment for melt curve steps), hold temperature, and hold time (duration) are set as part of the .tdd file. Data collection can also be turned on or off for the ramp or the hold parts of the step in each .tdd file. For cycling stages, a step is also defined by the AutoDelta status.
system dye	Dye supplied by Life Technologies and calibrated on the QuantStudio™ 6 and 7 Flex Systems. Before you use system dyes in your experiments, make sure the dye calibration is current in the Instrument Console.
target	The nucleic acid sequence to amplify and detect.
target color	From the software, a color assigned to a target to identify the target in the plate layout and analysis plots.
task	From the software, the type of reaction performed in the well for the target or SNP assay.
technical replicates	Wells containing identical reaction components, including sample; important for evaluating precision.
temperature plot	From the software, a display of temperatures for the instrument cover and instrument block during the instrument run.
template	The type of nucleic acid to add to the PCR reaction.
experiment	Refers to the entire process of performing a run, including setup, run, and analysis.
thermal profile	Part of the run method that specifies the temperature, time, ramp, and data collection points for all steps and stages of the instrument run.

experiment document file (.eds)	The Life Technologies name for the electronic records that comprise all information about a particular plate or array card, including metadata (name, barcode, comments), plate setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, analysis results, audit records, and other consumable-specific data. Experiment document files have the .eds suffix.
threshold	In Amplification Plots, the level of fluorescence above the baseline and within the exponential growth region.
threshold cycle (C_T)	The PCR cycle number at which the fluorescence meets the threshold in the Amplification Plot.
T_m	See melting temperature (Tm).
touchscreen	Instrument display that you touch to control the instrument.
uniformity calibration	Type of calibration in which the software measures sample block uniformity. The calibration generates data that compensate for the physical effects of the QuantStudio™ 6 and 7 Flex Systems filters on data collected during a experiment.
unknown	From the software, the task for the target in wells that contain the sample being tested. In quantification experiments, the task for the target in wells that contain a sample with unknown target quantities. In genotyping experiments, the task for the SNP assay in wells that contain a sample with an unknown genotype. In presence/absence experiments, the task for the target in wells that contain a sample in which the presence of the target is not known.In melt curve experiments, the task for the target in wells that contain a sample with an unknown melt curve profile.
variant	A sample (or group of samples) with a unique melt curve (that is, the melt curve is different from the melt curves of other samples or controls used in the experiment). The software determines melt curve differences by the melting temperature (T_m) and the shape of the melt curve.
y-intercept	From the standard curve, the value of y where the regression line crosses the y-axis. The y-intercept indicates the expected threshold cycle (C_T) for a sample with quantity equal to 1.

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