appliedbiosystems

QuantStudio™ 12K Flex Real–Time PCR System

USER GUIDE

Maintenance and Administration

for use with QuantStudio™ 12K Flex Software v1.4

Publication Number 4470689

Revision E

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: 4470689 E (English)

Revision	Date	Description
E	17 October 2024	• Sealing instructions were updated ("Seal the OpenArray™ Plate" on page 118). • The maintenance schedule for the OpenArray™ Plate sample block was updated to include cleaning the sample block ("Recommended calibration and maintenance" on page 95). Instructions were added to clean the OpenArray™ Plate sample block ("Clean the OpenArray™ Plate sample block" on page 133). • Minor verbiage and formatting updates made throughout document.
D	17 February 2022	• Information about network and password security requirements was added ("Network and password security requirements" on page 20). • Information about validating workflows before upgrading software was added ("Perform internal validations before software upgrades" on page 38). • References to the *QuantStudio™ 12K Flex Real–Time PCR System v1.5 or later Maintenance and Administration Guide* (Pub. No. MAN0018832, for QuantStudio™ 12K Flex Software v1.5) were added. • The specifications, electrical requirements, and environmental requirements for the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System were updated. • The site to download software was updated ("Software for the QuantStudio™ 12K Flex System" on page 37) and the instructions to update the software and firmware were updated ("Updating the software and the firmware" on page 140). • The software installation was updated to specify that OpenArray™ Sample Tracker Software is not applicable to OpenArray™ AccuFill™ Software v2.0 ("Software installation" on page 38). • The calibration status information was corrected in the following sections: – "Perform the background calibration" on page 57 – "Perform the uniformity calibration" on page 61 – "Perform the dye calibration" on page 68 • Information was added to specify that normalization calibration is required for all formats with QuantStudio™ 12K Flex Software v1.4 ("Normalization calibration" on page 71). • Instructions for OpenArray™ AccuFill™ Software v2.0 were added to load an OpenArray™ Plate for RNase P instrument verification ("Load the OpenArray™ Plate (OpenArray™ AccuFill™ Software v2.0)" on page 115). • The instructions to seal the OpenArray™ Plate for RNase P instrument verification were updated. The protective film on the outside of the lid is removed before running the plate on the QuantStudio™ 12K Flex Instrument. • Instructions to set up the computer were updated ("Set up the computer" on page 156). • The method of receiving supporting files was updated to a download instead of a CD ("Supporting files for experiment creation" on page 209 and "About the import file formats" on page 217). • The instructions to order parts and materials from the website were removed. • The list of consumables was updated to correct sizes and to update the TaqMan™ Array Card products ("Consumables" on page 253). • The firewall ports that must be open were added ("Firewall ports that must be open" on page 152). • The safety appendix was updated. • The list of related documents was updated ("Related documentation" on page 290). • The branding was updated.
C	4 March 2014	Baseline for this revision history.

The information in this guide is subject to change without notice.

Contents

About this guide

Purpose

The *QuantStudio™ 12K Flex Real–Time PCR System v1.4 Maintenance and Administration Guide* provides reference information for the QuantStudio™ 12K Flex Real–Time PCR System with the QuantStudio™ 12K Flex Software v1.4 and describes how to prepare, maintain, and troubleshoot the system.

For information about the QuantStudio™ 12K Flex Real–Time PCR System with the QuantStudio™ 12K Flex Software v1.5, see the *QuantStudio™ 12K Flex Real–Time PCR System v1.5 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

Audience

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

Assumptions

This guide assumes that your QuantStudio™ 12K Flex Real–Time PCR System has been installed by a Thermo Fisher Scientific service representative.

This guide also assumes that you have:

- Familiarity with Microsoft™ Windows™ 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.

User attention words

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

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

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

Safety alert words appear in this document with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instrument.

For descriptions of the safety symbols, see Appendix H, “Safety”.

Contents of this guide

You can use this guide to calibrate, service, network, and administrate the QuantStudio™ 12K Flex Real–Time PCR System with QuantStudio™ 12K Flex Software v1.4.

This user guide contains the following information:

- Chapter 1, “Get started”—Describes the QuantStudio™ 12K Flex Real–Time PCR System.
- Chapter 2, “Calibrate multi-well plate and TaqMan™ Array Card sample blocks”—Describes how to maintain a QuantStudio™ 12K Flex Real–Time PCR System with a 96-well plate, a 384-well plate, or a TaqMan™ Array Card sample block, including calibration and performance verification.
- Chapter 3, “Calibrating OpenArray™ Plate sample blocks”—Describes how to maintain a QuantStudio™ 12K Flex Real–Time PCR System with a TaqMan™ 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 Real–Time PCR System and resolve infrequent problems that can occur during normal use.
- Chapter 5, “Networking”—Describes how to install the QuantStudio™ 12K Flex Real–Time PCR 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 software.
- Appendix A, “Manual instrument operation”—Describes how to operate the QuantStudio™ 12K Flex Real–Time PCR System manually using the touchscreen interface.
- Appendix B, “Power on or off, store, and move the system”—Describes how to store, move, and reinstall the components of the system.
- Appendix C, “Prepare calibration consumables”—Describes how to prepare TaqMan™ Array Cards and TaqMan™ OpenArray™ Plates for calibration and verification of the QuantStudio™ 12K Flex Real–Time PCR System. 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 Thermo Fisher Scientific.
- Appendix D, “Command-line software operation”—Describes how to use the software command-line application.
- Appendix E, “File format reference”—Provides specifications for files that the software imports, exports, and stores.
- Appendix F, “Parts and materials”—Describes how to order parts, accessories, and consumables for the QuantStudio™ 12K Flex Real–Time PCR System.

Network and password security requirements

Network configuration and security

The network configuration and security settings of your laboratory or facility (such as firewalls, anti-virus software, network passwords) are the sole responsibility of your facility administrator, IT, and security personnel. This product does not provide any network or security configuration files, utilities, or instructions.

If external or network drives are connected to the software, it is the responsibility of your IT personnel to ensure that such drives are configured and secured correctly to prevent data corruption or loss. It is the responsibility of your facility administrator, IT, and security personnel to prevent the use of any unsecured ports (such as USB, Ethernet) and ensure that the system security is maintained.

Password security

Thermo Fisher Scientific strongly recommends that you maintain unique passwords for all accounts in use on this product. All passwords should be reset upon first sign in to the product. Change passwords according to your organization's password policy.

It is the sole responsibility of your IT personnel to develop and enforce secure use of passwords.

1 Get started

About the 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).

Overview of data collection

The 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 system performs.

Run type		Data collection point
Real-time PCR	Standard curve[1]	The QuantStudio™ 12K Flex Real–Time PCR System collects data following each extension step of the PCR.
	Relative standard curve[1]	
	Comparative C_t ($\Delta\Delta C_t$)	
	Melt curve[1]	
Post-PCR (endpoint)	Genotyping	The QuantStudio™ 12K Flex Real–Time PCR System 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 Real–Time PCR System 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[1]	

[1] Not available for TaqMan™ OpenArray™ Plate experiments.

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

1. Excitation—The instrument illuminates all wells of the reaction plate within the instrument, exciting the fluorophores in each reaction.
2. Emission—The 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 instrument assembles a digital representation of the residual fluorescence collected over a fixed time interval. The software stores the raw fluorescent image for analysis.

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

Instrument filters and supported dyes

System dyes

The QuantStudio™ 12K Flex Real–Time PCR System features a six-color filter set that supports all of the Thermo Fisher Scientific dyes. The following figure shows the emission spectrum for each dye, and the filter at which each dye is read.

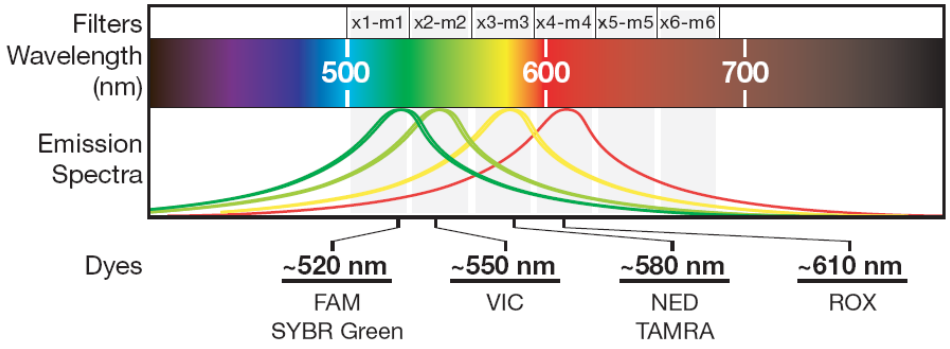


Figure 1 Emission spectra

Filter set	Color	Filter wavelength[1]		Supported dyes
		Excitation	Emission	
x1-m1	Blue	470 ± 15 nm	520 ± 15 nm	FAM™ dye and SYBR GREEN™ dye
x2-m2	Green	520 ± 10 nm	558 ± 12 nm	VIC™ dye, JOE™ dye , TET™ dye, and HEX™ dye
x3-m3	Yellow	549.5 ± 10 nm	586.5 ± 10 nm	NED™ dye, and TAMRA™ dye
x4-m4	Orange	580 ± 10 nm	623 ± 14 nm	ROX™ dye
x5-m5	Red	640 ± 10 nm	682 ± 14 nm	LIZ™ dye
x6-m6	Deep red	662 ± 10 nm	711 ± 12 nm	None[2]

[1] The central wavelengths are the optimized wavelengths.
[2] No Thermo Fisher Scientific supported dye currently available.

Custom dyes

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

Specifications and layout

System specifications

The figures below summarize the specifications and requirements for the QuantStudio™ 12K Flex Real–Time PCR System. For more information, see the *QuantStudio™ 12K Flex Real–Time PCR System Site Preparation Guide* (Pub. No. 4470654).

Component	Height	Depth	Width	Weight
QuantStudio™ 12K Flex Real–Time PCR System				
Instrument[1]	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[2]	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)
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System[3]				
OpenArray™ AccuFill™ Instrument	50 cm (20 in.)	70 cm (27.5 in.)	80 cm (31.5 in.)	80 kg (176 lbs)
Computer	41.1 cm (16.2 in.)	43.8 cm (17.2 in.)	18.7 cm (7.4 in.)	14.3 kg (31.5 lbs)
Monitor (with stand)	38.1 cm (15.0 in.)	14.0 cm (5.5 in.)	37.5 cm (14.8 in.)	4.6 kg (10.2 lbs)
Twister™ Robot				
Twister™ Robot[3]	97.0 cm (38.0 in.)	71.0 cm (28.0 in.)	52.0 cm (20.5 in.)	52.2 kg (115.0 lbs)

[1] Weight varies depending on the sample block installed.
[2] Computer specification differs depending on the computer ordered with the system (laptop or desktop).
[3] Optional component of the system. The system includes an instrument and a computer.

A typical layout of the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System is shown in Figure 2 on page 25 and Figure 3 on page 25.

The components require a width of 1.8 m (6 ft).

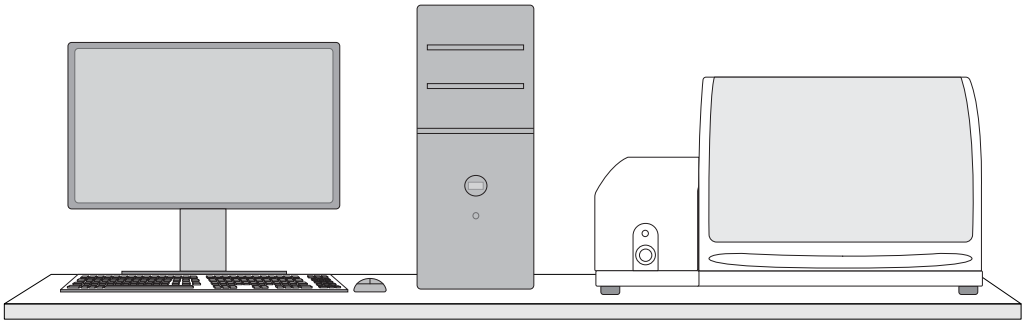


Figure 2 Typical layout of the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System

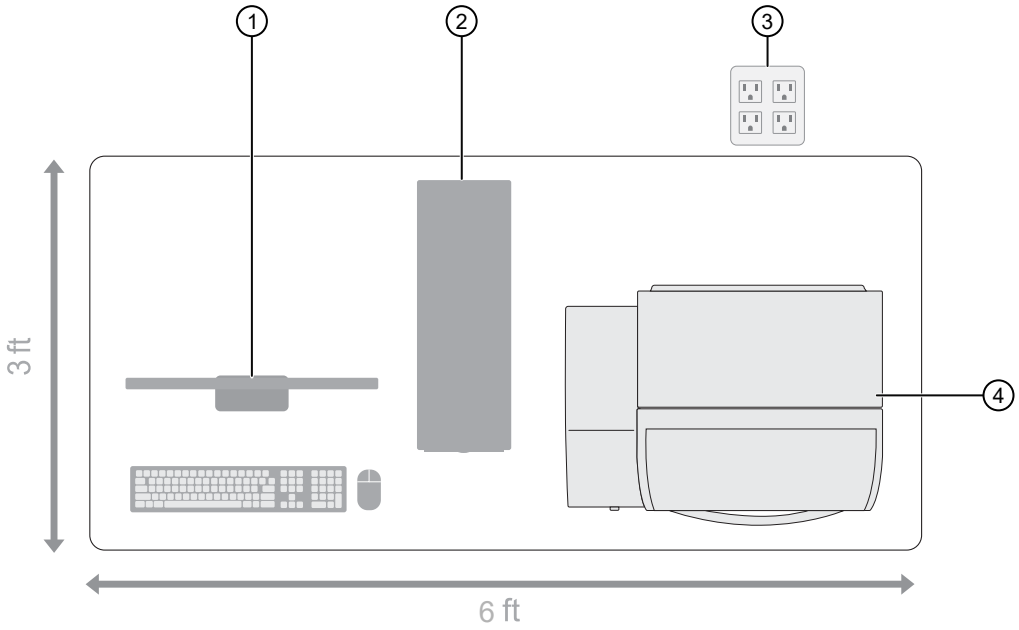


Figure 3 Dimensions of the typical layout of the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System

① Monitor and keyboard
② Computer tower
③ Electrical outlet
④ OpenArray™ AccuFill™ Instrument

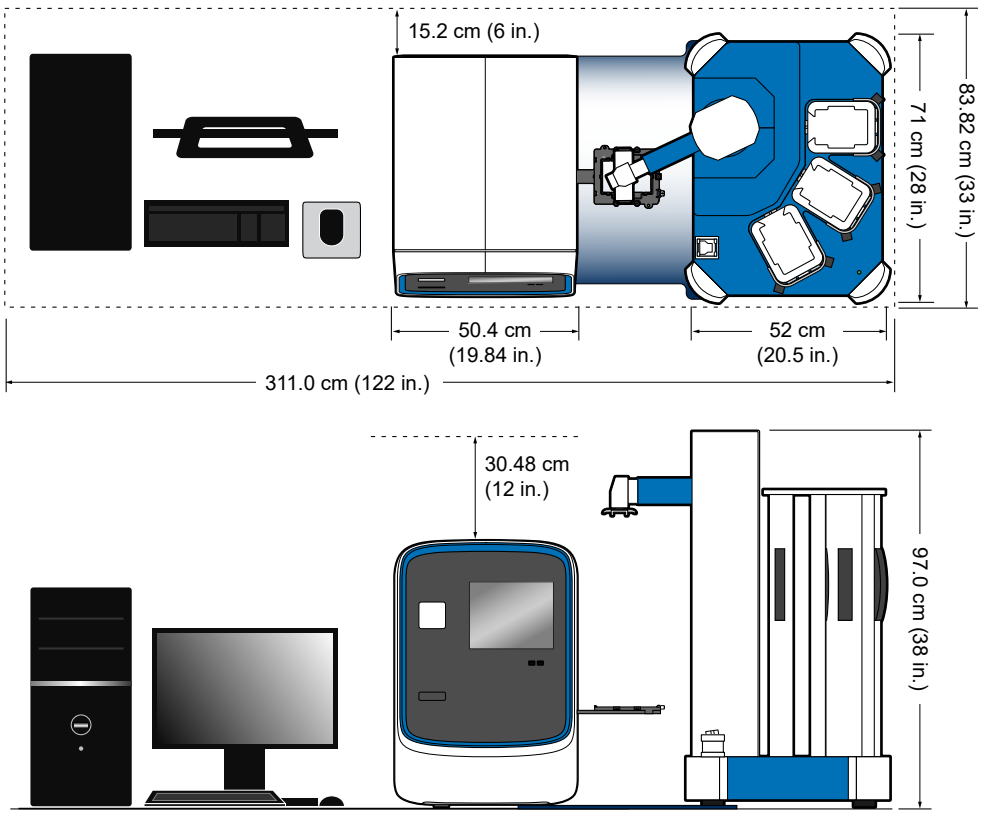


Figure 4 QuantStudio™ 12K Flex Real–Time PCR System with the Twister™ Robot

Required clearances

The QuantStudio™ 12K Flex Real–Time PCR System requires the following additional clearances.

Component	Top	Front	Sides	Back
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.)
QuantStudio™ 12K Flex 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 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 instrument and the 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.

Table 1 Electrical requirements for the QuantStudio™ 12K Flex System and the Twister™ Robot

Device	Rated current	Rated power	Rated voltage	Rated frequency
Instrument	12.5 A	950 VA	100–240 ± 10% VAC	50/60 Hz
Computer	2.1 A	125 VA		
Monitor	1.5 A	65 VA		
Twister™ Robot[1]	2.5 A	150 VA		

[1] Optional component of the QuantStudio™ 12K Flex Real–Time PCR System.

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

Table 2 Electrical requirements for the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System

Device	Rated voltage	Circuit required	Rated frequency	Rated power
Instrument	100–240 ±10% VAC[1]	4 A	50/60 Hz	75 W
Computer (laptop)	100–240 ±10% VAC	10 A	50/60 Hz	90 VA
Computer (desktop)	100–240 ±10% VAC	10 A	50/60 Hz	125 VA
Monitor				65 VA

[1] If the supplied power fluctuates beyond the rated voltage, a power line regulator may be required. High or low voltages can adversely affect the electronic components of the instrument.

Environmental requirements

Table 3 Environmental requirements for the QuantStudio™ 12K Flex Instrument

Requirement	Description
Altitude	Less than 2000 m (6500 ft) above sea level
Temperature	15–30°C (59–86°F) Do not place the instrument next to heaters, cooling ducts, or in direct sunlight. Temperature fluctuations can affect performance.
Humidity	QuantStudio™ 12K Flex Instrument, computer, and UPS unit: 20–80% (noncondensing)
Pollution	The instrument has a pollution degree rating of II.[1] 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.

[1] 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.

Table 4 Environmental requirements for the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System

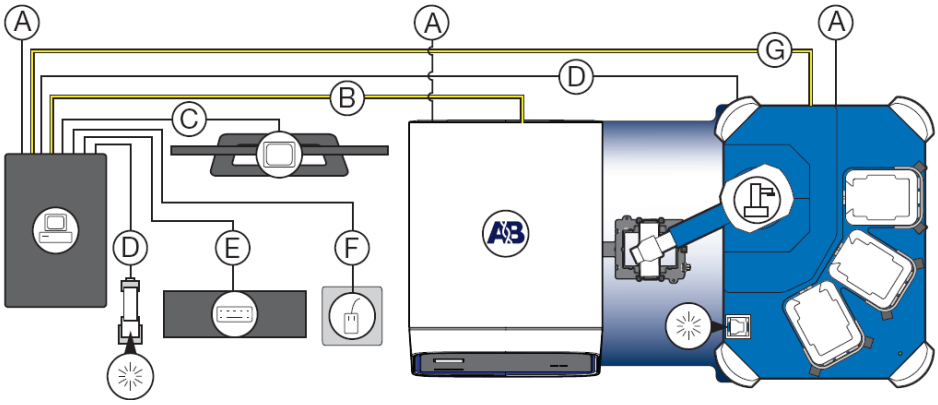
Condition	Acceptable range
Installation site	Indoor use only
Electromagnetic interference	Do not use this device in close proximity to sources of strong electromagnetic radiation (for example, unshielded intentional RF sources). Strong electromagnetic radiation may interfere with the proper operation of the device. This equipment has been designed and tested to CISPR 11 Class A. In a domestic environment it may cause radio interference. You may need to take measures to mitigate the interference.
Altitude	Located between sea level and 2,012 m (6,600 ft.) above sea level
Humidity (instrument and computer)	Operation: 80% relative humidity for temperatures up to 31°C, decreasing linearly to 50% relative humidity for 40°C
Temperature (instrument and computer)	15°C to 30°C (60°F to 85°F) **Note:** The room temperature must not fluctuate more than 2°C over a 2-hour period.
Transient category	Installation categories II
Overvoltage category	Installation categories II
Vibration	The instrument is not adjacent to strong vibration sources, such as a centrifuge, pump, or compressor. Excessive vibration will affect instrument performance.

Table 4 Environmental requirements for the QuantStudio 12K Flex OpenArray AccuFill System *(continued)*

Condition	Acceptable range
Pollution degree	II Install the instrument in an environment that has nonconductive pollutants such as dust particles or wood chips. Typical environments with a Pollution Degree II rating are laboratories and sales and commercial areas.
Liquid waste collection	Dispose of the polymer, buffer, reagents and any liquid waste as hazardous waste in compliance with local and national regulations.
Other conditions	Ensure the room is away from any vents that could expel particulate material on the components. Avoid placing the instrument and computer adjacent to heaters, cooling ducts, or in direct sunlight.

System layout and connections

The 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 perform the following functions: • Calibrate the instrument • Set up experiments • *(Optional)* Run experiments • Analyze experiments
	Monitor	
	Keyboard	
	Mouse	

(continued)

	Component	Description
	Barcode reader	Scans the barcodes of consumables before they are loaded into the instrument.
	Twister™ Robot[1]	Automates loading and unloading of consumables to and from the QuantStudio™ 12K Flex Instrument.

[1] Not for diagnostic use.

	Connection	Description
A	Power cables	Supply power to the computer, the Twister™ Robot, and the QuantStudio™ 12K Flex Instrument.[1]
B	LAN connection or Ethernet cable[2]	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).

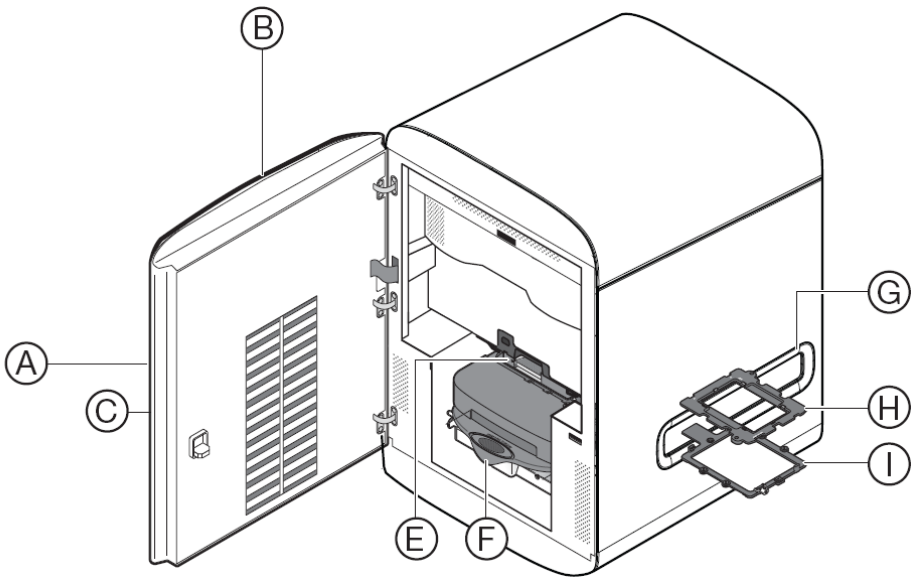
[1] Supplies 115/230 V depending on the geographic location of the installation.
[2] Supplied with the system.

System hardware

Instrument components

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

Front view

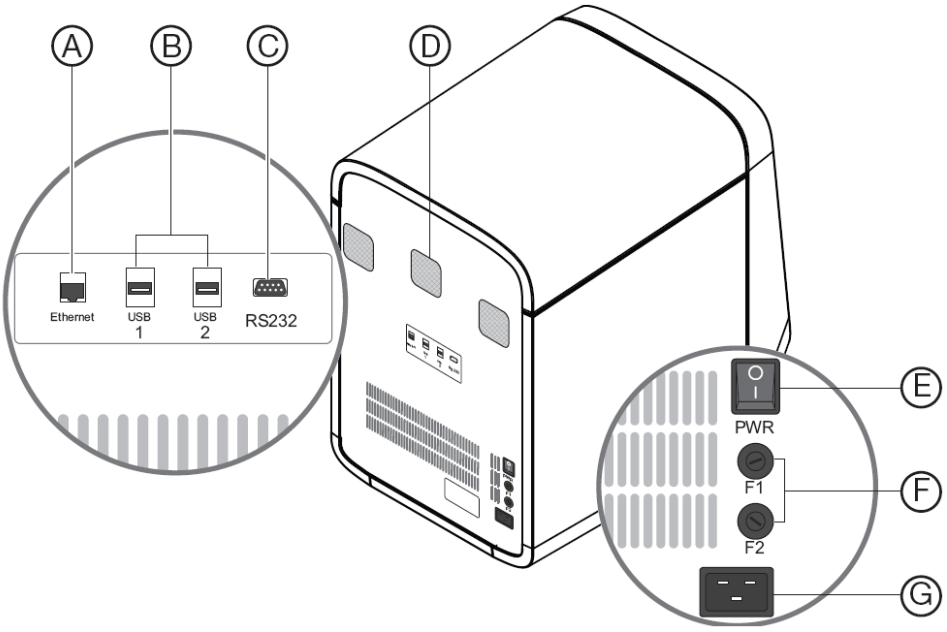


	Component	Description
A	USB ports	Provide USB communication with the QuantStudio™ 12K Flex Instrument. Can be used to transfer data to and from the instrument and to update the firmware. **Note:** If multiple USB drives are plugged into the QuantStudio™ 12K Flex Instrument, the instrument mounts only 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.

(continued)

	Component	Description
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.[1]
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 service use only.
D	Instrument fans	Cool the interior of the QuantStudio™ 12K Flex Instrument. **IMPORTANT!** The fans must be unobstructed to allow adequate cooling and proper function of the instrument.

(continued)

	Component	Description
E	On/Off switch	Power switch for the QuantStudio™ 12K Flex Instrument, where the states are on (|) or off (O).
F	Fuse cover	Dual 12.5A, Time-Lag T, 250VAC, 5 × 20–mm electrical fuses that protect the instrument from excessive electrical current.
		(Australia and New Zealand only) 6.3A, Time-Lag T, 250VAC, 5 × 20–mm electrical fuses that protect the instrument from excessive electrical current.
G	Power port	The 100–240 VAC port that provides power to the QuantStudio™ 12K Flex Instrument.

[1] 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 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.

Use the hand-held barcode reader

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

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.

1. In the 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.

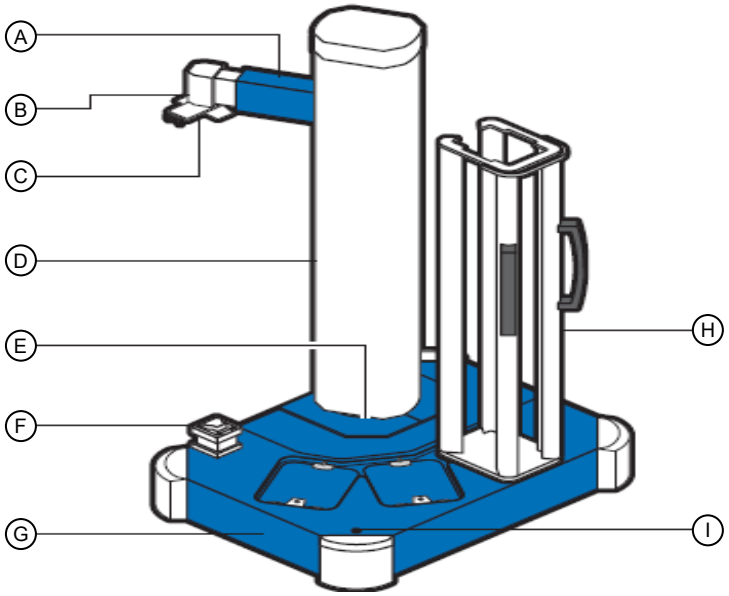
When the barcode reader scans a barcode, it automatically transmits the alphanumeric equivalent of the barcode to the software. The software enters the barcode text wherever the cursor is active. The barcode reader transmits a carriage-return character (the equivalent of pressing **Enter**).

Components of the Twister™ Robot

The QuantStudio™ 12K Flex System supports the use of the Twister™ Robot. It is an optional accessory that consists of the components shown below.

Note: For more information about operating, calibrating, maintaining, and integrating the Twister™ Robot, see the *Applied Biosystems™ Twister™ Robot Automation Accessory User Guide* (Pub. No. 4470693).

Front view

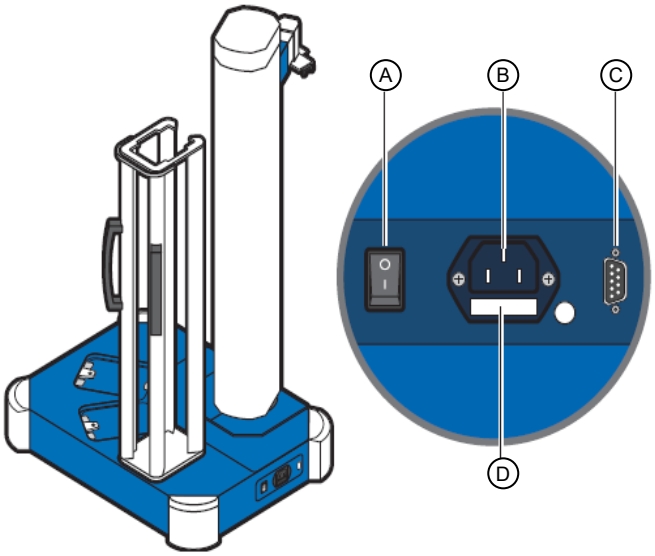


	Component	Description
A	Reach axis	Moves the grip horizontally 28.5–50.1 cm (11.25–19.75 in.) from the center of the robot post.
B	Wrist mechanism	Rotates materials to either the portrait or landscape positions, where the range of motion is ±135° (270° total).
C	Grip	Consists of two sets of fingers that 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.

(continued)

	Component	Description
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 (|) or off (O).
B	Power port	100–240 V 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 counterclockwise, with position 1 closest to the front of the Twister™ Robot (see Figure 5 on page 36). Each rack is labeled for a specific position and cannot be exchanged with the other racks.

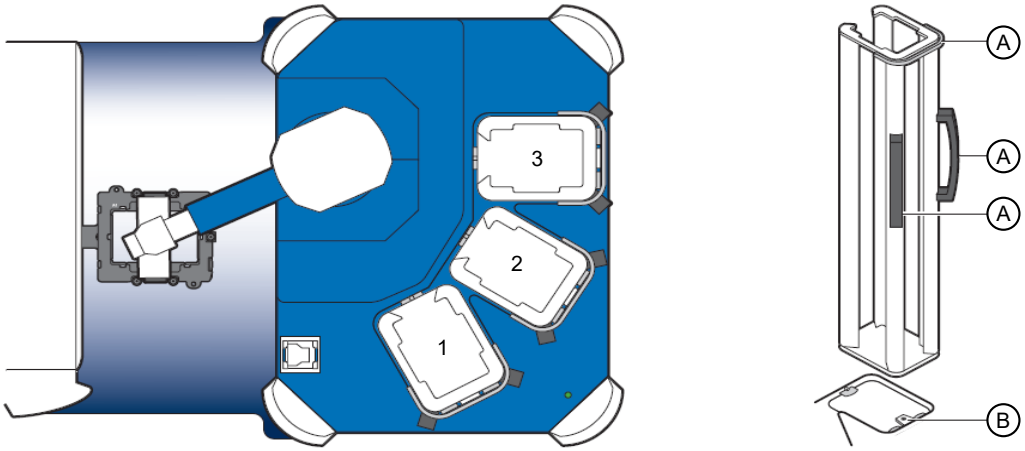


Figure 5 Parts of the rack

	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 instrument or the 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 system can corrupt data and possibly damage the computer or the instrument.

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

Surge protector

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

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

Software for the QuantStudio™ 12K Flex System

The QuantStudio™ 12K Flex System includes a suite of software applications that can be used to calibrate, run, automate, and integrate the system into a laboratory workflow.

The basic installation of the QuantStudio™ 12K Flex Software v1.4 contains the components described below.

Additional software may be available.

For a complete list of compatible software, go to **thermofisher.com/qpcrsoftware**.

Note: For updates and patches, go to **thermofisher.com/qpcrsoftware**.

Perform internal validations before software upgrades

IMPORTANT! If you have a validated workflow, you must perform all internal validations as required by your organization's standard operating procedures before performing a software upgrade.

Computer requirements

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

`C:\Applied Biosystems\QuantStudio 12K Flex\README.html`

Software installation

The default installation of the software partitions the computer hard drive to create the logical drives shown below.

Drive	Software	Description
C:	Microsoft™ Windows™ OS[1]	Operating system files.
	QuantStudio™ 12K Flex Software	Used to calibrate the instrument and perform experiments on the instrument.
	QuantStudio™ 12K Flex System Command-line Utility	Used to automate the creation of new experiments and the export of existing experiments.
	OpenArray™ Sample Tracker Software	Used to rapidly enter sample information for experiments with OpenArray™ Plates. **Note:** OpenArray™ Sample Tracker Software is not used with OpenArray™ AccuFill™ Software v2.0. The features in OpenArray™ Sample Tracker Software are included in OpenArray™ AccuFill™ Software v2.0.
	ExpressionSuite™ Software	Analyzes gene expression data generated by the QuantStudio™ 12K Flex Instrument.
	Software for the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System	Controls the OpenArray™ AccuFill™ Instrument used to load OpenArray™ Plates. **Note:** For OpenArray™ AccuFill™ Software v2.0, the software is used to enter sample information for experiments with OpenArray™ Plates.
	HRM Software Module	An optional module for the QuantStudio™ 12K Flex Software that allows you to set up, run, and analyze a high-resolution melt curve experiment.

(continued)

Drive	Software	Description
C:	TaqMan™ Genotyper Software	Analyzes genotyping data generated by the QuantStudio™ 12K Flex Instrument.
	Software for the Twister™ Robot	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.

[1] We recommend that you do not install programs to the C: drive.

Software for the Twister™ Robot

The software for the Twister™ Robot 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 computer for the QuantStudio™ 12K Flex System, and it consists of the components shown in the following table.

Software component	Function
QuantStudio™ 12K Flex Instrument Control Program (ICP)	Calibrates the Twister™ Robot and stores all the taught positions.
QuantStudio™ Adapter Driver for iLink™ PRO Software	Coordinates the operation of the Twister™ Robot and the QuantStudio™ 12K Flex Instrument.
Microsoft™ software	Provides the Microsoft™ services used by the software for the Twister™ Robot. The components include Microsoft™ Data Access Components (MDAC), Microsoft™ .NET Framework, Microsoft™ SQL 2005 Manager, and Microsoft™ VBA Service Packs.
Automation Controller II 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 Software storage for the Twister™ Robot racks is used with the iLink™ PRO Software automation control to set up the initial material layout.

Install 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

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 system performs each test experiment without producing errors.

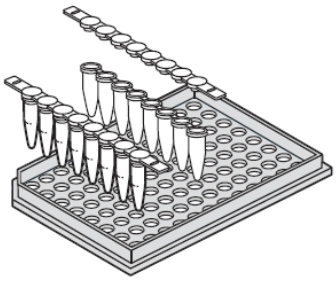
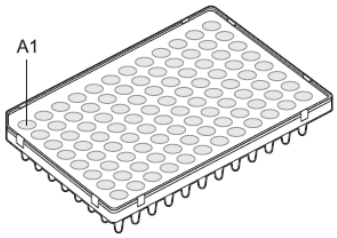
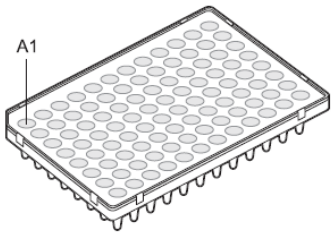
If the system performs the tests successfully, proceed with your experiments.

If the system encounters errors during the test runs, the third-party software may not be compatible with the QuantStudio™ 12K Flex Software.

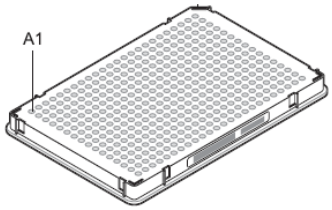
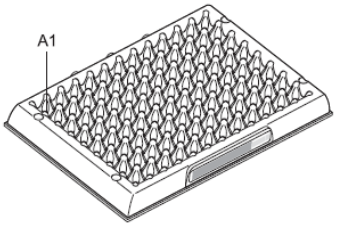
Consumables for the QuantStudio™ 12K Flex Instrument

Compatible consumables

The instrument supports a series of specialized consumables through interchangeable sample blocks. Use the consumables appropriate for the sample block on your instrument.

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, 0.1mL		• MicroAmp™ Optical Adhesive Film • MicroAmp™ Optical 96-Well Fast Reaction Plate with Bar Code	30 µL

(continued)

Sample block	Consumable		Reaction volume
384-well plate		• MicroAmp™ Optical Adhesive Film • MicroAmp™ Optical 384-Well Reaction Plate with Bar Code	20 µL
TaqMan™ Array Card		TaqMan™ Array Card	1 µL
TaqMan™ OpenArray™ Plate		TaqMan™ OpenArray™ Plate	33 nL

Guidelines for handling consumables

- 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 instrument 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.
- *(TaqMan™ OpenArray™ Plates only)* Hold the plate by the edges of the cases. Do not touch the through-holes.
- *(TaqMan™ OpenArray™ Plates only)* Load and seal the plate within one hour after opening the plate packaging.
- *(TaqMan™ OpenArray™ Plates only)* If you drop a loaded plate, discard it in the appropriate waste container.

2 Calibrate multi-well plate and TaqMan™ Array Card sample blocks

Recommended calibration and maintenance

The 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 support optimal instrument performance.

IMPORTANT! Calibrate the 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 system and, in extreme cases, influence experimental results.

IMPORTANT! Do not use organic solvents to clean the system.

Table 5 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 system, then after 30 seconds, power on the computer.
	Clean the surface of the instrument with a lint-free cloth.
	Perform an instrument self-test.

Table 5 Multi-well plate and array card sample block maintenance *(continued)*

Frequency	Maintenance task
Monthly	Perform a background calibration.[1]
	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.[2]
	Perform an instrument verification run.
As needed	Decontaminate the system.
	Replace the system fuses.
	Update the Windows™ operating system.
	Update the software and firmware.

[1] 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.

[2] Normalization calibration is required for all block types for QuantStudio™ 12K Flex Software v1.4.

Prepare TaqMan™ Array Cards for instrument calibration

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

Required materials

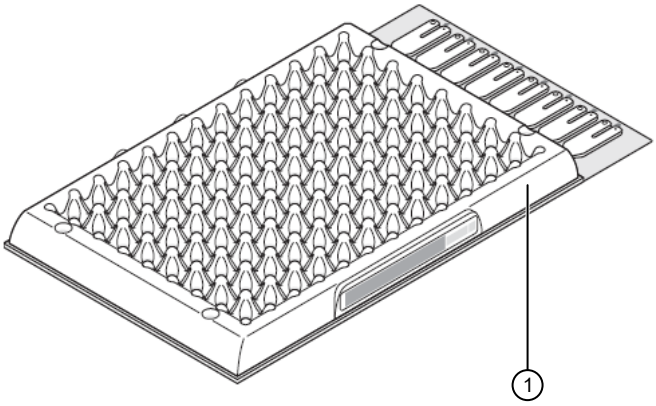
- ViiA™ 7 Array Card Spectral Calibration Kit
  The kit includes the following items:
  - Empty TaqMan™ Array Cards
  - FAM™ dye
  - VIC™ dye
  - ROX™ dye
  - ROI dye mix
  - Background buffer
  - FAM™/ROX™ dye
  - VIC™/ROX™ dye
- TaqMan™ Array Card Sealer
- Centrifuge with array card buckets and array card carrier clips
- Permanent marker or pen
- Pipettor, 200-µL (with pipette tips)
- Powder-free gloves
- Safety glasses

Fill the calibration TaqMan™ 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 43.

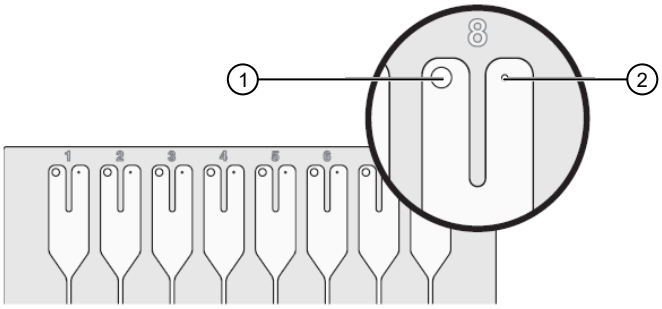
1. Remove the tubes of calibration solutions from the freezer, allow them to thaw, then vortex the tubes to mix the contents well.
2. Remove the array cards from their box and place them on a clean, dry surface.
3. Mark the side of the empty array cards with the following information.
   - Background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



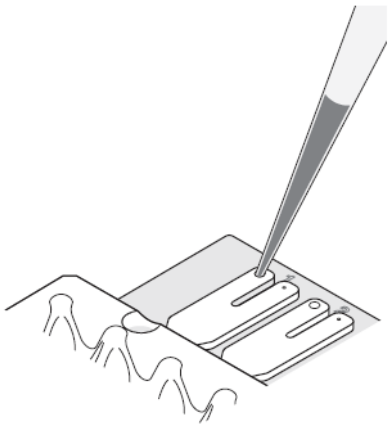
① Location to mark the information

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 (approximately 45 degrees) and place the tip into the fill port.

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

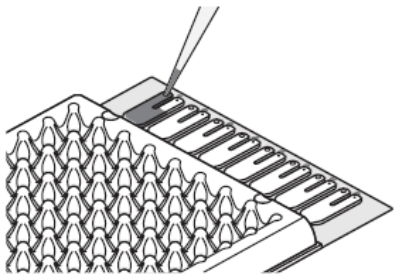


① Fill port
② Vent port



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

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

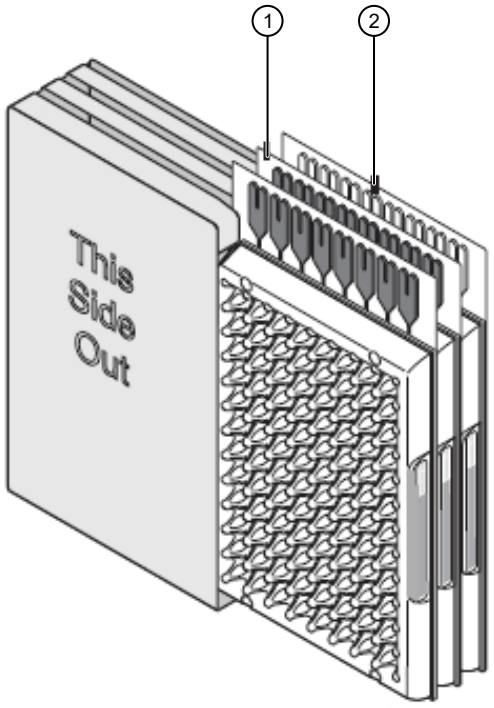


IMPORTANT! Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.

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.

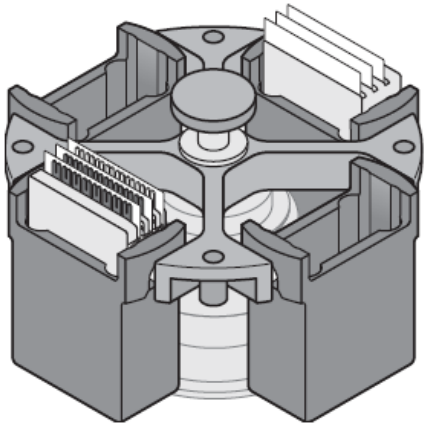


① Filled TaqMan™ Array Cards
② Empty TaqMan™ Array Cards

7. Place the filled carrier clips into the centrifuge buckets.

   Ensure 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.



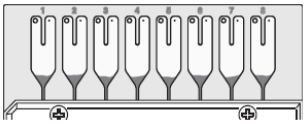
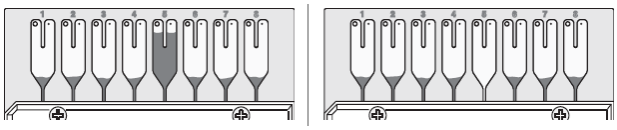
IMPORTANT! Balance the loads in opposite buckets in the centrifuge.

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.

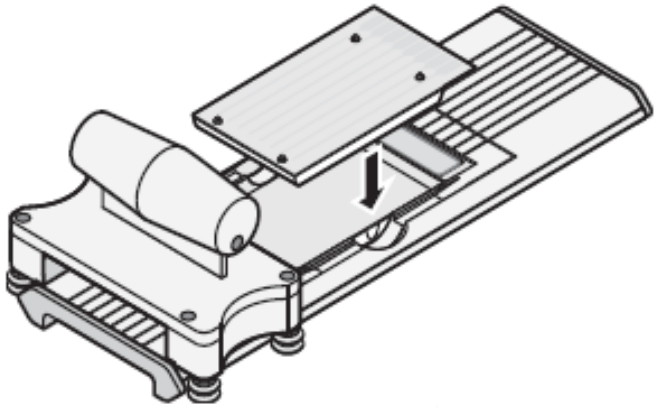
   IMPORTANT! Do not try to save time by doing one spin for 2 minutes. The two sets of ramps are important for a good fill into the array card.

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

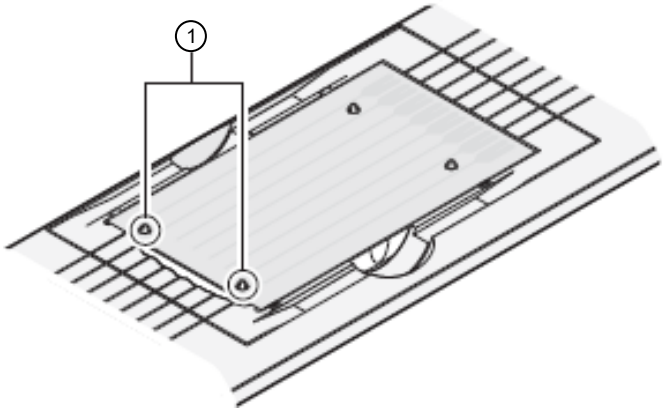
Correct fill	Incorrect/partial fill
	

   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).
    a. With the carriage (roller assembly) of the TaqMan™ Array Card 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.

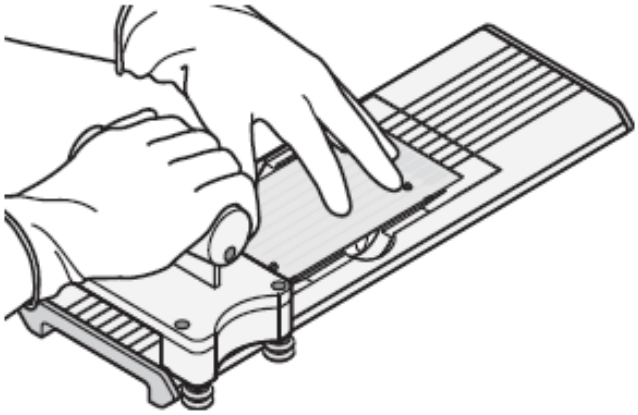


b. Press down on all four corners of the array card to ensure that it is fully seated within the fixture.

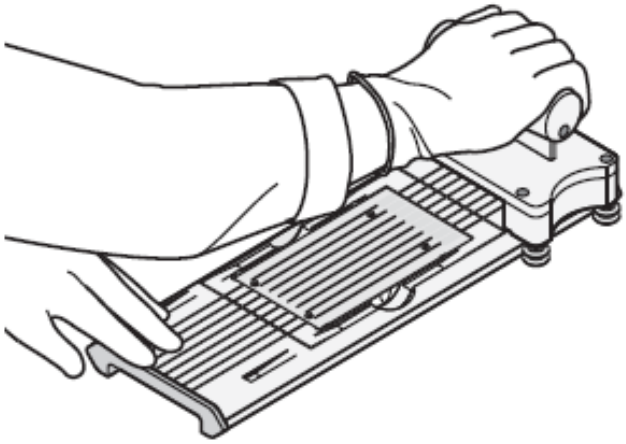


① Alignment pins

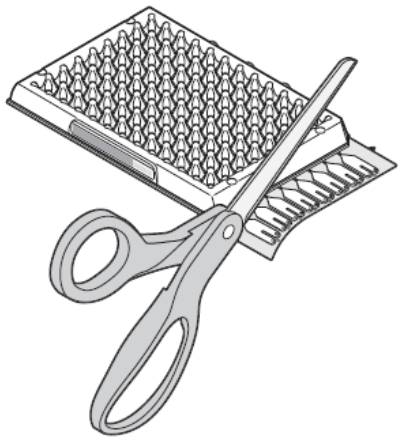
c. Use the two alignment pins in the fixture to position the array card correctly.



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



e. Remove the sealed array card from the fixture and trim the fill reservoirs from the array card assembly using scissors. Trim the foil array card so that the edge is even with the plastic carrier.



IMPORTANT! Completely remove the fill reservoirs from the array card so that the edge is free of residual plastic. The plastic from the fill reservoirs that extends beyond the edge of the card can prevent the array card from seating properly on the sample block and can affect amplification.

Correct trim	Incorrect trim
	

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 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 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

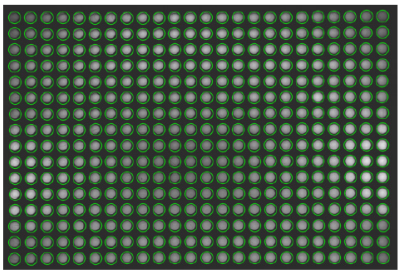
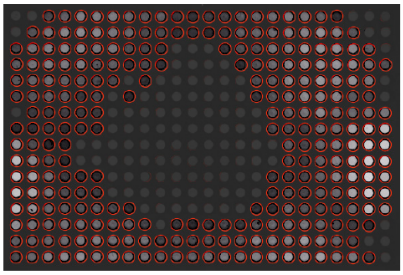
Perform the ROI calibration every year, or as often as necessary, depending on instrument use.

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.	

Prepare the calibration plate or the TaqMan™ 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 instrument.

- Prepare the ROI calibration plate (see page 52)
- Prepare the array cards for instrument calibration (see page 44)

Prepare 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.

Prepare the calibration plate

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.

1. Remove the calibration plate from its packaging.

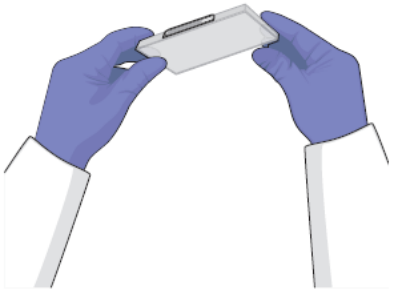
   Do not remove the optical film.

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

2. 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. Ensure 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.



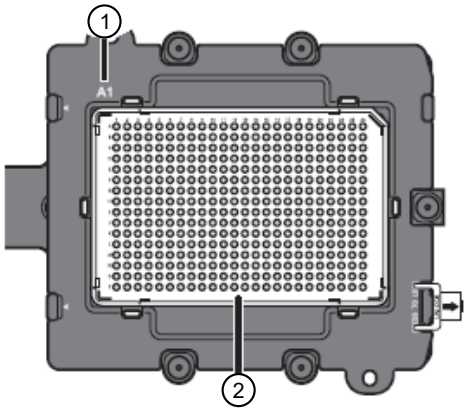
Correct	Incorrect
 Liquid is at bottom of well.	 • Not centrifuged with enough force, *or* • Not centrifuged for enough time

Perform the calibration

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

   Note: You must add an 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**, 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.



① Load 96- and 384-well plates with the A1 position at the top-left corner of the plate adapter.
② 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 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 **Analysis** screen is displayed, 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 instrument ejects the tray arm.
Failed	Troubleshoot the failed ROI calibration (see "Troubleshooting" on page 85).

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 instrument does not eject the plate, see "Troubleshooting" on page 85.

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. • If you plan to perform background and uniformity calibrations 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 an instrument 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 the following functions.

- Reads 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 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 the following:

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

Prepare the calibration plate or TaqMan™ Array Card

Prepare the background calibration consumable appropriate for your instrument.

- Calibration plate (see page 56)
- TaqMan™ Array Card (see page 44)

Prepare 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.

Prepare the calibration plate

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

1. Remove the background plate from its packaging.

   Do not remove the optical film.

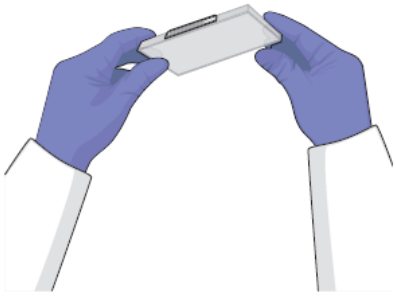
   IMPORTANT! Do not discard the packaging. You can use the background plate to calibrate a QuantStudio™ 12K Flex Instrument 3 times for up to 6 months if it is stored in its original packaging sleeve.

2. 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.



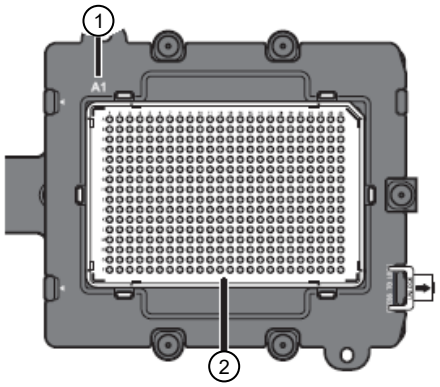
Correct	Incorrect
 Liquid is at bottom of well.	 • Not centrifuged with enough force, *or* • Not centrifuged for enough time

Perform the background calibration

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

   Note: You must add an 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, 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.



① Load 96- and 384-well plates with the A1 position at the top-left corner of the plate adapter.
② 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 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 software displays the **Analysis** screen, confirm the analysis status of the calibration, then select the **QC** tab and review the quality check summary.
   - **Data collection**—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.
   - **Calibration QC**—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.

     Note: If a calibration fails, the description of the failure is displayed in the **QC** tab.

Analysis status	Data collection	Calibration QC	Action
Pass	Passed	Passed (no information is displayed)	Click **Next**, then remove the plate or array card when the instrument ejects the tray arm.
Caution	Passed	Failed (warning message is displayed in the **QC** tab)	You can accept a calibration that passes the data collection check but fails the calibration QC check. We recommend using calibrations that yield passing results for *both* status reports. Troubleshoot the failed calibration (see “Troubleshooting” on page 85).
Fail	Failed	Failed (no information is displayed)	Troubleshoot the failed calibration (see “Troubleshooting” on page 85).

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 instrument does not eject the plate, see “Troubleshooting” on page 85.

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 an instrument 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 instrument 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.

Prepare the calibration plate or TaqMan™ Array Card

If you have an ROI plate or array card from a recent ROI calibration, you can use it for the uniformity calibration.

If you do not have a ROI plate or array card from a recent calibration, prepare the uniformity calibration plate or array card.

- Prepare the uniformity calibration plate (see page 60)
- Prepare the uniformity calibration array card (see page 44)

Prepare the calibration plate

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

Required materials

For a list of materials for the uniformity calibration, see “ROI calibration” on page 50.

Prepare the ROI calibration plate for uniformity calibration

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.

1. Remove the ROI calibration plate from its packaging.

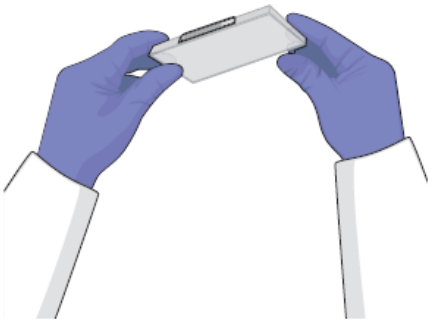
   Do not remove the optical film.

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

2. 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

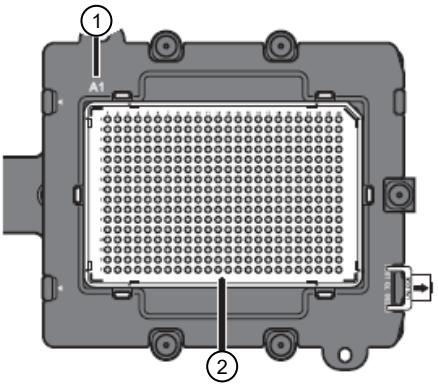
Perform the uniformity calibration

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

   Note: You must add an instrument to your list before you can manage it.

3. After the 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.



① Load 96- and 384-well plates with the A1 position at the top-left corner of the plate adapter.
② 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 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.
   - **Data collection**—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.

- **Calibration QC**—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.

Note: If a calibration fails, the description of the failure is displayed in the **QC** tab.

Analysis status	Data collection	Calibration QC	Action
Pass	Passed	Passed (no information is displayed)	Click **Next**, then remove the plate or array card when the instrument ejects the tray arm.
Caution	Passed	Failed (warning message is displayed in the **QC** tab)	You can accept a calibration that passes the data collection check but fails the calibration QC check. We recommend using calibrations that yield passing results for *both* status reports. Troubleshoot the failed calibration (see "Troubleshooting" on page 85).
Fail	Failed	Failed (no information is displayed)	Troubleshoot the failed calibration (see "Troubleshooting" on page 85).

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 instrument does not eject the plate, see "Troubleshooting" on page 85.

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 Instrument 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 QuantStudio™ 12K Flex Real–Time PCR System performs the following functions:

- 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 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 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 Instrument.

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 Instrument. For example, if you intend to run a TaqMan™ RNase P plate or array card to verify instrument performance, 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 QuantStudio™ 12K Flex System with QuantStudio™ 12K Flex Software v1.4 calibrates the following system dyes:

- FAM™ dye
- NED™ dye
- ROX™ dye
- SYBR GREEN™ dye
- TAMRA™ dye
- VIC™ dye

See Figure 1 on page 23.

Custom dyes

The QuantStudio™ 12K Flex System can be used to run assays designed with custom dyes (not supplied by Thermo Fisher Scientific). Before using custom dyes with the 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. For more information about custom dye calibrations, see “Creating a custom dye plate for calibration” on page 202.

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 represents 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 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 Figure 6 on page 65).

The 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 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.

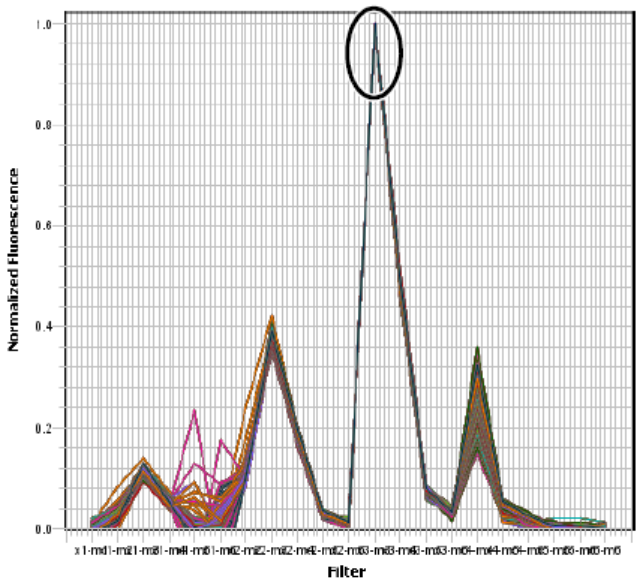


Figure 6 Acceptable spectra Spectra peak at the same wavelength and do not diverge significantly.

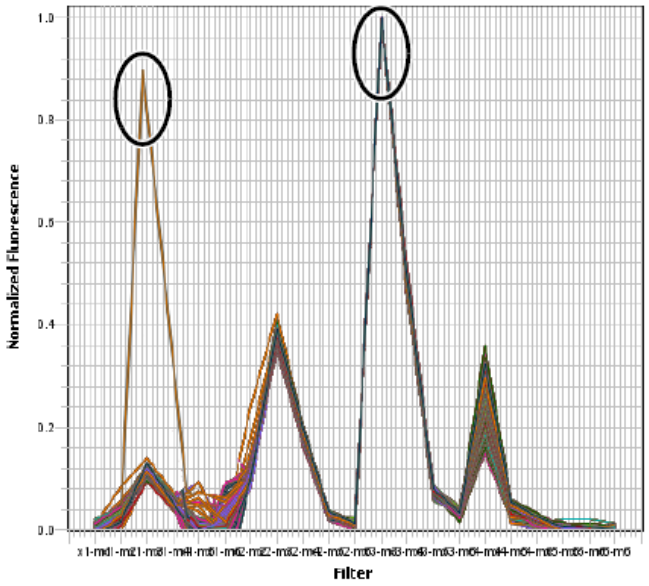


Figure 7 Unacceptable spectra Spectra peak different wavelengths.

Prepare the calibration plate or TaqMan™ Array Card

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

- Prepare the dye calibration plate (see page 67)
- Prepare the calibration TaqMan™ Array Card (see page 44)

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, see one of the following sections:
  - Calibration kits for the 96-well 0.2-mL plate block (page 251)
  - Calibration kits for the 96-well 0.1-mL plate block (page 252)
  - Calibration kits for the 384-well plate block (page 250)
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Prepare the dye calibration plate

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

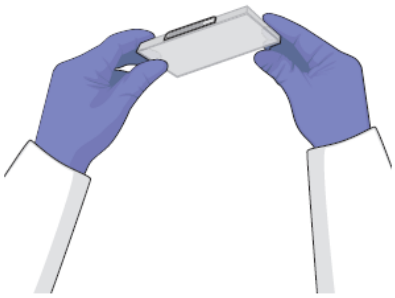
   IMPORTANT! Do not remove the dye plates from their packaging until you are ready to run them. The dyes in the dye plates are photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the plates.

   Note: If you store dye plates frozen and in their original packaging, you can use them to calibrate an instrument up to 3 times for 6 months after opening.

2. 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 the liquid in each dye plate is not at the bottom of the wells, centrifuge the plate again at a higher rpm and for a longer period of time.

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



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

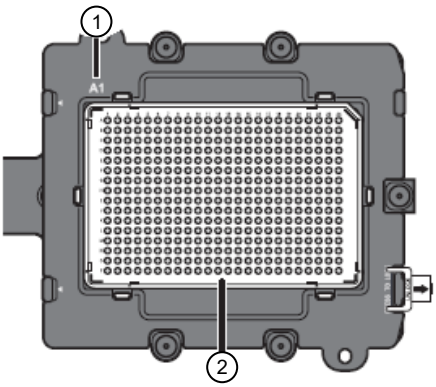
Perform the dye calibration

IMPORTANT! The 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 instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add an 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**, 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 instrument.
   a. Confirm that the dye plate or array card that you are about to load matches the dye selected in the 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.



① Load 96- and 384-well plates with the A1 position at the top-left corner of the plate adapter.
② 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 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 software displays the **Analysis** screen, confirm the grouping of the dye spectra.
   a. Select the **Plate Layout** tab, then review the raw data.
      For each spectrum, confirm that the peak meets the following criteria.
      - 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	Emission	System dyes
x1-m1 (Blue)	470±15 nm	520±15 nm	• FAM™ dye • SYBR GREEN™ dye
x2-m2 (Green)	520±10 nm	558±12 nm	• HEX™ dye • JOE™ dye • TET™ dye • VIC™ dye
x3-m3 (Yellow)	549.5±10 nm	586.5±10 nm	• NED™ dye • TAMRA™ dye
x4-m4 (Orange)	580±10 nm	623±14 nm	ROX™ dye
x5-m5 (Red)	640±10 nm	682±14 nm	LIZ™ dye
x6-m6 (Deep red)	662±10 nm	711±12 nm	—[1]

[1] No Thermo Fisher Scientific 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:
   - **Data collection**—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.
   - **Calibration QC**—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.

   Note: If a calibration fails, the description of the failure is displayed in the **QC** tab.

Analysis status	Data collection	Calibration QC	Action
Pass	Passed	Passed (no information is displayed)	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 instrument ejects the tray arm.
Caution	Passed	Failed (warning message is displayed in the **QC** tab)	You can accept a calibration that passes the data collection check but fails the calibration QC check. We recommend using calibrations that yield passing results for *both* status reports. Troubleshoot the failed calibration (see "Troubleshooting" on page 85).
Fail	Failed	Failed (no information is displayed)	Troubleshoot the failed calibration (see "Troubleshooting" on page 85).

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 instrument does not eject the plate, see "Troubleshooting" on page 85.

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 an instrument 3 times for up to 6 months after you open it.

11. Repeat step 4 to step 10 as needed to calibrate the instrument for the remaining dyes in the chemistries that you are running.

Normalization calibration

During the normalization calibration, the system performs the following functions:

- 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 software uses when comparing data from multiple instruments.

IMPORTANT! Normalization calibration is required for all block formats with QuantStudio™ 12K Flex Software v1.4.

Normalization calibration is not required for 96- and 384-well plate blocks with QuantStudio™ 12K Flex Software v1.5. For more information about calibration with QuantStudio™ 12K Flex Software v1.5, see *QuantStudio™ 12K Flex Real–Time PCR System v1.5 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

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.

Prepare the calibration plate or array card

Prepare the calibration consumables appropriate for your instrument:

- Prepare the normalization plates (see “Prepare the normalization plates” on page 71)
- Prepare the array cards (see “Prepare TaqMan™ Array Cards for instrument calibration” on page 44)

Prepare 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

Prepare the calibration plate

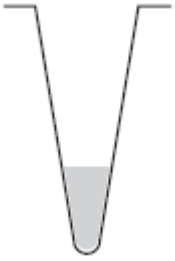
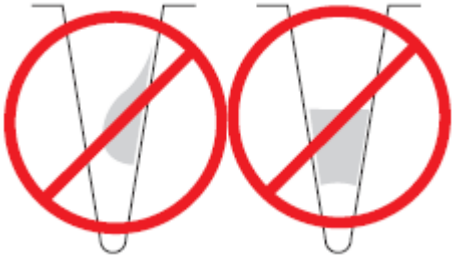
1. Remove the normalization plates from the freezer, then allow the plates to warm to room temperature (approximately 5 minutes).

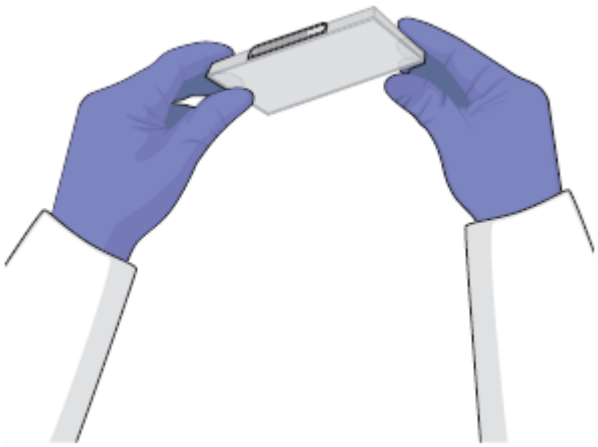
IMPORTANT! Do not remove the normalization plates from their packaging until you are ready to run them. The fluorescent dyes in the dye plates are photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the plates.

Note: If you store the normalization plates in their original packaging and in the freezer, you can use them to calibrate an instrument up to 3 times for 6 months after opening them.

2. Go to "Perform the calibration" on page 73.

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



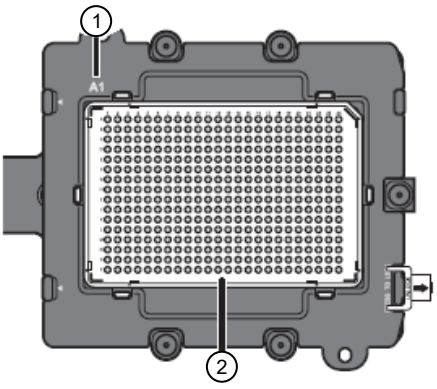
IMPORTANT! The normalization plates must be well mixed and centrifuged.

Perform the calibration

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

   Note: You must add an 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**, 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 instrument.
   a. Confirm that the normalization plate or array card matches the selection in the 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.



① Load 96- and 384-well plates with the A1 position at the top-left corner of the plate adapter.
② 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**.

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

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

8. When the run is complete and the software displays the **Analysis** screen, confirm the status of the calibration. The analysis status indicates the success of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or 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 instrument ejects the tray arm.
Failed	Troubleshoot the failed calibration (see "Troubleshooting" on page 85).

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 instrument does not eject the plate, see "Troubleshooting" on page 85.

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 an instrument 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 step 4 to step 10 to perform the remaining normalization calibration.

Verify the instrument performance

Perform the RNase P instrument verification experiment to verify the performance of a QuantStudio™ 12K Flex Instrument.

When to perform the RNase P experiment

We recommend performing an RNase P instrument verification experiment at the following times:

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

About the RNase P kits

The instrument verification experiment uses one of two instrument verification kits available from Thermo Fisher Scientific. The kits differ only in the consumable format for which they are designed.

- A TaqMan™ RNase P Instrument Verification Plate for instruments with 96- or 384-well sample blocks
- A TaqMan™ Array Card RNase P Kit for 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 the following items:

- TaqMan™ Fast Universal PCR Master Mix
- RNase P primers
- FAM™ dye-labeled probe
- Known concentration of human genomic DNA template

The RNase P plates contain five replicate groups of standards (1,250; 2,500; 5,000; 10,000; and 20,000 copies), two unknown populations (5,000 and 10,000 copies), and a no template control (NTC).

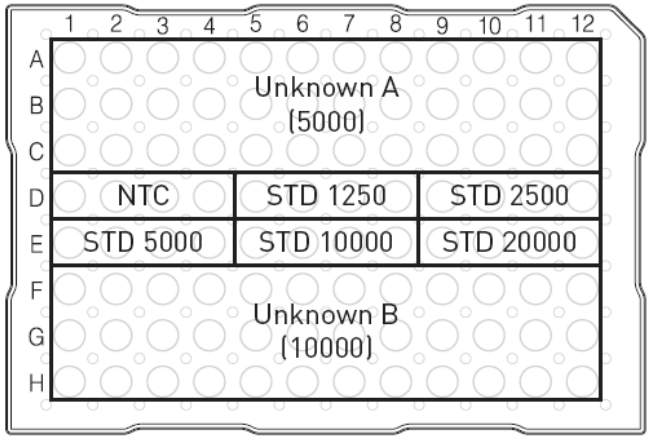


Figure 8 TaqMan™ RNase P Instrument Verification Plate, 96-well

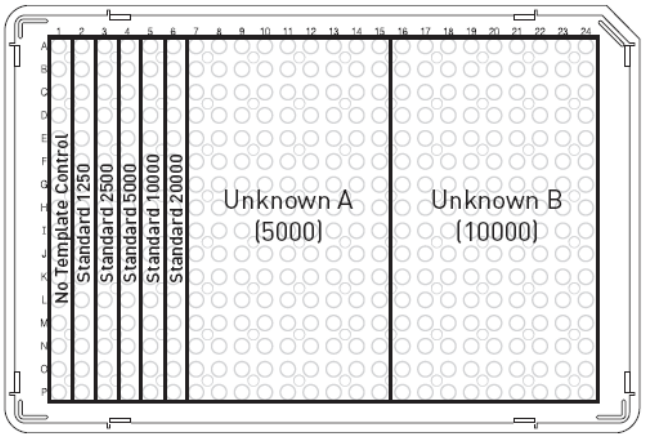


Figure 9 TaqMan™ RNase P Instrument Verification Plate, 384-well

TaqMan™ Array Card RNase P kits

The RNase P Kits include one empty array card and eight tubes of solution. Each tube contains reaction mix with the following components:

- TaqMan™ Universal PCR Master Mix
- RNase P primers
- FAM™-MGB dye-labeled probe
- 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 in the following figure. When complete, the array card contains five replicate groups of standards (200; 400; 800; 1,600; and 3,200 copies), two of unknown populations (800 and 1,600 copies), and one that serves as a no template control (NTC).

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

About the analysis

The QuantStudio™ 12K Flex Software performs the same analysis of data from an instrument verification runs for a 96-well 0.2-mL plate, 96-well 0.1-mL plate, 384-well plate, or array card block.

After the run, the software performs the following functions.

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 Real-Time PCR Instrument 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 Instrument Verification Plate or the 800-copy population in rows C and D of the loaded TaqMan™ Array Card.
Unknown population B refers to the 10,000-copy population in the wells of the TaqMan™ RNase P Instrument Verification Plate or the 1,600-copy population in rows E and F of the loaded TaqMan™ Array Card.

Installation specification

The QuantStudio™ 12K Flex System passes the installation specification if the inequality holds and the instrument successfully distinguishes between unknown populations A and B with a statistical confidence level of 99.7%.

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

Sample block	Maximum number of outlier wells that can be removed			
	Unknown population A[1]	Unknown population B[2]	Standards (STD)[3]	No template controls (NTC)
96-well plate[4]	6	6	1	0
384-well plate	10	10	2	0
TaqMan™ Array Card	4	4	4	0

[1] 5,000-copy population for 384-well plates. 800-copy population for array cards.
[2] 10,000-copy population for 384-well plates. 1,600-copy population for array cards.
[3] Maximum number of wells that can be removed from *each* standard population.
[4] 96-well 0.2-mL plates or 96-well 0.1-mL plates

Prepare the verification consumable

IMPORTANT! Use the following guidelines when performing the RNase P instrument verification experiment:

- Perform all calibrations beforehand.
- Run the plate or array card soon after you allow the plate or reagents to thaw. Minimizing the time between thaw and run supports 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:

- Prepare the TaqMan™ RNase P Instrument Verification Plate (see “Prepare the TaqMan™ RNase P Instrument Verification Plate” on page 78)
- Prepare a TaqMan™ Array Card for instrument verification (see “Prepare a TaqMan™ Array Card for instrument verification” on page 79)

Prepare the TaqMan™ RNase P Instrument Verification Plate

Required materials

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

Prepare the TaqMan™ RNase P Instrument Verification Plate

Obtain the plate from the freezer, then allow the plate to warm to room temperature (for approximately 5 minutes).

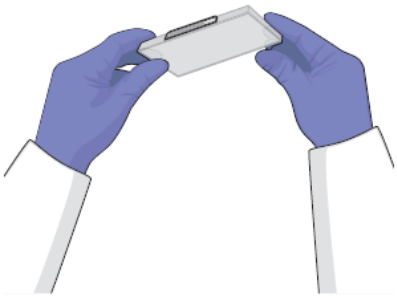
IMPORTANT! Do not remove the plate from its packaging until you are ready to run it. The fluorescent dyes in the dye plate are photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the plate.

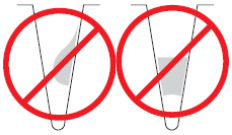
1. Remove the RNase P plate from its packaging.
2. Briefly vortex and centrifuge the RNase P plate.
   a. Vortex the plate for 5 seconds.
   b. Centrifuge the reaction plate for 2 minutes at less than 1500 rpm.

   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

Prepare a TaqMan™ Array Card for instrument verification

IMPORTANT! Perform the following procedure only if you are verifying the performance of a QuantStudio™ 12K Flex Instrument with a TaqMan™ Array Card sample block.

Required materials

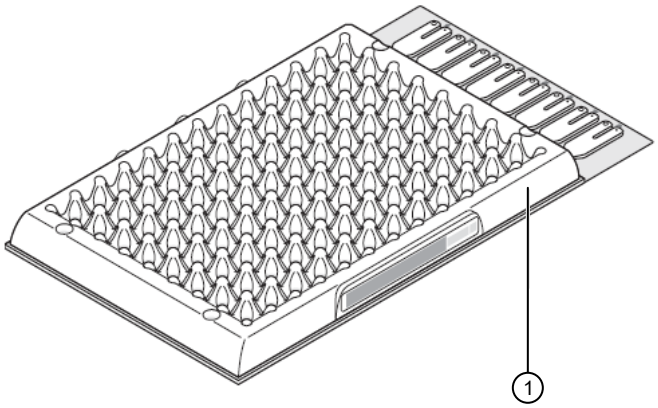
- TaqMan™ Array Card Sealer
- Centrifuge with centrifuge array card carrier clips and buckets
- Powder-free gloves
- Safety goggles
- Pipettor, 200- µL (with pipette tips)
- ViiA™ 7 Array Card RNaseP Verification Kit
  - TaqMan™ Array Card
  - Tubes with reagent mix for each port (8 tubes total)

Prepare the RNase P TaqMan™ Array Card

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

Remove the ViiA™ 7 Array Card RNaseP Verification Kit from the freezer, then allow it to thaw at room temperature.

1. Remove an array card from its box and place it on a clean, dry surface.
2. Using a permanent marker, mark the side of the empty array card with RNase P.



① Location to mark the information

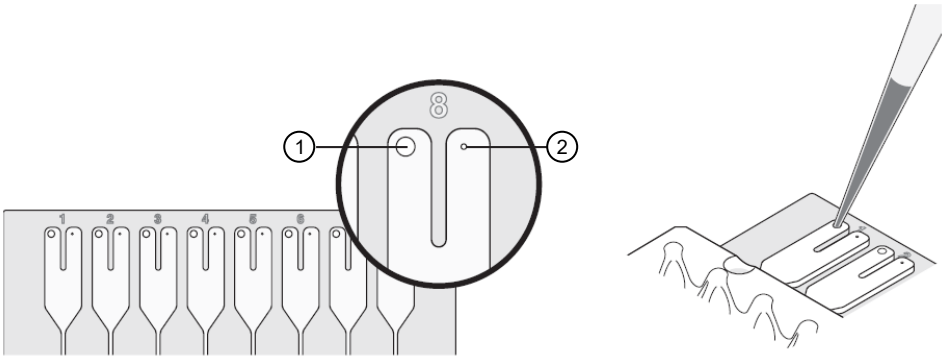
3. Transfer 100 µL of each solution into the appropriate port of the array card.

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

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 (approximately 45°) and place the tip into the fill port.

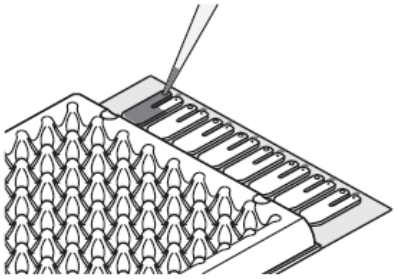


① Fill port
② Vent port

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.

4. Centrifuge and seal the array card.

   See “Fill the calibration TaqMan™ Array Cards” on page 45.

5. 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.

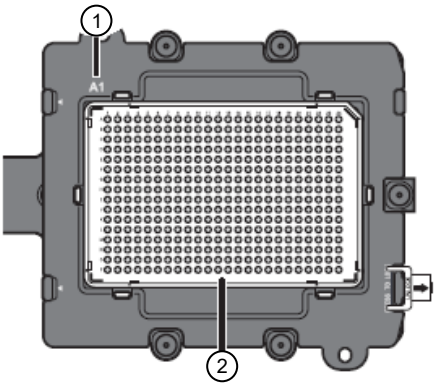
IMPORTANT! Do not expose the array card to light until you are ready to run it. The fluorescent dyes in the array card are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dye.

Run the experiment

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

Note: You must add an 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**, 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.



① Load 96- and 384-well plates with the A1 position at the top-left corner of the plate adapter.
② 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 **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 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 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.
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 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).
      - **TaqMan™ 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[1]	Unknown B[2]	Standard (STD)[3]	No template controls (NTC)
96-well plate[4]	6	6	1	0
384-well plate	10	10	2	0
TaqMan™ Array Card	4	4	4	0

[1] 5,000-copy population for 96- or 384-well plates. 800-copy population for array cards.
[2] 10,000-copy population for 96- or 384-well plates. 1,600-copy population for array cards.
[3] Maximum number of wells that can be removed from *each* standard population.
[4] 96-well 0.2-mL plates or 96-well 0.1-mL plates.

   d. Repeat substep 8a to substep 8c 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 (see "Troubleshooting" on page 85).
      - 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 step 8 and step 9, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Support.
11. Review the standard curve.
    a. Select **Standard Curve**.
    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 Support.
12. In the **Analysis** screen, click **Next**, remove the plate or array card when the 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 instrument does not eject the plate, see "Troubleshooting" on page 85.

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

Troubleshooting

Table 6 Troubleshooting ROI calibrations

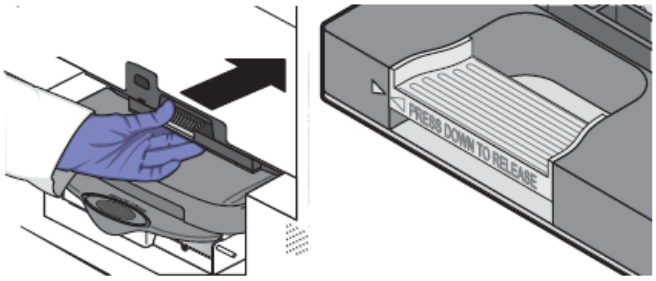
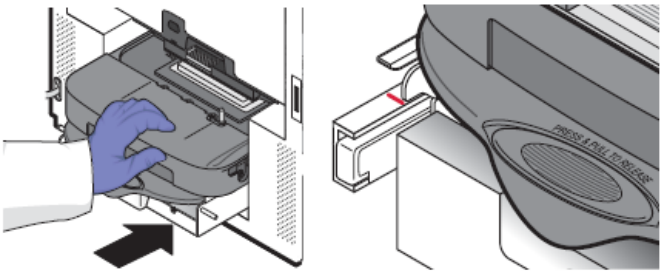
Problem/symptom	Possible cause	Action
ROI calibration failed.	The sample block or heated cover may not be seated correctly.	**1.** Power off and unplug the QuantStudio™ 12K Flex Instrument. **2.** Wait for 15 minutes, then open the access door. **3.** Firmly push the sample block and the heated cover toward the back of the 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 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 in the instrument, then replace the LEDs if necessary.
ROI image is faint.		
Instrument malfunction.	Multiple possible causes	Contact Support.

Table 6 Troubleshooting ROI calibrations *(continued)*

Problem/symptom	Possible cause	Action
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 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. 5. Perform a background calibration to confirm that the sample block has not been contaminated.

Table 7 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 93.
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 instrument and eject the plate. 3. If the plate does not eject, power off and unplug the 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. 5. Perform a background calibration to confirm that the sample block has not been contaminated.
Instrument malfunction.	Multiple possible causes	Contact Support.

Table 8 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 Support.

Table 8 Troubleshooting uniformity calibrations *(continued)*

Problem/symptom	Possible cause	Action
Uniformity calibration failed.	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 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. Contact 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 instrument and eject the plate. 3. If the plate does not eject, power off and unplug the 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. 5. Perform a background calibration to confirm that the sample block has not been contaminated.
Instrument malfunction.	Multiple possible causes	Contact Support.

Table 9 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.	1. Unload the dye calibration plate from the QuantStudio™ 12K Flex Instrument 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 Support.
	Dye calibration plate contains old or insufficient reagents.	

Table 9 Troubleshooting dye calibrations *(continued)*

Problem/symptom	Possible cause	Action
One or more raw spectra are at or below the detectable threshold for the calibration.	If you are running a custom dye calibration plate, the dye may not be present at a sufficient concentration.	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.	Verify that contaminants are not present by performing a background calibration (see "Background calibration" on page 55) 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.
	If you are running a custom spectral calibration plate, the dye may be too concentrated.	
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 instrument and eject the plate. 3. If the plate does not eject, power off and unplug the 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. 5. Perform a background calibration to confirm that the sample block has not been contaminated.
Instrument malfunction.	Multiple possible causes	Contact Support.

Table 10 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 a 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 Support.

Table 10 Troubleshooting normalization calibrations *(continued)*

Problem/symptom	Possible cause	Action
Normalization calibration failed.	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 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 Support.
Instrument does not eject the normalization plate or array card.	The adhesive cover may have adhered the plate to the heated cover within the instrument. An adhesive cover may have adhered the array card 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 or the array card. 3. If the plate or the array card does not eject, power off and unplug the instrument, then open the access door. 4. Wearing powder-free gloves, reach into the instrument and remove the plate or the array 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 Support.

Table 11 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	Contact Thermo Fisher Scientific to order a replacement TaqMan™ RNase P Instrument Verification Plate or a replacement ViiA™ 7 Array Card RNaseP Verification Kit. If the replacement plate or array card kit fails, contact Support for further assistance.
	Pipetting inaccuracy	
RNase P plate verification run failed.	Insufficient centrifugation	⚠ **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.

Table 11 Troubleshooting RNase P instrument verification experiments *(continued)*

Problem/symptom	Possible cause	Action
RNase P plate verification run failed.	Defective plate seal	1. Unload the RNase P plate or array card from the 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 Thermo Fisher Scientific to order a replacement TaqMan™ RNase P Instrument Verification Plate or a replacement ViiA™ 7 Array Card RNaseP Verification Kit. If the replacement plate or array card kit fails, contact Support 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 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 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. 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 Thermo Fisher Scientific to order a replacement TaqMan™ RNase P Instrument Verification Plate or a replacement ViiA™ 7 Array Card RNaseP Verification Kit. If the replacement plate or array card kit fails, contact Support for further assistance.

Table 11 Troubleshooting RNase P instrument verification experiments *(continued)*

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 Thermo Fisher Scientific to order a replacement TaqMan™ RNase P Instrument Verification Plate or a replacement ViiA™ 7 Array Card RNaseP Verification Kit. If the replacement plate or array card kit fails, contact Support 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 Thermo Fisher Scientific to order a replacement TaqMan™ RNase P Instrument Verification Plate or a replacement ViiA™ 7 Array Card RNaseP Verification Kit. If the replacement plate or array card kit fails, contact Support 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 Thermo Fisher Scientific to order a replacement TaqMan™ RNase P Instrument Verification Plate or a replacement ViiA™ 7 Array Card RNaseP Verification Kit. If the replacement plate or array card kit fails, contact Support 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 Thermo Fisher Scientific to order a replacement TaqMan™ RNase P Instrument Verification Plate or a replacement ViiA™ 7 Array Card RNaseP Verification Kit. If the replacement plate or array card kit fails, contact Support for further assistance.

Table 11 Troubleshooting RNase P instrument verification experiments *(continued)*

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 Thermo Fisher Scientific to order a replacement TaqMan™ RNase P Instrument Verification Plate or a replacement ViiA™ 7 Array Card RNaseP Verification Kit. If the replacement plate or array card kit fails, contact Support 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 Thermo Fisher Scientific to order a replacement TaqMan™ RNase P Instrument Verification Plate or a replacement ViiA™ 7 Array Card RNaseP Verification Kit. If the replacement plate or array card kit fails, contact Support 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 Thermo Fisher Scientific to order a replacement TaqMan™ RNase P Instrument Verification Plate or a replacement ViiA™ 7 Array Card RNaseP Verification Kit. Repeat the experiment, and make sure to properly seal and centrifuge the RNase P plate or array card. If the replacement plate or array card kit fails, contact Support for further assistance.

Table 11 Troubleshooting RNase P instrument verification experiments *(continued)*

Problem/symptom	Possible cause	Action
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 Thermo Fisher Scientific to order a replacement TaqMan™ RNase P Instrument Verification Plate or a replacement ViiA™ 7 Array Card RNaseP Verification Kit. If the replacement plate or array card kit fails, contact Support for further assistance.
Instrument malfunction.	Multiple possible causes	Contact Support.

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.

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 whether the sample block or the plate or array card is contaminated based on the location of the contaminated wells in step 1 and step 2.

Option	Description
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, perform the following steps.
   a. Cover a plate or array card with a piece of black paper.
   b. Perform the background run, while 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 instrument may be contaminated. Contact Support.
   - **Absent**—The sample block is contaminated. Decontaminate the sample block again and repeat the calibration.

3 Calibrating OpenArray™ Plate sample blocks

Recommended calibration and maintenance

The 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 enable optimal instrument performance.

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

IMPORTANT! Do not use organic solvents to clean the instrument.

Table 12 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 an instrument self-test.
Monthly	Perform a background calibration.[1]
	Run disk cleanup and disk defragmentation.

Table 12 OpenArray Plate sample block maintenance *(continued)*

Frequency	Maintenance task
Annually	Perform a major cleaning of the OpenArray™ Plate sample block.
	Perform a background calibration.
	Perform a uniformity calibration.
	Perform a dye calibration.
	Perform an instrument verification run.
As needed	Perform a major cleaning of the OpenArray™ Plate sample block or a minor cleaning of the OpenArray™ Plate sample block.
	Decontaminate the system.
	Replace the system fuses.
	Update the Windows™ operating system.
	Update the software and firmware.

[1] 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 Instrument 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	• Always wear powder-free gloves. • Grasp the tool by the edges. • Ensure that the tool does not become dirty or dusty.
Storing	When not in use, store the OpenArray™ Calibration Plaque under the following conditions: • 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: **1.** Place the calibration plaque on a clean, dry surface. **2.** Pipet a small volume of 95% ethanol or 95% isopropanol solution onto a lint-free wipe, then thoroughly swab the surface of the tool. **3.** 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 Instrument with an OpenArray™ sample block.

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

- Performs two reads of the 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

- 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.

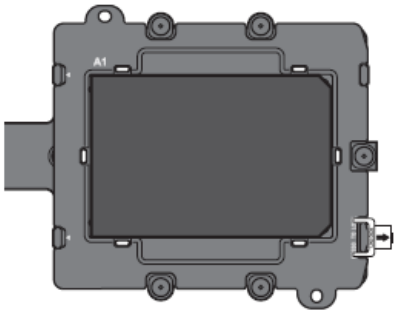
IMPORTANT! A user must be present throughout the duration of the calibration. Following the first read, the OpenArray™ Calibration Plaque must be rotated 180° before the instrument can complete the calibration.

Load the OpenArray™ Calibration Plaque

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

   IMPORTANT! Ensure that the 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 instrument is in operation.

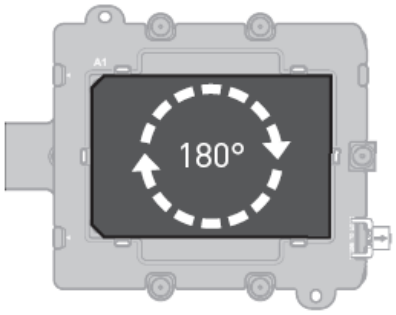
   Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach temperature.

Rotate the OpenArray™ Calibration Plaque

The instrument door opens and you are prompted to rotate the OpenArray™ Calibration Plaque.

1. Rotate the calibration plaque 180°, then place it back into the plate retainer (*black* side up).

IMPORTANT! Do not flip the 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 calibration plaque before you repeat the calibration (see “Caring for the OpenArray™ Calibration Plaque” on page 97). 2. If the calibration fails again, contact Support.

2. When the instrument door opens, remove the 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 calibration plaque to its original packaging or a clean plastic bag.

IMPORTANT! Do not expose the 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 instrument does not eject the calibration plaque, see “Troubleshooting” on page 123.

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 allow the QuantStudio™ 12K Flex Software to compensate for the physical effects of the QuantStudio™ 12K Flex System filters.

Required materials

- 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. The software uses the captured images to calibrate the optical uniformity of the QuantStudio™ 12K Flex Instrument.

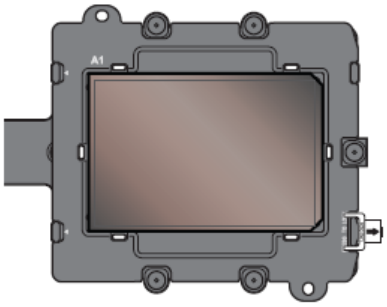
IMPORTANT! A user must be present throughout the duration of the calibration. Following the first read, the calibration plaque must be rotated 180 degrees before the instrument can complete the calibration.

Load the OpenArray™ Calibration Plaque

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

IMPORTANT! Ensure that the 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**.

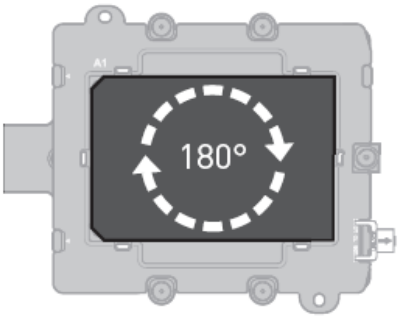
IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the instrument is in operation.

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

Rotate the OpenArray™ Calibration Plaque

The instrument door opens and you are prompted to rotate the OpenArray™ Calibration Plaque.

1. Rotate the calibration plaque 180°, then place it back into the plate retainer *(orange* side up).



IMPORTANT! Do not flip the 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.
Failed	1. Repeat the calibration. If necessary, clean the calibration plaque before you repeat the calibration (see “Caring for the OpenArray™ Calibration Plaque” on page 97). 2. If the calibration fails again, contact Support.

2. When the instrument door opens, remove the 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 calibration plaque to its original packaging or a clean plastic bag.

IMPORTANT! Do not expose the 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 instrument does not eject the calibration plaque, see “Troubleshooting” on page 123.

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 system performs the following functions:

- 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 instrument. After each run, the 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 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 system.

Required materials

- QuantStudio™ 12K Flex OpenArray™ Calibration Kit
  - FAM™ dye
  - OpenArray™ Calibration Cases (4)
  - Plugs (4)
  - Calibration syringe and tip
- QuantStudio™ 12K Flex 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 FAM™ dye

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 software extracts the dye calibration data, it evaluates the fluorescence signal generated by each 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 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 calibration cases (auto-repairing). The software allows only a few replacements, and it may reject the calibration if the spectra between neighboring wells vary significantly.

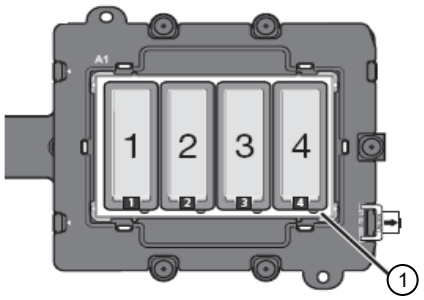
Guidelines for handling the OpenArray™ Calibration Cases

- 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 the calibration cases by the edges.
- If you drop a loaded calibration case, discard it in the appropriate waste container.

Perform the empty reading of the OpenArray™ Calibration Cases

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 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 instrument followed by the remaining cases in sequence as shown in the following figure.



① Plugs

IMPORTANT! Confirm that the 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 calibration 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 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 of the OpenArray™ Calibration Cases

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

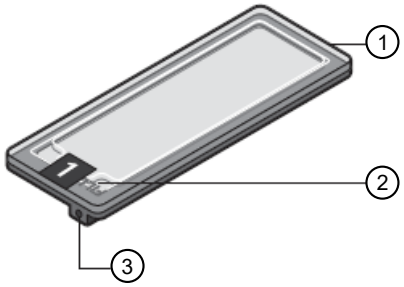
When the instrument door opens and you are prompted to perform the filled reading, load the OpenArray™ Calibration Cases with FAM™ dye.

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 FAM™ dye 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 calibration case.

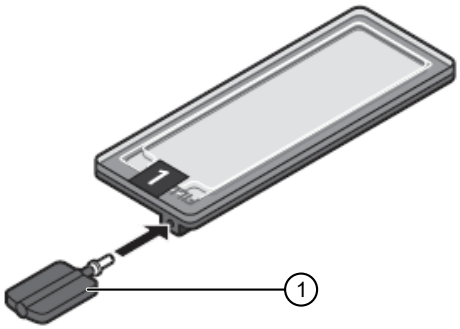
5. While holding the calibration case vertically, insert the syringe tip into the fill port at end of the case, then dispense the fluid completely in one gentle continuous motion.



① Empty OpenArray™ Calibration Case
② Fill label
③ Fill port

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.



① OpenArray™ Plug (insert and twist)

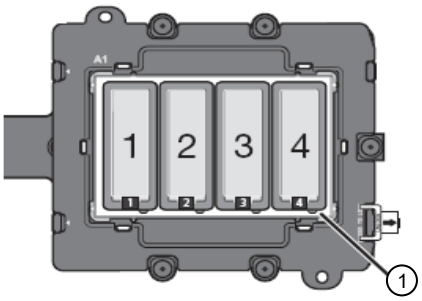
7. Load the sealed calibration case into the *same* position on the calibration carrier that it previously occupied (position 1).

IMPORTANT! You *must* load the filled calibration cases into the same positions on the 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 step 1 to step 7 to fill the remaining three calibration cases.

IMPORTANT! Confirm that the calibration cases are in their original positions and that their plugs are oriented away from the A1 position as shown.



① Plugs

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 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 the 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 Support for further assistance.

2. When the instrument door opens, remove the OpenArray™ Calibration 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 instrument does not eject the plate, (see “Troubleshooting” on page 123).

Verify 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 supports 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 OpenArray™ Calibration Cases.

When to perform the RNase P experiment

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

About the QuantStudio™ 12K Flex OpenArray™ Block RNase P Kit

The kit includes the following components:

- Empty OpenArray™ Plate (1)
- OpenArray™ RNase P Reaction Mix, contains the following components in a single tube:
  - TaqMan™ Universal PCR Master Mix
  - RNase P primers
  - FAM™-MGB dye-labeled probe
- 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 an 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 OpenArray™ Block RNase P Kit, including the following components:
  - 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
- QuantStudio™ 12K Flex OpenArray™ AccuFill™ System
- QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0
- Bleach (10%)
- Ethanol
- OpenArray™ 384-well Sample Plates
- OpenArray™ AccuFill™ System Tips
- Pipettes
- Powder-free gloves
- Safety glasses

Prepare for the verification experiment

IMPORTANT! Wear powder-free gloves while preparing the OpenArray™ Plate.

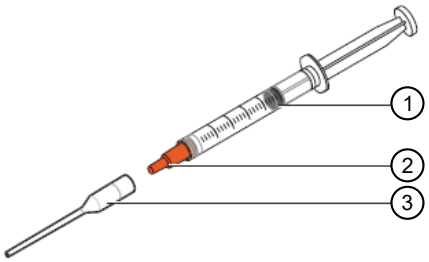
1. Confirm that the OpenArray™ 384-well Sample Plate, the OpenArray™ AccuFill™ System Tips, and the plate holder are completely clean and dry.

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.

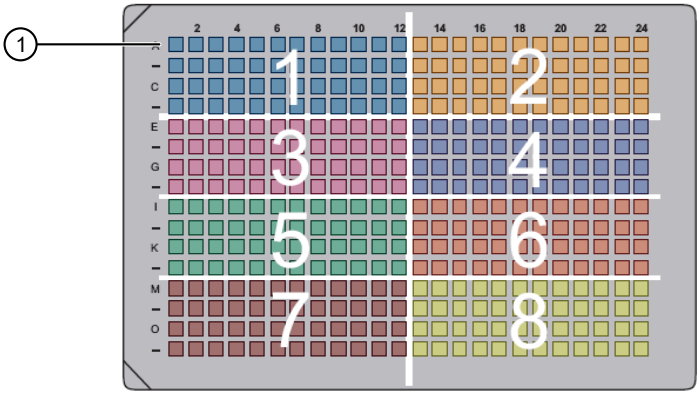
3. Prepare a syringe containing OpenArray™ Immersion Fluid. Attach the syringe tip to the syringe, then set the assembly on a clean surface.

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



① OpenArray™ Immersion Fluid
② Cap (remove)
③ Syringe tip (attach)

4. Pipet 5.0 µL of the RNase P solution into loading position 1 of the OpenArray™ 384-well Sample Plate.



① Loading position 1 of the OpenArray™ 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.

Proceed to one of the following sections, depending on the version of the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System:

- “Load the OpenArray™ Plate (OpenArray™ AccuFill™ Software v1.2)” on page 111
- “Load the OpenArray™ Plate (OpenArray™ AccuFill™ Software v2.0)” on page 115

Load the OpenArray™ Plate (OpenArray™ AccuFill™ Software v1.2)

Initialize the system

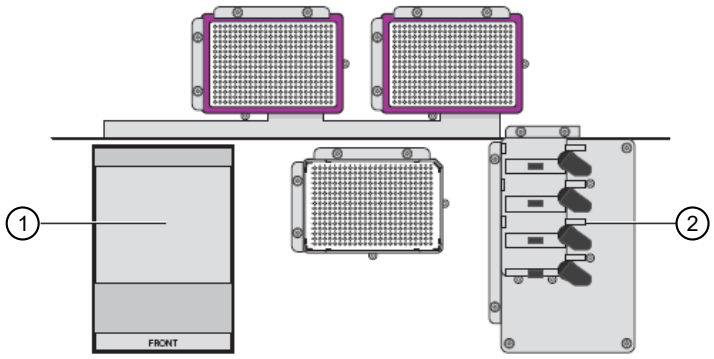
1. Close the enclosure door, then start the OpenArray™ AccuFill™ Software. The software checks the computer and connections as the system starts.

   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.



① Waste bin
② Plate holder

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. The 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.

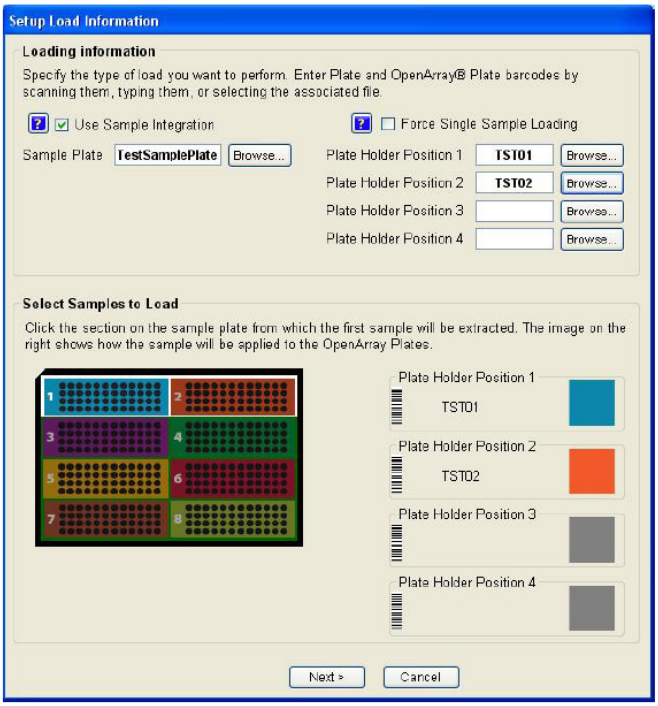
4. Close the door on the instrument.

5. Click **Proceed** to begin the system self-test. The application performs a number of tests and is then ready for you to continue.

Note: The 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 self-test utility is in the **Instrument** drop-down menu in the OpenArray™ AccuFill™ application.

Prepare 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 QuantStudio™ 12K Flex OpenArray™ Block RNase P 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. Use the following guidelines when handling an 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.

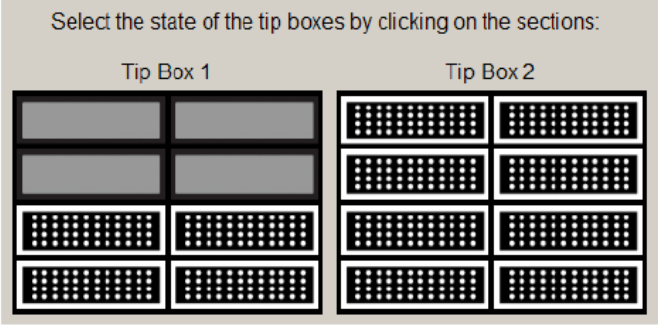
   Hold the OpenArray™ case by the edges and place it in the plate holder with the barcode facing up and to the left.

6. Click **Next**.

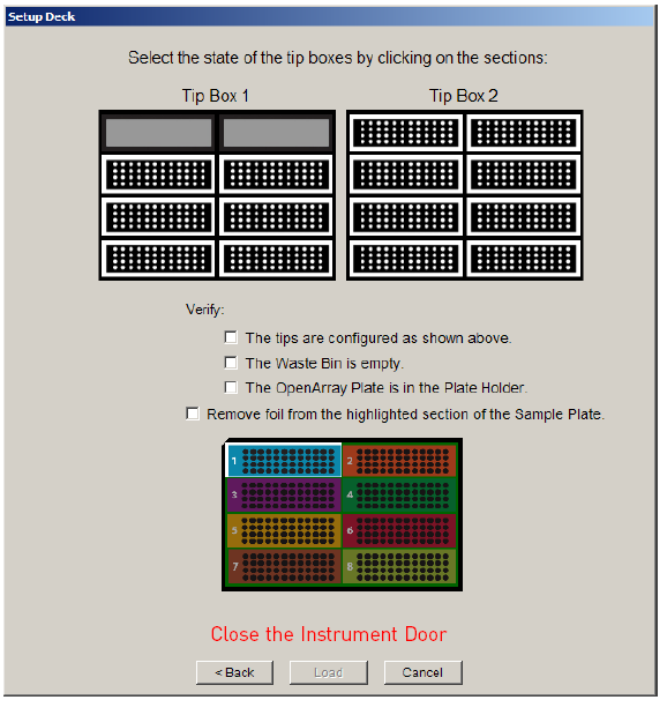
Load the OpenArray™ Plate

1. Verify that the **Tip Status** window in the software matches the state of the tips on the deck. Ensure that the following conditions are applicable.
   - 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:
   - The tips are configured.
   - The waste bin is empty.
   - The 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 (see “Seal the OpenArray™ Plate” on page 118).

   IMPORTANT! Once an OpenArray™ Plate has been filled, seal it within 90 seconds to prevent excessive evaporation.

8. Close the instrument door.

Seal the OpenArray™ Plate (see “Seal the OpenArray™ Plate” on page 118).

Note: After you run the plate, clean the OpenArray™ AccuFill™ Instrument.

Load the OpenArray™ Plate (OpenArray™ AccuFill™ Software v2.0)

There are multiple workflows available with OpenArray™ AccuFill™ Software v2.0. The quick run workflow without sample information is recommended to prepare an OpenArray™ Plate for RNase P instrument verification.

For other workflow options and more information, see *QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide* (Pub. No. MAN0025669).

Initialize the system

1. Ensure that the instrument door is closed.
2. Power on the instrument, if it is off.
3. Start the software ( ).
   The software checks the computer and connections as the system starts.

Proceed to set up the system (see “Set up the system” on page 115).

Set up the system

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

1. Open the instrument door, empty the waste bin, then place the waste bin back on the instrument deck.

CAUTION! Wear appropriate personal protective equipment while handling the waste bin.

2. Ensure that the sample plate holder and the OpenArray™ Plate holders are empty.
3. Replace the tip boxes, if necessary.
   Each tip box contains 384 tips, divided into 8 sections.
   When setting up a run, the status of the tip boxes is confirmed in the software. A full tip box is recommended when starting a run.
   Do not reuse tips.
4. Remove the cover from each tip box.

   Note: Ensure that the tip box covers are removed from the instrument deck.
5. Close the instrument door.

The system is ready to start a run. A self-test is initiated the first time that one of the following items is clicked after starting the software:

- **Full Run**
- **Quick Run**
- **Service ▸ Diagnostics**

Configure the run

Navigate to the **Quick Run** screen. Ensure that the **Load without sample plate information** radio button is selected.

1. *(Optional)* In the **Quick Run** screen, in the **Sample plate - optional** field, enter information to identify the sample plate.
   - Enter the information about the sample plate, for example, ***RNase P***.
   - Use a barcode scanner to scan the sample plate or manually enter the barcode text string.
2. Select one sample per subarray.
3. In the **OpenArray Plate name and position** field, enter information to identify the OpenArray™ Plate.

   We recommend using the serial number of the OpenArray™ Plate as the identifying information.

   The name and position are recorded in the loading history log.
4. Click a section of the sample plate to change the corresponding OpenArray™ Plate.

   Note: The first section of the sample plate is selected.

   The OpenArray™ Plate position displays the color that corresponds to the section of the sample plate.

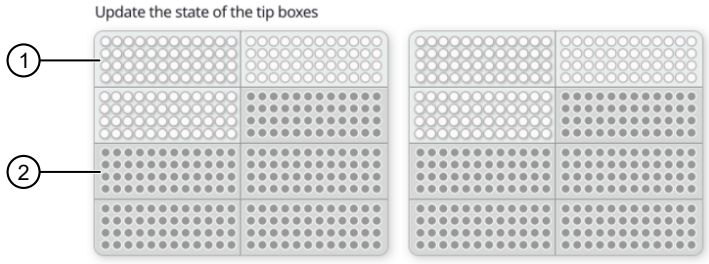
Proceed to “Verify the run setup and start the run” on page 116.

Verify the run setup and start the run

1. Click each tip box so that the status on the **Verify the run setup and start the run** section matches the physical tip box in the instrument.

   We recommend starting the run with full tip boxes.

   The instrument does not start the run if there are not enough tips on the deck.



① Section of the tip box is full
② Section of the tip box is empty

2. Select the section of the sample plate that will be used to fill the OpenArray™ Plate.
3. Remove the foil from the appropriate section of the sample plate, then click the checkbox to confirm.
4. Close the instrument door.
5. Click **Start Run**.

   The run does not begin under any of the following conditions:

   - The waste bin is not in position
   - The sample plate is not in position
   - The OpenArray™ Plates are not in position
   - There are more OpenArray™ Plates on the instrument deck than are defined in the experiment setup

The **Deck** screen is displayed.

IMPORTANT! Each OpenArray™ Plate must be prepared for PCR immediately after it is filled (see “Remove the OpenArray™ Plate from the instrument” on page 117).

Remove the OpenArray™ Plate from the instrument

After the OpenArray™ Plate in the run is filled, the **Remove plate** dialog box is displayed.

Remove the OpenArray™ Plate *immediately* after it has been filled.

1. Open the instrument door and remove the OpenArray™ Plate that is indicated by the blue box in the dialog box.

   IMPORTANT! Remove the OpenArray™ Plate within 30 seconds, to avoid evaporation within the plate.

2. Seal the case and fill the OpenArray™ Plate with immersion fluid.

   See “Seal the OpenArray™ Plate” on page 118.
3. Close the instrument door.

After the OpenArray™ Plate has been loaded, the **Deck** screen displays **Run completed successfully. Empty the waste bin before performing another run.**

Seal the OpenArray™ Plate

IMPORTANT! Throughout this procedure, handle the OpenArray™ Plate and the OpenArray™ Case only by the edges.

Wear snug-fitting gloves when working with OpenArray™ Plates.

Note: The OpenArray™ Case consists of the sealed OpenArray™ Plate and the OpenArray™ Case Lid.

1. Place the newly loaded OpenArray™ Plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

   Ensure that the barcode is facing left and the serial number is facing right.

2. From the OpenArray™ Lid, remove the clear protective film from the *inside* of the lid ① and the red adhesive-protective strip ② from around the edge of the lid.

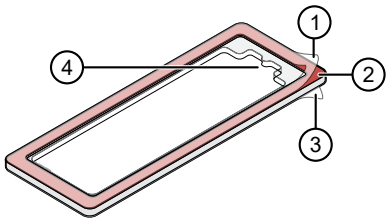
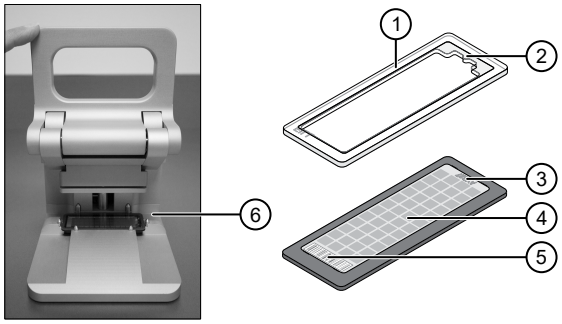


Figure 10 OpenArray™ Lid

① Protective film on inside of the lid (remove before *sealing*)
② Red adhesive-protective strip (remove before *sealing*)
③ Protective film on the outside of the lid (remove before *running*)
④ Notched end (align with serial number on plate)

3. Place the lid in the Plate Press using the alignment pins of the Plate Press for orientation.

IMPORTANT! The notched end of the case lid must be oriented towards the furthest back right-side of the Plate Press.



① OpenArray™ case lid
② Notched end of lid
③ Serial number of plate
④ OpenArray™ Plate
⑤ Barcode of plate
⑥ Alignment pins

4. Seat the lid on the OpenArray™ Plate with the lid adhesive against the plate.

5. Engage the press mechanism until the green flashing light changes to a steady green light (after 20 seconds).

   The status light turns solid green, indicating that the case is sealed.

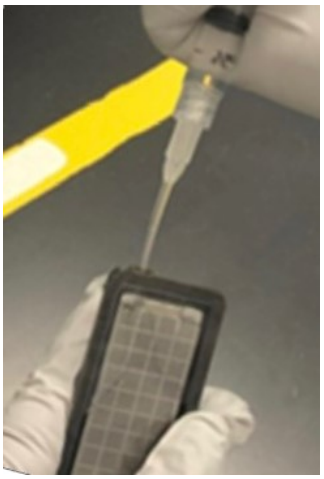
Note: Do not apply additional pressure onto the Plate Press during its actuation.

6. Disengage the press and carefully remove the OpenArray™ Case.

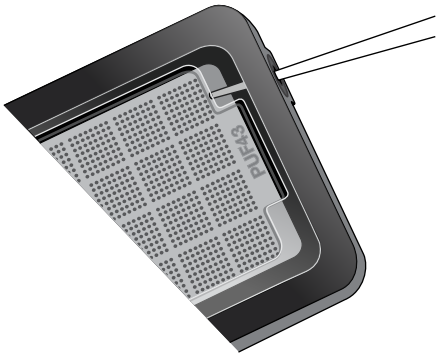
7. Prepare the immersion fluid. Remove the cap, insert the accompanying syringe tip, and prime the syringe by ejecting a small amount of immersion fluid onto a paper towel to ensure no air gap remains in the newly attached pipette tip.

IMPORTANT! If the syringe is not primed, the direct burst of air and fluid can negatively affect the assay(s) at the end of the array.

8. While holding the case upright by its edges at a 15–30 degree angle so that the port is at the highest point of the array, insert the prepared syringe tip into the port in the case.



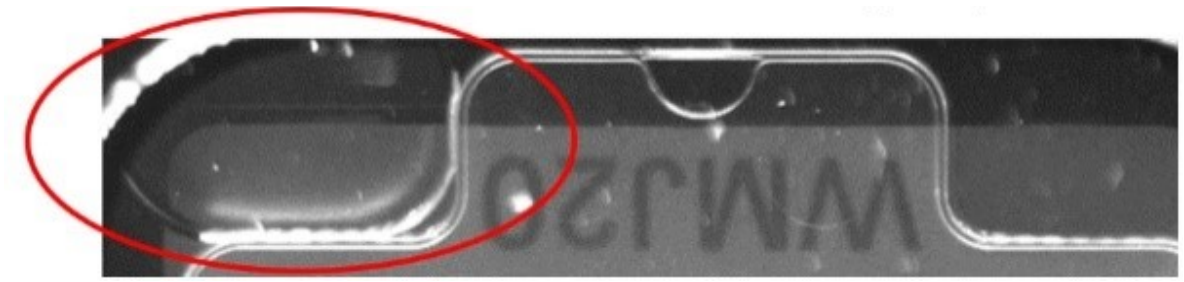
The syringe tip must be in front of the array when filling the case with immersion fluid.



9. Slowly inject the OpenArray™ Immersion Fluid until the case is filled, which should take about 10 seconds to fill. Minimize the creation of additional air bubbles when you dispense the fluid. Leave a small air bubble as shown below.

IMPORTANT! If injected too quickly, the fluid can flush out the samples that are suspended in the through-holes.

Overfilling the array and/or not leaving a small bubble may cause a leak during the PCR run.



10. While holding the case *vertically*, remove the syringe tip, insert the screw end of the OpenArray™ plug into the port of the case, then rotate clockwise until the black handle breaks off.

    Note: Ensure that you are screwing the plug in at the same angle the case base is at. If it is off, it can cause the plug to break off prematurely.

    IMPORTANT! To avoid leaking of immersion fluid, hold the case *vertically* and rotate the plug slowly to avoid cross-threading.

    If the plug handle breaks off prematurely, use a Phillips #0 screwdriver to complete this step. Do not overtighten. If plastic or adhesive remains attached to the screw due to premature breakout of the plug handle, remove it with forceps prior to loading it into the instrument.

11. If needed, clean the case with the lint-free cloth included with the OpenArray™ Plate or a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.

The plate is ready for PCR.

IMPORTANT! Run the prepared verification plates within one hour after loading them (see "Run the experiment" on page 121).

Discard the filled plate after a successful instrument verification.

Run 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 an 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. Remove the clear protective film from the outside of the OpenArray™ Case (sealed plate + lid).
   See Figure 10 on page 118.

7. 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 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 software displays the **Analysis** screen, verify the status of the run.

Analysis status	Action
Passed	Go to step 13.
Failed	Go to step 9 to review the data for outliers. If the run fails, the 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.

9. 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_t values 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.

10. 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 (see “Troubleshooting” on page 123).
   - 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

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

    If the status of the RNase P Run is “Failed” after performing step 9 to step 11, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Support.

12. Complete the calibration as instructed. When the instrument ejects the tray arm, 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 instrument does not eject the OpenArray™ Plate, see “Troubleshooting” on page 123.

13. 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.	Order and perform a replacement RNase P experiment. If the replacement RNase P OpenArray™ Plate fails, contact Support for further assistance.
	Pipetting inaccuracy.	

(continued)

Problem/symptom	Possible cause	Action
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 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 RNase P OpenArray™ Plate fails, contact Support 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 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 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 Support 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. If the replacement RNase P OpenArray™ Plate fails, contact Support for further assistance.

(continued)

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 Support 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 (see "View the ROX™ dye image files" on page 127) . Non-uniformity can indicate problems with plate loading. 5. Order and perform a replacement RNase P experiment. If the replacement RNase P OpenArray™ Plate fails, contact Support 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. If the replacement RNase P OpenArray™ Plate fails, contact Support for further assistance.

(continued)

Problem/symptom	Possible cause	Action
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 OpenArray™ Plate fails, contact Support for further assistance.
Instrument malfunction	Multiple possible causes.	Contact Support.

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.

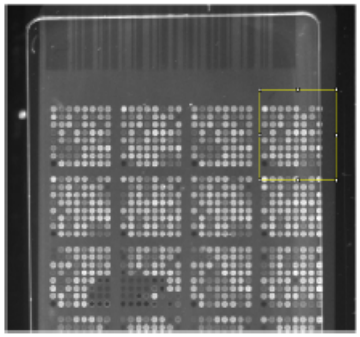
1. While viewing the background calibration data in the **Analysis** screen, select the **QC** tab and review the list of through-holes that failed the quality check.
2. Rotate the background plate 180°, then perform the background calibration again.
3. Determine the location of the contaminated through-holes 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 an 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. If the peak associated with the contamination is:
      - **Visible**—The optics of your instrument may be contaminated. Contact Support.
      - **Absent**—The sample block is contaminated. Decontaminate the sample block again and repeat the calibration.

View the ROX™ dye 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.

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 the following figure) 	The 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 instrument. 2. Hold the OpenArray™ Plate up to a light source, and ensure 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 Support for further assistance.

4 Maintenance

IMPORTANT! This chapter contains all user service procedures for the QuantStudio™ 12K Flex Real–Time PCR System. Procedures other than those described in this document must be performed by a qualified Thermo Fisher Scientific Field 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.

IMPORTANT! Do not run the disk management utilities and the 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 hours 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 instrument touchscreen to back up the instrument settings (instrument name, icon, standby time-out, and cover idle temperature). In the event that the instrument settings are reset, you can restore the settings from the backup.

For more information, see “Back up the instrument settings” on page 186.

Decontaminating the sample block

Perform this procedure to eliminate fluorescent contaminants from the 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 instrument cover. There are no components inside the instrument that you can safely service yourself. If you suspect a problem, contact Support.

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.

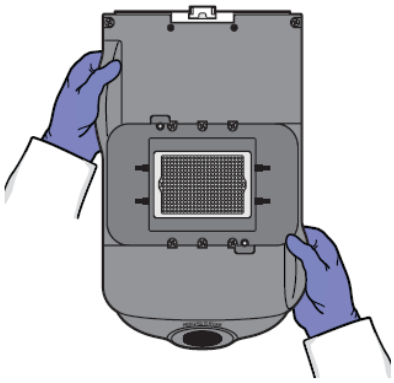
CAUTION! Before using a cleaning or decontamination method other than those recommended, verify with Thermo Fisher Scientific that the proposed method will not damage the equipment.

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. When the assembly has been removed from the instrument, place the sample block on a clean, dry surface or in its shipping container.



Cleaning the sample block

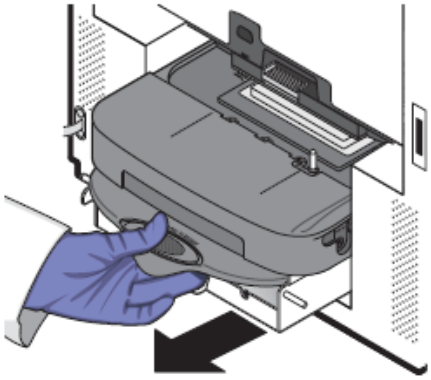
WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the sample block and heated cover can be heated to 100°C. Before removing the sample block, be sure to wait until it reaches room temperature.

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

1. Identify the contaminated wells of the sample block (see “Identifying contamination” on page 93).
2. Power off and unplug the instrument, then allow it to cool for 15 minutes.

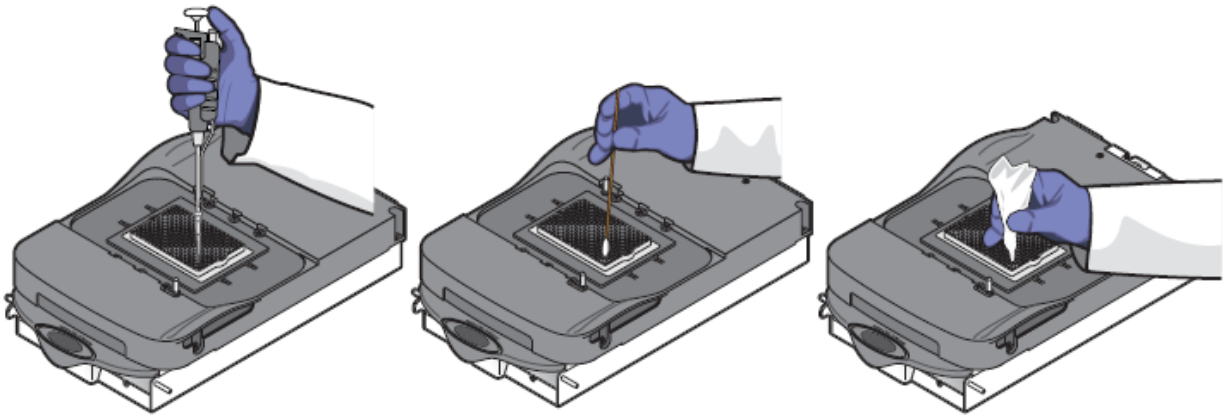
3. Open the access door.

4. Firmly press down on the handle of the sample block, then remove it from the instrument. Place the sample block on a clean, dry surface.



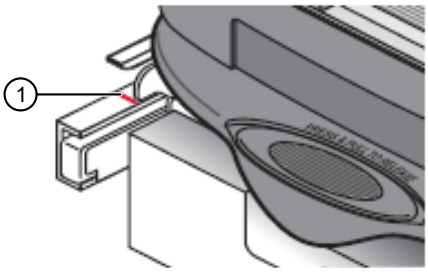
5. Clean the contaminated wells of the sample block using deionized water.
   a. Pipet a small volume of deionized water into each contaminated well.
   b. In each well, pipet the water up and down several times to rinse the well.
   c. Pipet the water to a waste beaker.
   d. Using a cotton swab, scrub inside of each contaminated well. If you are decontaminating a TaqMan™ Array Card block or an 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 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 instrument until it is seated correctly.



① Indicator

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 step 2 to step 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 a TaqMan™ Array Card block or an 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 step 5 to step 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 step 2 to step 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 a TaqMan™ Array Card block or an 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 step 5 to step 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 Support.

Clean the OpenArray™ Plate sample block

A minor cleaning procedure can be performed (see “Clean the OpenArray™ Plate sample block (minor cleaning procedure)” on page 134). Perform the minor cleaning procedure as needed, for example, after a minor leak of immersion fluid.

A major cleaning procedure can be performed (see “Clean the OpenArray™ Plate sample block (major cleaning procedure)” on page 136). The major cleaning procedure takes 30 minutes. Perform the major cleaning procedure as needed, for example, after a major leak of immersion fluid. Perform the major cleaning procdure annually.

Clean the OpenArray™ Plate sample block (minor cleaning procedure)

This procedure applies only to the OpenArray™ Plate sample block. It can be performed if there was a minor leak in immersion fluid.

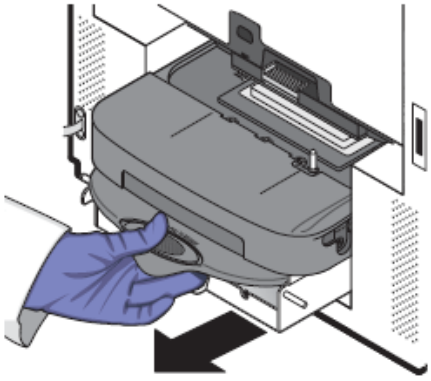
This procedure applies to the OpenArray™ Plate sample block due to the use of immersion fluid.

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. Touch to eject the instrument tray arm.
2. Remove the plate adapter, then place it on a clean, dry surface.
3. Touch to retract the instrument tray arm.
4. Power off and unplug the instrument, then allow it to cool for 15 minutes.
5. Open the access door.

6. Firmly press down on the handle of the sample block, then remove it from the instrument. Place the sample block on a clean, dry surface.

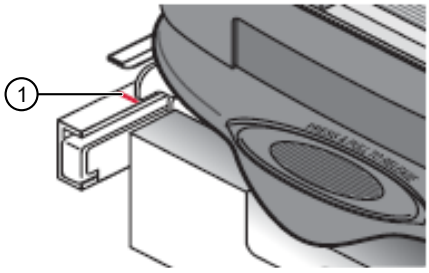


7. Wipe the surfaces of the sample block, heated cover, and plate adapter with a 95% ethanol solution using a lint-free cloth to remove contaminants, for example, fluid residue or dust.

8. Load the sample block into the instrument, then close the access door.

   Do not load the plate adapter during this step.

   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 instrument until it is seated correctly.



① Indicator

9. 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.

10. Plug in, then power on the instrument.

11. Touch ⏏ to eject the instrument tray arm.

12. Load the plate adapter, then touch ⏏ to retract the instrument tray arm.

Clean the OpenArray™ Plate sample block (major cleaning procedure)

This procedure applies only to the OpenArray™ Plate sample block. It should be performed annually. It can also be performed if there was a major leak in immersion fluid.

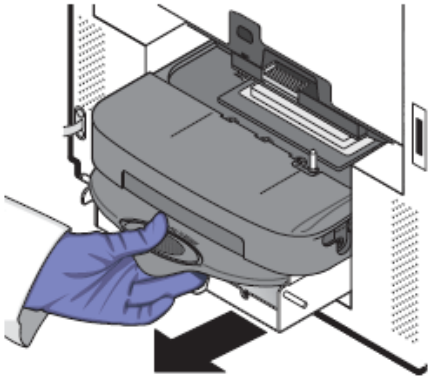
This procedure applies to the OpenArray™ Plate sample block due to the use of immersion fluid.

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. Touch to eject the instrument tray arm.
2. Remove the plate adapter, then place it on a clean, dry surface.
3. Touch to retract the instrument tray arm.
4. Power off and unplug the instrument, then allow it to cool for 15 minutes.
5. Open the access door.

6. Firmly press down on the handle of the sample block, then remove it from the instrument. Place the sample block on a clean, dry surface.

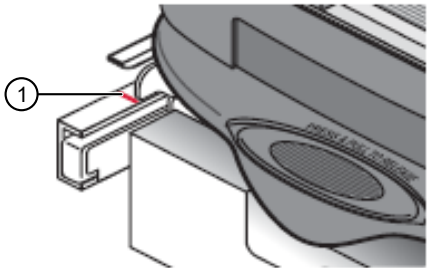


7. Wipe the surfaces of the sample block, heated cover, and plate adapter with a 95% ethanol solution using a lint-free cloth to remove contaminants, for example, fluid residue or dust.

8. Load the sample block into the instrument, then close the access door.

   Do not load the plate adapter during this step.

   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 instrument until it is seated correctly.



① Indicator

9. 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.

10. Plug in, then power on the instrument.

11. Touch ⏏ to eject the instrument tray arm.

12. Load the plate adapter, then touch ⏏ to retract the instrument tray arm.

13. Touch ⚡ **Start Cleaning**.

    The cleaning procedures takes 30 minutes.

14. *(Optional)* Perform one of the following actions during the cleaning procedure.

Touch	Action
	View an image of the sample block. This allows you to monitor the cleaning progress. Proceed to step 16
	Stop the cleaning procedure before it is complete. Proceed to step 17

15. When the procedure is complete, touch **OK** to view the image of the cleaned sample block.

16. Save an image of the sample block to a USB drive.
    a. Insert a USB drive into the instrument USB port.
    b. Touch .

17. *(Optional)* To repeat the cleaning procedure, touch **Start Cleaning**.

18. Touch **Close** to return to the **Clean Instrument** screen.

Replacing the instrument fuses

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

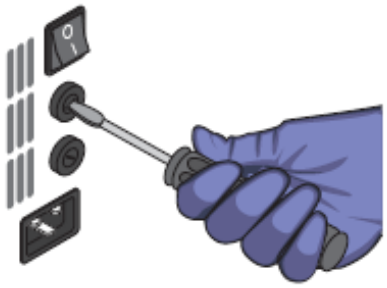
CAUTION! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with listed and certified fuses of the same type and rating as those currently in the QuantStudio™ 12K Flex Instrument.

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 instrument. The installation is successful if the instrument powers on.

Note: Fuse failure can result from fluctuations in the supplied power to the 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. Future versions and updates to the Windows™ operating system can conflict with the QuantStudio™ 12K Flex Software.

1. Go to `C:\Program Files\Applied Biosystems\QuantStudio 12K Flex\docs`, then open `README.html`.
2. Read the release notes for the compatibility of interest.
3. If the release notes do not mention the compatibility, go to **thermofisher.com/qpcrsoftware**, then search for the compatibility of interest.
4. If the website does not contain the information of interest, contact Support.

Updating the software and the firmware

Updates to the QuantStudio™ 12K Flex Software and the QuantStudio™ 12K Flex Instrument firmware that you can install without the aid of a Field Service Engineer can be released. You can obtain updates directly from the website.

1. Go to **thermofisher.com/qpcrsoftware**.
2. In the **Real-Time PCR** section, click **Applied Biosystems QuantStudio 12K Flex Real–Time PCR System**.

Updating the QuantStudio™ 12K Flex Software

Preparing for the software upgrade

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

1. Back up the application libraries.
   a. In the main menu of the QuantStudio™ 12K Flex Software, click **Tools**.
   b. Select the library.
   c. When the library dialog box opens, select the element(s) to export, then click **Export**.

d. 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\QuantStudio 12K Flex\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 Thermo Fisher Scientific. If you do not have a license file, see “Managing the software licenses” on page 141.

Updating the instrument firmware

You can use the instrument touchscreen to update the instrument firmware.

Managing the 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 the 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 Thermo Fisher Scientific 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: The 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 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 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 TXT 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.

IMPORTANT! An internet connection, a web browser, and a valid email account are required to activate a license. If the computer that contains the software is not connected to the internet or it lacks a web browser application, contact Support to request the license file.

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 Thermo Fisher Scientific website.
3. On the website, click **QuantStudio 12K Flex Software** from the list of products, then activate the license as instructed.

   After you successfully activate the license, the Thermo Fisher Scientific website emails you the activated license file (LIC) for you to install on your computer.

Install 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 file (LIC) 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.

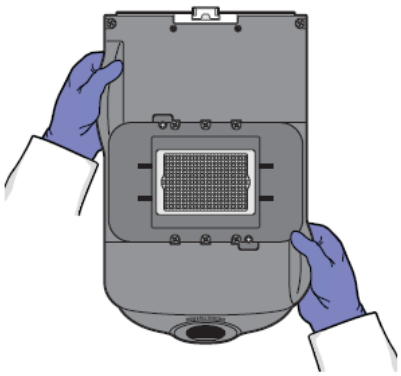
WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the sample block and heated cover can be heated to 100°C. Before removing the sample block, wait until it reaches room temperature.

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 instrument, place the sample block on a clean, dry surface or in its shipping container.

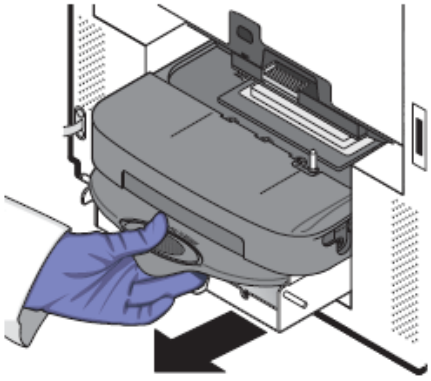


Replacing the sample block

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

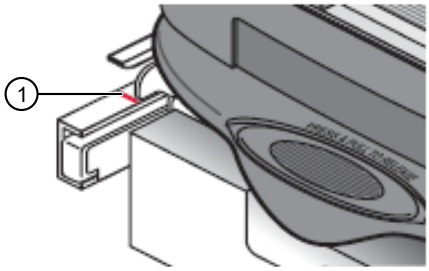
1. Power off and unplug the QuantStudio™ 12K Flex Instrument, then allow it to cool for 15 minutes.
2. Open the access door.

3. Firmly press down on the handle of the sample block, then remove it from the instrument. Place the sample block on a clean, dry surface.



4. Install the new sample block into the 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 instrument until it is seated correctly.



① Indicator

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 instrument.

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

9. In the **Instrument Console**, select your instrument from the list of instruments, then review the **Block Type** field in the **Instrument Properties** pane.

   The installation is successful if the 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 in the 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 (required for all blocks with QuantStudio™ 12K Flex Software v1.4)

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

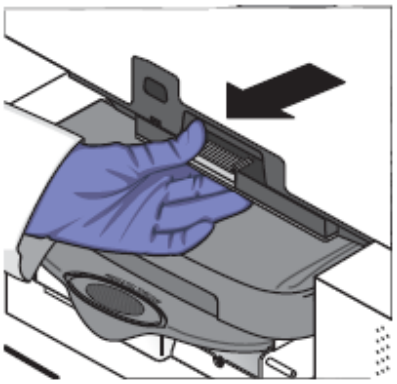
To prevent damaging or contaminating the heated cover, handle the assembly as shown below. After the assembly has been removed from the instrument, place the heated cover on a clean, dry surface or in its shipping container.

Replacing the heated cover

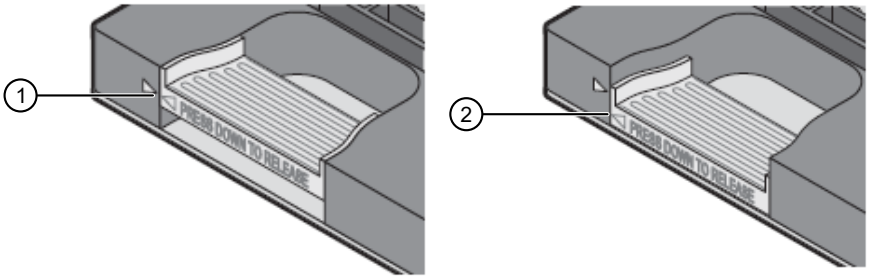
Note: Confirm that the replacement heated cover supports the consumable format that you want to use. Some heated covers support more than one consumable type.

1. Power off and unplug the instrument, 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 instrument and place it on a clean, dry surface.



4. Install the new heated cover into the instrument.



① Correct alignment
② Incorrect alignment

IMPORTANT! When the heated cover is seated correctly, the arrows on the front handle align as shown. If the arrows do not align, push the heated cover further into the instrument until the handle locks into place.

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 instrument.

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

9. In the **Instrument Console**, select your 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 instrument powers on and if the **Heated Cover Firmware Version** field displays a version number.

10. Perform the following calibrations in the specified order.
    a. ROI calibration
    b. Background calibration
    c. Uniformity calibration
    d. Dye calibration
    e. Normalization calibration (required for all blocks with QuantStudio™ 12K Flex Software v1.4)

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, wait until it reaches room temperature.

Required materials

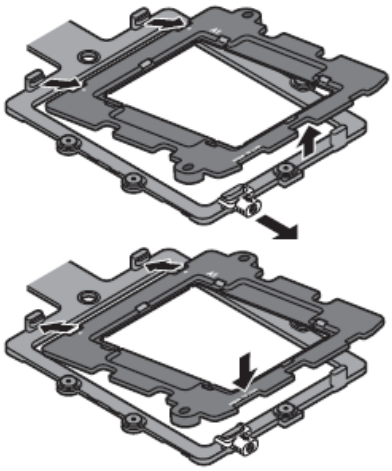
- Safety glasses
- Powder-free gloves
- Plate adapter

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

IMPORTANT! This chapter *does not* provide adequate detail to integrate the 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 system to your laboratory network.

Overview of networking

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

This chapter describes the following items:

- Set up the system for use on a network.
- Set up a computer for remote monitoring.
- Test the network connection by engaging the remote monitoring feature.

Control and monitor 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 software can control up to 4 instruments and monitor up to 15 instruments simultaneously. A networked 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.

For more information about remote monitoring, see “Monitor a QuantStudio™ 12K Flex Instrument” on page 156.

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 system is connected to a network, computers on the network that run the QuantStudio™ 12K Flex Software can perform the following functions:

- Send and download experiments to and from the system.
- Run experiments on the system.
- Remote monitor the system as it performs runs.

The Ethernet port of the instrument supports the following configurations:

- 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 system provides an IP address when no address is supplied by the DHCP server.

Firewall ports that must be open

Table 13 v1.4 and earlier

Ports	Condition
mDNS, 7000	Instrument-to-computer connection
mDNS, 5353	Instrument discovery

Table 14 v1.5 and later

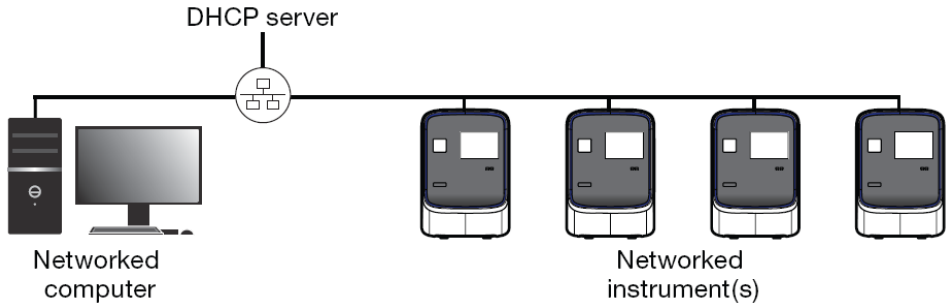
Ports	Condition
mDNS, 7443	Instrument-to-computer connection
mDNS, 5353	Instrument discovery

Example network layouts

Example 1

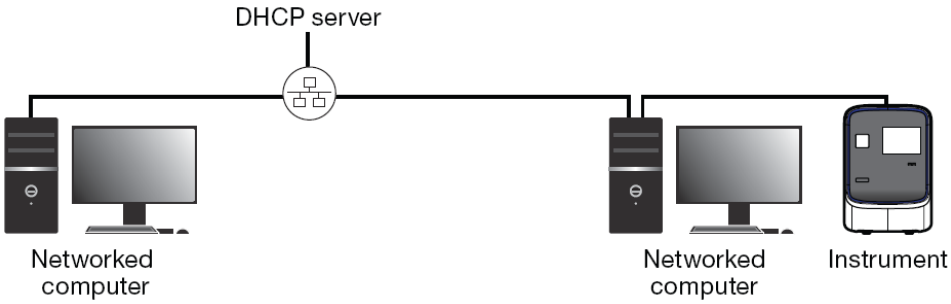
In the following example, one or more QuantStudio™ 12K Flex Instruments, which have been configured for dynamic host configuration protocol (DHCP) operation, are connected to a network by their Ethernet ports. In this layout, any computer on the network can monitor or control the instrument. Experiments can be started remotely from the networked computer or locally from the 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 system computer. 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 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 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 system by a router, hub, or another network device may not be able to access the instrument by its host name.

- Confirm the uniqueness of the instrument name.

  The instrument does support name resolution, but the instrument name must be unique within the subnet. The QuantStudio™ 12K Flex Software can automatically discover instruments on the link-local network that are configured for Autodiscovery (see “Define the network settings” on page 191).

  Note: The system does not test the uniqueness of the instrument name when it is set.

- Name instruments using lower-case letters.

  When you define the instrument settings, enter the instrument name using lowercase letters only (see “Define the instrument settings” on page 189).

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 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 instrument to the network

After deciding how to connect the QuantStudio™ 12K Flex System to a network, set up the 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)

Define 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 instrument.
3. Use the instrument touchscreen to configure the network settings (see “Define the network settings” on page 191).

Connecting the computer to the network

After connecting the 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

Set up the computer

IMPORTANT! We recommend that you arrange for a network administrator to connect your computer to the network. The following procedure does not provide adequate detail for all network architectures.

1. Connect an Ethernet cable from the instrument or a LAN to the computer.
2. Power on the computer, then log in using a Windows™ Administrator account.
3. Open the **Control Panel**, then open the **Network and Sharing Center**.
4. In the left pane of the **Network and Sharing Center** window, click **Change Adapter Settings**.
5. Right-click on **Broadband Connection**, then select **Properties**.
6. In the **Networking** tab, select **Internet Protocol Version 4 (TCP/IPv4)**.
7. Click **Properties**.
8. In the **Internet Protocol Version 4 (TCP/IPv4) Properties** dialog box, select one of the following options.
   - **Obtain an IP address automatically**
   - **Use the following IP address**
9. If **Use the following IP address** was selected, enter the ***IP address***.
10. Close all dialog boxes by clicking **OK**, then re-start the computer.

Install 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.

Monitor a QuantStudio™ 12K Flex Instrument

After connecting the 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 perform the following functions:

- Monitor the status of an instrument during a run (see page 157)
- Upload or download an experiment or a template (see page 158)
- Enable or change the calibration reminders (see page 158)

Guidelines for remote monitoring

To allow 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 an instrument be monitored by more than 10 computers simultaneously.
- Unless you are sure that your instrument and computer exist on the same subnet, we recommend that you use the IP address of the instrument to add it for remote monitoring.

Monitor 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 instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add an 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 perform one of the following functions:

- 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 instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add an 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 **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 instrument, then click **Open**.

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

   1. In the **Folders** field, select the folder that contains the files that you want to download.
   2. In the **Experiments** field, select the files to download. To select multiple files, control-click or shift-click files in the list.
   3. When you have selected the files that you want to download, click **Download**.
   4. 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 perform the following functions:

- Create or remove directories on the instrument
- Add, delete, or download experiments on the instrument

Enable or change 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 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).

Define 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** field and **Password** field—If the mail server requires authentication, enter the user name provided by your systems administrator.
4. Click **OK**.

Modify the notification settings for a monitored 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 instrument from the list of instruments on the network, then click **Add to My Instruments**.

      Note: You must add an instrument to your list before you can manage it.

   c. After the instrument is added to your list, select it, then click **Manage Instrument**.
   d. In the **Instrument Manager**, click **Maintenance** ▸ **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 instrument.
   b. In the **Send a Reminder** column, select the check box to configure the 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

Overview for administrators

IMPORTANT! The Security, Audit, and Electronic Signature (SAE) module is installed only with 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 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. 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.

Configure the system security

Access the Security screen and enable or disable security

Use the **Security** screen to disable and enable security, control restrictions and security policies for all user accounts, and set up notifications when certain security events occur.

IMPORTANT! If you disable security, you inactivate audit and electronic signature functions. 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 icon 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 “Enable or disable the instrument security” on page 193). The software cannot connect to instruments that do not match security settings.
4. Click **Apply Settings.**

Set 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 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 164.
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 software notifies the administrator of certain security events.

   For information, see “Set up the messaging notifications” on page 164.

7. Click **Apply Settings**.

The new settings are applied to the user account the next time that the user logs in.

Spaces in user names and/or passwords

If you allow spaces in user names and/or passwords, be aware of the following issues:

- Leading and trailing spaces in user names are difficult to detect on the screen or in printed reports.
- The number of consecutive spaces in a user name is difficult to determine on the screen or in printed reports.

Spaces in user names may cause confusion when a user searches for an audit record associated with a user name. To find a record associated with a user name, specify the user name exactly, including leading, consecutive, and trailing spaces.

Set up 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 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**.

Manage 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.

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**.

Determine 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**.

Create 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 a description.
   A description is optional.
5. Select permissions (see "Permissions and default user roles" on page 167).
   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

(continued)

Permissions		Default user roles		
Category	Function	Scientist	Technician	Administrator
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 a 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

Edit a user role

1. In the **Roles** screen, select a user role, then click **Edit**.
2. Edit settings as needed.

   Note: You cannot edit the Administrator user role.

3. Click **Save Role**.

View and print 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).
6. Close the report.

Manage audit functions

Enable and disable 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**.

Select 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**.

Create audit reason settings

You can create, modify, and delete the reasons that are available for selection in the **Audit Reason** dialog box (displayed when a user performs an audited action).

1. To require users to select a pre-defined reason in the **Audit Reason** dialog box (displayed when a user performs an audited action), enable **Require users to select a reason to change from the list**.

   Users are not permitted to enter a reason.
2. As needed, click **Create**, or select a reason from the list, then click **Edit** or **Delete**.

Generate 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.

- Display audit histories from the **Security Settings** dialog box (see page 170)
- Display audit histories for an experiment or a template (see page 173)

Display audit histories from the Security Settings dialog box

Display 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 this 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**.

Overview of 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

(continued)

Record type	Action	Corresponds to…
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[1]
	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

[1] Creates one role assignment record for each permission in a role.

Reviewing the Action Record 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 Record** 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)

View and print audit reports

1. Select the **System Configuration History** tab.
2. Display the records of interest.
3. Filter the list to decrease the time required to generate reports.

   IMPORTANT! You cannot cancel a report after you click a view button.

4. Click **View Report**.
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.

Archive, purge, and restore audit records

The audit archive function makes a copy of audit records. Purge makes a copy of audit records, and then deletes them. You can use the restore function to restore purged audit records.

Archive and purge audit records

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 audit records

Restore the system configuration or the action audit records.

1. Click **Restore**.
2. Select the ASZ file to restore.

Export audit records

You can export audit records to a TXT file for additional manipulation and reporting outside the QuantStudio™ 12K Flex Software.

1. Display the records of interest (see “Archive and purge audit records” on page 173).
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.

Display audit histories for an experiment or template

Display the audit history

1. Open an experiment file (EDS) or a template file (EDT).
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.

Export audit records

1. Open an experiment file (EDS) or a template file (EDT).
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.

Print audit records

1. Open an experiment file (EDS) or a template file (EDT).
2. In the open experiment or template, click **Audit**.
3. Click **View Summary Report** to open the **Print Preview** dialog box.
4. Preview, save or print the report.
   - Click **(Save)** to save the report as a PDF file or an 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 **(Close)** to close the **Print Preview** dialog box.

Manage the electronic signature

Enable or disable the 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 the experiment files (EDS) or the template files (EDT).

4. Click **Apply Settings**.

Configure 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

Add 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**.

Delete a meaning

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

Configure the electronic signature rights for user roles

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**.

Select the actions that require a signature

IMPORTANT! Do not change electronic signature settings during calibration.

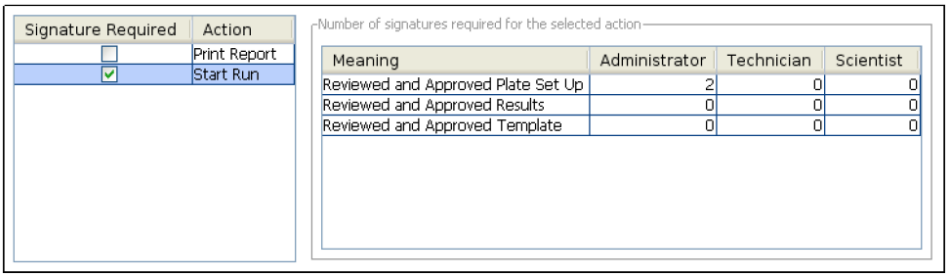
1. In the **Signature Required** column, select the check box next to each action for which you want to require electronic signatures.

   This selection causes the software to present an e-signature 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 the 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.

   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**.

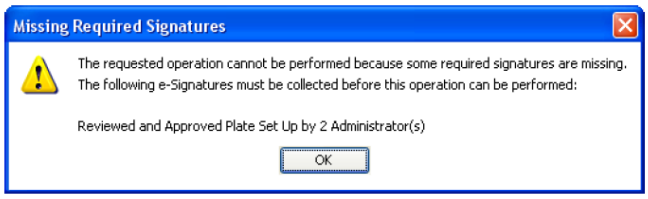
How the software prompts electronic signature

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.

Generate electronic signature reports

Use the QuantStudio™ 12K Flex Software to generate reports of e-signature history from open experiment files (EDS) or template files (EDT).

Display electronic signature records

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

Save or print electronic signature records

1. Open an experiment file (EDS) or a template file (EDT).
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**.
   - Click **(Print)**.
5. Click **OK** in the confirmation message.

Save or print the table of electronic signature events

1. Open an experiment file (EDS) or a template file (EDT).
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**.
   - Click **(Print)**.
4. Click **OK** in the confirmation message.

Export and import settings

Note: The export and 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.

Import 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.
3. *(Optional)* Click **Yes** to overwrite the current system configuration.
   If any imported user accounts already exist on the system, you are prompted to overwrite or skip each account.

Overview for users

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

Log 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.

1. Select **Tools** ▸ **Change Password**.
2. Enter your current password, then enter your new password twice.
3. 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. 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 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 (see “How the software prompts electronic signature” on page 177).

A Manual instrument operation

Note: This appendix describes how to operate the QuantStudio™ 12K Flex Instrument manually using the touchscreen interface. Although the 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 instrument features a touchscreen interface that you can use to run experiments, manage instrument settings, and configure the 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 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 instrument is operating in secure mode (see “Enable or disable the instrument security” on page 193).

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 instrument firmware	
Administration	Define the date and time	

(continued)

User role	Function	Available in secure mode?
Administration	Define the instrument settings	Yes
	Define the network settings	
	Define the maintenance reminders	
	Define the system shortcuts	
	Enable or disable instrument security	
	Review the instrument statistics	
	View the instrument log	

Operate the instrument from the touchscreen

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

- Create an experiment from a template (see page 182)
- Run an experiment (see page 183)
- Transfer experiments, templates, and results data (see page 184)

Note: If the instrument is operating in secure mode, users can only open and close the side door (see "Enable or disable the instrument security" on page 193).

Create an experiment from a template

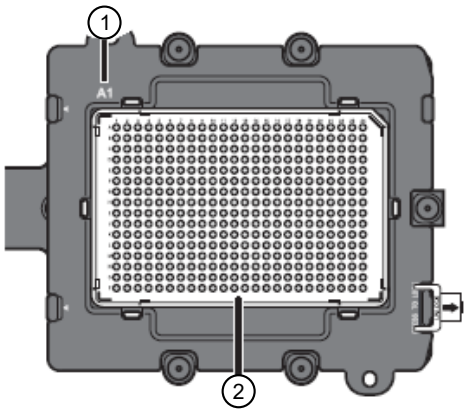
1. If necessary, download the experiment template to the instrument.
   See "Transfer experiments from a USB drive" on page 185.
2. If the instrument is in standby, tap the instrument touchscreen to activate it, then press .
3. In the **Main Menu**, tap **View Templates**.
4. In the **View Templates** screen, tap a template, then tap **+ New**.
   To view the parameters of a template, select the desired template, then tap **View**. When finished, tap to return to the **View Templates** screen.

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

5. In the **Create New Experiment** screen, tap each field to set the parameters:
   - Tap the **New Experiment Name** field and use the keypad to enter a name (up to 100 characters) for the experiment.
   - Tap the **Save to Folder** field to open the **Select Folder** screen.
   - Tap the **Reaction Volume** field to enter a reaction volume in µL.
   - *(Optional)* Tap the **Barcode Number** field to enter a barcode.
   - *(Optional)* Tap the **Notes** field to enter notes (up to 200 characters) about the experiment.
6. When finished, perform one of the following actions.
   - Tap **Save & Exit**.
   - Tap **Save & Start Run** to proceed to the **Start Run** screen.

Run an experiment

1. If the instrument is in standby, tap the instrument touchscreen to activate it, then press .
2. In the **Main Menu** screen, then tap .
3. When the side door opens, load the appropriate plate or array card.
   Ensure that the consumable is properly aligned in the holder.



① Load 96- and 384-well plates with the A1 position at the top-left corner of the plate adapter.
② 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.

4. In the **Main Menu**, tap **Browse Experiments**.
5. In the **Experiments** screen, tap the desired experiment, then tap one of the following options:
   - Tap **Start Run** to start the run immediately, then go to step 10 on page 184.
   - Tap **View/Edit** to view or edit the experiment before starting the run.

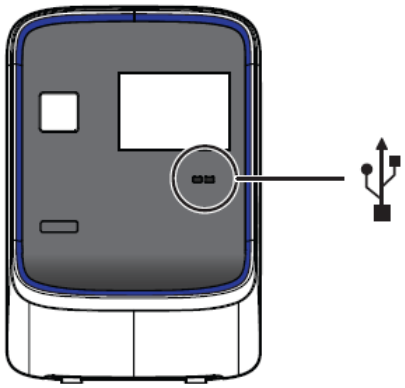
6. Modify the experiment parameters as needed.
   - To add a stage or step to the thermal profile, tap the stage or step to the left of where you want to add the stage or step, then tap **Add**.
   - To add a melt curve to the end of the thermal profile, tap **Add Melt Curve**.
   - To change the time or temperature of a stage or step, tap the time/temperature field of the stage or step, modify the settings as desired, then tap **Close**.
   - To change the cycle parameter of a stage, tap the cycle field, modify the setting as desired, then tap **Close**.
   - To delete a stage or step from the thermal profile, tap the stage or step you want to remove, then tap **Delete**.
7. When finished modifying the parameters, tap **Save**.
8. In the **Save Experiment** screen, tap each field to set the experiment, name, reaction volume, barcode, and any additional information to save to the experiment.
9. When finished, tap **Save & Start Run** to start the experiment.
10. In the **Start Run** screen, tap each field as needed to modify the associated parameter, then tap **Start Run Now** to start the experiment.

Note: When the run is complete, tap to unload the plate. You can download the experiment results to a computer if the instrument is connected to a network, or you can copy the data to a USB drive (see “Transfer experiments, templates, and results data” on page 184).

Transfer experiments, templates, and results data

You can transfer experiments, templates, and results data to and from the instrument using a USB drive. Before transferring the data, you must plug the drive into one of the USB ports behind the right side of the instrument touchscreen.

IMPORTANT! Do not use the USB ports on the rear panel of the instrument. The rear USB ports are for use by Thermo Fisher Scientific personnel only.



Transfer 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, tap the instrument touchscreen to activate it, then press .
3. In the **Main Menu**, tap **View Templates**.
4. In the **Browse Experiments** screen, select the template.
   a. Tap , then tap **USB**.

b. Tap the desired template, then tap **Save**.

5. In the **Save Experiment As** screen, set the name for the file.
   a. Tap the **New Template Name** field, then enter a name for the copied file.
   b. Tap the **Save to Folder** field, then select the folder to receive the file.
   c. Tap **Save**.
6. Tap to return to the **Main Menu**.
7. Unplug the USB drive.

Transfer experiments from a USB drive

1. Plug a USB drive into the USB port on the right side of the touchscreen.
2. If the instrument is in standby, tap the instrument touchscreen to activate it, then press .
3. In the **Main Menu**, tap **Browse Experiments**.
4. In the **Browse Experiments** screen, select the experiment.
   a. Tap , then tap **USB**.
   b. Tap the desired experiment, then tap **Save**.
5. In the **Save Experiment As** screen, tap the experiment that you want to transfer to the USB drive, then tap **Save**.
6. Tap to return to the **Main Menu**.
7. Unplug the USB drive.

Copy experiment results to a USB drive

1. Plug a USB drive into the USB port on the right side of the touchscreen.
2. If the instrument is in standby, tap the instrument touchscreen to activate it, then press .
3. In the **Main Menu**, tap **Collect Results**.
4. In the list of experiments, touch the row(s) for the experiment(s) of interest or tap **Select All**.
5. Tap **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 tap **Copy to USB**
7. Tap 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.

Maintain 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 instrument functions are performed as part of regular instrument maintenance:

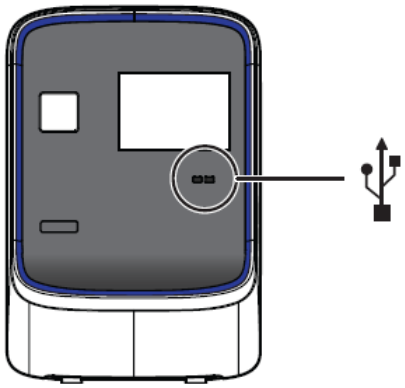
- Back up and restore the instrument settings (see page 186)
- Perform an instrument self-test (see page 187)
- Update the instrument firmware (see page 188)

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 instrument settings are reset, you can restore the settings from the backup.

The instrument backs up to and restores instrument settings from a USB drive. Before backing up or restoring settings, you must plug the drive into one of the USB ports behind the right side of the instrument touchscreen.



IMPORTANT! Do not use the USB ports on the rear panel of the instrument. The rear USB ports are for use by Thermo Fisher Scientific personnel, only to service the instrument.

Note: The backup feature can be used as an administrative tool to manage instruments. You can use the feature to create a standard “image” for an instrument that can then be restored on other instruments to bypass the manual set-up process.

Back up the instrument settings

1. Plug a USB drive into the USB port on the right side of the touchscreen.
2. If the instrument is in standby, tap the instrument touchscreen to activate it, then tap .
3. In the **Main Menu**, touch **Tools**, then tap **Back Up**.
4. In the **Backup Settings** screen, tap **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 instrument. You must configure the network settings for each instrument individually.

Restore the instrument settings

1. Plug the USB drive that contains the instrument settings into the USB port on the right side of the instrument touchscreen.

2. If the instrument is in standby, touch the instrument touchscreen to activate it, then press .

3. In the **Main Menu**, tap **Tools**, then tap **Restore Settings**.

4. In the **Restore Settings** screen, select the settings to restore.
   a. Tap the settings that you want to restore from the list.

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

   IMPORTANT! Do not remove the USB drive from the instrument until you are instructed to do so.

   Note: Alternatively, tap **Restore Default Settings** to restore the instrument to the factory settings.

5. After the instrument reboots, unplug the USB drive.

Perform an instrument self-test

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

Note: We recommend running the self-test as part of regular maintenance to support optimal performance of the instrument.

1. If the instrument is in standby, touch the instrument to activate it, then press .

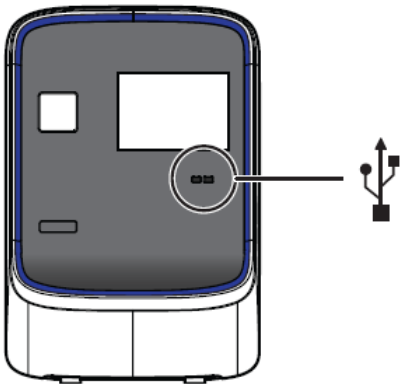
2. In the **Main Menu**, tap **Tools**, then tap **Run Self Test**.

3. In the **Self Test** screen, tap **Start Self Test**, then wait for the test to complete.

4. *(Optional)* When the 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 instrument touchscreen.
   b. Tap **Save**.

      IMPORTANT! Do not remove the USB drive from the instrument until instructed to do so.

   c. When the instrument finishes writing the results to the USB drive, tap **OK**, then remove the USB drive.



5. Tap to return to the **Main Menu**.

Update the instrument firmware

You can download the firmware updates directly from the service section of the Thermo Fisher Scientific website. After obtaining a firmware update, transfer the update to the instrument using a USB drive.

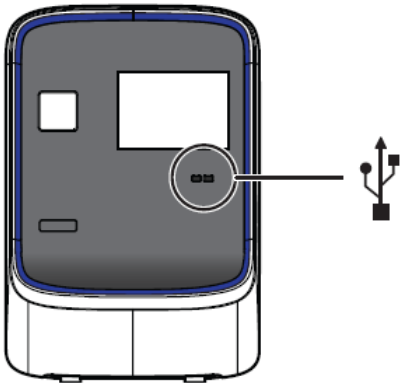
Update the firmware

1. Download the firmware update, then save it on a USB drive.

   Firmware updates are available at thermofisher.com/qpcrsoftware.

2. Plug the USB drive into the USB port on the right side of the instrument touchscreen.
3. If the instrument is in standby, touch the instrument touchscreen to activate it, then press .
4. In the **Main Menu**, tap **Tools**, then tap **Upgrade Firmware**.
5. In the **Upgrade Firmware** screen, select the update package, then tap **Upgrade Firmware**.

   Allow the instrument to complete the upgrade.



IMPORTANT! Do not remove the USB drive from the instrument until you are instructed to do so.

6. After the upgrade is complete and the instrument reboots, confirm the upgrade success.
   a. Unplug the USB drive.
   b. Tap **Settings**, then tap **About this instrument** to view the software version number to confirm that the firmware has been upgraded.

Administer 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 instrument settings and configure it for network use.

- Define the date and time (see page 189)
- Define the instrument settings (see page 189)
- Define the maintenance reminders (see page 191)
- Define the network settings (see page 191)
- Define the system shortcuts (see page 192)
- Review the instrument statistics (see page 193)
- Enable or disable the instrument security (see page 193)
- View the instrument log (see page 194)

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.

Define the date and time

1. If the instrument is in standby, touch the instrument touchscreen to activate it, then press .
2. In the **Main Menu**, tap **Settings**, then tap **Set Date & Time**.
3. Perform the following steps in the **Set Date & Time** screen.
   a. Tap the **Time zone** field, then tap the correct time zone in the list.
   b. Tap the **Date** field, enter the current date, then tap **Done**.
   c. Tap the **Date Format** drop-down list, then select the format for your region.
   d. Tap each **Time** field, enter the appropriate time units, then tap **Done**.
   e. Tap **12 Hour** or **24 Hour** to select the appropriate time format.
   f. Tap **Save** to save the settings, then tap **OK** when prompted.
4. Tap to return to the **Main Menu**.

Define the instrument settings

1. If the instrument is in standby, touch the instrument touchscreen to activate it, then press .
2. In the **Main Menu**, tap **Settings**, then tap **Configure the Instrument**.

3. Tap the **Instrument Name** field, enter up to a 16-character name for the instrument, then tap **Done**.

   The instrument name is the alphanumeric string used to identify the instrument on the network.

   IMPORTANT! To connect the 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.

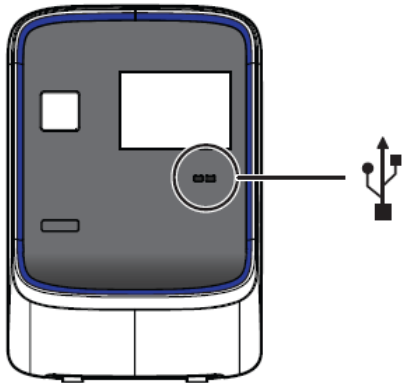
   The instrument icon is the graphic used to represent the instrument in the QuantStudio™ 12K Flex Software **Instrument Console**.

   a. Save the replacement graphic to a USB drive, then plug the drive into the USB port on the right side of the instrument touchscreen.

   b. Tap **Upload Icon**, select the desired graphic file, then tap **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. Tap the **Standby Time-out** field.

   c. Enter the number of minutes (1–300) that the instrument should remain idle until it enters standby mode, then tap **Done**.

      Note: When in standby mode, the 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. Tap the **Cover Idle Temperature** field.

   c. Enter the temperature (50–110°C) that the heated cover should maintain when the instrument is idle, then tap **Done**.

7. Tap **Save** to save the settings, then tap **OK** when prompted.

8. Tap to return to the **Main Menu**.

Define the maintenance reminders

You can use the instrument touchscreen screen to perform the following functions:

- Set the expiration period for the instrument calibrations and LED replacement.
- Activate, deactivate, or change the frequency of the maintenance reminders displayed by the instrument.

Setting the reminders

1. If the instrument is in standby, touch the instrument touchscreen to activate it, then press .
2. In the **Main Menu**, tap **Settings**, then tap **Set Maintenance Reminders**.
3. Configure the maintenance reminders. For each maintenance reminder, perform the following steps.
   a. Tap the **Calibration expires after** field, enter the number of days or hours that should elapse until the association calibration expires, then tap **Done**.
   b. Tap the check box to activate or deactivate reminders for the associated calibration.
   c. Tap the **Display reminders before** field, enter the number of days before the associated calibration expires that the instrument should start displaying warnings of the impending expiration, then tap **Done**.
4. Tap **Save** to save the settings, then tap **OK** when prompted.
5. Tap to return to the **Main Menu**.

Define the network settings

1. If the instrument is in standby, touch the instrument touchscreen to activate it, then press .
2. In the **Main Menu**, tap **Settings**, then tap **Set Network Information**.

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

   The smart monitoring feature allows Thermo Fisher Scientific service personnel to monitor the status of the 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 instrument.

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

Network service	Action
DHCP	Tap **Obtain an IP address automatically**, then tap **Save**.
Static IP	a. Tap **Use the following IP address**. b. Tap the **IP Address** field, enter the IP address using the keypad, then tap **Done**. c. Repeat the previous step to assign the following items: • IP addresses for the DNS Servers (primary and secondary) • Subnet Mask setting • Default Gateway setting d. Tap **Save** to save the settings, then tap **OK** when prompted.

6. Tap to return to the **Main Menu**.

Defining the system shortcuts

You can use the 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.

Define the shortcuts

1. If the instrument is in standby, touch the instrument touchscreen to activate it, then press .
2. In the **Main Menu**, configure the shortcuts as desired.

   To *add* a shortcut:

   a. Tap the shortcut of interest, then tap **Set Shortcut**.

   b. Tap **From Templates** to link to a specific template file or tap **From Folders** to link to a folder.

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

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

3. When you are finished configuring the shortcuts, tap to return to the **Main Menu**.

Review the instrument statistics

You can use the instrument touchscreen to view usage statistics on the heated cover, LEDs, and other system components.

View the statistics

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

Enable or disable the instrument security

The 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 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 instrument security, auditing, and electronic signature feature, you must similarly enable or disable the QuantStudio™ 12K Flex Software security (see “Configure the system security” on page 163). The software cannot connect to instruments that do not match security settings.

Note: Secure mode limits the number of features that are available from the instrument touchscreen. It does not provide user authentication functionality through the instrument touchscreen.

Enable or disable security

1. If the instrument is in standby, touch the instrument touchscreen to activate it, then press .
2. In the **Main Menu**, tap **Settings**, then tap **Set Administrator Options**.
3. In the **Set Administrator Options** screen, tap **Secure Environment** to enable (checked) or disable (unchecked) system security.
4. *(Optional)* To change the administrator password, perform the following steps.
   a. Tap **Change Password**.
   b. Enter the current password, then tap **Done**.
   c. Enter the new password, then tap **Done**.
   d. Reenter the password when prompted.
   e. Tap **OK** when prompted.

Note: The default password for the instrument touchscreen is *password;* however, the password can be changed during installation.

5. Tap **Save**.
6. Tap the **Administrator Password** field, enter the administrator password, then tap **Done**.
7. Tap to return to the **Main Menu**.

View the instrument log

You can use the instrument touchscreen to view a log that summarizes instrument activity from the last six months. For each recorded activity, the activity log provides a description of the activity and the date and time when it occurred.

View the log

1. If the instrument is in standby, touch the instrument touchscreen to activate it, then press .
2. In the **Main Menu**, tap **Tools**, then tap **View Log**.
3. In the **View Log** screen, configure the settings to display the records of interest.
   a. Select an option from the drop-down menu to filter the log.
   b. Select **Earliest First** or **Latest First** to determine the order to sort the records.
4. Tap to return to the **Main Menu**.

B Power on or off, store, and move the system

Place the QuantStudio™ 12K Flex System on standby

If left unattended, the system automatically enters standby mode to conserve power.

To enter standby mode manually, tap  on the instrument touchscreen.

Power on the QuantStudio™ 12K Flex System

1. Toggle the power button on the rear of the instrument, then wait for the instrument to start.

   Note: The instrument is ready to use when the touchscreen displays the **Main Menu**.

2. If you have a 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** screen appears, enter your user name and password, then click **OK**.

Power off the QuantStudio™ 12K Flex System

The QuantStudio™ 12K Flex System operates in low-power mode when not in use. The system can be powered off completely so that the components draw no power.

Note: If the system will be inactive for extended period of time, prepare it for storage (see in "Store the QuantStudio™ 12K Flex System" on page 196).

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

Store the QuantStudio™ 12K Flex System

The QuantStudio™ 12K Flex System can be powered off and stored for extended periods of time. The length of the period of inactivity determines the method you use to power off the system.

Required materials

MicroAmp™ Optical Reaction Plate (96-well or 384-well) or TaqMan™ Array Card (unused)

Prepare the instrument for storage

1. If you plan to store instrument for more than one week or if you plan to move it, load an unused plate or array card into the instrument.

   Note: The empty plate protects the internal components of the instrument during transport or during periods of inactivity lasting more than a week.

   a. Tap the instrument touchscreen to activate it, then tap  .
   b. Tap  to eject the tray arm, place a plate or array card onto the plate adapter, then tap  again to load the plate.
   c. Tap  to place the instrument into stand-by mode.
2. Toggle the power button on the rear of the instrument.
3. Power off the computer.

4. Power off the monitor.

5. If you have a Twister™ Robot, toggle the power button on the rear of the Twister™ Robot.

Move the QuantStudio™ 12K Flex System

Perform this procedure to safely move the system short distances (for example, between laboratories in 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 the instrument.

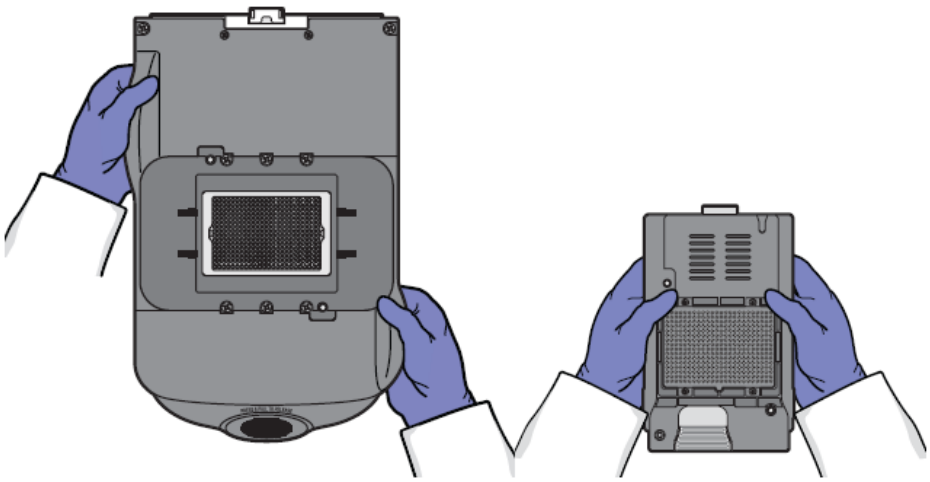
IMPORTANT! Moving the QuantStudio™ 12K Flex Instrument can create subtle changes in the alignment of the instrument optics. Recalibrate the instrument if necessary.

Required materials

None

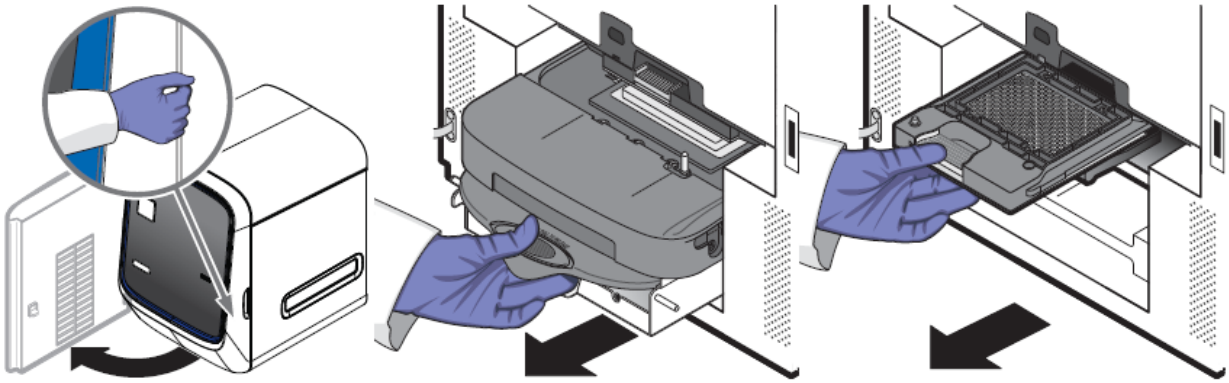
Handle the sample block and heated cover

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



Prepare the QuantStudio™ 12K Flex System components

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



Move 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.

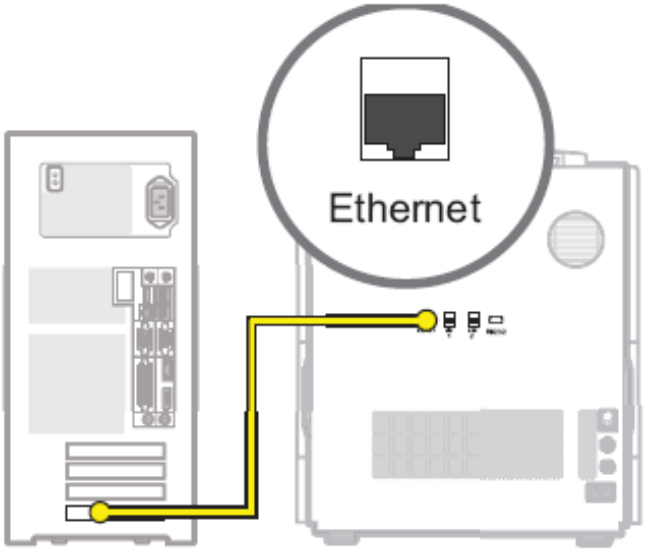
Reinstall the QuantStudio™ 12K Flex System

1. Reconnect the components of the QuantStudio™ 12K Flex System.

   Use the Ethernet cable supplied with the 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 instrument to the computer.

   IMPORTANT! Do not connect the Ethernet cable to the Ethernet 2 port on the instrument. The second port is for 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 system. No further action is necessary.
   - If the run fails, perform the following calibrations in the specified order: ROI, background, uniformity, dye, then normalization calibrations.

C Prepare calibration consumables

Create a background plate or a TaqMan™ Array Card

Whenever possible, use a Background Plate or the TaqMan™ Array Background Buffer that is included with the spectral calibration kit. The plates or 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 TaqMan™ Array Card from a spectral calibration kit is not available, you can create one as described below.

Required materials

96- or 384-well plate sample block

- Optical 96- or 384-well reaction plate
- Safety glasses
- Optical Adhesive Cover or Optical Flat Caps
- Pipettor, 200-µL (with pipette tips)
- Powder-free gloves
- Deionized water

TaqMan™ Array Card sample block

- TaqMan™ Array Cards
- TaqMan™ Array Card Sealer
- Centrifuge with array card buckets and array card carrier clips
- Permanent marker or pen
- Pipettor, 200-µL (with pipette tips)
- Powder-free gloves
- Safety glasses
- Deionized water

Create a background plate

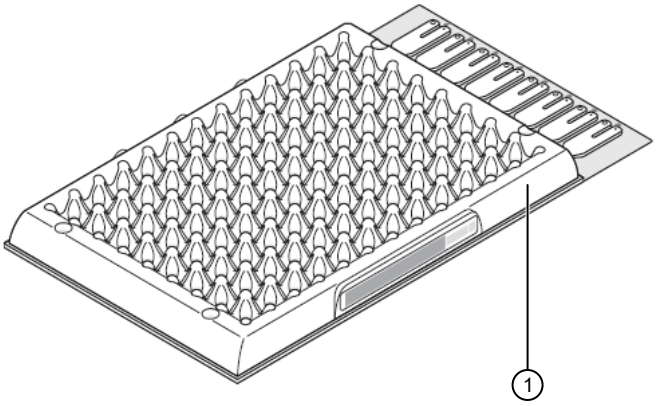
IMPORTANT! Wear powder-free gloves when you are creating the background plate.

1. Remove a 96- or 384-well optical reaction plate from its packaging, then 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.

Use the plate for background calibration as you would a background plate from the spectral calibration kit.

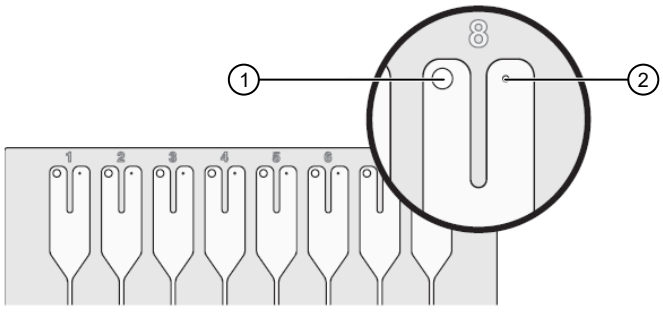
Create a background TaqMan™ Array Card

1. Remove a TaqMan™ Array Card from its packaging, then place it on a clean, dry surface.
2. Using a permanent marker, write “Background” on the side of the empty card.

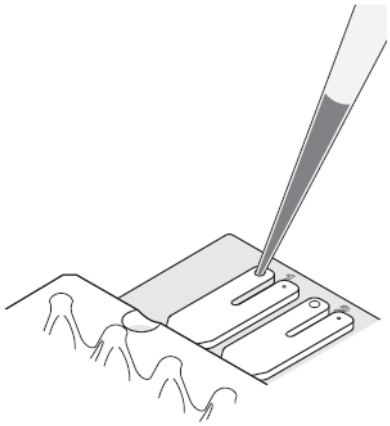


① Location to mark the information

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.

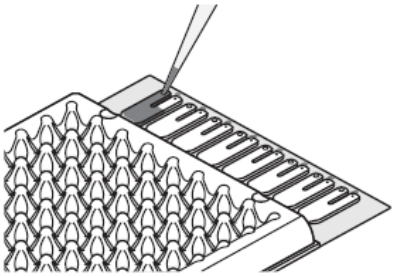


① Fill port ② Vent port



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

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



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

4. Centrifuge and seal the array card (see “Fill the calibration TaqMan™ Array Cards” on page 45).

Creating a custom dye plate for calibration

The QuantStudio™ 12K Flex Real–Time PCR System can be used to run assays designed with custom dyes (dyes not manufactured by Thermo Fisher Scientific). 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 complete the following tasks:

- Determine the 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- or 384-Well Reaction Plate
- Optical Adhesive Cover
- Pipettors and pipette tips (200-µL and 1000-µL)
- Tubes (2-mL and 10-mL)
- Deionized water

Determine the 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- or 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 both 96- and 384-well plates.
   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 instrument, then click **Add to My Instruments**.
   c. Select the 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 plate into the instrument.
   g. When the side door opens, load the sealed plate.
      Ensure that the plate or array card is properly aligned in the holder.
   h. 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 substep 3a and substep 3b for the remaining filters.
   d. After you determine the optimum concentration for each filter, determine the optimum concentration for the custom dye.
      Compare the results from all filters, then select the concentration that yields the highest possible signal in all filters.
4. Discard the plate.

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

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

Create a custom dye plate

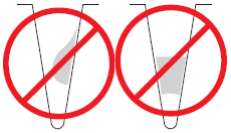
IMPORTANT! Wear powder-free gloves while creating the dye plate.

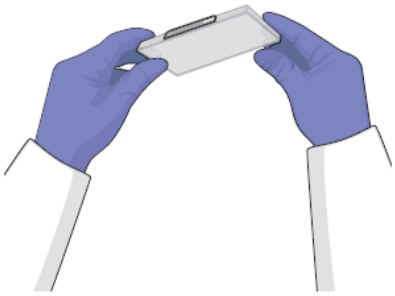
1. Prepare 2 mL of the custom dye at the optimal concentration determined (see “Determine the optimum dye concentration” on page 203).
2. Pipet 20 µL of the diluted custom dye to all wells of an optical reaction plate.
3. Seal the wells of the reaction plate using an optical adhesive cover.
4. Centrifuge the plate for 2 minutes at <1500 rpm.

Note: The custom dye calibration plate must be well mixed and centrifuged.

5. Ensure that the liquid in each well of the plate is at the bottom of the well.

   If the liquid is not at the bottom of each well, centrifuge the plate again at a higher rpm and for a longer period of time.

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



Add the custom dye to the software

1. Start the dye calibration.
   a. In the **Home** screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
   b. In the **Instrument Console**, select your instrument from the list of instruments on the network, then click **Add to My Instruments**.
   c. Select the 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, then click **OK**.

Field or option	Action
Name	Enter a name for the custom dye.
Wavelength	Enter the wavelength at which the dye fluoresces.
Type	Select one of the following options: • **Reporter**—the dye works in conjunction with a quencher dye to report an increase of PCR product. • **Quencher**—the dye suppresses the fluorescence of a reporter dye until amplification of PCR product. • **Both**—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 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 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. Ensure 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, ensure that the peak meets the following criteria:
      - 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. Ensure the status of the calibration.

   - If the calibration passed and all spectra are acceptable, finish the calibration.

   a. Click **Next**.

   b. Enter any comments in the **Comments** field, click **Finish**, then click **Yes** when prompted to save the calibration results.

   - If the calibration failed, created another custom dye plate using the next dye concentration greater than what was determined (see “Determine the optimum dye concentration” on page 203). Perform the calibration again.

D Command-line software operation

Overview of the command-line software operation

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.

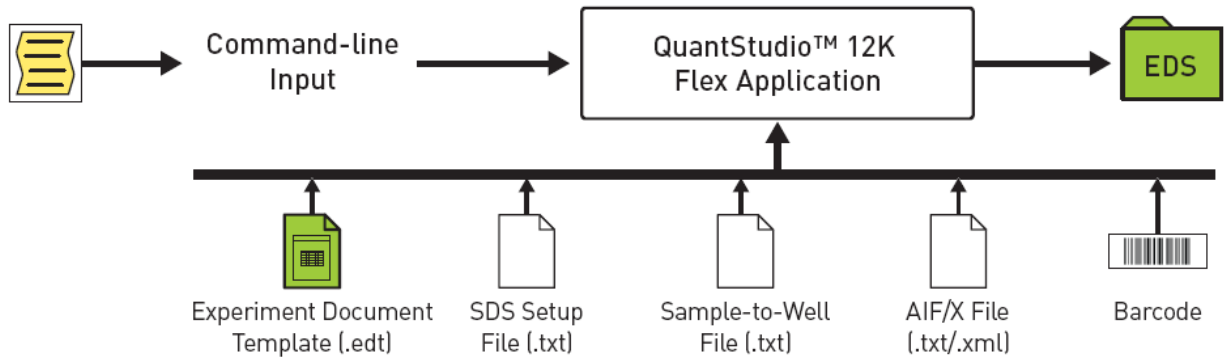


Figure 11 Single experiment file creation workflow

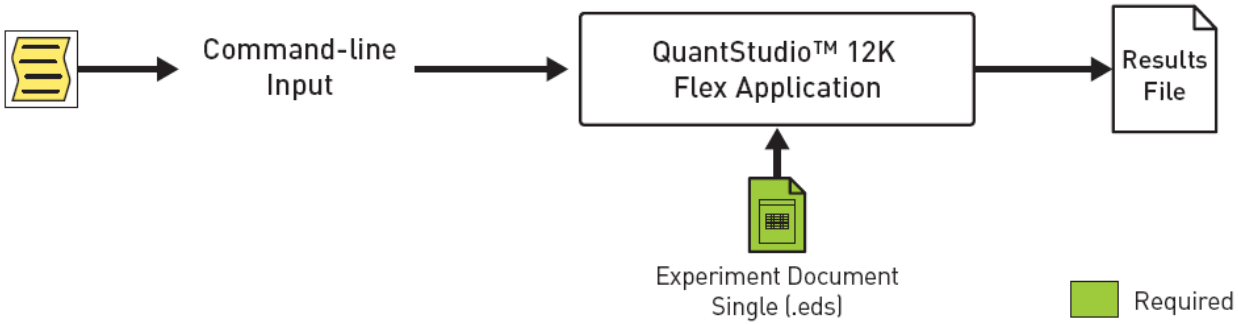


Figure 12 Export workflow

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 downloaded with each TaqMan™ Assay ordered from Thermo Fisher Scientific. The file, which contains data describing the assay, can be imported into the QuantStudio™ 12K Flex Software for use in related experiments. For more information, see “Assay information file” on page 226.
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. For more information, see “Barcode file format” on page 225.
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. For more information, see “Plate setup file format” on page 218.
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. For more information, see “Sample file format” on page 224.

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 the following values: • 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 or AIX)	All values in the template file take precedence, except for the following values: • 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 the following values: • 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

(continued)

Files used for experiment file (EDS) creation	Precedence rule
• Template file (EDT) • Plate setup file (TXT) • Assay information file (AIF or AIX)	All values in the template take precedence, except for the following values: 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

Run the command-line application

Run 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\QuantStudio 12KFlex\bin***, then press **Enter**.
   b. Enter ***cmdlineutil.exe***, then ***-expgen*** or ***-export***, then all applicable parameters and arguments.
      For a complete list of command-line parameters, see “Command syntax and arguments” on page 212.

View the command-line help

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

- To view the help for the entire application, enter ***cmdlineutil.exe –help***.
- To view the help for a particular function, enter ***cmdlineutil.exe –expgen -help*** to view the file generation help, or enter ***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. For an example of the experiment creation command, see “Examples” on page 215.

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. For examples of the experiment export command, see “Examples” on page 215.

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 (For the export file specifications, see “RDML export format” on page 247):

QuantStudio12KFlex—Exports data in a format compatible with the QuantStudio™ 12K Flex System.

SDS23—Exports data in a format compatible with the 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 (required and optional) to generate a set of experiment files. See "Command syntax and arguments" on page 212.

```
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

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 Appendix D, “Command-line software operation”) 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* (Pub. No. 4470693).

Note: The file specifications listed in this appendix are subject to change. For updated information, see the QuantStudio™ 12K Flex Software Release Notes at `C:\Program Files\Applied Biosystems\QuantStudio 12K Flex\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 218
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 224

(continued)

File format	Description	See
Assay information file (AIF or AIX)	A tab-delimited or XML data file that can be downloaded for each TaqMan™ Assay ordered from Thermo Fisher Scientific. The file, which contains data describing the assay, can be imported into the QuantStudio™ 12K Flex Software for use in related experiments.	page 225
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 226

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 Appendix D, “Command-line software operation”). 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 an 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 header	Defines the instrument model for which the experiment is designed and the dye used as the passive reference.	page 219
Plate setup file body	Defines the contents of a 96- or 384-well plate or array card, including target, SNP assay, sample, and task assignments.	page 219

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 the QuantStudio™ 12K Flex System for which the experiment is designed.	QuantStudio 12K Flex
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[1] or • *<blank>* if the consumable does not contain a passive reference.

[1] Custom dyes are allowed if they are in the **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- or 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. For a list of the data column headers, see “Plate setup data columns” on page 220.

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. For a list of the data column headers, see “Plate setup data columns” on page 220.

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)>*[1]
	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>*)”[2]
	Biogroup Name	*(Optional)* The name of the associated biological group.	*<100-character string>*

(continued)

	Column name	Description	Valid values
All experiments	**Biogroup Color**	*(Optional)* The RGB color of the biological group.	"RGB(*<r>*,*<g>*,*<b>*)"[2]
	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>*[3]
	Target Color	*(Optional)* The RGB color of the target.	"RGB(*<r>*,*<g>*,*<b>*)"[2]
	Task	The task assignment of the target assay at the well.[4]	<UNKNOWN | STANDARD | NTC | ENDOGENOUS | IPC | BlockedIPC>
	Reporter	The reporter dye used by the associated target assay.	*<dye name>*[3,5]
	Quencher	The quencher dye used by the associated target assay.	*<dye name>*[5]
	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>*[3]
	SNP Assay Color	*(Optional)* SNP assay color in RGB	"RGB(*<r>*,*<g>*,*<b>*)"[2]
	Task	The task assignment of the SNP assay at the well.[4]	<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>*[3]
	Allele1 Color	The RGB color used to represent data for the first allele.	"RGB(*<r>*,*<g>*,*<b>*)"[2]
	Allele1 Reporter	The reporter dye used to label the probe for the first allele.	*<dye name>*[3,5]
	Allele1 Quencher	The quencher dye used to label the probe for the first allele.	*<dye name>*[5]

(continued)

	Column name	Description	Valid values
Genotyping only	**Allele2 Name**	The name of the second allele detected by the SNP assay.	*<100-character string>*[3]
	Allele2 Color	The RGB color used to represent data for the second allele.	"RGB(*<r>*,*<g>*,*<b>*)"[2]
	Allele2 Reporter	The reporter dye used to label the probe for the second allele.	*<dye name>*[3,5]
	Allele2 Quencher	The quencher dye used to label the probe for the second allele.	*<dye name>*[5]

[1] Cannot be blank.

[2] 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.

[3] Can be empty if the Task field is empty. Otherwise, the field must contain a value.

[4] To determine the tasks applicable to your experiment, see the *QuantStudio™ 12K Flex Real–Time PCR System: Multi-Well Plates and Array Card Experiments User Guide* (Pub. No. 4470050.

[5] 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™ dye- 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 Al1 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 Al1 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 Al1 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 Appendix D, "Command-line software operation") 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 218).

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 assays from assay information files (AIF). An AIF can be downloaded for each assay order. The AIF contains technical details about all assays in the shipment. It includes the following information:

- Assay concentrations
- Reporters
- Quenchers
- Part number
- Lot number
- Assay ID numbers
- Vial ID numbers
- 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, see the QuantStudio™ 12K Flex Software Release Notes 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 QuantStudio™12K Flex-formatted text file that contains setup and/or results data exported from an experiment file (EDS).	page 227
7900 export file	A legacy 7900-formatted text file that contains setup and/or results data exported from an experiment file (EDS).	page 241
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 247

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 229
Sample setup data	Describes the configuration of samples on the experiment consumable, including sample location, target or SNP assay properties, and task assignments.	page 230
Raw data	Contains the raw data collected by the QuantStudio™ 12K Flex Instrument during the experiment run.	page 232
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 233
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 233
Results data	Contains the normalized, processed, and analyzed data generated by the QuantStudio™ 12K Flex Software.	page 234

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 on 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 the data.	Stage *<integer>*, Step *<integer>*

(continued)

Field	Description	Output
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 describe the sample configuration on the experiment consumable, including positions, sample names, task assignments, assay information, and color coding.

The data consist 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 vary depending on experiment type.

This section describes the following sample setup data formats:

- Quantification and presence/absence experiments (see page 230)
- Genotyping experiments (see page 231)

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 224.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)[1].
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>*)”[2]
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>*)”[2]

(continued)

Column	Description	Output
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

[1] Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom
[2] 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 230.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1–96/384)[1]
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>*)”[2]
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>*)”[2]
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>*,<6>)”[2]
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>*,<6>)”[2]
Allele2 Reporter	The reporter dye that labels the probe for the second allele.	100-character string

(continued)

Column	Description	Output
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

[1] Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

[2] 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)[1]
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

[1] 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)[1]
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

[1] 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)[1]
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

[1] 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 track 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 contain 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)[1]
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

[1] 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 depend 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 (see page 234)
- Biological replicate results (see page 235)
- Technical replicate results (see page 236)
- Genotyping (see page 237)
- Melt curve (see page 238)
- HRM (see page 239)
- Presence/absence (see page 240)

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)[1]
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
C_t	The calculated threshold cycle (C_t) for the target at the specified well.	Float
C_t Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float
C_t SD	The standard deviation of the average C_t of the replicate wells for the specified target.	Float

(continued)

Column	Description	Output
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 C_t Threshold	Whether the threshold was determined automatically (true) or manually (false).	`true` or `false`
C_t Threshold	The threshold cycle (C_t) for the sample at the well	Float
Automatic C_t 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`

[1] 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

(continued)

Column	Description	Output
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
C_t Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float
Delta C_t Mean	The average ΔC_t of the replicate wells for the specified target/sample combination.	Float
Delta C_t SD	The standard deviation of the ΔC_t for the replicate well. Depending on the analysis settings, this column may be replaced with "Delta Ct SE" (the standard error of the ΔC_t).	Float
Delta Delta C_t	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
C_t Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float
Delta C_t Mean	The average ΔC_t of the replicate wells for the specified target/sample combination.	Float

(continued)

Column	Description	Output
Delta C_t SD	The standard deviation of the ΔC_t for the replicate well. Depending on the analysis settings, this column may be replaced with "Delta Ct SE" (the standard error of the ΔC_t).	Float
Delta Delta C_t	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)[1]
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 C_t 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 C_t Threshold	Whether the allele 2 threshold was determined automatically (true) or manually (false).	`true` or `false`

(continued)

Column	Description	Output
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)

[1] 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)[1]
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
C_t	The calculated threshold cycle (C_t) for the target at the specified well.	Float
C_t Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float
C_t 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

(continued)

Column	Description	Output
Automatic C_t Threshold	Whether the threshold was determined automatically (true) or manually (false).	`true` or `false`
C_t Threshold	The threshold cycle (C_t) for the sample at the well.	Float
Automatic C_t 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 (Tm) 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)

[1] 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)[1]
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)
C_t	The calculated threshold cycle (C_t) for the target at the specified well.	Float
C_t Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float

(continued)

Column	Description	Output
C_t 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`

[1] 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)[1]
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
R_n	The raw fluorescence for the associated well normalized to the fluorescence of the passive reference dye.	Float
R_n Mean	The averaged normalized fluorescence (R_n) for the associated replicate wells that contain the same target/sample combination.	Float
R_n 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

(continued)

Column	Description	Output
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 C_t Threshold	Indicates whether the threshold was determined automatically (true) or manually (false).	`true` or `false`
C_t Threshold	The threshold cycle (C_t) for the sample at the well.	Float
Automatic C_t 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)

[1] 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 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 242
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 243
Results file	Contains the normalized, processed, and analyzed data generated by the QuantStudio™ 12K Flex Software.	page 244

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 Real-Time PCR 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 describe 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)[1]
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

[1] 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 that are 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 contain 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)[1]
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

[1] 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 depend 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 (see page 245)
- Genotyping experiments (see page 246)

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)[1]
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`
C_t	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
C_t Median	The median C_t of the replicate wells for the specified target/sample combination.	Float
C_t Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float
C_t SD	The standard deviation of the average C_t of the replicate wells for the specified target.	Float

(continued)

Column	Description	Output
Automatic C_t 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 C_t Threshold	Indicates whether the threshold was determined automatically (true) or manually (false).	`TRUE` or `FALSE`
C_t Threshold	The C_t for the sample at the well	Float

[1] 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)[1]
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

[1] 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.

More information about the RDML standard

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, go to the RDM organization website (**rdml.org**). The website features free data management tools, including an on-line RDML file generator and RDML software libraries.

F

Parts and materials

How to order

You can order materials and accessories from Thermo Fisher Scientific by ordering directly from the Thermo Fisher Scientific website.

Note: Product availability and pricing may vary according to your region or country. Online ordering through Thermo Fisher Scientific is not available in all countries. Contact your local 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 Reader™ software versions to use the Thermo Fisher Scientific website:

Operating system	Microsoft™ Internet Explorer™	Apple™ Safari™	Mozilla™ Firefox™	Adobe Reader™ Software
Microsoft™ Windows™ Operating System	v6.x or later	None[1]	v2.x or later	v4.0 or later
Macintosh™ Operating System	None[1]	v2.0.4 or later		

[1] Browser not available for this platform.

Note: Confirm that cookies and JavaScript are turned on for the website to function correctly.

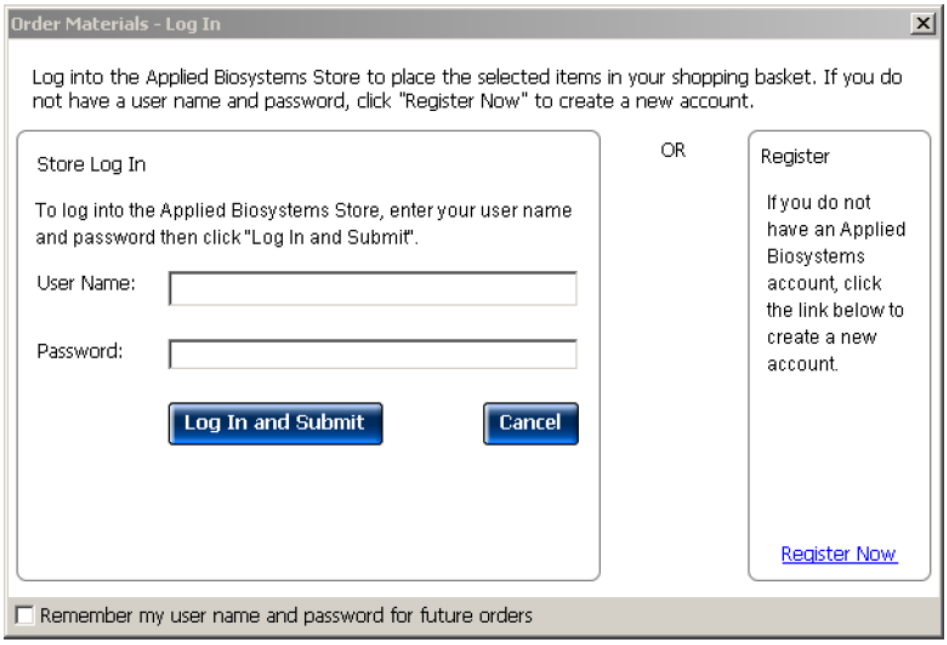
Order from the QuantStudio™ 12K Flex Software

1. To find your assay on the Thermo Fisher Scientific 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 step 1, 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 Thermo Fisher Scientific Store, then click **Log In and Submit**.



Note: If you do not have an account with the Thermo Fisher Scientific Store, click **Register Now** to create an account.

When you are connected to the Thermo Fisher Scientific Store, follow the prompts to complete your order.

Accessories

The following accessories are used with the QuantStudio™ 12K Flex Real–Time PCR System.

Accessories	Cat. 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

(continued)

Accessories	Cat. No.
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 materials are required to calibrate the QuantStudio™ 12K Flex Real–Time PCR System:

- 384-well plate block (see page 250)
- 96-well 0.2-mL plate block (see page 251)
- 96-well 0.1-mL plate block (see page 252)
- TaqMan™ Array Card block (see page 253)

Note: See the package label for reagent or consumable shelf-life expiration date.

Calibration kits for the 384-well plate block

The kits in the following table are for QuantStudio™ 12K Flex Software v1.4.

For QuantStudio™ 12K Flex Software v1.5 or later, see the *QuantStudio™ 12K Flex Real–Time PCR System v1.5 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

Kit	Cat. No.[1]	Storage
FAM™ Dye Spectral Calibration Plate, 384-well	4432271	–25°C to –15°C
VIC™ Dye Spectral Calibration Plate, 384-well	4432278	
ROX™ Dye Spectral Calibration Plate, 384-well	4432284	
NED™ Dye Spectral Calibration Plate, 384-well	4432302	

(continued)

Kit	Cat. No.[1]	Storage
SYBR™ Green Dye Spectral Calibration Plate, 384-well	4432290	–25°C to –15°C
TAMRA™ Dye Spectral Calibration Plate, 384-well	4432296	
Region of Interest (ROI) and Background Plates, 384-well	4432320	
FAM™/ROX™ and VIC™/ROX™ Dye Normalization Plates, 384-well	4432308	
TaqMan™ RNase P Instrument Verification Plate, 384-well	4455280	

[1] Catalog numbers that appear as links open the web pages for those products.

Calibration kits for the 96-well 0.2-mL plate block

The kits in the following table are for QuantStudio™ 12K Flex Software v1.4.

For QuantStudio™ 12K Flex Software v1.5 or later, see the *QuantStudio™ 12K Flex Real–Time PCR System v1.5 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

Kit	Cat. No.[1]	Storage
FAM™ Dye Spectral Calibration Plate, 96-well	4432327	–25°C to –15°C
VIC™ Dye Spectral Calibration Plate, 96-well	4432334	
ROX™ Dye Spectral Calibration Plate, 96-well	4432340	
NED™ Dye Spectral Calibration Plate, 96-well	4432358	
SYBR™ Green Dye Spectral Calibration Plate, 96-well	4432346	
TAMRA™ Dye Spectral Calibration Plate, 96-well	4432352	
Region of Interest (ROI) and Background Plates, 96-Well 0.2-mL (2 plates)	4432364	
Normalization Plates with FAM™/ROX™ and VIC™/ROX™ Dyes , 96-well	4432370	
TaqMan™ RNase P Instrument Verification Plate, 96-Well 0.2-mL	4432382	

[1] Catalog numbers that appear as links open the web pages for those products.

Calibration kits for the 96-well 0.1-mL plate block

The kits in the following table are for QuantStudio™ 12K Flex Software v1.4.

For QuantStudio™ 12K Flex Software v1.5 or later, see the *QuantStudio™ 12K Flex Real–Time PCR System v1.5 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

Kit	Cat. No.[1]	Storage
FAM™ Dye Spectral Calibration Plate, Fast 96-well	4432389	–25°C to –15°C
VIC™ Dye Spectral Calibration Plate, Fast 96-well	4432396	
ROX™ Dye Spectral Calibration Plate, Fast 96-well	4432402	
NED™ Dye Spectral Calibration Plate, Fast 96-well	4432420	
SYBR™ Green Dye Spectral Calibration Plate, Fast 96-well	4432408	
TAMRA™ Dye Spectral Calibration Plate, Fast 96-well	4432414	
Region of Interest (ROI) and Background Plates, Fast 96-Well 0.1-mL (2 plates)	4432426	
FAM™/ROX™ and VIC™/ROX™ Dye Normalization Plates, Fast 96-well	4432432	
TaqMan™ RNase P Instrument Verification Plate, Fast 96-Well	4351979	

[1] Catalog numbers that appear as links open the web pages for those products.

Calibration kits for the TaqMan™ Array Card block

Note: The calibration procedure for the TaqMan™ Array Card block is the same for the QuantStudio™ 12K Flex Software v1.4 and the QuantStudio™ 12K Flex Software v1.5 or later.

Kit	Cat. No.	Storage
ViiA™ 7 Array Card Spectral Calibration Kit The kit includes the following items: • Empty TaqMan™ Array Cards • FAM™ dye • VIC™ dye • ROX™ dye • ROI dye mix • Background buffer • FAM™/ROX™ dye • VIC™/ROX™ dye	4432314	–25°C to –15°C
ViiA™ 7 Array Card RNaseP Verification Kit The kit includes the following items: • 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 used with the QuantStudio™ 12K Flex Real–Time PCR System.

Item		Cat. No.[1]
MicroAmp™ Fast 8-Tube Strip, 0.1 mL	125 strips	4358293
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	20 plates	4346906
	200 plates	4366932
MicroAmp™ Optical 96-Well Reaction Plate	10 plates	N8010560

(continued)

Item		Cat. No.[1]
MicroAmp™ Optical 96-Well Reaction Plate	500 plates	4316813
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	20 plates	4306737
	500 plates	4326659
MicroAmp™ Optical 384-Well Reaction Plate	1,000 plates	4343370
MicroAmp™ Optical 384-Well Reaction Plate with Barcode	1,000 plates	4343814
	500 plates	4326270
	50 plates	4309849
MicroAmp™ Optical 8-Cap Strips	300 strips	4323032
MicroAmp™ Optical 8-Tube Strip, 0.2 mL	1,000 tubes	4316567
MicroAmp™ Optical Adhesive Film	100 covers	4311971
	25 covers	4360954

[1] Catalog numbers that appear as links open the web pages for those products.

The following accessories are required for TaqMan™ Array Cards. Contact your local sales office for details.

- Centrifuge with custom buckets and card holders, one of the following:
  - Sorvall™ centrifuge
  - Multifuge™ centrifuge
  - Megafuge™ centrifuge
- TaqMan™ Array Card Sealer (referred to as the Stylus Staker in some documents)
- Blank balance TaqMan™ Array Cards (included with the installation kit)

Glossary

AIF

See “Assay information file” on page 226.

AIX

XML version of the assay information file.

See also “Assay information file” on page 226.

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 one of the following:

- 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” on page 259.

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 to TaqMan™ Assays by Thermo Fisher Scientific.

assay information file (AIF)

Tab-delimited data file on available for download with each assay order. The AIF contains technical details about all assays in the shipment. It includes the following information:

- Assay concentrations
- Reporters and quenchers
- Catalog numbers and lot numbers
- 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 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” on page 257.

automatic threshold

An analysis setting in which the software calculates the baseline start and end cycles and the threshold in the amplification plot. The software uses the baseline and threshold to calculate the threshold cycle (C_t).

See also “threshold cycle (Ct)” on page 274 (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)” on page 265.

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 experiment 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” on page 274.

blocked IPC

In presence/absence experiments, a reaction that contains IPC blocking agent, which blocks amplification of the internal positive control (IPC). In the 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” on page 264.

calibrator

See “reference sample” on page 268.

chemistry

See “reagents” on page 268.

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 (CRT)” on page 269.

C_{rt} algorithm

See “Relative Threshold algorithm” on page 269.

C_t

See “threshold cycle (Ct)” on page 274.

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 (Ct)” on page 274.

cycling stage

In the thermal profile, a stage that is repeated. A cycling stage is also called an amplification stage.

See also “amplification stage” on page 256.

C_q

See “quantification cycle (Cq)” on page 267.

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)” on page 257.

diluent

A reagent used to dilute a sample or standard before it is added to the PCR reaction.

dilution factor

See “serial factor” on page 271.

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%)” on page 255.

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” on page 266.

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 Thermo Fisher Scientific name for the electronic records that comprise all information about a particular plate or array card consumable, including the following information:

- Metadata (name, bar code, comments)
- Plate setup (well contents, assay definitions)
- Run method (thermal cycling protocol)
- Run results
- Analysis protocol
- Analysis results
- Audit records
- Other plate-specific data

Experiment documents have the following suffixes:

- EDS—Single experiment document
- EDT—Template
- EDM—Multiple experiment document

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 with QuantStudio™ 12K Flex Software v1.4 includes a six-color filter set that supports the following dyes:

- FAM™ dye
- NED™ dye
- ROX™ dye
- SYBR GREEN™ dye
- TAMRA™ dye
- VIC™ dye

The QuantStudio™ 12K Flex System with QuantStudio™ 12K Flex Software v1.5 includes a six-color filter set that supports the following dyes:

- FAM™ dye
- NED™ dye
- ROX™ dye
- SYBR GREEN™ dye
- TAMRA™ dye
- VIC™ dye
- ABY™ dye
- JUN™ dye
- MUSTANG PURPLE™ dye
- Cy™5 dye

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** screen, the **Well Table** screen, and the **QC Summary** screen.

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 has one of the following characteristics:

- 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” on page 261.

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” on page 261.

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. Their constancy should always be validated experimentally.

See also “endogenous control” on page 260.

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 perform the following functions:

- Monitor the status of any instrument on the network
- View calibration, maintenance, and instrument properties for a selected instrument
- 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 perform the following functions:

- 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)” on page 263.

IPC blocking agent

Reagent added to PCR reactions to block amplification of the internal positive control (IPC).

IPC+

See “negative control-IPC wells” on page 264.

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” on page 257.

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” on page 264.

no template control (NTC)

See “negative control (NC)” on page 264.

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.

Normalization calibration is not required for plate blocks with QuantStudio™ 12K Flex Software v1.5. It is required for TaqMan™ Array Card blocks.

Normalization calibration is required for all blocks with QuantStudio™ 12K Flex Software v1.4.

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 Applied Biosystems™ 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” on page 273.

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 Thermo Fisher Scientific website for a TaqMan™ 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” on page 272.

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” on page 268.

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™ dye 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)” on page 265.

ROX™ dye

A dye supplied by Thermo Fisher Scientific and precalibrated on the instrument. ROX™ dye is used as the passive reference.

rs number

See “refSNP ID” on page 268.

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 10X, the difference between any two adjacent points in the curve is 10-fold.

slope

Regression coefficient calculated from the regression line in the standard curve. The slope indicates the PCR amplification efficiency for the assay. A slope of -3.32 indicates 100% amplification efficiency.

See also “amplification efficiency (EFF%)” on page 255 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 following information:

- SNP assay name
- SNP assay color
- The allele name or bases for each allele
- The reporter and quencher for each allele
- The allele colors for each allele

The SNP assays in the library can also contain the assay ID and comments about the SNP assay.

stage

In the thermal profile, a group of one or more steps.

Examples include the following stages:

- PCR stage
- Cycling stage (also called the amplification stage
- 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” on page 272.

standard curve

- The best-fit line in a plot of the C_t values from the standard reactions plotted against standard quantities.

  See also “regression line” on page 269.
- 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 curve” on page 272.

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” on page 272.

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 Thermo Fisher Scientific and precalibrated on the QuantStudio™ 12K Flex System. Before you use system dyes in your experiments, ensure that the system dye calibration is current in the **Instrument Console**.

The following dyes are system dyes with QuantStudio™ 12K Flex Software v1.4:

- FAM™ dye
- ROX™ dye
- NED™ dye
- SYBR GREEN™ dye
- TAMRA™ dye
- VIC™ dye

The following dyes are system dyes with QuantStudio™ 12K Flex Software v1.5:

- FAM™ dye
- NED™ dye
- ROX™ dye
- SYBR GREEN™ dye
- TAMRA™ dye
- VIC™ dye
- ABY™ dye
- JUN™ dye
- MUSTANG PURPLE™ dye
- Cy™5 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.

The following tasks are available:

- 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 or it can be set manually (see “automatic threshold” on page 257 and “manual threshold” on page 263).
- 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 (Tm)” on page 264).

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.

- Quantification experiments—The task for the target in wells that contain a sample with unknown target quantities.
- Genotyping experiments—The task for the SNP assay in wells that contain a sample with an unknown genotype.
- Presence/absence experiments—The task for the target in wells that contain a sample in which the presence of the target is not known.
- 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.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **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.

Standard safety symbols

Symbol and description
 **CAUTION!** Risk of danger. Consult the manual for further safety information.
 **CAUTION!** Risk of electrical shock.
 **CAUTION!** Hot surface.
 **CAUTION!** Potential biohazard.
 **CAUTION!** Ultraviolet light.

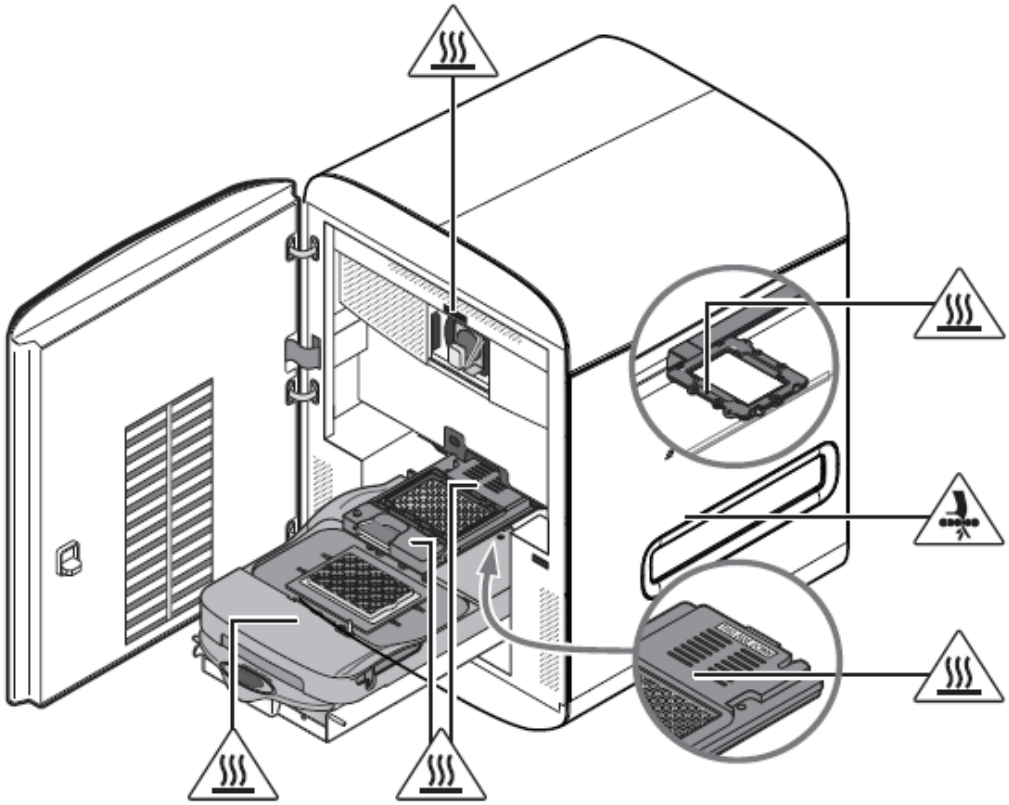
Symbole et description
 **MISE EN GARDE !** Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
 **MISE EN GARDE !** Risque de choc électrique.
 **MISE EN GARDE !** Surface chaude.
 **MISE EN GARDE !** Danger biologique potentiel.
 **MISE EN GARDE !** Rayonnement ultraviolet.

Additional safety symbols

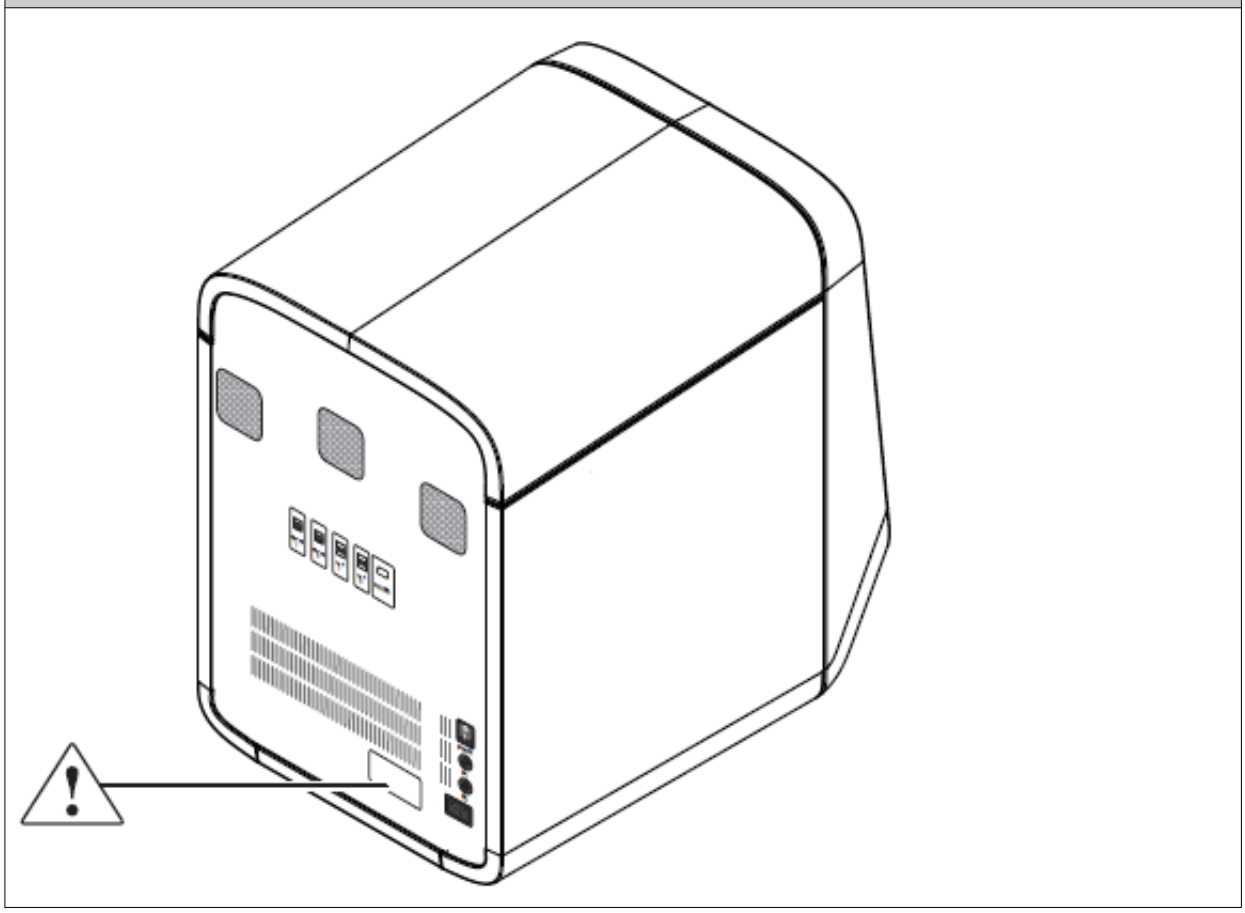
Symbol and description
 **CAUTION!** Laser radiation
 **CAUTION!** Moving parts.
 **CAUTION!** Potential slipping hazard.

Symbole et description
 **MISE EN GARDE !** Laser radiation
 **MISE EN GARDE !** Parties mobiles.
 **MISE EN GARDE !** Danger de glisser potentiel.

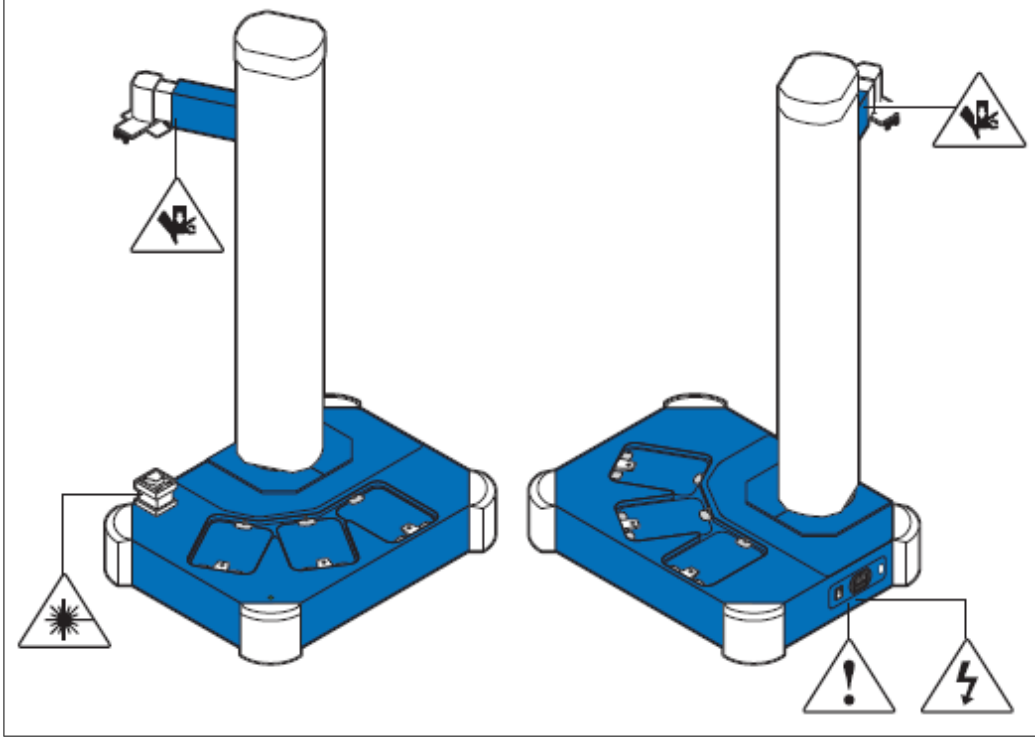
Location of safety labels

Label and location


(continued)

Label and location


(continued)

Label and location


Control and connection symbols

Symbols and descriptions	
	On (Power)
	Off (Power)
	Standby
	Earth (ground) terminal
	Protective conductor terminal (main ground)
	Direct current
	On/Off

(continued)

Symbols and descriptions	
~	Alternating current
⎓	Both direct and alternating current

Conformity symbols

Conformity mark	Description
c UL us	Indicates conformity with safety requirements for Canada and U.S.A.
25	Indicates conformity with China RoHS requirements.
CE	Indicates conformity with European Union requirements.
	Indicates conformity with Australian standards for electromagnetic compatibility.
PS E PS E	Indicates conformity with requirements for safety with Japanese requirements.
	Indicates conformity with the WEEE Directive 2012/19/EU. ⚠ **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Physical injury

CAUTION! Moving and Lifting Injury. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- 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.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Consider including language specific to the moving parts hazard associated with the instrument. The alert word will change based on potential severity of the injury should it occur. If this is the case for your product, use the "z_safety_Physical injury" template to create a new topic.

Electrical safety

WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

AVERTISSEMENT ! Cordons d'alimentation électrique. Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Instrument component and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Laser safety

The optional barcode scanners included with the QuantStudio™ 12K Flex Real–Time PCR Systems are categorized as Class 2 (II) lasers.

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.

The following table lists laser safety symbols and alerts that may be present on the instrument.

Alert
CAUTION! LASER HAZARD, Bar Code Scanner. The bar code scanner included with the instrument system is a Class 2 laser. To avoid damage to eyes, do not stare directly into the beam or point into another person's eyes.

Alerte
MISE EN GARDE ! RISQUE LIÉ AU RAYONNEMENT LASER, Lecteur de code-barres. Le lecteur de code-barres inclut dans l'instrument est un appareil laser de classe 2. Pour éviter toute lésion oculaire, ne regardez pas directement le faisceau et ne le dirigez pas vers les yeux d'une autre personne.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

CAUTION! MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety standards

Reference	Description
EU Directive 2014/35/EU	European Union "Low Voltage Directive"
IEC 61010-1 EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010 EN 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*

EMC standards

Reference	Description
EU Directive 2014/30/EU	European Union "EMC Directive"
EN 61326-1 IEC 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 18 (47 CFR)	U.S. Standard "Industrial, Scientific, and Medical Equipment"
AS/NZS CISPR 11	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 4	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union "WEEE Directive"—Waste electrical and electronic equipment
Directive 2011/65/EU & Commission Delegated Directive (EU) 2015/863	European Union "RoHS Directive"—Restriction of hazardous substances in electrical and electronic equipment
SJ/T 11364-2014	"China RoHS" Standard—Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products For instrument specific certificates, visit our customer resource page at **www.thermofisher.com/us/en/home/technical-resources/rohs-certificates.html**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Related documentation

Document	Pub. No.
QuantStudio™ 12K Flex Real–Time PCR System Site Preparation Guide	4470654
QuantStudio™ 12K Flex Real–Time PCR System: Multi-Well Plates and Array Card Experiments User Guide	4470050
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Plate Quick Reference	4478673
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
High Resolution Melt Software for QuantStudio™ 12K Flex Real–Time PCR System Getting Started Guide	4470692
QuantStudio™ 12K Flex Real–Time PCR System OpenArray™ Digital PCR Experiments User Guide	4471926
QuantStudio™ 12K Flex Software v1.5 or later	
QuantStudio™ 12K Flex Real–Time PCR System v1.5 or later Maintenance and Administration Guide	MAN0018832
QuantStudio™ 12K Flex Real–Time PCR System v1.5 or later Multi-Well Plates and Array Card Quick Reference	MAN0018833
QuantStudio™ 12K Flex Software v1.4	
QuantStudio™ 12K Flex Real–Time PCR System: Multi-Well Plates and Array Card Quick Reference	4470688
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System with OpenArray™ AccuFill™ Software v1.2	
OpenArray™ AccuFill™ System User Guide	4456986
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System with OpenArray™ AccuFill™ Software v2.0	
QuantStudio™ 12K Flex OpenArray™ AccuFill™ System User Guide	MAN0025669

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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