USER GUIDE

applied biosystems® by life technologies™

Applied Biosystems® QuantStudio™ 12K Flex Real-Time PCR System

MAINTENANCE AND ADMINISTRATION

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TRADEMARKS

Contents

About This Guide

Purpose

The *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* provides reference information for the QuantStudio™ 12K Flex Instrument and describes how to prepare, maintain, and troubleshoot the system.

Audience

This user guide is written for laboratory staff who operate and maintain the QuantStudio™ 12K Flex System.

Assumptions

This guide assumes that your QuantStudio™ 12K Flex System has been installed by a Life Technologies service representative.

This guide also assumes that you have:

- Familiarity with Microsoft® Windows® 7 operating system.
- Knowledge of techniques for handling and preparing DNA samples for PCR.
- A general understanding of data storage, file transfers, and copying and pasting.

Safety information

Note: For general safety information, see this section and Appendix G, "Safety" on page 237. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Safety alert words

Four safety alert words appear in Life Technologies user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

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. This signal word is to be limited to the most extreme situations.

Except for IMPORTANTs, each safety alert word in a Life Technologies document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard symbols that are affixed to Life Technologies instruments* (see "Safety symbols" on page 238).

Safety data sheets (SDSs)

The SDSs for any chemicals supplied by Life Technologies or Ambion are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 246.

IMPORTANT! For the SDSs of chemicals not distributed by Life Technologies or Ambion contact the chemical manufacturer.

Safety labels on instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Life Technologies instruments in combination with the safety symbols described in the preceding section.

Hazard symbol	English	Français
	**CAUTION!** Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION!** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
	**WARNING!** Hot lamp.	**AVERTISSEMENT!** Lampe brûlante.
	WARNING! Hot. Do not remove lamp until **15** min after disconnecting supply.	**AVERTISSEMENT!** Lampe brûlante, après avoir déconnecté le câble d'alimentation de l'appareil, attendre environ **15** minutes avant d'effectuer un remplacement de la lampe.
	WARNING! Hot. Replace lamp with an Life Technologies lamp.	**AVERTISSEMENT!** Composants brûlants. Remplacer la lampe par une lampe Life Technologies.
	CAUTION! Hot surface.	**ATTENTION!** Surface brûlante.
	**DANGER!** High voltage.	**DANGER!** Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Life Technologies qualified service personnel.	**AVERTISSEMENT!** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Life Technologies.
	**CAUTION!** Moving parts. Crush/ pinch hazard.	**ATTENTION!** Pièces en mouvement, risque de pincement et/ou d'écrasement.

Using this guide

You can use this guide to calibrate, service, network, and administrate the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System.

This user guide contains the following information:

- Chapter 2, "Calibrating Multi-Well Plate and Array Card Sample Blocks" – Describes how to maintain a QuantStudio™ 12K Flex System with a 96/384-well plate or array card sample block, including calibration and performance verification.
- Chapter 3, "Calibrating OpenArray® Plate Sample Blocks" – Describes how to maintain a QuantStudio™ 12K Flex System with an OpenArray® plate sample block, including calibration and performance verification.
- Chapter 4, "Maintenance" – Describes how to replace the user-serviceable parts of the QuantStudio™ 12K Flex Instrument and resolve infrequent problems that can occur during normal use.
- Chapter 5, "Networking" – Describes how to install the QuantStudio™ 12K Flex System to a local area network for remote monitoring and control.
- Chapter 6, "Security, Audit, and Electronic Signature" – Describes how to configure the security, audit, and electronic signature functions of the QuantStudio™ 12K Flex Software.
- Appendix A, "Manual Instrument Operation" – Describes how to operate the QuantStudio™ 12K Flex Instrument manually using the touchscreen interface.
- Appendix B, "Powering On or Off, Storing, and Moving the System" – Describes how to store, move, and reinstall the components of the system.
- Appendix C, "Calibration Consumable Preparation" – Describes how to prepare array cards and OpenArray® plates for calibration and verification of the QuantStudio™ 12K Flex Instrument. The appendix also describes how to create a background plate or array card in the event that one is unavailable, and how to create a dye plate or array card that can be used to calibrate the system for a dye not manufactured by Life Technologies.
- Appendix D, "Command-line Software Operation" – Describes how to use the QuantStudio™ 12K Flex Software command-line application.
- Appendix E, "File Format Reference" – Provides specifications for files that the QuantStudio™ 12K Flex Software imports, exports, and stores.
- Appendix F, "Parts and Materials" – Describes how to order parts, accessories, and consumables for the QuantStudio™ 12K Flex System.

1 Getting Started

This chapter covers:

About the QuantStudio™ 12K Flex System

The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System uses 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).

About data collection

The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System collects raw fluorescence data at different points during a PCR, depending on the type of run that the QuantStudio™ 12K Flex Instrument performs:

Run type		Data collection point
Real-time	Standard curve‡	The QuantStudio™ 12K Flex Instrument collects data following each extension step of the PCR.
	Relative standard curve‡	
	Comparative C_T ($\Delta\Delta C_T$)	
	Melting curve‡	
Post-PCR (endpoint)	Genotyping	The QuantStudio™ 12K Flex Instrument collects data: • Before the PCR. For presence/absence experiments, data collection before the PCR is optional, but recommended. • (Optional) During the PCR. The QuantStudio™ 12K Flex Instrument can collect data during the run (real-time); collecting real-time data during the run can be helpful for troubleshooting endpoint results. • After the PCR.
	Presence/absence‡	

‡ Not available for OpenArray® experiments.

Regardless of the run type, a data collection point or read consists of three phases:

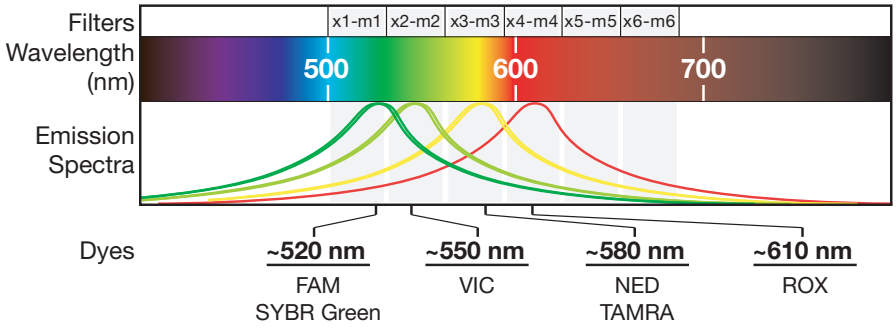
1. **Excitation** – The QuantStudio™ 12K Flex Instrument illuminates all wells of the reaction plate within the instrument, exciting the fluorophores in each reaction.
2. **Emission** – The QuantStudio™ 12K Flex Instrument optics collect the residual fluorescence emitted from the wells of the reaction plate. The resulting image collected by the device consists only of light that corresponds to the range of emission wavelengths.
3. **Collection** – The QuantStudio™ 12K Flex Instrument assembles a digital representation of the residual fluorescence collected over a fixed time interval. The QuantStudio™ 12K Flex Software stores the raw fluorescent image for analysis.

After a run, the QuantStudio™ 12K Flex Software uses calibration data 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 Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System features a six-color filter set that supports all Life Technologies dyes. The following figure shows the emission spectrum for each dye, and the filter at which each dye is read.



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

‡ The central wavelengths are the optimized wavelengths.
§ No Life Technologies supported dye currently available.

Custom dyes

The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System can run assays designed with custom dyes (dyes not supplied by Life Technologies) that are excited between 455–672 nm and read between 505–723 nm.

Specifications and layout

QuantStudio™ 12K Flex System specifications

The figures below summarize the specifications and requirements for the QuantStudio™ 12K Flex System. For more information, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Site Preparation Guide* (Part no. 4470654).

Component	Height	Depth	Width	Weight
QuantStudio™ 12K Flex Instrument‡	73.8 cm (29.0 in.)	66.0 cm (26.0 in.)	50.4 cm (19.8 in.)	70.0 kg (154.3 lbs)
Computer§	56.5 cm (22.3 in.)	54.7 cm (22.4 in.)	21.6 cm (8.5 in.)	24.9 kg (55.0 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	5.0 cm (2.0 in.)	15.25 cm (6.0 in.)	44.7 cm (17.5 in.)	0.1 kg (0.2 lbs)
OpenArray® Accufill™ System#	76.0 cm (30.0 in.)	64.0 cm (25.0 in.)	79.0 cm (31.0 in.)	55.0 kg (120.0 lbs)
Twister® Robot#	97.0 cm (38.0 in.)	71.0 cm (28.0 in.)	52.0 cm (20.5 in.)	52.2 kg (115.0 lbs)

‡ Weight varies depending on the sample block installed.

§ Computer specification differs depending on the computer ordered with the QuantStudio™ 12K Flex System (laptop or desktop).

Optional component of the QuantStudio™ 12K Flex System.

Figure 1 QuantStudio™ 12K Flex System with Twister® Robot

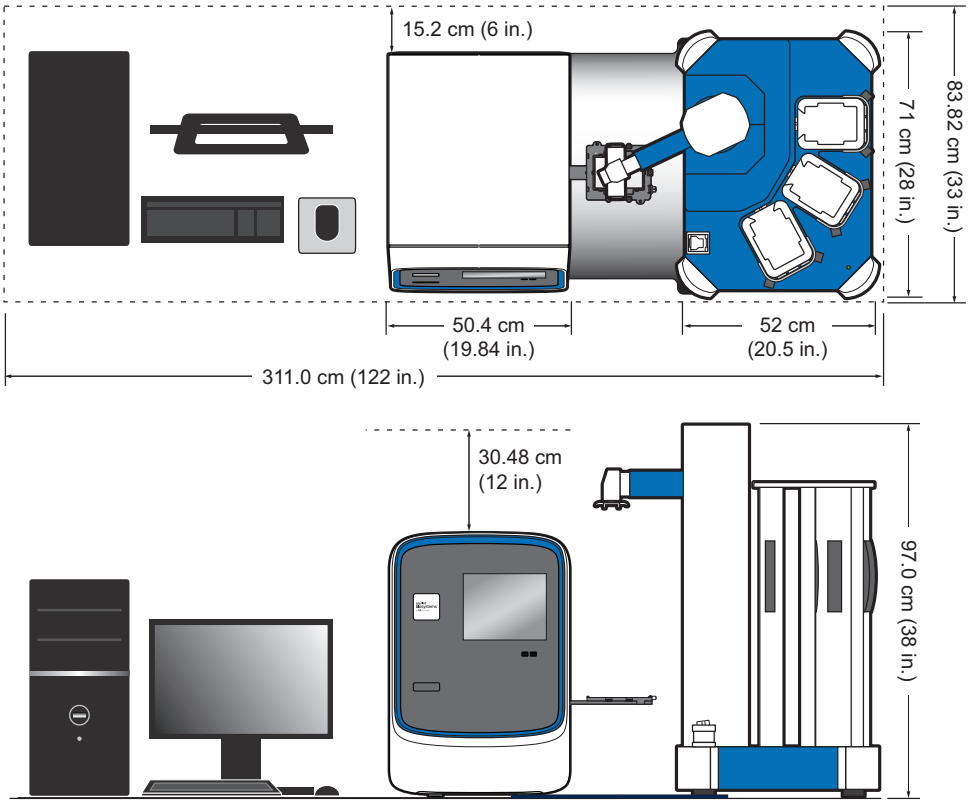
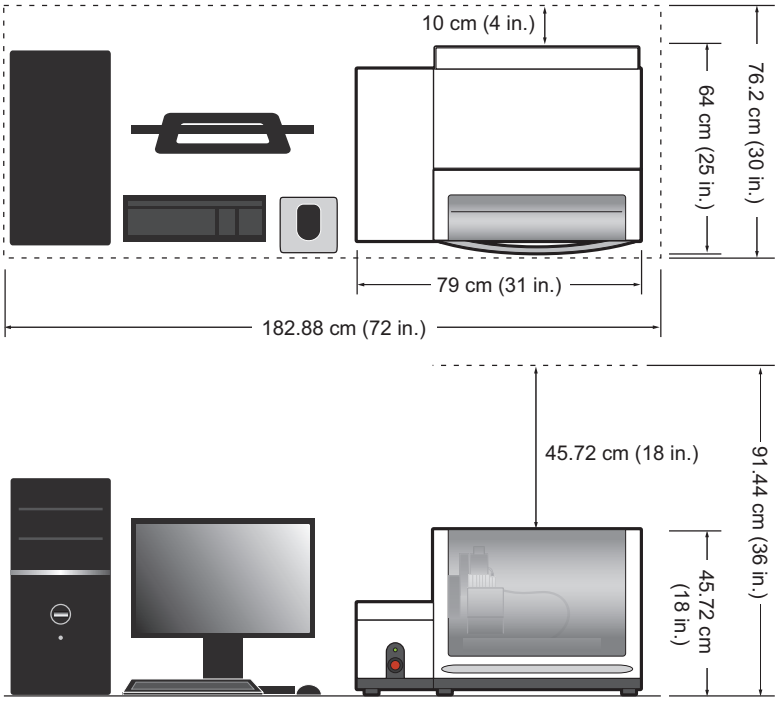


Figure 2 OpenArray® Accufill™ System



Required clearances

The QuantStudio™ 12K Flex System requires the following additional clearances:

Component	Top	Front	Sides	Back
QuantStudio™ 12K Flex Instrument	30.48 cm (12.0 in.)	122.0 cm (48.0 in.)	51.0 cm (20.0 in.)	15.2 cm (6.0 in.)
Twister® Robot	15.2 cm (6.0 in.)	15.2 cm (6.0 in.)	15.2 cm (6.0 in.)	15.2 cm (6.0 in.)
OpenArray® Accufill™ System	190.0 cm (76.0 in.)	—	—	10.0 cm (4.0 in.)
Computer and optional UPS	—	30.48 cm (12.0 in.)	—	15.24 cm (6.0 in.)

Instrument hot-air exhaust venting

The maximum thermal output of the QuantStudio™ 12K Flex Instrument is 2731 BTU/hr (800 W) vented directly into the room air from the hot-air waste port on the rear panel.

Electrical requirements

Note: We recommend placing the QuantStudio™ 12K Flex Instrument 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
QuantStudio™ 12K Flex 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		
Twister® Robot‡	2.5 A	150 VA		
OpenArray® Accufill™ System‡	0.6 A	75 VA		

‡ Optional component of the QuantStudio™ 12K Flex System.

Note: The instrument, monitor, desktop computer, Twister® Robot, and laptop computer self-adjust for 100–240V input voltages of 50/60 Hz.

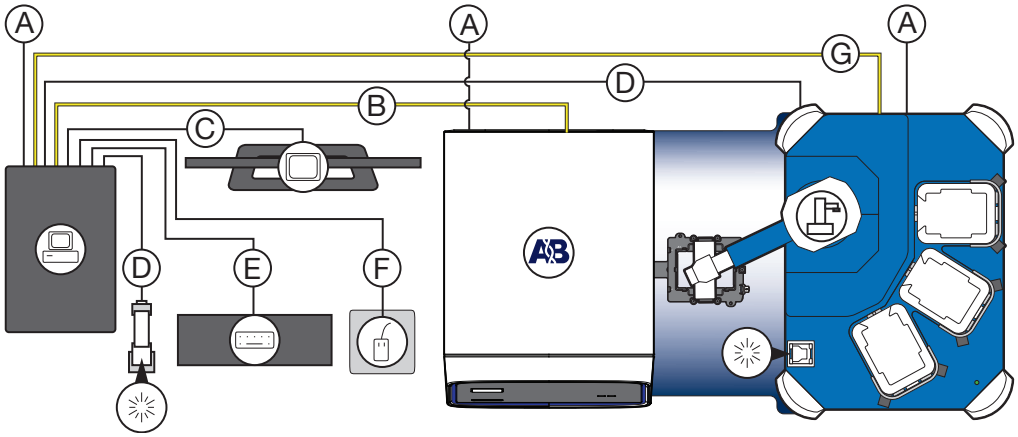
Environmental requirements

Requirement	Description
Altitude	Less than 2000 m (6500 ft) above sea level
Temperature	15–30°C (59–86°F) Do not place the QuantStudio™ 12K Flex Instrument next to heaters, cooling ducts, or in direct sunlight. Temperature fluctuations can affect performance.
Humidity	QuantStudio™ 12K Flex Instrument, computer, and the UPS unit: 20–80% (noncondensing) OpenArray® Accufill™ System maximum humidity: • 80% at 31°C • 50% at 40°C
Pollution	The instrument has a pollution degree rating of II.‡ The noise output of the instrument is <60 dB at idle.
Location	For indoor use only IMPORTANT! Do not place the QuantStudio™ 12K Flex Instrument next to electrically noisy devices, such as a refrigeration unit, or vibration sources, such as a centrifuge, pump, or compressor. Excessive vibration can affect instrument performance.

‡ The QuantStudio™ 12K Flex Instrument can be used in an environment that contains nonconductive pollutants only (dust particles or wood chips). Typical environments with a Pollution Degree II rating are laboratories, sales, and commercial areas.

QuantStudio™ 12K Flex System layout and connections

The QuantStudio™ 12K Flex System consists of the components shown in the following figure.



	Component	Description
	QuantStudio™ 12K Flex Instrument	Performs fluorescence research detection and data collection of experiment and calibration consumables.
	Computer	Run the QuantStudio™ 12K Flex Software that is used to: • Calibrate the QuantStudio™ 12K Flex Instrument. • Set up experiments. • (Optional) Run experiments. • Analyze experiments.
	Monitor	
	Keyboard	
	Mouse	
	Barcode reader	Scans the barcodes of consumables before they are loaded into the QuantStudio™ 12K Flex Instrument.
	Twister® Robot‡	Automates loading and unloading of consumables to and from the QuantStudio™ 12K Flex Instrument.

‡ Not for diagnostic use.

	Connection	Description
A	Power cables	Supply power to the computer, the Applied Biosystems Twister® Robot, and the QuantStudio™ 12K Flex Instrument.‡
B	LAN connection or Ethernet cable§	Connects the QuantStudio™ 12K Flex Instrument (Ethernet port) to the Ethernet port on the network interface card in the computer.
C	DVI cable	Connects the monitor to the computer (DVI port).
D	Barcode reader cable	Connects the barcode reader 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).
G	Serial cable	Connects the Twister® Robot to the computer (serial port).

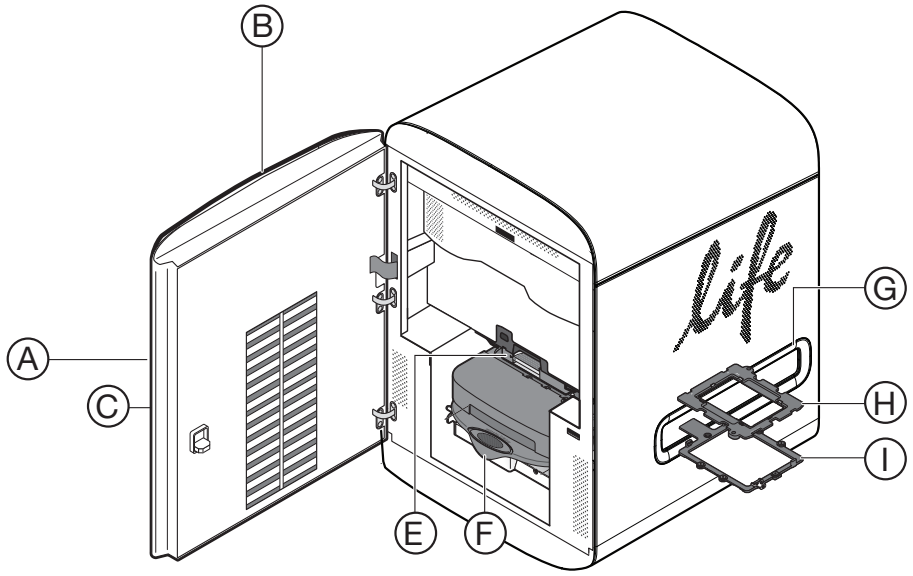
‡ Supplies 115/230 V depending on the geographic location of the installation.
§ Supplied with the QuantStudio™ 12K Flex System.

QuantStudio™ 12K Flex System hardware

Instrument components

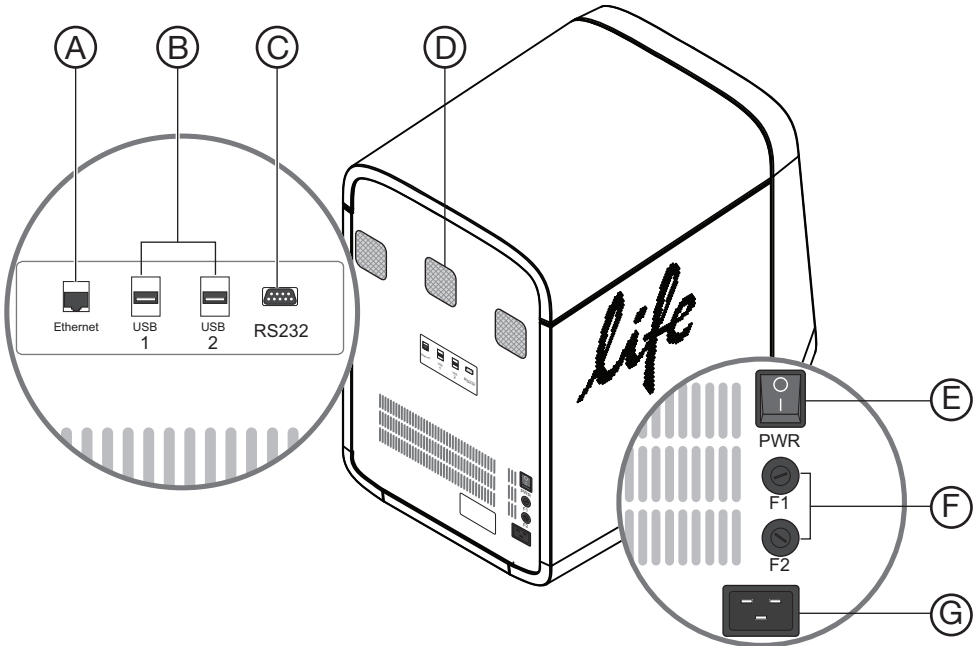
The QuantStudio™ 12K Flex System consists of the components shown in the following figures.

Front view



	Component	Description
A	USB ports	Provide USB communication with the QuantStudio™ 12K Flex Instrument. Can be used to transfer data to and from the instrument and to update the firmware. Note: If multiple USB drives are plugged into the QuantStudio™ 12K Flex Instrument, the instrument mounts only the first drive that is installed, regardless of the USB port used.
B	Instrument touchscreen	Provides access to the QuantStudio™ 12K Flex Instrument functions. Can be used to run experiments, transfer data, and operate the instrument functions without the use of the computer.
C	Access door	Provides access to the QuantStudio™ 12K Flex Instrument LED, the heated cover, and the sample block.
D	LED	Illuminates the reaction 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 consumable 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 plates or array cards to the tray arm.
I	Tray arm	Conveys plates or array cards to and from the sample block in the interior of the QuantStudio™ 12K Flex Instrument.

Rear view



	Component	Description
A	Ethernet port	An RJ45 port that provides Ethernet (Gigabit) communication with the QuantStudio™ 12K Flex Instrument.‡
B	USB ports	Provide USB communication with the QuantStudio™ 12K Flex Instrument. They can be used to transfer data to/from the instrument and to update the firmware. Note: If multiple USB drives are plugged into the QuantStudio™ 12K Flex 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 QuantStudio™ 12K Flex Instrument and the computer. IMPORTANT! The serial port is reserved for Life Technologies use only.
D	Instrument fans	Cool the interior of the QuantStudio™ 12K Flex Instrument. IMPORTANT! The fans must be unobstructed to ensure adequate cooling and proper function of the QuantStudio™ 12K Flex Instrument.
E	On/Off switch	Power switch for the QuantStudio™ 12K Flex Instrument, where the states are on (**I**) or off (**O**).
F	Fuse cover	Dual 12.5A, Time-Lag T, 250VAC, 5 × 20-mm electrical fuses that protect the QuantStudio™ 12K Flex Instrument from excessive electrical current.
G	Power port	The 100-240VAC port that provides power to the QuantStudio™ 12K Flex Instrument.

‡ Use the Ethernet cable supplied with the QuantStudio™ 12K Flex System to connect the QuantStudio™ 12K Flex Instrument (Ethernet port) to the network interface card in the computer.

Barcode reader

The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System can include two barcode readers for data entry and plate recognition:

- A hand-held barcode reader for scanning plates manually.
- A fixed-position barcode reader for automatically scanning plates as they are loaded into the instrument (available only with the Twister® Robot).

Both barcode readers use 670 nm Class II lasers to scan plates, and both readers are capable of reading Code 128 (alphanumeric), which supports 128 ASCII character barcodes. The barcode readers are optional and available depending on the system configuration.

About the hand-held barcode reader

WARNING! LASER HAZARD. 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 reader functions as an extension of the keyboard. You can use the reader to scan barcodes into the QuantStudio™ 12K Flex Software.

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

1. In the QuantStudio™ 12K Flex Software, select the field where you want to enter the barcode.
2. Hold the barcode reader 20–30 cm away from a plate and aim at the center of the barcode, then press the trigger. Slowly move the scanning beam across the barcode until the reader emits a high-pitched tone.

When the reader scans a barcode, it automatically:

- Transmits the alphanumeric equivalent of the barcode to the QuantStudio™ 12K Flex Software. The software enters the barcode text wherever the cursor is active.
- Transmits a carriage-return character (the equivalent of pressing the Enter key).

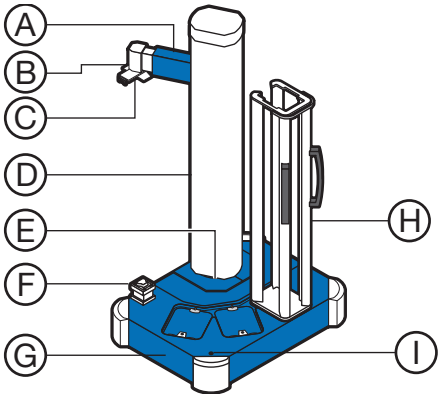
For more information on the hand-held barcode reader, see the barcode reader user documentation shipped with the QuantStudio™ 12K Flex System.

Twister® Robot components

The QuantStudio™ 12K Flex System supports the use of the Applied Biosystems Twister® Robot, an optional QuantStudio™ 12K Flex System accessory that consists of the components shown below.

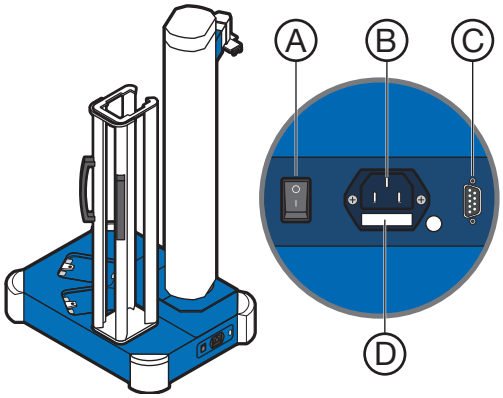
Note: See the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Automation Guide* (Part no. 4470693) for information on operating, calibrating, maintaining and integrating the Twister® Robot.

Front view



	Component	Description
A	Reach axis	Moves the grip horizontally 28.5–50.1 cm (11.25–19.75 in.) 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 54.6 cm (21.5 in.), from 16.5–71.1 cm (6.5–28 in.) above the table.
E	Rotary axis	Rotates the arm 340° around the base of the Twister® Robot. Mechanical stops prevent continuous rotation.
F	Fixed-position barcode reader	Scans the barcodes of consumables as they are loaded into the QuantStudio™ 12K Flex Instrument.
G	Base cover	Removable cover that contains four access bolts, which secure the Twister® Robot to the Sciclone ALH 3000 base.
H	Racks	Provides storage for PCR consumables before and after they are run by the QuantStudio™ 12K Flex Instrument (one of three shown).
I	Power LED	When lit, indicates the Twister® Robot is powered on.

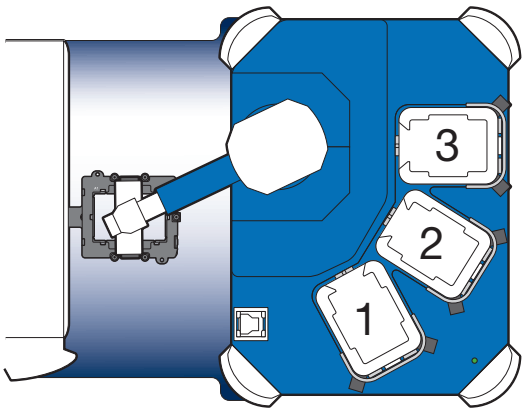
Rear view

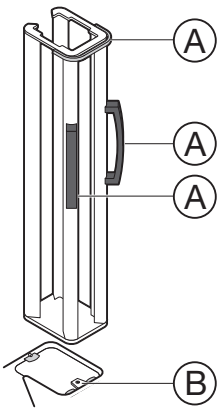


	Component	Description
A	On/Off switch	Power switch for the Twister® Robot, where the states are on (**I**) or off (**O**).
B	Power port	100–240V port that provides power to the Twister® Robot.
C	RS232 port	Provides serial communication with the computer.
D	Fuse cover	Two T1.6A 250VAC, 5 × 20-mm electrical 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 devices to protect the QuantStudio™ 12K Flex System 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 QuantStudio™ 12K Flex System.

Note: A power line regulator monitors the input current and adjusts the power supplied to the QuantStudio™ 12K Flex System 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 QuantStudio™ 12K Flex Instrument.

Note: A dedicated line and ground between the QuantStudio™ 12K Flex System/ computer and the building's main electrical service can also prevent problems caused by power fluctuations.

QuantStudio™ 12K Flex System Software

The QuantStudio™ 12K Flex System includes a suite of software applications that can be used to calibrate, run, automate, and integrate the QuantStudio™ 12K Flex System into a laboratory workflow. The basic installation of the QuantStudio™ 12K Flex Software contains the components described below; however, additional software may be available for the QuantStudio™ 12K Flex System. Visit the QuantStudio™ 12K Flex System website for a complete list of compatible software:

www.lifetechnologies.com/quantstudio12Kflex/

Note: Visit the QuantStudio™ 12K Flex System website for updates and patches for the QuantStudio™ 12K Flex Software and QuantStudio™ 12K Flex Instrument Firmware.

Computer requirements

The requirements for the computer used to operate the QuantStudio™ 12K Flex Instrument can vary depending on the version of the QuantStudio™ 12K Flex Software that you are running. To determine the computer requirements for your QuantStudio™ 12K Flex System, check the QuantStudio™ 12K Flex Software release notes at the following location:

```
C:\Applied Biosystems\QuantStudio12KFlex\README.html
```

Software installation

The default installation of the QuantStudio™ 12K Flex System partitions the computer hard drive to create the logical drives shown below.

Drive	Software	Description
C	Microsoft® Windows® OS‡	Operating system files.
	QuantStudio™ 12K Flex Software	Used to calibrate and perform experiments on the QuantStudio™ 12K Flex Instrument.
	QuantStudio™ 12K Flex System Command-line Utility	Used to automate the creation of new experiments and the export of existing experiments.
	SampleTracker Software	Used to rapidly enter sample information into OpenArray® experiments.
	ExpressionSuite Software	Analyzes gene expression data generated by the QuantStudio™ 12K Flex Instrument.
	AccuFill™ Software	Controls the AccuFill™ instrument used to load OpenArray® plates.
	HRM Software Module	An optional QuantStudio™ 12K Flex Software module that allows you to set up, run, and analyze an high-resolution melting curve experiment.
	TaqMan® Genotyper™ Software	Analyzes genotyping data generated by the QuantStudio™ 12K Flex Instrument.
	Twister® Robot Software	Controls the Twister® Robot, stores all of the taught positions for the robot, and includes the Visual Basic code required to operate the Twister® Robot with the automation control software.

‡ We recommend that you do not install programs to the C drive.

Twister® Robot software

The Twister® Robot Software consists of several applications that are used to calibrate, program, and operate the Twister® Robot. By default, the software is installed to the C drive of the QuantStudio™ 12K Flex System computer, and it consists of the components shown below.

- **QuantStudio™ 12K Flex Instrument Control Program (ICP) –** Calibrates the Twister® Robot and stores all taught positions.
- **QuantStudio Adapter Driver for iLink®** PRO Software – Coordinates the operation of the Twister® Robot and QuantStudio™ 12K Flex Instrument.
- **Microsoft® software** – Provides the Microsoft® services used by the Twister® Robot Software. The components include: Microsoft® Data Access Components (MDAC), Microsoft® .NET Framework, Microsoft® SQL 2005 Manager, and Microsoft® VBA Service Packs.
- **Automation Controller Software and iLink®** PRO Software – Software and automation controller software applications that can be used to automate the operation of the Twister® Robot and the QuantStudio™ 12K Flex Instrument.

  Note: The iLink® PRO Storage software for the Twister® Robot racks is used with the iLink® PRO automation control software to set up the initial material layout.

Third-party software

Before you install third-party software to the computer running the QuantStudio™ 12K Flex Software, confirm that the software will not:

- Restrict Ethernet communication
- Interfere with QuantStudio™ 12K Flex Software operation (see below)

To confirm that third-party software does not interfere with the QuantStudio™ 12K Flex Software:

1. Install the software to the computer that contains the QuantStudio™ 12K Flex Software.
2. Perform several dry-run test experiments using plates that do not contain reagents.

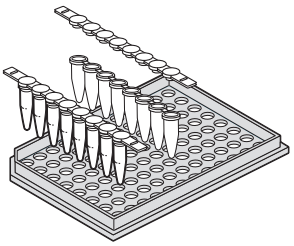
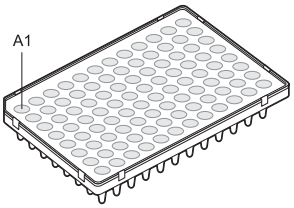
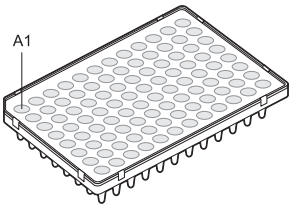
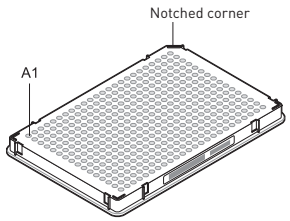
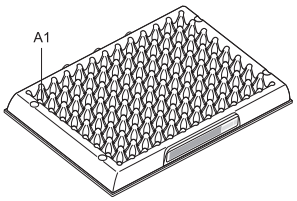
   Note: The goal of the test experiments is to run plates under conditions that match normal instrument operation. Therefore, the characteristics of the test experiments (plate layout and run method) must closely resemble your actual experiments.
3. Confirm that the QuantStudio™ 12K Flex System performs each test experiment without producing errors.

   If the QuantStudio™ 12K Flex System performs the tests successfully, proceed with your experiments. If the QuantStudio™ 12K Flex System encounters errors during the test runs, the software may not be compatible with the QuantStudio™ 12K Flex Software.

QuantStudio™ 12K Flex System consumables

Compatible consumables

The QuantStudio™ 12K Flex System supports a series of specialized consumables through interchangeable sample blocks. Use the consumables appropriate for the sample block of your QuantStudio™ 12K Flex System.

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	50 µL
		• MicroAmp® Optical Adhesive Film • MicroAmp® Optical 96-Well Reaction Plate with Bar Code	50 µL
96-well plate, 0.1 mL		• MicroAmp® Optical Adhesive Film • MicroAmp® Optical 96-Well Fast Reaction Plate with Bar Code	50 µL
384-well plate		• MicroAmp® Optical Adhesive Film • MicroAmp® Optical 384-Well Reaction Plate with Bar Code	20 µL
Array card		Array card	1 µL
OpenArray® plate		OpenArray® plate	33 nL

Guidelines for handling consumables

Observe the following guidelines when using tubes, plates, or array cards:

- Store the 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 tubes or plates 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 the centrifuge you use is clean. Before centrifugation, wipe down the bucket using a tissue.
- (*Plates only*) Vortex all calibration plates to ensure complete mixing, then centrifuge them to ensure that all reagents are contained in the bottom of the wells. The calibration plates must be well mixed and centrifuged before use.
- (*Plates only*) Do not discard the packaging for the calibration plates. Each plate can be used to calibrate the QuantStudio™ 12K Flex System 3 times for up to 6 months if it is stored in its packing sleeve.
- (*Plates only*) Handle the calibration plates with care to prevent contamination. Do not place the plates on a lab bench, to avoid contaminating them. Always put calibration plates back into their packaging sleeves.
- (*96-well plates only*) If you are using cap strips to seal your plates, firmly seal all wells before running the plate. Partially seated caps can leak during the experiment, causing evaporation.
- (*Tubes only*) Firmly seal all individual tubes and tube strips. Partially seated caps can leak during the experiment, causing evaporation.
- (*OpenArray® plates only*) Hold OpenArray® plates by the edges of the cases. Do not touch the through-holes.
- (*OpenArray® plates only*) Load and seal the TaqMan® OpenArray® plate *within one hour* after opening the plate packaging.
- (*OpenArray® plates only*) If you drop a loaded OpenArray® plate, discard it in the appropriate waste container.

2 Calibrating Multi-Well Plate and Array Card Sample Blocks

This chapter covers:

Recommended calibration and maintenance

The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System requires regular calibration and maintenance for proper operation. The following table displays the recommended maintenance schedule that you must perform to ensure optimal instrument performance.

IMPORTANT! Calibrate the QuantStudio™ 12K Flex System at the same ambient temperature at which you will run experiments. Extreme variations in ambient temperature can affect the heating and cooling of the QuantStudio™ 12K Flex System and, in extreme cases, influence experimental results.

IMPORTANT! Do not use organic solvents to clean the QuantStudio™ 12K Flex System.

Table 1 Multi-well plate and array card sample block maintenance

Frequency	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 QuantStudio™ 12K Flex System, then after 30 seconds, power on the computer.
	Clean the surface of the QuantStudio™ 12K Flex System with a lint-free cloth.
	Perform a QuantStudio™ 12K Flex Instrument self test.
Monthly	Perform a background calibration.‡
	Run disk cleanup and disk defragmentation.
Annually	Perform a regions of interest (ROI) calibration.
	Perform a background calibration.
	Perform a uniformity calibration.
	Perform a dye calibration.
	Perform a normalization calibration.
	Perform an instrument verification run.
As needed	Decontaminate the QuantStudio™ 12K Flex System.
	Replace the QuantStudio™ 12K Flex System fuses.
	Update the Windows® operating system.
	Update the QuantStudio™ 12K Flex Software and firmware.

‡ You can perform a background calibration to check for contamination. If any parts of the optics are replaced or moved, you must perform all calibrations, including an RNase P instrument verification run.

Preparing array cards for instrument calibration

IMPORTANT! Perform the following procedure only if you are verifying the performance of a QuantStudio™ 12K Flex System with an array card sample block.

Required materials

- QuantStudio™ 12K 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
- 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

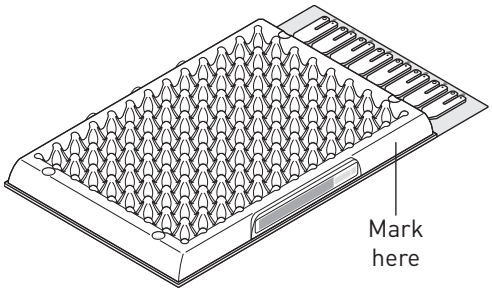
Filling the calibration array cards

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

Note: This procedure explains how to create *all* of the array cards required to calibrate the QuantStudio™ 12K Flex System, but not all of them are required for a monthly maintenance. Before preparing array cards for calibration, see "Recommended calibration and maintenance" on page 36 to determine which calibrations are required.

Note: You can view a video of the array card loading procedure on the Life Technologies website. To view the demonstration, go to:
www2.appliedbiosystems.com/lib/multimedia/taqman_tlda/tlda_1.cfm

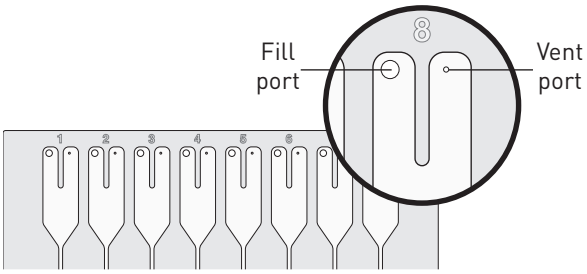
1. Remove the tubes of calibration solutions from the freezer, allow them to thaw, then vortex the tubes to mix the contents well.
2. Remove the array cards from their box and place them on a clean, dry surface.
3. Mark the side of the empty array cards with:
   - Background
   - FAM™
   - ROI
   - ROX™
   - VIC®
   - FAM™/ROX™
   - VIC®/ROX™

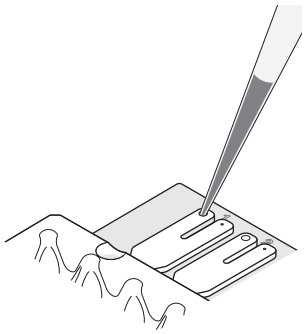


4. For each array card, pipet 100 µL of the appropriate calibration solution into each of the eight reservoirs in the array card:
   a. Place the array card on a lab bench, with the foil side down.
   b. Load 100 µL of the calibration solution into a pipette.

c. Hold the pipette in an angled position (~45 degrees) and place the tip into the fill port.

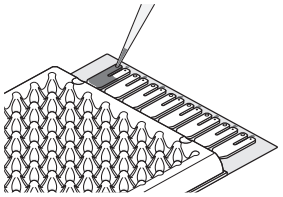
There is a fill port on the left arm of each fill reservoir – the larger of the two holes.





d. Dispense the fluid so that it sweeps in and around the fill reservoir toward the vent port.

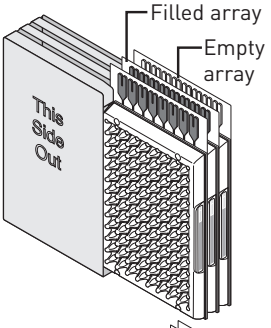
When pipetting the reagents into the array card, pipet the entire 100-µL volume into the fill reservoir, but *do not* go past the first stop of pipettor plunger or you may blow the solution out of the port.



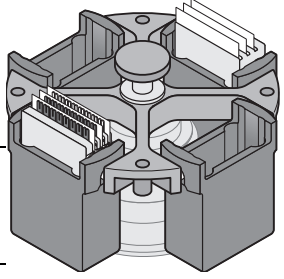
IMPORTANT! Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.

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

6. 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 face the same way.



7. 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 card into unfilled slots.

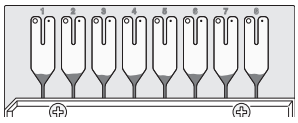
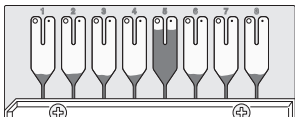
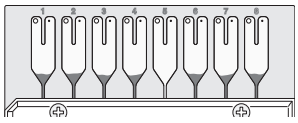
IMPORTANT! Balance the loads in opposite buckets in the centrifuge.

8. Close the centrifuge cover, then spin the array card(s) for 1 minute at 1200 rpm.

9. 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.

10. 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 for the formation of bubbles in all wells and note possible problems.

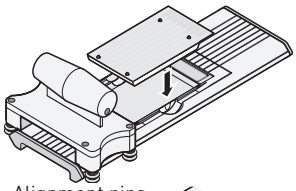
Correct fill	Incorrect/partial fill	
		

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.

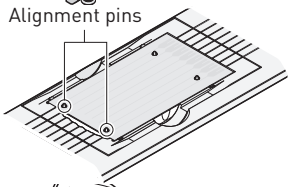
11. Seal the array card(s):

⚠ Staker Seal Use

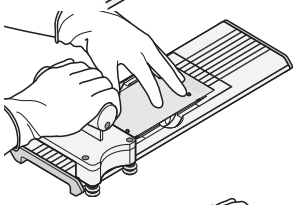
a. With the carriage (roller assembly) of the Array Card Staker/Sealer in the Start position, place a filled array card into the fixture with the foil side up so that the fill reservoirs are the farthest away from the carriage.



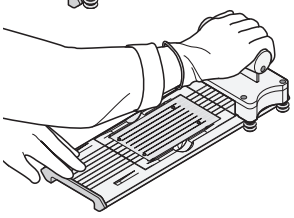
b. Press down on all four corners of the array card to ensure that it is fully seated within the fixture.



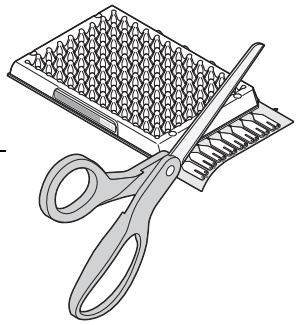
c. Use the two alignment pins in the fixture to position the array card correctly.



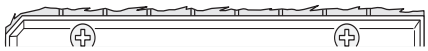
d. Seal the array card by running the carriage slowly over it. Run the carriage over the array card in one direction only. Do not apply downward force on the carriage as you move it forward over the card.



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
	

12. Repeat step 11 to seal the remaining array cards.

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.

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

ROI calibration

A regions of interest (ROI) calibration maps the positions of the wells on the sample block of the QuantStudio™ 12K Flex Instrument. The QuantStudio™ 12K Flex Software uses the ROI calibration data to associate increases in fluorescence during a run with specific wells on the plate. The QuantStudio™ 12K Flex Instrument uses a set of optical filters to distinguish the fluorescence emissions gathered during runs. You must generate a calibration image for each filter to account for minor differences in the optical path.

When to perform the calibration

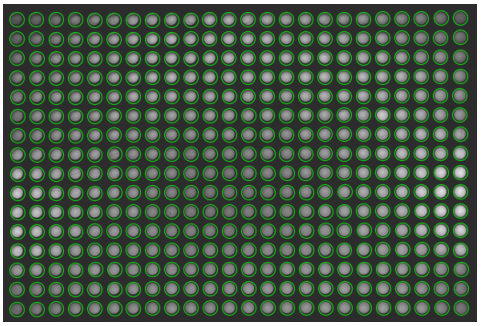
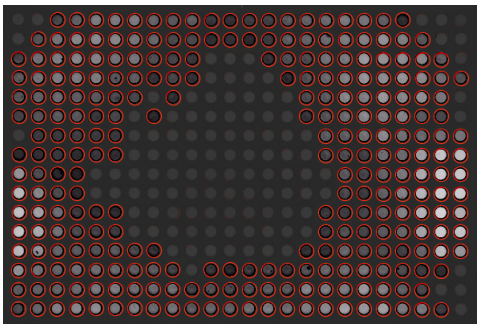
Perform the ROI calibration every year, or as often as necessary, depending on instrument use.

IMPORTANT! After every ROI calibration, you must perform a background calibration, uniformity calibration, dye calibration, normalization calibration, and RNase P instrument verification experiment.

About the ROI calibration data

During the ROI calibration, the QuantStudio™ 12K Flex Software captures images of the ROI calibration plate at each instrument filter. An ROI calibration passes if the collected image for each filter shows 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.

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.

Status	Image
Passing image Green circles appear around *all* wells indicating that the wells calibrated successfully. Each green circle indicates that the region of interest for the well position is sufficiently bright.	
Failing image Red circles appear around some or none of the wells indicating 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 ROI calibration consumable appropriate for your QuantStudio™ 12K Flex Instrument:

- Preparing the ROI calibration plate 42
- Preparing array cards for instrument calibration 37

Preparing the ROI calibration plate

Required materials

- 96- or 384-Well Region of Interest (ROI) and Background Plates
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Note: Only the ROI plate is required for this calibration.

Preparing the calibration plate

1. Remove the ROI calibration plate from the freezer, then allow it to warm to room temperature (approximately 5 minutes).

   IMPORTANT! 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 dye.

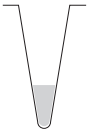
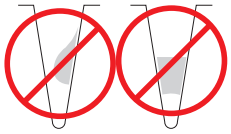
2. Remove the calibration plate from its packaging. Do not remove the optical film.

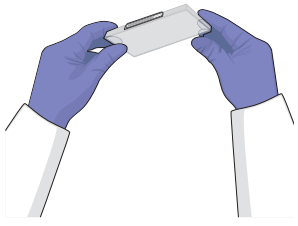
   IMPORTANT! Do not discard the packaging for the plate. You can use the plate to calibrate a QuantStudio™ 12K Flex System 3 times for up to 6 months if it is stored in its sleeve.

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. Verify 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 greater rpm and for longer.

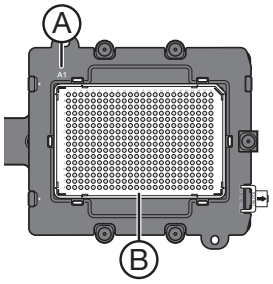
Correct	Incorrect
	
Liquid is at bottom of well.	• Not centrifuged with enough force, *or* • Not centrifuged for enough time



Performing the calibration

1. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
2. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add a QuantStudio™ 12K Flex Instrument to your list before you can manage it.
3. After the QuantStudio™ 12K Flex Instrument is added to your list, select it, then click **Manage Instrument**.
4. In the Instrument Manager, start the calibration wizard:
   a. Click **Maintenance**, then click **ROI**.
   b. In the ROI Calibration screen, click **Start Calibration**.
5. Click **Next**, then perform the calibration as instructed. When the side door opens, load the ROI calibration plate or array card. Ensure that the plate or array card 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.



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

6. After loading the plate or array card, start the calibration:
   a. In the Setup tab, select **Check the box when the ROI calibration plate has been loaded**, then click **Next**.
   b. In the Run screen, click **START RUN**.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Instrument is in operation.

Note: Before starting the calibration, the QuantStudio™ 12K Flex Instrument may pause (up to 10 minutes) to allow the heated cover to reach temperature.

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

8. After you inspect all ROI images, verify 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 the data it collected is unusable.

Analysis status	Action
Passed	Click **Next**, then remove the plate or array card when the QuantStudio™ 12K Flex Instrument ejects the tray arm.
Failed	Troubleshoot the failed calibration as described in "Troubleshooting ROI calibrations" on page 72.

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the plate can reach 100°C. Allow the plate to reach room temperature before removing.

IMPORTANT! If the QuantStudio™ 12K Flex Instrument does not eject the plate, remove the plate according to "Troubleshooting ROI calibrations" on page 72.

9. Discard or store the plate or array card.

Consumable	Action
Array card	Discard the array card if you *do not* plan to perform a uniformity calibration soon. Note: You can reuse the array card if the ROI and uniformity calibrations are performed on the same day.
Plate	Return the ROI calibration plate to its packaging sleeve. If you plan to perform background and uniformity calibrations: • Within 8 hours, keep the ROI calibration plate at room temperature. (The ROI calibration plate is used in the uniformity calibration.) • After 8 hours, return the packaged plate to the freezer. IMPORTANT! Do not discard the calibration plate. If the plate is stored in its original packaging sleeve, you can use it to calibrate a QuantStudio™ 12K Flex System 3 times for up to 6 months after you open it.

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

Background calibration

During a background calibration, the QuantStudio™ 12K Flex System:

- Performs reads of a background plate containing PCR buffer for 10 minutes at 60°C.
- Averages the spectra recorded during the run and extracts the resulting spectral component to a calibration file.

The QuantStudio™ 12K Flex Software then uses the calibration file during subsequent runs to remove background fluorescence from the run data.

When to perform the calibration

Perform the background calibration monthly or as often as necessary, depending on instrument use.

About the background calibration data

During the background calibration, the QuantStudio™ 12K Flex Software captures a series of images of the background plate using each instrument filter. The software compares the fluorescence from each well to the average for the plate. A background calibration passes if the collected images for all filters are free of abnormal fluorescence.

About the data

After the calibration, you can review the calibration data in the Background tab of the Instrument Manager. The Analysis Data plot (left-side) displays the fluorescence data in all filters. The Well Table tab (right-side) displays the data collected for the current calibration. The QC tab displays a summary of quality check performed by the QuantStudio™ 12K Flex Software on the calibration data.

Background fluorescence

Fluorescence data collected by the QuantStudio™ 12K Flex Instrument includes a fluorescence signal inherent to the system, 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

Prepare the background calibration consumable appropriate for your instrument:

- Preparing the calibration plate 46
- Preparing array cards for instrument calibration 37

Preparing the background plate

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

Required materials

- 96- or 384-Well Region of Interest (ROI) and Background Plates
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Note: Only the background plate is required for this calibration.

Preparing the calibration plate

1. Remove the background plate from the freezer, then allow it to warm to room temperature (approximately 5 minutes).
2. Remove the background plate from its packaging. Do *not* remove the optical film.

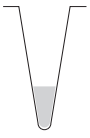
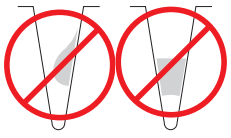
   IMPORTANT! Do not discard the packaging. You can use the background plate to calibrate a QuantStudio™ 12K Flex System 3 times for up to 6 months if it is stored in its original packaging sleeve.

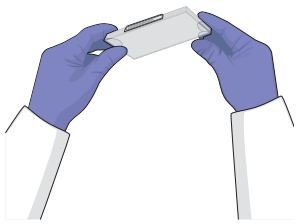
3. Vortex and centrifuge the background plate:
   a. Vortex the background plate for 5 seconds.
   b. Centrifuge the plate for 2 minutes at < 1500 rpm.

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

   c. Confirm that the liquid in each well of the background 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.

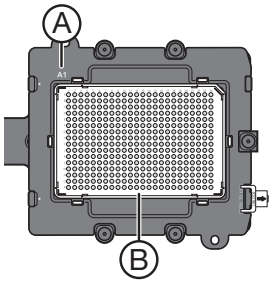
Correct	Incorrect
	
Liquid is at bottom of well.	• Not centrifuged with enough force, *or* • Not centrifuged for enough time



Performing the calibration

1. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
2. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add a QuantStudio™ 12K Flex Instrument to your list before you can manage it.
3. After the QuantStudio™ 12K Flex Instrument is added to your list, select it, then click **Manage Instrument**.
4. In the Instrument Manager, start the calibration wizard:
   a. Click **Maintenance**, then click **Background**.
   b. In the Background Calibration screen, click **Start Calibration**.
5. Click **Next**, then perform the calibration as instructed. When the side door opens, load the background plate or array card. Ensure that the plate or array card 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.



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

6. After loading the plate or array card, start the calibration:
   a. In the Setup tab, select **Check the box when the background calibration plate has been loaded**, then click **Next**.
   b. In the Run screen, click **START RUN**.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Instrument is in operation.

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

7. When the run is complete and the QuantStudio™ 12K Flex Software displays the Analysis screen, confirm the analysis status of the calibration, then select the **QC** tab and review the quality check summary.
   - **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 the data it collected is unusable.

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

Analysis status	QC status	Action
Passed	Passed	Click **Next**, then remove the plate or array card when the QuantStudio™ 12K Flex Instrument ejects the tray arm.
Passed	Failed	Troubleshoot the failed calibration as described in "Troubleshooting background calibrations" on page 73. Note: You can accept a calibration that passes the Analysis Status check, but fails the QC Status check. We recommend using calibrations that yield passing results for *both* status reports.
Failed	Failed	

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the plate can reach 100°C. Allow the plate to reach room temperature before removing.

IMPORTANT! If the QuantStudio™ 12K Flex Instrument does not eject the plate, remove the plate according to "Troubleshooting background calibrations" on page 73.

8. Discard or store the plate or array card.

Consumable	Action
Array card	Discard the array card.
Plate	Return the background plate to its packaging sleeve, then return the packaged plate to the freezer. IMPORTANT! Do not discard the calibration plate. If the plate is stored in its original packaging sleeve, you can use it to calibrate a QuantStudio™ 12K Flex System 3 times for up to 6 months after you open it.

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

Uniformity calibration

The uniformity calibration generates data that allows the QuantStudio™ 12K Flex Software to compensate for the physical effects of the QuantStudio™ 12K Flex System filters.

When to perform the calibration

Perform a uniformity calibration every year, or as often as necessary, depending on instrument use.

About the uniformity calibration data

During the uniformity calibration, the QuantStudio™ 12K Flex Software captures a series of images of the ROI plate using each instrument filter. After the calibration, you can review the data in the Uniformity tab of the Instrument Manager. The Analysis Data plot (left-side) displays the fluorescence data in all filters. The Well Table tab (right-side) displays the data collected for the current calibration in all well positions. The QC tab displays a summary of quality check performed by the QuantStudio™ 12K Flex Software on the calibration data.

Preparing the calibration plate or array card

If you have an ROI plate or array card from a recent ROI calibration, go to step b on page 50 (plates), or go to "Performing the calibration" on page 51 (array cards). Otherwise, prepare the ROI calibration consumable appropriate for your QuantStudio™ 12K Flex Instrument:

- Preparing the ROI calibration plate 42
- Preparing array cards for instrument calibration 37

Preparing the calibration plate

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

Required materials

See "ROI calibration" on page 41 for a complete list of materials for the calibration.

Preparing the ROI calibration plate

1. Remove the ROI calibration plate from the freezer, then allow it to warm to room temperature (approximately 5 minutes).

   IMPORTANT! Do not remove a 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 dye.

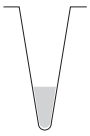
2. Remove the ROI calibration plate from its packaging. Do not remove the optical film.

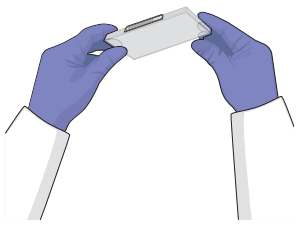
   IMPORTANT! Do not discard the packaging for the calibration plate. You can use the plate to calibrate a QuantStudio™ 12K Flex System 3 times for up to 6 months if it is stored in its sleeve.

3. Vortex and centrifuge the plate:
   a. Vortex the ROI calibration plate for 5 seconds.
   b. Centrifuge the plate for 2 minutes at less than 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.

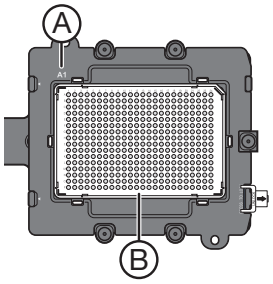
Correct	Incorrect
	
Liquid is at bottom of well.	• Not centrifuged with enough force, *or* • Not centrifuged for enough time



Performing the calibration

1. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
2. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add a QuantStudio™ 12K Flex Instrument to your list before you can manage it.
3. After the QuantStudio™ 12K Flex Instrument is added to your list of instruments, select it, then click **Manage Instrument**.
4. In the Instrument Manager, start the calibration wizard:
   a. Click **Maintenance**, then click **Uniformity**.
   b. In the Uniformity Calibration screen, click **Start Calibration**.
5. Click **Next**, then perform the calibration as instructed. When the side door opens, load the ROI calibration plate or array card. Ensure that the plate or array card 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.



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

6. After loading the plate or array card, start the calibration:
   a. In the Setup tab, select **Check the box when the Uniformity Calibration plate has been loaded**, then click **Next**.
   b. In the Run screen, click **START RUN**.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Instrument is in operation.

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

7. When the run is complete and the QuantStudio™ 12K Flex Software displays the Analysis screen, confirm the analysis status of the calibration. Select the **QC** tab to review the quality check summary.
   - **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 the data it collected is unusable.

     Note: A calibration can fail if wells produce spectra that deviate significantly from the other wells of the plate, or if all wells produce abnormally low spectra. Abnormal spectra can indicate the presence of fluorescent contamination on the plate or array card or sample block.
   - **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.

Analysis status	QC status	Action
Passed	Passed	Click **Next**, then remove the plate or array card when the QuantStudio™ 12K Flex Instrument ejects the tray arm.
Passed	Failed	Troubleshoot the failed calibration as described in Table 4, "Troubleshooting uniformity calibrations" on page 74. Note: You can accept a calibration that passes the Analysis Status check, but fails the QC Status check. We recommend using calibrations that yield passing results for *both* status reports.
Failed	Failed	

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the plate can reach 100°C. Allow the plate to reach room temperature before removing.

IMPORTANT! If the QuantStudio™ 12K Flex Instrument does not eject the plate, remove the plate according to Table 4, "Troubleshooting uniformity calibrations" on page 74.

8. Discard or store the plate or array card.

Consumable	Action
Array card	Discard the array card.
Plate	Return the ROI calibration plate to its packaging sleeve, then return the packaged plate to the freezer. IMPORTANT! Do not discard the calibration plate. If the plate is stored in its original packaging sleeve, you can use it to calibrate a QuantStudio™ 12K Flex System 3 times for up to 6 months after you open it.

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

Dye calibration

During a dye calibration, the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System:

- Collects spectral data from a series of dye standards.
- Stores the spectral information for the dye standards in a dye calibration file.

The QuantStudio™ 12K Flex Software uses the pure spectra data during experiment runs to characterize and distinguish the individual contribution of each dye in the total fluorescence collected by the QuantStudio™ 12K Flex Instrument. After each run, the QuantStudio™ 12K Flex Software receives data in the form of a raw spectra signal for each reading. It determines the contribution of each fluorescent dye used in the sample by comparing the raw spectra to the pure spectra calibration data. When you save an experiment after analysis, the QuantStudio™ 12K Flex Software stores the pure spectra with the collected fluorescence data for that experiment.

IMPORTANT! Calibrate only those dyes that are present in the chemistries that you intend to run on your QuantStudio™ 12K Flex System.

When to perform the dye calibration

Perform a dye calibration every year, or as often as necessary, depending on instrument use.

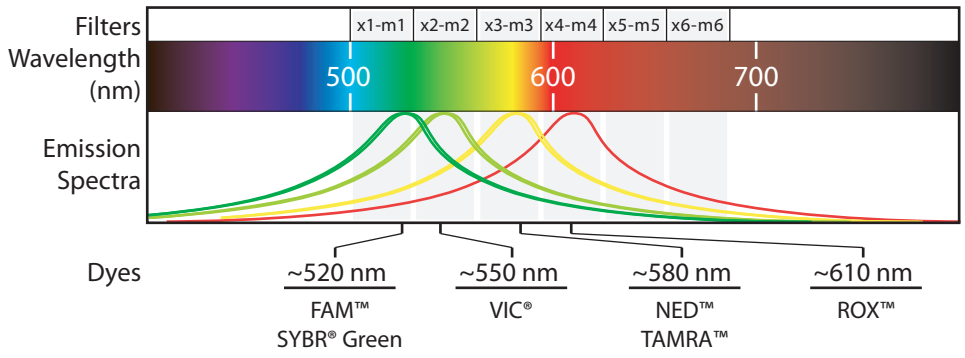
IMPORTANT! Calibrate only dyes that are present in the chemistries that you intend to run on the QuantStudio™ 12K Flex System. For example, if you intend to run a TaqMan® RNase P plate or array card to verify instrument performance (see page 63), you must calibrate the FAM™ dye, TAMRA™ dye, and ROX™ dye because all three are present in the TaqMan® assay chemistry.

IMPORTANT! Perform a background calibration before every series of dye calibrations. Because the age and use of instrument components can affect spectra readings, we recommend performing a dye calibration at least every year.

About the dye calibration

System dyes

The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System calibrates the following system dyes: FAM™ dye, NED™ dye, ROX™ dye, SYBR® Green dye, TAMRA™ dye, and VIC® dye. The following figure shows the emission spectrum for each dye, and the filters and wavelengths at which each dye is read.



Custom dyes

The QuantStudio™ 12K Flex System can be used to run assays designed with custom dyes (not supplied by Life Technologies); however, before using custom dyes with the QuantStudio™ 12K Flex System, you must create and run a custom calibration plate. The QuantStudio™ 12K Flex Software uses the custom calibration plate to create a spectral standard to distinguish the custom dye in the fluorescence data collected during the run. See "Creating a custom dye plate for calibration" on page 184 for information on custom dye calibrations.

IMPORTANT! A custom dye must excite between 455 and 672 nm and read between 505 and 723 nm.

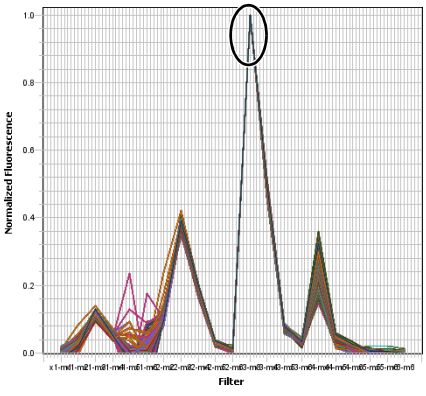
About the dye calibration data

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 spectral calibration plate. The QuantStudio™ 12K Flex Software plots the resulting data for each spectral profile in a graph of fluorescence versus filter.

When the QuantStudio™ 12K Flex 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. Dye spectra are generally acceptable if they peak within the same filter as their group but diverge slightly at other wavelengths (see below).

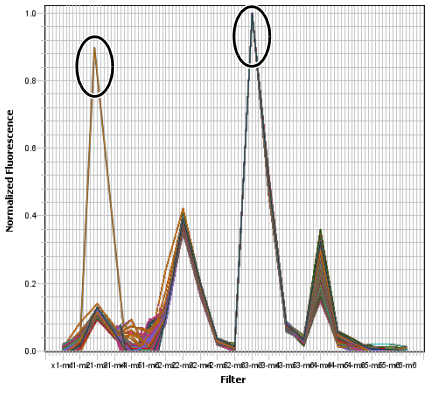
The QuantStudio™ 12K Flex Software can compensate for some differences in a spectral profile by replacing the spectra of unacceptable wells with the spectra of other wells on the reaction plate (auto-repairing). The QuantStudio™ 12K Flex Software allows only a few replacements, and it may reject the calibration if the spectra between neighboring wells vary significantly.

Note: Because the wells of a calibration plate 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.



Acceptable spectra

Spectra peak at the same wavelength and do not diverge significantly.



Unacceptable spectra

Spectra peak at the different wavelengths.

Preparing the calibration plate or array card

Prepare the Dye calibration consumables appropriate for your QuantStudio™ 12K Flex Instrument:

Preparing the calibration plates

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 plates or array cards.

Required materials

- 96- or 384-Well Spectral Calibration Plates (FAM™ Dye, VIC® Dye, ROX™ Dye, NED™ Dye, TAMRA™ Dye, and SYBR® Green Dye)
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Preparing the dye calibration plate

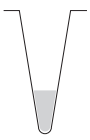
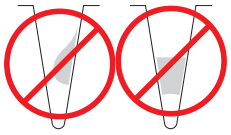
1. Remove the dye plates from the freezer, then allow them to warm to room temperature (approximately 5 minutes).

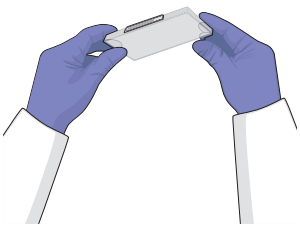
   IMPORTANT! Do not remove the dye plates from their packaging until you are ready to run them. The dyes in the dye plates are photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the plates.

   Note: If you store dye plates frozen and in their original packaging, you can use them to calibrate a QuantStudio™ 12K Flex System up to 3 times for 6 months after opening.

2. Go to "Performing the calibration" on page 56.

   Before using each dye plate, vortex the plate for 5 seconds, centrifuge it for 2 minutes at less than 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



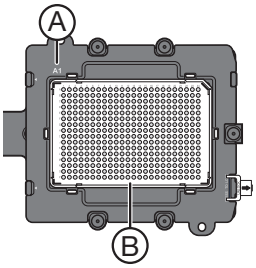
IMPORTANT! The dye plates must be well mixed and centrifuged.

Performing the calibration

IMPORTANT! The QuantStudio™ 12K Flex Software guides you through the calibration of each dye separately. You must set up, run, and analyze each dye independently.

1. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
2. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add a QuantStudio™ 12K Flex Instrument to your list before you can manage it.
3. After the QuantStudio™ 12K Flex Instrument is added to your list, select it, then click **Manage Instrument**.
4. In the Instrument Manager, start the calibration wizard:
   a. Click **Maintenance**, then click **Dye**.
   b. In the Dye Calibration screen, select **System Dye Calibration**, then click **Start Calibration**.
5. In the Dye Calibration screen, select the dye to calibrate from the Dye Name drop-down list, then perform the calibration as instructed.
6. Load the calibration plate or array card into the QuantStudio™ 12K Flex Instrument:
   a. Confirm that the dye plate or array card that you are about to load matches the dye selected in the QuantStudio™ 12K Flex Software. The name of the dye contained by the consumable is next to the barcode on the front of the plate or array card.
   b. Load the dye plate or array card into the plate adapter. Ensure that the plate or array card 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.



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

7. After loading the plate or array card, start the calibration:
   a. In the Dye Calibration screen, select **Check the box when the dye calibration plate has been loaded**, then click **Next**.
   b. In the Run screen, click **START RUN**.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Instrument is in operation.

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

8. When the run is complete and the QuantStudio™ 12K Flex 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, verify that the peak is:
      - Within the detectable range for the QuantStudio™ 12K Flex System.
      - Free of irregular spectral peaks.
      - Present at the correct filter for the dye (see the following table).

Filter set	Excitation (nm)	Emission (nm)	System dyes
x1-m1 (Blue)	470 ± 15	520 ± 15	• FAM™ dye • SYBR® Green dye
x2-m2 (Green)	520 ± 10	558 ± 12	• HEX™ dye • JOE™ dye • TET™ dye • VIC® dye
x3-m3 (Yellow)	549.5 ± 10	586.5 ± 10	• NED™ dye • TAMRA™ dye
x4-m4 (Orange)	580 ± 10	623 ± 14	ROX™ dye
x5-m5 (Red)	640 ± 10	682 ± 14	LIZ™ dye
x6-m6 (Deep red)	662 ± 10	711 ± 12	—‡

‡ No Life Technologies fluorescent dyes are collected at the x6-m6 filter set.

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, verify the status of the calibration:
   - **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 the data it collected is unusable.
   - **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.

Analysis status	QC status	Action
Passed	Passed	1. Click **Next**. 2. Enter any comments you have in the Comments field, click **Finish**, then click **Yes** when prompted to save the results. 3. Remove the plate or array card when the QuantStudio™ 12K Flex Instrument ejects the tray arm.
Passed	Failed	Troubleshoot the failed calibration as described in Table 5, "Troubleshooting dye calibrations" on page 75. Note: You can accept a calibration that passes the Analysis Status check but fails the QC Status check. We recommend using calibrations that yield passing results for *both* status reports.
Failed	Failed	

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the plate can reach 100°C. Allow the plate to reach room temperature before removing.

IMPORTANT! If the QuantStudio™ 12K Flex Instrument does not eject the plate, remove the plate according to Table 5, "Troubleshooting dye calibrations" on page 75.

10. Discard or store the consumable:

Consumable	Action
Array card	Discard the array card.
Plate	Store the dye calibration plate in the freezer, in its packaging sleeve. IMPORTANT! Do not discard the calibration plate. If the plate is stored frozen in its packaging sleeve, you can use it to calibrate a QuantStudio™ 12K Flex System 3 times for up to 6 months after you open it.

11. Repeat steps 4 to 10 as needed to calibrate the QuantStudio™ 12K Flex System for the remaining dyes in the chemistries that you are running.

Normalization calibration

During the normalization calibration, the QuantStudio™ 12K Flex System:

- Collects data from the normalization standards.
- Stores the information for the normalization standards in a normalization calibration file.

The normalization calibration generates factors that the QuantStudio™ 12K Flex Software uses when comparing data from multiple QuantStudio™ 12K Flex Instruments.

When to perform the calibration

Perform a normalization calibration every year, or as often as necessary, depending on instrument use.

About the normalization calibration data

During the normalization calibration, the QuantStudio™ 12K Flex Software captures a series of images of each normalization plate using each instrument filter. The normalization calibration yields a "Pass" or "Fail" result for each normalization plate used.

Preparing the calibration plate or array card

Prepare the calibration consumables appropriate for your QuantStudio™ 12K Flex Instrument:

- Preparing the normalization plates 59
- Preparing array cards for instrument calibration 37

Preparing the normalization plates

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

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

Required materials

- 96- or 384-Well Normalization Plates with FAM™/ROX™ and VIC®/ROX™ Dyes
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Preparing the calibration plate

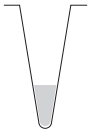
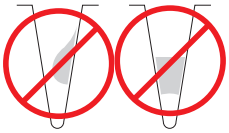
1. Remove the normalization plates from the freezer, then allow the plates to warm to room temperature (approximately 5 minutes).

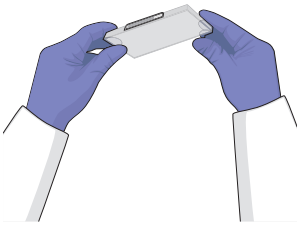
   IMPORTANT! Do not remove the normalization plates from their packaging until you are ready to run them. The fluorescent dyes in the dye plates are photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the plates.

   Note: If you store the normalization plates in their original packaging and in the freezer, you can use them to calibrate a QuantStudio™ 12K Flex System up to 3 times for 6 months after opening them.

2. Go to "Performing the calibration" on page 60.

   Before using each normalization plate, vortex the plate for 5 seconds, centrifuge it for 2 minutes at < 1500 rpm, then verify 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



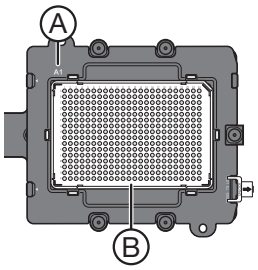
IMPORTANT! The normalization plates must be well mixed and centrifuged.

Performing the calibration

1. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
2. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add a QuantStudio™ 12K Flex Instrument to your list before you can manage it.
3. After the QuantStudio™ 12K Flex Instrument is added to your list, select it, then click **Manage Instrument**.
4. In the Instrument Manager, start the calibration wizard:
   a. Click **Maintenance**, then click **Normalization**.
   b. In the Normalization Calibration screen, click **Start Calibration**.
5. In the Normalization Calibration screen, select the reporter/passive dye combination that you want to calibrate, then perform the calibration as instructed.

6. Load the calibration plate or array card into the QuantStudio™ 12K Flex Instrument:
   a. Verify that the normalization plate or array card matches the selection in the QuantStudio™ 12K Flex Software. The name of the dyes contained by each consumable appears next to the barcode on the front of the plate or array card.
   b. Load the appropriate normalization plate or array card into the plate adapter. Ensure that the plate or array card 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.



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

7. After loading the plate or array card, start the calibration:
   a. In the Dye Calibration screen, select **Check the box when the normalization calibration plate has been loaded**, then click **Next**.
   b. In the Run screen, click **START RUN** to start the calibration.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Instrument is in operation.

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

8. When the run is complete and the QuantStudio™ 12K Flex Software displays the Analysis screen, verify 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 the data it collected is unusable.

Analysis status	Action
Passed	Enter any comments you have in the Comments field, click **Next**, then remove the plate or array card when the QuantStudio™ 12K Flex Instrument ejects the tray arm.
Failed	Troubleshoot the failed calibration as described in Table 6, "Troubleshooting normalization calibrations" on page 76.

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the plate can reach 100°C. Allow the plate to reach room temperature before removing.

IMPORTANT! If the QuantStudio™ 12K Flex Instrument does not eject the plate, remove the plate according to Table 6, "Troubleshooting normalization calibrations" on page 76.

9. Discard or store the plate or array card:

Consumable	Action
Array card	Discard the array card.
Plate	Return the normalization calibration plate to its packaging sleeve, then return the packaged plate to the freezer. IMPORTANT! Do not discard the calibration plate. If the plate is stored in its original packaging sleeve, you can use it to calibrate a QuantStudio™ 12K Flex System 3 times for up to 6 months after you open it.

10. In the Normalization Calibration screen, click **Finish** to complete the calibration, then click **Yes** when prompted to save the results.
11. Repeat steps 4 through 10 to perform the remaining normalization calibration.

Verifying the instrument performance

Perform the RNase P instrument verification experiment to verify the performance of an Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System.

When to perform the RNase P experiment

We recommend performing an RNase P instrument verification experiment:

- After moving the QuantStudio™ 12K Flex Instrument to another location.
- As needed to verify the function of the QuantStudio™ 12K Flex System.

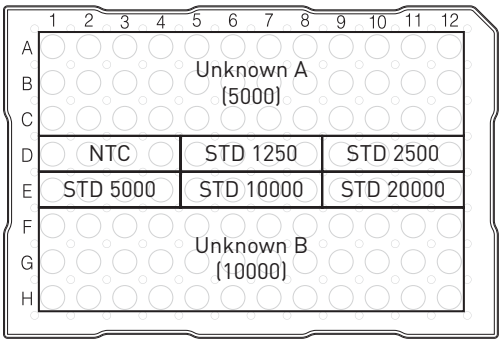
About the RNase P kits

The instrument verification experiment uses one of two instrument verification kits available from Life Technologies. The kits differ only in the consumable format for which they are designed: a TaqMan® RNase P Instrument Verification Plate for QuantStudio™ 12K Flex Instruments with 96/384-well sample blocks and an Array Card RNase P Kit for QuantStudio™ 12K Flex Instruments with array card sample blocks.

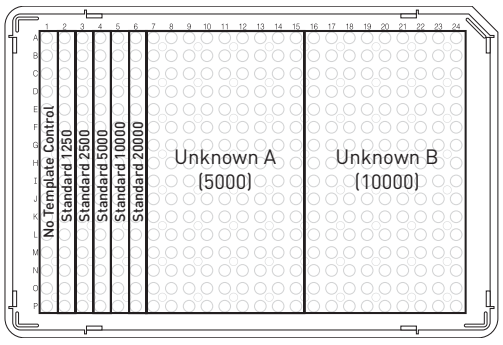
TaqMan® RNase P Instrument Verification Plates

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: TaqMan® Fast Universal 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).



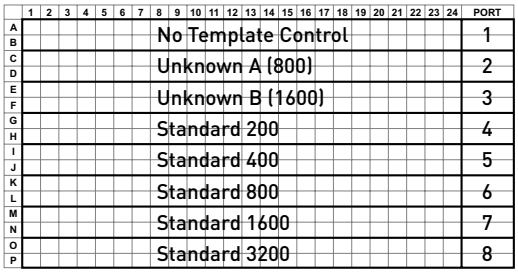
The figure to the right illustrates the arrangement of the standard and unknown populations on a 384-well RNase P plate. The RNase P plate contains five replicate groups of standards (1250, 2500, 5000, 10000, and 20000 copies), two unknown populations (5,000 and 10,000 copies), and a no template control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



About the analysis

The QuantStudio™ 12K Flex Software performs the same analysis of data from an instrument verification runs for 96-well plate, Fast 96-well plate, 384-well plate, or array card blocks.

After the run, the QuantStudio™ 12K Flex 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™ 12K 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 in columns 7–15 of the TaqMan® RNase P Plate or the 800-copy population in rows C and D of the loaded array card. Unknown population B refers to the 10,000-copy population in the wells of the TaqMan® RNase P Plate or the 1,600-copy population in rows E and F of the loaded array card.

Installation specification

The QuantStudio™ 12K Flex System passes the installation specification if the inequality holds and the QuantStudio™ 12K Flex 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

Preparing the verification consumable

IMPORTANT! When performing the RNase P instrument verification experiment:

- Perform all calibrations beforehand.
- Run the TaqMan® RNase P plate or array card soon after you allow the plate or reagents to thaw. Minimizing the time between thaw and run ensures optimal performance.
- Wear powder-free gloves and safety glasses when you prepare plates or array cards.

Prepare the instrument verification consumable appropriate for your instrument:

■ Preparing the TaqMan® RNase P Instrument Verification Plate 65
■ Preparing an array card for instrument verification 66

Preparing the TaqMan® RNase P Instrument Verification Plate

Required materials

- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles
- TaqMan® RNase P Fast 96-Well Instrument Verification Plate

Prepare the TaqMan® RNase P plate

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

   IMPORTANT! Do not remove the plate from its packaging until you are ready to run it. The fluorescent dyes in the dye plate are photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the plate.

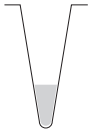
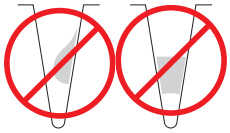
2. Remove the RNase P plate from its packaging.

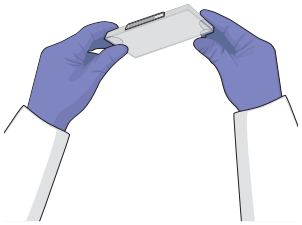
3. Briefly vortex and centrifuge the RNase P plate:
   a. Vortex the plate for 5 seconds.
   b. Centrifuge the reaction plate for 2 minutes at less than 1500 rpm.

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

   c. Verify 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 time.

   IMPORTANT! Do not allow the bottom of the RNase P 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 signal.

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™ 12K Flex System with an array card sample block.

Required materials

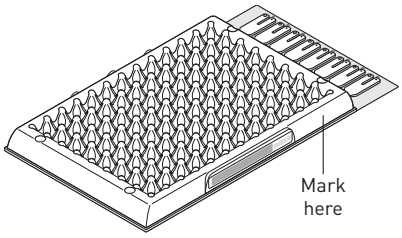
- Applied Biosystems® Array Card Staker/Sealer
- Centrifuge with centrifuge array card carrier clips and buckets
- Powder-free gloves
- Safety goggles
- Pipettor, 200-µL (with pipette tips)
- TaqMan® RNase P Array Card Instrument Verification Reagents Kit:
  - Array Card
  - TaqMan® RNase P Array Card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

Preparing the TaqMan® 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 thaw at room temperature.
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.



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								No Template Control																	1
B																									
C								Unknown A (800)																	2
D																									
E								Unknown B (1600)																	3
F																									
G								Standard 200																	4
H																									
I								Standard 400																	5
J																									
K								Standard 800																	6
L																									
M								Standard 1600																	7
N																									
O								Standard 3200																	8
P																									

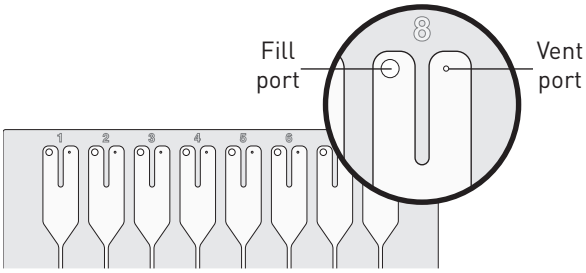
For each transfer:

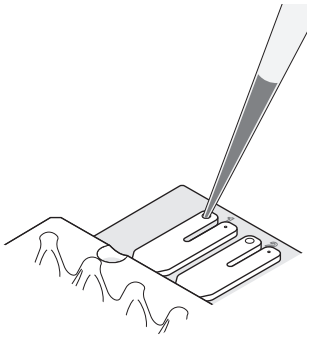
a. Place the array card on a lab bench, with the foil side down.

b. Load 100 µL of fluid into a pipette.

c. Hold the pipette in an angled position (~45°) 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.

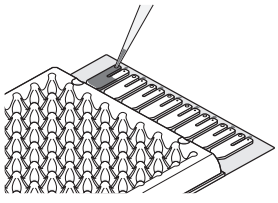




IMPORTANT! Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.

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 below the fill port.

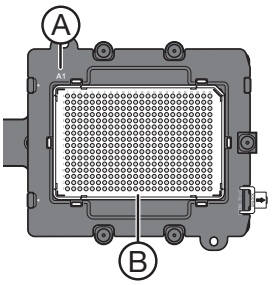
5. Centrifuge and seal the array card as explained in steps 6 through 11 on page 38.
6. Run the prepared array card as soon as possible after filling it. Store the array card in a dark place until you are ready to run it.

IMPORTANT! Do not expose the array card to light 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.

Running the experiment

1. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
2. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add a QuantStudio™ 12K Flex Instrument to your list before you can manage it.
3. After the QuantStudio™ 12K Flex Instrument is added to your list, select it, then click **Manage Instrument**.
4. In the Instrument Manager, start the RNase P wizard:
   a. Click **Maintenance**, then click **RNase P Run**.
   b. In the RNase P Run screen, click **Start RNase P Run**.
5. Complete the calibration as instructed by the wizard. When the side door opens, load the RNase P plate or array card. Ensure that the plate or array card 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, array cards, and OpenArray® plates with the barcode facing the front of the instrument.



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

6. After loading the plate or array card, start the calibration:
   a. In the Overview screen, select **Check the box when the RNase P calibration plate has been loaded**, then click **Next**.
   b. In the Run screen, click **START RUN** to start the calibration.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Instrument is in operation.

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

7. When the run is complete and the QuantStudio™ 12K Flex Software displays the Analysis screen, verify the status of the run.

Analysis status	Action
Passed	The QuantStudio™ 12K Flex System passed the RNase P run. Go to step 12 on page 70.
Failed	The QuantStudio™ 12K Flex System failed the RNase P run. Go to step 8 to review the data for outliers. If the run fails, the QuantStudio™ 12K Flex Software may have included outliers that caused the initial analysis to fail. Experimental 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.

8. In the Amplification Plot, select **Ct vs. Well** from the Plot Type menu, then verify the uniformity of each replicate population (controls, standards, and unknowns) on the reaction plate by comparing the groupings of C_T values:
   a. In the plate layout, select the wells containing Unknown Population A:
      - **96-well plate** – Select rows A–C (5,000-copy population).
      - **384-well plate** – Select columns 7–15 (5,000-copy population).
      - **Array card** – Select rows C and D (800-copy population).
   b. In the plot, verify that the C_T values of the replicate population are equivalent.

      Note: The numbers on the X-axis of the plot correspond to the wells of the reaction plate. 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 experiment using another RNase P plate or array card.

Sample block	Maximum number of outlier wells that can be removed			
	Unknown A‡	Unknown B§	Standard (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 96/384-well plates; 800-copy population for array cards.
§ 10,000-copy population for 96/384-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.

   d. Repeat step 3a through 3c for each replicate population (unknowns, standards, and no template controls) on the plate or array card.

9. Review the Results Table for quality flags generated by the experiment:
   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 Table 7, "Troubleshooting RNase P instrument verification experiments" on page 77.
      - 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 in plate
      - NOSIGNAL - No signal in well
      - OFFSCALE - Fluorescence is offscale
      - OUTLIERRG - Outlier in replicate group
      - SPIKE - Noise spikes
      - THOLDFAIL - Thresholding algorithm failed
10. If you omitted outliers, click **Reanalyze** to analyze the run.

    If the status of the RNase P Run is "Failed" after performing steps 8 through 10, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Life Technologies.
11. 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. Verify that the R2 value is ≥ **0.990**.

    If the R2 value is less than 0.990, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Life Technologies.
12. In the Analysis screen, click **Next**, remove the plate or array card when the QuantStudio™ 12K Flex Instrument ejects the tray arm, then 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.

    IMPORTANT! If the QuantStudio™ 12K Flex Instrument does not eject the plate, remove the plate according to Table 7, "Troubleshooting RNase P instrument verification experiments" on page 77.
13. Click **Finish**, then click **Yes** when prompted to save the experiment.

Troubleshooting

Table 2 Troubleshooting ROI calibrations

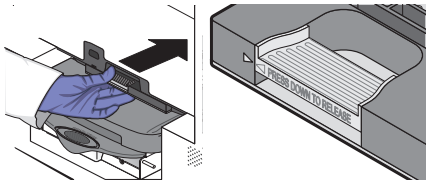
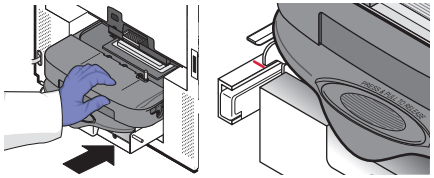
Problem/symptom	Possible cause	Action
ROI calibration failed.	The sample block or heated cover may not be seated correctly.	1. Power off and unplug the QuantStudio™ 12K Flex Instrument. 2. Wait for 15 minutes, then open the access door. 3. Firmly push the sample block and the heated cover toward the back of the QuantStudio™ 12K Flex Instrument to confirm that they are seated correctly. IMPORTANT! Confirm that the arrows on the front handle of the heated cover align as shown below. If the arrows do not align, push the heated cover further into the QuantStudio™ 12K Flex 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 the indicator is forward of the red line, push the sample block into the instrument until it is seated correctly.  4. If the ROI calibration continues to fail, check the status of the LEDs within the QuantStudio™ 12K Flex System, then replace the LEDs if necessary.
ROI image is faint.		
Instrument malfunction.	Multiple possible causes	Contact a local Life Technologies Field Service Office.
Instrument does not eject the ROI plate.	The adhesive cover may have adhered the plate to the heated cover within the instrument.	1. Power off the QuantStudio™ 12K Flex Instrument. 2. Wait for 15 minutes, then power on the instrument and eject the plate. 3. If the plate does not eject, power off and unplug the QuantStudio™ 12K Flex Instrument, then open the access door. 4. Wearing powder-free gloves, reach into the QuantStudio™ 12K Flex Instrument and remove the plate from the heated cover, then close the access door. 5. Perform a background calibration to confirm that the sample block has not been contaminated.

Table 3 Troubleshooting background calibrations

Problem/symptom	Possible cause	Action
Background calibration failed.	One or more wells of the background plate produced spectra that exceed the maximum limit for the instrument.	1. Repeat the calibration using the same background plate. 2. If the calibration fails again, repeat the calibration using a different background plate. 3. If the calibration fails again, determine the source of the contamination, as explained in "Identifying contamination" on page 80.
Instrument does not eject the background plate.	The adhesive cover may have adhered the plate to the heated cover within the instrument.	1. Power off the QuantStudio™ 12K Flex Instrument. 2. Wait for 15 minutes, then power on the QuantStudio™ 12K Flex Instrument and eject the plate. 3. If the plate does not eject, power off and unplug the QuantStudio™ 12K Flex Instrument, then open the access door. 4. Wearing powder-free gloves, reach into the QuantStudio™ 12K Flex Instrument and remove the plate from the heated cover, then close the access door. 5. Perform a background calibration to confirm that the sample block has not been contaminated.
Instrument malfunction.	Multiple possible causes	Contact a local Life Technologies Field Service Office.

Table 4 Troubleshooting uniformity calibrations

Problem/symptom	Possible cause	Action
Uniformity calibration failed.	Abnormally low spectra across all wells of the plate or array card.	1. Confirm that you loaded an ROI plate or array card into the QuantStudio™ 12K Flex Instrument. If not, perform the calibration again using the correct ROI plate or array card. 2. If you are using the correct plate or array card, perform the calibration again using a different ROI plate or array card. 3. If the calibration fails again, contact Life Technologies technical support.
	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 calibration 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 step 1 and step 2 are: • **Identical** – The sample block is contaminated. Decontaminate the sample block. • **Reversed** – The ROI plate or array card is contaminated. Discard the plate or array card, then perform the uniformity calibration using a new ROI plate or array card. 4. If the calibration fails again, contact Life Technologies technical support.
Instrument does not eject the ROI plate.	The adhesive cover may have adhered the plate to the heated cover within the instrument.	1. Power off the QuantStudio™ 12K Flex Instrument. 2. Wait for 15 minutes, then power on the QuantStudio™ 12K Flex Instrument and eject the plate. 3. If the plate does not eject, power off and unplug the QuantStudio™ 12K Flex Instrument, then open the access door. 4. Wearing powder-free gloves, reach into the QuantStudio™ 12K Flex Instrument and remove the plate from the heated cover, then close the access door. 5. Perform a background calibration to confirm that the sample block has not been contaminated.
Instrument malfunction.	Multiple possible causes	Contact a local Life Technologies Field Service Office.

Table 5 Troubleshooting dye calibrations

Problem/symptom	Possible cause	Action
One or more raw spectra are at or below the detectable threshold for the calibration.	Dye calibration plate was centrifuged insufficiently. Dye calibration plate contains old or insufficient reagents. If you are running a custom dye calibration plate, the dye may not be present at a sufficient concentration.	1. Unload the QuantStudio™ 12K Flex System and view the wells of the dye calibration plate. If the liquid in the wells is not: • At the bottom of the wells, centrifuge the plate for a longer time, then repeat the calibration. • Equivalent in volume, the plate is not sealed and the reagents have evaporated. Discard the plate and run another. 2. If the dye calibration plate appears to be normal, discard the plate and run another. 3. If the problem persists, contact Life Technologies. If you are running a custom dye calibration plate, create another plate but increase the concentration of the dye that produced insufficient signal.
• Spectra contain peaks in more than one filters. • One or more raw spectra exceed the maximum limit for the QuantStudio™ 12K Flex System.	Fluorescent contaminants are present on the sample block or dye calibration plate. If you are running a custom spectral calibration plate, the dye may be too concentrated.	Verify that contaminants are not present by performing a background calibration (see "Background calibration" on page 45) If the background calibration does not show sample block contamination, the dye calibration plate may be contaminated. Note: If you are running a custom dye calibration plate, create another plate but decrease the concentration of the dye that exceeded the detectable limit.
Instrument does not eject the dye plate.	The adhesive cover may have adhered the plate to the heated cover within the instrument.	1. Power off the QuantStudio™ 12K Flex Instrument. 2. Wait for 15 minutes, then power on the QuantStudio™ 12K Flex Instrument and eject the plate. 3. If the plate does not eject, power off and unplug the QuantStudio™ 12K Flex Instrument, then open the access door. 4. Wearing powder-free gloves, reach into the QuantStudio™ 12K Flex Instrument and remove the plate from the heated cover, then close the access door. 5. Perform a background calibration to confirm that the sample block has not been contaminated.
Instrument malfunction.	Multiple possible causes	Contact a local Life Technologies Field Service Office.

Table 6 Troubleshooting normalization calibrations

Problem/symptom	Possible cause	Action
Normalization calibration failed.	Abnormally low spectra across all wells of the plate or array card.	1. Confirm that you loaded an normalization plate or array card into the QuantStudio™ 12K Flex Instrument. If not, perform the calibration again using the correct normalization plate or array card. 2. If you are using the correct plate or array card, perform the calibration again using a different normalization plate or array card. 3. If the calibration fails again, contact Life Technologies technical support.
	One or more wells produced spectra that deviate significantly from the rest of the plate or array card.	1. While viewing the calibration data, locate the well(s) with abnormal signal in the Plate Layout tab. 2. Rotate the calibration 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 steps 1 and 2 are: • **Identical** – The sample block is contaminated. Decontaminate the sample block. • **Reversed** – The normalization plate or array card is contaminated. Discard the plate or array card, then perform the normalization calibration using a new normalization plate or array card. 4. If the calibration fails again, contact Life Technologies technical support.
Instrument does not eject the normalization plate.	The adhesive cover may have adhered the plate to the heated cover within the instrument.	1. Power off the QuantStudio™ 12K Flex Instrument. 2. Wait for 15 minutes, then power on the QuantStudio™ 12K Flex Instrument and eject the plate. 3. If the plate does not eject, power off and unplug the QuantStudio™ 12K Flex Instrument, then open the access door. 4. Wearing powder-free gloves, reach into the QuantStudio™ 12K Flex Instrument and remove the plate from the heated cover, then close the access door. 5. Perform a background calibration to confirm that the sample block has not been contaminated.
Instrument malfunction.	Multiple possible causes	Contact a local Life Technologies Field Service Office.

Table 7 Troubleshooting RNase P instrument verification experiments

Problem/symptom	Possible cause	Action
More than the maximum number of outliers are present in RNase P data.	Possible contamination Pipetting inaccuracy	Contact Life Technologies to order a replacement TaqMan® RNase P plate or array card kit. If the replacement RNase P plate or array card fails, contact Life Technologies for further assistance.
RNase P plate verification run failed.	Insufficient centrifugation Defective plate seal	**CAUTION! PHYSICAL INJURY HAZARD**. During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, wait until the sample block reaches room temperature. 1. Unload the RNase P plate or array card from the QuantStudio™ 12K Flex Instrument. 2. Hold the plate or array card up to a light source to verify that all wells contain the same volume of fluid. 3. 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. If the well positions coincide, the heat seal on the plate may be defective, resulting in the evaporation of the associated samples. 4. Contact Life Technologies to order a replacement TaqMan® RNase P plate or array card kit. If the replacement RNase P plate or array card fails, contact Life Technologies for further assistance.
Instrument does not eject the RNase P plate.	Adhesive cover may have adhered the plate to the heated cover within the instrument	1. Power off the QuantStudio™ 12K Flex Instrument. 2. Wait for 15 minutes, then power on the QuantStudio™ 12K Flex Instrument and eject the plate. 3. If the plate does not eject, power off and unplug the QuantStudio™ 12K Flex Instrument, then open the access door. 4. Wearing powder-free gloves, reach into the QuantStudio™ 12K Flex Instrument and remove the plate from the heated cover, then close the access door. 5. Perform a background calibration to confirm that the sample block has not been contaminated.
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 reaction plate for evaporation or low reaction volume. 3. Contact Life Technologies to order a replacement TaqMan® RNase P plate or array card kit. If the replacement RNase P plate or array card fails, contact Life Technologies for further assistance.

Problem/symptom	Possible cause	Action
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 reaction plate	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 reaction plate to check for condensation and/or inconsistent reaction volumes. 4. Contact Life Technologies to order a replacement TaqMan® RNase P plate or array card kit. Repeat the experiment, and make 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 further assistance.
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 ΔR_n vs. Cycle) and check for early, late, low, or no amplification. 3. Contact Life Technologies to order a replacement TaqMan® RNase P plate or array card kit. Repeat the experiment, making sure to properly seal and centrifuge the RNase P plate. If the replacement RNase P plate or array card fails, contact Life Technologies for further assistance.
Well displays the CTFAIL flag, indicating that the software cannot calculate the threshold cycle (C_T).	• Amplification too early • Amplification too late • Low amplification • No amplification	
Well displays the EXPFAIL flag, indicating that the software cannot identify the exponential region of the amplification plot.		
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 plate or array card fails, contact Life Technologies for further 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 reaction plate 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 TaqMan® RNase P plate or array card kit. If the replacement RNase P plate or array card fails, contact Life Technologies for further assistance.

Problem/symptom	Possible cause	Action
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 reaction plate • Inconsistent volumes across the plate	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 TaqMan® RNase P plate or array card kit. Repeat the experiment, and make sure to properly seal and centrifuge the RNase P plate. If the replacement RNase P plate or array card fails, contact Life Technologies for further 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.	• Droplets on the sides of the wells • Improper sealing or seal leaks • Condensation on the reaction plate	
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 TaqMan® RNase P plate or array card kit. Repeat the experiment, and make sure to properly seal the RNase P plate or array card. If the replacement RNase P plate or array card fails, contact Life Technologies for further 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 TaqMan® RNase P plate or array card kit. Repeat the experiment, and make 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 further 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 (Rn vs. Cycle and ΔRn vs. Cycle), and check for early, late, low, or no amplification. 3. Contact Life Technologies to order a replacement TaqMan® RNase P plate or array card kit. If the replacement RNase P plate or array card fails, contact Life Technologies for further assistance.
Instrument malfunction.	Multiple possible causes	Contact a local Life Technologies Field Service Office.

Identifying contamination

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

To determine the source and location of the contamination:

1. While viewing the background calibration data in the Analysis screen, select the **QC** tab and review the list of wells that failed the quality check.
2. Rotate the background plate 180°, then perform the background calibration again.
3. Determine the location of the contaminated wells again.

   If the position(s) of the contaminated well(s) in step 1 and step 2 are:
   - **Identical** – The sample block is contaminated. Decontaminate the sample block.
   - **Reversed** – The background plate or array card is contaminated. Discard the plate or array card, then perform the background calibration using a new background plate or array card.
4. If the calibration fails after you replace the background plate and decontaminate the sample block:
   a. Cover a plate or array card with a piece of black paper.
   b. Perform the background run as explained in this chapter, substituting the plate or array card covered with paper for the background plate or array card.
   c. After the run is complete and while viewing the calibration data, select all wells in the Plate Layout tab, then view the Spectral plot for the peak(s). If the peak associated with the contamination is:
      - **Visible** – The optics of your QuantStudio™ 12K Flex System may be contaminated. Contact Life Technologies for further support.
      - **Absent** – The sample block is contaminated. Decontaminate the sample block again and repeat the calibration.

3 Calibrating OpenArray® Plate Sample Blocks

This chapter covers:

Recommended calibration and maintenance

The Applied Biosystems® QuantStudio™ 12K Flex Real-Time PCR System requires regular calibration and maintenance for proper operation. The following table displays the recommended maintenance schedule that you must perform to ensure optimal instrument performance.

IMPORTANT! Calibrate the QuantStudio™ 12K Flex System at the same ambient temperature at which you will run experiments. Extreme variations in ambient temperature can affect the heating and cooling of the QuantStudio™ 12K Flex System and, in extreme cases, influence experimental results.

IMPORTANT! Do not use organic solvents to clean the QuantStudio™ 12K Flex System.

Table 8 OpenArray® plate sample block maintenance

Frequency	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 QuantStudio™ 12K Flex System, then after 30 seconds, power on the computer.
	Clean the surface of the QuantStudio™ 12K Flex System with a lint-free cloth.
	Perform a QuantStudio™ 12K Flex Instrument self test.
Monthly	Perform a background calibration.‡
	Run disk cleanup and disk defragmentation.
Annually	Perform a background calibration.
	Perform a uniformity calibration.
	Perform a dye calibration.
	Perform an instrument verification run.
As needed	Decontaminate the QuantStudio™ 12K Flex System.
	Replace the QuantStudio™ 12K Flex System fuses.
	Update the Windows® operating system.
	Update the QuantStudio™ 12K Flex Software and firmware.

‡ You can perform a background calibration to check for contamination. If any parts of the optics are replaced or moved, you must perform all calibrations, including an RNase P instrument verification run.

About the OpenArray® Calibration Plaque

The OpenArray® Calibration Plaque is a specialized tool that is used to perform background and uniformity calibrations of the QuantStudio™ 12K Flex System with an OpenArray® sample block. The plaque consists of a thin sheet of black plastic that has two distinct sides shown below.

Black side	Orange side
• Dull, matte black in color. • Completely smooth. • Performs the background calibration.	• Glossy, dark orange in color. • Textured with a faint lattice pattern. • Performs the uniformity calibration.
	

Caring for the OpenArray® Calibration Plaque

The OpenArray® Calibration Plaque is sensitive to light and must be kept clean at all times. Adhere to the following handling, storage, and cleaning guidelines when using the tool.

Action	Guidelines
Handling	When handling the OpenArray® Calibration Plaque: • Always wear powder-free gloves. • Grasp the tool by the edges. • Ensure the tool does not become dirty or dusty.
Storing	When not in use, store the OpenArray® Calibration Plaque: • At room temperature. • In the original packaging sleeve or in a clean plastic bag. • In a dark, clean place, such as a drawer or cabinet.
Cleaning	If the OpenArray® Calibration Plaque becomes dirty, clean the tool as follows: a. Place the OpenArray® Calibration Plaque on a clean, dry surface. b. Pipet a small volume of 95% ethanol or 95% isopropanol solution onto a lint-free wipe, then thoroughly swab the surface of the tool. c. Use a lint-free wipe to absorb the excess solution.

Background calibration

IMPORTANT! Perform the following procedure only if you are calibrating a QuantStudio™ 12K Flex System with an OpenArray® plate sample block.

During a background calibration, the QuantStudio™ 12K Flex System:

- Performs two reads of the QuantStudio™ 12K Flex OpenArray™ Calibration Plaque for 10 minutes at 60°C.
- Averages the spectra recorded during the run and extracts the resulting spectral component to a calibration file.

The QuantStudio™ 12K Flex Software then uses the calibration file during subsequent runs to remove background fluorescence from the run data.

Required materials

- QuantStudio™ 12K Flex OpenArray® Calibration Plaque
- Powder-free gloves
- Safety goggles

When to perform the calibration

Perform the background calibration monthly or as often as necessary, depending on instrument use.

About the background calibration data

During the background calibration, the QuantStudio™ 12K Flex Software captures a series of images of the *black* side of the OpenArray® Calibration Plaque using each instrument filter. The software measures the fluorescence across the image. A background calibration passes if the collected images for all filters have signals that are within normal range.

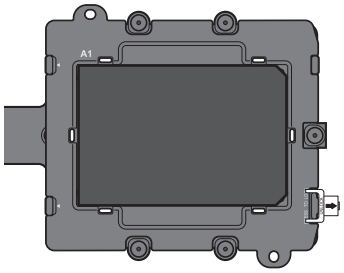
IMPORTANT! A user must be present throughout the duration of the calibration. Following the first read, the OpenArray® Calibration Plaque must be rotated 180° before the instrument can complete the calibration.

Load the plaque

1. When the instrument door opens, load the OpenArray® Calibration Plaque (*black* side up) into the plate retainer.

   IMPORTANT! Ensure that the OpenArray® Calibration Plaque is loaded into the plate retainer so that the *black* side of the tool is facing up.

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



2. Start the calibration:
   a. Select **Check the box when the calibration plaque has been loaded**, then click **Next**.
   b. In the Run screen, click **START RUN**.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Instrument is in operation.

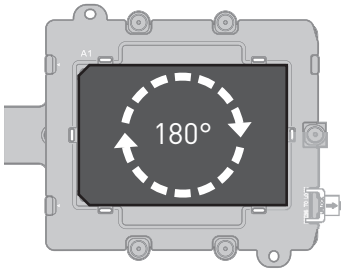
Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach temperature.

Rotate the plaque

When the instrument door opens and you are prompted to rotate the OpenArray® Calibration Plaque:

1. Rotate the OpenArray® Calibration Plaque 180°, then place it back into the plate retainer (*black* side up).

IMPORTANT! Do not flip the OpenArray® Calibration Plaque over. The *black* side of the tool must face up.



2. Click **OK** to close this dialog box, then click **START RUN** in the Run screen to perform the second reading.

Complete the calibration

IMPORTANT! Wear powder-free gloves and safety glasses when you handle the OpenArray® Calibration Plaque.

1. Verify the status of the calibration:

The Analysis Status displayed by the QuantStudio™ 12K Flex Software 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 the data it collected is unusable.

Analysis status	Action
Passed	1. Click **Next**. 2. Enter any comments you have in the Comments field, click **Finish**, then click **Yes** when prompted to save the results.
Failed	1. Repeat the calibration. If necessary, clean the OpenArray® Calibration Plaque before you repeat the calibration as described in "Caring for the OpenArray® Calibration Plaque" on page 83. 2. If the calibration fails again, contact Life Technologies for further assistance.

2. When the instrument door opens, remove the OpenArray® Calibration Plaque from the instrument tray.

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the plates or plaque can reach 100°C. Ensure the plate or plaque is at room temperature before removing.

3. Return the OpenArray® Calibration Plaque to its original packaging or a clean plastic bag.

IMPORTANT! Do not expose the OpenArray® Calibration Plaque to sunlight for extended periods of time. When not in use, store the plaque at room temperature within the original packaging in a clean, dark location.

IMPORTANT! If the QuantStudio™ 12K Flex Instrument does not eject the OpenArray® Calibration Plaque, remove the plate as explained in "Troubleshooting" on page 105.

Uniformity calibration

IMPORTANT! Perform the following procedure only if you are calibrating a QuantStudio™ 12K Flex System with an OpenArray® plate sample block.

The uniformity calibration generates data that allows the QuantStudio™ 12K Flex Software to compensate for the physical effects of the QuantStudio™ 12K Flex System filters.

Required materials

- QuantStudio™ 12K Flex OpenArray® Calibration Plaque
- Powder-free gloves
- Safety goggles

When to perform the calibration

Perform a uniformity calibration at least once per year or more often, depending on use.

IMPORTANT! You must perform a uniformity calibration before a dye calibration.

About the uniformity calibration

During the uniformity calibration, the QuantStudio™ 12K Flex Software captures a series of images of the *orange* side of the OpenArray® Calibration Plaque using each instrument filter using each instrument filter. The QuantStudio™ 12K Flex Software uses the captured images to calibrate the optical uniformity of the QuantStudio™ 12K Flex Instrument.

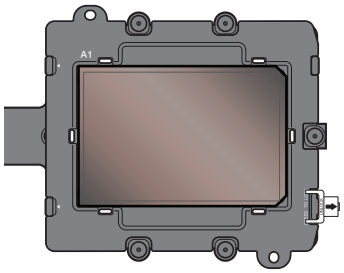
IMPORTANT! A user must be present throughout the duration of the calibration. Following the first read, the OpenArray® Calibration Plaque must be rotated 180 degrees before the instrument can complete the calibration.

Load the plaque

1. When the instrument door opens, load the OpenArray® Calibration Plaque (*orange* side up) into the plate retainer.

   IMPORTANT! Ensure that the OpenArray® Calibration Plaque is loaded into the plate retainer so that the *orange* side of the tool is facing up.

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



2. Start the calibration:
   a. Select **Check the box when the calibration plaque has been loaded**, then click **Next**.
   b. In the Run screen, click **START RUN**.

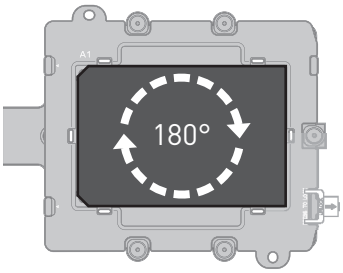
IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Instrument is in operation.

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

Rotate the plaque

When the instrument door opens and you are prompted to rotate the OpenArray® Calibration Plaque:

1. Rotate the OpenArray® Calibration Plaque 180°, then place it back into the plate retainer (*orange* side up).



IMPORTANT! Do not flip the OpenArray® Calibration Plaque over. The *orange* side of the tool must be facing up.

2. Click **OK** to close this dialog box, then click **START RUN** in the Run screen to perform the second reading.

Complete the calibration

IMPORTANT! Wear powder-free gloves and safety glasses when you handle the OpenArray® Calibration Plaque.

1. Verify the status of the calibration:

   The Analysis Status displayed by the QuantStudio™ 12K Flex Software 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 the data it collected is unusable.

Analysis status	Action
Passed	1. Click **Next**. 2. Enter any comments you have in the Comments field, click **Finish**, then click **Yes** when prompted to save the results.

Analysis status	Action
Failed	1. Repeat the calibration. If necessary, clean the OpenArray® Calibration Plaque before you repeat the calibration as described in "Caring for the OpenArray® Calibration Plaque" on page 83. 2. If the calibration fails again, contact Life Technologies for further assistance.

2. When the instrument door opens, remove the OpenArray® Calibration Plaque from the instrument tray.

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the plates or plaque can reach 100°C. Ensure the plate or plaque is at room temperature before removing.

3. Return the OpenArray® Calibration Plaque to its original packaging or a clean plastic bag.

IMPORTANT! Do not expose the OpenArray® Calibration Plaque to sunlight for extended periods of time. When not in use, store the plaque at room temperature, in the original packaging, in a clean, dark location.

IMPORTANT! If the QuantStudio™ 12K Flex Instrument does not eject the OpenArray® Calibration Plaque, remove the plate as explained in "Troubleshooting" on page 105.

Dye calibration

IMPORTANT! Perform the following procedure only if you are calibrating a QuantStudio™ 12K Flex System with an OpenArray® plate sample block.

During a dye calibration, the Applied Biosystems® QuantStudio™ 12K Flex Real-Time PCR System:

- Collects spectral data from the FAM™ dye standard.
- Stores the spectral information for the dye standard in a dye calibration file.

The QuantStudio™ 12K Flex Software uses the pure spectra data during experiment runs to characterize and distinguish the individual contribution of dyes in the total fluorescence collected by the QuantStudio™ 12K Flex Instrument. After each run, the QuantStudio™ 12K Flex Software receives data in the form of a raw spectra signal for each reading. It determines the contribution of each fluorescent dye used in the sample by comparing the raw spectra to the pure spectra calibration data. When you save an experiment after analysis, the QuantStudio™ 12K Flex Software stores the pure spectra with the collected fluorescence data for that experiment.

IMPORTANT! Calibrate only those dyes that are present in the chemistries that you intend to run on your QuantStudio™ 12K Flex System.

Required materials

- QuantStudio™ 12K Flex System Installation/Calibration Kit:
  - OpenArray® FAM™ Dye Solution
  - QuantStudio™ 12K Flex System OpenArray® Calibration Cases (4)
  - QuantStudio™ 12K Flex System OpenArray® Plugs (4)
  - QuantStudio™ 12K Flex System OpenArray® Calibration Syringe and Tip
- OpenArray® Plate Press 2.0
- Pipettes
- Powder-free gloves
- Safety glasses

When to perform the dye calibrations

Perform a dye calibration at least once per year or more often, depending on use.

IMPORTANT! You must perform a background calibration before every dye calibration. Because the age and use of instrument components can affect spectra readings, we recommend performing a dye calibration at least every year.

About the dye calibration

The dye calibration is a two-part procedure in which the QuantStudio™ 12K Flex Instrument performs two readings of the OpenArray® Calibration Cases:

- A preread of the empty OpenArray® Calibration Cases
- A postread of the OpenArray® Calibration Cases filled with OpenArray® FAM™ Dye Solution

About the dye calibration data

The product of the dye calibration is a spectral profile that represents the fluorescence signature of the FAM™ dye standard. The profile consists of a set of spectra that correspond to the fluorescence collected from the OpenArray™ calibration cases. The QuantStudio™ 12K Flex Software plots the resulting data for the spectral profile in a graph of fluorescence versus filter.

When the QuantStudio™ 12K Flex Software extracts the dye calibration data, it evaluates the fluorescence signal generated by each OpenArray™ calibration case in terms of the collective spectra for the entire tool. Dye spectra are generally acceptable if they peak within the same filter as their group but diverge slightly at other wavelengths.

The QuantStudio™ 12K Flex Software can compensate for some differences in a spectral profile by replacing the spectra of unacceptable wells with the spectra of other regions of the OpenArray™ Calibration Case (auto-repairing). The QuantStudio™ 12K Flex Software allows only a few replacements, and it may reject the calibration if the spectra between neighboring wells vary significantly.

Guidelines for handling the OpenArray® Calibration Cases

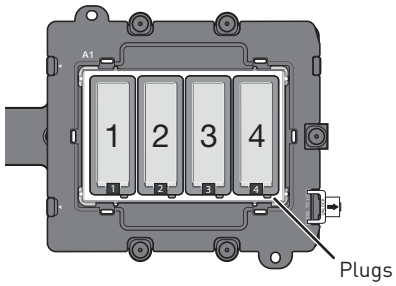
- Wear gloves that are one size smaller than the size you typically wear, to help prevent excess glove material from contacting the OpenArray® Calibration Cases while loading.
- Hold OpenArray® Calibration Cases by the edges.
- If you drop a loaded OpenArray® calibration case, discard it in the appropriate waste container.

Perform the empty reading

IMPORTANT! Wear powder-free gloves while preparing the OpenArray® Calibration Cases.

1. Load the empty OpenArray® Calibration Cases into the OpenArray® Calibration Carrier according to the labels on the cases:
   a. Remove the OpenArray® Calibration Cases from their packaging.
   b. Remove the protective film from all of the OpenArray® Calibration Cases.

c. Load case 1 into the position closest to the QuantStudio™ 12K Flex Instrument followed by the remaining cases in sequence as shown in the following figure.



IMPORTANT! Confirm that the OpenArray® Calibration Cases are positioned so that the plugs are oriented away from the A1 position as shown.

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

2. After loading the OpenArray® cases, start the calibration:
   a. In the Dye Calibration screen, select **Check the box when the dye calibration cases have been loaded**, then click **Next**.
   b. In the Run screen, click **START RUN**.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Instrument is in operation.

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

Perform the filled reading

IMPORTANT! Wear powder-free gloves while preparing the OpenArray® Calibration Cases.

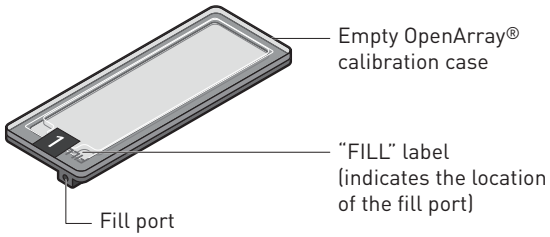
When the instrument door opens and you are prompted to perform the filled reading, load the OpenArray® Calibration Cases with OpenArray® FAM™ Dye Solution:

1. Attach a syringe tip to the syringe, then place the assembly on a clean surface.

   IMPORTANT! The application of the syringe tip requires force. Confirm that the tip is locked firmly in place before proceeding.

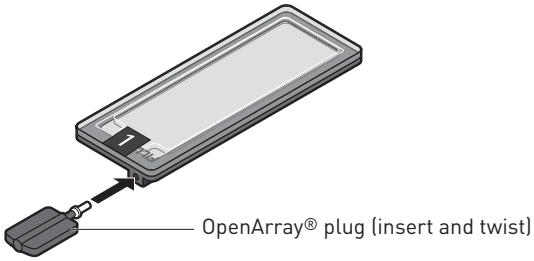
2. Carefully draw approximately 2 mL of OpenArray® FAM™ Dye Solution into the syringe.
3. Grasp the OpenArray® calibration case in position 1 by the edges, then remove it from the OpenArray® Calibration Carrier.
4. Remove the "RUN EMPTY FIRST" label that covers the fill port of the OpenArray® calibration case.

5. While holding the OpenArray® calibration case vertically, insert the syringe tip into the fill port at end of the case, then dispense the fluid completely in one gentle continuous motion.



Note: Try to minimize creating air bubbles when you dispense the fluid. You can leave one small air bubble at the fill port to prevent overfilling.

6. Seal the loading port by inserting an OpenArray® Plug into the port and twisting it clockwise until hand-tight, then remove the handle from the plug.



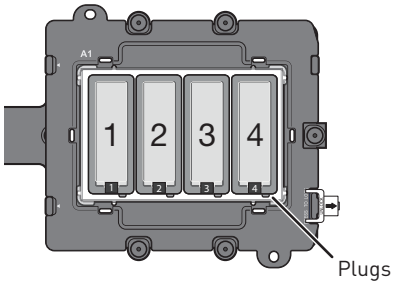
7. Load the sealed OpenArray® calibration case into the *same* position on the OpenArray® Plate Carrier that it previously occupied (position 1).

IMPORTANT! You *must* load the filled OpenArray® Calibration Cases into the same positions on the OpenArray® Calibration Carrier.

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

8. Repeat steps 1 – 7 to fill the remaining three OpenArray® Calibration Cases.

IMPORTANT! Confirm that the OpenArray® Calibration Cases are in their original positions and that their plugs are oriented away from the A1 position as shown.



9. Click **OK** to close this dialog box, then click **START RUN** in the Run screen to start the filled reading.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Instrument is in operation.

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

Complete the calibration

IMPORTANT! Wear powder-free gloves while preparing the OpenArray® calibration cases.

1. Verify the status of the calibration:
   - **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 the data it collected is unusable.
   - **QC Status** – Indicates the quality of the calibration data, where *passed* indicates that all OpenArray® calibration cases produced data that passed the quality check, and *failed* indicates that one or more cases produced dye spectra that vary significantly.

Analysis status	Action
Passed	1. Click **Next**. 2. Enter any comments you have in the Comments field, click **Finish**, then click **Yes** when prompted to save the results.
Failed	Discard the OpenArray® calibration cases, then prepare and run replacement cases. If the calibration fails again, contact Life Technologies for further assistance.

2. When the instrument door opens, remove the OpenArray® Plate Carrier from the instrument tray.

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the cases can reach 100°C. Ensure the cases are at room temperature before removing.

3. Discard the OpenArray® Calibration Cases.

IMPORTANT! If the QuantStudio™ 12K Flex Instrument does not eject the plate, remove the plate as explained in "Troubleshooting" on page 105.

Verifying the instrument performance

IMPORTANT! Perform the following procedure only if you are performing a verification experiment for a QuantStudio™ 12K Flex System with an OpenArray® plate sample block.

IMPORTANT! When performing the RNase P instrument verification experiment:

- Perform all calibrations first.
- Run the OpenArray® plate soon after you allow the plate or reagents to thaw. Minimizing the time between thaw and run ensures optimal performance.
- Wear powder-free gloves and safety glasses when you prepare OpenArray® plates.

Perform the RNase P instrument verification experiment to verify the performance of the Applied Biosystems® QuantStudio™ 12K Flex Real-Time PCR System.

When to perform the RNase P experiment

We recommend performing an RNase P instrument verification experiment:

- After moving the QuantStudio™ 12K Flex Instrument to another location.
- As needed to verify the function of the QuantStudio™ 12K Flex System.

About the OpenArray® Plate RNase P Kit

The RNase P Kit includes one empty OpenArray® plate and a single tube that contains OpenArray® RNase P Reaction Mix (TaqMan® Universal PCR Master Mix, RNase P primers, and FAM™-MGB dye-labeled probe) and a known concentration of human genomic DNA template.

Installation specification

The QuantStudio™ 12K Flex System passes the installation specification if the standard deviation of the C_T values for all through-holes on the OpenArray® plate is ≤0.25. The data from up to 48 through-holes can be omitted from the population to meet the installation specification.

Guidelines for handling the OpenArray® plate

- Hold the OpenArray® case by the edges.
- Do not touch the through-holes of the OpenArray® plate.
- Load and seal a OpenArray® plate within *one hour* after opening the packaging.
- If you drop a loaded OpenArray® plate, discard it in the appropriate waste container.

Required materials

- QuantStudio™ 12K Flex System RNase P Kit, including:
  - OpenArray® RNase P Reaction Mix
  - QuantStudio™ 12K Flex System OpenArray® Lid
  - QuantStudio™ 12K Flex System OpenArray® Plug
  - QuantStudio™ 12K Flex System OpenArray® Immersion Fluid
  - QuantStudio™ 12K Flex System OpenArray® Immersion Fluid Tip
  - OpenArray® Digital PCR Plate
  - OpenArray® 384-Well Sample Plate
- OpenArray® AccuFill™ System

- OpenArray® Plate Press 2.0
- Bleach (10%)
- Ethanol
- OpenArray® 384-Well Sample Plates
- OpenArray® AccuFill™ System Loader Tips
- Pipettes
- Powder-free gloves
- Safety glasses

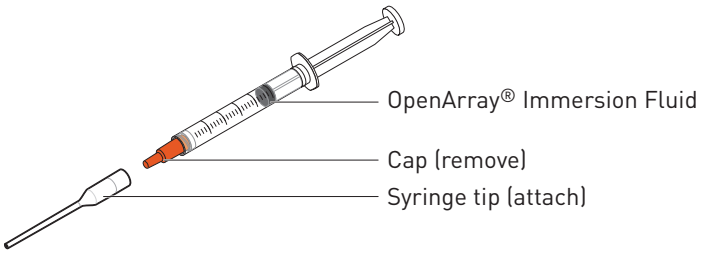
Preparing for the verification experiment

IMPORTANT! Wear powder-free gloves while preparing the OpenArray® plates.

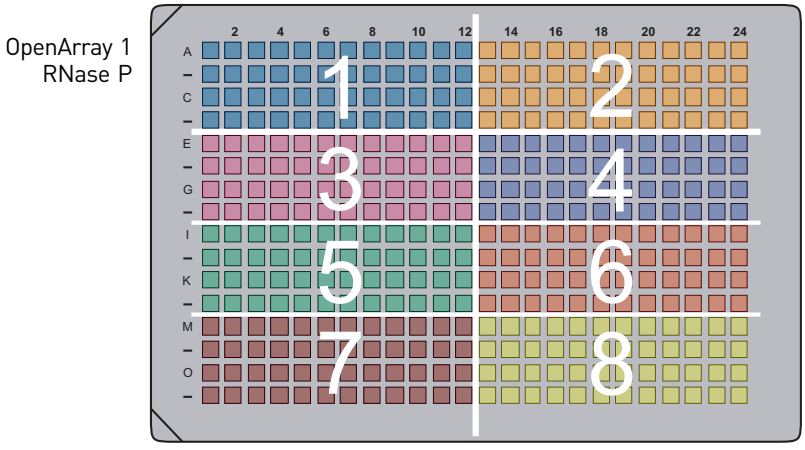
1. Confirm that the OpenArray® 384-well sample plate, OpenArray® AccuFill™ Loader Tips, and plate holder are completely clean and dry.
2. Remove an OpenArray® plate from the freezer, but do not open the packaging. Allow the plate to thaw at room temperature (approximately 15 minutes).

   Note: Unopened OpenArray® plates can remain at room temperature for up to 24 hours.
3. Prepare a syringe containing OpenArray® Immersion Fluid. Attach the syringe tip to the syringe, then set the assembly on a clean surface.

   IMPORTANT! The application of the syringe tip requires force. Confirm that the tip is locked firmly in place before proceeding.



4. Pipet 5.0 µL of the RNase P solution into loading position 1 of the 384-well sample plate.



5. Cover the sample plate with a foil seal, then score or cut the foil into the 8 sections shown above.
6. Centrifuge the plate for 1 minute at 1500 rpm, then place the plate on ice to keep the samples cold.

Initializing the system

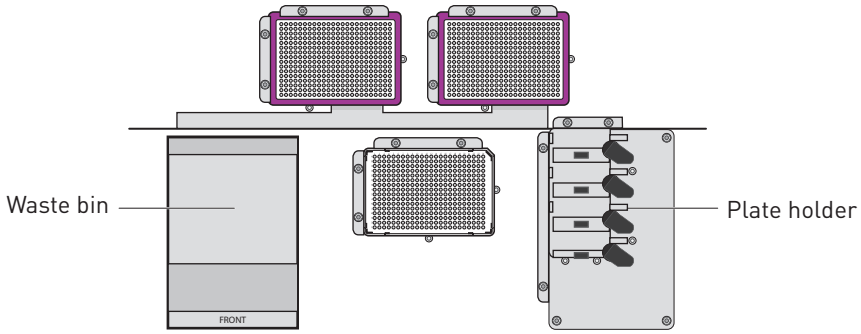
1. Close the enclosure door, then start the OpenArray® Accufill™ software. The software checks the computer and connections as the system starts.

   When prompted, clear the deck and empty the waste bin of used tips:

   a. Open the instrument by grasping the enclosure door handle and gently, but firmly, pulling the enclosure door up.

   IMPORTANT! To safely operate the instrument, keep the deck clear and have enough room in the waste bin to eject the used pipette tips.

   b. Empty the waste bin and place it back on the deck.



2. Remove any OpenArray® plates from the deck.
3. If necessary, replace the tip boxes.

   Note: Tip boxes contain 384 tips, divided into 8 sections. When you click **Load**, the OpenArray® Accufill™ instrument loads as though a new, full box of tips is on the deck. OpenArray® Accufill™ software prompts you to verify that tips are in the locations shown in the Setup Deck screen. Clicking a section in the Setup Deck window confirms that tips are in that section of the tip box. We recommend using a full tip box.

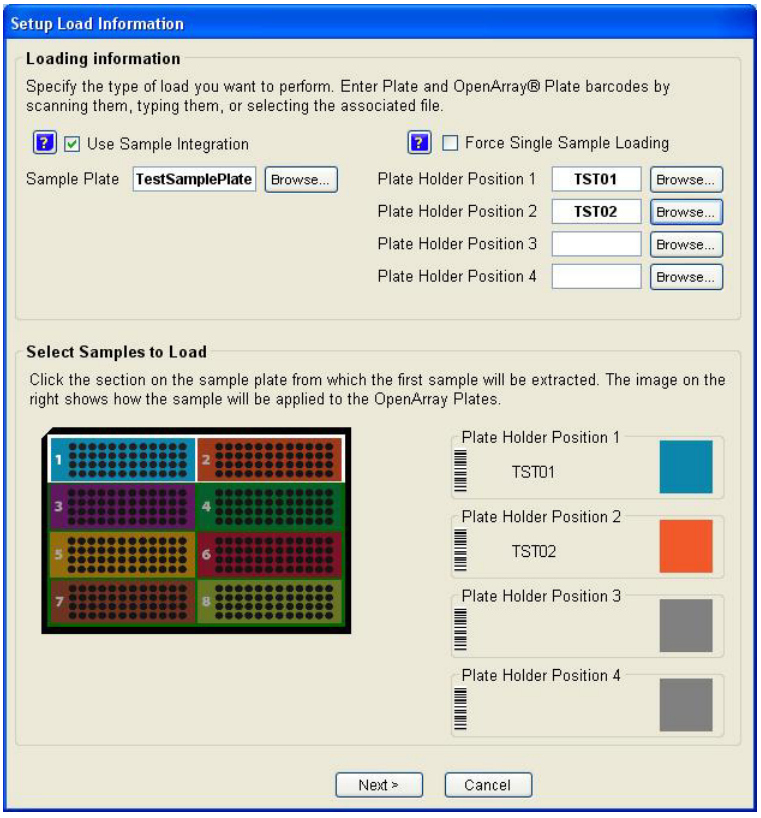
   a. Place tip boxes into the assigned locations.
   b. Place tip boxes on the deck in the two side-by-side recessed rectangular platforms.
   c. Remove the cover before using the tips for loading.

4. Close the door on the instrument.
5. Click **Proceed** to begin the System Self Test. The application performs a number of self tests and is then ready for you to continue.

   Note: System Self Test runs only at start up. The test does not run again unless the system is restarted or a self test is intentionally run. The System Self Test utility is in the Instrument drop-down menu in the OpenArray® Accufill™ application.

Preparing for loading

1. Click **Setup & Load**.



2. Open the enclosure door of the OpenArray® Accufill™ instrument by grasping and lifting up the door handle.
3. In the Setup Load Information window, enter or scan the barcode of your TaqMan® RNase P OpenArray® Plate Instrument Verification Reagents Kit into the Sample Plate field.
4. Insert the 384-well sample plate with the foil cover still in place. Press on the plate until it snaps into place.

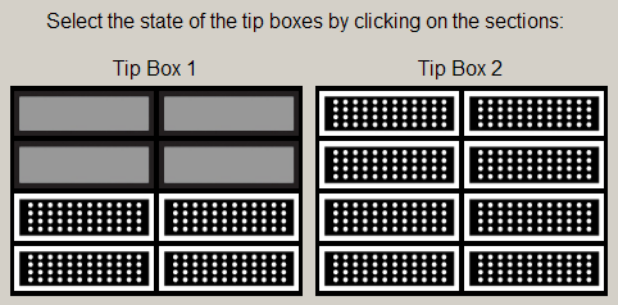
   Note: Do not remove the foil from the 384-well sample plate at this stage.
5. Enter the data for the OpenArray® plate:
   a. Select **1** from the Samples Per Subarray drop-down list.
   b. In the plate holder Position 1 text field, enter **RNase P** (the sample loaded into first position of the plate holder).
   c. Place a thawed OpenArray® plate into the plate holder. When handling the OpenArray® plate:
      - Always hold the OpenArray® case by the edges.
      - If you drop a loaded OpenArray® plate, discard it in the sharps waste container.
      - Load the OpenArray® plate within an hour after you open it.

      Hold the OpenArray® case by the edges and place it in the plate holder with the barcode facing up and to the left.
6. Click **Next**.

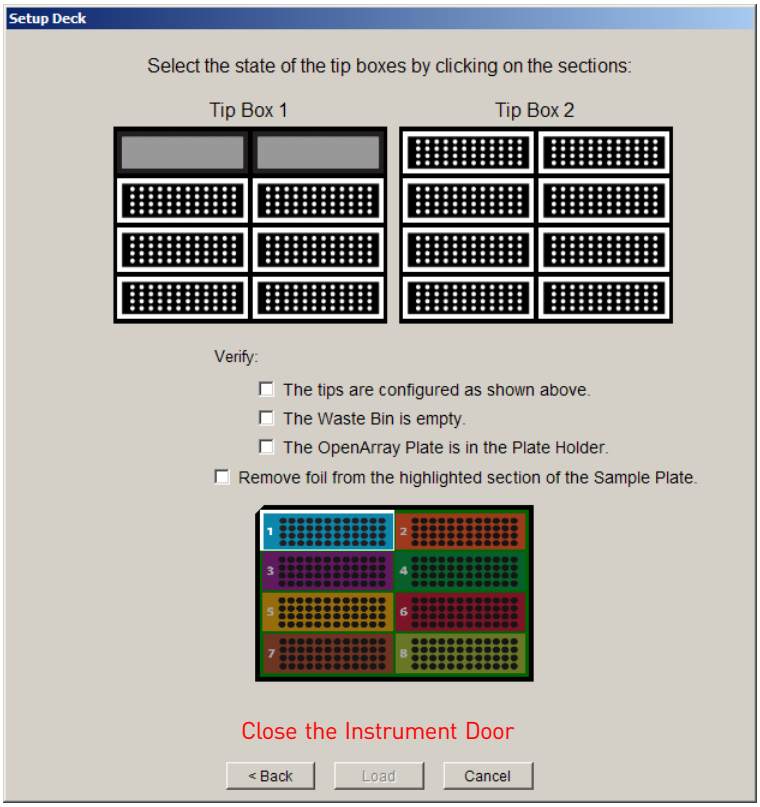
Loading the OpenArray® plate

1. Verify that the Tip Status window in the software matches the state of the tips on the deck. Ensure that:
   - Gray areas in the Tip Status window indicate that tips are not present.
   - White areas indicate that tips are present.

   If the software and the tips on the deck do not match, click the appropriate section in the Tip Status window.



2. Verify each of the following conditions and select each check box:
   - Tips are configured.
   - Waste bin is empty.
   - OpenArray® plate is in the plate holder.



   Note: The software will not continue until you select all the check boxes.

3. With forceps, peel off the foil covering the area of the sample plate containing the samples to be loaded on the OpenArray® plate.

4. Select **Remove foil from the highlighted section of the Sample Plate**.
5. Close the instrument door.
6. Click **Load**.

   Note: If the number of OpenArray® plates in the instrument differs from the number that is entered in the Setup Load Information window, an error message instructs you to remove any extra plates. Correct the error and continue.
7. When the Remove OpenArray® Plate window appears, open the instrument door, carefully remove the indicated OpenArray® plate, then immediately seal the plate as explained in "Sealing the OpenArray® plate" on page 100.

   IMPORTANT! Once an OpenArray® plate has been filled, seal it within 90 seconds to prevent excessive evaporation.
8. Close the instrument door.

   Note: After you run the plate, clean the OpenArray® Accufill™ instrument.

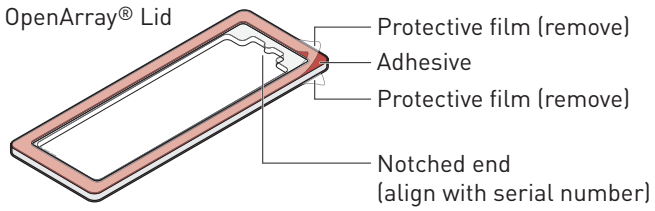
Sealing the OpenArray® plate

IMPORTANT! Wear snug-fitting gloves when working with OpenArray® plates.

1. Remove the protective film from the top *and* bottom of an OpenArray® Case Lid.

   Note: The protective film at the bottom fo the Case Lid is covered by red tape that needs to be removed first to access the protective film and the adhesive.

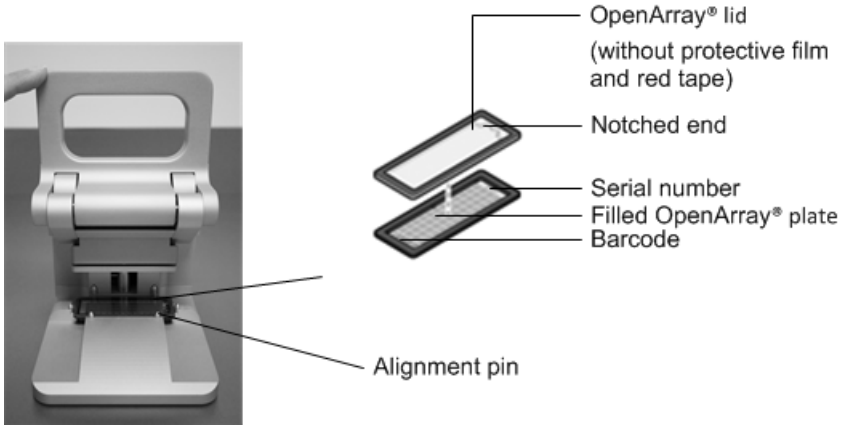
   IMPORTANT! The protective film at the bottom of the Case Lid is covered by a red tape that needs to be removed first to access the protective film. Make sure to remove the protective film from *both* sides of the lids.



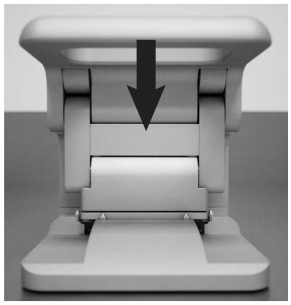
2. Using the thumb and index finger, grasp the OpenArray® case by the top (nearest the barcode), gently lift the case from the plate holder, then load it into the OpenArray® Plate Press 2.0.

3. Seal the plate using the Plate Press:
   a. Place the Case Lid with protective film removed (both top and bottom) onto the Plate Press using the alignment pins of the Plate Press for orientation.

   IMPORTANT! The notched end of the lid must be oriented toward the right side of the Plate Press.



   b. Actuate the Plate Press by pulling down the lever.



   The status light flashes green for 20 seconds. After 20 seconds, the status light turns solid green indicating that the case is ready.

   Note: Do not apply additional pressure onto the Plate Press during its actuation.

   c. Release the lever.

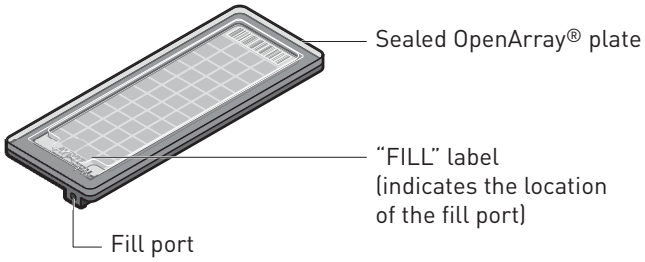
4. Load the Case with OpenArray® Immersion Fluid:

   IMPORTANT! Do not expose the Immersion fluid in the OpenArray® cases to air for more than 60 seconds.

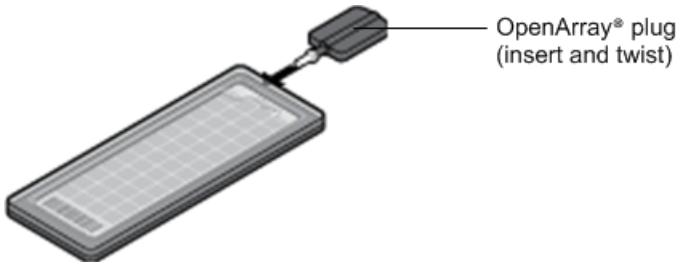
   a. Remove the sealed plate from the Plate Press, grasping the case by the edges.
   b. Insert the syringe tip into the loading port at end of the sealed Case, then dispense the fluid completely in one gentle continuous motion.

   IMPORTANT! Expel the OpenArray® Immersion Fluid slowly. If injected too quickly, the fluid can flush out the samples suspended in the through holes.

Note: Try to minimize creating air bubbles when you dispense the fluid; one small air bubble is acceptable.



c. While holding the OpenArray® plate vertically, seal the loading port by inserting the with OpenArray® Plug into the port and twisting the plug clockwise, applying sufficient pressure until the handle breaks off.



d. Clean the case with a laboratory wipe that has been thoroughly sprayed with ethanol. To dry the case, wipe the case downward with a clean laboratory wipe. Gently handle the case; do not apply pressure on the plate within the case.

The sealed plate is ready to be loaded into the QuantStudio™ 12K Flex System.

Note: Dust or excess sample on the case may interfere with thermal uniformity and can fluoresce. Make sure you thoroughly clean each case.

IMPORTANT! Run the prepared calibration plates within one hour after loading them. Discard the filled plate after a successful calibration.

Running the experiment

1. In the QuantStudio™ 12K Flex Software Home screen, click **Instrument Console**.
2. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add a QuantStudio™ 12K Flex Instrument to your list before you can manage it.
3. After the QuantStudio™ 12K Flex Instrument is added to your list, select it, then click **Manage Instrument**.
4. In the Instrument Manager, start the RNase P wizard:
   a. Click **Maintenance**, then click **RNase P Run**.
   b. In the RNase P Run screen, click **Start RNase P Run**.

5. Complete the calibration as instructed by the wizard. When the instrument door opens, load the OpenArray® plate into *any* position on the plate carrier. Confirm that the OpenArray® plate is positioned so that the barcode is closest to the A1 position on the plate retainer and that the plug is oriented toward the front of the instrument.

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

   Note: The OpenArray® plate can be loaded into *any* position on the plate carrier.

6. After loading the OpenArray® plate, start the calibration:
   a. In the Overview screen, select **Check the box when the RNase P calibration plate has been loaded**, then click **Next**.
   b. In the Run screen, click **START RUN** to start the calibration.

   IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the QuantStudio™ 12K Flex Instrument is in operation.

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

7. When the run is complete and the QuantStudio™ 12K Flex Software displays the Analysis screen, verify the status of the run.

Analysis status	Action
Passed	Go to step 12 on page 104.
Failed	Go to step 8 to review the data for outliers. If the run fails, the QuantStudio™ 12K Flex Software may have included outliers that caused the initial analysis to fail. Experimental error may cause some through-holes to be amplified insufficiently or not at all. These through-holes typically produce C_T values that differ significantly from the average for the associated replicate through-holes. If included in the calculations, these outlying data (outliers) can result in erroneous measurements.

8. In the Amplification Plot, select **C_{RT} vs. Well** from the Plot Type menu, then verify the uniformity of the C_T values for the replicate population:
   a. In the plate layout, select all through-holes.
   b. In the plot, verify that the C_Ts of the replicate population are equivalent.
   c. If an outlier is present in the population, select the corresponding through-hole of the plate layout, then click **Omit** to remove the through-hole from the analysis. If the total number of outliers for the replicate population exceeds 48 through-holes, repeat the experiment using another OpenArray® plate.

9. Review the Results Table for quality flags generated by the experiment:
   a. Select the **Results Table** tab.
   b. Review the Flag column for through-holes that generated quality flags.

c. Troubleshoot each through-hole that generated a flag as explained in "Troubleshooting" on page 105.
   - 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 in plate
   - NOSIGNAL - No signal in through-hole
   - OFFSCALE - Fluorescence is offscale
   - OUTLIERRG - Outlier in replicate group
   - SPIKE - Noise spikes
   - THOLDFAIL - Thresholding algorithm failed

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

    If the status of the RNase P Run is "Failed" after performing steps 8 through 10, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Life Technologies.

11. Complete the calibration as instructed. When the QuantStudio™ 12K Flex Instrument ejects the tray arm, then discard the OpenArray® plate.

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the plate can reach 100°C. Allow the plate to reach room temperature before removing.

IMPORTANT! If the QuantStudio™ 12K Flex Instrument does not eject the OpenArray® plate, remove the plate according to "Troubleshooting" on page 105.

12. Click **Finish**, then click **Yes** when prompted to save the experiment.

Troubleshooting

Problem/symptom	Possible cause	Action
More than the maximum number of outliers are present in RNase P data.	Possible contamination Pipetting inaccuracy	Order and perform a replacement RNase P experiment. If the replacement RNase P OpenArray® plate fails, contact Life Technologies for further assistance.
RNase P plate verification run failed.	Defective plate seal	**CAUTION! PHYSICAL INJURY HAZARD**. During instrument operation, the sample block can reach 100°C. Allow the plate to reach room temperature before removing. 1. Unload the OpenArray® plate from the QuantStudio™ 12K Flex Instrument. 2. Hold the OpenArray® plate up to a light source, and verify that the plate contains fluid and that bubbles are not present. 3. Order and perform a replacement RNase P experiment. If the replacement OpenArray® plate fails, contact Life Technologies for further assistance.
Instrument does not eject the RNase P plate.	Adhesive cover may have adhered the plate to the heated cover within the instrument.	1. Power off the QuantStudio™ 12K Flex Instrument. 2. Wait for 15 minutes, then power on the QuantStudio™ 12K Flex Instrument and eject the plate. 3. If the plate does not eject, power off and unplug the QuantStudio™ 12K Flex Instrument, then open the access door. 4. Wearing powder-free gloves, reach into the instrument and remove the plate from the heated cover, then close the access door.
Through-hole displays the NOSIGNAL flag (the through-hole produced very low or no fluorescence signal.	Missing reaction mix resulting from pipetting error.	If a through-hole is flagged, confirm the results: 1. Consider omitting the through-hole from the analysis. 2. Note the location for each flagged through-hole, and check each corresponding through-hole in the reaction plate for evaporation or low reaction volume. 3. Order and perform a replacement RNase P experiment. If the replacement RNase P OpenArray® plate fails, contact Life Technologies for further assistance.
Through-hole displays the SPIKE flag (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	Order and perform a replacement RNase P experiment. Properly seal and centrifuge the RNase P OpenArray® plate. If the replacement RNase P OpenArray® plate fails, contact Life Technologies for further assistance.

Problem/symptom	Possible cause	Action
Through-hole displays the OFFSCALE flag (the fluorescence signal for one or more dyes in the through-hole exceeds the instrument's maximum detectable range for one or more cycles).	• Fluorescent contaminant on the reaction plate or sample block • Fluorescent contaminant in the reaction	1. Perform a background calibration. If you detect fluorescent contamination, decontaminate the sample block. 2. Order and perform a replacement RNase P experiment. If the replacement RNase P OpenArray® plate fails, contact Life Technologies for further assistance.
Through-hole displays the HIGHSD flag (the C_T standard deviation for the replicate group exceeds the current flag setting).	• Droplets on the sides of the through-holes • Improper sealing or seal leaks • Condensation on the reaction plate • Inconsistent volumes across the plate	If a through-hole is flagged, confirm the results: 1. Select the flagged through-hole(s) and the associated replication group(s) in the plate layout or through-hole table. 2. View the amplification plot (R_n vs. Cycle), and review the data for abnormalities. 3. Hold the OpenArray® plate up to a light source, and check for leaks and bubbles. 4. Check the ROX image files for non-uniformity as explained in "Viewing the ROX™ image files" on page 107. Non-uniformity can indicate problems with plate loading. 5. Order and perform a replacement RNase P experiment. Properly seal and centrifuge the RNase P plate. If the replacement RNase P plate fails, contact Life Technologies for further assistance.
Through-hole displays the NOAMP flag (the sample did not amplify).	• Missing template • Excitation source in the instrument stopped functioning	
Through-hole displays the NOISE flag (the through-hole produced more noise in the amplification plot than other through-holes on the plate).	• Droplets on the sides of the through-holes • Improper sealing or seal leaks • Condensation on the reaction plate	
Through-hole displays the OUTLIERRG flag (the C_T of the through-hole 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. Order and perform a replacement RNase P experiment. Properly seal the RNase P plate. If the replacement RNase P plate fails, contact Life Technologies for assistance.
Through-hole displays the THOLDFAIL flag (the software cannot calculate the threshold).	• Amplification too early • Amplification too late • Low amplification • No amplification	If a through-hole is flagged, confirm the results: 1. Select the flagged through-hole(s) in the plate layout or through-hole table. 2. View the amplification plot (R vs. Cycle and ΔR vs. Cycle), and check for early, late, low, or no amplification. 3. Order and perform a replacement RNase P experiment. If the replacement RNase P plate fails, contact Life Technologies for assistance.
Instrument malfunction	Multiple possible causes	Contact a local Life Technologies Field Service Office.

Identifying contamination

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

To determine the source and location of the contamination:

1. While viewing the background calibration data in the Analysis screen, select the **QC** tab and review the list of through-holes that failed the quality check.
2. Rotate the background plate 180° , then perform the background calibration again.
3. Determine the location of the contaminated through-holes again.

   If the position(s) of the contaminated through-hole(s) in step 1 and step 2 are:

   - **Identical** – The sample block is contaminated. Decontaminate the sample block.
   - **Reversed** – The background plate is contaminated. Discard the plate, then perform the background calibration using a new background plate.
4. If the calibration fails after you replace the background plate and decontaminate the sample block:
   a. Cover a OpenArray® plate with a piece of black paper.
   b. Perform the background calibration as explained in this chapter, substituting the OpenArray® plate covered with paper for the background plate.
   c. After the run is complete and while viewing the calibration data, select all through-holes in the Plate Layout tab, then view the Spectral plot for the peak(s). If the peak associated with the contamination is:
      - **Visible** – The optics of your QuantStudio™ 12K Flex System may be contaminated. Contact Life Technologies for further support.
      - **Absent** – The sample block is contaminated. Decontaminate the sample block again and repeat the calibration.

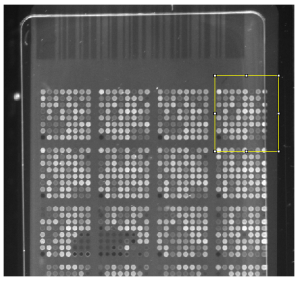
Viewing the ROX™ image files

You can export quality control (QC) images from RNase P experiments. The QC images include: calibration images, a barcode image, and images taken during the run. You can view the images to check that calibration was correct or to validate the data.

To export QC images from an RNase P experiment:

1. In the Instrument Manager, click Export while viewing the results of the RNase P run.
2. In the Export screen, select a location for the file. Click **Browse** if you do not want to save the image files to the default export folder.

   Note: To set up the Export File Location, go to **Tools ▸ Preferences**, select the **Export** tab, then select **Use Last File Location** or **Use Default Folder**. If you do not specify a directory to receive the image files, the software exports the files to the default directory (`C:\Applied Biosystems\QuantStudio\user files\experiments`).
3. Click **Export QC Images**.
4. Using a graphics editor program (such as Microsoft® Paint), open and review each QC image.

Problem/symptom	Possible cause	Action
No amplification in the through-holes along the long edge of the OpenArray® plate. QC image shows misalignment of the OpenArray® plate (see below). 	OpenArray case is sealed improperly (case lid is askew).	**CAUTION! PHYSICAL INJURY HAZARD**. During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature. 1. Unload the OpenArray® plate from the QuantStudio™ 12K Flex Instrument. 2. Hold the OpenArray® plate up to a light source, and verify that the lid is positioned correctly. If the lid is not seated correctly on the case, then the through-holes along the unsealed edge of the OpenArray® plate will fail to amplify. 3. Order and perform a replacement RNase P experiment. If the replacement OpenArray® plate fails, contact Life Technologies for further assistance.

4 Maintenance

This chapter covers:

IMPORTANT! This chapter contains all user service procedures for the Applied Biosystems® QuantStudio™ 12K Flex Real-Time PCR System. Procedures other than those described in this document must be performed by a qualified Life Technologies service engineer.

Regular data maintenance

Maintaining the computer hard drives

Defragment and clean up the hard drive:

- At least once every month.
- When a message is displayed by the Windows® operating system instructing you to defragment.

For more information on maintaining the hard drives, see the Windows® Operating System Help, then search the Help to find information on the Disk Cleanup and Disk Defragment utilities.

IMPORTANT! Do not run the disk management utilities and QuantStudio™ 12K Flex Software at the same time.

Archiving and backing up experiment files

Archive experiment files regularly

To conserve space on the computer hard drive, older EDS files can be archived using a data compression utility. Several commercial compression utilities are available to store experiment files in the ZIP or ARC archive format.

Back up experiment files

We strongly recommend that you back up your experiments. Backing up data:

- Protects against potential loss of data caused by failure of the computer or its hard drive(s).
- Conserves space on the hard drive and optimizes performance.

Develop a data management strategy

We recommend developing a strategy for managing the files produced by the QuantStudio™ 12K Flex Software.

Note: Real-time runs generate significantly more data than genotyping or presence/absence experiments. During 24 hrs of real-time operation, the QuantStudio™ 12K Flex System can generate more than 10 MB of data.

Check disk space

If you perform real-time experiments on your QuantStudio™ 12K Flex System, check the amount of available space on your hard drive weekly. When the hard drive is within 20% of maximum capacity, transfer the older data to another storage device.

Backing up the instrument settings

You can use the QuantStudio™ 12K Flex Instrument touchscreen to back up the instrument settings (instrument name, icon, standby time-out, and cover idle temperature). In the event that the QuantStudio™ 12K Flex Instrument settings are reset, you can restore the settings from the backup.

See "Backing up the QuantStudio™ 12K Flex Instrument settings" on page 164 for more information.

Decontaminating the sample block

Perform this procedure to eliminate fluorescent contaminants from the QuantStudio™ 12K 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™ 12K Flex Instrument 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 be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

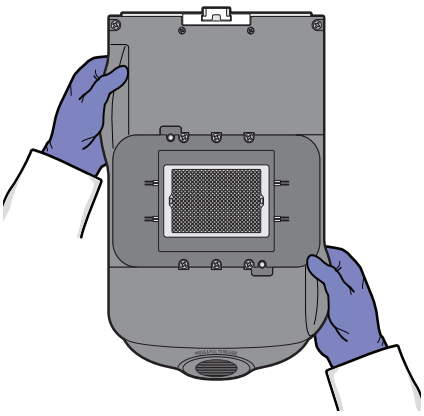
CAUTION! Before using a cleaning or decontamination method other than those recommended, verify with Life Technologies that the proposed method will not damage the equipment.

Required materials

- 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

Handling 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™ 12K Flex Instrument, place the sample block on a clean, dry surface or in its shipping container.



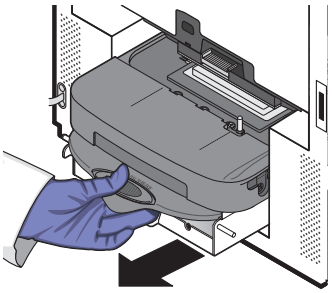
Cleaning the sample block

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the sample block and heated cover can be heated to 100°C. Before removing the sample block, be sure to wait until it reaches room temperature.

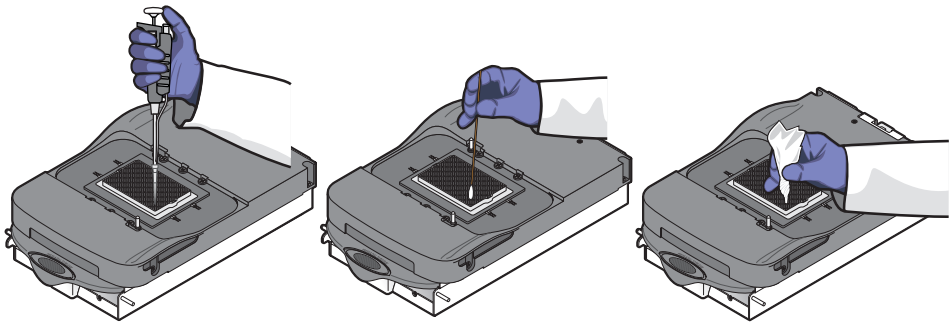
IMPORTANT! Wear powder-free gloves when you perform this procedure.

1. Identify the contaminated wells of the sample block (see "Identifying contamination" on page 80).
2. Power off and unplug the QuantStudio™ 12K Flex Instrument, 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™ 12K Flex Instrument. Place the sample block on a clean, dry surface.

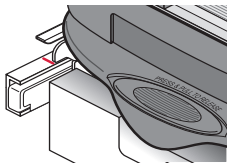


5. Clean the contaminated wells of the sample block using deionized water:
   a. Pipet a small volume of deionized water into each contaminated well.
   b. In each well, pipet the water up and down several times to rinse the well.
   c. Pipet the water to a waste beaker.
   d. Using a cotton swab, scrub inside of each contaminated well. If you are decontaminating an array card or OpenArray® plate sample block, swab the surface of the block that contacts the consumable.
   e. Using a lint-free cloth, absorb the excess deionized water.



6. Load the sample block into the QuantStudio™ 12K Flex Instrument, 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™ 12K Flex Instrument until it is seated correctly.



7. Close the access door.

IMPORTANT! Confirm that the access door is completely closed. The QuantStudio™ 12K Flex 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™ 12K Flex System.
9. Perform a background calibration to confirm that you have eliminated the contamination.
10. If the contamination remains, repeat steps 2 through 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. If you are decontaminating an array card or OpenArray® plate sample block, swab the surface of the block that contacts the consumable.
    c. Pipet the ethanol solution to a waste beaker.
11. Repeat steps 5 through 9 to rinse the wells of the sample block and to verify 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 through 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. If you are decontaminating an array card or OpenArray® plate sample block, swab the surface of the block that contacts the consumable.
    c. Pipet the bleach solution to a waste beaker.
13. Repeat steps 5 through 9 to rinse the wells of the sample block and to verify 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.

Replacing the instrument fuses

Replace the QuantStudio™ 12K Flex System fuses when the fuses fail.

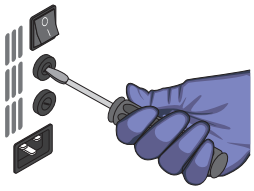
CAUTION! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with listed and certified fuses of the same type and rating as those currently in the QuantStudio™ 12K Flex Instrument.

Required materials

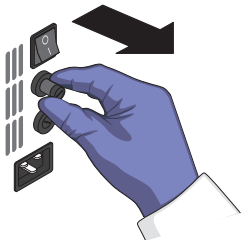
- Fuses, 12.5A, Time-Lag T, 250VAC, 5 × 20-mm (2)
- Safety glasses
- Powder-free gloves
- Screwdriver, flathead

Replacing the fuses

1. Power off, then unplug the QuantStudio™ 12K Flex Instrument. 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.
5. Install the fuse holder.
6. Plug in, then power on the QuantStudio™ 12K Flex Instrument. The installation is successful if the instrument powers on.

Note: Fuse failure can result from fluctuations in the supplied power to the QuantStudio™ 12K Flex Instrument. To prevent further failures, consider installing an electrical protective device, such as a UPS or a surge protector.

Updating the Windows® operating system

Do not upgrade or update the Microsoft® Windows® operating system of the computer running the QuantStudio™ 12K Flex Software without first consulting the software release notes or the Life Technologies website. Future versions and updates to the Windows® operating system can conflict with the QuantStudio™ 12K Flex Software.

To determine compatibility of an upgrade or update:

1. Open **C:\Program Files\Applied Biosystems\QuantStudio12KFlex\docs**, double-click **README.html**, then read the QuantStudio™ 12K Flex Software Release Notes for the compatibility of interest.
2. If the release notes do not mention the compatibility, visit **www.lifetechnologies.com/quantstudio**, then search the website for the compatibility of interest.
3. If the website does not contain the information of interest, contact Life Technologies.

Updating the QuantStudio™ 12K Flex Software and Firmware

Life Technologies may release updates to the QuantStudio™ 12K Flex Software and QuantStudio™ 12K Flex Instrument firmware 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™ 12K Flex System:

1. Go to **www.lifetechnologies.com/support/software/**
2. In the Software Downloads page, select **Applied Biosystems®** QuantStudio™ 12K Flex Real-Time PCR System from the menu.
3. In the QuantStudio™ 12K Flex Instrument Software Downloads page, click **Updates - Patches**.

The website opens the page describing the latest software and firmware updates for the QuantStudio™ 12K Flex Software and QuantStudio™ 12K Flex Instrument.

Updating the QuantStudio™ 12K Flex Software

Preparing for the upgrade

To update the QuantStudio™ 12K Flex Software, prepare your computer by exporting the application libraries and backing up your experiment files.

To prepare for the software update:

1. Back up the application libraries. For each library:
   a. In the main menu of the QuantStudio™ 12K Flex Software, select **Tools** ▸ ***<library>***.
   b. When the library dialog box opens, select the element(s) to export, then click **Export**.
   c. In the Export dialog box, click **Save** to archive the selected records.
2. Back up all experiment files by creating a copy of the directory that you are using to store files.

   The default directory for experiments is:

   `C:\Applied Biosystems\QuantStudio12KFlex\User Files\experiments`

Installing 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™ 12K Flex Software, the update automatically acquires the software license from the existing installation. If you are installing the QuantStudio™ 12K Flex 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 "Managing QuantStudio™ 12K Flex Software licenses" on page 117.

Updating the QuantStudio™ 12K Flex Instrument firmware

You can use the QuantStudio™ 12K Flex Instrument touchscreen to update the QuantStudio™ 12K Flex Instrument firmware. See "Updating the QuantStudio™ 12K Flex Instrument firmware" on page 116 for more information.

Managing QuantStudio™ 12K Flex Software licenses

You can use the License Central feature to monitor, activate, or install the licenses that control access to the QuantStudio™ 12K Flex Software base application and associated modules.

About QuantStudio™ 12K Flex Software license keys and files

The QuantStudio™ 12K Flex 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™ 12K Flex Software on the target computer.

Note: QuantStudio™ 12K Flex Software licenses are valid for a limited time and they must be renewed regularly. If a license has expired or is nearing expiry, the QuantStudio™ 12K Flex Software displays a warning when the software is started.

Note: License keys are found on the QuantStudio™ 12K Flex Software CD packaging, or they can be supplied by Life Technologies support.

Managing licenses

Monitoring the current licenses

You can use the QuantStudio™ 12K Flex Software to review the status and expiration date of the licenses currently installed to the software.

1. In the main menu of the QuantStudio™ 12K Flex Software, select **Tools ▸ License Central**.
2. In the License Central dialog box, review the status of your licenses.

   The software displays the status of all installed licenses, where possible states include Current and Expired, and the date at which it expires.

   Note: The License Central dialog box lists the QuantStudio™ 12K Flex Software core application and modules on different rows because the licenses are maintained separately.
3. (Optional) Save the license information to a text file:
   a. Select the license that you want to export from the table, then click **Save License Request Info**.
   b. Navigate to the appropriate location, then click **Save**.
4. When you are done, click **OK**.

Renewing a license

If you have a valid license key for the QuantStudio™ 12K Flex Software or an associated module, or if your license file has expired, you can use the License Central feature to activate the license as explained below.

IMPORTANT! An internet connection, a web browser, and a valid email account are required to activate a QuantStudio™ 12K Flex Software license. If the computer that contains the QuantStudio™ 12K Flex Software is not connected to the internet or it lacks a web browser application, contact Life Technologies support to request the license file.

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1. In the main menu of the QuantStudio™ 12K Flex Software, select **Tools ▸ License Central**.
2. In the License Central dialog box, select the license of interest from the table, click **Renew License**, then wait for the default web browser application to connect to the Life Technologies website.
3. In the Life Technologies Software License Activation website, click **QuantStudio™ 12K Flex 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) for you to install on your computer.

Installing a license file

After you activate your license and receive an activated license file (.lic), install the file as explained below to unlock the QuantStudio™ 12K Flex 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™ 12K Flex Software.
2. In the main menu of the QuantStudio™ 12K Flex Software, select **Tools ▸ License Central**.
3. In the License Central dialog box, click **Install License**.
4. In the Open dialog box, navigate to and select the license file, then click **Open**.
5. Click **OK** to close the License Central dialog box.

Replacing the sample block

Replace the sample block in the event of a hardware failure or to change the consumable format of the QuantStudio™ 12K Flex Instrument.

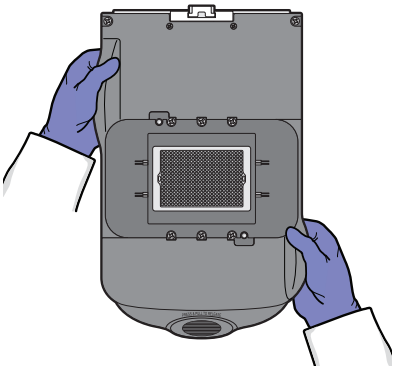
WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the sample block and heated cover can be heated to 100°C. Before removing the sample block, be sure to wait until it reaches room temperature.

Required materials

- Safety glasses
- Powder-free gloves
- Sample block

Handling 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™ 12K Flex Instrument, place the sample block on a clean, dry surface or in its shipping container.

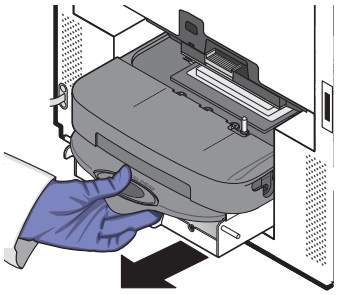


Replacing the sample block

IMPORTANT! If you are installing a sample block of a different format (for example, 96/384-well plate to array card), you must also change the plate adapter to match the new consumable format.

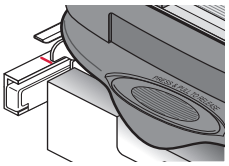
1. Power off and unplug the QuantStudio™ 12K Flex Instrument, then allow it to cool for 15 minutes.
2. Open the access door.

3. Firmly press down on the handle of the sample block, then remove it from the QuantStudio™ 12K Flex Instrument. Place the sample block on a clean, dry surface.



4. Install the new sample block into the QuantStudio™ 12K Flex Instrument.

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™ 12K Flex Instrument 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 to match the new consumable format.

IMPORTANT! If you are installing a sample block of a different format, you must also change the plate adapter to match the new consumable format.

6. Close the access door.

IMPORTANT! Confirm that the access door is completely closed. The QuantStudio™ 12K Flex 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™ 12K Flex System.
8. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
9. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument from the list of instruments, then review the Block Type field in the Instrument Properties pane.

   The installation is successful if the QuantStudio™ 12K Flex Instrument 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™ 12K Flex Instrument.
10. Perform the following calibrations in the specified order:
    a. ROI calibration
    b. Background calibration
    c. Uniformity calibration
    d. Dye calibration
    e. Normalization calibration.

Replacing the heated cover

Replace the heated cover in the event of a hardware failure or if you want to change the consumable format of the QuantStudio™ 12K Flex Instrument.

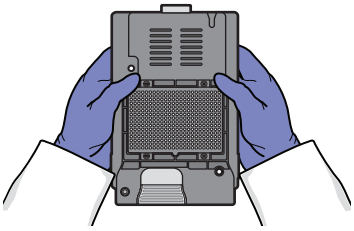
WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the sample block and heated cover can be heated to 100°C. Before removing the heated cover, be sure to wait until it reaches room temperature.

Required materials

- Safety glasses
- Powder-free gloves
- Heated cover

Handling 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™ 12K Flex Instrument, place the heated cover on a clean, dry surface or in its shipping container.

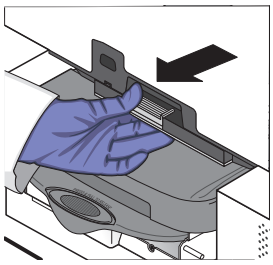


Replacing the heated cover

Note: Confirm that the replacement heated cover supports the consumable format that you want to use. Some heated covers support more than one consumable type.

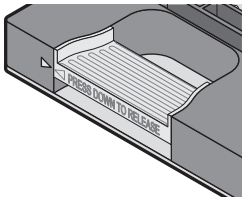
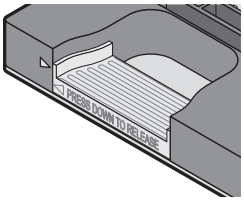
1. Power off and unplug the QuantStudio™ 12K Flex System, then allow it to cool for 15 minutes.
2. Open the access door.

3. Unlock the heated cover by pinching the handle together, then pull the assembly from the QuantStudio™ 12K Flex Instrument and place it on a clean, dry surface.



4. Install the new heated cover into the QuantStudio™ 12K Flex Instrument.

   IMPORTANT! When the heated cover is seated correctly, the arrows on the front handle align as shown below. If the arrows do not align, push the heated cover further into the QuantStudio™ 12K Flex Instrument 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.

   IMPORTANT! If you are installing a heated cover of a different format, you must also change the sample block and plate adapter to match the new consumable format.

6. Close the access door.

   Confirm that the access door is completely closed. The QuantStudio™ 12K Flex 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™ 12K Flex System.

8. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.

9. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument 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™ 12K Flex Instrument powers on and if the Heated Cover Firmware Version field displays a version number.

10. Perform the following calibrations in the specified order: ROI calibration, Background calibration, Uniformity calibration, Dye calibration, then Normalization calibration.

Replacing the plate adapter

Replace the plate adapter in the event of a hardware failure or if you want to change the consumable format of the QuantStudio™ 12K Flex Instrument.

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the sample block and heated cover can be heated to 100°C. Before removing the heated cover, be sure to wait until it reaches room temperature.

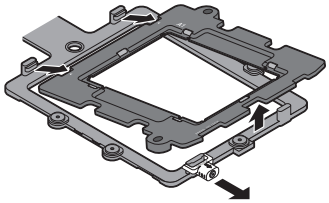
Required materials

- Safety glasses
- Powder-free gloves
- Plate adapter

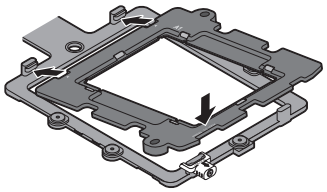
Replacing the plate adapter

IMPORTANT! If you are installing a plate adapter of a different format, you may also be required to change the sample block to match the new consumable format.

1. Touch the instrument touchscreen to activate it, then press  .
2. In 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. In the Main Menu, touch  .
6. If you are installing a tray adapter of a different consumable format, replace the sample block if necessary.

5 Networking

This chapter covers:

IMPORTANT! This chapter *does not* provide adequate detail to integrate the Applied Biosystems® QuantStudio™ 12K Flex Real-Time PCR System into all possible network architectures. Because your network may contain advanced features (such as a firewall or network domains), we recommend that you consult a network administrator before connecting the QuantStudio™ 12K Flex System to your laboratory network.

Networking overview

After installing the QuantStudio™ 12K Flex System, you can connect the QuantStudio™ 12K Flex System to a local area network to enhance its functionality.

This chapter describes how to:

- Set up the QuantStudio™ 12K 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 instruments

When the QuantStudio™ 12K Flex Instrument is connected to a network, computers on the network that are running the QuantStudio™ 12K Flex Software can control or monitor it. The QuantStudio™ 12K Flex Software can control up to 4 instruments and monitor up to 15 instruments simultaneously. A networked QuantStudio™ 12K Flex Instrument can be controlled by only one computer at a time. A networked computer running the QuantStudio™ 12K Flex 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. See "Monitoring a QuantStudio™ 12K Flex Instrument" on page 132 for more information on remote monitoring.

Note: Remote monitoring does not allow you to control the QuantStudio™ 12K Flex System.

About the Ethernet port

The QuantStudio™ 12K Flex Instrument features a Gigabit Ethernet port for direct communication with the QuantStudio™ 12K Flex System computer and for network communication. When the QuantStudio™ 12K Flex System is connected to a network, computers on the network that run the QuantStudio™ 12K Flex Software can:

- Send and download experiments to and from the QuantStudio™ 12K Flex System.
- Run experiments on the QuantStudio™ 12K Flex System.
- Remote monitor the QuantStudio™ 12K Flex System as it performs runs.

The Ethernet port of the QuantStudio™ 12K Flex Instrument 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™ 12K 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])

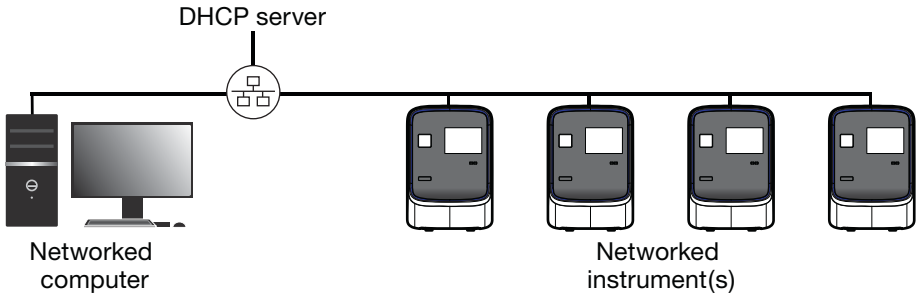
  Note: When the QuantStudio™ 12K Flex System is set for DHCP, APIPA is automatically enabled, and the QuantStudio™ 12K Flex System provides an IP address when no address is supplied by the DHCP server.

Example network layouts

Example 1

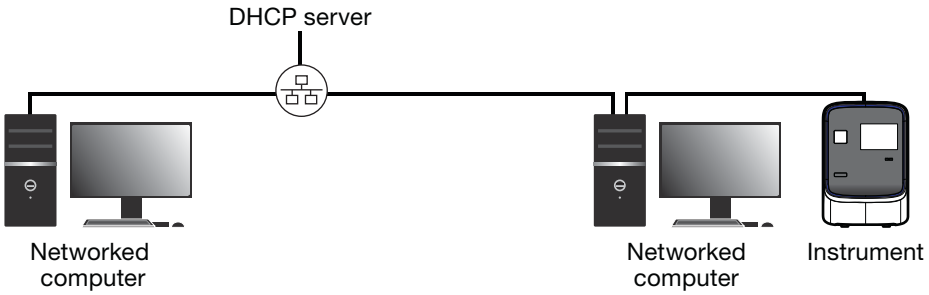
In the following example, one or more QuantStudio™ 12K Flex Instruments, which have been configured for dynamic host configuration protocol (DHCP) operation, are connected to a network by their Ethernet ports. In this layout, any computer on the network can monitor or control the QuantStudio™ 12K Flex Instrument. Experiments can be started remotely from the networked computer or locally from the QuantStudio™ 12K Flex Instrument touchscreen.

Note: A networked computer running the QuantStudio™ 12K Flex Software can simultaneously control up to 4 instruments and monitor up to 15 instruments that have been connected to the network.



Example 2

The QuantStudio™ 12K Flex System computer can be connected to the network. In the configuration shown below, computers on the network can exchange experiment data with the QuantStudio™ 12K Flex System computer; however, the QuantStudio™ 12K Flex Instrument can be neither monitored nor controlled remotely because it is physically isolated from the network.



Networking guidelines and best practices

- Consult a network administrator.
  - We recommend that you consult a network administrator before connecting the QuantStudio™ 12K Flex System to your laboratory network.
  - To enable the full functionality of the QuantStudio™ 12K Flex Software, the computer requires a network connection.
- Limit remote monitoring to 10 computers.

  Avoid using more than 10 computers to simultaneously monitor the QuantStudio™ 12K Flex Instrument remotely. Although the QuantStudio™ 12K Flex System supports remote monitoring from multiple computers, each connection taxes the instrument microprocessor. Too many connections can overburden the QuantStudio™ 12K Flex System and result in instrument errors.

  Note: The effects of an overburdened QuantStudio™ 12K 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™ 12K Flex System supports mDNS but only when the QuantStudio™ 12K Flex Instrument and computer share a direct network connection and are within the same subnet. Consequently, network computers that are separated from the QuantStudio™ 12K Flex System by a router, hub, or another network device may not be able to access the QuantStudio™ 12K Flex Instrument by its host name.
- Confirm the uniqueness of the instrument name.

  The QuantStudio™ 12K Flex Instrument does support name resolution but the instrument name must be unique within the subnet. The QuantStudio™ 12K Flex Software can automatically discover QuantStudio™ 12K Flex Instruments on the link-local network that are configured for Autodiscovery (see "Defining the network settings" on page 170).

  Note: The QuantStudio™ 12K Flex System does not test the uniqueness of the instrument name when it is set.
- Name QuantStudio™ 12K Flex Instruments using lower-case letters.

  When you define the QuantStudio™ 12K Flex Instrument settings (see "Defining the instrument settings" on page 168), enter the instrument name using lower-case letters only.

Network setup workflow

1. Collect the required network information.
2. Connect the QuantStudio™ 12K Flex Instrument to the network.
3. Connect the computer to the network.
4. Monitor the QuantStudio™ 12K Flex Instrument (to test the network connection).

Collecting 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™ 12K Flex System is set for DHCP, APIPA is automatically enabled and the QuantStudio™ 12K 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™ 12K Flex Instrument.

Connecting the QuantStudio™ 12K Flex Instrument to the network

After deciding how to connect the QuantStudio™ 12K Flex System to a network, set up the QuantStudio™ 12K Flex System according to your network policies.

Required materials

Ethernet cable with RJ45 connectors (a CAT6 Ethernet cable for a 1000 Mbit/s network connection or a CAT5 for 100 Mbit/s connection)

Defining the internet protocol settings

1. Use the Ethernet cable to connect the Ethernet port of the QuantStudio™ 12K Flex Instrument to the nearest network port.
2. Power on the QuantStudio™ 12K Flex Instrument.
3. Use the QuantStudio™ 12K Flex Instrument touchscreen to configure the network settings as described in “Defining the network settings” on page 170.

Connecting the computer to the network

After connecting the QuantStudio™ 12K Flex Instrument to the network, connect the computer to the network and install the QuantStudio™ 12K Flex Software for remote monitoring.

Required materials

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.

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

Setting 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® XP 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. In the computer 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.

Installing the QuantStudio™ 12K Flex Software

1. If you are using a computer that you have provided, install the QuantStudio™ 12K Flex Software using the Applied Biosystems® QuantStudio™ 12K Flex Software CD.
   Note: You must install the QuantStudio™ 12K Flex Software to monitor the QuantStudio™ 12K Flex System over the network.
2. (Optional) Install protective software to the computer.

Monitoring a QuantStudio™ 12K Flex Instrument

After connecting the QuantStudio™ 12K Flex System and a computer to the network, you can enable remote monitoring in the QuantStudio™ 12K Flex Software to observe the instrument status remotely.

About remote monitoring

When the QuantStudio™ 12K Flex System is connected to the network, any computer on the network that is running the QuantStudio™ 12K Flex Software can:

- Monitoring the status of an instrument during a run . . . . . see below
- Uploading or downloading an experiment or template 133
- Enabling or changing the calibration reminders. 134

Guidelines for remote monitoring

To ensure optimal performance of the remote monitoring feature, observe the following guidelines:

- The QuantStudio™ 12K Flex Software can monitor up to 15 instruments.
- We do not recommend that a QuantStudio™ 12K Flex Instrument be monitored by more than 10 computers simultaneously.
- Unless you are sure that your QuantStudio™ 12K Flex Instrument and computer exist on the same subnet, we recommend that you use the IP address of the QuantStudio™ 12K Flex Instrument to add it for remote monitoring.

Monitoring the status of an instrument during a run

1. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
2. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add a QuantStudio™ 12K Flex Instrument to your list before you can manage it.
3. After the instrument is added to your list, select it, then click **Manage Instrument**.
4. In the Instrument Manager, click **Monitor**, then click **Information**.
5. In the Monitor Instrument screen, click **Monitor Running Experiment**.

   The QuantStudio™ 12K Flex Software displays the status, attributes, calibration status, and plot data for the selected QuantStudio™ 12K Flex System. If a communications warning appears, contact your network administrator to troubleshoot the problem.

You can lose the software connection to the QuantStudio™ 12K Flex Instrument if you:

- Change the QuantStudio™ 12K Flex Instrument 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™ 12K Flex Software.

Uploading or downloading an experiment or template

Note: The QuantStudio™ 12K Flex Instrument can store up to 100 gene expression experiments. Before sending an experiment, confirm that the instrument contains sufficient storage space.

1. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
2. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add a QuantStudio™ 12K Flex Instrument to your list before you can manage it.
3. After the QuantStudio™ 12K Flex Instrument is added to your list, select it, then click **Manage Instrument**.
4. In the Instrument Manager, click **Manage Files**, then click **File Manager**:
5. In the File Manager screen, transfer the file(s):

   To *upload* a file to the QuantStudio™ 12K Flex Instrument:

   a. In the Folders field, select the folder to which you want to upload the file. To create a new folder, click **Create**, then enter a name for the new folder.
   b. Click **Upload**, select the experiment or template file to send to the QuantStudio™ 12K Flex Instrument, then click **Open**.

   To *download* a file from the QuantStudio™ 12K Flex Instrument:

   a. In the Folders field, select the folder that contains the files that you want to download.
   b. In the Experiments field, select the files to download. To select multiple files, **Ctrl**-click or **Shift**-click files in the list.
   c. When you have selected the files that you want to download, click **Download**.
   d. In the Send experiment to instrument dialog box, select the folder to which you want to download the selected file(s), then click **Open**.

Note: You can also use the Folders and Experiments fields to:

- Create or remove directories on the QuantStudio™ 12K Flex Instrument
- Add, delete, or download experiments on the QuantStudio™ 12K Flex Instrument

Enabling or changing the calibration reminders

The calibration reminders settings allow you to configure the QuantStudio™ 12K Flex Software to alert you by email when the QuantStudio™ 12K Flex Instrument requires calibration. The notifications settings feature is optional, and it does not affect performance.

IMPORTANT! The QuantStudio™ 12K Flex Software transmits email only while the QuantStudio™ 12K Flex Instrument is monitored. If the network connection is interrupted, the software stops transmitting updates.

Required information

The QuantStudio™ 12K Flex 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).

Defining the mail server settings

1. In the QuantStudio™ 12K Flex Software, select **Tools ▸ Preferences**.
2. In the Preferences dialog box, select the **SMTP Settings** tab.
3. In 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™ 12K Flex 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**.

Modifying the notification settings for a monitored instrument

1. Open the Calibration Reminders screen for the QuantStudio™ 12K Flex Instrument:
   a. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
   b. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.

      Note: You must add a QuantStudio™ 12K Flex Instrument to your list before you can manage it.

   c. After the QuantStudio™ 12K Flex Instrument is added to your list, select it, then click **Manage Instrument**.
   d. In the Instrument Manager, click **Maintenance**, then click **Calibration Reminders**.
2. In 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™ 12K Flex Instrument.
   b. In the Send a Reminder column, select the check box to configure the QuantStudio™ 12K Flex 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. In 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.

6 Security, Audit, and Electronic Signature

This chapter covers:

Administrators overview

IMPORTANT! The Security, Audit, and Electronic Signature (SAE) module is installed only with Applied Biosystems® QuantStudio™ 12K Flex Real-Time PCR Systems that were purchased with the SAE module.

IMPORTANT! Enabling the Security, Audit, and Electronic Signature module alone does not make the QuantStudio™ 12K Flex System compliant with any particular standard. You must modify the module settings according to your requirements to ensure compliance.

The Security, Audit, and Electronic Signature (SAE) module is an optional component of the QuantStudio™ 12K Flex Software that can allow you to configure the QuantStudio™ 12K Flex System 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: The default password for the Administrator user account is Administrator; however, the password can be changed during installation.

  Note: You can enable or disable system security globally.

- **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. It is disabled globally by default.

- **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: Electronic signature can be enabled or disabled globally. It is disabled globally by default.

Example applications

You can configure the SAE 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.

Configuring the system security

Accessing the Security screen and enabling or disabling 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.

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.

Note: Security is enabled by default.

To enable or disable security:

1. In the QuantStudio™ 12K Flex 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:
   - Disabling Security inactivates Auditing and E-Signature.
   - 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.
   - The software requires you to enter your user name and password when you enable security.

   IMPORTANT! If you enable or disable the QuantStudio™ 12K Flex Software security, auditing, and electronic signature feature, you must similarly enable or disable the QuantStudio™ 12K Flex Instrument security (see page 172). The QuantStudio™ 12K Flex Software cannot connect to QuantStudio™ 12K Flex Instruments that do not match security settings.
4. Click **Apply Settings**.

Setting the account and security policies

Note: Security policies apply to all user accounts.

1. In the QuantStudio™ 12K Flex Software, select **Tools** ▸ **Security** ▸ **Settings**.
2. In Account Setup, specify the user name limits.

   IMPORTANT! The QuantStudio™ 12K Flex 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 140.
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™ 12K Flex Software notifies the administrator of certain security events. For information, see "Setting up the messaging notifications" on page 140.

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.

Setting up the messaging notifications

1. In the QuantStudio™ 12K Flex 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™ 12K Flex 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**.

Managing user accounts

Creating and editing 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.

Action	Procedure
Create a user account	1. In the QuantStudio™ 12K Flex Software, select **Tools** ▸ **Security** ▸ **Settings**. 2. In the Security Settings dialog box, select the **Users** tab. 3. Click **Create** to display the New User dialog box. 4. 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. 5. 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. 6. Select the user role and the electronic signature state (determines if a user account has permission to electronically sign objects). Leave the status set to **ACTIVE**. 7. (Optional) Enter email (for information only), phone, and comments. 8. Click **Save**. A grayed Save button indicates an invalid entry in a field. Click a field to display the limits for the field, then enter a valid entry.
Edit a user account	1. In 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. In 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. In the Users screen, select the user. 2. Click **Edit**. 3. Change the Status from **ACTIVE** to **INACTIVE**, then click **Save**.

Determining the name of the logged-in user

The title bar of the QuantStudio™ 12K Flex 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™ 12K Flex Software includes three default user roles:

- Administrator (cannot be edited or deleted)
- Scientist
- Technician

You can modify the Scientist and Technician roles, and you can create your own roles with customized settings as needed. To determine the permissions for a default role or to edit it, select the role, then click **Edit**.

Creating a user role

1. In the QuantStudio™ 12K Flex Software, select **Tools** ▸ **Security** ▸ **Settings**.
2. In the Security Settings dialog box, select the **Roles** tab.
3. Click **Create**.
4. Enter a role name and (optional) description.
5. Select permissions (see "Permissions and default user roles" on page 144). 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.
6. Click **Save Role**.

Permissions and default user roles

The following table shows all user-configurable permissions and the settings for the default user accounts.

Permissions		Default user roles		
Category	**Function**	**Scientist**	**Technician**	**Administrator**
Setup	Create and edit experiments or experiment templates (includes running experiments)	Yes	Yes	Yes
Run	Perform a run using the Quickstart function	Yes	Yes	Yes
	Start a run	Yes	Yes	Yes
	Stop a run	Yes	Yes	Yes
Targets (Library)	Create targets	Yes	Yes	Yes
	Edit targets	Yes	Yes	Yes
	Delete targets	Yes	**No**	Yes
Analysis Settings (Library)	Create analysis settings (includes default settings)	Yes	Yes	Yes
	Edit analysis settings (includes default settings)	Yes	Yes	Yes
	Delete analysis settings	Yes	**No**	Yes
Run Methods (Library)	Create a run method	Yes	Yes	Yes
	Delete a run method	Yes	**No**	Yes
Dye (Library)	Create a custom dye	Yes	Yes	Yes
	Delete a dye	Yes	**No**	Yes
Preferences	Edit the system preferences	Yes	**No**	Yes
	Export the system preferences	**No**	**No**	Yes
	Import the system preferences	**No**	**No**	Yes
Calibrations	Perform calibrations	Yes	Yes	Yes
RNase P	Perform an RNase P experiment	Yes	**No**	Yes
Instrument Configuration	Add or remove QuantStudio™ 12K Flex Instrument from monitoring	**No**	**No**	Yes
Security Configuration	Configure the security and audit feature	**No**	**No**	Yes
	Log into user sessions that have timed out	**No**	**No**	Yes
	Perform E-Signing	Yes	Yes	Yes

Editing a user role

1. In 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**.

Viewing and printing a user report

1. In the QuantStudio™ 12K Flex Software, select **Tools** ▸ **Security** ▸ **Settings**.
2. In the Security Settings dialog box, select the **Users** or **Roles** tab.
3. Click **View Report**.
4. In 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.

Managing auditing

Enabling/disabling auditing

Use the Audit screen to control the auditing state (enabled or disabled), the events that are audited, and the reasons available to users when audit mode is set to Prompt or Required. Auditing is disabled by default.

IMPORTANT! If you disable security, you inactivate audit functions. No audit record is generated for the inactivation of audit and electronic signature functions when you disable security.

1. In the QuantStudio™ 12K Flex Software, select **Tools** ▸ **Security** ▸ **Settings**.
2. In the Security Settings dialog box, select the **Audit** tab.
3. Select or deselect **Enable Audit**.
4. Click **Apply Settings**.

Selecting objects to audit

1. Select the objects to audit and the mode for each enabled item.
   - Experiments
   - Experiment Templates
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**.

Creating 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**.

Generating audit reports

You can use the QuantStudio™ 12K Flex Software to generate reports of audit history from both the Security Settings dialog box and open experiments, templates, or studies.

Displaying audit histories from the Security Settings dialog box

Displaying audit histories

1. In the QuantStudio™ 12K Flex Software, select **Tools** ▸ **Security** ▸ **Settings**.
2. In 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 specify the Configure SAE permission. Users without the Configure SAE permission can view object audit histories for individual entries in the libraries by selecting entries, then clicking **View Audit History**.
3. 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.
4. (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**.

Reviewing the system configuration history

The System Configuration History lists security, audit, and electronic signature configuration records.

Record type	Action	Corresponds to...
Security Settings	Update	Disable, enable, or modify security policies: session timeout 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

‡ Creates one role assignment record for each permission in a role.

Reviewing 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)

Viewing and printing 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**.
5. In 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.

Archiving, purging, and restoring 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.

Action	Procedure
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.

Exporting audit records

You can export audit records to a txt file for additional manipulation and reporting outside the QuantStudio™ 12K Flex 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 are not in their original location (samples have been deleted or moved), an error message is displayed. Return sample data files to their original location, then export again.

Displaying audit histories for an experiment or template

Displaying the audit history

1. Open an experiment (.eds) or template (.edt) file.
2. In the open experiment or template, click  **Audit**, then click **Audit Records**.
3. (Optional) Filter the table:

   To view fewer records:

   a. Check the **Filter by** check box.
   b. Enter criteria for the records of interest, such as a date range, a user name, or a type of action.
   c. Click **Refresh**.

   To view details for a specific record:

   a. Click the row in the list on the left to view the details of the record in the table on the upper right.
   b. Click any row to view details for individual records in the table on the bottom right.

Exporting audit records

1. Open an experiment (.eds) or template (.edt) file.
2. In the open experiment or template, click  **Audit**.
3. In 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.
4. Export the records:
   - Click **Export Summary** to export only the records in the left-hand table.
   - Click **Export Details** to export the records in the left-hand table and the associated details.
5. Select a location for the export file, enter a name for the file, then click **Save**.
6. Click **OK** in the confirmation message.

Printing audit records

1. Open an experiment (.eds) or template (.edt) file.
2. In the open experiment or template, click **Audit**.
3. Click **View Report** to open the Print Preview dialog box.
4. 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. In the Print dialog box, select the printer and print options, then click **OK**.
5. Click to close the Print Preview dialog box.

Managing electronic signature

Enabling/disabling electronic signature

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.

1. In the QuantStudio™ 12K Flex Software, select **Tools** ▸ **Security** ▸ **Settings**.
2. In 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 (.eds) or template (.edt) files.

4. Click **Apply Settings**.

Configuring the meanings of the electronic signatures

Use the Security Settings dialog box to add or remove electronic signature meanings and to determine the data types to which they apply. The e-signature meanings are the text that a user can select to describe a reason for an electronic signature.

The QuantStudio™ 12K Flex Software is installed with the following default meanings.

Electronic Signature definition	Default data types			
	Plate setup	Thermocycler Protocol	Analysis Protocol	Analysis Results
Reviewed and Approved Plate Set Up	Yes	Yes	No	No
Reviewed and Approved Results	Yes	Yes	Yes	Yes
Reviewed and Approved Template	Yes	Yes	No	No

Adding a meaning

1. In the e-Signature tab of the Security Settings dialog box, click **Add** in the e-Signature Meanings settings.

2. In 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**.

Deleting a meaning

1. Select the meaning from the e-Signature Meanings list, then click **Remove**.
2. Click **Apply Settings**.

Configuring the electronic signature rights for user roles

To determine the user roles that can perform an electronic signature:

1. In 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**.

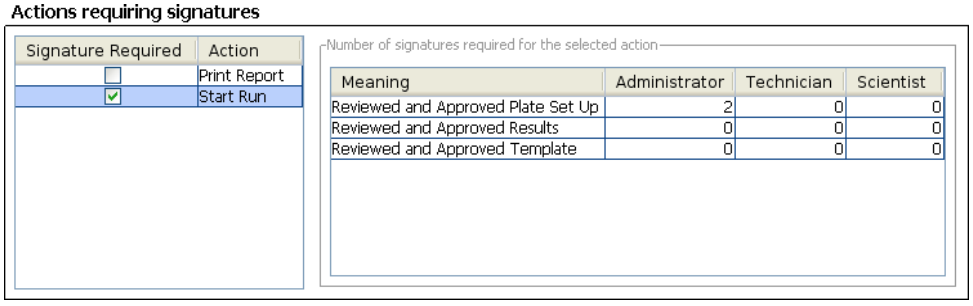
Selecting the actions that require signature

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 QuantStudio™ 12K Flex Software requires electronic signatures when a user...
Print Report	Prints a report from an experiment
Start Run	Initiates a run from the QuantStudio™ 12K Flex Software or QuantStudio™ 12K Flex Instrument

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.



3. Click **Apply Settings**.

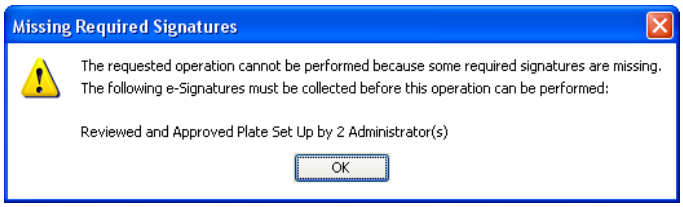
How the software prompts electronic signature

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™ 12K Flex 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:



Before the run can start, two administrators must sign. If a user with an incorrect user role signs, the message is displayed again.

Generating electronic signature reports

You can use the QuantStudio™ 12K Flex Software to generate reports of e-signature history from open experiment (.eds) or template (.edt) files.

Displaying electronic signature records

1. Open an experiment (.eds) or template (.edt) file.
2. In the open experiment or template, click **Audit**, then click **E-Signatures**.
3. (Optional) Click any row to view details for individual signatures.

Saving or printing electronic signature records

1. Open an experiment (.eds) or template (.edt) file.
2. In the open experiment or template, click **Audit**, then click **E-Signatures**.
3. In 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.

Saving or printing the table of electronic signature events

1. Open an experiment (.eds) or template (.edt) file.
2. In the open experiment or template, 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.

Exporting and importing 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™ 12K Flex Software that can then be imported by other copies of the software to bypass manual setup.

Exporting settings

1. In any screen of the Security Settings dialog box, click **Export**.
2. Select the items to export:
   - **All** – Contains all settings.
   - **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.

Importing settings

1. In any screen in the Security Settings dialog box, click **Import** in the navigation pane.
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.

Users overview

The Security, Audit, and Electronic Signature (SAE) module is an optional component of the QuantStudio™ 12K Flex Software. The module 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.

Security

Logging in

If security is enabled on your system, you must provide a user name and password to access the software.

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 grayed.

Note: If the QuantStudio™ 12K Flex Software is configured for password expiration, you are periodically prompted to change your password.

Note: If the QuantStudio™ 12K Flex Software is configured to monitor failed log in attempts, you will be locked out of the software if you incorrectly enter your user name or password for a specified number of times.

Permissions

If your user account does not have permission to perform any function in the software, menu commands are grayed.

Changing 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**.

Account suspension

If the QuantStudio™ 12K Flex Software is configured to suspend a user account for failed logins, and you enter an incorrect user name and password for more than the allowed number of times, 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™ 12K Flex 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.

Audit

If the QuantStudio™ 12K Flex 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™ 12K Flex Software configuration, you can either select a reason from the list or enter a reason for change.

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 as described in "How the software prompts electronic signature" on page 152.

A Manual Instrument Operation

This appendix covers:

Note: This appendix describes how to operate the QuantStudio™ 12K Flex Instrument manually using the touchscreen interface. Although the QuantStudio™ 12K Flex Instrument can be used without a physical attachment to a computer, the touchscreen allows you to perform only a subset of the total instrument functions.

Instrument touchscreen functions

The QuantStudio™ 12K Flex Instrument features a touchscreen interface that you can use to run experiments, manage instrument settings, and configure the QuantStudio™ 12K Flex Instrument for network use. The touchscreen does not provide access to all instrument functions. Features such as experiment analysis, instrument calibration, and remote notification are available only through the QuantStudio™ 12K Flex Software.

List of instrument functions

The following table summarizes the functions that are available from the QuantStudio™ 12K Flex Instrument touchscreen. The table organizes the functions by user role, where operational functions are for users that perform experiments, maintenance functions are for users who maintain the instrument, and administration functions are for systems administrators or for information technology personnel. The right-most column indicates whether a function is available when the QuantStudio™ 12K Flex Instrument is operating in secure mode (see "Enabling/disabling instrument security" on page 172 for more information).

User role	Function	Available in secure mode?
Operational	Create experiments from templates	No
	Run experiments	
	Transfer experiments, templates, and results to/from a USB drive	
Maintenance	Back up and restore the instrument settings	Yes
	Perform an instrument self test	
	Update the QuantStudio™ 12K Flex 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	
	View the QuantStudio™ 12K Flex Instrument log	

Operating the instrument from the touchscreen

The touchscreen provides limited control of the QuantStudio™ 12K Flex Instrument to run experiments and transfer data. You can perform the following functions from the touchscreen to operate the QuantStudio™ 12K Flex Instrument without using the QuantStudio™ 12K Flex Software:

Note: If the QuantStudio™ 12K Flex Instrument is operating in secure mode (see "Enabling/disabling instrument security" on page 172), users can only open and close the side door.

Creating an experiment from a template

1. If necessary, download the experiment template to the QuantStudio™ 12K Flex Instrument as described in "Transferring experiments from a USB drive" on page 161.
2. If the instrument is in standby, touch the QuantStudio™ 12K Flex Instrument 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**:

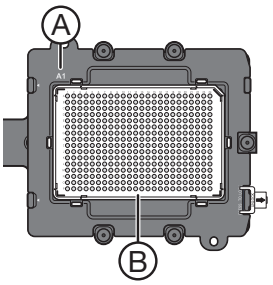
   To view the parameters of a template, select the desired template, then touch  **View**. When finished, touch  to return to the View Templates screen.

   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.

Running an experiment

1. If the instrument is in standby, touch the QuantStudio™ 12K Flex Instrument 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.



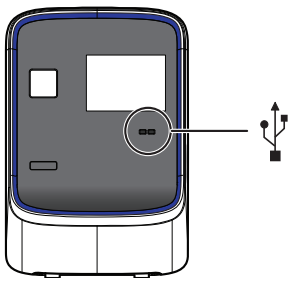
4. In the Main Menu, touch **Browse Experiments**.
5. In the Experiments screen, touch the desired experiment, then touch either:
   - **Start Run** to start the run immediately, then go to step 10.

     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™ 12K Flex Instrument is connected to a network, or you can copy the data to a USB device (see "Transferring experiments, templates, and results data" on page 161).

Transferring experiments, templates, and results data

You can transfer experiments, templates, and results data to/from the QuantStudio™ 12K Flex Instrument using a USB flash drive. Before transferring data, you must plug the drive into one of the USB ports behind the right side of the QuantStudio™ 12K Flex Instrument touchscreen.



IMPORTANT! Do not use the USB ports on the rear panel of the QuantStudio™ 12K Flex Instrument. The rear USB ports are for use by Life Technologies personnel only.

Transferring templates from a USB drive

1. Plug a USB drive into the USB port on the right side of the touchscreen.
2. If the instrument is in standby, touch the QuantStudio™ 12K Flex Instrument 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.

Transferring experiments from a USB drive

1. Plug a USB drive into the USB port on the right side of the touchscreen.
2. If the instrument is in standby, touch the QuantStudio™ 12K Flex Instrument 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.

Copying experiment results to a USB drive

1. Plug a USB drive into the USB port on the right side of the touchscreen.
2. If the instrument is in standby, touch the QuantStudio™ 12K Flex Instrument touchscreen to activate it, then press .
3. In the Main Menu, touch **Collect Results**.
4. In the list of experiments, touch the row(s) for the experiment(s) of interest or touch **Select All**.
5. Touch **Copy to USB**.
6. In the Copy Results To USB screen, check that the name of the USB drive is correct to ensure that it is mounted, then touch **Copy 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.

Maintaining the instrument from the touchscreen

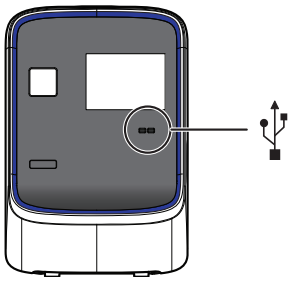
The QuantStudio™ 12K Flex Instrument touchscreen provides access to several maintenance functions that cannot be accessed remotely from the QuantStudio™ 12K Flex Software. The following local QuantStudio™ 12K Flex Instrument functions are performed as part of regular QuantStudio™ 12K Flex Instrument maintenance:

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™ 12K Flex Software.

Backing up and restoring the instrument settings

You can use the QuantStudio™ 12K Flex Instrument 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). In the event that the QuantStudio™ 12K Flex Instrument settings are reset, you can restore the settings from the backup.

The QuantStudio™ 12K Flex Instrument backs 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 behind the right side of the QuantStudio™ 12K Flex Instrument touchscreen.



IMPORTANT! Do not use the USB ports on the rear panel of the QuantStudio™ 12K Flex Instrument. The rear USB ports are for use by Life Technologies personnel, only to service the instrument.

Note: The backup feature can be used as an administrative tool to manage QuantStudio™ 12K Flex Instruments. You can use the feature to create a standard "image" for a QuantStudio™ 12K Flex Instrument that can then be restored on other instruments to bypass the manual setup process.

Backing up the QuantStudio™ 12K Flex Instrument settings

1. Plug a USB drive into the USB port on the right side of the QuantStudio™ 12K Flex Instrument touchscreen.
2. If the instrument is in standby, touch the QuantStudio™ 12K Flex Instrument touchscreen to activate it, then touch .
3. In the Main Menu, touch **Tools**, then touch **Back Up**.
4. In the Backup Settings screen, touch **Backup**.
5. Touch to return to the Main Menu.
6. 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™ 12K Flex Instruments. Note that you must configure the network settings for each instrument individually.

Restoring the instrument settings

1. Plug the USB drive that contains the instrument settings into the USB port on the right side of the QuantStudio™ 12K Flex Instrument touchscreen.
2. If the instrument is in standby, touch the QuantStudio™ 12K Flex Instrument touchscreen to activate it, then press .
3. In the Main Menu, touch **Tools**, then touch **Restore Settings**.

4. In 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™ 12K Flex Instrument until you are instructed to do so.

Note: Alternatively, touch **Restore Default Settings** to restore the QuantStudio™ 12K Flex Instrument to the factory settings.

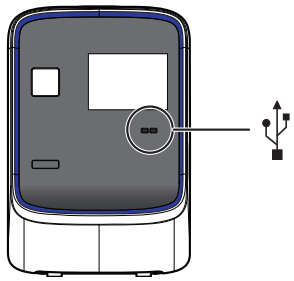
5. After the QuantStudio™ 12K Flex Instrument reboots, unplug the USB drive.

Performing an instrument self test

You can use the QuantStudio™ 12K Flex Instrument touchscreen to perform a comprehensive self test of the QuantStudio™ 12K Flex Instrument subsystems. After the self test is complete, the QuantStudio™ 12K Flex Instrument 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™ 12K Flex Instrument.

1. If the instrument is in standby, touch the QuantStudio™ 12K Flex System touchscreen to activate it, then press .
2. In the Main Menu, touch **Tools**, then touch **Run Self Test**.
3. In the Self Test screen, touch **Start Self Test**, then wait for the test to complete.
4. (Optional) When the QuantStudio™ 12K Flex Instrument completes the self test, save the results to a USB drive:
   a. Plug a USB drive into the USB port on the right side of the QuantStudio™ 12K Flex Instrument touchscreen.



   b. Touch **Save**.

IMPORTANT! Do not remove the USB drive from the QuantStudio™ 12K Flex Instrument until instructed to do so.

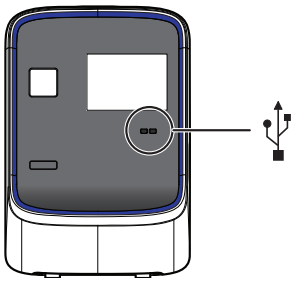
   c. When the QuantStudio™ 12K Flex Instrument finishes writing the results to the USB drive, touch **OK**, then remove the USB drive.
5. Touch to return to the Main Menu.

Updating the instrument firmware

You can download QuantStudio™ 12K Flex Instrument firmware updates directly from the service section of the Life Technologies website. After obtaining a firmware update, transfer the update to the QuantStudio™ 12K Flex Instrument using a USB drive.

Updating the firmware

1. Download the firmware update:
   a. Go to **www.lifetechnologies.com/support/software/**
   b. In the Software Downloads page, select **Applied Biosystems® QuantStudio™ 12K Flex Software** from the menu.
   c. In the Software Downloads page for your QuantStudio™ 12K Flex Instrument, click **Updates - Patches**.
   d. Download the QuantStudio™ 12K Flex Instrument firmware to a USB drive.
2. Plug the drive into the USB port on the right side of the QuantStudio™ 12K Flex Instrument touchscreen.



3. If the instrument is in standby, touch the QuantStudio™ 12K Flex Instrument 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™ 12K Flex Instrument to complete the upgrade.

   IMPORTANT! Do not remove the USB drive from the QuantStudio™ 12K Flex Instrument until you are instructed to do so.

6. After the upgrade is complete and the QuantStudio™ 12K Flex Instrument 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.

Administering the instrument from the touchscreen

The touchscreen provides access to several administrative functions that you can use to integrate the QuantStudio™ 12K Flex Instrument into a laboratory workflow. The following functions are available from the touchscreen and can be used after installation to customize the QuantStudio™ 12K Flex 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™ 12K Flex Software.

Defining the date and time

1. If the instrument is in standby, touch the QuantStudio™ 12K Flex System touchscreen to activate it, then press .
2. In the Main Menu, touch **Settings**, then touch **Set Date & Time**.
3. In 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.
4. Touch to return to the Main Menu.

Defining the instrument settings

1. If the instrument is in standby, touch the QuantStudio™ 12K Flex System touchscreen to activate it, then press .
2. In the Main Menu, touch **Settings**, then touch **Configure the Instrument**.
3. Touch the **Instrument Name** field, enter up to a 16-character name for the QuantStudio™ 12K Flex Instrument, then touch **Done**.

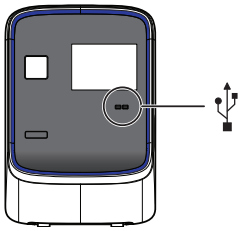
   The instrument name is the alphanumeric string used to identify the QuantStudio™ 12K Flex Instrument on the network.

   IMPORTANT! To connect the QuantStudio™ 12K Flex Instrument to a network, the name must be unique.

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

4. Upload the instrument icon:

   The instrument icon is the graphic used to represent the QuantStudio™ 12K Flex Instrument in the QuantStudio™ 12K Flex Software Instrument Console.

   a. Save the replacement graphic to a USB drive, then plug the drive into the USB port on the right side of the QuantStudio™ 12K Flex Instrument touchscreen.
   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.

   c. Unplug the USB drive.
5. 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™ 12K Flex Instrument should remain idle until it enters standby mode, then touch **Done**.

Note: When in standby mode, the QuantStudio™ 12K Flex Instrument powers off the LCD screen backlight and enters low-power mode.

6. 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 (50–110°C) that the heated cover should maintain when the QuantStudio™ 12K Flex Instrument is idle, then touch **Done**.
7. Touch **Save** to save the settings, then touch **OK** when prompted.
8. Touch  to return to the Main Menu.

Defining the maintenance reminders

You can use the QuantStudio™ 12K Flex Instrument touchscreen screen to:

- Set the expiration period for the instrument calibrations and LED replacement.
- Activate, deactivate, or change the frequency of the maintenance reminders displayed by the QuantStudio™ 12K Flex Instrument.

Setting the reminders

1. If the instrument is in standby, touch the QuantStudio™ 12K Flex System touchscreen to activate it, then press  .
2. In the Main Menu, touch **Settings**, then touch **Set Maintenance Reminders**.
3. Configure the maintenance reminders. For each maintenance reminder:
   a. Touch the **Calibration expires after** field, enter the number of days or hours that should elapse until the association calibration expires, then touch **Done**.
   b. Touch the check box to activate or deactivate reminders for the associated calibration.
   c. Touch the **Display reminders before** field, enter the number of days before the associated calibration expires that the QuantStudio™ 12K Flex Instrument should start displaying 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.

Defining the network settings

1. If the instrument is in standby, touch the QuantStudio™ 12K Flex System touchscreen to activate it, then press  .
2. In the Main Menu, touch **Settings**, then touch **Set Network Information**.

   Note: The Set Network Information screen displays the Media Access Control (MAC) address of the QuantStudio™ 12K Flex Instrument below the Autodiscovery and Smart Monitoring check boxes. The MAC address can be used to uniquely identify the QuantStudio™ 12K Flex Instrument on the network.
3. Touch **Autodiscovery** to make the QuantStudio™ 12K Flex Instrument discoverable by computers that are running the QuantStudio™ 12K Flex Software.
4. Touch **Smart Monitoring** to enable the feature on the QuantStudio™ 12K Flex Instrument.

   The Smart Monitoring feature allows Life Technologies service personnel to monitor the status of the QuantStudio™ 12K Flex Instrument 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™ 12K Flex 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).
5. Set the Internet Protocol (TCP/IP) Properties for either DHCP or Static IP communication.

Network service	Action
DHCP	Touch **Obtain an IP address automatically**, then touch  **Save**.
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. Repeat step 2 to assign the: • IP addresses for the DNS Servers (primary and secondary) • Subnet Mask setting • Default Gateway setting 4. Touch **Save** to save the settings, then touch **OK** when prompted.

6. Touch  to return to the Main Menu.

Defining the system shortcuts

You can use the QuantStudio™ 12K Flex Instrument 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.

Defining the shortcuts

1. If the instrument is in standby, touch the QuantStudio™ 12K 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.

Reviewing the instrument statistics

You can use the QuantStudio™ 12K Flex Instrument touchscreen to view usage statistics on the heated cover, LEDs, and other system components.

Viewing the statistics

1. If the instrument is in standby, touch the QuantStudio™ 12K Flex System touchscreen to activate it, then press .
2. In the Main Menu, touch **Tools**, then touch **Show Statistics**.
3. When you are finished, touch to return to the Main Menu.

Enabling/disabling instrument security

The QuantStudio™ 12K Flex Instrument features 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™ 12K Flex Instrument 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™ 12K Flex Instrument security, auditing, and electronic signature feature, you must similarly enable or disable the QuantStudio™ 12K Flex Software security (see page 139). The QuantStudio™ 12K Flex Software cannot connect to QuantStudio™ 12K Flex Instruments that do not match security settings.

Note: Secure mode limits the number of feature that are available from the QuantStudio™ 12K Flex Instrument touchscreen; it does not provide user authentication functionality through the instrument touchscreen.

Enabling or disabling security

1. If the instrument is in standby, touch the QuantStudio™ 12K 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™ 12K Flex Instrument 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.

Viewing the instrument log

You can use the QuantStudio™ 12K Flex Instrument 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.

Viewing the log

1. If the instrument is in standby, touch the QuantStudio™ 12K Flex System touchscreen to activate it, then press  .
2. In the Main Menu, touch **Tools**, then touch **View Log**.
3. In 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.
4. Touch  to return to the Main Menu.

B

Powering On or Off, Storing, and Moving the System

This appendix covers:

Placing the QuantStudio™ 12K Flex System on standby

If left unattended, the QuantStudio™ 12K Flex Instrument automatically enters standby mode to conserve power. To enter standby mode manually, touch  on the QuantStudio™ 12K Flex Instrument touchscreen.

Powering on the QuantStudio™ 12K Flex System

To power on the QuantStudio™ 12K Flex System from a powered-off state:

1. Toggle the power button on the rear of the QuantStudio™ 12K Flex Instrument, then wait for it to start.

   Note: The QuantStudio™ 12K Flex Instrument is ready to use when the touchscreen displays the Main Menu.

2. 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.

3. Power on the monitor.

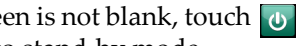
4. Power on the QuantStudio™ 12K Flex System computer:
   a. Press the power button of the computer, then wait for it to start.
   b. When the Login screen appears, enter your user name and password, then click **OK**.
   c. In the desktop, double-click **QuantStudio™ 12K Flex System** (or select **Start** ▸ **All Programs** ▸ **Applied Biosystems** ▸ **QuantStudio™ 12K Flex System** ▸ **QuantStudio™ 12K Flex Software**.
   d. If the QuantStudio™ 12K Flex Software Login appears, enter your user name and password, then click **OK**.

Powering off the QuantStudio™ 12K Flex System

The Applied Biosystems® QuantStudio™ 12K Flex Real-Time PCR System operates in low-power mode when not in use; however, the QuantStudio™ 12K Flex System can be powered off completely so that the components draw no power.

Note: If the QuantStudio™ 12K Flex System will be inactive for extended period of time, prepare it for storage as explained in "Storing the QuantStudio™ 12K Flex System" on page 178.

To power off the QuantStudio™ 12K Flex System components:

1. Power off the QuantStudio™ 12K Flex Instrument:
   a. If the QuantStudio™ 12K Flex Instrument touchscreen is not blank, touch  to place the QuantStudio™ 12K Flex Instrument into stand-by mode.
   b. Toggle the power button on the rear of the QuantStudio™ 12K Flex Instrument.
2. Power off the QuantStudio™ 12K Flex System computer:
   a. In the desktop, select **Start ▸ Shut Down**.
   b. In the Shut Down Windows dialog box, select **Shut Down**, then click **OK**.
3. Power off the monitor.
4. If you have an Applied Biosystems Twister® Robot, toggle the power button on the rear of the Twister® Robot.

Storing the QuantStudio™ 12K Flex System

The Applied Biosystems® QuantStudio™ 12K Flex Real-Time PCR 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™ 12K Flex Instrument.

Required materials

MicroAmp® Optical 96/384-Well Reaction Plate or array card (unused)

Preparing the QuantStudio™ 12K Flex Instrument

1. If you plan to store the QuantStudio™ 12K Flex System for more than a week or you plan to move it, load an unused plate or array card into the QuantStudio™ 12K Flex Instrument:

   Note: The empty plate protects the internal components of the QuantStudio™ 12K Flex System during transport or during periods of inactivity lasting more than a week.

   a. Touch the QuantStudio™ 12K Flex Instrument touchscreen to activate it, then touch  .

   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™ 12K Flex Instrument into stand-by mode.

2. Toggle the power button on the rear of the QuantStudio™ 12K Flex Instrument.

3. Power off the computer:

   a. Select **Start ▸ Shut Down**.

   b. In the Shut Down Windows® dialog box, select **Shut Down**, then click **OK**.

4. Power off the monitor.

5. If you have an Applied Biosystems Twister® Robot, toggle the power button on the rear of the Twister® Robot.

Moving the QuantStudio™ 12K Flex System

Perform this procedure to safely move the QuantStudio™ 12K Flex System short distances (for example, between laboratories of the same building).

CAUTION! PHYSICAL INJURY HAZARD. Do not attempt to lift the QuantStudio™ 12K Flex Instrument 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™ 12K Flex Instrument. At least two people are required to lift it.

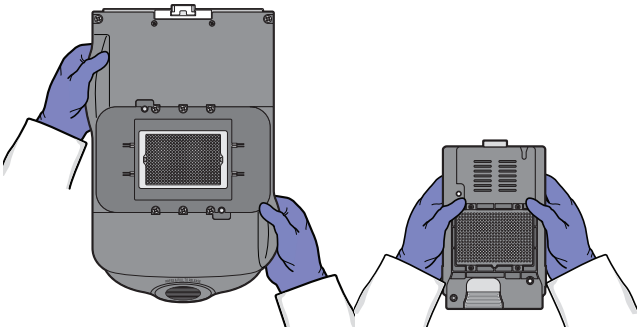
IMPORTANT! Moving your QuantStudio™ 12K Flex System can create subtle changes in the alignment of the instrument optics. Recalibrate the instrument if necessary.

Required materials

None

Handling the sample block and heated cover

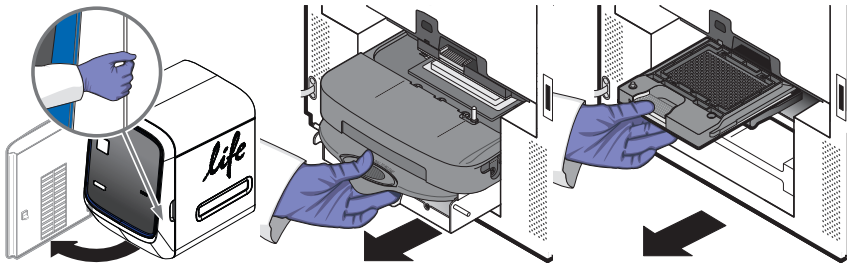
To prevent damaging or contaminating the sample block or the heated cover, handle the assemblies as shown below. After you remove each assembly from the QuantStudio™ 12K Flex Instrument, place them on a clean, dry surface or in its shipping container.



Preparing the QuantStudio™ 12K Flex System components

1. Power off the QuantStudio™ 12K Flex Instrument and computer.
2. When the QuantStudio™ 12K Flex System and computer are powered off, disconnect all QuantStudio™ 12K Flex System components and package the cabling for the move.
3. Prepare the QuantStudio™ 12K Flex Instrument for the move:
   a. Open the QuantStudio™ 12K Flex System access door.
   b. Firmly press down on the sample block handle, pull the sample block from the QuantStudio™ 12K Flex Instrument, then place it on a clean, dry surface.
   c. Pinch the handle of the heated cover together, then pull the assembly from the QuantStudio™ 12K Flex Instrument and place it on a clean, dry surface.

d. Package the sample block and heated cover assemblies in a clean, dust-free container for the move.



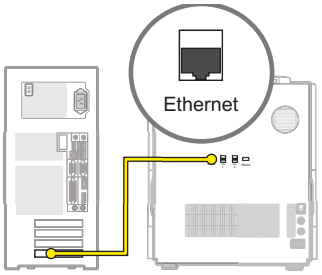
Moving the QuantStudio™ 12K Flex System

Move the QuantStudio™ 12K Flex System according to the following guidelines:

- Verify that the surface on which you will place the QuantStudio™ 12K Flex System can support at least 77.9 ± 0.6 kg (171.5 ± 0.13 lbs).
- Verify that the path to transport the QuantStudio™ 12K Flex Instrument is clear of obstructions.
- Enlist at least one other person to lift and carry the QuantStudio™ 12K Flex Instrument.
- 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.

Reinstalling the QuantStudio™ 12K Flex System

1. Reconnect the components of the QuantStudio™ 12K Flex System. Use the Ethernet cable supplied with the QuantStudio™ 12K Flex System to connect the QuantStudio™ 12K Flex Instrument (Ethernet port) to the network interface card in the computer.



IMPORTANT! Do not use a standard Ethernet cable to connect the QuantStudio™ 12K Flex Instrument to the computer.

IMPORTANT! Do not connect the Ethernet cable to the Ethernet 2 port on the QuantStudio™ 12K Flex Instrument. The second port is for Life Technologies service use only.

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™ 12K Flex System. No further action is necessary.

   Fails – Perform the following calibrations in the specified order: ROI, background, uniformity, dye, then normalization calibrations.

C

Calibration Consumable Preparation

This appendix covers:

Creating 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

- Applied Biosystems® 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

- 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

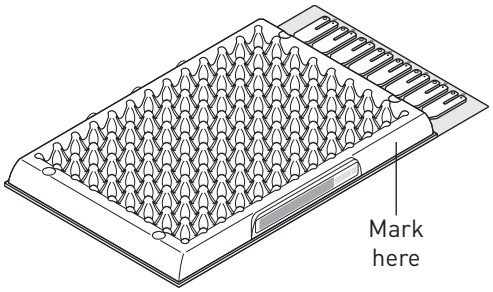
Creating a background plate

IMPORTANT! Wear powder-free gloves while creating the background plate.

1. Remove an Applied Biosystems® 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.

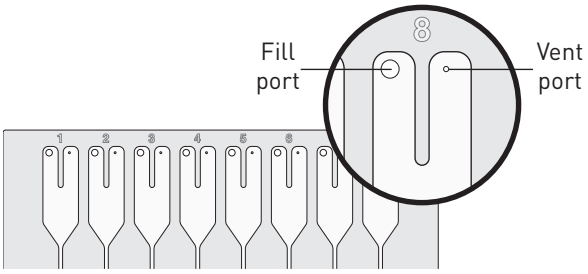
Creating a background array card

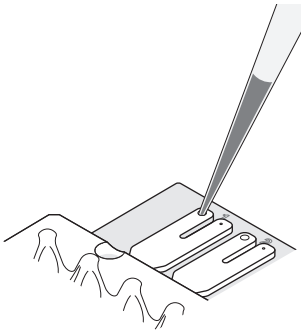
1. Remove an array card from its box and place it on a clean, dry surface.
2. Using a permanent marker, write "Background" on the side of the empty card.



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.

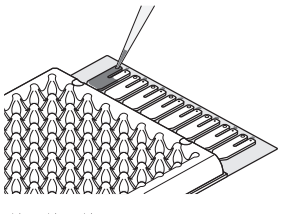
   There is a fill port on the left arm of each fill reservoir – the larger of the two holes.





   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 37.

Creating a custom dye plate for calibration

The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR 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™ 12K 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

Determining 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™ 12K Flex Software, click **Instrument Console**.
    b. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument, then click **Add to My Instruments**.
    c. Select the QuantStudio™ 12K Flex Instrument, 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™ 12K Flex Instrument. 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.

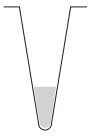
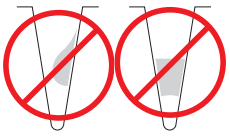
5. In the ROI dialog box, click **Finish** to complete the calibration, then click **No** when prompted to save the results.

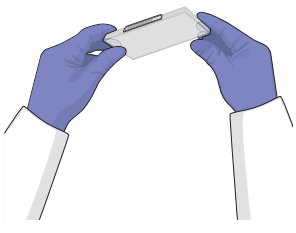
Creating a custom dye plate

IMPORTANT! Wear powder-free gloves while creating the dye plate.

1. Prepare 2 mL of the custom dye at the concentration determined in "Determining optimum dye concentration" on page 184.
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



Adding the custom dye to the software

1. Start the dye calibration:
   a. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
   b. In the Instrument Console, select your QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.
   c. Select the QuantStudio™ 12K Flex Instrument, 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™ 12K Flex Instrument 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™ 12K Flex 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™ 12K Flex System.
   - 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 "Determining optimum dye concentration" on page 184, then perform the calibration again.

D Command-line Software Operation

This appendix covers:

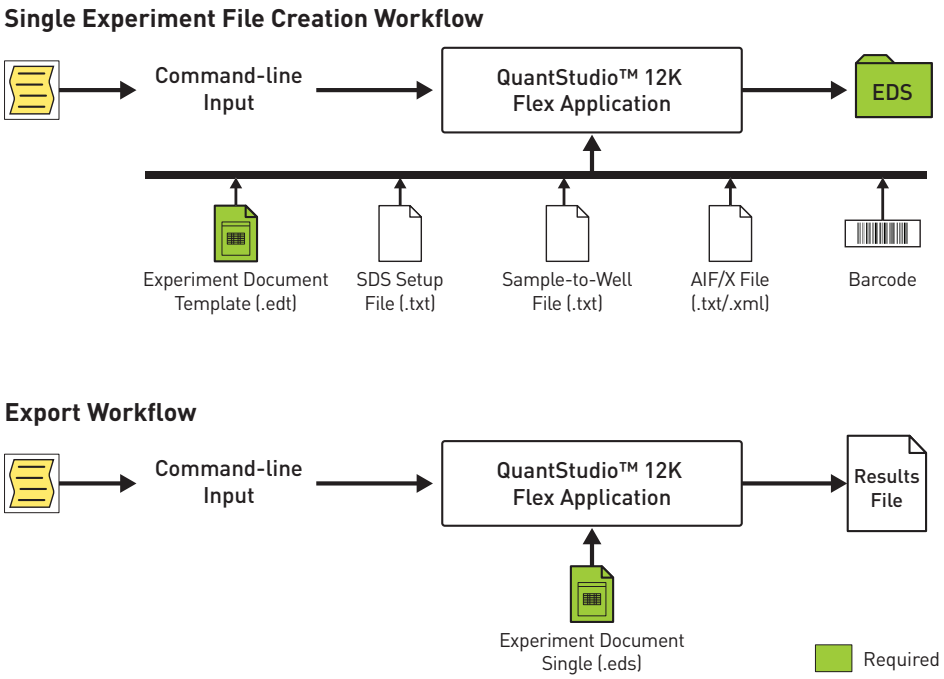
Overview

The QuantStudio™ 12K Flex Software includes a command-line application that allows you to generate and export batches of experiment files from an MS DOS prompt or a batch file. The application is intended for advanced users who choose to create or export experiments using a scripting language.

IMPORTANT! After you use the command-line application to generate experiment files, validate the contents of the files by opening them in the QuantStudio™ 12K Flex Software.

Command-line workflows

The command-line interface supports the workflows in the following figure. For each workflow, the figure shows both the required and optional supporting files.



Supporting files for experiment creation

The file generation function (`cmdlineutil.exe -expgen`) can use the files shown below. The command does not require all input files.

File	Description
assay information file (.aif or .aix)	A tab-delimited or XML data file that is shipped on a CD with each TaqMan® assay ordered from Life Technologies. (For some products, assay information files are available for download from the Life Technologies website following delivery.) The file, which contains data describing the assay, can be imported into the QuantStudio™ 12K Flex Software for use in related experiments. See "Assay information file" on page 207 for more information.
barcode file (.txt)	A user-created, line-separated text file that contains the barcode of each consumable for which you want to create an experiment file. See "Barcode file format" on page 207 for more information.
experiment document single file (.eds)	A QuantStudio™ 12K Flex Software file that contains all information about a particular plate or array card consumable, 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 plate-specific data.
experiment document template file (.edt)	A QuantStudio™ 12K Flex Software file used as a template to create experiment files. The file can contain plate setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, and other plate-specific data.
plate setup file (.txt)	A user-created, tab-delimited text file that describes the layout of a consumable for an experiment to be run on the QuantStudio™ 12K Flex System. The file defines the arrangement of assays and samples on the consumable. See "Plate setup file format" on page 201 for more information.
sample file (.txt)	A user-created, tab-delimited text file containing sample data that can be imported into the QuantStudio™ 12K Flex Software for use in related experiments. See "Sample file format" on page 206 for more information.

Precedence rules for experiment file generation

When generating experiment files (.eds), the QuantStudio™ 12K Flex Software command-line interface relies on a set of precedence rules to resolve conflicts that arise from the data supplied by some input files. Assay information files (.aif or .aix), plate setup files (.txt), and template files (.edt) can contain data used to populate the same fields of new experiment files. For example, both template and plate setup files can contain location data for samples and assays.

Files used for experiment file (.eds) creation	Precedence rule
Template file (.edt)	The values in the template take precedence except for: • Experiment Name – Determined by the File Name Convention preference. • Barcode – Determined by the barcode, if present. Otherwise, the value is null. • Experiment File Name – Determined by the File Name Convention preference.
• Template file (.edt) • Assay information file (.aif/.aix)	All values in the template file take precedence, except for: • Gene Expression Targets/Assay Definition • Genotyping Assay/SNP Definition • Passive Reference If any conflicts exist between the assay information file and the template for the attributes above, then the assay information file values always take precedence.
• Template file (.edt) • Plate setup file (.txt)	All values in the template file take precedence, except for: • Target/Assay/SNP to Well Assignment • Sample to Well Assignment • Task to Well Assignment • Biological Group to Well Assignment • Well Quantity to Well Assignment • Sample Color • Biological Group Color • Target Color • Gene Expression Targets Definition • Genotyping Assay Definition • Passive Reference
• Template file (.edt) • Plate setup file (.txt) • Assay information file (.aif/.aix)	All values in the template take precedence, except for the following. The following assay information file values take precedence over Plate Setup and Template: • Gene Expression Targets/Detectors Definition • GT Assay/Marker Definition • Passive Reference The following Plate Setup values take precedence over the template: • Block Type • Target/Assay/Marker to Well Assignment • Sample to Well Assignment • Task to Well Assignment • Biological Group to Well Assignment • Well Quantity to Well Assignment • Sample Color • Biological Group Color • Target Color

Running the command-line application

Running the application

1. In the desktop, select **Start ▸ Run**.
2. In the Run dialog box, enter **cmd** in the Open field, then click **OK**.
3. In the DOS prompt, change to the installation directory and enter the command:
   a. Enter **cd C:\Program Files\Applied Biosystems\QuantStudio12KFlex\bin**, then press **Enter**.
   b. Enter **cmdlineutil.exe**, followed by **-expgen** or **-export**, then all applicable parameters and arguments. See "Command syntax and arguments" on page 194 for a complete list of command-line parameters.

Viewing the command-line help

The command-line application includes a help function that provides the information in this chapter. To view help for:

- The entire application, enter **cmdlineutil.exe –help**
- A particular function, enter **cmdlineutil.exe –expgen -help** to view the file generation help, or **cmdlineutil.exe –export -help** to view the file export help.

Command syntax and arguments

Batch file creation

The command used to create batches of files uses the following syntax:

```
cmdlineutil.exe -expgen [ parameters ]
```

The following table lists the acceptable parameters that can be included in any order. See "Examples" on page 197 for an example of the experiment creation command.

IMPORTANT! Enclose file paths in double quotes to allow spaces in the string.

`-a <filepath>`

(Optional) Specifies the path and name (<*filepath*>) of the assay information file (.aif or .aix) that the software uses to create new experiment files.

Example:

```
-a "D:\assayfiles\assayfile.aif"
```

`-b <filepath>`

(Optional) Specifies the path and name (<*filepath*>) of the barcode file that the software uses to create new files. If the `-b` parameter is not used, then the software creates the number of experiment specified by the `-n` parameter.

Example:

```
-b "D:\barcodefiles\barcodefile.txt"
```

`-c <string>`

(Optional) When the `-f` parameter is included, specifies the alphanumeric string that the software includes in the file names of the new experiments. If no value is supplied, "custom" is used as the default value.

Example:

```
-c "Batch001_"
```

`-f <option>`

(Optional) Specifies the convention that the software uses to name the new files. The convention can consist of all or some of the following interchangeable arguments, in any order:

Custom Name Field – The alphanumeric string specified by the `-c` parameter.

ID – The barcode of the plate specified in the barcode file specified by the `-b` parameter.

Example:

```
-f "Custom Name Field_ID"
```

If the `-f` parameter is used without arguments, then the software names files according to the following convention: `"Custom Name Field_ID"`

`-l <dirpath>`

(Required) Specifies the path of the directory (<*dirpath*>) to which the software saves the new files.

Example:

```
-l "C:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\
   experiments"
```

Before creating experiment files, the software confirms whether the export location exists and aborts if the location does not exist.

`-m <filepath>`

(Optional) Specifies the path and name (<*filepath*>) of the sample file that the software uses to create new files.

Example:

```
-m "C:\samplefiles\samplefile.txt"
```

`-n <integer>`

(Optional) If the `-b` parameter is not included, specifies number of experiments (`<integer>`) that the software will create. If no value is supplied, the software creates 25 experiments by default.

Example:

```
-n 31
```

`-s <filepath>`

(Optional) Specifies the path and name (<*filepath*>) of the setup file that the software uses to create new files.

Example:

```
-s "C:\setupfiles\setupfile.txt"
```

`-t <filepath>`

(Required) Specifies the path and name (<*filepath*>) of the QuantStudio™ 12K Flex Software template file that the software uses to create new files.

Example:

```
-t "C:\Applied Biosystems\QuantStudio 12K Flex Software\
   User Files\experiments\templatefile.edt"
```

`-v`

(Optional) Configures the software to operate in verbose mode, where the software displays each operation as it is performed.

Results export

The command used to export the results from experiment files uses the following syntax:

```
cmdlineutil.exe -export [ parameters ]
```

The following table lists the acceptable parameters that can be included in any order. See "Examples" on page 197 for and examples of the experiment export command.

IMPORTANT! Enclose file paths in double quotes to allow spaces in the string.

`-e <dirpath>`

(Required) Specifies the path to the directory (<*dirpath*>) that contains the experiment files (.eds) for which the software exports data.

Example:

```
-e "C:\Applied Biosystems\QuantStudio 12K Flex Software\
   User Files\experiments\"
```

`-f <option>`

(Required) Specifies the format of the exported data (see "RDML export format" on page 228 for the export file specifications):

QuantStudio12KFlex – Exports data in a format compatible with the QuantStudio™ 12K Flex System.

SDS23 – Exports data in a format compatible with the Applied Biosystems® 7900HT Real-Time PCR System.

RDML – Exports data in the real-time data markup language (RDML) format.

Example:

```
-f "RDML"
```

`-l <path>`

(Optional) Specifies the path (<*path*>) of the directory to which the software saves the exported files.

Example:

```
-l "C:\exports\"
```

`-s <option>`

(Optional) Specifies the data spanning option (<*option*>) that determines how the software exports data from multiple experiments:

single – Exports data for all experiments into one contiguous data file.

multiple – Exports data for each experiment to a separate data file.

Example:

```
-s "multiple"
```

`-x <filepath>`

(Required) Specifies the file format of the exported file:

QuantStudio12KFlex export format: .txt, .xls, or .xlsx

SDS23 export format: .txt

RDML export format: .rdml

Example:

```
-x "rdml"
```

Examples

Batch file creation

The following example uses all parameters described in "Command syntax and arguments" on page 194 (required and optional) to generate a set of experiment files.

```
cmdlineutil.exe -expgen -t "C:\Applied Biosystems\QuantStudio
   12K Flex Software\User Files\experiments\templates\
   standard_curve.edt" -a "C:\Applied Biosystems\QuantStudio 12K
   Flex Software\User Files\experiments\examples\AIF\
   AIF_820629.txt" -s "C:\Applied Biosystems\QuantStudio 12K
   Flex Software\User Files\experiments\examples\Plate Setup
   Files\SDS_820629.txt" -m "C:\Applied Biosystems\QuantStudio
   12K Flex Software\User Files\experiments\examples\
   SampleNames\SampleFileNames.txt" -c "alloptionsused"
   -f "Plate Barcode_Custom Name Field" -b "C:\barcodes.txt"
   -v -l "C:\Experiment"
```

For this example, the command-line application:

- Imports assay definitions from the `AIF_820629.txt` assay information file.
- Imports sample names from the `SampleFileNames.txt` sample file.
- Generates an experiment for each barcode in the `barcodes barcodes.txt` barcode file, where each new experiment uses the settings found in the standard_curve.edt template file and the `SDS_820629.txt` setup file.

  Note: The setup file links the information from the `AIF_820629.txt` and `SampleFileNames.txt` to each new experiment file.
- Saves all generated files using the following naming convention:

  <*barcode*>_alloptionsused
- Saves all generated files to:

  `C:\Experiment\`<*date/time*>

  Note: The command-line application automatically creates a time-stamped folder at the export location for each batch operation. For example, the folder created for files generated on April 7, 2010 at 12:48:35 would be: `2010-04-07 124835`

Results export

The following example performs a real-time data markup language (RDML) export of experiments in the QuantStudio™ 12K Flex Software experiments directory to the exports directory of the C drive. The software generates an RDML file for each individual experiment file.

```
cmdlineutil.exe -export -e "C:\Applied Biosystems\QuantStudio
   12K Flex Software\User Files\experiments\" -f "SDS23"
   -l "C:\exports\" -s "single" -x "rdml"
```

E

File Format Reference

This appendix covers:

Import formats and file specifications

The QuantStudio™ 12K Flex Software supports several import file formats that can be used to automate experiment creation and assay and sample data import. The files can be used with the command-line application (see page 189) or the QuantStudio™ 12K Flex Software application programming interface (API) to integrate the QuantStudio™ 12K Flex System into a laboratory information management system (LIMS). For a detailed explanation of the API, or for information on integrating the QuantStudio™ 12K Flex System into a laboratory workflow, see the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Automation Guide* (Part no. 4470693).

Note: The file specifications listed in this appendix are subject to change. For updated information, review the QuantStudio™ 12K Flex Software Release Notes found at: C:\Program Files\Applied Biosystems\QuantStudio12KFlex\docs\README.html.

About the import file formats

File format	Description	See...
Plate setup file (.txt)	A user-created, tab-delimited text file that describes the layout of a consumable for an experiment to be run on the QuantStudio™ 12K Flex System. The file defines the arrangement of assays and samples on the consumable, and provides other experiment data, such as the thermal profile and data collection settings.	page 201
Sample file (.txt)	A user-created, tab-delimited text file containing sample data that can be imported into the QuantStudio™ 12K Flex Software for use in related experiments.	page 206
Assay information file (.aif or .aix)	A tab-delimited or XML data file that is shipped on a CD with each TaqMan® assay ordered from Life Technologies. The file, which contains data describing the assay, can be imported into the QuantStudio™ 12K Flex Software for use in related experiments.	page 207
Barcode file (.txt)	A user-created, text file containing the barcodes of consumables for which you want to create experiment files using the command-line utility.	page 207

Conventions

The following conventions are used in the rest of this section:

- `normal` – Normal text must be entered exactly as it appears.
- <*italic*> – Italicized text between brackets must be substituted with custom values.
- `[ required text ]` – Text appearing between brackets is required information. All information inside the brackets must be present for the QuantStudio™ 12K Flex Software to import it.
- `{ optional text }` – Text appearing between braces is optional.
- Unless noted otherwise, separate all fields in a row using a tab character (U+0009).
- Unless noted otherwise, end all rows using a carriage-return character (U+000D).

Plate setup file format

You can use plate setup files to automatically populate setup information into an open experiment in the QuantStudio™ 12K Flex Software or into new experiments created by the command-line application (see page 189). A plate setup file is a tab-delimited ASCII text file (.txt) that contains data that describes the location experiment data information. The files can be created manually using a text processor or generated automatically by third-party applications.

IMPORTANT! To guarantee successful import of the plate setup file into a experiment, the file must contain all the elements described in the following section and in the order that they appear.

File structure

The plate setup file consists of a header, which specifies the instrument model for which the experiment is designed, and a sample setup section.

Section	Description	See...
Plate setup file header	Defines the instrument model for which the experiment is designed and the dye used as the passive reference.	page 201
Plate setup file body	Defines the contents of a 96/384-well plate or array card, including target, SNP assay, sample, and task assignments.	page 202

Plate setup file header

The plate setup file begins with a header that consists of two lines. Each line starts with an asterisk (*) and ends with a carriage return in the following pattern:

** <field name> = <field value>*

The header must contain the lines shown in the following table.

Field	Description	Valid values
Instrument Type	The model of QuantStudio™ 12K Flex System for which the experiment is designed.	QuantStudio12KFlex
Passive Reference	The dye that the experiment will use as a passive reference.	• The name of a dye in the Dye Library of the QuantStudio™ 12K Flex Software‡, or • <*blank*> if the consumable does not contain a passive reference.

‡ Custom dyes are allowed if they are in Dye Library.

Note: The QuantStudio™ 12K Flex Software automatically removes any leading and trailing white space around the field name and field value.

Example:

```
* Instrument Type = QuantStudio12KFlex
* Passive Reference = ROX
```

Plate setup file body

The body of a plate setup file contains either target information, which can be imported into all experiments except genotyping, or SNP assay information. This information can be imported into genotyping experiments only. The body consists of three required elements (the header, the column header, and the body) that describe the contents of a 96/384-well plate or array card. The sample setup column header and body can appear in any order.

IMPORTANT! Observe the following guidelines when creating a plate setup file:

- Do not insert blank lines between the sample setup header and the column header.
- Do not use illegal characters, including backslash (\), tab, asterisk (*), hard return, soft return, brackets([or]), or comma (,).

Sample setup header

The header contains the label that defines the beginning of the sample setup data.

Example:

```
[Sample Setup]
```

Sample setup column header

The column header contains the headings that define the positions of the data columns in the sample setup body. The headings are separated by tab characters. See "Plate setup data columns" on page 203 for a list of the data column headers.

Example:

```
Well Sample Name Sample Color  Biogroup Name Biogroup Color Target Name…
```

Sample setup body

Contains the sample setup data where each row defines the contents of a single well on the consumable, including the: well contents (sample, target, or SNP assay added to the well), task assignments, and comments. If a well contains multiple assays (multiplex PCR), the data for the additional assays are defined on separate lines by repeating the well designation. See "Plate setup data columns" on page 203 for a list of the data column headers.

Note: The sample setup data rows can occur in any order.

Example:

```
Well Sample Name Sample Color  Biogroup Name Biogroup Color Target Name…
1    Liver  cDNA "RGB(25,0,0)"
2    Liver  cDNA "RGB(25,0,0)"
3    Liver  cDNA "RGB(25,0,0)"
4    Heart  cDNA "RGB(0,25,0)"
5    Heart  cDNA "RGB(0,25,0)"
…
```

Plate setup data columns

The following table lists the headings and columns that are present in the plate setup file body of all experiment types followed by the columns that are specific to genotyping experiments and non-genotyping experiments.

	Column name	Description	Valid values
All experiments	Well	The number of the well on the consumable, where the well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.	*<Positive integer (1–96/384)>*‡
	Sample Name	The name of the sample contained by the associated well.	*<100-character string>*
	Sample Color	(Optional) The RGB color of the associated sample.	`"RGB(`*<r>*`,`*<g>*`,`*<b>*`)"`§
	Biogroup Name	(Optional) The name of the associated biological group.	*<100-character string>*
	Biogroup Color	(Optional) The RGB color of the biological group.	`"RGB(`*<r>*`,`*<g>*`,`*<b>*`)"`§
	Comments	(Optional) Additional text that describes the well.	*"<1024-character string>"*
All except genotyping	Target Name	The name of the target detected or amplified by the assay in the associated well.	*<100-character string>*#
	Target Color	(Optional) The RGB color of the target.	`"RGB(`*<r>*`,`*<g>*`,`*<b>*`)"`§
	Task	The task assignment of the target assay at the well.‡‡	`<UNKNOWN` | `STANDARD` | `NTC` | `ENDOGENOUS` | `IPC` | `BlockedIPC>`
	Reporter	The reporter dye used by the associated target assay.	*<dye name>*#§§
	Quencher	The quencher dye used by the associated target assay.	*<dye name>*§§
	Quantity	(Optional) The quantity of standard present in the given well expressed as a float or integer. If the associated well is not assigned the `STANDARD` task, then the field is blank.	*<float or integer>*
Genotyping only	SNP Assay Name	The name of the SNP assay detected or amplified by the assay in the associated well.	*<100-character string>*#
	SNP Assay Color	(Optional) SNP assay color in RGB	`"RGB(`*<r>*`,`*<g>*`,`*<b>*`)"`§
	Task	The task assignment of the SNP assay at the well.‡‡	`<UNKNOWN` | `NTC` | `PC_ALLELE_1` | `PC_ALLELE_2` | `PC_ALLELE_BOTH>`
	Allele1 Name	The name of the first allele detected by the SNP assay.	*<100-character string>*#
	Allele1 Color	The RGB color used to represent data for the first allele.	`"RGB(`*<r>*`,`*<g>*`,`*<b>*`)"`§
	Allele1 Reporter	The reporter dye used to label the probe for the first allele.	*<dye name>*#§§
	Allele1 Quencher	The quencher dye used to label the probe for the first allele.	*<dye name>*§§
	Allele2 Name	The name of the second allele detected by the SNP assay.	*<100-character string>*#
	Allele2 Color	The RGB color used to represent data for the second allele.	`"RGB(`*<r>*`,`*<g>*`,`*<b>*`)"`§
	Allele2 Reporter	The reporter dye used to label the probe for the second allele.	*<dye name>*#§§
	Allele2 Quencher	The quencher dye used to label the probe for the second allele.	*<dye name>*§§

‡ Cannot be blank.

§ Contains (r)ed, (b)lue, and (g)reen color values between 0–255. The field must be set within double quotes with no spaces between the values.

Can be empty if the Task field is empty. Otherwise, the field must contain a value.

‡‡ See the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Getting Started Guide* to determine the tasks applicable to your experiment.

§§ The dye must already exist in the QuantStudio™ 12K Flex Software Dye Library. The dye name must be 100 characters or less.

Examples

Quantitative PCR experiments

The following example shows a plate setup file created for a quantitative PCR experiment to be run on a QuantStudio™ 12K Flex System. The experiment evaluates the expression of two targets (CCKAR and GH1) in three samples (cDNA from the liver, heart, and brain). For both TaqMan® assays, the probes are labeled with the FAM™ reporter dye and the non-fluorescent quencher (NFQ-MGB). Biological groups are not used in this experiment.

Example:

```
* Instrument Type = QuantStudio12KFlex
* Passive Reference = ROX
[Sample Setup]
Well  Sample Name  Sample Color   Biogroup Name  Biogroup Color  Target Name  Target Color    Task        Reporter  Quencher  Quantity  Comments
1     Liver cDNA   "RGB(25,0,0)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
2     Liver cDNA   "RGB(25,0,0)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
3     Liver cDNA   "RGB(25,0,0)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
4     Heart cDNA   "RGB(0,25,0)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
5     Heart cDNA   "RGB(0,25,0)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
6     Heart cDNA   "RGB(0,25,0)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
7     Brain cDNA   "RGB(0,0,25)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
…
378   Liver cDNA   "RGB(25,0,0)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
379   Heart cDNA   "RGB(0,25,0)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
380   Heart cDNA   "RGB(0,25,0)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
381   Heart cDNA   "RGB(0,25,0)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
382   Brain cDNA   "RGB(0,0,25)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
383   Brain cDNA   "RGB(0,0,25)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
384   Brain cDNA   "RGB(0,0,25)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
```

The following example shows a plate setup file for a multiplex version of the experiment above, where the assays for the two targets (CCKAR and GH1 targets) are added to the same well. For both TaqMan® assays, the probes are labeled with the FAM™ reporter dye and the non-fluorescent quencher (NFQ-MGB).

Example:

```
* Instrument Type = QuantStudio12KFlex
* Passive Reference = ROX
[Sample Setup]
Well  Sample Name  Sample Color   Biogroup Name  Biogroup Color  Target Name  Target Color    Task        Reporter  Quencher  Quantity  Comments
1     Liver cDNA   "RGB(25,0,0)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
1     Liver cDNA   "RGB(25,0,0)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
2     Liver cDNA   "RGB(25,0,0)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
2     Liver cDNA   "RGB(25,0,0)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
3     Liver cDNA   "RGB(25,0,0)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
3     Liver cDNA   "RGB(25,0,0)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
4     Heart cDNA   "RGB(0,25,0)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
4     Heart cDNA   "RGB(0,25,0)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
5     Heart cDNA   "RGB(0,25,0)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
5     Heart cDNA   "RGB(0,25,0)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
6     Heart cDNA   "RGB(0,25,0)"                                 CCKAR        "RGB(98,25,0)"  ENDOGENOUS  FAM       NFQ-MGB
6     Heart cDNA   "RGB(0,25,0)"                                 GH1          "RGB(0,0,105)"  UNKNOWN     FAM       NFQ-MGB
…
```

Presence/absence experiments

The following example shows a plate setup file created for a presence/absence experiment to be run on a QuantStudio™ 12K Flex System. The experiment screens samples for the presence of a pathogen (E. coli O157:H7). The detection assay uses FAM™ and VIC® dye-labeled TaqMan® probes to amplify a unique genomic sequence and an internal positive control (IPC).

Example:

```
* Instrument Type = QuantStudio12KFlex
* Passive Reference = ROX
[Sample Setup]
Well  Sample Name  Sample Color   Biogroup Name  Biogroup Color  Target Name  Target Color    Task       Reporter  Quencher  Quantity  Comments
1     Control      "RGB(25,0,0)"                                 E.coli       "RGB(98,25,0)"  NTC        FAM       NFQ-MGB
1     Control      "RGB(25,0,0)"                                 IPC          "RGB(98,25,0)"  NTC        VIC       NFQ-MGB
2     Control      "RGB(25,0,0)"                                 E.coli       "RGB(98,25,0)"  NTC        FAM       NFQ-MGB
2     Control      "RGB(25,0,0)"                                 IPC          "RGB(98,25,0)"  NTC        VIC       NFQ-MGB
3     Control      "RGB(25,0,0)"                                 E.coli       "RGB(98,25,0)"  NTC        FAM       NFQ-MGB
3     Control      "RGB(25,0,0)"                                 IPC          "RGB(98,25,0)"  NTC        VIC       NFQ-MGB
4     Pos Control  "RGB(0,25,0)"                                 E.coli       "RGB(98,25,0)"  IPC        FAM       NFQ-MGB
4     Pos Control  "RGB(0,25,0)"                                 IPC          "RGB(98,25,0)"  IPC        VIC       NFQ-MGB
5     Pos Control  "RGB(0,25,0)"                                 E.coli       "RGB(98,25,0)"  IPC        FAM       NFQ-MGB
5     Pos Control  "RGB(0,25,0)"                                 IPC          "RGB(98,25,0)"  IPC        VIC       NFQ-MGB
6     Pos Control  "RGB(0,25,0)"                                 E.coli       "RGB(98,25,0)"  IPC        FAM       NFQ-MGB
6     Pos Control  "RGB(0,25,0)"                                 IPC          "RGB(98,25,0)"  IPC        VIC       NFQ-MGB
…
```

Genotyping experiments

The following example shows a plate setup file created for a genotyping experiment to be run on a QuantStudio™ 12K Flex System. The experiment screens samples for one SNP targets (rs15934), using a set of allele-specific TaqMan® probes labeled with the FAM™ and VIC® reporter dyes and the non-fluorescent quencher (NFQ-MGB).

Example:

```
* Instrument Type = QuantStudio12KFlex
 * Passive Reference = ROX
 [Sample Setup]
 Well Sample Name Sample Color  SNP Assay Name SNP Assay Color Task       Allele1 Name Allele1 Color Allele1 Reporter Allele1 Quencher Allele2 Name Allele2 Color Allele2 Reporter Allele2
Quencher Comments
 1    Neg Control "RGB(25,0,0)" SNP rs15934    "RGB(0,75,0)"   NTC         G            "RGB(0,0,50)" VIC              NFQ-MGB          A            "RGB(0,50,0)" FAM              NFQ-MGB
 2    Neg Control "RGB(25,0,0)" SNP rs15934    "RGB(0,75,0)"   NTC         G            "RGB(0,0,50)" VIC              NFQ-MGB          A            "RGB(0,50,0)" FAM              NFQ-MGB
 3    Neg Control "RGB(25,0,0)" SNP rs15934    "RGB(0,75,0)"   NTC         G            "RGB(0,0,50)" VIC              NFQ-MGB          A            "RGB(0,50,0)" FAM              NFQ-MGB
 4    All Control "RGB(25,0,0)" SNP rs15934    "RGB(0,75,0)"   PC_ALLELE_1 G            "RGB(0,0,50)" VIC              NFQ-MGB          A            "RGB(0,50,0)" FAM              NFQ-MGB
 5    All Control "RGB(25,0,0)" SNP rs15934    "RGB(0,75,0)"   PC_ALLELE_1 G            "RGB(0,0,50)" VIC              NFQ-MGB          A            "RGB(0,50,0)" FAM              NFQ-MGB
 6    All Control "RGB(25,0,0)" SNP rs15934    "RGB(0,75,0)"   PC_ALLELE_1 G            "RGB(0,0,50)" VIC              NFQ-MGB          A            "RGB(0,50,0)" FAM              NFQ-MGB
 7    Al2 Control "RGB(25,0,0)" SNP rs15934    "RGB(0,75,0)"   PC_ALLELE_2 G            "RGB(0,0,50)" VIC              NFQ-MGB          A            "RGB(0,50,0)" FAM              NFQ-MGB
 8    Al2 Control "RGB(25,0,0)" SNP rs15934    "RGB(0,75,0)"   PC_ALLELE_2 G            "RGB(0,0,50)" VIC              NFQ-MGB          A            "RGB(0,50,0)" FAM              NFQ-MGB
 9    Al2 Control "RGB(25,0,0)" SNP rs15934    "RGB(0,75,0)"   PC_ALLELE_2 G            "RGB(0,0,50)" VIC              NFQ-MGB          A            "RGB(0,50,0)" FAM              NFQ-MGB
 10   Sample01    "RGB(25,0,0)" SNP rs15934    "RGB(0,75,0)"   UNKNOWN     G            "RGB(0,0,50)" VIC              NFQ-MGB          A            "RGB(0,50,0)" FAM              NFQ-MGB
 …
```

Sample file format

The QuantStudio™ 12K Flex Software can import sample files to populate sample information into an open experiment. A sample file is a tab-delimited ASCII text file (.txt) that contains sample/well designations, and custom sample properties. The files can be created manually using a text processor or generated automatically by third-party applications.

IMPORTANT! To guarantee successful import, the file must contain all the elements described in the following section and in the order that they appear.

Note: The command-line application (see page 189) does not import sample files. If you are using the application to create experiments, use plate setup files to import sample information into the new experiments (see "Plate setup file format" on page 201).

File structure

Sample file header row

The sample file begins with an optional header row that contains column headers for well number ("Well"), sample name ("Sample Name"), and optional custom properties names. The order of the columns is important and cannot be changed.

Sample file body

A body of rows, containing the sample data, follows the optional header row. Each body row defines the sample information for a single well on the consumable, including: well number, sample name, and any applicable custom fields. The body can contain data for a subset of wells on the consumable, so the rows for empty wells can be omitted from the file. The sample body rows can occur in any order.

Column name	Description	Valid values
Well	The number of the well on the consumable, where the well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.	*<Positive integer (1–96/384)>*
Sample Name	The name of the sample contained by the associated well.	*<100-character string>*
Custom1… Custom6	(Optional) Additional text that describes the sample in the well.	*<1024-character string>*

Example file

```
Well  Sample Name  Custom1  Custom2  Custom3  Custom4  Custom5  Custom6
21    Sample       1test1   test2    test3    test4    test5    test6
22    Sample       2test1   test2    test3    test4    test5    test6
23    Sample       3test1   test3    test4    test5    test6
1     Sample       5test1   test2    test3    test4    test5    test6
2     Sample       6test1   test2    test3    test4    test5    test6
3     Sample       7test1   test2    test3    test4    test5    test6
4     Sample       8test1   test2    test3    test4    test5    test6
…
```

Barcode file format

The QuantStudio™ 12K Flex Software command-line application can import barcode files to populate experiment files (.eds) it generates with barcode information. A barcode file is a tab-delimited ASCII text file (.txt) that contains a list of barcodes. The files can be created manually using a text processor or generated automatically by third-party applications.

IMPORTANT! To guarantee successful import, the file must contain all the elements described in the following section and in the order that they appear.

File structure

The barcode file contains a list of barcodes, where each line defines a single barcode terminated by a carriage return. The barcodes can occur in any order and cannot contain starting or trailing white space.

Note: The QuantStudio™ 12K Flex Software command-line application does not validate the barcodes.

Example file

```
HA996346102
IB894812348
DD834814679
EK209825848
AF092387348
FF225676243
```

Assay information file

The QuantStudio™ 12K Flex Software command-line application can import data for Life Technologies assays from assay information files (.aif), which is shipped on a CD with each assay order. The .aif contains technical details about all assays in the shipment. It includes information about assay concentrations; reporters and quenchers used; part and lot numbers; and assay, vial, and plate ID numbers. The file name includes the number from the barcode on the plate.

Export formats and file specifications

This section describes the export formats supported by the QuantStudio™ 12K Flex Software. The information provided in this appendix is intended for users who want to integrate the QuantStudio™ 12K Flex Software with third-party applications, including downstream analysis software and laboratory information management system (LIMS) tools.

Note: The file specifications listed in this appendix are subject to change. For updated information, review the QuantStudio™ 12K Flex Software Release Notes found at: C:\Program Files\Applied Biosystems\QuantStudio12KFlex\docs\README.html.

Export formats

The QuantStudio™ 12K Flex Software can export setup and results data from experiment files (.eds) in several file formats that allow further downstream analysis. The export formats feature standardized data structures and markup to maximize accessibility by downstream applications.

The QuantStudio™ 12K Flex Software supports the following export formats:

File format	Description	See...
QuantStudio12KFlex export file	A QuantStudio12KFlex-formatted text file that contains setup and/or results data exported from an experiment file (.eds).	page 209
7900 export file	A legacy 7900-formatted text file that contains setup and/or results data exported from an experiment file (.eds).	page 223
RDML export file	A compressed XML file that contains setup and/or results data exported from an experiment file (.eds) and parsed in Real-time PCR Data Markup Language (RDML). The file is stored as a compressed file using the PKZIP archive format.	page 228

Export formats and the QuantStudio™ 12K Flex Software API

The export formats can be used in combination with the QuantStudio™ 12K Flex Software application programming interface (API) to integrate the QuantStudio™ 12K Flex System into a laboratory information management system (LIMS) workflow.

QuantStudio12KFlex export format

The QuantStudio™ 12K Flex Software can export setup and results data from experiment files (.eds) to tab-delimited text files (.txt) in a native QuantStudio™ 12K Flex System export format. Data exported in the QuantStudio12KFlex export format can be opened by common spreadsheet applications, such as Microsoft® Excel®, or imported by laboratory information management system (LIMS) applications or databases that have been configured to parse the file format.

File structure

The following table shows the data structure common to data exported in the QuantStudio12KFlex export format, regardless of experiment type. Each row represents one or more lines of data in the exported file corresponding to a common functional group. The QuantStudio12KFlex export format allows the user to customize and/or omit columns. The columns and orders described below are the default configuration: all columns in their natural order. Actual files may contain fewer columns if the user modified the configuration.

Section	Description	See...
File header	Describes the qualities of the QuantStudio™ 12K Flex Instrument used to run the experiment and several general experiment properties, such as the date and time of the run and the dye used as the passive reference.	page 210
Sample setup data	Describes the configuration of samples on the experiment consumable, including sample location, target or SNP assay properties, and task assignments.	page 211
Raw data	Contains the raw data collected by the QuantStudio™ 12K Flex Instrument during the experiment run.	page 213
Amplification data	Contains the normalized data collected during the cycling stage of PCR amplification, which the QuantStudio™ 12K Flex Software uses to generate the amplification plot. Note: Not applicable for presence/absence, genotyping, or melting curve experiments that are run without a PCR (cycling) stage.	page 214
Multicomponent data	Contains the spectral data used by the QuantStudio™ 12K Flex Software to generate the multicomponent plot that displays the contribution of each dye over the duration of the PCR run.	page 215
Results data	Contains the normalized, processed, and analyzed data generated by the QuantStudio™ 12K Flex Software.	page 215

File header

The plate setup file begins with a header that describes the qualities of the QuantStudio™ 12K Flex Instrument used to run the experiment and several other general experiment properties. Each line starts with an asterisk (*) and ends with a carriage return in the following pattern:

`* <field name> = <field value>`

Note: The QuantStudio™ 12K Flex Software automatically removes any leading and trailing white space around the field name and field value.

The header contains the lines listed in the following table.

Field	Description	Output
Block Type	The model of the sample block installed to the QuantStudio™ 12K Flex Instrument at the time the experiment was run.	`96/384-well` or `array card`
Calibration Expired	Expiration status of the calibration. Indicates whether the calibration of the QuantStudio™ 12K Flex Instrument was current at the time that the experiment was run.	`Yes` or `No`
Chemistry	The chemistry of the experiment.	<*100-character string*>
Experiment File Name	The path to the experiment file on the local computer hard drive.	<*filepath*>
Experiment Name	The name of experiment entered into the Experiment Name field.	<*100-character string*>
Experiment Run End Time	The date and time that the QuantStudio™ 12K Flex Instrument finished running the experiment.	<*date and time*>
Experiment Type	The type of chemistry application for which the experiment is designed.	`Standard Curve`, `Presence/Absence`, `Relative Standard Curve`, or `DDCt Quantification`
Instrument Type	The model of the QuantStudio™ 12K Flex Instrument that ran the experiment.	`QuantStudio12KFlex`
Passive Reference	The dye used as a passive reference (or blank if the consumable did not contain one).	<*100-character string*>
Signal Smoothing On	The smoothing setting status for the experiment. Indicates whether smoothing is turned on for the experiment.	`true` or `false`
Stage\Cycle where Analysis is performed	The stage and cycle during the thermal cycling protocol when the QuantStudio™ 12K Flex Instrument collected data.	Stage <*integer*>, Step <*integer*>
Calibration Date	The date and time that the current background, ROI, uniformity, or pure dye calibration was performed and when it will expire.	<*date and time*>
Calibration Expiration Date		<*date and time*>
Instrument serial number	The serial number of the QuantStudio™ 12K Flex Instrument that ran the experiment.	<*100-character string*>
Quantification cycle method	The method of quantification for the associated experiment.	<*100-character string*>

Sample setup data

When selected as an export option, the QuantStudio™ 12K Flex Software exports sample setup data after the file header. The sample setup data describes the sample configuration on the experiment consumable, including positions, sample names, task assignments, assay information, and color coding.

The data consists of a column header followed by the sample data fields, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the QuantStudio™ 12K Flex Software lists the data for each additional assay on separate rows, repeating the well number and sample information. The data included in the sample setup data export varies depending on experiment type.

This section describes the following sample setup data formats:

Quantification and presence/absence experiments

The table below describes the sample setup data that can be exported from absolute quantification, relative quantification, or presence/absence experiments. The body can contain all or some of the data columns below depending on the export configuration.

Note: For genotyping experiments, see"Genotyping experiments" on page 212.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Sample Name	The name of the sample contained by the well.	100-character string
Sample Color	The RGB color of the associated sample.	`"RGB(<r>,<g>,<b>)"`§
Target Name	The name of one target in the well, if applicable. If a well contains multiple targets one row is used per target.	100-character string
Target Color	The RGB color of the associated SNP assay.	`"RGB(<r>,<g>,<b>)"`§
Task	The task the target is used for in this well.	`UNKNOWN`, `STANDARD`, `IPC`, `NTC`, or `BlockedIPC`
Reporter	The reporter dye that labels the probe for the target assay.	100-character string
Quencher	The quencher dye that labels the probe for the target assay.	100-character string
Quantity	Standard quantity (if applicable). This column only appears for Standard Curve and Relative Standard Curve experiments	Float or Integer
Comments	Additional text that describes the well.	1024-character string

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

§ Contains (r)ed, (b)lue, and (g)reen color values, each between 0–255. The field is enclosed in double quotes with no spaces between the values.

Genotyping experiments

The table below describes the sample setup data that can be exported from a genotyping experiment. The body can contain all or some of the data columns below depending on the export configuration.

Note: For all other experiments, see"Quantification and presence/absence experiments" on page 211.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Sample Name	The name of the sample contained by the well.	100-character string
Sample Color	The RGB color of the associated sample.	`"RGB(<r>,<g>,<b>)"`§
SNP Assay Name	The name of the SNP assay applied to the well. If the well contains multiple assays, the data for each SNP assay are exported in an additional row.	100-character string
SNP Assay Color	The RGB color of the associated SNP assay.	`"RGB(<r>,<g>,<b>)"`§
Task	The task assignment of the SNP assay at the well.	`UNKNOWN` or `NTC`
Allele1 Name	The name of the first allele for the associated SNP assay.	100-character string
Allele1 Color	The RGB color of the first allele for the associated SNP assay.	`"RGB(<r>,<g>,<b>)"`§
Allele1 Reporter	The reporter dye that labels the probe for the first allele.	100-character string
Allele1 Quencher	The quencher dye that labels the probe for the first allele.	100-character string
Allele2 Name	The name of the second allele for the associated SNP assay.	100-character string
Allele2 Color	The RGB color of the second allele for the associated SNP assay.	`"RGB(<r>,<g>,<b>)"`§
Allele2 Reporter	The reporter dye that labels the probe for the second allele.	100-character string
Allele2 Quencher	The quencher dye that labels the probe for the second allele.	100-character string
Comments	Additional text that describes the well	1024-character string

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

§ Contains (r)ed, (b)lue, and (g)reen color values, each between 0–255. The field is enclosed in double quotes with no spaces between the values.

Raw data

The QuantStudio™ 12K Flex Software can export the unprocessed raw data (R) collected by the QuantStudio™ 12K Flex Instrument during the experiment run. The raw data consists of fluorescence readings collected by the QuantStudio™ 12K Flex Instrument that have not been normalized to the passive reference.

The section begins with a column header followed by the raw data, where each row contains the data for a single well separated by tab characters. Each line of raw data consists of readings sorted by bin, where each bin represents an excitation/emission filter pair that was selected during experiment setup. The bins are named for the corresponding filter combination according to the following convention:

<excitation filter name>-<emission filter name>

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Cycle	The cycle of the run during which the QuantStudio™ 12K Flex Instrument recorded the fluorescence.	Integer
<Bin #>	The raw fluorescence for the well measured by the QuantStudio™ 12K Flex Instrument for the associated bin at the designated cycle.	Float

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

HRM Raw

The following table describes the raw data exported from high resolution melting curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Well	The number of the well on the consumable	Integer (1–96/384)‡
Reading	1-based index of the reading	Integer
Temperature	Temperature in Celsius	Float
Fluorescence	Fluorescence value	Float
Derivative	Value of the fluorescence curve derivative for this reading point	Float

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Amplification data

The QuantStudio™ 12K Flex Software can export the processed amplification data used to generate the amplification plot of a real-time PCR experiment. The amplification data (R_n) are the raw fluorescence readings collected by the QuantStudio™ 12K Flex Instrument normalized to the fluorescence from the passive reference. If available, the exported amplification data also exports the baseline-compensated normalized fluorescence data (ΔR_n) calculated by the QuantStudio™ 12K Flex Software.

The section begins with a column header followed by the amplification data, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the QuantStudio™ 12K Flex Software lists the data for each additional assay on separate rows, repeating the well number and sample information.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Cycle	The cycle of the run during which the QuantStudio™ 12K Flex Instrument recorded the fluorescence.	Integer
Target Name	Genotyping experiments – The name of the SNP assay assigned to the well and the allele name.	`<SNP assay name>-<allele name>`
	All other experiments – The name of the target assigned to the well.	Name of the target
Rn	The raw fluorescence for the associated well normalized to the fluorescence of the passive reference dye (reporter signal or passive reference signal).	Float
Delta Rn	The baseline compensated R_n value for the associated well	Float

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Multicomponent data

The QuantStudio™ 12K Flex Software can export the data used to generate the multicomponent plot of a real-time PCR experiment. The multicomponent data tracks the raw fluorescence of all reporter dyes present on the reaction consumable throughout the duration of the experiment run.

The section begins with a column header followed by the multicomponent data, where each row contains the data for a single well separated by tab characters. The multicomponent data contains a dye column for each dye present on the reaction consumable, including reporter dyes, quencher dyes (except non-fluorescent dyes), and the passive reference.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Cycle	The cycle of the run during which the QuantStudio™ 12K Flex Instrument recorded the fluorescence data.	Integer
<*Dye name*>	The raw fluorescence for the designated dye measured by the QuantStudio™ 12K Flex Instrument at the specified well and cycle.	Float

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Results data

The QuantStudio™ 12K Flex Software can export the results data from an analyzed experiment file. The format and content of the results data depends on the experiment type and the analysis settings.

The section begins with a column header followed by the results data, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the QuantStudio™ 12K Flex Software lists the data for each additional assay on separate rows, repeating the well number and sample information.

This section describes the following results data formats:

Standard curve, relative standard curve and comparative C_T

The following table describes the results data exported from standard curve, relative standard curve and comparative C_T experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Sample Name	The name of the sample contained by the well.	100-character string
Target Name	The name of the target assay added to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN`, `NTC`, or `STANDARD`
Reporter	The reporter dye that labels the probe for the target assay.	100-character string
Quencher	The quencher dye that labels the probe for the target assay.	100-character string
CT	The calculated threshold cycle (C_T) for the target at the specified well.	Float
Ct Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Ct SD	The standard deviation of the average C_T of the replicate wells for the specified target.	Float
Quantity	• Unknown wells – The calculated quantity for the sample at the well. • Standard wells – The quantity assigned to the standard at the well.	Float
Quantity Mean	• Unknown wells – The average quantity of the replicate wells for the target/sample. • Standard wells – The quantity assigned to the replicate wells for the target/sample.	Float
Quantity SD	The standard deviation of the average quantity of the replicate wells for the target/sample combination	Float
Automatic Ct Threshold	Whether the threshold was determined automatically (true) or manually (false).	`true` or `false`
Ct Threshold	The threshold cycle (C_T) for the sample at the well	Float
Automatic Ct Baseline	Whether the baseline was determined automatically (true) or manually (false).	`true` or `false`
Baseline Start	The first cycle used to calculate the baseline.	Integer
Baseline End	The last cycle used to calculate the baseline.	Integer
Custom1... Custom6	The contents of the custom text fields found in the Results table of the experiment.	1024-character string (per field)
If analysis flags are present, results data is present in additional columns named for the associated flags.		`true` or `false`

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Biological replicate results

The following table describes the results data exported from high resolution melting curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Biogroup Name	The name of the biological replicate group.	100-character string
Target Name	The name of the target assay assigned to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
RQ	The relative quantity calculated for the replicate wells of the target/sample combination.	Float
RQ Min	The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.	Float
RQ Max	The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.	Float
Ct Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Delta Ct Mean	The average ΔC_T of the replicate wells for the specified target/sample combination.	Float
Delta Ct SD	The standard deviation of the ΔC_T for the replicate well. Depending on the analysis settings, this column may by replaced with "Delta Ct SE" (the standard error of the ΔC_T).	Float
Delta Delta Ct	The $\Delta\Delta C_T$ value of the replicate wells for the specified target/sample combination.	Float

Technical replicate results

The following table describes the results data exported from high resolution melting curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Sample Name	The name of the sample contained by the well.	100-character string
Target Name	The name of the target assay assigned to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
RQ	The relative quantity calculated for the replicate wells of the target/sample combination.	Float
RQ Min	The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.	Float
RQ Max	The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.	Float
Ct Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Delta Ct Mean	The average ΔC_T of the replicate wells for the specified target/sample combination.	Float
Delta Ct SD	The standard deviation of the ΔC_T for the replicate well. Depending on the analysis settings, this column may by replaced with "Delta Ct SE" (the standard error of the ΔC_T).	Float
Delta Delta Ct	The $\Delta\Delta C_T$ value of the replicate wells for the specified target/sample combination.	Float

Genotyping

The following table describes the results data exported from genotyping experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Sample Name	The name of the sample contained by the well.	100-character string
SNP Assay Name	The name of the SNP assay added to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
Allele1 Rn	The raw fluorescence associated with the allele 1 probe of the SNP assay at the well normalized to the fluorescence of the passive reference dye.	Float
Allele2 Rn	The raw fluorescence associated with the allele 2 probe of the SNP assay at the well normalized to the fluorescence of the passive reference dye.	Float
Pass. Ref	The raw fluorescence of the passive reference at the well.	Float
Quality(%)	The confidence of the automatic allele call.	Float (1–100)
Call	The allele call assigned to the sample at the specified well.	`Homozygous` <*allele x*/*allele x*>, `Heterozygous` <*allele x*/*allele y*>, or `Negative Control (NC)`
Method	The method used to call alleles.	`Auto` or `Manual`
Allele1 Automatic Ct Threshold	Whether the allele 1 threshold was determined automatically (true) or manually (false).	`true` or `false`
Allele1 Baseline Start	The start cycle used to calculate the baseline section of allele 1.	Float
Allele1 Baseline End	The end cycle used to calculate the baseline section of allele 1.	Float
Allele2 Automatic Ct Threshold	Whether the allele 2 threshold was determined automatically (true) or manually (false).	`true` or `false`
Allele2 Baseline Start	The first cycle used to calculate the baseline for allele 2.	Float
Allele2 Baseline End	The last cycle used to calculate the baseline for allele 2.	Float
Custom1... Custom6	The contents of the custom text fields found in the Results table of the experiment.	1024-character string (per field)

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Melting curve

The following table describes the results data exported from melting curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Sample Name	The name of the sample contained by the well.	100-character string
Target Name	The name of the target assay assigned to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
Reporter	The reporter dye that labels the probe for the target assay.	100-character string
Quencher	The quencher dye that labels the probe for the target assay.	100-character string
CT	The calculated threshold cycle (C_T) for the target at the specified well.	Float
Ct Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Ct SD	The standard deviation of the average C_T of the replicate wells for the specified target.	Float
Quantity	• Unknown wells – The calculated quantity for the sample at the well. • Standard wells – The quantity assigned to the standard at the well.	Float
Quantity Mean	• Unknown wells – The average quantity of the replicate wells for the target/sample. • Standard wells – The quantity assigned to the replicate wells for the target/sample.	Float
Quantity SD	The standard deviation of the average quantity of the replicate wells for the target/sample.	Float
Automatic Ct Threshold	Whether the threshold was determined automatically (true) or manually (false).	`true` or `false`
Ct Threshold	The threshold cycle (C_T) for the sample at the well.	Float
Automatic Ct Baseline	Whether the baseline was determined automatically (true) or manually (false).	`true` or `false`
Baseline Start	The first cycle used to calculate the baseline.	Integer
Baseline End	The last cycle used to calculate the baseline.	Integer
Tm1... Tm3	The first, second, and third melting temperatures (T_m) calculated in degrees Celsius.	Float
Comments	Additional text that describes the well.	1024-character string
Custom1... Custom6	The contents of the custom text fields found in the Results table of the experiment.	1024-character string (per field)

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

HRM

The following table describes the results data exported from high resolution melting curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Sample Name	The name of the sample contained by the well.	100-character string
Target Name	The name of the target assay assigned to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
Reporter	The reporter dye that labels the probe for the target assay.	100-character string
Quencher	The quencher dye that labels the probe for the target assay.	100-character string
Variant Calls	The variant call assigned to the sample at the specified well.	`Hetero`, `Homo 1`, or `Homo 2`
Confidence Value	The calculated confidence of the automatic variant call.	Float (1–100)
CT	The calculated threshold cycle (C_T) for the target at the specified well.	Float
Ct Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Ct SD	The standard deviation of the average C_T of the replicate wells for the specified target.	Float
Number of Flags	The number of quality flags generated by the sample during the analysis.	Integer
Tm	Melting point.	Float
Tm1... Tm3	The first, second, and third melting temperatures (T_m) calculated in degrees Celsius.	Float
Comments	Additional text that describes the well.	1024-character string
Custom1... Custom6	The contents of the custom text fields found in the Results table of the experiment.	1024-character string (per field)
If analysis flags are present, results data is present in additional columns named for the associated flags.		`true` or `false`

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Presence/absence

The following table describes the results data exported from presence/absence experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Sample Name	The name of the sample contained by the well.	100-character string
Target Name	The name of the target assay assigned to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
Reporter	The reporter dye that labels the probe for the target assay.	100-character string
Quencher	The quencher dye that labels the probe for the target assay.	100-character string
Rn	The raw fluorescence for the associated well normalized to the fluorescence of the passive reference dye.	Float
Rn Mean	The averaged normalized fluorescence (R_n) for the associated replicate wells that contain the same target/sample combination.	Float
Rn SD	The standard deviation of the normalized fluorescence (R_n) for the associated replicate wells that contain the same target/sample combination.	Float
Threshold Value	The calculated value of the threshold for a positive call.	Float
Call	The presence/absence call assigned to the sample at the specified well.	`Negative Control`, `Blocked IPC Control`, `IPC Failed`, `Positive`, or `Negative`
Comments	Additional text that describes the well	1024-character string
Automatic Ct Threshold	Indicates whether the threshold was determined automatically (true) or manually (false).	`true` or `false`
Ct Threshold	The threshold cycle (C_T) for the sample at the well.	Float
Automatic Ct Baseline	Indicates whether the baseline was determined automatically (true) or manually (false).	`true` or `false`
Baseline Start	The first cycle used to calculate the baseline.	Float
Baseline End	The last cycle used to calculate the baseline.	Float
Custom1... Custom6	The contents of the custom text fields found in the Results table of the experiment.	1024-character string (per field)

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

7900 export format

The QuantStudio™ 12K Flex Software can export setup and results data from experiment files (.eds) to tab-delimited text files (txt) in a legacy export format of the Applied Biosystems® 7900HT Real-Time PCR System. The 7900 export format features a standardized data structure and markup to maximize accessibility by downstream applications. Data exported in the QuantStudio12KFlex export format can be opened by common spreadsheet applications, such as Microsoft® Excel®, or imported by laboratory information management system (LIMS) applications that have been configured to parse the file format.

Note: Due to the very different nature of the QuantStudio™ 12K Flex Instrument some export types are not available.

Note: Column customization (sorting and omission) is not available. Only multiple tab-delimited text files are supported.

Exportable files

The following table shows the data files that the QuantStudio™ 12K Flex Software can export in the 7900 export format. Each row represents a single exportable data file.

File	Description	See...
Setup file	Describes the configuration of samples on the experiment consumable, including sample location, target or SNP assay properties, and task assignments.	page 223
Multicomponent file	Contains the spectral data used by the QuantStudio™ 12K Flex Software to generate the multicomponent plot that displays the contribution of each dye over the duration of the PCR run.	page 225
Results file	Contains the normalized, processed, and analyzed data generated by the QuantStudio™ 12K Flex Software.	page 226

Setup file

When setup file is selected as an export option, the QuantStudio™ 12K Flex Software exports sample setup data to a stand-alone file. The sample setup file describes the sample configuration on the experiment consumable, including sample and assay data, positions, and task assignments.

File header

The file begins with several lines, shown in the following table, that describe the experiment file and the QuantStudio™ 12K Flex Instrument for which it is designed.

Category	Component	Output
File Version	Defines the version of Setup File format used to generate the document.	Integer
Plate Size	Defines the number of wells in the plate modeled by the file (for example, 96/384).	Integer
Plate ID	Defines the ID of the Assay Plate. Normally this is a barcode printed on the plate.	100-character string

```
*** Setup File Version  <version number>
*** Output Plate Size    <number of wells>
*** Output Plate ID      <plate id>
```

Assay (detector) data

The assay data describes the qualities of the target assays present on the consumable. (In the context of the 7900HT System, target assays are referred to as "detectors".) The section consists of multiple lines that define the total target assays followed by a column header and tab-separated data. The first line defines the total number of target assays on the consumable formatted as follows:

```
*** Number of Detectors   <number of assays>
```

The column header defines the columns of exported data followed by one or more lines, where each row defines the properties of a single assay separated by tab characters.

Column	Description	Output
Detector	The name of one target in the well, if applicable. If a well contains multiple targets one row is used per target.	100-character string
Reporter	The reporter dye that labels the probe for the target assay.	100-character string
Quencher	The quencher dye that labels the probe for the target assay.	100-character string
Description	The standard.	1024-character string
Comments	The additional text that describes the well.	1024-character string

Well data

After the assay data, the QuantStudio™ 12K Flex Software exports the well data that describes the configuration of samples and assays on the experiment consumable. The table below describes the well data that can be exported from absolute quantification, relative quantification, or presence/absence experiments. If a well contains more than one assay, the QuantStudio™ 12K Flex Software lists the setup data for each additional assay in additional columns to the right of the existing data.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Sample Name	The name of the sample contained by the well.	100-character string
Detector Name	The name of one target assay applied to the well, if applicable.	100-character string
Task	Task the target is used for in this well.	`UNKNOWN`, `STANDARD`, or `NTC`
Quantity	The standard quantity (if applicable). This column only appears for Standard Curve and Relative Standard Curve experiments	Float or Integer

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Multicomponent file

The QuantStudio™ 12K Flex Software can export the data used to generate the multicomponent plot of a real-time PCR experiment. The multicomponent data tracks the raw fluorescence of all reporter dyes present on the reaction consumable throughout the duration of the experiment run.

The file begins with a line that names the export format (SDS 2.3) and the type of data contained by the file (multicomponent). A column header occurs next followed by the multicomponent data, where each row contains the data for a single well separated by tab characters. The multicomponent data contains a dye column for each dye present on the reaction consumable, including reporter dyes, quencher dyes (except non-fluorescent dyes), and the passive reference.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Time	The time in milliseconds after the start of the run when the reading was taken.	Integer
Temp	The temperature (°C) of the sample when the QuantStudio™ 12K Flex Instrument recorded the fluorescence data.	Integer
Cycle	The cycle of the run during which the QuantStudio™ 12K Flex Instrument recorded the fluorescence data.	Integer
<*Dye name*>	The raw fluorescence for the designated dye measured by the QuantStudio™ 12K Flex Instrument at the specified well and cycle.	Float

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Results file

When selected as an export option, the QuantStudio™ 12K Flex Software exports sample setup data to a stand-alone file. The sample setup file describes the sample configuration on the experiment consumable, including sample and assay data, positions, and task assignments.

File header

The file begins with a line that names the export format (SDS 2.3) and the type of data contained by the file (Std Results). The following lines, listed in the table below, describe the qualities of the QuantStudio™ 12K Flex Instrument and several other general experiment properties.

Field	Description	Output
Filename	The path to the experiment file on the local computer hard drive.	<*filename*>
PlateID	The plate identifier entered into the barcode filed of the experiment.	<*100-character string*>
Assay Type	The type of chemistry application for which the experiment is designed.	`Standard Curve`, `Presence/Absence`, `Relative Standard Curve`, or `DDCt Quantification`
Run Datetime	The date and time that the QuantStudio™ 12K Flex Instrument finished running the experiment.	<*date and time*>
Operator	The user logged into the QuantStudio™ 12K Flex Software at the time the experiment was run.	<*100-character string*>
ThermalCycleParams	The thermal cycling profile for the experiment.	`96/384-well` or `array card`

The QuantStudio™ 12K Flex Software can export the results data from an analyzed experiment file. The format and content of the results data depends on the experiment type and the analysis settings.

The section begins with a column header followed by the results data, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the QuantStudio™ 12K Flex Software lists the data for each additional assay on separate rows, repeating the well number and sample information.

This section describes the following results data formats:

Standard Curve, Relative Standard Curve, and Comparative C_T experiments

The following table describes the results data exported from standard curve, relative standard curve and comparative C_T experiments.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Sample Name	The name of the sample contained by the well.	100-character string
Detector Name	The name of the target assay added to the well.	100-character string
Reporter	The reporter dye that labels the probe for the target assay.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN`, `NTC`, or `STANDARD`
CT	The calculated threshold cycle (C_T) for the target at the specified well.	Float
Quantity	• Unknown wells – The calculated quantity for the sample at the well. • Standard wells – The quantity assigned to the standard at the well.	Float
Quantity Mean	• Unknown wells – The average quantity of the replicate wells for the target/sample. • Standard wells – The quantity assigned to the replicate wells for the target/sample.	Float
Quantity SD	The standard deviation of the average quantity of the replicate wells for the target/sample combination.	Float
Ct Median	The median C_T of the replicate wells for the specified target/sample combination.	Float
Ct Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Ct SD	The standard deviation of the average C_T of the replicate wells for the specified target.	Float
Automatic Ct Baseline	Indicates whether the baseline was determined automatically (true) or manually (false).	`TRUE` or `FALSE`
Baseline Start	The first cycle used to calculate the baseline.	Integer
Baseline End	The last cycle used to calculate the baseline.	Integer
Automatic Ct Threshold	Indicates whether the threshold was determined automatically (true) or manually (false).	`TRUE` or `FALSE`
Ct Threshold	The C_T for the sample at the well	Float

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Genotyping experiments

The following table describes the results data exported from genotyping experiments.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)‡
Sample Name	The name of the sample contained by the well.	100-character string
SNP Assay Name	The name of the SNP assay added to the well.	100-character string
Allele1 Rn	The raw fluorescence associated with the allele 1 probe of the SNP assay at the well normalized to the fluorescence of the passive reference dye.	Float
Allele2 Rn	The raw fluorescence associated with the allele 2 probe of the SNP assay at the well normalized to the fluorescence of the passive reference dye.	Float
Call	The allele call assigned to the sample at the specified well.	`Homozygous` <*allele x*/*allele x*>, `Heterozygous` <*allele x*/*allele y*>, or `Negative Control (NC)`
Quality(%)	The confidence of the automatic allele call.	Float (1–100)
Method	The method used to call alleles.	`Auto` or `Manual`
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
Pass. Ref	The raw fluorescence of passive reference at the well.	Float

‡ Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

RDML export format

The QuantStudio™ 12K Flex Software can export data from real-time quantitative PCR experiments as well-formed Real-time PCR Data Markup Language (RDML), a structured extensible markup language (XML) standard for quantitative PCR (qPCR) data. In combination with the Minimal Information (MIQPCR) guidelines, the RDML element structure describes all aspects of a qPCR experiment, including setup, analysis, and data interpretation. The exported RDML data is saved as a flat text file that can be used to transfer qPCR data between the QuantStudio™ 12K Flex Software and third-party applications.

IMPORTANT! The RDML export format is available only for standard curve, gene expression, and relative standard curve experiments.

For more information

The RDML standard is maintained by the RDML consortium, an organization that consists of key developer groups and a member community. For more information on the RDML format, visit the RDM organization website (**www.rdml.org**). The website features free data management tools, including an on-line RDML file generator and RDML software libraries.

F

Parts and Materials

This appendix covers:

How to order

You can order materials and accessories from Life Technologies by ordering directly from the Life Technologies online store.

Note: Product availability and pricing may vary according to your region or country. Online ordering through Life Technologies is not available in all countries. Contact your local Life Technologies representative for help.

To order through the website or the QuantStudio™ 12K Flex 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:

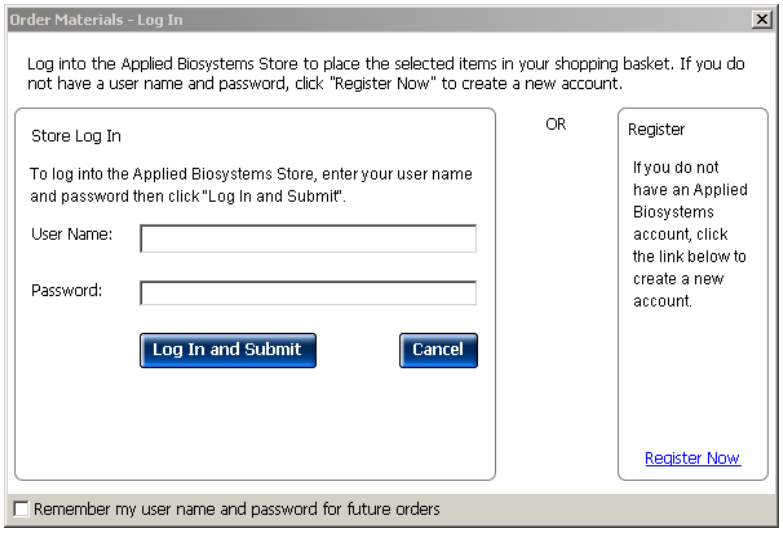
Operating system	Microsoft® Internet Explorer®	Apple® Safari®	Mozilla® Firefox®	Adobe® Acrobat® Reader®
Microsoft® Windows®	v6.x or later	None†	v2.x or later	v4.0 or later
Macintosh®	None†	v2.0.4 or later		

† Browser not available for this platform.

Note: Confirm that cookies and Javascript are turned on for the website to function correctly.

Ordering from the QuantStudio™ 12K Flex Software

1. To find your assay on the Life Technologies Store, complete the Find Assay pane in the QuantStudio™ 12K Flex 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 Applied Biosystems® Store, follow the prompts to complete your order.

Ordering 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	1. Go to **www.lifetechnologies.com/quantstudio** 2. Click **Parts and accessories**. 3. Select the desired components, the complete the order as instructed. See "Consumables" on page 236 for a complete list of compatible instrument parts, accessories, and kits.
Calibration kits	

Accessories

The following accessories are to be used with the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System.

QuantStudio™ 12K Flex System accessories	Part no.
384-Well Plate Adapter	4457087
384-Well/Array Card Heated Cover	4453555
96-Well Heated Cover	4453560
96-Well Plate Adapter	4459845
96-Well Tube Adapter	4462077
Array Card Plate Adapter	4454166
Fast 96-Well Heated Cover	4459838
Fast 96-Well Plate Adapter	4459846
Fast 96-Well Tube Adapter	4462078
OpenArray® Heated Cover	4471049
OpenArray® Plate Adapter	4454166
QuantStudio™ 12K Flex System 384-Well Sample Block	4453553
QuantStudio™ 12K Flex System 96-Well Sample Block	4453556
QuantStudio™ 12K Flex System Array Card Sample Block	4453554
QuantStudio™ 12K Flex System Fast 96-Well Sample Block	4453559
QuantStudio™ 12K Flex System OpenArray® Sample Block	4471025

Calibration and verification kits

The following kits are to be used with the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System.

The following materials are required to calibrate the QuantStudio™ 12K Flex System:

Note: For reagent or consumable shelf-life expiration date, see the package label.

384-well sample block kits

QuantStudio™ 12K Flex System consumable	Part no.	Storage (°C)
384-Well Spectral Calibration Plate with FAM™ Dye	4432271	–15 to –25
384-Well Spectral Calibration Plate with VIC® Dye	4432278	
384-Well Spectral Calibration Plate with ROX™ Dye	4432284	
384-Well Spectral Calibration Plate with NED™ Dye	4432302	
384-Well Spectral Calibration Plate with SYBR® Green Dye	4432290	
384-Well Spectral Calibration Plate with TAMRA™ Dye	4432296	
384-Well Region of Interest (ROI) and Background Plates • 384-Well Region of Interest (ROI) Calibration Plate • 384-Well Background Plate	4432320	
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	4432308	
Kit, TaqMan® RNase P Fast 384-Well Instrument Verification Plate • 384-Well TaqMan® RNase P Fast Instrument Verification Plate	4455280	

96-well sample block kits

QuantStudio™ 12K Flex System consumable	Part no.	Storage (°C)
96-Well Spectral Calibration Plate with FAM™ Dye	4432327	–15 to –25
96-Well Spectral Calibration Plate with VIC® Dye	4432334	
96-Well Spectral Calibration Plate with ROX™ Dye	4432340	
96-Well Spectral Calibration Plate with SYBR® Green Dye	4432346	
96-Well Spectral Calibration Plate with TAMRA™ Dye	4432352	
96-Well Spectral Calibration Plate with NED™ Dye	4432358	
96-Well Region of Interest (ROI) and Background Plates • 96-Well Region of Interest (ROI) Calibration Plate • 96-Well Background Plate	4432364	
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	4432370	
Kit, TaqMan® RNase P 96-Well Instrument Verification Plate • TaqMan® RNase P 96-Well Instrument Verification Plate	4432382	

Fast 96-well sample block kits

QuantStudio™ 12K Flex System consumable	Part no.	Storage (°C)
Fast 96-Well Spectral Calibration Plate with FAM™ Dye	4432389	–15 to –25
Fast 96-Well Spectral Calibration Plate with VIC® Dye	4432396	
Fast 96-Well Spectral Calibration Plate with ROX™ Dye	4432402	
Fast 96-Well Spectral Calibration Plate with SYBR® Green Dye	4432408	
Fast 96-Well Spectral Calibration Plate with TAMRA™ Dye	4432414	
Fast 96-Well Spectral Calibration Plate with NED™ Dye	4432420	
Fast 96-Well Region of Interest (ROI) and Background Plates • Fast 96-Well Region of Interest (ROI) Calibration Plate • Fast 96-Well Background Plate	4432426	
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	4432432	
Kit, TaqMan® RNase P Fast 96-Well Instrument Verification Plate • TaqMan® RNase P Fast 96-Well Instrument Verification Plate	4351979	

Array card sample block kits

QuantStudio™ 12K Flex System consumable	Part no.	Storage (°C)
Array Card Spectral Calibration Dye Kit • TaqMan® Array Calibration with FAM™ Dye • TaqMan® Array Calibration with VIC® Dye • TaqMan® Array Calibration with ROX™ Dye • TaqMan® Array Calibration with ROI Dye • TaqMan® Array Calibration with FAM™/ROX™ Dye • TaqMan® Array Calibration with VIC®/ROX™ Dye • TaqMan® Array Background Buffer	4432376	–15 to –25
Kit, TaqMan® 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	4432265	

Consumables

Note: For consumable shelf-life expiration date, see the package label.

The following consumables are to be used with the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System.

QuantStudio™ 12K Flex System consumable		Part no.
Applied Biosystems® Array Card Staker/Sealer		4331770
Array Card Bucket/Clip Set	1st Generation	4337762
	2nd Generation	4442571
Array Cards, 8-Port (Empty)	Empty Array Card Kit, 4-pk	4334812
	Empty Array Card Kit	4351471
Centrifuge Buckets, Array Card	1st Generation	4337230
	2nd Generation	4442573
Clip, Array Card Centrifuge Adapter		4334682
MicroAmp® Fast 8-Tube Strip, 0.1-mL	125 strips	4358293
MicroAmp® Fast Optical 96-Well Reaction Plate with Bar Code, 0.1-mL	10 plates	4346906
	200 plates	4366932
MicroAmp® Optical 96-Well Reaction Plate, 0.2-mL	10 plates	N8010560
	500 plates	4316813
MicroAmp® Optical 96-Well Reaction Plate with Bar Code, 0.2-mL	10 plates	4306737
	500 plates	4326659
MicroAmp® Optical 384-Well Reaction Plate	1000 plates	4343370
MicroAmp® Optical 384-Well Reaction Plate with Bar Code	1000 plates	4343814
	500 plates	4326270
	50 plates	4309849
MicroAmp® Optical 8-Cap Strip	300 strips	4323032
MicroAmp® Optical 8-Tube Strip, 0.2-mL	1000 tubes	4316567
MicroAmp® Optical Adhesive Film		4311971

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Safety

This appendix covers:

Instrumentation safety

Symbols on instruments

Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on Life Technologies instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates that the device receives or supplies direct current or voltage.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety symbols

The following table describes the safety symbols that may be displayed on Life Technologies devices. Each symbol may appear by itself or with text that explains the relevant hazard. These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should proceed with appropriate caution and 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.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.

Symbol	Description
	Indicates the presence of a pinching hazard and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.
	Indicates the presence of a biological hazard and to proceed with appropriate caution.
	Indicates the presence of a laser light in the instrument and to proceed with appropriate caution.
	Indicates the presence of an ultraviolet light and to proceed with appropriate caution.
	Indicates the presence of a slipping hazard and to proceed with appropriate caution.
	Indicates the presence of a radiological hazard and to proceed with appropriate caution.

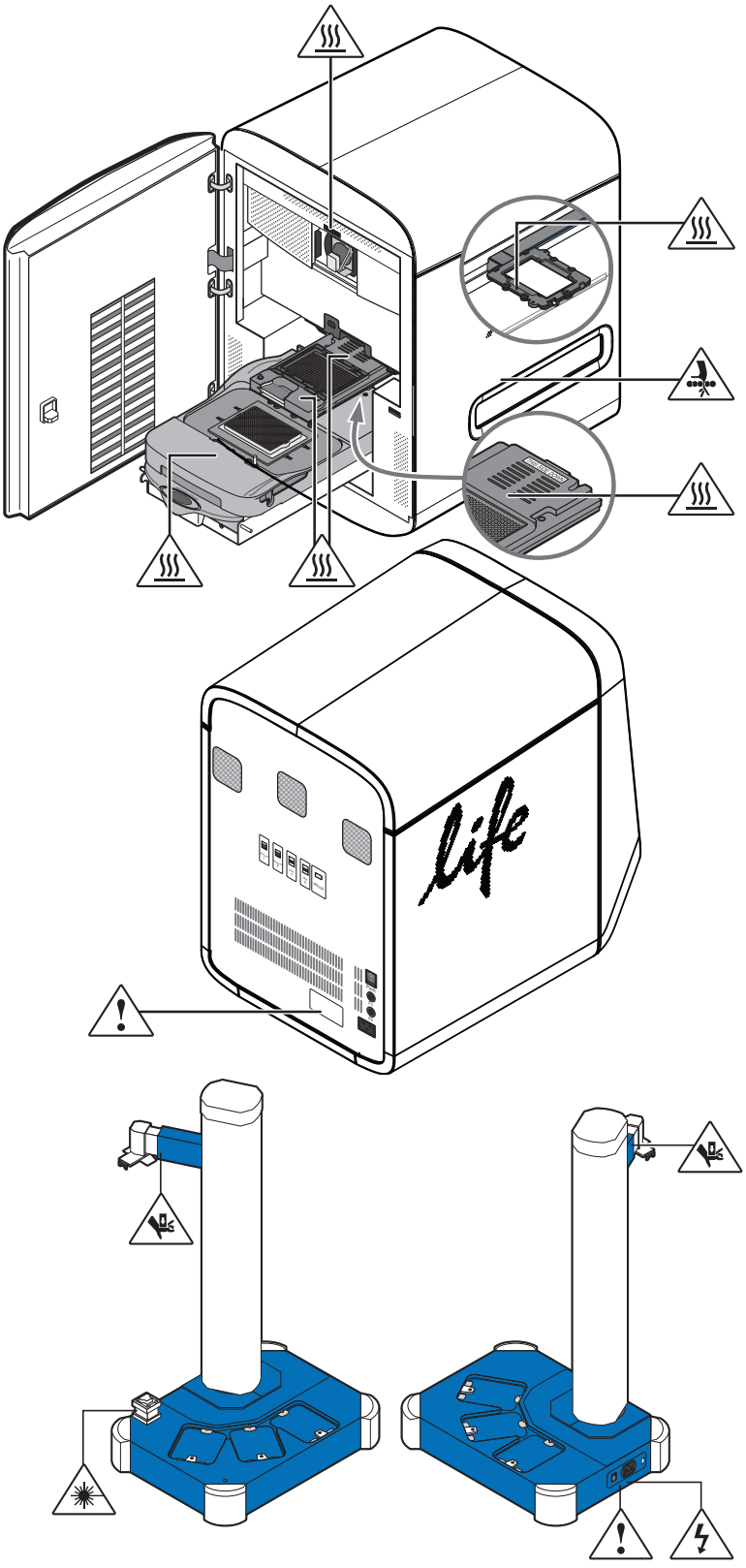
Environmental symbols on instruments

The following symbol applies to all Life Technologies electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your local Life Technologies Customer Service office for equipment pick-up and recycling. See **www.lifetechnologies.com** for a list of customer service offices in the European Union.

Locations of safety labels on instruments

The QuantStudio™ 12K Flex Instrument contains warnings at the locations shown below:



General instrument safety

WARNING! PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by Life Technologies may result in personal injury or damage to the instrument.

Moving and lifting the instrument

CAUTION! PHYSICAL INJURY HAZARD. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and lifting stand-alone computers and monitors

WARNING! Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure 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.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs).

Cleaning or decontaminating the instrument

CAUTION! Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Physical hazard safety

Ultraviolet light

WARNING! ULTRAVIOLET LIGHT HAZARD. Looking directly at a UV light source can cause serious eye damage. Never look directly at a UV light source and always prevent others from UV exposure. Follow the manufacturer's recommendations for appropriate protective eyewear and clothing.

Moving parts

WARNING! PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Electrical safety

WARNING! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the QuantStudio™ 12K Flex Instrument without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

WARNING! FIRE HAZARD. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

WARNING! ELECTRICAL HAZARD. Grounding circuit continuity is required for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

WARNING! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

WARNING! ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage rating

The QuantStudio™ 12K Flex System has an installation (overvoltage) category of II, and is classified as portable equipment.

Bar code scanner laser safety

Laser classification

The bar code scanners included with the QuantStudio™ 12K Flex Instrument 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

This section provides information on:

- U.S. and Canadian safety standards
- Canadian EMC standard
- European safety and EMC standards
- Australia and New Zealand EMC standards

U.S. and Canadian safety standards

The instrument has been tested to and complies with standard:

UL 61010-1:2nd Edition/CSA C22.2 No. 61010-1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

UL 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

Canadian EMC standard

This instrument has been tested to and complies with standard:

ICES-001, Issue 3: "Industrial, Scientific, and Medical Radio Frequency Generators."
Cet appareil numerique de la classe B est conforme a la norme NMB-001 du Canada.

European safety and EMC standards

This instrument meets European requirements for safety (Low Voltage Directive 2006/95/EC). This instrument has been tested to and complies with standards:

EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements."

EN 61010-2-010:2003, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

EN 61010-2-081:2002+A1:2003, "Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes."

The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR Instrument meets European requirements for emission and immunity (EMC Directive 2004/108/EC).

EN 61326-1:2006 "Electrical equipment for measurement, control and laboratory use-Part 1 General EMC requirements." (Group 1, Class B)

Australia and New Zealand EMC standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. 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. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- 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.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

Obtaining SDSs

The SDS for any chemical supplied by Life Technologies is available to you free 24 hours a day. To obtain SDSs:

1. Go to **www.lifetechnologies.com**, click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Life Technologies instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide 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.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument 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.

Biological hazard safety

General biohazard

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* (**www.cdc.gov/biosafety/publications/index.htm**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **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 **http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Safety alerts

General alerts for all chemicals

Avoid contact with (skin, eyes, and/or clothing). Read the SDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

General alerts for instrumentation

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

WARNING! This instrument is designed for 12 V, 75 W halogen LED only.

Specific alerts for instrumentation

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 instrument.

CAUTION! PHYSICAL INJURY HAZARD. Do not attempt to lift the instrument 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 instrument. At least two people are required to lift the instrument.

CAUTION! PHYSICAL INJURY HAZARD. Do not remove the instrument cover. There are no components inside the instrument that you can safely service yourself. If you suspect a problem, contact an Life Technologies Service Representative.

WARNING! PHYSICAL INJURY HAZARD. The QuantStudio™ 12K Flex System and LED are hot! The LED can become very hot while in use. Allow the LED to cool for 15 minutes and put on protective, powder-free gloves before handling it.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

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

Documentation and Support

Related documentation

The following related documents are provided with the system:

Document	Part no.	Description
Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Multi-well Plates and Array Card Experiments User Guide	4470050	Contains five individual booklets that explain how to perform the six different experiments on the QuantStudio™ 12K Flex Instrument The experiments include Standard Curve, Relative Standard Curve and Comparative C_T, Genotyping, Presence/ Absence and Melt Curve. Each Getting Started Guide booklet functions as both: • A tutorial, using example experiment data provided with the QuantStudio™ 12K Flex Software. • A guide for your own experiments. Intended for laboratory staff and principal investigators who perform experiments using the QuantStudio™ 12K Flex System.
Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Quick Reference Guide	4470688	Explains how to install and maintain the QuantStudio™ 12K Flex Instrument Intended for laboratory staff responsible for the use and maintenance of the QuantStudio™ 12K Flex Instrument.
Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Site Preparation Guide	4470654	Explains how to prepare your site to receive and install the QuantStudio™ 12K Flex Instrument Intended for personnel who schedule, manage, and perform the tasks required to prepare your site for installation of the QuantStudio™ 12K Flex Instrument.
Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Installation Guide	4442662	Explains how to install and maintain the ViiA™ 7 Real-Time PCR Instrument Intended for laboratory staff responsible for the installation and maintenance of the ViiA™ 7 Real-Time PCR Instrument.
QuantStudio™ 12K Flex Software Help	NA	Explains how to use the QuantStudio™ 12K Flex Software to: • Set up, run, and analyze experiments. • Monitor a networked QuantStudio™ 12K Flex Instrument. • Calibrate the QuantStudio™ 12K Flex Instrument. • Verify the performance of QuantStudio™ 12K Flex Instrument with an RNase P run. • Intended for: – Laboratory staff and principal investigators who perform experiments using the QuantStudio™ 12K Flex System. – Laboratory staff responsible for the installation and maintenance of the QuantStudio™ 12K Flex Instrument.

Note: For additional documentation, see "Obtaining support" on page 252.

Obtaining information from the Help system

The QuantStudio™ 12K Flex System has 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™ 12K Flex Software window.
- Select **Help** ▸ **QuantStudio™ 12K Flex 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/support

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.
- Obtain information about customer training.
- Download software updates and patches.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Glossary

AIF	See assay information file (AIF).
AIX	XML version of the assay information file. See also assay information file (AIF).
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).
allelic discrimination plot	Display of genotyping data collected during the post-PCR read. The allelic discrimination plot is a graph of the normalized reporter signal from the allele 1 probe, plotted against the normalized reporter signal from the allele 2 probe.
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 TaqMan chemistry, the last two steps of a PCR stage are typically combined. See also cycling stage.
Analysis Settings Library	In the software, a collection of analysis settings to use in experiments. You can save settings and reuse them. You cannot edit or import settings into the library.

assay	In a PCR reaction mix, two target-specific primers or two primers and a probe used to amplify a target.
Assay ID	Identifier assigned by Life Technologies to TaqMan® assays.
assay information file (AIF)	Tab-delimited data file on a CD shipped with each assay order. The AIF contains technical details about all assays in the shipment. It includes information about assay concentrations; reporters and quenchers used; part and lot numbers; and assay, vial, and plate ID numbers. The file name includes the number from the bar code on the plate.
assay mix	PCR reaction component in Applied Biosystems TaqMan® assays. The assay mix contains primers designed to amplify a target and a TaqMan® probe designed to detect amplification of the target.
AutoDelta	In the run method, a setting to increase or decrease the temperature and/or time for a step with each subsequent cycle in a cycling stage. When AutoDelta is enabled for a cycling stage, the settings are indicated by an icon in the thermal profile: • AutoDelta on: ▲ • AutoDelta off: ▲
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 (C_T).
background calibration	Type of calibration in which the instrument performs reads of a background plate, 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	In the amplification plot, a cycle-to-cycle range that defines background fluorescence. This range can be set manually on an assay-by-assay basis, or automatically to set each individual well.

baseline-corrected normalized reporter (ΔRn)	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. In the ΔRn vs Cycle amplification plot, ΔRn is calculated at each cycle as: ΔRn (cycle) = Rn (cycle) –Rn (baseline), where Rn = normalized reporter In genotyping experiments and presence/absence experiments, the difference in normalized fluorescence signal generated by the reporter between the pre-PCR read and the post-PCR read. In the allelic discrimination plot (genotyping experiments) and the presence/absence plot (presence/absence experiments), ΔRn is calculated as: ΔRn = Rn (post-PCR read) –Rn (pre-PCR read), where Rn = normalized reporter See also normalized reporter (Rn).
baseline threshold algorithm	Expression estimation algorithm (C_T) that subtracts a baseline component and sets a fluorescent threshold in the exponential region for gene quantification.
biological replicates	Reactions that contain identical components and volumes, but evaluate separate samples of the same biological source (for example, samples from three different mice of the same strain, or separate extractions of the same cell line or tissue sample). When an experiments uses biological replicate groups in a gene expression study, the values displayed in the Biological Replicates tab are calculated by combining the results of the separate biological samples and treating this collection as a single population (that is, as one sample). For ΔC_T computations (normalizing by the endogenous control) in a singleplex experiment, the software treats separate biological samples as unpaired data when computing variability estimates of the single biological replicate. Individual contributions of the separate biological samples to the single biological replicate results are observed in the Technical Replicates tab. See also technical replicates.
blocked IPC	In presence/absence experiments, a reaction that contains IPC blocking agent, which blocks amplification of the internal positive control (IPC). In QuantStudio™ 12K Flex 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.
calibrator	See reference sample.
chemistry	See reagents.
comparative C_T ($\Delta\Delta C_T$) method	Method for determining relative target quantity in samples. The software measures amplification of the target and of the endogenous control in samples and in a reference sample. Measurements are normalized using the endogenous control. The software determines the relative quantity of target in each sample by comparing normalized target quantity in each sample to normalized target quantity in the reference sample.
C_{RT}	See relative threshold cycle (C_{RT}).
C_{RT} algorithm	See Relative Threshold algorithm.
C_T	See threshold cycle (C_T).

C_T algorithm	Algorithm used to determine the threshold cycle. The software provides two C_T algorithms: Baseline Threshold and Relative Threshold.
custom dye	Dye that is not precalibrated for an instrument. Custom dyes that fall within the emission wavelength range of the instrument can be added and adapted for use in experiments on the QuantStudio™ 12K Flex Instrument. To use a custom dye, add the dye to the Dye Library and perform a dye calibration.
cycle threshold	See threshold cycle (C_T).
cycling stage	In the thermal profile, a stage that is repeated. A cycling stage is also called an amplification stage. See also amplification stage.
C_q	See quantification cycle (C_q).
data collection	During the instrument run, a process in which an instrument detects fluorescence data from each well of the reaction plate. The instrument transforms the signal to electronic data and saves the data in the experiment file. In the QuantStudio™ 12K Flex Software, a data collection point is indicated by an icon in the thermal profile: • Data collection on:  • Data collection off: 
delta R_n (ΔR_n)	See baseline-corrected normalized reporter (ΔRn).
diluent	A reagent used to dilute a sample or standard before it is added to the PCR reaction.
dilution factor	See serial factor.
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.
Dye Library	In the software, a collection of dyes to use in experiments. Custom dyes can be added to the library, but system dyes cannot be removed. Before using a dye, make sure that the dye calibration is current in the Instrument Console.
EFF%	See amplification efficiency (EFF%).
efficiency correction	In Comparative C_T experiments, a feature that allows you to manually enter previously-determined amplification efficiencies for each experiment, following the experimental run. The real-time software mathematically compensates for differences in efficiency between each target assay and the endogenous control when calculating sample-to-sample relative quantities. This method can be employed as a substitute for the Relative Standard Curve Method.

endogenous control	A gene that is used to normalize template differences and sample-to-sample or run-to-run variation.
endpoint read	See post-PCR read.
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.
experiment	Refers to the entire process of performing a run, including setup, run, and analysis. You can perform the following types of experiments: • Quantification - Standard curve • Quantification - Relative standard curve • Quantification - Comparative C_T ($\Delta\Delta C_T$) • Melt Curve • Genotyping • Presence/absence
experiment document	The Life Technologies name for the electronic records that comprise all information about a particular plate or array card consumable, including metadata (name, bar code, comments), plate setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, analysis results, audit records, and other plate-specific data. Experiment documents have the suffixes .eds (experiment document single), .edt (template), and .edm (multiple).
experiment name	Entered during experiment setup, the name that is used to identify the experiment.
Experiment Setup	A software feature that allows you to set up an experiment according to your experiment design. Experiment Setup provides you with maximum flexibility in the design and setup of your experiment.
experiment type	The type of experiment to perform: • Standard curve • Comparative C_T ($\Delta\Delta C_T$) • Relative standard curve • Genotyping • Presence/absence • Melt curve The experiment type that you select affects setup, run, and analysis.
export	A software feature that allows you to export experiment setup files, experiment results, instrument information, and security and auditing settings to spreadsheet, presentation, or text files. You can edit the default location of the exported file.

filter	Dye excitation and emission filter combination that you select for an experiment. The QuantStudio™ 12K Flex System includes a six-color filter set that supports FAM™, NED™, ROX™, SYBR® Green, TAMRA™, and VIC® dyes.
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.
genotyping experiment	An experiment used to identify known mutations in a DNA sample. With this experiment type, you can determine if a DNA sample is: • Homozygous (samples having only allele 1). Also called wild type homozygote. • Homozygous (samples having only allele 2). Also called variant homozygote. • Heterozygous (samples having both allele 1 and allele 2).
heterozygote	Samples having both allele 1 and allele 2. See also genotyping experiment.
holding stage	In the thermal profile, the stage that holds the temperature constant for a defined period of time. A stage that includes one or more steps. You can add a holding stage to the thermal profile to activate enzymes, to inactivate enzymes, or to incubate a reaction.
homozygote	Samples having only allele 1 or only allele 2. See also genotyping experiment.
housekeeping gene	A gene that is involved in basic cellular functions and that may be constitutively expressed. Housekeeping genes may be candidates for use as endogenous controls; however, their constancy should always be validated experimentally. See also endogenous control.
import	A software feature that allows you to import plate setup information or security settings before an experiment run. You can also import information into some libraries in the system.
Instrument Console	A software feature that allows you to view information about instruments on the network. In the Instrument Console, you can 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 view information about instrument available on the network. In the Instrument Manager, you can 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.
inventoried assays	TaqMan® Gene Expression Assays and TaqMan® SNP Genotyping Assays that have been previously manufactured, passed quality control specifications, and stored in inventory.
IPC	See internal positive control (IPC).
IPC blocking agent	Reagent added to PCR reactions to block amplification of the internal positive control (IPC).
IPC+	See negative control-IPC wells.
made-to-order assays	TaqMan® Gene Expression Assays that are manufactured at the time of order. Only assays that pass manufacturing quality control specifications are shipped.
manual baseline	An analysis setting for the Baseline Threshold algorithm. You enter the baseline start and end cycles for the amplification plot. See also baseline.
manual threshold	An analysis setting for the Baseline Threshold algorithm. You enter the threshold value and select whether to use automatic baseline or manual baseline values. The software uses the baseline and the threshold values to calculate the threshold cycle (C_T).
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. In a high resolution melting experiment, you can view the melt curve as fluorescence vs. temperature. Also called dissociation curve.
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 (T_m).
multicomponent plot	A plot of the complete spectral contribution of each dye for the selected well(s) over the duration of the PCR run.

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 amplification control (NAC)	See negative control-blocked IPC wells.
no template control (NTC)	See negative control (NC).
nonfluorescent quencher-minor groove binder (NFQ-MGB)	Molecules that are attached to the 3′ end of TaqMan® 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. This file is used in comparisons of data from multiple instruments within a study.
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 (Rn)	Fluorescence signal from the reporter dye normalized to the fluorescence signal of the passive reference dye (usually ROX dye on Life Technologies instruments).
omit well	An action that you perform before reanalysis to omit one or more wells from analysis. Because no algorithms are applied to omitted wells, omitted wells contain no results. You can add wells back in to the analysis; no information is permanently discarded.
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 reaction plate. The number of rows and columns in the grid depends on the sample block that you use. In 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.
plate setup file	A file (.txt, .csv, .xml, or .sds) that contains setup information such as the well number, sample name, sample color, target name, dyes, and other reaction plate contents.
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.
post-PCR read	In genotyping and presence/absence experiments, the part of the instrument run that occurs after amplification. In genotyping experiments, fluorescence data collected during the post-PCR read are displayed in the allelic discrimination plot and used to make allele calls. In presence/absence experiments, fluorescence data collected during the post-PCR read are displayed in the presence/absence plot and used to make detection calls. Also called endpoint read.
pre-PCR read	In genotyping and presence/absence experiments, the part of the instrument run that occurs before amplification. The pre-PCR read is optional but recommended. Fluorescence data collected during the pre-PCR read can be used to normalize fluorescence data collected during the post-PCR read.
primer mix	PCR reaction component that contains the forward primer and reverse primer designed to amplify the target.
primer/probe mix	PCR reaction component that contains the primers designed to amplify the target and a TaqMan® probe designed to detect amplification of the target.
pure dye	Fluorescent compound used to calibrate the instrument. See system dye.
quantification cycle (C_q)	The fractional PCR cycle used for quantification, according to the Real-time PCR Data Markup Language (RDML) data standard. C_T and C_{RT} are the algorithm-specific calculations of C_q.

quantification method	In quantification experiments, the method used to determine the quantity of target in the samples.
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 TaqMan® probes to prevent the reporter from emitting fluorescence signal while the probe is intact. With TaqMan® reagents, a nonfluorescent quencher-minor groove binder (NFQ-MGB) can be used as the quencher. With SYBR® Green reagents, no probe (and therefore no quencher) is used.
QuickStart	A feature that allows you to run an experiment without entering plate setup information, if your instrument and computer are in the same network. QuickStart requires an experiment template file.
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.
ramp	The step at which the temperature changes during the instrument run. The ramp rate is defined as °C per second. In the graphical view of the thermal profile, the ramp rate is indicated by a diagonal line.
ramp speed	Speed at which the temperature ramp occurs during the instrument run. Available ramp speeds include fast and standard.
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.
Real-time PCR Data Markup Language (RDML)	A reporting format that is compliant with the Minimum Information for Publication for Quantitative Real-Time Experiments (MIQE) guidelines.
reference	In an HRM experiment, the melt curve selected by a user in the difference plot to use as a basis for comparison. The software displays the aligned data as the difference in fluorescence between the reference curve and the other melt curves.

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.
refSNP ID	The reference SNP (refSNP) cluster ID. Generated by the Single Nucleotide Polymorphism Database of Nucleotide Sequence Variation (dbSNP) at the National Center for Biotechnology Information (NCBI). The refSNP ID can be used to search the Life Technologies Store for an Applied Biosystems SNP Genotyping Assay. Also called an rs number.
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. 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	An experimental 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.
Relative Threshold algorithm	Expression estimation algorithm (C_{RT}) which calculates a relative threshold from a fitted efficiency model for gene quantification.
relative threshold cycle (C_{RT})	The PCR cycle number for the threshold calculated from the modeled amplification efficiency profile.
replicate group	A user-defined biological grouping. A replicate group may be a set of identical reactions in an experiment.
replicates	Total number of identical reactions containing identical components and identical volumes.

reporter	A fluorescent dye used to detect amplification. With TaqMan® reagents, the reporter dye is attached to the 5′ end. With SYBR® Green reagents, the reporter dye is SYBR® Green dye. SYBR® and HRM-specific dyes are DNA-binding dyes.
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 and precalibrated on the instrument. ROX dye is used as the passive reference.
rs number	See refSNP ID.
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 *.csv) that contains the following setup information: well number, sample name, and custom sample properties.
Sample Library	In the software, an editable collection of sample names to use in experiments. The samples in the library contain the sample name and the sample color. The samples in the library may also contain comments about the sample.
sample/SNP assay reaction	In genotyping experiments, the combination of the sample to test and the SNP assay to perform in one PCR reaction. Each PCR reaction can contain only one sample and one SNP assay.
sample/target reaction	In quantification experiments, the combination of the sample to test and the target to detect and quantify in one PCR reaction.

security, auditing and eSignature

An optional 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 (eSignature)** – 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 10×, 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.

SNP

Single nucleotide polymorphism. The SNP can consist of a base difference or an insertion or deletion of one base.

SNP assay

Used in genotyping experiments, a PCR reaction that contains primers to amplify the SNP and two probes to detect different alleles.

SNP Assay Library

In the software, an editable collection of SNP assays to add to genotyping experiments. The SNP assays in the library contain the SNP assay name; SNP assay color; and for each allele, the allele name or base(s), reporter, quencher, and allele colors. The SNP assays in the library may also contain the assay ID and comments about the SNP assay.

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. In 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). You can turn data collection on or off for the ramp or the hold parts of the step. For cycling stages, a step is also defined by the AutoDelta status.

SYBR® Green reagents

PCR reaction components that consist of two primers designed to amplify the target and SYBR® Green dye to facilitate detection of the PCR product.

system dye — Dye supplied by Life Technologies and precalibrated on the QuantStudio™ 12K Flex System. Before you use system dyes in your experiments, make sure the system dye calibration is current in the Instrument Console.

The system dyes are:

- FAM™ dye
- JOE™ dye
- ROX™ dye
- NED™ dye
- SYBR® Green dye
- TAMRA™ dye
- VIC® dye

TaqMan® reagents — PCR reaction components that consist of primers designed to amplify the target and a TaqMan® probe designed to detect amplification of the target.

target — The nucleic acid sequence to amplify and detect.

target color — In the software, a color assigned to a target to identify the target in the plate layout and analysis plots.

Target Library — In the software, an editable collection of targets to use in experiments. Targets in the library contain the target name, reporter, quencher, and target color. The targets in the library may also contain comments about the target.

task — In the software, the type of reaction performed in the well for the target or SNP assay. Available tasks:

- Unknown
- Negative Control
- Standard (standard curve and relative standard curve experiments)
- Positive control (genotyping experiments)
- IPC (presence/absence experiments)
- Blocked IPC (presence/absence experiments)

technical replicates — Wells containing identical reaction components, including sample; important for evaluating precision.

temperature plot — In 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.

template file — A user-created file that contains experiment setup information (experiment type, sample names, target name, and thermal conditions) to be used as a starting point for new experiment setup. Template files have an .edt extension.

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.

threshold	• In amplification plots, the level of fluorescence above the baseline and within the exponential growth region. For the Baseline Threshold algorithm, the threshold can be determined automatically (see automatic threshold) or can be set manually (see manual threshold). • In presence/absence experiments, the level of fluorescence above which the software assigns a presence call.
threshold cycle (C_T)	The PCR cycle number at which the fluorescence meets the threshold in the amplification plot.
Tm	See melting temperature (T_m).
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™ 12K Flex System filters on data collected during an experiment.
unknown	In the software, the task for the target or SNP assay 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.
unknown-IPC wells	In presence/absence experiments, wells that contain a sample and internal positive control (IPC).
y-intercept	In 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.

Index

Numerics

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

Q

R

S

T

U

V

W

Y

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