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Applied Biosystems™ ViiA™ 7 Real-Time PCR System

USER GUIDE

Calibration, Maintenance, Networking, and Security

for use with QuantStudio™ Real-Time PCR Software v1.6.1

Publication Number MAN0018830

Revision C.0

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Revision	Date	Description
C.0	15 July 2020	Removed Twister™ Robot.
B.0	16 January 2020	Update QuantStudio™ Real-Time PCR Software to v1.6.1. No changes to workflow or user interface compared to v1.6.
A.0	30 September 2019	New document for QuantStudio™ Real-Time PCR Software v1.6. Based on *Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security* (Pub. No. 4442661, Rev. C). Updated calibration workflow, safety information, software name, branding, template and licensing information.

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About this guide

Purpose

The Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide provides reference information for the ViiA™ 7 Instrument. The guide describes how to prepare, maintain, and troubleshoot a ViiA™ 7 System that is running QuantStudio™ Real-Time PCR Software v1.6.1.

Audience

This user guide is written for laboratory staff who operate and maintain the ViiA™ 7 System.

Assumptions

This guide assumes that your ViiA™ 7 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.

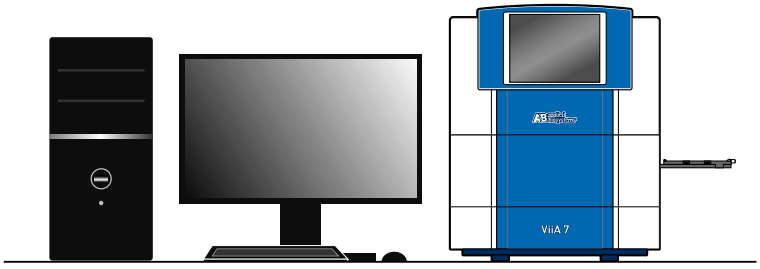
1 Getting started

Access the Help system by pressing **F1**, by clicking in the toolbar of the QuantStudio™ Real-Time PCR Software v1.6.1 window, or by selecting **Help ▸ Contents and Index**.

About the ViiA™ 7 system

The Applied Biosystems™ ViiA™ 7 Real-Time PCR System uses fluorescent-based polymerase chain reaction (PCR) reagents to provide:

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



About data collection

The ViiA™ 7 Real-Time PCR System collects raw fluorescence data at different points during a PCR, depending on the type of run that the instrument performs:

Run type	Data collection point
Real-time runs • Standard curve • Relative standard curve • Comparative C_t ($\Delta\Delta C_t$) • Melting curve	The instrument collects data following each extension step of the PCR.
Post-PCR (endpoint) runs • Genotyping • Presence/absence	1. Before the PCR. (For presence/absence experiments, data collection before the PCR is optional, but recommended.) 2. (*Optional*) During the PCR. The instrument can collect data during the run (realtime); collecting data during the run can be helpful for troubleshooting. 3. After the PCR.

Regardless of the run type, a data collection point, or read, on the ViiA™ 7 Real-Time PCR System 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 that is collected by the device consists only of light that corresponds to the range of emission wavelengths.

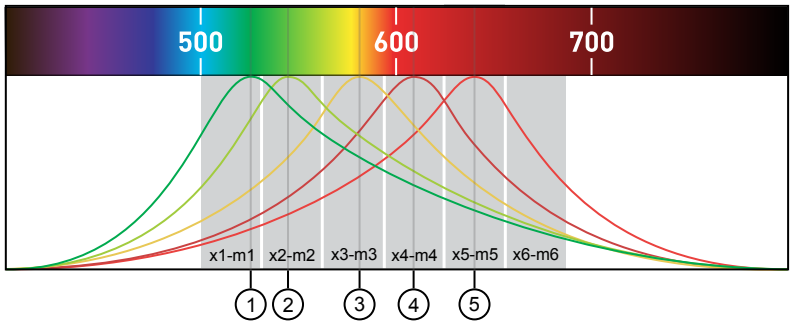
3. **Collection** – The instrument assembles a digital representation of the residual fluorescence collected over a fixed time interval. The QuantStudio™ Real-Time PCR Software stores the raw fluorescent image for analysis.

After a run, the QuantStudio™ Real-Time PCR Software uses calibration data (ROI, background, uniformity, dye, and normalization) to determine the location and intensity of the fluorescent signals in each read, the dye associated with each fluorescent signal, and the significance of the signal.

Instrument filters and supported dyes

System dyes

The ViiA™ 7 Real-Time PCR System features a six-color filter set that supports all Thermo Fisher Scientific dyes. The following figure shows the emission spectrum for each dye, and the filter at which each dye is read.



① x1-m1 — FAM™, SYBR™ Green
② x2-m2 — VIC™
③ x3-m3 — ABY™, NED™, TAMRA™
④ x4-m4 — JUN™, ROX™
⑤ x5-m5 — Cy®5, MUSTANG PURPLE™

Peak filter	Color	Filter wavelength (nm)[1]		Factory-calibrated dyes
		Excitation	Emission	
x1-m1	Blue	470 ± 15	520 ± 15	FAM™, SYBR™ Green
x2-m2	Green	520 ± 10	558 ± 12	VIC™
x3-m3	Yellow	550 ± 10	587 ± 10	NED™, TAMRA™, ABY™
x4-m4	Orange	580 ± 10	623 ± 14	ROX™, JUN™
x5-m5	Red	640 ± 10	682 ± 14	MUSTANG PURPLE™, Cy®5
x6-m6	Deep-Red	662 ± 10	711 ± 12	None [2]

[1] The central wavelengths are the optimized wavelengths.
[2] This filter set currently does not support any dyes supplied by Thermo Fisher Scientific.

Custom dyes

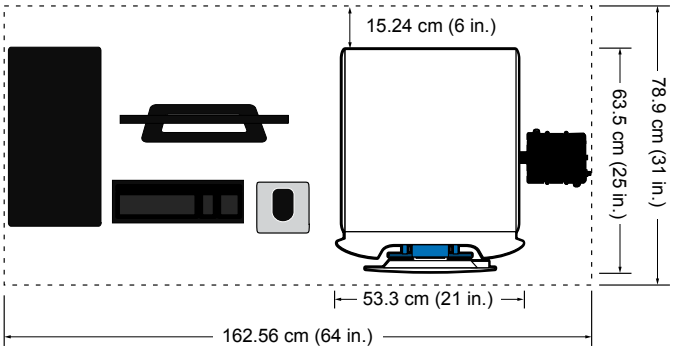
The ViiA™ 7 Real-Time PCR System can run assays that are designed with custom dyes (dyes that are not supplied by Thermo Fisher Scientific) that are excited between 455 nm to 672 nm and read between 505 nm to 723 nm.

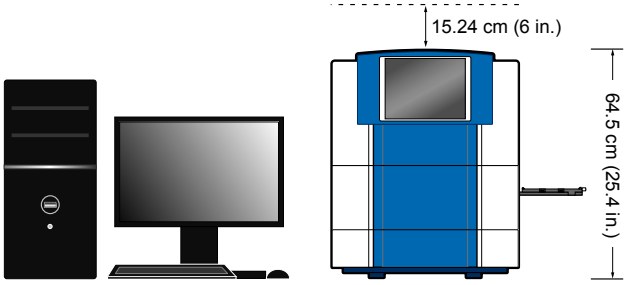
Specifications and layout

ViiA™ 7 Real-Time PCR System specifications

The figures below summarize the specifications and requirements for the ViiA™ 7 Real-Time PCR System. For more information, refer to the *Applied Biosystems™ ViiA™ 7 Real-Time PCR System Site Preparation Guide* (Pub. No. 4445302).

ViiA™ 7 Real-Time PCR System dimensions





Component		Width		Depth		Height		Weight	
		cm	in	cm	in	cm	in	kg	lbs
Instrument[1]		53.3	21.0	63.5	25.0	64.5	25.4	60.7	133.5
Computer[2]	Laptop	35.8	14.1	25.7	10.1	35.8	14.1	2.6	5.7
	Desktop	18.7	7.3	44.5	17.5	41.0	16.1	10.9	24.0
Monitor		44.7	17.5	19.3	7.6	36.6	14.4	6.9	15.2
Keyboard		44.7	17.5	15.25	6.0	5.0	2.0	0.1	0.2
Total footprint		233	91.7	86	33.8	79.7	31.4	77.9	171.5

[1] Weight varies depending on the sample block installed.

[2] Computer properties differ depending on the computer ordered with the ViiA™ 7 System (laptop or desktop).

Required clearance

The ViiA™ 7 Instrument requires the following additional clearances:

- **Clearance on all sides** – At least 15.2 cm (6 in) of clearance for ventilation, service access, and cable routing.
- **Vertical clearance** – At least 30.5 cm (12 in) of unobstructed vertical clearance above the ViiA™ 7 Instrument to allow removal of the cover during service.

Instrument hot-air exhaust venting

The maximum thermal output of the ViiA™ 7 Instrument is 2731BTU/hr (800W) vented directly into the room air from the hot-air waste port on the rear panel.

Electrical requirements

Note: We recommend placing the ViiA™ 7 Instrument and computer power receptacle on an electrical circuit that is not shared with electrically noisy devices or devices that can cause power surges, such as refrigeration units.

The following table provides electrical specifications for the instrument and associated devices. For all indicated input voltages, a 15 A circuit is required.

Device		Rated current (A)	Rated power (VA)	Rated voltage (VAC)	Rated frequency (Hz)
Instrument		12.5	950	100-240±10 %	50/60
Computer	Desktop	2.1	125		
	Laptop	1.5	90		
Monitor		1.5	65		

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

Environmental requirements

Requirement	Description
Altitude	Less than 2,000 m (6,500 ft) above sea level
Temperature	15–30°C (59–86°F) Do not place the ViiA™ 7 Instrument next to heaters, cooling ducts, or in direct sunlight. Temperature fluctuations can affect performance.
Humidity	20–80% relative humidity, noncondensing
Pollution	Pollution Degree rating of 2[1]

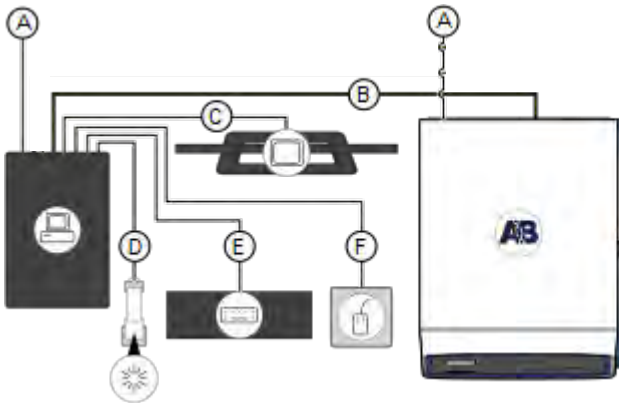
(continued)

Requirement	Description
Location	For indoor use only **IMPORTANT!** Do not locate the ViiA™ 7 Instrument next to: • Vibration sources, such as a centrifuge, pump, or compressor. Excessive vibration affects instrument performance. • Electrically noisy devices, such as a refrigeration unit.

[1] The ViiA™ 7 Instrument can be used in an environment that contains nonconductive pollutants only (dust particles or wood chips). Typical environments with a Pollution Degree 2 rating are laboratories, sales, and commercial areas.

ViiA™ 7 system layout and connections

The ViiA™ 7 System consists of the components shown in the following figure.



	Component	Description
	ViiA™ 7 Instrument	Performs fluorescence research detection and data collection of experiment and calibration consumables.
	Computer	Runs the software that is used to: • Calibrate the ViiA™ 7 Instrument. • Set up experiments. • (*Optional*) Run experiments. • Analyze experiments.
	Monitor	
	Keyboard	
	Mouse	
	Bar code reader	Scans the bar codes of consumables before and after they are loaded into the ViiA™ 7 Instrument.

	Connection	Description
A	Power cables	Supply power to the computer and the ViiA™ 7 Instrument.[1]
B	LAN connection or Ethernet cable[2]	Connects the ViiA™ 7 Instrument (Ethernet 1 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	Bar code reader cable	Connects the bar code 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).

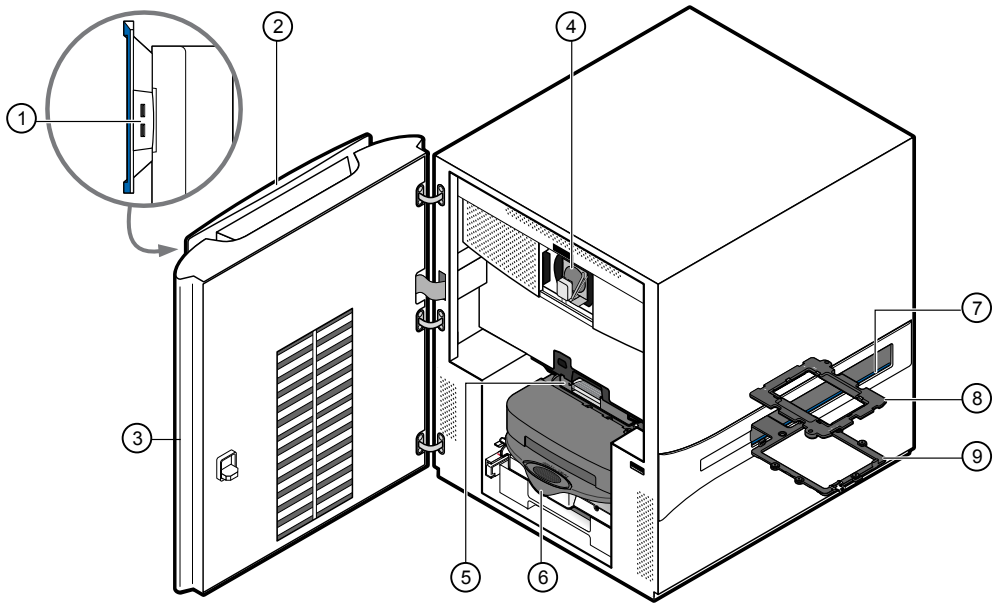
[1] Supplies 115/230 V depending on the geographic location of the installation.
[2] Supplied with the ViiA™ 7 System.

ViiA™ 7 Real-Time PCR System hardware

Instrument components

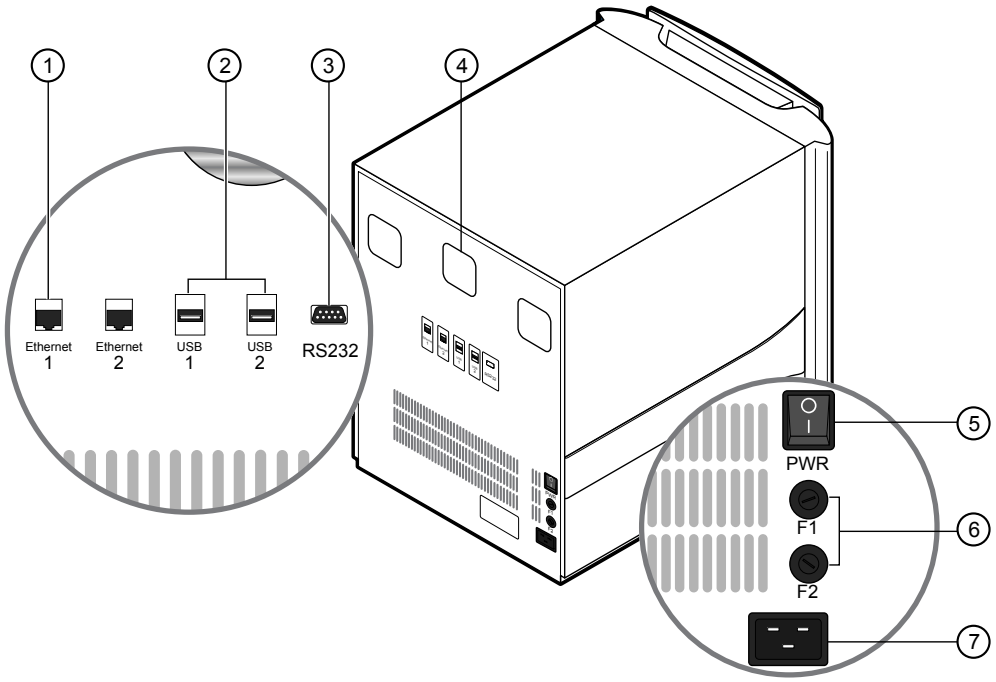
The ViiA™ 7 System consists of the components shown in the following figures.

Front view



	Component	Description
1	USB ports	Provide USB communication with the ViiA™ 7 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 ViiA™ 7 Instrument, the instrument mounts only the first drive that is installed, regardless of the USB port used.
2	Instrument touchscreen	Provides access to the instrument functions. Can be used to run experiments, transfer data, and operate the instrument functions without the use of the computer.
3	Access door	Provides access to the instrument lamp, the heated cover, and the sample block.
4	Lamp	Illuminates the reaction plate or array card during a run.
5	Heated cover	Covers the plate or array card during a run to prevent condensation and leakage through the consumable cover.
6	Sample block	Heats the plate or array card during a run.
7	Side door	Opens to allow extension of the tray arm.
8	Plate adapter	Secures plates or array cards to the tray arm.
9	Tray arm	Conveys plates or array cards to and from the sample block in the interior of the instrument.

Rear view



Component		Description
1	Ethernet 1 port	An RJ45 port that provides Ethernet (Gigabit) communication with the instrument.[1] **IMPORTANT!** The Ethernet 2 port is for Thermo Fisher Scientific use only.
2	USB ports	Provide USB communication with the 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 ViiA™ 7 Instrument, the instrument mounts only the first drive that is installed, regardless of the USB port used.
3	RS232 port	Provides serial communication between the instrument and the computer. **IMPORTANT!** The serial port is reserved for Thermo Fisher Scientific use only.
4	Instrument fans	Cool the interior of the ViiA™ 7 Instrument. **IMPORTANT!** The fans must be unobstructed to ensure adequate cooling and proper function of the ViiA™ 7 Instrument.
5	On/Off switch	Power switch for the ViiA™ 7 Instrument, where the states are on (**|**) or off (**O**).
6	Fuse cover	Dual 12.5A, Time-Lag T, 250VAC, 5 × 20-mm electrical fuses that protect the ViiA™ 7 Instrument from excessive electrical current.
7	Power port	The 100-240VAC port that provides power to the ViiA™ 7 Instrument.

[1] Use the Ethernet cable supplied with the ViiA™ 7 System to connect the instrument (Ethernet 1 port) to the network interface card in the computer.

Bar code reader

The Applied Biosystems™ ViiA™ 7 Real-Time PCR System can include a bar code reader for data entry and plate recognition:

- A hand-held bar code reader for scanning plates manually.

The bar code reader uses 670 nm Class II lasers to scan plates, and is capable of reading Code 128 (alphanumeric), which supports 128 ASCII character bar codes. The bar code reader is optional and available depending on the system configuration.

About the hand-held bar code reader

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

The optional hand-held bar code reader functions as an extension of the keyboard that you can use to scan bar codes into the software.

To scan a bar code using the hand-held bar code reader:

1. Select the field in the software where you want to enter the bar code.
2. Hold the hand-held bar code reader 20 to 30 cm away from a plate and aim at the center of the bar code, then press the trigger. Slowly move the scanning beam across the bar code until the reader emits a high-pitched tone.

When the reader scans a bar code, it automatically:

- Transmits the alphanumeric equivalent of the bar code to the software. The software enters the bar code text wherever the cursor is active.
- Transmits a carriage-return character (the equivalent of pressing the Enter key).

For more information on the hand-held bar code reader, see the bar code reader user documentation shipped with the ViiA™ 7 System.

Electrical protective devices

We recommend several protective devices to protect the ViiA™ 7 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 ViiA™ 7 System.

Note: A power line regulator monitors the input current and adjusts the power supplied to the ViiA™ 7 System or computer. It does not protect against a power surge or failure.

Uninterruptible power supply (UPS)

We recommend the use of a 1.5-kVA uninterruptible power supply (UPS), especially in areas prone to power failure. Power failures and other events that abruptly terminate the function of the ViiA™ 7 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 ViiA™ 7 Instrument.

Note: A dedicated line and ground between the ViiA™ 7 System/computer and the building's main electrical service can also prevent problems caused by power fluctuations.

ViiA™ 7 Real-Time PCR System software

The ViiA™ 7 Real-Time PCR System System includes a suite of software applications that can be used to calibrate, run, automate, and integrate the ViiA™ 7 System into a laboratory workflow. The basic installation of the QuantStudio™ Real-Time PCR Software contains the components that are described below. Additional software may be available for the ViiA™ 7 System. Go to **www.thermofisher.com/viia7** for a complete list of compatible software.

Note: For QuantStudio™ Real-Time PCR Software, go to **thermofisher.com/qpcrsoftware**.

Computer requirements

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

1. Go to **thermofisher.com/qpcrsoftware**.
2. In the Real-Time PCR section, click **Applied Biosystems™ ViiA™ 7 Real-Time PCR System**.
3. Click **Read Me** to download the release notes.

Software installation

The default installation of the ViiA™ 7 System partitions the computer hard drive to create the logical drives shown below.

Drive	Software	Description
C drive	Microsoft™ Windows™ OS[1]	Operating system files.
C or D drive	QuantStudio™ Real-Time PCR Software	Used to calibrate and perform experiments on the ViiA™ 7 Instrument. Stores the installation files, application data files, and user files (experiments, exports, and imports).
	ViiA™ 7 System Command-line Utility	Used to automate the creation of new experiments and the export of existing experiments.

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

Third-party software

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

- Restrict Ethernet communication
- Interfere with QuantStudio™ Real-Time PCR Software operation (see below)

To confirm that third-party software does not interfere with the system:

1. Install the third-party software to the computer that contains the QuantStudio™ Real-Time PCR Software.

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

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

3. Confirm that the ViiA™ 7 System performs each test experiment without producing errors.

   If the ViiA™ 7 System performs the tests successfully, perform experiments normally. If the ViiA™ 7 System encounters errors during the test runs, the third-party software may not be compatible with the QuantStudio™ Real-Time PCR Software.

Using this guide

You can use this guide to calibrate, service, network, and administrate the ViiA™ 7 Real-Time PCR System.

This user guide contains the following information:

- Chapter 2, “Calibration and maintenance”—Describes how to perform regular maintenance of the ViiA™ 7 System, including calibrating the ViiA™ 7 Instrument and verifying instrument performance.
- Chapter 3, “Networking”—Describes how to install the ViiA™ 7 System to a local area network for remote monitoring and control.
- Chapter 4, “Security, audit, and electronic signature”—Describes how to configure the security, audit, and e-signature functions of the QuantStudio™ Real-Time PCR Software.
- Chapter 5, “Service”—Describes how to replace the user-serviceable parts of the ViiA™ 7 Instrument and resolve infrequent problems that can occur during normal use.
- Appendix A, “Manual instrument operation”—Describes how to operate the ViiA™ 7 Instrument manually using the touchscreen interface.
- Appendix B, “Power on or off, store, and move the ViiA™ 7 system” – Describes how to store, move, and reinstall the components of the system.
- Appendix C, “Creating custom calibration plates and array cards”—Describes how to create a background plate in the event that one is unavailable, and how to create a dye plate that can be used to calibrate the system for a dye not manufactured by Thermo Fisher Scientific.

- Appendix D, “Parts and materials”—Describes how to order parts, accessories, and consumables for the ViiA™ 7 System.
- Appendix E, “Software reference”—Describes how to use the QuantStudio™ Real-Time PCR Software command line application, and provides specifications for files that the software imports, exports, and stores.

2 Calibration and maintenance

Access the Help system by pressing **F1,** by clicking in the toolbar of the QuantStudio™ Real-Time PCR Software window, or by selecting **Help ▸ Contents and Index**.

Note: After the system software is upgraded, the instrument must be calibrated before starting a run with a 96-well plate block or a 384-well plate block.
After the system software is upgraded, the instrument does not need to be calibrated before starting a run with a TaqMan™ Array Card block.

Calibrate and maintain the ViiA™ 7 Real-Time PCR System

The ViiA™ 7 Real-Time PCR System requires regular calibration and maintenance to ensure optimal instrument performance.

Recommended calibration and maintenance schedule

Note: After the software is upgraded to QuantStudio™ Real-Time PCR Software v1.6.1, the instrument must be calibrated before starting a run with a 96-well plate block or a 384-well plate block. The instrument does not need to be calibrated before starting a run with a TaqMan™ Array Card block.

IMPORTANT! Calibrate the ViiA™ 7 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 ViiA™ 7 System and, in extreme cases, influence experimental results.

IMPORTANT! Do not use organic solvents to clean the ViiA™ 7 System.

Frequency	User-performed maintenance task
Weekly	Check the computer disk space. If necessary, archive or back up your experiment files and instrument settings.
	Power off the computer that controls the ViiA™ 7 System, then after 30 seconds, power on the computer.
	Clean the surface of the ViiA™ 7 System with a lint-free cloth.
	Perform a ViiA™ 7 Instrument self test.
Monthly	Check the lamp status. If necessary, replace the lamp.

(continued)

Frequency	User-performed maintenance task
Monthly	Perform a background calibration.[1]
	Run disk cleanup and disk defragmentation.
Semi-annually (6 months)	Perform a regions of interest (ROI) calibration.
	Perform a background calibration.
	Perform a uniformity calibration.
	Perform a dye calibration.
	(*TaqMan™ Array Card block only*) Perform a normalization calibration.
	Perform an instrument verification run.
As needed	Decontaminate the ViiA™ 7 System.
	Replace the ViiA™ 7 System fuses.
	Update the Windows™ operating system.
	Update the QuantStudio™ Real-Time PCR 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.

Calibration with QuantStudio™ Real-Time PCR Software v1.6.1

QuantStudio™ Real-Time PCR Software v1.6.1 does not require a normalization calibration for the 96-well plate blocks or the 384-well plate block. For this version of the software, normalization calibration is only required for the TaqMan™ Array Card block.

After the software is upgraded, the instrument must be calibrated before starting a run with a 96-well plate block or a 384-well plate block.

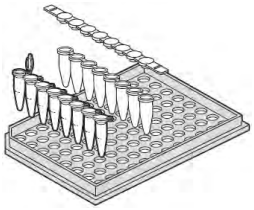
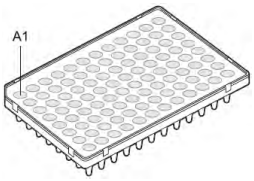
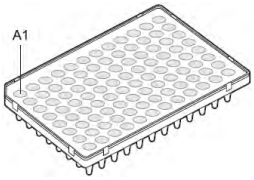
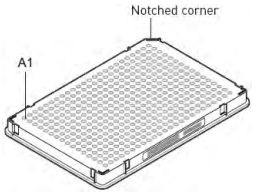
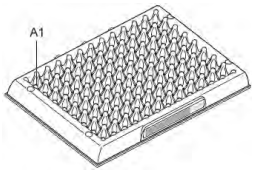
After the software is upgraded, the instrument does not need to be calibrated before starting a run with a TaqMan™ Array Card block.

The feature to override a calibration can only be used for a data file with compatible calibration data.

- A data file generated with a previous version of the software cannot have the calibration overridden after an upgrade to QuantStudio™ Real-Time PCR Software v1.6.1.
- A data file generated with QuantStudio™ Real-Time PCR Software v1.6.1 cannot have the calibration overridden if it is opened using a previous version of the software.

About the consumables

The ViiA™ 7 System uses a series of specialized consumables for calibration. The calibration plates and array cards can be ordered from **thermofisher.com**. Use the consumables appropriate for the sample block of your ViiA™ 7 System.

Sample block	Consumable		Reaction volume
96-well plate, 0.2 mL		• MicroAmp™ Optical 8-Cap Strip • MicroAmp™ 8-Tube Strips (0.2-mL) • MicroAmp™ Reaction Tubes without Caps (0.2-mL) • MicroAmp™ 96-Well Tray/Retainer Set	50 µL
		• MicroAmp™ Optical Adhesive Film • MicroAmp™ Optical 96-Well Reaction Plate with Bar Code	50 µL
96-well plate, 0.1 mL		• MicroAmp™ Optical Adhesive Film • MicroAmp™ Optical 96-Well Fast Reaction Plate with Bar Code	50 µL
384-well plate		• MicroAmp™ Optical Adhesive Film • MicroAmp™ Optical 384-Well Reaction Plate with Bar Code	20 µL
TaqMan™ Array Card		TaqMan™ Array Card	1 µL

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

- Store the calibration plates or array cards in a dark place until you are ready to use them. The fluorescent dyes in the wells of calibration consumables are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dyes.
- Do not allow the bottom of the tubes, plates, or array cards to become dirty. Fluids and other contaminants that adhere to the bottoms of the consumables can contaminate the sample block and cause an abnormally high background signal.
- Confirm that 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 ViiA™ 7 System 3 times for up to 6 months if it is stored in its packing sleeve.
- *(Plates only)* Handle the calibration plates with care to prevent contamination. Do not place the plates on a lab bench, to avoid contaminating them. Always put calibration plates back into their packaging sleeves.
- *(96-well plates only)* If you are using cap strips to seal your plates, firmly seal all wells before running the plate. Partially seated caps can leak during the experiment, causing evaporation.
- *(Tubes only)* Firmly seal all individual tubes and tube strips. Partially seated caps can leak during the experiment, causing evaporation.

Perform regular data maintenance

Maintain the computer hard drives

Defragment and clean up the hard drive:

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

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

IMPORTANT! Do not run the disk management utilities and QuantStudio™ Real-Time PCR Software at the same time.

Archive and back 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 an unforeseen 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 data files.

Note: Real-time runs generate significantly more data than genotyping or presence/absence experiments. During one day of real-time operation, the ViiA™ 7 System can generate more than 10 MB of data.

Check disk space

If you perform real-time experiments on your ViiA™ 7 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.

Back up the instrument settings

You can use the ViiA™ 7 Instrument touchscreen to back up the instrument settings (instrument name, icon, standby time-out, and cover idle temperature). In the event that the ViiA™ 7 Instrument settings are reset, you can restore the settings from the backup.

See “Back up and restore the instrument settings” on page 133 for more information.

Fill the array cards

IMPORTANT! Perform the following procedure only if you are calibrating a ViiA™ 7 System with an array card sample block. Otherwise, go to "Perform the ROI calibration" on page 34 to begin the calibrations.

Materials required

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

Fill the calibration array cards

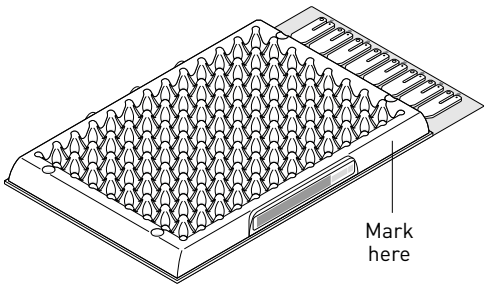
IMPORTANT! Wear powder-free gloves while creating the calibration array cards.

Note: This procedure explains how to create *all* of the array cards that are required to calibrate the ViiA™ 7 System, but not all of them are required for monthly maintenance. Before preparing array cards for calibration, see "Recommended calibration and maintenance schedule" on page 25 to determine which calibrations are required.

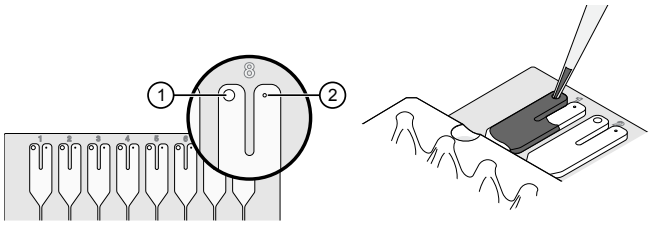
1. Remove the tubes of calibration solutions from –20°C, allow them to thaw, then vortex the tubes to mix the contents well.

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

3. Using a permanent marker, mark the side of the empty array cards with:
   - Background
   - FAM™
   - ROI
   - ROX™
   - VIC™
   - FAM™/ROX™
   - VIC™/ROX™



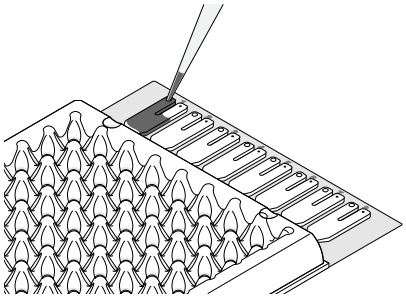
4. For each array card, pipette 100 µL of the appropriate calibration solution into each of the eight reservoirs in the array card:
   a. Place the array card on a lab bench, with the foil side down.
   b. Load 100 µL of the calibration solution into a pipette.
   c. Hold the pipette in an angled position (approximately 45°), then place the tip into the fill port.



① Fill port
② Vent port

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

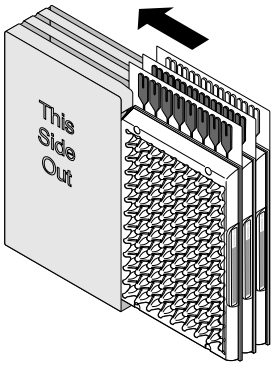
   When pipetting the reagents into the array card, pipette the entire 100-µL volume into the fill reservoir, but *do not* go past the first stop of pipettor plunger or you can blow the solution out of the port.



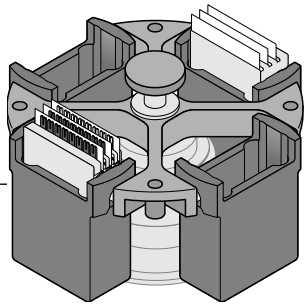
IMPORTANT! Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.

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

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



7. Place the filled carrier clips into the centrifuge buckets. 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 that are filled with array cards. Place empty array card into unfilled slots.

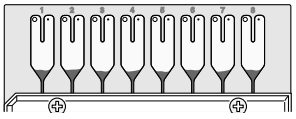
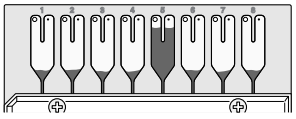
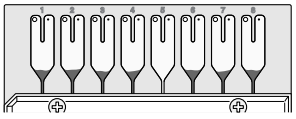
IMPORTANT! Balance the loads in opposite buckets in the centrifuge.

8. Close the centrifuge cover, then spin the array cards for 1 minute at 1,200 rpm.

9. When the run is finished, stop the centrifuge, then spin the array cards again for 1 minute at 1,200 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.

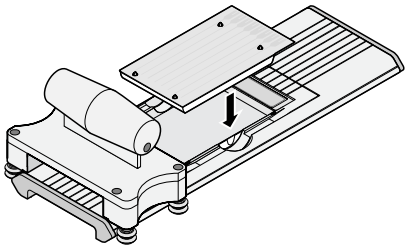
10. When the second run is finished, open the centrifuge, then check that the fluid levels in the reservoirs of each array card have decreased by the same amount. Also, check for the formation of bubbles in all wells, then note possible problems.

Correct fill	Incorrect/partial fill	
		

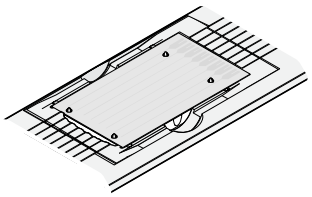
If needed, centrifuge the array cards for an extra minute to fill any unfilled wells. Do not exceed three 1-minute runs or centrifuge the array card for longer than 1 minute at a time.

11. Seal the array card(s):

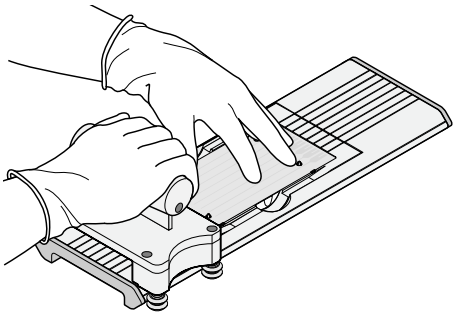
    a. With the carriage (roller assembly) of the Array Card Staker/Sealer in the Start position, place a filled array card into the fixture with the foil side up so that the fill reservoirs are the farthest away from the carriage.



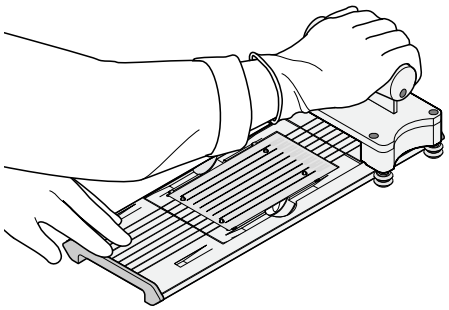
b. Press down on all four corners of the array card to ensure that it is fully seated within the fixture.



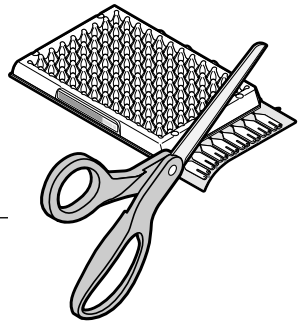
c. Use the two alignment pins in the fixture to position the array card correctly.



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



e. Remove the sealed array card from the fixture, then 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 can leak and contaminate the sample block and/or it can cause the associated calibration or RNase P experiment to fail.

Perform the ROI calibration

A regions of interest (ROI) calibration maps the positions of the wells on the sample block of the ViiA™ 7 Instrument. The software uses the ROI calibration data to associate increases in fluorescence during a run with specific wells on the plate. The ViiA™ 7 Instrument uses a set of optical filters to distinguish the fluorescence emissions gathered during runs. You must generate a calibration image for each individual filter to account for minor differences in the optical path.

Materials required

96-Well plate sample block

- 96-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.

384-Well plate sample block

- 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.

Array card sample block

- Array Card filled with ROI Calibration Mix
- Array Card Staker/Sealer
- Centrifuge with centrifuge array card carrier clips and buckets
- Powder-free gloves
- Safety goggles
- Pipettor, 200-µL (with pipette tips)

When to perform the calibration

Perform the ROI calibration:

- Every 6 months, or as often as necessary, depending on instrument use.
- After replacing the lamp.

IMPORTANT! After every ROI calibration, you must perform the following:

- Background calibration
- Uniformity calibration
- Dye calibration
- (*TaqMan™ Array Card block only*) Normalization calibration
- RNase P instrument verification

About the ROI calibration data

During the ROI calibration, the QuantStudio™ Real-Time PCR 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.

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.

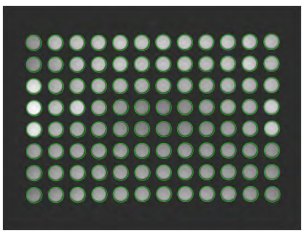


Figure 1 Passing Image

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.

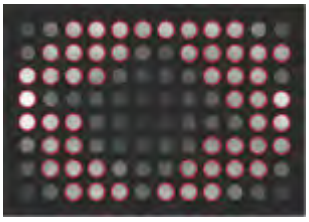


Figure 2 Failing Image

Prepare the calibration plate or array card

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

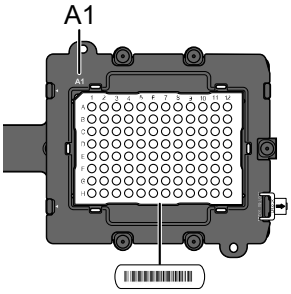
Prepare the ROI calibration consumable appropriate for your ViiA™ 7 Instrument:

- "Prepare the ROI calibration plate" on page 48
- "Fill the array cards" on page 30

Perform the calibration

1. In the Home screen of the software, click **Instrument Console.**
2. In the Instrument Console, select your ViiA™ 7 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.



   - Load 96/384-well plates with the A1 position at the top-left corner of the plate adapter.
   - Load both plates and array cards with the bar code 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 displays, select each filter from the Filter Set drop-down list, then verify that the corresponding ROI Image displays a green circle around each well area

8. After you inspect all ROI images, verify the status of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or that the data it did collect 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 calibration as described in “Troubleshoot the ROI calibration” on page 39.

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

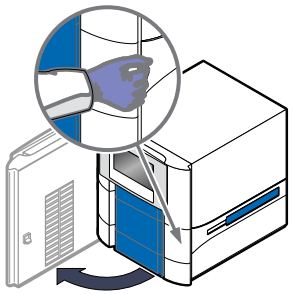
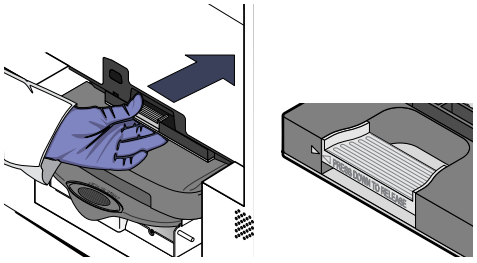
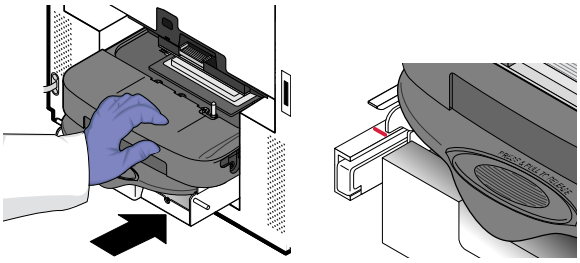
IMPORTANT! If the instrument does not eject the plate, remove the plate as explained in “Troubleshoot the ROI calibration” on page 39.

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: • During the next 8 hours, keep the ROI calibration plate at room temperature. (The ROI calibration plate is used in the uniformity calibration.) • Another day, 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 ViiA™ 7 System 3 times for up to 6 months after you open it.

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

Troubleshoot the ROI calibration

Problem/symptom	Possible cause	Action
ROI calibration failed ROI image is faint	The sample block or heated cover may not be seated correctly.	**1.** Power off and unplug the ViiA™ 7 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 ViiA™ 7 Instrument until it is seated correctly.  **4.** If the ROI calibration continues to fail, check the status of the halogen lamp within the ViiA™ 7 System, then replace the lamp if necessary (see “Replace the halogen lamp” on page 114).

(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 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 Technical Support.

Perform the background calibration

During a background calibration, the ViiA™ 7 System:

- Performs reads of a background plate containing PCR buffer for 10 minutes at 60°C.
- Averages the spectra that are 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 background fluorescence from the run data.

Materials required

96-Well plate sample block

- 96-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.

384-Well plate sample block

- 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.

Array card sample block

- Array card filled with Background Mix *or* deionized water
- Array card Staker/Sealer
- Centrifuge with centrifuge array card carrier clips and buckets
- Powder-free gloves
- Safety goggles
- Pipettor, 200-µL (with pipette tips)

When to perform the calibration

Perform the background calibration:

- Monthly or as often as necessary, depending on instrument use.
- After replacing the lamp.

About the background calibration data

During the background calibration, the QuantStudio™ Real-Time PCR 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™ Real-Time PCR Software on the calibration data.

Background fluorescence

Fluorescence data collected by the ViiA™ 7 Instrument includes a fluorescence signal inherent to the system, referred to as “background fluorescence.” Background fluorescence is a composite signal found in all spectral data that consists of fluorescence from several sources, including:

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

Prepare the background plate or array card

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

Prepare the background calibration consumable appropriate for your instrument:

- "Prepare the background calibration plate" on page 42
- "Fill the array cards" on page 30

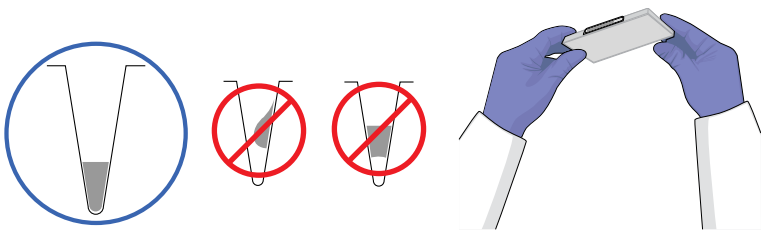
Prepare the background calibration plate

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

   IMPORTANT! Do not discard the packaging. You can use the background plate to calibrate a ViiA™ 7 System 3 times for up to 6 months if it is stored in its original packaging sleeve.
3. Vortex and centrifuge the background plate:
   a. Vortex the background plate for 5 seconds.
   b. Centrifuge the plate for 2 minutes at less than 1,500 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.



Perform the calibration

1. In the Home screen of the QuantStudio™ Real-Time PCR Software, click **Instrument Console**.

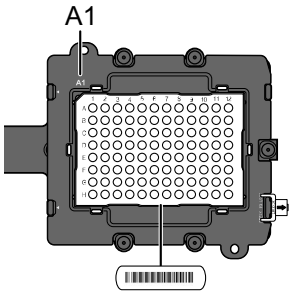
2. In the Instrument Console, select your ViiA™ 7 Instrument from the list of instruments on the network, then click **Add to My Instruments**.

   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.



   - Load 96/384-well plates with the A1 position at the top-left corner of the plate adapter.
   - Load both plates and array cards with the bar code 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 QuantStudio™ Real-Time PCR Software displays the Analysis screen, confirm the analysis status of the calibration, then select the **QC** tab and review the quality check summary.
   - **Analysis Status**—Indicates the success of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data, or that the data it did collect is unusable.

     Note: Abnormal spectra or abnormally high background fluorescence can indicate the presence of contamination on the plate, array card, or sample block, which can cause the calibration to fail.
   - **QC Status**—Indicates the quality of the calibration data, where *passed* indicates that all wells produced data that passed the quality check, and *failed* indicates that one or more wells produced spectra that deviate significantly from the other wells on the plate.

Analysis status	QC status	Action
Passed	Passed	Click **Next**, then remove the plate or array card when the instrument ejects the tray arm.
Passed	Failed	Troubleshoot the failed calibration as described in "Troubleshoot the background calibration" on page 45. **Note:** You can accept a calibration that passes the Analysis Status check, but fails the QC Status check. We recommend using calibrations that yield passing results for *both* status reports.
Failed	Failed	

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

IMPORTANT! If the instrument does not eject the plate, remove the plate as explained in "Troubleshoot the background calibration" on page 45.

8. Discard or store the plate or array card.

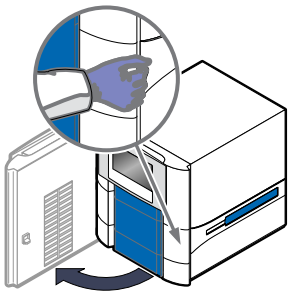
Consumable	Action
Array card	Discard the array card.
Plate	Return the background plate to its packaging sleeve, then return the packaged plate to the freezer. **IMPORTANT!** Do not discard the calibration plate. If the plate is stored in its original packaging sleeve, you can use it to calibrate a ViiA™ 7 System 3 times for up to 6 months after you open it.

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

Troubleshoot the background calibration

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 ViiA™ 7 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 “How to identify contamination” on page 46.

(continued)

Problem/symptom	Possible cause	Action
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 ViiA™ 7 Instrument. 2. Wait for 15 minutes, then power on the ViiA™ 7 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 Technical Support.

How to identify contamination

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

To determine the source and location of the contamination:

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

   If the position(s) of the contaminated well(s) in step 1 and step 2 are:

   - **Identical**—The sample block is contaminated. Decontaminate the sample block (see “Decontaminate the sample block” on page 111).
   - **Reversed**—The background plate or array card is contaminated. Discard the plate or array card, then perform the background calibration using a new background plate or array card.

4. If the calibration fails after you replace the background plate and decontaminate the sample block:
   a. Cover a plate or array card with a piece of black paper.
   b. Perform the background run as explained in this chapter, substituting the plate or array card covered with paper for the background plate or array card.
   c. After the run is complete and while viewing the calibration data, select all wells in the Plate Layout tab, then view the Spectral plot for the peak(s). If the peak associated with the contamination is:
      - **Visible**—The optics of your ViiA™ 7 System may be contaminated. Contact Technical Support.
      - **Absent**—The sample block is contaminated. Decontaminate the sample block again and repeat the calibration.

Perform the uniformity calibration

The uniformity calibration generates data that allows the QuantStudio™ Real-Time PCR Software to compensate for the physical effects of the ViiA™ 7 System filters.

Materials required

See “Perform the ROI calibration” on page 34 for a complete list of materials for the calibration.

When to perform the calibration

Perform a uniformity calibration every 6 months, or as often as necessary, depending on instrument use.

About the uniformity calibration data

During the uniformity calibration, the QuantStudio™ Real-Time PCR 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 (leftside) 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™ Real-Time PCR Software on the calibration data.

Prepare the calibration plate or array card

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

If you have an ROI plate or array card from a recent ROI calibration, go to substep 3b (plates), or go to "Perform the calibration" on page 49 (array cards). Otherwise, prepare the ROI calibration consumable appropriate for your ViiA™ 7 Instrument:

- "Prepare the ROI calibration plate" on page 48
- "Fill the array cards" on page 30

Prepare the ROI calibration plate

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

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

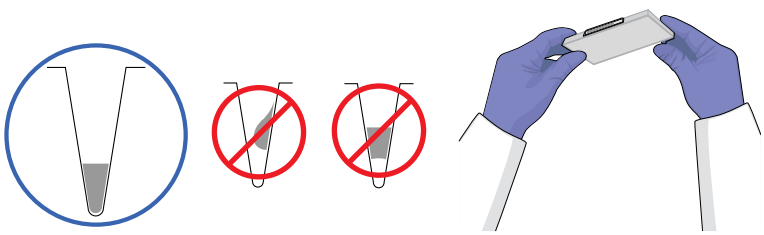
2. Remove the ROI calibration plate from its packaging. Do not remove the optical film.

   IMPORTANT! Do not discard the packaging for the ROI calibration plate. You can use the plate to calibrate a ViiA™ 7 System 3 times for up to 6 months if it is stored in its sleeve.

3. Vortex and centrifuge the plate:
   a. Vortex the ROI calibration plate for 5 seconds.
   b. Centrifuge the plate for 2 minutes at less than 1,500 rpm.

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

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



Perform the calibration

1. In the Home screen of the QuantStudio™ Real-Time PCR Software, click **Instrument Console.**

2. In the Instrument Console, select your ViiA™ 7 Instrument from the list of instruments on the network, then click **Add to My Instruments.**

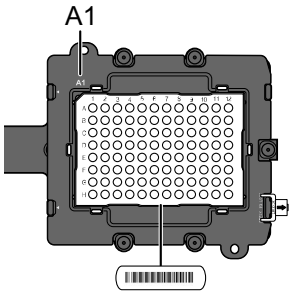
   Note: You must add an instrument to your list before you can manage it.

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.

   - Load 96/384-well plates with the A1 position at the top-left corner of the plate adapter.
   - Load both plates and array cards with the bar code 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 ViiA™ 7 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™ Real-Time PCR Software displays the Analysis screen, confirm the analysis status of the calibration, then select the **QC** tab and review the quality check summary.
   - **Analysis Status**—Indicates the success of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or that the data it did collect is unusable.

     Note: A calibration can fail if wells produce spectra that deviate significantly from the other wells of the plate, or if all wells produce abnormally low spectra. Abnormal spectra can indicate the presence of fluorescent contamination on the plate or array card or sample block.
   - **QC Status**—Indicates the quality of the calibration data, where *passed* indicates that all wells produced data that passed the quality check, and *failed* indicates that one or more wells produced spectra that deviate significantly from the other wells on the plate.

Analysis status	QC status	Action
Passed	Passed	Click **Next**, then remove the plate or array card when the instrument ejects the tray arm.
Passed	Failed	Troubleshoot the failed calibration as described in "Troubleshoot the uniformity calibration" on page 51. **Note:** You can accept a calibration that passes the Analysis Status check, but fails the QC Status check. We recommend using calibrations that yield passing results for *both* status reports.
Failed	Failed	

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

IMPORTANT! If the instrument does not eject the plate, remove the plate as explained in "Troubleshoot the uniformity calibration" on page 51.

8. Discard or store the plate or array card.

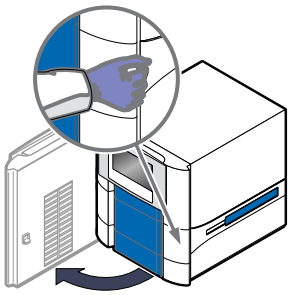
Consumable	Action
Array card	Discard the array card.
Plate	Return the ROI calibration plate to its packaging sleeve, then return the packaged plate to the freezer. **IMPORTANT!** Do not discard the calibration plate. If the plate is stored in its original packaging sleeve, you can use it to calibrate a ViiA™ 7 System 3 times for up to 6 months after you open it.

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

Troubleshoot the uniformity calibration

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 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 Technical Support.
	One or more wells produced spectra that deviate significantly from the rest of the plate or array card.	1. While viewing the calibration data in the Analysis screen, locate the well(s) with abnormal signal in the Plate Layout tab. 2. Rotate the calibration plate or array card 180°, then perform the calibration again. 3. Determine the location of the contaminated wells again. If the position(s) of the well(s) identified in step 1 and step 2 on page 51 are: • **Identical**—The sample block is contaminated. Decontaminate the sample block (see "Decontaminate the sample block" on page 111). • **Reversed**—The ROI plate or array card is contaminated. Discard the plate or array card, then perform the uniformity calibration using a new ROI plate or array card. 4. If the calibration fails again, contact Technical Support.

(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 ViiA™ 7 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 a local Thermo Fisher Scientific Field Service Office.

Perform the dye calibration

During a dye calibration, the ViiA™ 7 Real-Time PCR System:

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

The QuantStudio™ Real-Time PCR 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 ViiA™ 7 Instrument. After each run, the QuantStudio™ Real-Time PCR Software receives data in the form of a raw spectra signal for each reading. It determines the contribution of each fluorescent dye used in the sample by comparing the raw spectra to the pure spectra calibration data. When you save an experiment after analysis, the QuantStudio™ Real-Time PCR Software stores the pure spectra with the collected fluorescence data for that experiment.

IMPORTANT! You must calibrate only those dyes that are present in the chemistries that you intend to run on your ViiA™ 7 System.

Materials required

96-Well plate sample block

- Spectral calibration plates

Block	Plate
96-well 0.2-mL block	QuantStudio™ 3/5 Spectral Calibration Plate 1 (FAM™, VIC™, ROX™, and SYBR™ dyes), 96-Well 0.2-mL
	QuantStudio™ 3/5 Spectral Calibration Plate 2, 96-Well 0.2-mL (ABY™, JUN™, and MUSTANG PURPLE™ dyes)
	QuantStudio™ 3/5 Spectral Calibration Plate 3, 96-Well 0.2-mL (TAMRA™, NED™, and Cy®5 dyes)
96-well 0.1-mL block	QuantStudio™ 3/5 Spectral Calibration Plate 1, (FAM™, VIC™, ROX™, and SYBR™ dyes), 96-well, 0.1 mL
	QuantStudio™ 3/5 Spectral Calibration Plate 2 (ABY™, JUN™, MUSTANG PURPLE™ dyes), 96-well Fast (0.1-mL) Plate
	QuantStudio™ 3/5 Spectral Calibration Plate 3 (TAMRA™, NED™ and Cy®5 dyes) 96-well Fast (0.1-mL) Plate

- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

384-Well plate sample block

- Spectral calibration plates
  - QuantStudio™ 5 Spectral Calibration Plate 1, (FAM™, VIC™, ROX™ TAMRA™, and SYBR™ dyes), 384-well
  - QuantStudio™ 5 Spectral Calibration Plate 2 (ABY™, JUN™, MUSTANG PURPLE™, NED™, and Cy®5 dyes), 384-well
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Array card sample block

- Array Cards filled with:
  - FAM™ Dye
  - VIC™ Dye
  - ROX™ Dye
- Array Card Staker/Sealer
- Centrifuge with centrifuge array card carrier clips and buckets
- Powder-free gloves

- Safety goggles
- Pipettor, 200-µL (with pipette tips)

When to perform the dye calibrations

Perform a dye calibration every 6 months, or as often as necessary, depending on instrument use.

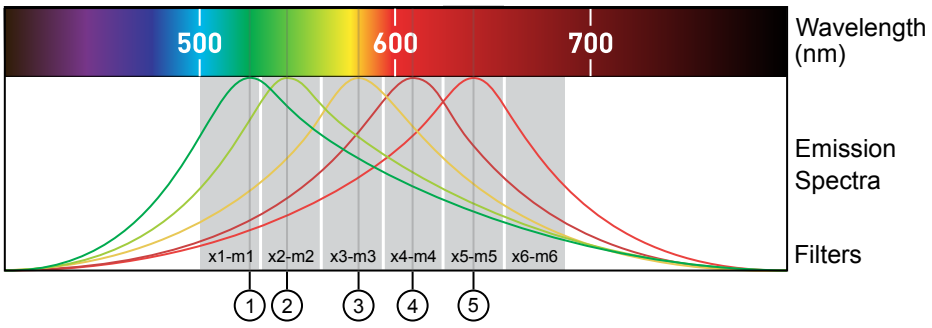
IMPORTANT! You must calibrate only dyes that are present in the chemistries that you intend to run on the ViiA™ 7 System. For example, if you intend to run a TaqMan™ RNase P plate or array card to verify instrument performance (see "Verify the instrument performance" on page 66), you must calibrate the FAM™ dye, TAMRA™ dye, and ROX™ dye because all three are present in the TaqMan™ assay chemistry present on the consumable.

IMPORTANT! You must 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 6 months.

About the dye calibration

System dyes

The ViiA™ 7 Real-Time PCR System features a six-color filter set that supports all Thermo Fisher Scientific dyes. The following figure shows the emission spectrum for each dye, and the filter at which each dye is read.



1. x1-m1 — FAM™, SYBR™ Green
2. x2-m2 — VIC™
3. x3-m3 — ABY™, NED™, TAMRA™
4. x4-m4 — JUN™, ROX™
5. x5-m5 — Cy®5, MUSTANG PURPLE™

Peak filter	Color	Filter wavelength (nm)[1]		Factory-calibrated dyes
		Excitation	Emission	
x1-m1	Blue	470 ± 15	520 ± 15	FAM™, SYBR™ Green
x2-m2	Green	520 ± 10	558 ± 12	VIC™
x3-m3	Yellow	550 ± 10	587 ± 10	NED™, TAMRA™, ABY™
x4-m4	Orange	580 ± 10	623 ± 14	ROX™, JUN™
x5-m5	Red	640 ± 10	682 ± 14	MUSTANG PURPLE™, Cy®5
x6-m6	Deep-Red	662 ± 10	711 ± 12	None [2]

[1] The central wavelengths are the optimized wavelengths.

[2] This filter set currently does not support any dyes supplied by Thermo Fisher Scientific.

Custom dyes

The ViiA™ 7 System can be used to run assays designed with custom dyes (dyes not supplied by Thermo Fisher Scientific). Before using custom dyes with the ViiA™ 7 System, you must create and run a custom calibration plate. The QuantStudio™ Real-Time PCR Software uses the custom calibration plate to create a spectral standard to distinguish the custom dye in the fluorescence data collected during the run. See “Create a custom dye plate for calibration” on page 150 for information on custom dye calibrations.

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

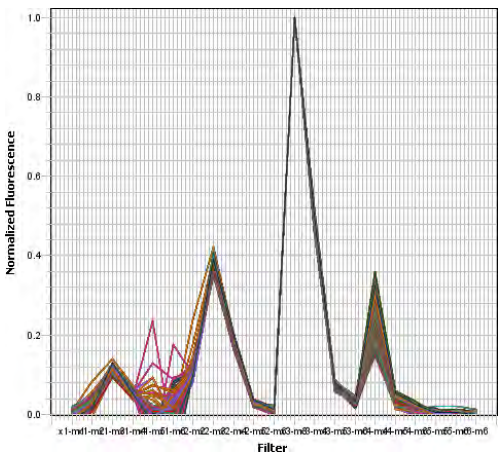
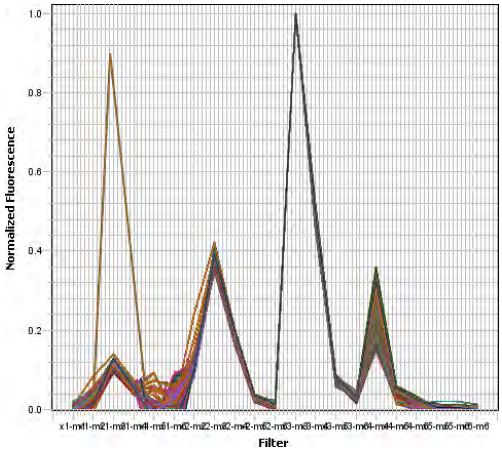
About dye calibration data

The product of a dye calibration is a collection of spectral profiles that represent the fluorescence signature of each dye standard. Each profile consists of a set of spectra that correspond to the fluorescence collected from the wells of the spectral calibration plate. The QuantStudio™ Real-Time PCR 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 that is generated by each well in terms of the collective spectra for the entire calibration plate. Dye spectra are generally acceptable if they peak within the same filter as their group but diverge slightly at other wavelengths (see below).

The 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.

Acceptable spectra	Unacceptable spectra
Spectra peak at the same wavelength and do not diverge significantly 	Spectra peak at the different wavelengths 

Prepare the calibration plates or array cards

Spectral calibration plates for the QuantStudio™ 3 and 5 Instruments are used for the calibration procedure with the QuantStudio™ Real-Time PCR Software v1.6.1.

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.

Prepare the dye calibration consumables appropriate for your instrument:

- Prepare the dye calibration plate (see page 56)
- Fill the array cards (see page 30)

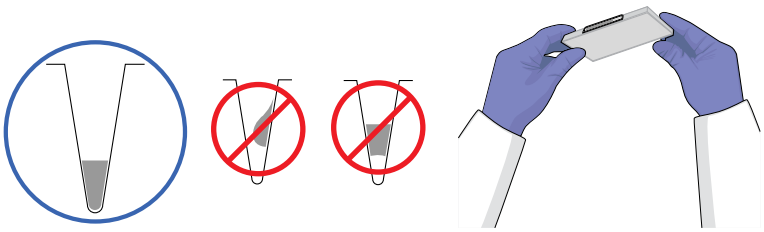
Prepare the dye calibration plate

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 in their original packaging and in the freezer, you can use them to calibrate a ViiA™ 7 System up to 3 times for 6 months after opening them.

2. Before using each dye plate, vortex the plate for 5 seconds, centrifuge it for 2 minutes at less than 1,500 rpm, then confirm that the liquid in each dye plate is at the bottom of the wells. If not, centrifuge the plate again at a higher rpm and for a longer period of time.



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

Perform the calibration

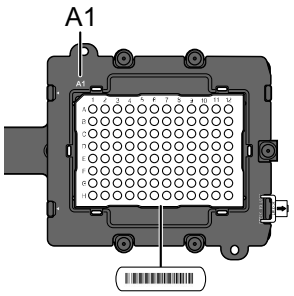
IMPORTANT! The QuantStudio™ Real-Time PCR Software guides you through the calibration of each dye separately. You must set up, run, and analyze each dye independently.

1. In the Home screen of the QuantStudio™ Real-Time PCR Software, click **Instrument Console.**
2. In the Instrument Console, select your ViiA™ 7 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 dropdown 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 QuantStudio™ Real-Time PCR Software. The name of the dye contained by the consumable is next to the bar code 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.
      - Load 96/384-well plates with the A1 position at the top-left corner of the plate adapter.
      - Load both plates and array cards with the bar code 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 QuantStudio™ Real-Time PCR Software displays the Analysis screen, confirm the grouping of the dye spectra:
   a. Select the **Plate Layout** tab, then review the raw data. For each spectrum, verify that the peak is:
      - Within the detectable range for the ViiA™ 7 System.
      - Free of irregular spectral peaks.
      - Present at the correct filter for the dye (see the following table).

Peak filter	Color	Filter wavelength (nm)[1]		Factory-calibrated dyes
		Excitation	Emission	
x1-m1	Blue	470 ± 15	520 ± 15	FAM™, SYBR™ Green
x2-m2	Green	520 ± 10	558 ± 12	VIC™
x3-m3	Yellow	550 ± 10	587 ± 10	NED™, TAMRA™, ABY™
x4-m4	Orange	580 ± 10	623 ± 14	ROX™, JUN™
x5-m5	Red	640 ± 10	682 ± 14	MUSTANG PURPLE™, Cy®5
x6-m6	Deep-Red	662 ± 10	711 ± 12	None [2]

[1] The central wavelengths are the optimized wavelengths.
[2] This filter set currently does not support any dyes supplied by Thermo Fisher Scientific.

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

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

9. After you inspect the dye spectra, verify the status of the calibration:
   - **Analysis Status**—Indicates the success of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or that the data it did collect is unusable.
   - **QC Status**—Indicates the quality of the calibration data, where *passed* indicates that all wells produced data that passed the quality check, and *failed* indicates that one or more wells produced dye spectra that differ significantly from the other wells on the plate.

Analysis status	QC status	Action
Passed	Passed	1. Click **Next.** 2. Enter any comments you have in the Comments field, click **Finish,** then click **Yes** when prompted to save the results. 3. Remove the plate or array card when the instrument ejects the tray arm.
Passed	Failed	Troubleshoot the failed calibration as described in "Troubleshoot the dye calibration" on page 61. **Note:** You can accept a calibration that passes the Analysis Status check but fails the QC Status check. We recommend using calibrations that yield passing results for *both* status reports.
Failed	Failed	

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

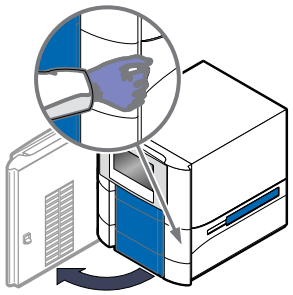
IMPORTANT! If the instrument does not eject the plate, remove the plate as explained in "Troubleshoot the dye calibration" on page 61.

10. Discard or store the plate or array card:

Consumable	Action
Array card	Discard the array card.
Plate	Return the dye 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 ViiA™ 7 System 3 times for up to 6 months after you open it.

11. Repeat the calibration and review (step 4 through step 10) to calibrate your ViiA™ 7 System for the remaining dyes that are present in the chemistries that you will be running.

Troubleshoot the dye calibration

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 ViiA™ 7 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 Technical Support. If you are running a custom dye calibration plate, create another plate but increase the concentration of the dye that produced insufficient signal.
	Dye calibration plate contains old or insufficient reagents.	
	If you are running a custom dye calibration plate, the dye may not be present at a sufficient concentration.	
Spectra contain peaks in more than one filters	Fluorescent contaminants are present on the sample block or dye calibration plate.	Verify that contaminants are not present by performing a background calibration (see "Perform the background calibration" on page 40) 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.
One or more raw spectra exceed the maximum limit for the ViiA™ 7 System	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 ViiA™ 7 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 a local Thermo Fisher Scientific Field Service Office.

Perform the normalization calibration

IMPORTANT! Normalization calibration is only required for the TaqMan™ Array Card block. Normalization calibration is not required for the 96-well plate blocks or the 384-well plate block.

During the normalization calibration, the ViiA™ 7 System:

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

The normalization calibration generates factors that the QuantStudio™ Real-Time PCR Software uses when comparing data from multiple ViiA™ 7 Instruments within a study.

Materials required

- Array Cards filled with:
  - FAM™/ROX™ dye mix
  - VIC™/ROX™ dye mix
- Array Card Staker/Sealer
- Centrifuge with centrifuge array card carrier clips and buckets
- Powder-free gloves
- Safety goggles
- Pipettor, 200-µL (with pipette tips)

When to perform the calibration

TaqMan™ Array Card block, perform a normalization calibration every 6 months, or as often as necessary, depending on instrument use.

About the normalization calibration data

During the normalization calibration, the QuantStudio™ Real-Time PCR 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 array card

IMPORTANT!

- Normalization calibration is only required for the TaqMan™ Array Card block. Normalization calibration is not required for the 96-well plate blocks or the 384-well plate block.
- Wear powder-free gloves and safety glasses when you prepare plates or array cards.
- Before performing a normalization calibration, you must perform ROI, background, uniformity, and dye calibrations.

Prepare the calibration array card (see "Fill the array cards" on page 30).

Perform the calibration

1. In the Home screen of the QuantStudio™ Real-Time PCR Software, click **Instrument Console.**
2. In the Instrument Console, select your ViiA™ 7 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 array card into the ViiA™ 7 Instrument:
   a. Verify that the normalization array card matches the selection in the QuantStudio™ Real-Time PCR Software. The name of the dyes contained by each consumable appears next to the bar code on the front of the array card.
   b. Load the appropriate normalization array card into the plate adapter with the bar code facing the front of the instrument.

      IMPORTANT! Arrays cards 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 array card, start the calibration:
   a. In the Dye Calibration screen, select **Check the box when the normalization calibration plate has been loaded,** then click **Next.**
   b. In the Run screen, click **START RUN** to start the calibration.

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

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

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

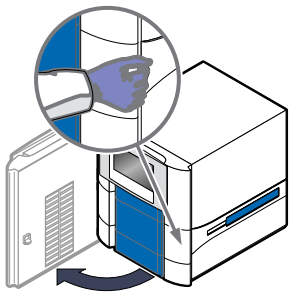
Analysis status	Action
Passed	Enter any comments you have in the Comments field, click **Next,** then remove the array card when the instrument ejects the tray arm.
Failed	Troubleshoot the failed calibration as described in “Troubleshoot the normalization calibration” on page 65.

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

IMPORTANT! If the instrument does not eject the array card, see “Troubleshoot the normalization calibration” on page 65.

9. Discard the array card.
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 through step 10 to perform the remaining normalization calibration.

Troubleshoot the normalization calibration

Problem/symptom	Possible cause	Action
Normalization calibration failed	Abnormally low spectra across all wells of the array card.	**1.** Confirm that you loaded an normalization array card into the ViiA™ 7 Instrument. If not, perform the calibration again using the correct normalization array card. **2.** If you are using the correct array card, perform the calibration again using a different normalization array card. **3.** If the calibration fails again, contact Technical Support.
	One or more wells produced spectra that deviate significantly from the rest of the array card.	**1.** While viewing the calibration data, locate the well(s) with abnormal signal in the Plate Layout tab. **2.** Rotate the calibration array card 180°, then perform the calibration again. **3.** Determine the location of the contaminated wells again. If the position(s) of the well(s) identified in both calibrations are: • **Identical**—The sample block is contaminated. Decontaminate the sample block (see "Decontaminate the sample block" on page 111). • **Reversed**—The normalization card is contaminated. Discard the array card, then perform the normalization calibration using a new normalization array card. **4.** If the calibration fails again, contact Technical Support.
Instrument does not eject the calibration array card	An adhesive cover may have adhered the array card to the heated cover within the instrument.	**1.** Power off the ViiA™ 7 Instrument. **2.** Wait for 15 minutes, then power on the instrument and eject the array card. **3.** If 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 array card from the heated cover, then close the access door. **5.** Perform a background calibration to confirm that the sample block has not been contaminated.
Instrument malfunction	Multiple possible causes	Contact a local Thermo Fisher Scientific Field Service Office.

Verify the instrument performance

Perform the RNase P instrument verification experiment to verify the performance of an Applied Biosystems™ ViiA™ 7 Real-Time PCR System.

Materials required

96-Well plate sample block

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

384-Well plate sample block

- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles
- TaqMan™ RNase P Fast 384-Well Instrument Verification Plate

Array card sample block

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

When to perform the RNase P instrument verification experiment

We recommend performing an RNase P instrument verification experiment:

- After moving the ViiA™ 7 Instrument to another location.
- As needed to verify the function of the ViiA™ 7 System.

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 ViiA™ 7 Instruments with 96/384-well sample blocks and an Array Card RNase P Kit for ViiA™ 7 Instruments with array card sample blocks.

About the TaqMan™ RNase P fast 96/384-well instrument verification plate

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

TheTaqMan™ RNase P fast 96-well instrument verification plate contains five replicate groups of standards (1250, 2500, 5000, 10000, and 20000 copies), two unknown populations (5000 and 10000 copies), and a no template control (NTC).

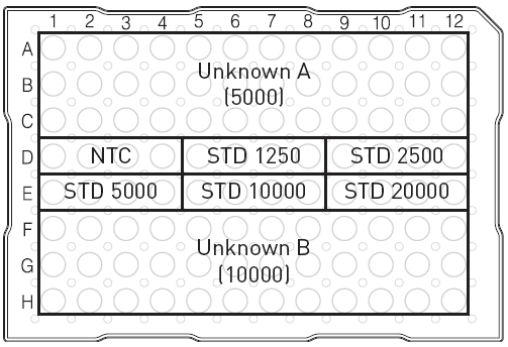


Figure 3 TaqMan™ RNase P fast 96-well instrument verification plate

The TaqMan™ RNase P fast 384-well instrument verification plate contains five replicate groups of standards (1250, 2500, 5000, 10000, and 20000 copies), two unknown populations (5000 and 10000 copies), and a no template control (NTC).

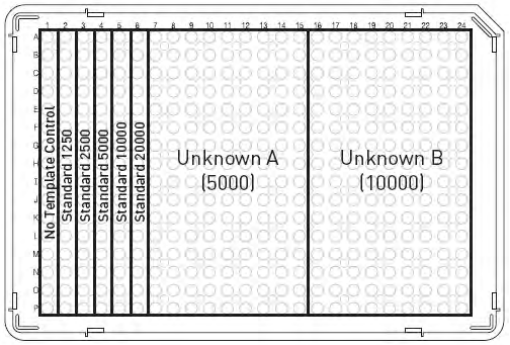


Figure 4 TaqMan™ RNase P fast 384-well instrument verification plate

About the array card RNase P kit

The Array Card RNase P Kit includes one empty array card and eight tubes of solution. Each tube contains reaction mix (TaqMan™ Universal PCR Master Mix, RNase P primers, and FAM™-MGB dye-labeled probe) and a known concentration of human genomic DNA template.

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

	1–24	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

About the analysis

The QuantStudio™ Real-Time PCR Software performs the same analysis of data from an instrument verification run regardless of the type of consumable used (96/384-well plate or array card).

After the run, the software:

1. Generates a standard curve from the averaged threshold cycle (C_t) values of the replicate groups of standards.
2. Calculates the concentration of the two unknown populations using the standard curve.
3. Calculates the following to assess the ViiA™ 7 System performance:

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

   where:

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

   Note: Unknown population A refers to the 5000-copy population in columns 7 through 15 of the TaqMan™ RNase P Plate or the 800-copy population in rows C and D of the loaded array card. Unknown population B refers to the 10000-copy population in the wells of the TaqMan™ RNase P Plate or the 1600-copy population in rows E and F of the loaded array card.

Installation specification

The ViiA™ 7 System passes the installation specification if the inequality holds and the ViiA™ 7 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)	No template controls (NTC)
96-well plate	6	6	1[3]	0
384-well plate	10	10	2[3]	0
Array card	4	4	4[3]	0

[1] 5000-copy population for 384-well plates; 800-copy population for array cards.
[2] 10000-copy population for 384-well plates; 1600-copy population for array cards.
[3] Maximum number of wells that can be removed from *each* standard population.

Prepare the TaqMan™ RNase P plate or array card

IMPORTANT! When performing the RNase P instrument verification experiment:

- Perform all calibrations beforehand.
- Run the TaqMan™ RNase P plate or array card soon after you allow the plate or reagents to thaw. Minimizing the time that the prepared consumable sits on the bench ensures optimal performance.
- Wear powder-free gloves and safety glasses when you prepare plates or array cards.

Prepare the instrument verification consumable appropriate for your instrument:

- "Prepare the TaqMan™ RNase P Instrument Verification Plate" on page 69
- "Prepare the array card for the instrument verification run" on page 70

Prepare the TaqMan™ RNase P Instrument Verification Plate

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

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

2. Remove the RNase P plate from its packaging.

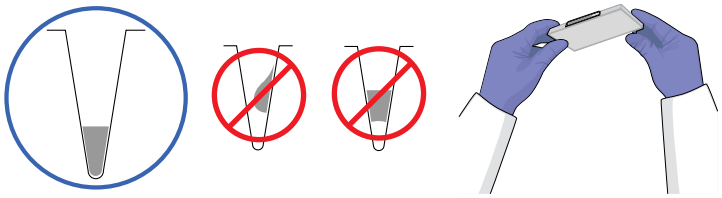
3. Briefly vortex and centrifuge the RNase P plate:
   a. Vortex the plate for 5 seconds.

b. Centrifuge the reaction plate for 2 minutes at less than 1,500 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.



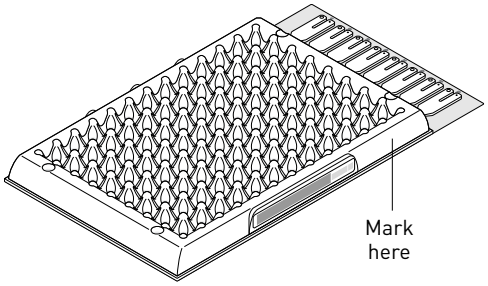
Prepare the array card for the instrument verification run

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

1. Remove the Array Card RNase P Kit from the freezer, then allow it to thaw at room temperature.

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

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



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

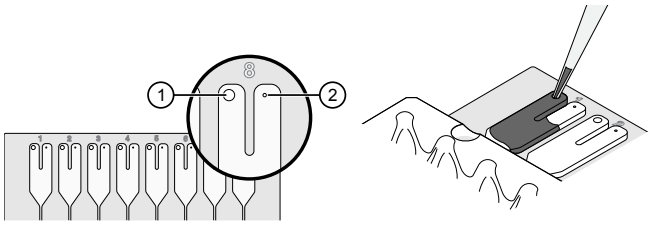
   For each transfer, do the following:

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

   b. Load 100 µL of fluid into a pipette.

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

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

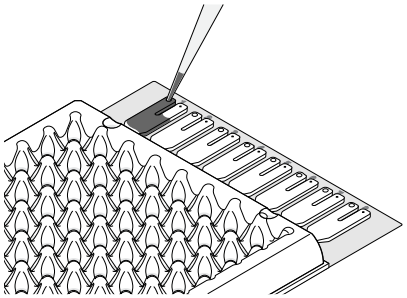


① Fill port
② Vent port

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, pipette 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. Centrifuge and seal the array card (see "Fill the calibration array cards" on page 30).

6. Run the prepared array card as soon as possible after filling it. Store the array card in a dark place until you are ready to run it.

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

Run the experiment

1. In the Home screen of the QuantStudio™ Real-Time PCR Software, click **Instrument Console.**

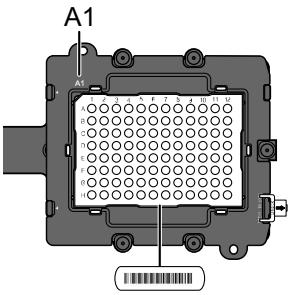
2. In the Instrument Console, select your ViiA™ 7 Instrument from the list of instruments on the network, then click **Add to My Instruments.**

   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.



   - Load 96/384-well plates with the A1 position at the top-left corner of the plate adapter.
   - Load both plates and array cards with the bar code 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 ViiA™ 7 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™ Real-Time PCR Software displays the Analysis screen, verify the status of the run:
   - **Passed**—The ViiA™ 7 System passed the RNase P run. Go to step 12.
   - **Failed**—The ViiA™ 7 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 on the reaction plate (controls, standards, and unknowns) 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 through C (5000-copy population).
      - **384-well plate**—Select columns 7 through 15 (5000-copy population).
      - **Array card**—Select rows C and D (800-copy population).

   b. In the plot, verify that the C_ts of the replicate population are equivalent.

      Note: The numbers on the X-axis of the plot correspond to the wells of the 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 population A[1]	Unknown population B[2]	Standard (STD)	No template controls (NTC)
96-well plate	6	6	1[3]	0
384-well plate	10	10	2[3]	0
Array card	4	4	4[3]	0

[1] 5000-copy population for 96/384-well plates; 800-copy population for array cards.
[2] 10000-copy population for 96/384-well plates; 1600-copy population for array cards.
[3] Maximum number of wells that can be removed from *each* standard population.

   d. Repeat substep 8a through 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 as explained in “Troubleshoot the RNase P experiment” on page 75.
      - AMPNC—Amplification in negative control
      - BADROX™—Bad passive reference signal
      - BLFAIL—Baseline algorithm failed
      - CTFAIL—C_t algorithm failed
      - EXPFAIL—Exponential algorithm failed

- HIGHSD—High standard deviation in replicate group
- NOAMP—No amplification
- NOISE—Noise higher than others 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 through step 10, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Technical Support.

11. Review the standard curve:
    a. Select the **Standard Curve** tab.
    b. Click the upper-left corner of the Plate Layout to select all wells.
    c. Verify that the R2 value is greater than or equal to **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 Technical Support.

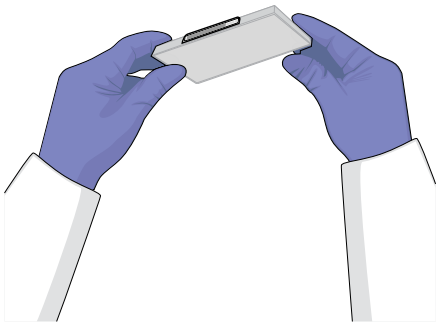
12. In the Analysis screen, click **Next,** remove the plate or array card when the ViiA™ 7 Instrument ejects the tray arm, then discard the plate or array card.

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

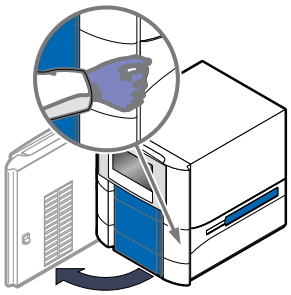
IMPORTANT! If the instrument does not eject the plate, remove the plate as explained in "Troubleshoot the RNase P experiment" on page 75.

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

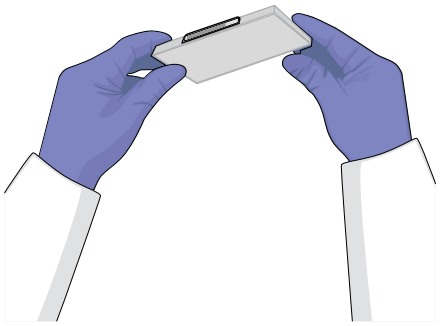
Troubleshoot the RNase P experiment

Problem/symptom	Possible cause	Action
More than the maximum number of outliers are present in RNase P data	Possible contamination	Order a replacement TaqMan™ RNase P plate or array card kit at **thermofisher.com**. If the replacement RNase P plate or array card fails, contact Technical 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, be sure to wait until the sample block reaches room temperature. **1.** Unload the RNase P plate or array card from the ViiA™ 7 Instrument. **2.**  Hold the plate or array card up to a light source, and verify that all wells contain the same volume of fluid. If there are differences in fluid volumes, check the heat seal of the wells with lower volumes for signs of damage or evaporation. Also, 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. **3.** Order a replacement TaqMan™ RNase P plate or array card kit at **thermofisher.com**. If the replacement RNase P plate or array card fails, contact Technical Support for further assistance.
	Defective plate seal	

(continued)

Problem/symptom	Possible cause	Action
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.
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	Order a replacement TaqMan™ RNase P plate or array card kit at **thermofisher.com**. If the replacement RNase P plate or array card fails, contact Technical Support for further assistance.
Well displays the BADROX™ flag, indicating 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. Order a replacement TaqMan™ RNase P plate or array card kit at **thermofisher.com**. Repeat the experiment, and make sure to properly seal and centrifuge the RNase P plate or array card. If the replacement RNase P plate or array card fails, contact Technical Support for further assistance.
Well displays the BLFAIL flag, indicating the software cannot calculate the best fit baseline for the data	• Amplification too late • No amplification	If a well is flagged, confirm the results: 1. Select the flagged well(s) in the plate layout or well table. 2. View the amplification plot (R_n vs. Cycle and DR_n vs. Cycle) and check for early, late, low, or no amplification. 3. Order a replacement TaqMan™ RNase P plate or array card kit at **thermofisher.com**. Repeat the experiment, and make sure to properly seal and centrifuge the RNase P plate or array card. If the replacement RNase P plate or array card fails, contact Technical Support for further assistance.

(continued)

Problem/symptom	Possible cause	Action
Well displays the CTFAIL flag, indicating the software cannot calculate the threshold cycle (C_t)	• Amplification too early • Amplification too late • Low amplification • No amplification	If a well is flagged, confirm the results: **1.** Select the flagged well(s) in the plate layout or well table. **2.** View the amplification plot (R_n vs. Cycle and DR_n vs. Cycle) and check for early, late, low, or no amplification. **3.** Order a replacement TaqMan™ RNase P plate or array card kit at **thermofisher.com**. Repeat the experiment, and make sure to properly seal and centrifuge the RNase P plate or array card. If the replacement RNase P plate or array card fails, contact Technical Support for further assistance.
Well displays the EXPFAIL flag, indicating the software cannot identify the exponential region of the amplification plot		
Well displays the HIGHSD flag, indicating 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.** Order a replacement TaqMan™ RNase P plate or array card kit at **thermofisher.com**. Repeat the experiment, and make sure to properly seal and centrifuge the RNase P plate or array card. If the replacement RNase P plate or array card fails, contact Technical Support for further assistance.
Well displays the NOAMP flag, indicating the sample did not amplify	• Missing template • Excitation source in the instrument stopped functioning	
Well displays the NOISE flag, indicating 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	

(continued)

Problem/symptom	Possible cause	Action
Well displays the NOSIGNAL flag, indicating 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.** Order a replacement TaqMan™ RNase P plate or array card kit at **thermofisher.com**. If the replacement RNase P plate or array card fails, contact Technical Support for further assistance.
Well displays the OFFSCALE flag, indicating 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.** Order a replacement TaqMan™ RNase P plate or array card kit at **thermofisher.com**. If the replacement RNase P plate or array card fails, contact Technical Support for further assistance.
Well displays the OUTLIERRG flag, indicating 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.** Order a replacement TaqMan™ RNase P plate or array card kit at **thermofisher.com**. Repeat the experiment, and make sure to properly seal and centrifuge the RNase P plate or array card. If the replacement RNase P plate or array card fails, contact Technical Support for further assistance.
Well displays the SPIKE flag, indicating 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 a replacement TaqMan™ RNase P plate or array card kit at **thermofisher.com**. Repeat the experiment, and make sure to properly seal and centrifuge the RNase P plate or array card. If the replacement RNase P plate or array card fails, contact Technical Support for further assistance.

(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 DRn vs. Cycle), and check for early, late, low, or no amplification. **3.** Order a replacement TaqMan™ RNase P plate or array card kit at **thermofisher.com**. If the replacement RNase P plate or array card fails, contact Technical Support for further assistance.
Instrument malfunction	Multiple possible causes	Contact a local Thermo Fisher Scientific Field Service Office.

3 Networking

Access the Help system by pressing **F1,** by clicking in the toolbar of the QuantStudio™ Real-Time PCR Software window, or by selecting **Help ▸ Contents and Index.**

IMPORTANT! This chapter *does not* provide adequate detail to integrate the ViiA™ 7 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 ViiA™ 7 System to your laboratory network.

Networking overview

After installing the ViiA™ 7 System, you can connect the ViiA™ 7 System to a local area network to enhance its functionality.

This chapter describes how to:

- Set up the ViiA™ 7 System for use on a network.
- Set up a computer for remote monitoring.
- Test the network connection by engaging the remote monitoring feature.

Controlling and monitoring networked ViiA™ 7 instruments

When the ViiA™ 7 Instrument is connected to a network, computers on the network that are running the QuantStudio™ Real-Time PCR Software can control or monitor it. The QuantStudio™ Real-Time PCR Software can control up to 4 instruments and monitor up to 15 instruments simultaneously. A networked ViiA™ 7 Instrument can be controlled by only one computer at a time. A networked computer running the QuantStudio™ Real-Time PCR Software can transfer experiments to and from an instrument, begin or stop a run, and perform some maintenance functions. During a run, the Remote Monitoring feature of the software can be used to view the run status, temperature, and amplification data in real-time. See “Monitor the ViiA™ 7 instrument” on page 86 for more information on remote monitoring.

Note: Remote monitoring does not allow you to control the ViiA™ 7 System.

About the ethernet 1 port

The ViiA™ 7 Instrument features a Gigabit Ethernet 1 port for direct communication with the ViiA™ 7 System computer and for network communication. When the ViiA™ 7 System is connected to a network, computers on the network that run the software can:

- Send and download experiments to/from the ViiA™ 7 System.
- Run experiments on the ViiA™ 7 System.
- Remote monitor the ViiA™ 7 System as it performs runs.

The Ethernet 1 port of the ViiA™ 7 Instrument supports:

- Static IP network service with subnet mask, primary and secondary data network service (DNS), and default gateway settings, or dynamic host configuration protocol (DHCP) network service
- mDNS/DNS for local domains

  Note: Because mDNS is limited to direct network connections, a ViiA™ 7 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])

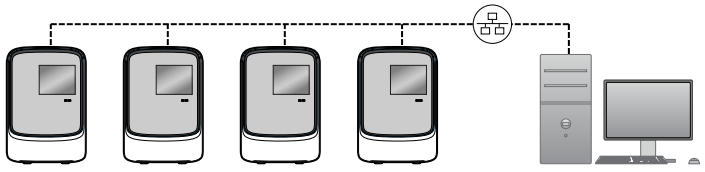
  Note: When the ViiA™ 7 System is set for DHCP, APIPA is automatically enabled, and the ViiA™ 7 System provides an IP address when no address is supplied by the DHCP server.

Example network layouts

Example 1

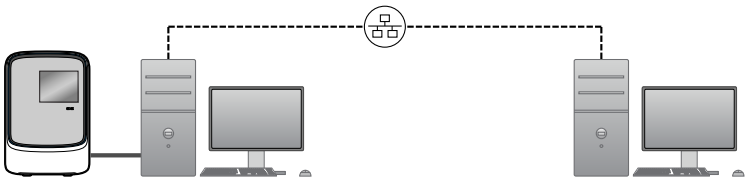
In the following example, a one or more ViiA™ 7 Instruments, which have been configured for dynamic host configuration protocol (DHCP) operation, are connected to a network by their Ethernet 1 ports. In this layout, any computer on the network can monitor or control the ViiA™ 7 Instrument. Experiments can be started remotely from the networked computer or locally from the ViiA™ 7 Instrument touchscreen.

Note: A networked computer running the software can simultaneously control up to 4 instruments and monitor up to 15 instruments that have been connected to the network.



Example 2

The ViiA™ 7 System computer can be connected to the network. In the configuration shown below, computers on the network can exchange experiment data with the ViiA™ 7 System computer; however, the ViiA™ 7 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 ViiA™ 7 System to your laboratory network.
  - To enable the full functionality of the software, the computer requires a network connection.
- Limit remote monitoring to 10 computers.

  Avoid using more than 10 computers to simultaneously monitor the ViiA™ 7 Instrument remotely. Although the ViiA™ 7 System supports remote monitoring from multiple computers, each connection taxes the instrument microprocessor. Too many connections can overburden the ViiA™ 7 System and result in instrument errors.

  Note: The effects of an overburdened ViiA™ 7 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 ViiA™ 7 System supports mDNS but only when the ViiA™ 7 Instrument and computer share a direct network connection and are within the same subnet. Consequently, network computers that are separated from the ViiA™ 7 System by a router, hub, or another network device may not be able to access the ViiA™ 7 Instrument by its host name.
- Confirm the uniqueness of the instrument name.

  The ViiA™ 7 Instrument does support name resolution but the instrument name must be unique within the subnet. The software can automatically discover ViiA™ 7 Instruments on the link-local network that are configured for Autodiscovery (see “Define the network settings” on page 139).

  Note: The ViiA™ 7 System does not test the uniqueness of the instrument name when it is set.
- Name ViiA™ 7 Instruments using lower-case letters.

  When you define the ViiA™ 7 Instrument settings (see “Define the instrument settings” on page 137), enter the instrument name using lower-case letters only.

Network setup workflow

1. Collect the required network information.
2. Connect the ViiA™ 7 Instrument to the network.
3. Connect the computer to the network.
4. Monitor the ViiA™ 7 Instrument (to test the network connection).

Collect the required network information

Obtain the following information from your network administrator:

- Network policy for obtaining IP addresses (DHCP or static IP).

  IMPORTANT! When the ViiA™ 7 System is set for DHCP, APIPA is automatically enabled and the ViiA™ 7 System will self assign 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 ViiA™ 7 Instrument.

Connect the ViiA™ 7 instrument to the network

After deciding how to connect the ViiA™ 7 System to a network, set up the ViiA™ 7 System according to your network policies.

Materials required

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

Define the ViiA™ 7 instrument internet protocol settings

1. Use the Ethernet cable to connect the Ethernet 1 port of the ViiA™ 7 Instrument to the nearest network port.

   IMPORTANT! Do not connect the Ethernet cable to the Ethernet 2 port on the ViiA™ 7 Instrument. The second port is for Applied Biosystems™ use only.

2. Power on the ViiA™ 7 Instrument.
3. Use the ViiA™ 7 Instrument touchscreen to configure the network settings as described in “Define the network settings” on page 139.

Connect the computer to the network

After connecting the ViiA™ 7 Instrument to the network, connect the computer to the network and install the QuantStudio™ Real-Time PCR Software for remote monitoring.

Materials required

Ethernet cable with RJ45 connectors

Computer requirement

If you are connecting a computer that you provided to a network, confirm that the computer contains a free network port.

Collect required information

Obtain the following information from your network administrator:

- Network policy for obtaining IP addresses (DHCP or static IP)
- If the network requires static IP addresses, obtain the IP address, subnet mask, and gateway address for the computer

Set up the computer

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

1. Use the Ethernet cable to connect the computer to the nearest network port.
2. Power on the computer, then log in using a user account that belongs to the Administrators user group.
3. In the computer desktop, right-click **My Network Places,** then select **Properties.**
4. Right-click **Local Area Connection**, then select **Properties**.
5. Select **Internet Protocol (TCP/IP)**, then click **Properties**.

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

Network configuration	Action
DHCP	1. Select **Obtain an IP address automatically.** 2. Set the DNS address. If the computer obtains DNS addresses: • Automatically – Select **Obtain DNS server address automatically.** • Statically – Select **Use the following DNS address,** enter the address of the preferred and alternate DNS servers if available.
Static IP	1. Select **Use the following IP address.** 2. In the IP Address field, enter the static IP address. 3. If necessary, enter a subnet mask. 4. If necessary, enter a static gateway address in the Default Gateway™ field.

7. If your network requires advanced TCP/IP setup (such as WINS), define the settings:
   a. Click **Advanced** in the Internet Protocol (TCP/IP) Properties dialog box.
   b. Define the IP Settings, DNS, and WINS tabs as instructed by your systems administrator, then click **OK.**
8. Close all dialog boxes by clicking **OK.**
9. Restart the computer.
   The computer is now visible to other computers on the network.

Install the QuantStudio™ Real-Time PCR Software

Note: If you are installing the 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, obtain one as explained in “Manage QuantStudio™ Real-Time PCR Software licenses” on page 120. If you are installing a

software update, the update automatically acquires the software license from the existing installation.

Note: You must install the QuantStudio™ Real-Time PCR Software to monitor the ViiA™ 7 System over a network.

1. Go to **thermofisher.com/qpcrsoftware**.
2. In the Real-Time PCR section, **Applied Biosystems™ ViiA™ 7 Real-Time PCR System**.
3. Click **Download**.
4. Install the QuantStudio™ Real-Time PCR Software according to the instructions that download with the software.
5. (*Optional*) Install protective software to the computer.

Monitor the ViiA™ 7 instrument

After connecting the ViiA™ 7 System and a computer to the network, you can enable remote monitoring in the QuantStudio™ Real-Time PCR Software to observe the instrument status remotely.

About remote monitoring

When the ViiA™ 7 System is connected to the network, any computer on the network that is running the QuantStudio™ Real-Time PCR Software can:

- “Monitor the status of ViiA™ 7 instrument during a run” on page 87
- “Upload or download an experiment or template to a ViiA™ 7 instrument” on page 87
- “Enable or change the calibration reminders” on page 88

Guidelines for remote monitoring

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

- The software can monitor up to 15 instruments.
- We do not recommend that a ViiA™ 7 Instrument be monitored by more than 10 computers simultaneously.
- Unless you are sure that your ViiA™ 7 Instrument and computer exist on the same subnet, we recommend that you use the IP address of the ViiA™ 7 Instrument to add it for remote monitoring.

Monitor the status of ViiA™ 7 instrument during a run

1. In the Home screen of the QuantStudio™ Real-Time PCR Software, click **Instrument Console.**
2. In the Instrument Console, select your ViiA™ 7 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™ Real-Time PCR Software displays the status, attributes, calibration status, and plot data for the selected ViiA™ 7 System. If a communications warning appears, contact your network administrator to troubleshoot the problem.

You can lose the software connection to the ViiA™ 7 Instrument if you:

- Change the ViiA™ 7 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™ Real-Time PCR Software.

Upload or download an experiment or template to a ViiA™ 7 instrument

Note: The ViiA™ 7 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 software, click **Instrument Console.**
2. In the Instrument Console, select your ViiA™ 7 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 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 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 that you want to download. To select multiple files, **Ctrl**-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:

- 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™ Real-Time PCR Software to alert you by email when the ViiA™ 7 Instrument requires calibration. The notifications settings feature is optional, and it does not affect performance.

IMPORTANT! The QuantStudio™ Real-Time PCR Software transmits email only while the ViiA™ 7 Instrument is monitored. If the network connection is interrupted, the software stops transmitting updates.

Collect the required information

The 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 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 software uses the default port number (25).
   - **Encryption Required?** – Select if the mail server has SSL enabled.
   - **Authentication Required?** – Select if the mail server requires a user name and password.
   - **User Name and Password fields** – If the mail server requires authentication, enter the user name provided by your systems administrator.
4. Click **OK.**

Modify the notification settings for a monitored ViiA™ 7 Instrument

1. Open the Calibration Reminders screen for the ViiA™ 7 Instrument:
   a. In the Home screen of the software, click **Instrument Console.**
   b. In the Instrument Console, select your ViiA™ 7 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,** then click **Calibration Reminders.**
2. In the Calibration Reminders Setting table, configure the notification settings for the calibrations in interest. For each calibration that you want to monitor:
   a. In the Expiry Interval column, enter the number of days that elapse before the type of calibration expires on the 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.

4 Security, audit, and electronic signature

Administrators

Administrators overview

IMPORTANT! The Security, Audit, and Electronic Signature (SAE) module is installed only with ViiA™ 7 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 ViiA™ 7 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™ Real-Time PCR Software that can allow you to configure the ViiA™ 7 System to meet specific requirements. The module provides the following functionality:

- **Security**—Controls user access to the software. A default Administrator user account is provided, and additional user accounts and permissions can be user-defined.

  Note: The default password for the Administrator user account is *Administrator;* however, the password can be changed during installation.

  Note: You can enable or disable system security globally.

- **Auditing**—Tracks changes made to library items, actions performed by users, and changes to the Security, Audit, and Electronic Signature settings. The software automatically audits some actions silently. You can select other items for auditing and specify the audit mode. The Auditing function provides reports for audited library items, Security, Audit, and Electronic Signature changes, and actions.

  Note: You can enable or disable auditing globally and by record type. It is disabled globally by default.

- **Electronic signature (e-sig)**—Determines if users are required to provide a user name and password when performing certain functions. You can configure e-sig so that a user can print a report or start a run only if the associated data are signed. You can also configure each e-sig event to require multiple signatures and to require users with specific permissions to sign.

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

Example applications

You can configure the SAE module in a variety of ways. For example, you can:

- Require users to log in, and leave audit disabled.
- Allow only certain users to create or modify protocols.
- Allow only certain users to approve reviewed samples.
- Require experiments to be signed before users can run or print them.

Configure the security system

Access the security screen and enable or disable security

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

IMPORTANT! If you disable security, you inactivate audit and electronic signature functions; however, no audit record is generated to indicate that audit and electronic signature functions are disabled.

Note: Security is enabled by default.

To enable or disable security:

1. In the software, select **Tools ▸ Security ▸ Settings.**
2. In the Security Settings dialog box, select the **System** tab.
3. Select or deselect **Enable Security.** Note the following:
   - Disabling Security inactivates Auditing and E-Signature.
   - The enable commands are grayed when a run is in process.
   - When security is disabled, the  is not active in lower parts of the screen.
   - The software requires you to enter your user name and password when you enable security.

IMPORTANT! If you enable or disable the software security, auditing, and electronic signature feature, you must similarly enable or disable the ViiA™ 7 Instrument security (see "Enable or disable instrument security" on page 141). The software cannot connect to ViiA™ 7 Instruments that do not match security settings.

4. Click **Apply Settings**.

Set account setup and security policies

Note: Security policies apply to all user accounts.

1. In the software, select **Tools ▸ Security ▸ Settings.**
2. In Account Setup, specify User Name limits.

   IMPORTANT! The software allows spaces in user names. Use spaces in user names with caution. For information, see "Spaces in user names and/or passwords" on page 93.

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 messaging notifications" on page 93.
7. Click **Apply Settings.**

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

Spaces in user names and/or passwords

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

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

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

Set up messaging notifications

1. In the 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 doftware 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

Create or edit a user account

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 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 (described in “Create or edit a user role” on page 95) 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 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 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™ Real-Time PCR Software, select **Tools ▸ Security ▸ Settings.**
2. In the Security Settings dialog box, select the **Roles** tab.
3. Click **Create.**
4. Enter a role name and (optional) description.

5. Select permissions (see "Permissions and default user roles" on page 96). 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 QuantStudio™ Real-Time PCR Software 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
	Print, create slides, and print report	Yes	Yes	Yes
	Export experimental results, audit reports and security settings	Yes	Yes	Yes
	Create, edit, and import sample attributes	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
	Edit a dye	Yes	Yes	Yes
	Delete a dye	Yes	No	Yes

(continued)

Permissions		Default user roles		
Category	**Function**	**Scientist**	**Technician**	**Administrator**
Study	Create or edit a study (for example, edit a plate or analysis settings)	Yes	Yes	Yes
	Add (transfer) experiments to a study	Yes	Yes	Yes
	Remove experiments from study	Yes	Yes	Yes
Preferences	Edit the system preferences	Yes	No	Yes
Calibrations	Perform calibrations	Yes	Yes	Yes
RNaseP	Perform an RNase P experiment	Yes	Yes	Yes
Instrument Configuration	Add or remove ViiA™ 7 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
Libraries	Manage Control Library (add, edit, delete, delete all, import, or export)	Yes	Yes	Yes
	Manage Sample Library (add, edit, delete, delete all, import, or export)	Yes	Yes	Yes
	Manage SNP Assay Library (add, edit, delete, delete all, import AIF, or export)	Yes	Yes	Yes
	Manage Target Library (add, edit, delete, delete all, import AIF, or export)	Yes	Yes	Yes
	Manage Analysis Settings Library (delete or delete all)	Yes	Yes	Yes

Edit a user role

1. In the Roles screen, select a user role, then click **Edit.**
2. Edit settings as needed. You cannot edit the Administrator user role.
3. Click **Save Role.**

View and print a user report

1. In the software, select **Tools ▸ Security ▸ Settings.**
2. In the Security Settings dialog box, select the **Users** or **Roles** tab.
3. Click **View Report.**
4. In the Report screen, click tool bar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.
5. Click (Print) to print the report, or click (Save) to save the report electronically (PDF). Close the report.

Manage auditing

Access the audit screen and enable or disable auditing

Use the Audit screen to control the auditing state (enabled/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™ Real-Time PCR 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
   - Study

2. Set the Audit Mode for each item you enable for auditing:
   - **Optional** – The event is audited, a reason prompt is displayed, but the user can cancel and continue without entering a reason.
   - **Required** – The event is audited, a reason prompt is displayed, and the user must specify a reason.
   - **Silent** – The event is audited, no reason prompt is displayed.
3. Click **Apply Settings.**

Create audit reason settings

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

1. To require users to select a pre-defined reason in the Audit Reason dialog box (displayed when a user performs an audited action), enable **Require users to select a reason to change from the list.** Users are not permitted to enter a reason.
2. As needed, click **Create,** or select a reason from the list, then click **Edit** or **Delete.**

Generate audit reports

You can use the 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" on page 99
- "Display audit histories for an experiment, template, or study" on page 103

Display audit histories from the security settings dialog box

Display audit histories

1. In the software, select **Tools ▸ Security ▸ Settings.**
2. In the Security Settings dialog box, select the **Audit** tab, then click **View Reports.**

   Note: To access the Audit Reports screen, the user role for an account must specify the Configure SAE permission. Users without the Configure SAE permission can view object audit histories for individual entries in the libraries by selecting entries, then clicking **View Audit History**.
3. Select a tab to display:
   - **System Configuration History**—Security, audit, and electronic signature configuration records, including audit history for each user account.
   - **Action Record**—System-specified audit events.

4. (*Optional*) Select **Filter by,** then filter the table:
   - Sort the table.
   - Specify filters (date range, user name, action, object or record type, object or record name, reason), then click **Refresh.**

     Note: The Reason field in System Configuration History is not used.
   - Select one or more records, then click **View Report.**

Review the system configuration history

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

Record type	Action	Corresponds to…
Security Settings	Update	Disable, enable, or modify security policies: session timeout settings.
Account Settings	Update	Modify password settings, security policies (password expiration and account suspension), or user name settings
User Group Manager	Update	Create, delete, or modify reason for change
User Role	Create	Create user role
	Delete	Delete user role
	Update	Modify user role
User Account	Create	Create new user account
	Update	Edit or suspend a user account
Role Assignment	Delete	Assign a different user role to an existing user account
	Update	Create a user account, or assign a different user role to an existing account
Audit Settings	Update	Enable or disable auditing
Audit Type	Update	Modify audit settings
Function Management Settings	Update	Update function management
Function Access Manager	Update	Update function access management
Function	Create	Create function
	Delete	Delete function
	Update	Update function

(continued)

Record type	Action	Corresponds to…
Role Permissions	Create	Create a user role[1]
	Delete	Delete a user role[1]
	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.

Review the action log

The Action Record log lists system-specified audit events.

All items in the action log are audited silently, except for the items noted as configurable. Configurable items may include comments in the action log.

- Audit Settings (Update)
- Auditing Event (Archive, Restore, Purge)
- Configuration (Import, Export)
- Data Audit (Archive, Restore, Purge)
- Login (Success, Failure)
- Logout (Success)
- Run (Start, Stop, Completed, Failed, Aborted, Error)
- User Account (Create, Update)

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

To selectively archive or purge (delete) system configuration or action audit records:

1. Select the **System Configuration History** tab.
2. Select records in the appropriate screen.
3. Click **Archive** or **Purge.**
4. If you select Archive, specify a location and name for the archive file (.asz).

Restore

To restore system configuration or action audit records, click **Restore,** then select the ASZ file to restore.

Export audit records

You can export audit records to a TXT file for additional manipulation and reporting outside the software.

1. Display the records of interest as described above.
2. Click **Export**.
3. Specify a name and location for the export TXT file, then click **Save**.

   Note: If you export audit records for samples that are not in their original location (samples have been deleted or moved), an error message is displayed. Return sample data files to their original location, then export again.

Display audit histories for an experiment, template, or study

Display the audit history

1. In the software, open an experiment (EDS), template (EDT), or study (EDM) file.
2. In the open experiment, template, or study, click **Audit,** then click **Audit Records.**
3. (*Studies only*) Select the audit records of interest:
   - **Study** to view the audit records for the study.
   - The name of an experiment to view the audit records for the experiment.
4. (*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:

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

Export audit records

1. In the software, open an experiment (EDS) or template (EDT) file.
2. In the open experiment, template, or study, 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. In the software, open an experiment (EDS) or template (EDT) file.
2. In the open experiment, template, or study, click **Audit**.
3. Click **View Report** to open the Print Preview dialog box.
4. Preview, save or print the report:
   - Click (Save) to save the report as a PDF or HTML file. Enter the file name, select a location, select the file type, then click **Save.**
   - Click (Print) to send the report to the printer. In the Print dialog box, select the printer and print options, then click **OK.**
5. Click to close the Print Preview dialog box.

Manage electronic signature

Access the e-Signature settings screen and enable or disable e-sig

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 software, select **Tools** ▸ **Security** ▸ **Settings**.
2. In the Security Settings dialog box, select the **e-Signature** tab.
3. Select or deselect **Enable e-Signature**.

   IMPORTANT! Enabling the electronic signature feature can substantially increase the size of experiment (EDS), template (EDT), and study (EDM) files.
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 software is installed with the following default meanings.

E-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 e-signature rights for user roles

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

1. In the e-Signature tab of the Security Settings dialog box, select the check box next to the appropriate user roles in the User Role signature rights table.
2. Click **Apply Settings.**

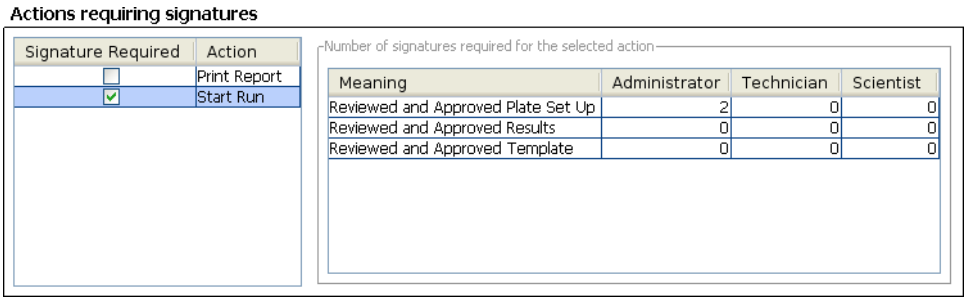
Select the actions that require signature

IMPORTANT! Do not change electronic signature settings during calibration.

1. In the Signature Required column, select the check box next to each action for which you want to require electronic signatures (see below). This selection causes the software to present an e-sig prompt if a user performs the action on a data file that does not have the required signatures. The data must be signed before the user can perform the action.

Action	The software requires e-signatures when a user…
Print Report	Prints a report from an experiment or study
Start Run	Initiates a run from the software or ViiA™ 7 Instrument

2. For each selected action, enter the number of e-signatures from each user role that are required for each meaning before the software can execute the action. For example, in the following figure, at least two users from the Administrator user role must sign an experiment using the “Reviewed and Approved Plate Set Up” meaning before a user can start the associated run.



3. Click **Apply Settings**.

How the software prompts electronic signature

If the system is configured to check that data is signed before starting a run or printing a report and the data is not signed, the 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 e-signature reports

You can use the software to generate reports of e-signature history from open experiment (EDS) or template (EDT) files.

Display the e-sig records

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

Save or print e-sig records

1. In the software, open an experiment (EDS) or template (EDT) file.
2. In the open experiment or template, click **Audit,** then click **E-Signatures.**
3. In the table, select the record to be saved or printed.

4. Save or print the record:
   - Click (Save), select a location for the export file, enter a name for the file, then click **Save.**

     or
   - Click (Print).
5. Click **OK** in the confirmation message.

Save or print the table of e-signature events

1. In the software, open an experiment (EDS) or template (EDT) file.
2. In the open experiment or template, click **Audit,** then click **Print E-Signatures**.
3. Save or print the record:
   - Click (Save), select a location for the export file, enter a name for the file, then click **Save**.

     or
   - Click (Print).
4. Click **OK** in the confirmation message.

Export and import user, security, audit, and e-signature settings

Note: The export/import feature can be used to replicate identical security, audit, and e-signature settings across multiple computers. The feature allows you to create a standard security, audit, and e-signature settings "image" for the software that can then be imported by other copies of the software to bypass manual setup.

Export

1. In any screen of the SAE module, 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**—Contains all system settings and all user roles and associated permissions.
3. Click **Export** or **OK**.
4. When prompted, specify the name and location for the exported file (DAT), then click **Save.** A message is displayed when the export completes.

Import

1. In any screen in the SAE module, click **Import** in the navigation pane.
2. Select the DAT file to import, then click **Open**. A message is displayed asking if you want to overwrite the current system configuration. Click **Yes**.

   If any imported user accounts already exist on the system, you are prompted to overwrite or skip each account.

Users

Users overview

The Security, Audit, and Electronic Signature (SAE) module is an optional component of the 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 software is configured for password expiration, you will periodically be prompted to change your password.

Note: If the 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.

Change your password when it expires

When your password is about to expire, a message is displayed when you log in.

To change your password, select **Tools ▸ Change Password.** Enter your current password, then enter the new password two times, then click **OK.**

Account suspension

If the software is configured to suspend a user account for failed logins, and you enter an incorrect user name and password for more than the allowed number of times, your user account is suspended, and the Log In dialog box indicates that your account is inactive.

There are two ways to activate a suspended account:

- You can wait until the suspension period ends.
- An administrator can change the account status from Suspended to Active.

Note: While a user is suspended, another user can click Reset, then log in and replace the suspended user.

Session time-out

If the 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 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 as described in “How the software prompts electronic signature” on page 107.

5 Service

IMPORTANT! This chapter contains all user service procedures for the Applied Biosystems™ ViiA™ 7 Real-Time PCR System. Procedures other than those described in this document must be performed by a qualified Applied Biosystems™ service engineer.

Decontaminate the sample block

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

CAUTION! PHYSICAL INJURY HAZARD. Do not remove the ViiA™ 7 Instrument cover. There are no components inside the instrument that you can safely service yourself. If you suspect a problem, contact a Technical Support.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

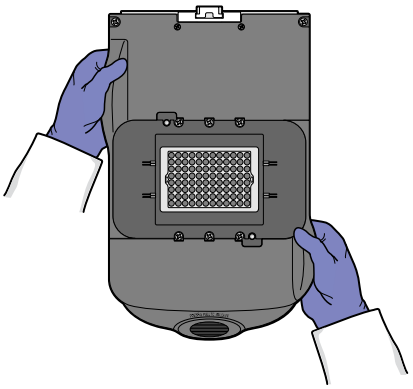
CAUTION! Before using a cleaning or decontamination method other than those recommended in this guide, verify with Thermo Fisher Scientific that the proposed method will not damage the equipment.

Materials required

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

How to handle the sample block

To prevent damaging or contaminating the sample block, handle the assembly as shown below. After the assembly has been removed from the ViiA™ 7 Instrument, place the sample block on a clean, dry surface or in its shipping container.

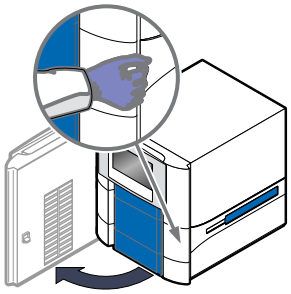


Clean the sample block

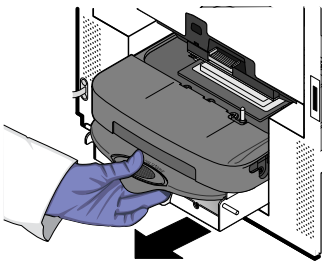
WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the sample block and heated cover can be heated to 100°C. Before removing the sample block, be sure to wait until it reaches room temperature.

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

1. Identify the contaminated wells of the sample block.
   For more information, see “How to identify contamination” on page 46
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.

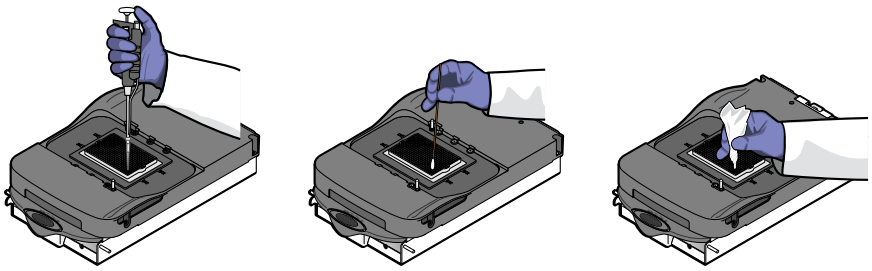


5. Clean the contaminated wells of the sample block using deionized water:
   a. Pipette a small volume of deionized water into each contaminated well.
   b. In each well, pipette the water up and down several times to rinse the well.

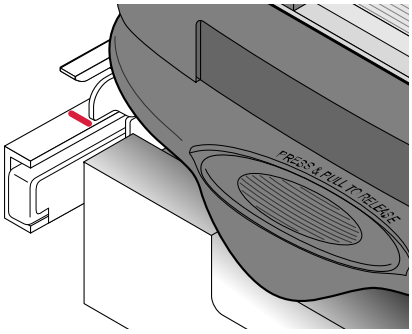
c. Pipette the water to a waste beaker.

d. Using a cotton swab, scrub inside of each contaminated well.

e. Using a lint-free cloth, absorb the excess deionized water.



6. Load the sample block into the ViiA™ 7 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.

7. Close the access door.

IMPORTANT! Confirm that the access door is completely closed. The 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 ViiA™ 7 System.

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

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

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

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

    c. Pipette the ethanol solution to a waste beaker.

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

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

12. If the contamination remains, repeat steps 2 through 5, then clean the contaminated wells of the sample block using 10% bleach solution:
    a. Pipette a small volume of 10% bleach solution into each contaminated well.
    b. In each contaminated well, pipette the solution up and down several times to rinse the well.
    c. Pipette the bleach solution to a waste beaker.

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

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

14. If the contamination remains, contact Technical Support.

Replace the halogen lamp

Replace the halogen lamp after approximately 2,000 hours of life.

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

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

WARNING! This ViiA™ 7 Instrument is designed for 12 V, 75 W halogen lamps only. Replace with halogen bulbs supplied by Thermo Fisher Scientific.

Materials required

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

Halogen lamp warnings

The QuantStudio™ Real-Time PCR Software can display the following warnings before or during a run:

- The lamp current is below the acceptable level at the start of the run.
  You cannot proceed with the run until you replace the halogen bulb as explained in “Replace the lamp” on page 115.
- The QuantStudio™ Real-Time PCR Software stopped the run because the lamp current decreased below the acceptable level during the run.
  You cannot proceed with the run until you replace the halogen bulb as explained in “Replace the lamp” on page 115. Click **OK** in the message box, then replace the lamp bulb.
- The lamp usage exceeds 2,000 hours at the start of a run.
  Click **Cancel Run,** then replace the lamp, or click **Continue Run**.

Check the lamp status

1. In the Home screen of the software, click **Instrument Console**.
2. In the Instrument Console, select your ViiA™ 7 Instrument from the list of instruments, then review the Lamp Life and Last Serviced readings in the Maintenance Info pane.
   If the lamp usage is greater than 2,000 hours, we recommend that you replace the lamp (see “Replace the lamp” on page 115).
   Note: The Lamp Life report displays the total number of hours that the halogen lamp has been illuminated. The Last Serviced report displays the date that the lamp was installed.

Replace the lamp

IMPORTANT! Wear powder-free gloves when you handle the lamp.

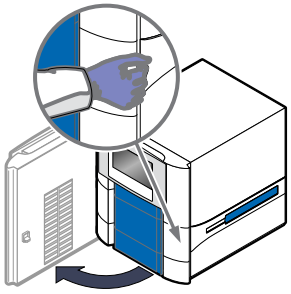
IMPORTANT! Do not touch the lamp without powder-free gloves. Finger prints shorten the lamp life.

1. (*Optional*) Record the lamp installation:
   a. Touch the ViiA™ 7 Instrument touchscreen to awaken it, then press .
   b. In the Main Menu, touch **Tools,** then touch **Record Lamp Installation.**
   c. Touch the **Name** field, enter any relevant information (such as the make, model, or serial number of the lamp), then touch **Done.**
   d. Touch the **Comments** field, then enter any additional information that you want to record (such as the make and model of the replacement lamp).
   e. When you are finished, touch **Record New Lamp,** then touch **OK.**

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

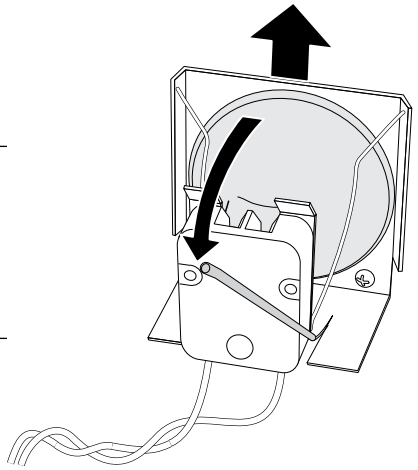
2. Power off and unplug the instrument, then allow it to cool for 15 minutes.

3. Open the access door.



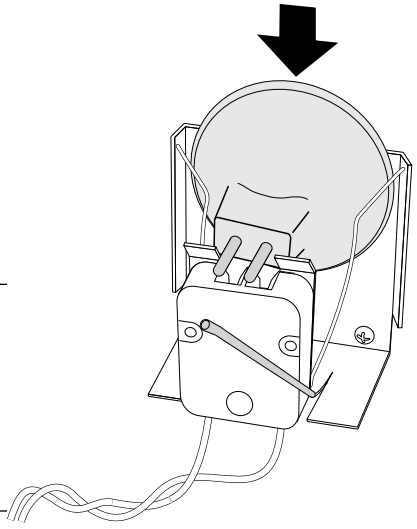
4. Remove the lamp from the instrument:
   a. Slide the lamp release lever downward.
   b. Firmly grasp the lamp and lift it up and out of the slotted mount.

5. Inspect the lamp for damage (carbon usually coats the inside of a failed lamp). If necessary, dispose of the lamp and obtain a replacement.



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

6. Install the new lamp into the instrument:
   a. Slide the lamp release lever upward.
   b. Firmly grasp the lamp, place it into the slotted mount, then carefully slide the lamp downward into place.



7. Close the access door.

8. Plug in and power on the instrument.

IMPORTANT! Confirm that the access door is completely closed. The software displays an error message if the door is not completely closed and latched, or if the lamp is not seated correctly.

9. Start the QuantStudio™ Real-Time PCR Software, then perform an ROI calibration.

   While the ViiA™ 7 Instrument is running, look through grating of the access door and verify that the lamp is illuminated.

   - If the lamp is illuminated, the lamp has been installed successfully.
   - If the lamp is not illuminated, the replacement halogen lamp may be defective. Replace the lamp again. If the second lamp does not illuminate, check the ViiA™ 7 Instrument fuses for failure (see "Replace the instrument fuses" on page 117).

10. After replacing the lamp, perform the following calibrations in the specified order:
    a. Background calibration
    b. Uniformity calibration
    c. Dye calibration
    d. (*TaqMan™ Array Card block only*) Normalization calibration

Replace the instrument fuses

Replace the ViiA™ 7 System fuses when the fuses fail.

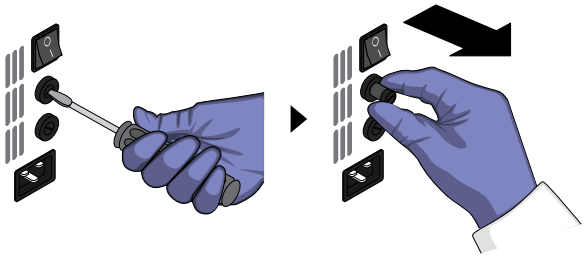
CAUTION! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with listed and certified fuses of the same type and rating as those currently in the ViiA™ 7 Instrument.

Materials required

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

Replace the fuses

1. Power off, then unplug the ViiA™ 7 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.

Update the Windows™ operating system

Do not upgrade or update the Microsoft™ Windows™ operating system of the computer running the QuantStudio™ Real-Time PCR Software without first consulting the software release notes or **thermofisher.com**. Future versions and updates to the Windows™ operating system can conflict with the QuantStudio™ Real-Time PCR Software.

To determine compatibility of an upgrade or update:

1. Go to **thermofisher.com/qpcrsoftware**.
2. In the Real-Time PCR section, click **Applied Biosystems™ ViiA™ 7 Real-Time PCR System**.
3. Click **Read Me** to download the release notes.

If the release notes do not contain the information of interest, contact Technical Support.

Update the system software

Thermo Fisher Scientific may release updates to the QuantStudio™ Real-Time PCR Software that you can install without the aid of Thermo Fisher Scientific service personnel. You can obtain updates directly from **thermofisher.com/qpcrsoftware**.

Prepare for the software upgrade

To prepare for the software update, back up all data files and experiments by creating a copy of the directory that you are using to store files. The default directories are:

- **Installation directory**—`<drive>:\Program Files (x86)\Applied Biosystems\QuantStudio Real-Time PCR Software\`
- **Data directory**—`<drive>:\Applied Biosystems\AppData\QuantStudio Real-Time PCR Software`
- **User files directory**—`<drive>:\Applied Biosystems\QuantStudio Real-Time PCR Software\User Files`

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

Install the QuantStudio™ Real-Time PCR Software

Note: If you are installing the 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, obtain one as explained in “Manage QuantStudio™ Real-Time PCR Software licenses” on page 120. If you are installing a

software update, the update automatically acquires the software license from the existing installation.

Note: You must install the QuantStudio™ Real-Time PCR Software to monitor the ViiA™ 7 System over a network.

1. Go to **thermofisher.com/qpcrsoftware**.
2. In the Real-Time PCR section, **Applied Biosystems™ ViiA™ 7 Real-Time PCR System**.
3. Click **Download**.
4. Install the QuantStudio™ Real-Time PCR Software according to the instructions that download with the software.
5. (*Optional*) Install protective software to the computer.

Update the ViiA™ 7 Instrument firmware

You can use the ViiA™ 7 Instrument touchscreen to update the ViiA™ 7 Instrument firmware. See “Update the firmware” on page 135 for more information.

Manage QuantStudio™ Real-Time PCR Software licenses

You can use the License Central feature to monitor, activate, or install software licenses.

Note: For the QuantStudio™ Real-Time PCR Software, licenses are only required for the HRM Module and the SAE Module.

About software license keys and files

The QuantStudio™ Real-Time PCR Software HRM Module and the SAE Module 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 software on the target computer.

Note: The 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 supplied by Technical Support.

Manage licenses

Monitor current licenses

Use the software to review the status and expiration date of the current licenses.

1. In the main menu of the 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 software modules on different rows because the licenses are maintained separately.
3. (*Optional*) If necessary, save the license information to a text file:
   a. Select the license that you want to export from the table, then click **Save License Request Info**.
   b. Navigate to the appropriate location, then click **Save**.
4. When you are done, click **OK**.

Activate or renew a license

If you have a valid licence key for a QuantStudio™ Real-Time PCR Software module, or if your license file has expired, you can use the License Central feature to activate the license as explained below.

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

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1. In the main menu of the QuantStudio™ Real-Time PCR 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. In the Thermo Fisher Scientific Software License Activation website, select the license 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) to install on the 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 software module.

Note: Each license file is generated specifically for the computer that was used to activate the license key.

1. Save the license (LIC) file to the computer that contains the QuantStudio™ Real-Time PCR Software.
2. In the main menu of the QuantStudio™ Real-Time PCR 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.

Replace the sample block

Replace the sample block in the event of a hardware failure or to change the consumable format of the ViiA™ 7 Instrument.

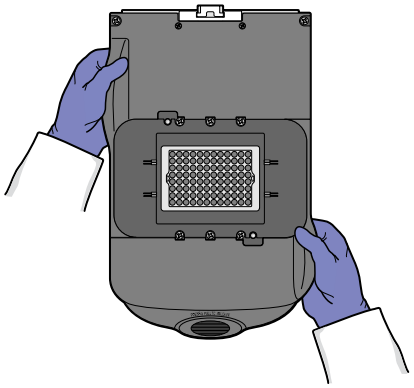
WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the sample block and heated cover can be heated to 100°C. Before removing the sample block, be sure to wait until it reaches room temperature.

Materials required

- Safety glasses
- Powder-free gloves
- Sample block

How to handle the sample block

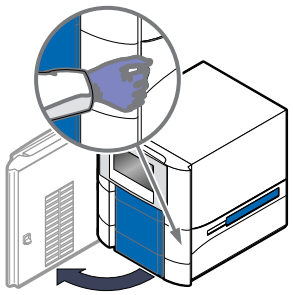
To prevent damaging or contaminating the sample block, handle the assembly as shown below. After the assembly has been removed from the ViiA™ 7 Instrument, place the sample block on a clean, dry surface or in its shipping container.



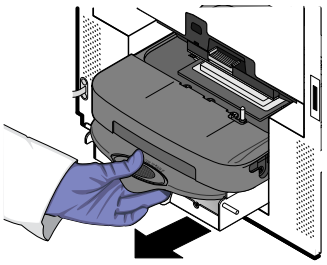
Replace the sample block

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

1. Power off and unplug the ViiA™ 7 Instrument, then allow it to cool for 15 minutes.
2. Open the access door.

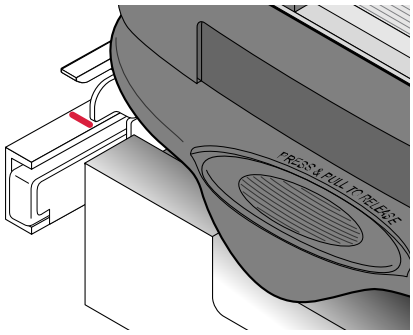


3. Firmly press down on the handle of the sample block, then remove it from the instrument. Place the sample block on a clean, dry surface.



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.



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 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 ViiA™ 7 System.

8. In the Home screen of the QuantStudio™ Real-Time PCR Software, click **Instrument Console**.

9. In the Instrument Console, select your ViiA™ 7 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 to the instrument.

10. Perform the following calibrations in the specified order:
    1. ROI calibration
    2. Background calibration
    3. Uniformity calibration
    4. Dye calibration
    5. (*TaqMan™ Array Card block only*) Normalization calibration

Replace 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 ViiA™ 7 Instrument.

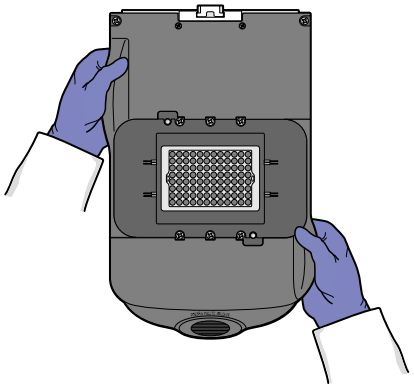
WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the sample block and heated cover can be heated to 100°C. Before removing the heated cover, be sure to wait until it reaches room temperature.

Materials required

- Safety glasses
- Powder-free gloves
- Heated cover

How to handle the heated cover

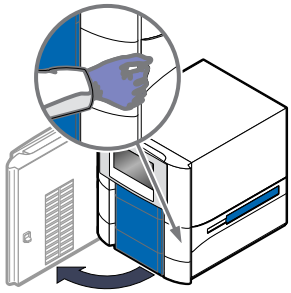
To prevent damaging or contaminating the heated cover, handle the assembly as shown below. After the assembly has been removed from the ViiA™ 7 Instrument, place the heated cover on a clean, dry surface or in its shipping container.



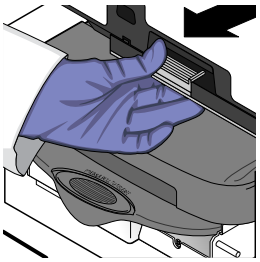
Replace the heated cover

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 ViiA™ 7 System, then allow it to cool for 15 minutes.
2. Open the access door.

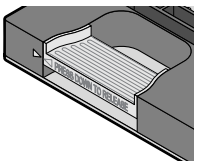
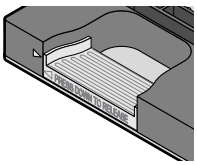


3. Unlock the heated cover by pinching the handle together, then pull the assembly from the ViiA™ 7 Instrument and place it on a clean, dry surface.



4. Install the new heated cover into the instrument.

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

Correct	Incorrect
	

5. If you are installing a heated cover of a different consumable format, replace the sample block and plate adapter if necessary.

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

6. Close the access door.

   Confirm that the access door is completely closed. The 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 ViiA™ 7 System.

8. In the Home screen of the QuantStudio™ Real-Time PCR Software, click **Instrument Console**.

9. In the Instrument Console, select your ViiA™ 7 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 ViiA™ 7 Instrument powers on and if the Heated Cover Firmware Version field displays a version number.

10. Perform the following calibrations in the specified order:

    1. ROI calibration
    2. Background calibration
    3. Uniformity calibration
    4. Dye calibration
    5. (*TaqMan™ Array Card block only*) Normalization calibration

Replace 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 ViiA™ 7 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.

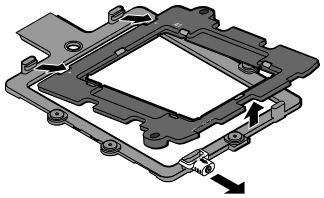
Materials required

- Safety glasses
- Powder-free gloves
- Plate adapter

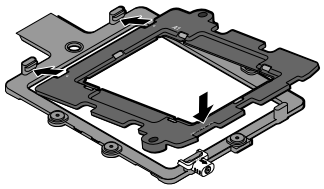
Replace the plate adapter

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 awaken 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.

A Manual instrument operation

Note: This appendix describes how to operate the ViiA™ 7 Instrument manually using the touchscreen interface. Although the ViiA™ 7 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.

Overview

The ViiA™ 7 Instrument features a touchscreen interface that you can use to run experiments, manage instrument settings, and configure the ViiA™ 7 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™ Real-Time PCR Software.

Functions available from the instrument touchscreen

The following table summarizes the functions that are available from the ViiA™ 7 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 ViiA™ 7 Instrument is operating in secure mode (see “Enable or disable instrument security” on page 141 for more information).

User role	Function	Available in secure mode?
Operational	Create experiments from templates	No
	Run experiments	
	Transfer experiments, templates, and results to/from a USB drive	
Maintenance	Back up and restore the instrument settings	Yes
	Perform an instrument self test	
	Update the ViiA™ 7 Instrument firmware	
Administration	Define the date and time	
	Define the instrument settings	

(continued)

User role	Function	Available in secure mode?
Administration	Define the network settings	Yes
	Define the maintenance reminders	
	Define the system shortcuts	
	Enable or disable instrument security	
	Review the instrument statistics	
	View the ViiA™ 7 Instrument log	

Operate the instrument from the touchscreen

The touchscreen provides limited control of the ViiA™ 7 Instrument to run experiments and transfer data. You can perform the following functions from the touchscreen to operate the ViiA™ 7 Instrument without using the software:

- "Create an experiment from a template" on page 129
- "Run an experiment" on page 130
- "Transfer experiments, templates, and results data" on page 131

Note: If the ViiA™ 7 Instrument is operating in secure mode (see "Enable or disable instrument security" on page 141), users can only open and close the side door.

Create an experiment from a template

1. If necessary, download the experiment template to the ViiA™ 7 Instrument as described in "Transfer experiments from a USB drive" on page 132.
2. If the instrument is in standby, touch the ViiA™ 7 Instrument touchscreen to awaken it, then press .
3. In the Main Menu, touch **View Templates.**
4. In the View Templates screen, create the experiment:
   a. Touch , then touch the folder that contains the desired template.
   b. Touch the desired template.
   c. Touch **Create Experiment.**

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

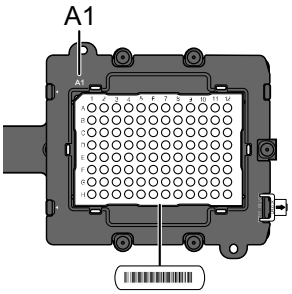
   Note: You cannot modify the experiment parameters of a template.

5. In the Create New Experiment screen, touch each field to set the:
   - Experiment name
   - Folder to receive the experiment
   - Reaction volume
   - Bar code
   - Any additional information to save to the experiment
6. When finished, either:
   - Touch **Save & Exit,** then touch to return to the Main Menu.

     or
   - Touch **Save & Start Run** to proceed to the Start Run screen.

Run an experiment

1. If the instrument is in standby, touch the ViiA™ 7 Instrument touchscreen to awaken it, then press .
2. In the Main Menu screen, then touch .
3. When the side door opens, load the appropriate plate or array card. Ensure that the consumable is properly aligned in the holder.
   - Load 96/384-well plates with the A1 position at the top-left corner of the plate adapter.
   - Load both plates and array cards with the bar code facing the front of the instrument.



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

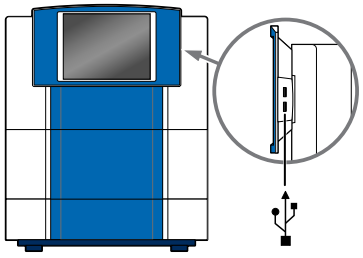
     or
   - **View/Edit** to view or edit the experiment before starting the run.

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

    Note: When the run is complete, touch to unload the plate. You can download the experiment results to a computer if the ViiA™ 7 Instrument is connected to a network, or you can copy the data to a USB device (see “Transfer experiments, templates, and results data” on page 131).

Transfer experiments, templates, and results data

You can transfer experiments, templates, and results data to/from the ViiA™ 7 Instrument using a USB flash drive. Before transferring data, you must plug the drive into one of the USB ports behind the right side of the ViiA™ 7 Instrument touchscreen.



IMPORTANT! Do not use the USB ports on the rear panel of the ViiA™ 7 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, touch the ViiA™ 7 Instrument touchscreen to awaken it, then press .
3. In the Main Menu, touch **View Templates**.
4. In the Browse Experiments screen, select the template:
   a. Touch , then touch **USB**.
   b. Touch the desired template, then touch .
5. In the Save Experiment As screen, set the name for the file.
   a. Touch the New Template Name field, then enter a name for the copied file.
   b. Touch the Save to Folder field, then select the folder to receive the file.
   c. Touch **Save**.
6. Touch to return to the Main Menu.
7. Unplug the USB drive.

Transfer experiments from a USB drive

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

Copy experiment results to a USB drive

1. Plug a USB drive into the USB port on the right side of the touchscreen.
2. If the instrument is in standby, touch the ViiA™ 7 Instrument touchscreen to awaken it, then press .
3. In the Main Menu, touch **Collect Results**.
4. In the Collect Results screen, touch the desired experiment, touch **Copy to USB**.
5. In the Copy Results To USB screen, touch **Copy to USB**.
6. Touch to return to the Main Menu.
7. 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 ViiA™ 7 Instrument touchscreen provides access to several maintenance functions that cannot be accessed remotely from the software. The following local instrument functions are performed as part of regular instrument maintenance:

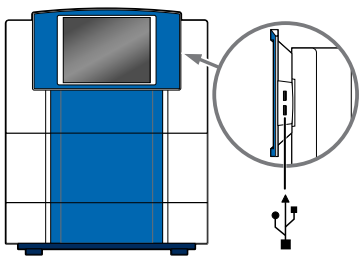
- “Restore the instrument settings” on page 134
- “Perform an instrument self test” on page 135
- “Update the firmware” on page 135

Note: The touchscreen does not provide access to all instrument functions. Features such as instrument calibration and remote notification are available only through the software.

Back up and restore the instrument settings

You can use the ViiA™ 7 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 ViiA™ 7 Instrument backs up to and restores instrument settings from a USB flash drive. Before backing up or restoring settings, you must plug the drive into one of the USB ports behind the right side of the 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 ViiA™ 7 Instruments. You can use the feature to create a standard "image" for a ViiA™ 7 Instrument that can then be restored on other instruments to bypass the manual setup process.

Back up the ViiA™ 7 instrument settings

1. Plug a USB drive into the USB port on the right side of the ViiA™ 7 Instrument touchscreen.
2. If the instrument is in standby, touch the ViiA™ 7 Instrument touchscreen to awaken it, then touch .
3. In the Main Menu, touch **Tools,** then touch **Back Up Settings.**
4. In the Backup Settings screen, touch **Backup.**
5. Touch to return to the Main Menu.
6. Unplug the USB drive.

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

Restore the instrument settings

1. Plug the USB drive that contains the instrument settings into the USB port on the right side of the ViiA™ 7 Instrument touchscreen.
2. If the instrument is in standby, touch the ViiA™ 7 Instrument touchscreen to awaken it, then press .
3. In the Main Menu, touch **Tools,** then touch **Restore Settings.**
4. In the Restore Settings screen, select the settings to restore:
   a. Touch the settings that you want to restore from the list.
   b. Touch **Restore** to upload the instrument settings from the USB drive.

   IMPORTANT! Do not remove the USB drive from the ViiA™ 7 Instrument until you are instructed to do so.

   Note: Alternatively, touch **Restore Default Settings** to restore the ViiA™ 7 Instrument to the factory settings.
5. After the ViiA™ 7 Instrument reboots, unplug the USB drive.

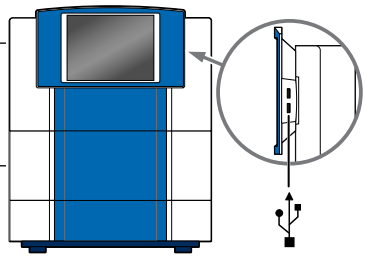
Perform an instrument self test

You can use the ViiA™ 7 Instrument touchscreen to perform a comprehensive self test of the ViiA™ 7 Instrument subsystems. After the self test is complete, the ViiA™ 7 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 Technical Support for a diagnosis.

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

1. If the instrument is in standby, touch the ViiA™ 7 System touchscreen to awaken it, then press .
2. In the Main Menu, touch **Tools,** then touch **Run Self Test**.
3. In the Self Test screen, touch **Start Self Test**, then wait for the test to complete.
4. (*Optional*) When the ViiA™ 7 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. Touch **Save to USB**.

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

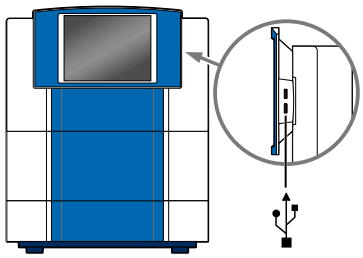
      

   c. When the instrument finishes writing the results to the USB drive, touch **OK,** then remove the USB drive.
5. Touch to return to the Main Menu.

Update the firmware

1. Download the firmware update:
   a. Go to **thermofisher.com/qpcrsoftware**.
   b. In the **Real-Time PCR** section, select ViiA™ 7 Real-Time PCR System.
   c. In the **Software Downloads** page, click **Updates - Patches**.
   d. Download the firmware to a USB drive.

2. Plug the drive into the USB port on the right side of the ViiA™ 7 Instrument touchscreen.



3. If the instrument is in standby, touch the ViiA™ 7 Instrument touchscreen to awaken it, then press .

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

5. In the Upgrade Firmware screen, select the update package, then touch **Upgrade Firmware**. Allow the instrument to complete the upgrade.

   IMPORTANT! Do not remove the USB drive from the ViiA™ 7 Instrument until you are instructed to do so.

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

Administrate the instrument from the touchscreen

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

- "Define the date and time" on page 137
- "Define the instrument settings" on page 137
- "Define the maintenance reminders" on page 138
- "Define the network settings" on page 139
- "Define the system shortcuts" on page 140
- "Review the instrument statistics" on page 141
- "Enable or disable instrument security" on page 141
- "View the instrument log" on page 142

Note: The touchscreen does not provide access to all instrument functions. Features such as instrument calibration and remote notification are available only through the software.

Define the date and time

1. If the instrument is in standby, touch the ViiA™ 7 System touchscreen to awaken it, then press .
2. In the Main Menu, touch **Settings,** then touch **Set Date & Time.**
3. In the Set Date & Time screen:
   a. Touch the **Time zone** field, then touch the correct time zone from the list.
   b. Touch the **Date** field, enter the current date, then touch **Done.**
   c. Touch the **Date Format** dropdown list, then select the format for your region.
   d. Touch each Time field, enter the appropriate time units, then touch **Done.**
   e. Touch **12 Hour** or **24 Hour** to select the appropriate time format.
   f. Touch **Save** to save the settings, then touch **OK** when prompted.
4. Touch to return to the Main Menu.

Define the instrument settings

1. If the instrument is in standby, touch the ViiA™ 7 System touchscreen to awaken it, then press .
2. In the Main Menu, touch **Settings,** then touch **Configure the Instrument**.
3. Touch the **Instrument Name** field, enter up to a 16-character name for the ViiA™ 7 Instrument, then touch **Done**.

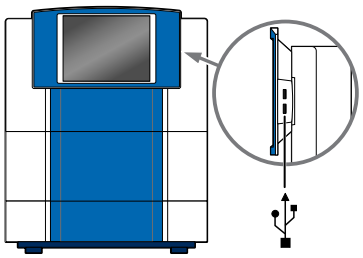
   The instrument name is the alphanumeric string used to identify the ViiA™ 7 Instrument on the network.

   IMPORTANT! To connect the ViiA™ 7 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 ViiA™ 7 Instrument in the 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. Touch **Upload Icon**, select the desired graphic file, then touch **Done**.

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

c. Unplug the USB drive.

5. Define the standby time-out setting:
   a. Select **Standby Time-out** to activate the feature.
   b. Touch the **Standby Time-out** field.
   c. Enter the number of minutes (1–300) that the instrument should remain idle until it enters standby mode, then touch **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. Touch the **Cover Idle Temperature** field.
   c. Enter the temperature (50–110°C) that the heated cover should maintain when the instrument is idle, then touch **Done**.
7. Touch **Save** to save the settings, then touch **OK** when prompted.
8. Touch  to return to the Main Menu.

Define the maintenance reminders

You can use the ViiA™ 7 Instrument touchscreen screen to:

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

Set the reminders

1. If the instrument is in standby, touch the ViiA™ 7 System touchscreen to awaken it, then press  .
2. In the Main Menu, touch  **Settings,** then touch **Set Maintenance Reminders.**
3. Configure the maintenance reminders. For each maintenance reminder:
   a. Touch the Calibration expires after field, enter the number of days or hours that should elapse until the association calibration expires, then touch **Done.**

b. Touch the check box to activate or deactivate reminders for the associated calibration.

c. Touch the Display reminders before field, enter the number of days before the associated calibration expires that the ViiA™ 7 Instrument should start displaying warnings of the impending expiration, then touch **Done.**

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

5. Touch  to return to the Main Menu.

Define the network settings

1. If the instrument is in standby, touch the ViiA™ 7 System touchscreen to awaken it, then press  .

2. In the Main Menu, touch  **Settings,** then touch **Set Network Information.**

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

3. Touch **Autodiscovery** to make the ViiA™ 7 Instrument discoverable by computers that are running the software.

4. Touch **Smart Monitoring** to enable the feature on the ViiA™ 7 Instrument.

   The Smart Monitoring feature allows Thermo Fisher Scientific service personnel to monitor the status of the ViiA™ 7 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. For a detailed description of the Smart Monitoring Service, see the *Smart Monitoring Service Product Bulletin: Leveraging the power of the Internet while maintaining system security* (Pub. No. 121PB07-03).

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

Network service	Action
DHCP	Touch **Obtain an IP address automatically,** then touch **Save.**
Static IP	1. Touch **Use the following IP address.** 2. Touch the **IP Address** field, enter the IP address using the keypad, then touch **Done.** 3. Repeat List item. to assign the: • IP addresses for the DNS Servers (primary and secondary) • Subnet Mask setting • Default Gateway setting 4. Touch **Save** to save the settings, then touch **OK** when prompted.

6. Touch to return to the Main Menu.

Define the system shortcuts

You can use the ViiA™ 7 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 ViiA™ 7 System touchscreen to awaken it, then press .

2. In the Main Menu, access shortcut settings:
   - Touch **Edit.**
     or
   - Touch **Settings**, then touch **Set Up Shortcuts**.

3. Configure the shortcuts as desired:
   To *add* a shortcut:
   a. Touch the shortcut of interest, then touch **Set Shortcut.**
   b. Touch **From Templates** to link to a specific template file or touch **From Folders** to link to a folder.
   c. Touch the desired template file or folder to configure the shortcut.

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

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

Review the instrument statistics

You can use the ViiA™ 7 Instrument touchscreen to view usage statistics on the heated cover, halogen lamp, and other system components.

View the statistics

1. If the instrument is in standby, touch the ViiA™ 7 System touchscreen to awaken it, then press .
2. In the Main Menu, touch **Tools,** then touch **Show Statistics.**
3. When you are finished, touch to return to the Main Menu.

Enable or disable instrument security

The ViiA™ 7 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 ViiA™ 7 Instrument security, auditing, and electronic signature feature, you must similarly enable or disable the software security (see “Configure the security system” on page 91). The software cannot connect to ViiA™ 7 Instruments that do not match security settings.

Note: Secure mode limits the number of feature that are available from the ViiA™ 7 Instrument touchscreen. Secure mode does not provide user authentication functionality through the instrument touchscreen.

Enable or disable security

1. If the instrument is in standby, touch the ViiA™ 7 System touchscreen to awaken it, then press .
2. In the Main Menu, touch **Settings,** then touch **Set Administrator Options.**
3. In the Set Administrator Options screen, touch **Secure Environment** to enable (checked) or disable (unchecked) system security.
4. (Optional) To change the administrator password:
   a. Touch **Change Password**.

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

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

d. Reenter the password when prompted.

e. Touch **OK** when prompted.

Note: The default password for the ViiA™ 7 Instrument touchscreen is *password;* however, the password can be changed during installation.

5. Touch **Save.**

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

7. Touch to return to the Main Menu.

View the instrument log

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

View the log

1. If the instrument is in standby, touch the ViiA™ 7 System touchscreen to awaken it, then press .

2. In the Main Menu, touch **Tools,** then touch **View Log.**

3. In the View Log screen, configure the settings to display the records of interest:
   - Select an option from the drop-down menu to filter the log.
   - Select **Earliest First** or **Latest First** to determine the order to sort the records.

4. Touch to return to the Main Menu.

B Power on or off, store, and move the ViiA™ 7 system

Place the ViiA™ 7 system on standby

If left unattended, the ViiA™ 7 Instrument automatically enters standby mode to conserve power. To enter standby mode manually, touch on the ViiA™ 7 Instrument touchscreen.

Power on the ViiA™ 7 System

To power on the ViiA™ 7 System from a powered-off state:

1. Toggle the power button on the rear of the ViiA™ 7 Instrument, then wait for it to boot.

   Note: The ViiA™ 7 Instrument is ready to use when the touchscreen displays the Main Menu.

2. Power on the monitor.

3. Power on the ViiA™ 7 System computer:
   a. Press the power button of the computer, then wait for it to boot.

   b. When the Login screen appears, enter your user name and password, then click **OK**.

4. Start the QuantStudio™ Real-Time PCR Software.
   a. On the desktop, double-click **QuantStudio™ Real-Time PCR Software**(or select .

      Note: If the shortcut is not present on the desktop, select **Start ▸ All Programs ▸ Applied Biosystems ▸ QuantStudio Real-Time PCR Software** to start the software.

      IMPORTANT! If the software does not start, confirm that no other instances of the software are open. If any instance of the software is open, close it before starting the software.

   b. If the QuantStudio™ Real-Time PCR Software login appears, enter your user name and password, then click **Log In**.

Power off the ViiA™ 7 Real-Time PCR System

The ViiA™ 7 Real-Time PCR System operates in low-power mode when not in use. However, the ViiA™ 7 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 as explained in "Store the ViiA™ 7 system" on page 144.

To power off the ViiA™ 7 System components:

1. Power off the ViiA™ 7 Instrument:
   a. If the ViiA™ 7 Instrument touchscreen is not blank, touch to place the ViiA™ 7 Instrument into stand-by mode.
   b. Toggle the power button on the rear of the instrument.
2. Power off the ViiA™ 7 System computer:
   a. In the desktop, select **Start ▸ Shut Down**.
   b. In the Shut Down Windows™ dialog box, select **Shut Down**, then click **OK**.
3. Power off the monitor.

Store the ViiA™ 7 system

The Applied Biosystems™ ViiA™ 7 Real-Time PCR System can be powered off and stored for extended periods of time. The length of the period of inactivity determines the method you use to power off the ViiA™ 7 Instrument.

Materials required

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

Prepare the ViiA™ 7 instrument

1. If you plan to store the ViiA™ 7 System for more than a week or you plan to move it, load an unused plate or array card into the ViiA™ 7 Instrument:

   Note: The empty plate protects the internal components of the ViiA™ 7 System during transport or during periods of inactivity lasting more than a week.

   a. Touch the ViiA™ 7 Instrument touchscreen to awaken it, then touch .
   b. Touch to eject the tray arm, place a plate or array card onto the plate adapter, then press again to load the plate.
   c. Touch to place the ViiA™ 7 Instrument into stand-by mode.
2. Toggle the power button on the rear of the ViiA™ 7 Instrument.

3. Power off the computer:
   a. Select **Start ▸ Shut Down.**
   b. In the Shut Down Windows™ dialog box, select **Shut Down**, then click **OK**.
4. Power off the monitor.

Move the ViiA™ 7 system

Perform this procedure to safely move the ViiA™ 7 System short distances (for example, between laboratories of the same building).

CAUTION! PHYSICAL INJURY HAZARD. Do not attempt to lift the ViiA™ 7 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 ViiA™ 7 Instrument. At least two people are required to lift it.

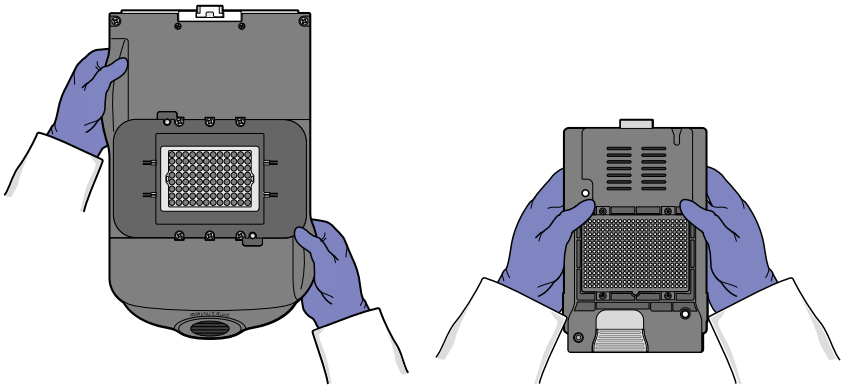
IMPORTANT! Moving your ViiA™ 7 System can create subtle changes in the alignment of the instrument optics. Recalibrate the instrument if necessary.

Materials required

None

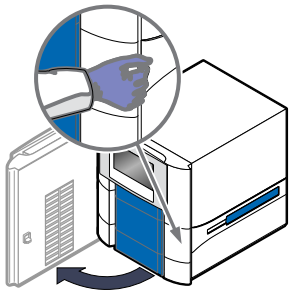
How to handle the sample block and heated cover

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

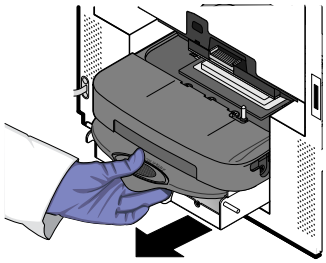


Prepare for the ViiA™ 7 system components

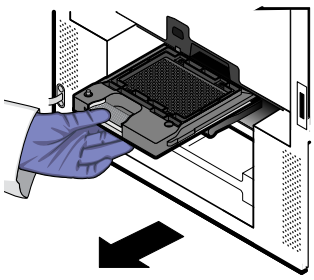
1. Power off the ViiA™ 7 Instrument and computer.
2. When the ViiA™ 7 System and computer are powered off, disconnect all ViiA™ 7 System components and package the cabling for the move.
3. Prepare the ViiA™ 7 Instrument for the move:
   a. Open the ViiA™ 7 System access door.



   b. Firmly press down on the sample block handle, pull the sample block from the ViiA™ 7 Instrument, then place it on a clean, dry surface.



   c. Pinch the handle of the heated cover together, then pull the assembly from the ViiA™ 7 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.

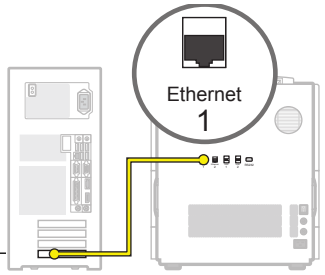
Move the ViiA™ 7 system

Move the ViiA™ 7 System according to the following guidelines:

- Verify that the surface on which you will place the ViiA™ 7 System can support at least 60.1±0.6 kg (132.5±0.13 lbs).
- Verify that the path to transport the ViiA™ 7 Instrument is clear of obstructions.
- Enlist at least one other person to lift and carry the ViiA™ 7 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 ViiA™ 7 System

1. Reconnect the components of the ViiA™ 7 System. Use the Ethernet cable supplied with the ViiA™ 7 System to connect the ViiA™ 7 Instrument (Ethernet 1 port) to the network interface card in the computer.



IMPORTANT! Do not use a standard Ethernet cable to connect the ViiA™ 7 Instrument to the computer.

IMPORTANT! Do not connect the Ethernet cable to the Ethernet 2 port on the ViiA™ 7 Instrument. The second port is for Thermo Fisher Scientific 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 ViiA™ 7 System. No further action is necessary.

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

   1. a. ROI calibration
      b. Background calibration
      c. Uniformity calibration
      d. Dye calibration
      e. (*TaqMan™ Array Card block only*) Normalization calibration

C Creating custom calibration plates and array cards

Create a background plate or array card

Whenever possible, use a Background Plate or the TaqMan™ Array Background Buffer that is included with the spectral calibration kit. The plates/array cards supplied in the kit contain a buffer that accurately simulates the reagents used for PCR, and, therefore, produces high-quality calibration data. If a background plate or array card from a spectral calibration kit is not available, you can create one as described below.

Materials required

96/384-Well plate sample block

- Applied Biosystems™ Optical 96/384-Well Reaction Plate
- Safety glasses
- Optical Adhesive Cover or Optical Flat Caps
- Pipettor, 200-µL (with pipette tips)
- Powder-free gloves
- Deionized water

Array card sample block

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

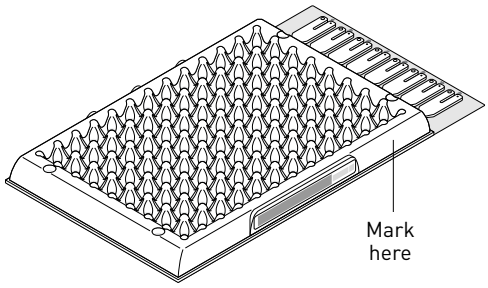
Create a background plate

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

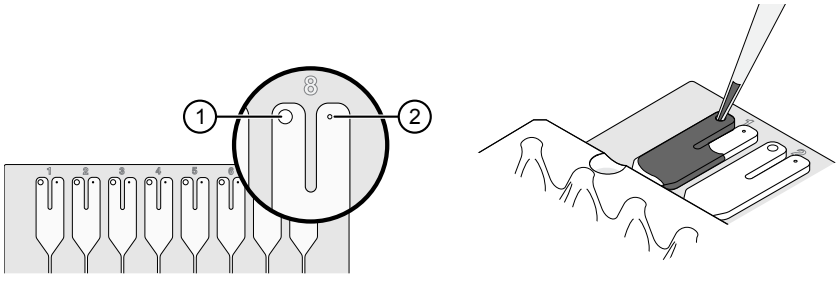
1. Remove an 96/384-Well Optical Reaction Plate from its box and place it on a clean, dry surface.
2. Aliquot 20 µL deionized water to each well of the reaction plate.
3. Seal the plate using an optical adhesive cover or optical flat caps.
4. Use the plate for background calibration as you would a background plate from the spectral calibration kit.

Create a background array card

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



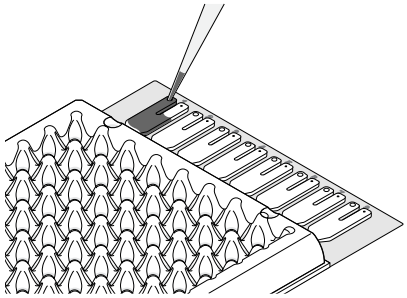
3. Pipette 100 µL of deionized water into each of the eight reservoirs in the card:
   a. Place the array card on a lab bench, with the foil side down.
   b. Load 100 µL of the solution into a pipette.
   c. Hold the pipette in an angled position (approximately 45°) and place the tip into the fill port.



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



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

4. Centrifuge and seal the array card as explained in "Fill the array cards" on page 30.

Create a custom dye plate for calibration

TheViiA™ 7 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 nm and 672 nm and read between 505 nm and 723 nm.

Before you use custom dyes

Before using custom dyes with the ViiA™ 7 System, you must:

- Determine optimum dye concentration.
- Create a custom dye plate.
- Add the custom dye to the software.
- Perform a dye calibration.

Materials required

- Centrifuge with plate adapter
- Custom dye(s)
- Safety glasses
- Powder-free gloves
- MicroAmp™ Optical 96/384-Well Reaction Plate
- Optical Adhesive Cover
- Pipettors and pipette tips (200-µL and 1000-µL)
- Tubes (2-mL and 10-mL)
- Deionized water

Determine optimum dye concentration

Note: Wear powder-free gloves while creating the dye plate.

1. Prepare, then load the custom dye plate:
   a. In the center of a 96/384-well plate, prepare a dilution series of the custom dye (for example, 25, 50, 100, 200, 400, 800, 1,600, and 3,200 nM) using 20-µL volumes for a 96/384-well plate.

   b. Seal the reaction plate using an optical adhesive cover.

   c. Load the prepared reaction plate.

2. Start the calibration wizard:
   a. In the Home screen of the software, click **Instrument Console**.

   b. In the Instrument Console, select your ViiA™ 7 Instrument, then click **Add to My Instruments**.

   c. Select the ViiA™ 7 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 ViiA™ 7 Instrument. When the side door opens, load the sealed plate. Ensure that the plate/array card is properly aligned in the holder.

   g. In the ROI dialog box, select **Check the box when the ROI calibration plate has been loaded**, click **Next** twice, then click **START RUN** to start the calibration.

3. When the run is complete, inspect the ROI images:
   a. Select the first filter from the Filter dropdown list.

   b. Record the coordinate of the well that contains the lowest concentration of dye and that is encircled by a ring. This well contains the optimal concentration of the custom dye at the given filter.

   c. Repeat steps 3a and 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 be heated to 100°C. Before removing the plate, wait until it reaches room temperature.

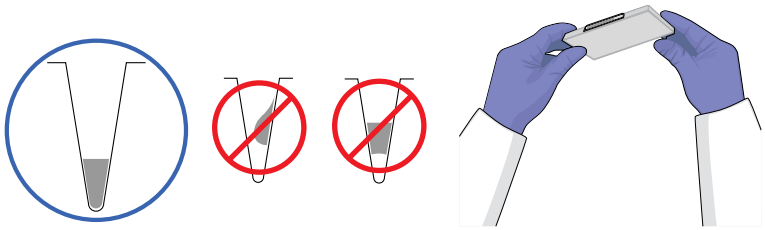
5. In the ROI dialog box, click **Finish** to complete the calibration, then click **No** when prompted to save the results.

Create a custom dye plate

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

1. Prepare 2 mL of the custom dye at the concentration determined in “Determine optimum dye concentration” on page 151.
2. Pipette 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 less than 1,500 rpm.

   Note: The custom dye calibration plate must be well mixed and centrifuged.
5. Verify that the liquid in each well of the plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.



Add the custom dye to the QuantStudio™ Real-Time PCR Software

1. Start the dye calibration:
   a. In the Home screen of the QuantStudio™ Real-Time PCR Software, click **Instrument Console.**
   b. In the Instrument Console, select your ViiA™ 7 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 box, then click **OK**.

Field/option	Action
Name	Enter a name for the custom dye.
Wavelength	Enter the wavelength at which the dye fluoresces.
Type	Select: • **Reporter** if the dye works in conjunction with a quencher dye to report an increase of PCR product. • **Quencher** if the dye suppresses the fluorescence of a reporter dye until amplification of PCR product. • **Both** if the dye reports an increase of PCR product without the aid of a quencher dye.

   d. Click **Close.**

3. In the Dye window, enter a temperature setting for the calibration. Set the temperature to match the temperature at which you intend to collect data. For example, the temperature for all Thermo Fisher Scientific 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 be heated to 100°C. Before removing the plate, be sure to wait until it reaches room temperature.

6. In the Dye dialog box of the QuantStudio™ Real-Time PCR Software, click **Next**.

7. Verify the grouping of the dye spectra:
   a. In the plate layout, select the wells of the plate.
   b. Inspect the raw data. For each spectrum, verify that the peak is:
      • Within the detectable range for the ViiA™ 7 System.
      • Free of irregular spectral peaks.
      • Present at the correct filter for the dye.

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

8. Verify the status of the calibration. If the calibration:

   Passed—If all spectra are acceptable, finish the calibration:

   a. Click **Next.**

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

   Failed—Create another custom dye plate using the next dye concentration greater than the concentration determined in “Determine optimum dye concentration” on page 151, then perform the calibration again.

D

Parts and materials

How to order

You can order materials and accessories directly from **thermofisher.com**.

Note: Product availability and pricing may vary according to your region or country. Online ordering through **thermofisher.com** is not available in all countries. Contact your local Thermo Fisher Scientific representative for help.

To order through the website or the QuantStudio™ Real-Time PCR Software:

- Confirm that your computer has an Internet connection.
- We recommend the following browsers and Adobe™ Acrobat™ Reader versions to use **thermofisher.com**:

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

How to order from the QuantStudio™ Real-Time PCR Software

1. To find your assay, complete the Find Assay pane in the QuantStudio™ Real-Time PCR 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 is added to your shopping list.

2. Check that the Experiment Shopping List contains the desired materials, other than the assay selected in the previous step, and that the quantities are correct, then click **Order Materials in List**.

3. In the Order Materials - Login dialog box, enter your user name and password for **thermofisher.com**, then click **Log In and Submit**.

Note: If you do not have an account with **thermofisher.com**, click **Register Now** to create an account.

When you are connected to the **thermofisher.com**, follow the prompts to complete your order.

Accessories

The following accessories are to be used with the Applied Biosystems™ ViiA™ 7 Real-Time PCR System.

System accessories	Part number
ViiA™ 7 System 384-Well Plate Adapter	4457087
ViiA™ 7 System 384-Well Sample Block	4453553
ViiA™ 7 System 384-Well/Array Card Heated Cover	4453555
ViiA™ 7 System 96-Well Heated Cover	4453560
ViiA™ 7 System 96-Well Plate Adapter	4459845
ViiA™ 7 System 96-Well Sample Block	4453556

(continued)

System accessories	Part number
ViiA™ 7 System 96-Well Tube Adapter	4462077
ViiA™ 7 System Array Card Plate Adapter	4454166
ViiA™ 7 System Array Card Sample Block	4453554
ViiA™ 7 System Fast 96-Well Heated Cover	4459838
ViiA™ 7 System Fast 96-Well Plate Adapter	4459846
ViiA™ 7 System Fast 96-Well Sample Block	4453559
ViiA™ 7 System Fast 96-Well Tube Adapter	4462078
Array Card Staker/Sealer	4456102
Array Card Bucket/Clip Set	4452205
Replacement Lamp for OptiFlex System	4443978

Calibration and verification consumables

Note: For reagent or consumable shelf-life expiration date, see the package label.

96-well 0.2-mL consumables

Note: Spectral calibration plates for the QuantStudio™ 3 and 5 Instruments are used for the calibration procedure with QuantStudio™ Real-Time PCR Software v1.6.1.

Consumable	Cat. No.	Storage	Shelf life at storage temperature
QuantStudio™ 3/5 10-Dye Spectral Calibration Kit, 96-Well 0.2-mL (Contains all 3 spectral calibration plates listed below)	A26343	–25°C to –15°C	Use the consumable by the expiration date on the packaging.
QuantStudio™ 3/5 Spectral Calibration Plate 1 (FAM™, VIC™, ROX™, and SYBR™ dyes), 96-Well 0.2-mL	A26331		
QuantStudio™ 3/5 Spectral Calibration Plate 2, 96-Well 0.2-mL (ABY™, JUN™, and MUSTANG PURPLE™ dyes)	A26332		
QuantStudio™ 3/5 Spectral Calibration Plate 3, 96-Well 0.2-mL (TAMRA™, NED™, and Cy®5 dyes)	A26333		

(continued)

Consumable	Cat. No.	Storage	Shelf life at storage temperature
Region of Interest (ROI) and Background Plates, 96-Well 0.2-mL (2 plates)	4432364	–25°C to –15°C	Use the consumable by the expiration date on the packaging.
TaqMan™ RNase P Instrument Verification Plate, 96-Well 0.2-mL	4432382		

96-well 0.1-mL consumables

Note: Spectral calibration plates for the QuantStudio™ 3 and 5 Instruments are used for the calibration procedure with QuantStudio™ Real-Time PCR Software v1.6.1.

Consumable	Cat. No.	Storage	Shelf life at storage temperature
QuantStudio™ 3/5 10-Dye Spectral Calibration Kit, 96-well, 0.1-mL (Contains all 3 spectral calibration plates listed below)	A26342	–25°C to –15°C	Use the consumable by the expiration date on the packaging
QuantStudio™ 3/5 Spectral Calibration Plate 1, (FAM™, VIC™, ROX™, and SYBR™ dyes), 96-well, 0.1 mL	A26336		
QuantStudio™ 3/5 Spectral Calibration Plate 2 (ABY™, JUN™, MUSTANG PURPLE™ dyes), 96-well Fast (0.1-mL) Plate	A26337		
QuantStudio™ 3/5 Spectral Calibration Plate 3 (TAMRA™, NED™ and Cy®5 dyes) 96-well Fast (0.1-mL) Plate	A26340		
Region of Interest (ROI) and Background Plates, Fast 96-Well 0.1-mL (2 plates)	4432426		
TaqMan™ RNase P Instrument Verification Plate, Fast 96-Well	4351979		

384-well consumables

Note: Spectral calibration plates for the QuantStudio™ 3 and 5 Instruments are used for the calibration procedure with the QuantStudio™ Real-Time PCR Software v1.6.1.

Consumable	Cat. No.	Storage	Shelf life at storage temperature
QuantStudio™ 5 10-Dye Spectral Calibration Kit, 384-well (Contains the 2 spectral calibration plates listed below)	A26341	–25°C to –15°C	Use the consumable by the expiration date on the packaging.
QuantStudio™ 5 Spectral Calibration Plate 1, (FAM™, VIC™, ROX™ TAMRA™, and SYBR™ dyes), 384-well	A26334		
QuantStudio™ 5 Spectral Calibration Plate 2 (ABY™, JUN™, MUSTANG PURPLE™, NED™, and Cy®5 dyes), 384-well	A26335		
Region of Interest (ROI) and Background Plates, 384-well	4432320		
TaqMan™ RNase P Instrument Verification Plate, 384-well	4455280		

Array card sample block consumables

Consumable	Cat. No.	Storage	Shelf life at storage temperature
ViiA™ 7 Array Card Spectral Calibration Kit Includes FAM™ Dye, VIC™ Dye, ROX™ Dye, ROI Dye, FAM™/ROX™ Dye, VIC™/ROX™ Dye, and Background Buffer	4432314	–25°C to –15°C	Use the consumable by the expiration date on the packaging.
ViiA™ 7 Array Card RNaseP Verification Kit	4432265		

Empty plate and array card consumables

Note: For consumable shelf-life expiration date, see the package label.

The following consumables are to be used with the Applied Biosystems™ ViiA™ 7 Real-Time PCR System.

Consumable		Part number
Array Cards, 8-Port (Empty)	Empty Array Card Kit, 4-pk	4334812
	Empty Array Card Kit	4351471
MicroAmp™ Fast 8-Tube Strip, 0.1-mL	125 strips	4358293
MicroAmp™ Fast Optical 96-Well Reaction Plate with Bar Code, 0.1-mL	10 plates	4346906
	200 plates	4366932
MicroAmp™ Optical 96-Well Reaction Plate, 0.2-mL	10 plates	N8010560
	500 plates	4316813
MicroAmp™ Optical 96-Well Reaction Plate with Bar Code, 0.2-mL	10 plates	4306737
	500 plates	4326659
MicroAmp™ Optical 384-Well Reaction Plate, 1000 plates		4343370
MicroAmp™ Optical 384-Well Reaction Plate with Bar Code	1000 plates	4343814
	500 plates	4326270
	50 plates	4309849
MicroAmp™ Optical 8-Cap Strip	300 strips	4323032
MicroAmp™ Optical 8-Tube Strip, 0.2-mL	1000 tubes	4316567
MicroAmp™ Optical Adhesive Film		4311971

Software reference

Software command-line application

The QuantStudio™ Real-Time PCR 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™ Real-Time PCR 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.

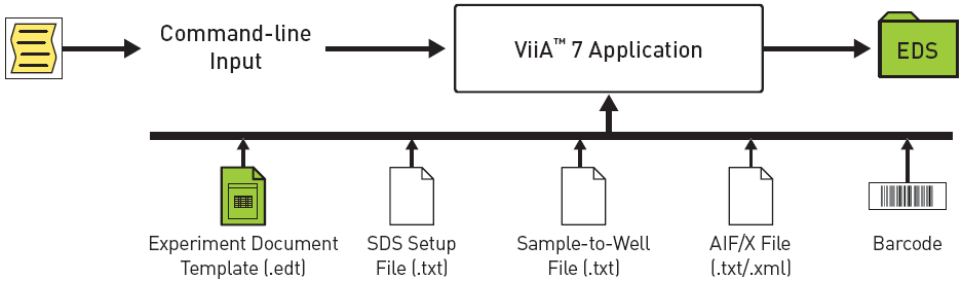


Figure 5 Single Experiment File Creation Workflow

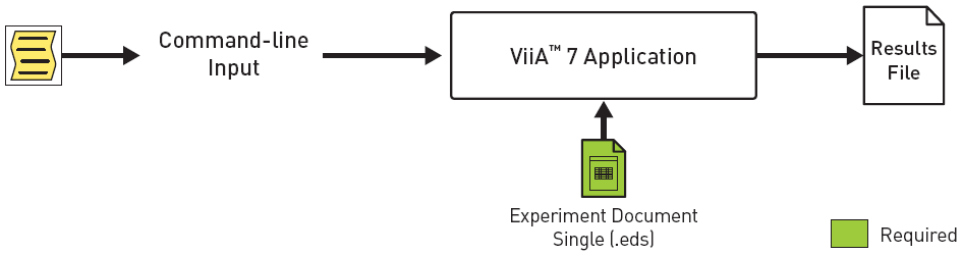


Figure 6 Export Workflow

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 for each TaqMan™ assay ordered from Thermo Fisher Scientific. For some products, assay information files are available for download from the Thermo Fisher Scientific website following delivery. The file, which contains data describing the assay, can be imported into the software for use in related experiments. See “Assay information file” on page 178 for more information.
barcode file (TXT)	A user-created, line-separated text file that contains the bar code of each consumable for which you want to create an experiment file. See “Bar code file format” on page 178 for more information.
experiment document single file (EDS)	A QuantStudio™ Real-Time PCR Software file that contains all information about a particular plate or array card consumable, including metadata (name, bar code, comments), plate setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, analysis results, audit records, and other plate-specific data.
experiment document template file (EDT)	A QuantStudio™ Real-Time PCR Softwaree 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 ViiA™ 7 System. The file defines the arrangement of assays and samples on the consumable. See “Plate setup file format” on page 170 for more information.
sample file (TXT)	A user-created, tab-delimited text file containing sample data that can be imported into the QuantStudio™ Real-Time PCR Software for use in related experiments. See “Sample file format” on page 177 for more information.

Precedence rules for experiment file generation

When generating experiment files (EDS), the software command-line interface relies on a set of precedence rules to resolve conflicts that arise from the data supplied by some input files. Assay information files (AIF or AIX), plate setup files (TXT), and template files (EDT) can contain data used to populate the same fields of new experiment files. For example, both template and plate setup files can contain location data for samples and assays.

Files used for experiment file (.eds) creation	Precedence rule
Template file (EDT)	The values in the template take precedence except for: • Experiment Name—Determined by the File Name Convention preference. • Bar Code—Determined by the bar code, if present. Otherwise, the value is null. • Experiment File Name—Determined by the File Name Convention preference.
• Template file (EDT) • Assay information file (AIF/AIX)	All values in the template file take precedence, except for: • Gene Expression Targets/Assay Definition • Genotyping Assay/SNP Definition • Passive Reference If any conflicts exist between the assay information file and the template for the attributes above, then the assay information file values always take precedence.
• Template file (EDT) • Plate setup file (TXT)	All values in the template file take precedence, except for: • Target/Assay/SNP to Well Assignment • Sample to Well Assignment • Task to Well Assignment • Biological Group to Well Assignment • Well Quantity to Well Assignment • Sample Color • Biological Group Color • Target Color • Gene Expression Targets Definition • Genotyping Assay Definition • Passive Reference

(continued)

Files used for experiment file (.eds) creation	Precedence rule
	All values in the template take precedence, except for the following. The following assay information file values take precedence over Plate Setup and Template: • Gene Expression Targets/Detectors Definition • GT Assay/Marker Definition • Passive Reference
• Template file (EDT) • Plate setup file (TXT) • Assay information file (AIF/AIX)	The following Plate Setup values take precedence over the template: • Block Type • Target/Assay/Marker to Well Assignment • Sample to Well Assignment • Task to Well Assignment • Biological Group to Well Assignment • Well Quantity to Well Assignment • Sample Color • Biological Group Color • Target Color

Running the command-line application from a command prompt

Running the application

1. In the desktop, select **Start ▸ Run**.
2. In the Run dialog box, enter **cmd** in the Open field, then click **OK.**
3. In the DOS prompt, change to the installation directory and enter the command:
   a. Enter **cd D:\applied biosystems\ViiA7**, then press **Enter**.
   b. Enter **cmdlineutil.exe**, followed by **-expgen** or **-export**, then all applicable parameters and arguments. See “Command syntax and arguments” on page 165 for a complete list of command-line parameters.

Viewing the command-line help

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

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

Command syntax and arguments

Batch file creation

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

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

The following table lists the acceptable parameters that can be included in any order. See “Examples” on page 167 for an example of the experiment creation command.

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

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

Parameter	Description
`-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 "<drive>:\assayfiles\assayfile.aif"`
`-b <filepath>`	(*Optional*) Specifies the path and name (<*filepath*>) of the bar code 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 "<drive>:\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 bar code of the plate specified in the bar code 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 "<drive>:\Applied Biosystems\QuantStudio Real-Time PCR Software\experiments"` Before creating experiment files, the software confirms whether the export location exists and aborts if the location does not exist.

(continued)

Parameter	Description
`-m <filepath>`	(*Optional*) Specifies the path and name (<*filepath*>) of the sample file that the software uses to create new files. Example:`-m "<drive>:\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 "<drive>:\setupfiles\setupfile.txt"`
`-t <filepath>`	(*Required*) Specifies the path and name (<*filepath*>) of the ViiA™ 7 Software template file that the software uses to create new files. Example:`-t "<drive>:\Applied Biosystems\QuantStudio Real-Time PCR Software\experiments\templatefile.edt"`
`-v`	(*Optional*) Configures the software to operate in verbose mode, where the software displays each operation as it is performed.

Results export

The command used to export the results from experiment files uses the following syntax:

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

The following table lists the acceptable parameters that can be included in any order. See “Examples” on page 167 for and examples of the experiment export command.

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

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

Parameter	Description
`-e <dirpath>`	(Required) Specifies the path to the directory (*<dirpath>*) that contains the experiment files (EDS) for which the software exports data. Example:`-e "<drive>:\Applied Biosystems\QuantStudio Real-Time PCR Software\experiments\"`
`-f <option>`	(Required) Specifies the format of the exported data (see "Export formats and file specifications" on page 179 for the export file specifications): • ViiA7—Exports data in a format compatible with the ViiA™ 7 System. • SDS23—Exports data in a format compatible with the Applied Biosystems™ 7900HT Real-Time PCR System. • RDML—Exports data in the real-time data markup language (RDML) format. Example:`-f "RDML"`
`-l <path>`	(*Optional*) Specifies the path (*<path>*) of the directory to which the software saves the exported files. Example:`-l "<drive>:\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: • ViiA™ 7 export format: TXT, XLS, or XLSX • SDS23 export format: TXT • RDML export format: RDML Example:`-x "rdml"`

Examples

Batch file creation

The following example uses all parameters described in "Command syntax and arguments" on page 165 (required and optional) to generate a set of experiment files.

```
cmdlineutil.exe -expgen -t "<drive>:\Applied Biosystems\QuantStudio Real-Time PCR
Software
\experiments\templates\standard_curve.edt" -a "D:\Applied
Biosystems\QuantStudio Real-Time PCR Software\experiments\examples\AIF
\AIF_820629.txt"
-s "<drive>:\Applied Biosystems\QuantStudio Real-Time PCR Software\experiments
\examples\Plate
Setup Files\SDS_820629.txt" -m "<drive>:\Applied Biosystems\QuantStudio Real-Time
PCR Software\experiments\examples\SampleNames\SampleFileNames.txt"
-c "alloptionsused" -f "Plate Barcode_Custom Name Field"
```

```
-b "C:\QuantStudio Real-Time PCR Software\barcodes - v12.txt" -l "C:\QuantStudio
Real-Time PCR Software\Experiment"
```

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

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 bar code in the `bar codes - v12.txt` bar code 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:\QuantStudio Real-Time PCR Software\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™ Real-Time PCR 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 "<drive>:\Applied Biosystems\QuantStudio Real-Time PCR
Software\experiments\" -f "SDS23" -l "C:\exports\" -s "single" -x "rdml"
```

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

Import formats and file specifications

The 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 "Software command-line application" on page 161) or the software application programming interface (API) to integrate the ViiA™ 7 System into a laboratory information management system (LIMS). For a detailed explanation of the API, or for information on integrating the ViiA™ 7 System into a laboratory workflow, see the *Applied Biosystems™ ViiA™ 7 Real-Time PCR System Robotics User Guide* (Pub. No. 4442663).

Note: The file specifications listed in this appendix are subject to change. For updated information, review the software release notes available at the software download page (**thermofisher.com/qpcrsoftware**).

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 ViiA™ 7 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.	"Plate setup file format" on page 170
Sample file (TXT)	A user-created, tab-delimited text file containing sample data that can be imported into the software for use in related experiments.	"Sample file format" on page 177
Assay information file (AIF or AIX)	A tab-delimited or XML data file for each TaqMan™ assay ordered from Thermo Fisher Scientific. The file, which contains data describing the assay, can be imported into the software for use in related experiments.	"Assay information file" on page 178
Bar code file (TXT)	A user-created, text file containing the bar codes of consumables for which you want to create experiment files using the command-line utility.	"Bar code file format" on page 178

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 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 software or into new experiments created by the command-line application (see “Software command-line application” on page 161). A plate setup file is a tab-delimited ASCII text file (TXT) that contains data that describes the location experiment data information. The files can be created manually using a text processor or generated automatically by third-party applications.

IMPORTANT! To guarantee successful import of the plate setup file into a experiment, the file must contain all the elements described in the following section and in the order that they appear.

File structure

The plate setup file consists of a header, which specifies the instrument model for which the experiment is designed, and a sample setup section.

Section	Description	See…
Plate setup file header	Defines the instrument model for which the experiment is designed and the dye used as the passive reference.	“Plate setup file header” on page 171
Plate setup file body	Defines the contents of a 96/384-well plate or array card, including target, SNP assay, sample, and task assignments.	“Plate setup file body” on page 171

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 ViiA™ 7 System for which the experiment is designed.	ViiA 7
Passive Reference	The dye that the experiment will use as a passive reference.	• The name of a dye in the Dye Library[1], or • <*blank*> if the consumable does not contain a passive reference.

[1] Custom dyes are allowed as long as they are in the Dye Library.

Note: The software automatically removes any leading and trailing white space around the field name and field value.

Example:

```
* Instrument Type = ViiA 7
* Passive Reference = ROX
```

Plate setup file body

The body of a plate setup file contains either target information, which can be imported into all experiments except genotyping, or SNP assay information. This information can be imported into genotyping experiments only. The body consists of three required elements (the header, the column header, and the body) that describe the contents of a 96/384-well plate or array card. The sample setup column header and body can appear in any order.

IMPORTANT! Observe the following guidelines when creating a plate setup file:

- Do not insert blank lines between the sample setup header and the column header.
- Do not use illegal characters, including backslash (\), tab, asterisk (*), hard return, soft return, brackets([or]), or comma (,).

Sample setup header

The header contains the label that defines the beginning of the sample setup data.

Example:

```
[Sample Setup]
```

Sample setup column header

The column header contains the headings that define the positions of the data columns in the sample setup body. The headings are separated by tab characters. See “Plate setup data columns” on page 172 for a list of the data column headers.

Example:

```
Well Sample Name Sample Color Biogroup Name Biogroup Color Target Name…
```

Sample setup body

Contains the sample setup data where each row defines the contents of a single well on the consumable, including the: well contents (sample, target, or SNP assay added to the well), task assignments, and comments. If a well contains multiple assays (multiplex PCR), the data for the additional assays are defined on separate lines by repeating the well designation. See “Plate setup data columns” on page 172 for a list of the data column headers.

Note: The sample setup data rows can occur in any order.

Example:

```
Well Sample Name Sample Color Biogroup Name Biogroup Color Target Name…
1 Liver cDNA “RGB(25,0,0)”
2 Liver cDNA “RGB(25,0,0)”
3 Liver cDNA “RGB(25,0,0)”
4 Heart cDNA “RGB(0,25,0)”
5 Heart cDNA “RGB(0,25,0)”
…
```

Plate setup data columns

The following table lists the headings and columns that are present in the plate setup file body of all experiment types followed by the columns that are specific to genotyping experiments and non-genotyping experiments.

Column name	Description	Valid values
All experiments		
Well	The number of the well on the consumable, where the well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.	*<Positive integer (1 to 96/384)>*[1]

(continued)

Column name	Description	Valid values
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>*
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>*[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>*
SNP Assay Name	The name of the SNP assay detected or amplified by the assay in the associated well.	*<100-character string>*[3]
Genotyping only		
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]

(continued)

Column name	Description	Valid values
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]
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 to 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] See the *Applied Biosystems™ ViiA™ 7 Real-Time PCR System Getting Started Guide* to determine the tasks applicable to your experiment.

[5] The dye must already exist in the 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 ViiA™ 7 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.

```
* Instrument Type = ViiA 7
* 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
8 Brain cDNA "RGB(0,0,25)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
9 Brain cDNA "RGB(0,0,25)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
…
376 Liver cDNA "RGB(25,0,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
377 Liver cDNA "RGB(25,0,0)" GH1 "RGB(0,0,105)" UNKNOWN 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).

```
* Instrument Type = ViiA 7
* 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
7 Brain cDNA "RGB(0,0,25)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
7 Brain cDNA "RGB(0,0,25)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
8 Brain cDNA "RGB(0,0,25)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
8 Brain cDNA "RGB(0,0,25)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
9 Brain cDNA "RGB(0,0,25)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
9 Brain cDNA "RGB(0,0,25)" 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 ViiA™ 7 System. The experiment screens samples for the presence of a pathogen (E. coli O157:H7). The detection assay uses FAM™ and VIC™ dye-labeled TaqMan™ probes to amplify a unique genomic sequence and an internal positive control (IPC).

```
* Instrument Type = ViiA 7
* 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
7 Blocked IPC "RGB(0,0,25)" E.coli "RGB(98,25,0)" BlockedIPC FAM NFQ-MGB
7 Blocked IPC "RGB(0,0,25)" IPC "RGB(0,0,105)" BlockedIPC VIC NFQ-MGB
8 Blocked IPC "RGB(0,0,25)" E.coli "RGB(0,0,105)" BlockedIPC FAM NFQ-MGB
8 Blocked IPC "RGB(0,0,25)" IPC "RGB(0,0,105)" BlockedIPC VIC NFQ-MGB
9 Blocked IPC "RGB(0,0,25)" E.coli "RGB(0,0,105)" BlockedIPC FAM NFQ-MGB
9 Blocked IPC "RGB(0,0,25)" IPC "RGB(0,0,105)" BlockedIPC VIC NFQ-MGB
10 Sample01 "RGB(90,0,0)" E.coli "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
10 Sample01 "RGB(90,0,0)" IPC "RGB(0,0,105)" UNKNOWN VIC NFQ-MGB
11 Sample01 "RGB(90,0,0)" E.coli "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
11 Sample01 "RGB(90,0,0)" IPC "RGB(0,0,105)" UNKNOWN VIC NFQ-MGB
12 Sample01 "RGB(90,0,0)" E.coli "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
12 Sample01 "RGB(90,0,0)" IPC "RGB(0,0,105)" UNKNOWN VIC NFQ-MGB
…
```

Genotyping experiments

The following example shows a plate setup file created for a genotyping experiment to be run on a ViiA™ 7 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).

```
* Instrument Type = ViiA 7
* 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
11 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
12 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
13 Sample02 "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
14 Sample02 "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
15 Sample02 "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™ Real-Time PCR 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 "Software command-line application" on page 161) 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 170).

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 to 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
…
```

Bar code file format

The software command-line application can import bar code files to populate experiment files (EDS) it generates with bar code information. A bar code file is a tab-delimited ASCII text file (TXT) that contains a list of bar codes. 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 bar code file contains a list of bar codes, where each line defines a single bar code terminated by a carriage return. The bar codes can occur in any order and cannot contain starting or trailing white space.

Note: The software command-line application does not validate the bar codes.

Example file

```
HA996346102
IB894812348
DD834814679
EK209825848
AF092387348
FF225676243
```

Assay information file

The software command-line application can import data for Thermo Fisher Scientific assays from assay information files (AIF). The AIF contains technical details about all assays in the shipment. It includes information about assay concentrations; reporters and quenchers used; part and lot numbers; and assay, vial, and plate ID numbers. The file name includes the number from the bar code on the plate.

Export formats and file specifications

This section describes the export formats supported by the software. The information provided in this appendix is intended for users who want to integrate the software with third-party applications, including downstream analysis software and laboratory information management system (LIMS) tools.

Note: The file specifications listed in this appendix are subject to change. For updated information, review the software release notes available at the software download page (**thermofisher.com/qpcrsoftware**).

Export formats

The 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 software supports the following export formats:

File format	Description	See…
ViiA™ 7 export file	A ViiA™ 7-formatted text file that contains setup and/or results data exported from an experiment file (EDS).	"ViiA™ 7 export format" on page 180
7900 export file	A legacy 7900-formatted text file that contains setup and/or results data exported from an experiment file (EDS).	"7900 export format" on page 199
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.	"RDML export format" on page 206

Export formats and the software API

The export formats can be used in combination with the software application programming interface (API) to integrate the ViiA™ 7 System into a laboratory information management system (LIMS) workflow.

ViiA™ 7 export format

The software can export setup and results data from experiment files (EDS) to tab-delimited text files (TXT) in a native ViiA™ 7 System export format. Data exported in the ViiA™ 7 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 ViiA™ 7 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. Because the ViiA™ 7 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 ViiA™ 7 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.	"File header" on page 181
Sample setup data	Describes the configuration of samples on the experiment consumable, including sample location, target or SNP assay properties, and task assignments.	"Sample setup data" on page 182
Raw data	Contains the raw data collected by the ViiA™ 7 Instrument during the experiment run.	"Raw data" on page 185
Amplification data	Contains the normalized data collected during the cycling stage of PCR amplification, which the 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.	"Amplification data" on page 186
Multicomponent data	Contains the spectral data used by the software to generate the multicomponent plot that displays the contribution of each dye over the duration of the PCR run.	"Multicomponent data" on page 186
Results data	Contains the normalized, processed, and analyzed data generated by the software.	"Results data" on page 187

File header

The plate setup file begins with a header that describes the qualities of the ViiA™ 7 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 software automatically removes any leading and trailing white space around the field name and field value.

The header contains the lines listed in the following table.

Field	Description	Output
Block Type	The model of the sample block installed to the ViiA™ 7 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 ViiA™ 7 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 ViiA™ 7 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 ViiA™ 7 Instrument that ran the experiment.	`ViiA™ 7`
Passive Reference	The dye used as a passive reference (or blank if the consumable did not contain one).	*<100-character string>*

(continued)

Field	Description	Output
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 ViiA™ 7 Instrument collected data.	Stage <*integer*>, Step <*integer*>
Calibration Date	The date and time that the current background, ROI, uniformity, or pure dye calibration was performed and when it will expire.	<*date and time*>
Calibration Expiration Date		<*date and time*>
Instrument serial number	The serial number of the ViiA™ 7 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 software exports sample setup data after the file header. The sample setup data describes the sample configuration on the experiment consumable, including positions, sample names, task assignments, assay information, and color coding.

The data consists of a column header followed by the sample data fields, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the software lists the data for each additional assay on separate rows, repeating the well number and sample information. The data included in the sample setup data export varies depending on experiment type.

This section describes the following sample setup data formats:

- “Quantification and presence/absence experiments” on page 183
- “Genotyping experiments” on page 184

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 184

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1 to 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*>,<*r*>,)"[2]
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 to 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 183.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1 to 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*>,<*b*>)"[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*>,<*b*>)"[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 to 255. The field is enclosed in double quotes with no spaces between the values.

Raw data

The software can export the unprocessed raw data (R) collected by the ViiA™ 7 Instrument during the experiment run. The raw data consists of fluorescence readings collected by the ViiA™ 7 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 to 96/384)[1]
Cycle	The cycle of the run during which the ViiA™ 7 Instrument recorded the fluorescence.	Integer
<Bin #>	The raw fluorescence for the well measured by the ViiA™ 7 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.

Amplification data

The 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 ViiA™ 7 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 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 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 to 96/384)[1]
Cycle	The cycle of the run during which the 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 software can export the data used to generate the multicomponent plot of a real-time PCR experiment. The multicomponent data tracks the raw fluorescence of all reporter dyes present on the reaction consumable throughout the duration of the experiment run.

The section begins with a column header followed by the multicomponent data, where each row contains the data for a single well separated by tab characters. The multicomponent data contains a dye column for each dye present on the reaction

consumable, including reporter dyes, quencher dyes (except non-fluorescent dyes), and the passive reference.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1 to 96/384)[1]
Cycle	The cycle of the run during which the ViiA™ 7 Instrument recorded the fluorescence data.	Integer
<Dye name>	The raw fluorescence for the designated dye measured by the ViiA™ 7 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 software can export the results data from an analyzed experiment file. The format and content of the results data depends on the experiment type and the analysis settings.

The section begins with a column header followed by the results data, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the 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 Ct experiments” on page 188
- “Genotyping” on page 191
- “Melting curve” on page 192
- “Presence/absence” on page 194
- “Study data” on page 195
- “Technical replicate results” on page 190
- “Technical analysis result (study)” on page 197
- “BioGroup analysis results (study)” on page 198
- “Technical analysis result (study)” on page 197

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. 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 to 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
Ct	The calculated threshold cycle (C_t) for the target at the specified well.	Float
Ct Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float
Ct SD	The standard deviation of the average C_t of the replicate wells for the specified target.	Float
Quantity	• Unknown wells—The calculated quantity for the sample at the well. • Standard wells—The quantity assigned to the standard at the well.	Float
Quantity Mean	• Unknown wells—The average quantity of the replicate wells for the target/sample. • Standard wells—The quantity assigned to the replicate wells for the target/sample.	Float
Quantity SD	The standard deviation of the average quantity of the replicate wells for the target/sample combination	Float

(continued)

Column	Description	Output
Automatic Ct Threshold	Whether the threshold was determined automatically (true) or manually (false).	`true` or `false`
Ct Threshold	The threshold cycle (C_t) for the sample at the well	Float
Automatic Ct Baseline	Whether the baseline was determined automatically (true) or manually (false).	`true` or `false`
Baseline Start	The first cycle used to calculate the baseline.	Integer
Baseline End	The last cycle used to calculate the baseline.	Integer
Custom1… Custom6	The contents of the custom text fields found in the Results table of the experiment.	1024-character string (per field)
If analysis flags are present, results data is present in additional columns named for the associated flags.		`true` or `false`

[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
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

(continued)

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

Technical replicate results

The following table describes the results data exported from high resolution melting curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Sample Name	The name of the sample contained by the well.	100-character string
Target Name	The name of the target assay assigned to the well.	100-character string
Task	The task assigned to the target in the well.	`UNKNOWN` or `NTC`
RQ	The relative quantity calculated for the replicate wells of the target/sample combination.	Float
RQ Min	The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.	Float
RQ Max	The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.	Float
Ct Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float
Delta Ct Mean	The average ΔC_t of the replicate wells for the specified target/sample combination.	Float

(continued)

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

Genotyping

The following table describes the results data exported from genotyping experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1 to 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 to 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`

(continued)

Column	Description	Output
Allele1 Automatic Ct Threshold	Whether the allele 1 threshold was determined automatically (true) or manually (false).	`true` or `false`
Allele1 Baseline Start	The start cycle used to calculate the baseline section of allele 1.	Float
Allele1 Baseline End	The end cycle used to calculate the baseline section of allele 1.	Float
Allele2 Automatic Ct Threshold	Whether the allele 2 threshold was determined automatically (true) or manually (false).	`true` or `false`
Allele2 Baseline Start	The first cycle used to calculate the baseline for allele 2.	Float
Allele2 Baseline End	The last cycle used to calculate the baseline for allele 2.	Float
Custom1… Custom6	The contents of the custom text fields found in the Results table of the experiment.	1024-character string (per field)

[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 to 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

(continued)

Column	Description	Output
Ct	The calculated threshold cycle (C_t) for the target at the specified well.	Float
Ct Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float
Ct SD	The standard deviation of the average C_t of the replicate wells for the specified target.	Float
Quantity	• Unknown wells—The calculated quantity for the sample at the well. • Standard wells—The quantity assigned to the standard at the well.	Float
Quantity Mean	• Unknown wells—The average quantity of the replicate wells for the target/sample. • Standard wells—The quantity assigned to the replicate wells for the target/sample.	Float
Quantity SD	The standard deviation of the average quantity of the replicate wells for the target/sample.	Float
Automatic Ct Threshold	Whether the threshold was determined automatically (true) or manually (false).	`true` or `false`
Ct Threshold	The threshold cycle (C_t) for the sample at the well.	Float
Automatic Ct Baseline	Whether the baseline was determined automatically (true) or manually (false).	`true` or `false`
Baseline Start	The first cycle used to calculate the baseline.	Integer
Baseline End	The last cycle used to calculate the baseline.	Integer
Tm1… Tm3	The first, second, and third melting temperatures (T_m) calculated in degrees Celsius.	Float
Comments	Additional text that describes the well.	1024-character string
Custom1… Custom6	The contents of the custom text fields found in the Results table of the experiment.	1024-character string (per field)

[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 to 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
Rn	The raw fluorescence for the associated well normalized to the fluorescence of the passive reference dye.	Float
Rn Mean	The averaged normalized fluorescence (R_n) for the associated replicate wells that contain the same target/sample combination.	Float
Rn SD	The standard deviation of the normalized fluorescence (R_n) for the associated replicate wells that contain the same target/sample combination.	Float
Threshold Value	The calculated value of the threshold for a positive call.	Float
Call	The presence/absence call assigned to the sample at the specified well.	`Negative Control`, `Blocked IPC Control`, `IPC Failed`, `Positive`, or `Negative`
Comments	Additional text that describes the well	1024-character string

(continued)

Column	Description	Output
Automatic Ct Threshold	Indicates whether the threshold was determined automatically (true) or manually (false).	`true` or `false`
Ct Threshold	The threshold cycle (C_t) for the sample at the well.	Float
Automatic Ct Baseline	Indicates whether the baseline was determined automatically (true) or manually (false).	`true` or `false`
Baseline Start	The first cycle used to calculate the baseline.	Float
Baseline End	The last cycle used to calculate the baseline.	Float
Custom1… Custom6	The contents of the custom text fields found in the Results table of the experiment.	1024-character string (per field)

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

Study data

The following table describes the results data exported from a study of relative quantification experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

Column	Description	Output
Experiment Name	The name of the of the experiment.	100-character string
Well	The number of the well on the consumable.	Integer (1 to 96/384)[1]
Omitted	Whether the well was omitted from the analysis (true) or included (false).	`true` or `false`
Sample	The name of the sample contained by the well.	100-character string
Target	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

(continued)

Column	Description	Output
RQ	The relative quantity calculated for the replicate wells of the target/sample combination.	Float
RQ Min	The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.	Float
RQ Max	The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.	Float
Ct	The calculated threshold cycle (C_t) for the target at the specified well.	Float
Ct Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float
Delta Ct	The ΔC_t value of the replicate wells for the specified target/sample combination.	Float
Delta Ct Mean	The average ΔC_t of the replicate wells for the specified target/sample combination.	Float
Delta Ct SD	The standard deviation of the ΔC_t for the replicate well. Depending on the analysis settings, this column may by replaced with “Delta Ct SE” (the standard error of the ΔC_t).	Float
Delta Delta Ct	The $\Delta\Delta C_t$ value of the replicate wells for the specified target/sample combination.	Float
Automatic Ct Threshold	Indicates whether the threshold was determined automatically (true) or manually (false).	`true` or `false`
Ct Threshold	The threshold cycle (C_t) for the sample at the well.	Float
Automatic Ct Baseline	Whether the baseline was determined automatically (true) or manually (false).	`true` or `false`
Baseline Start	The first cycle used to calculate the baseline.	Float

(continued)

Column	Description	Output
Baseline End	The last cycle used to calculate the baseline.	Float
Efficiency	The calculated efficiency of the target assay for the specified target/sample combination.	Float (1 to 100)

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

Technical analysis result (study)

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`
Omitted	Indicates whether the well was omitted from the analysis (true) or included (false).	`true` or `false`
RQ	The relative quantity calculated for the replicate wells of the target/sample combination.	Float
RQ Min	The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.	Float
RQ Max	The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.	Float
Ct Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float
Delta Ct Mean	The average ΔC_t of the replicate wells for the specified target/sample combination.	Float

(continued)

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

BioGroup analysis results (study)

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	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`
Omitted	Indicates whether the well was omitted from the analysis (true) or included (false).	`true` or `false`
# Tech Replicates	The number of technical replicates in the associated biological replicate group.	Integer
RQ	The relative quantity calculated for the replicate wells of the target/sample combination.	Float
RQ Min	The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.	Float
RQ Max	The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.	Float
Ct Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float
Delta Ct Mean	The average ΔC_t of the replicate wells for the specified target/sample combination.	Float

(continued)

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

7900 export format

The software can export setup and results data from experiment files (EDS) to tab-delimited text files (TXT) in a legacy export format of the Applied Biosystems™ 7900HT Real-Time PCR System. The 7900 export format features a standardized data structure and markup to maximize accessibility by downstream applications. Data exported in the ViiA™ 7 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 ViiA™ 7 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 spftware 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.	"Setup file" on page 200
Multicomponent file	Contains the spectral data used by the software to generate the multicomponent plot that displays the contribution of each dye over the duration of the PCR run.	"Multicomponent file" on page 202
Results file	Contains the normalized, processed, and analyzed data generated by the software.	"Results file" on page 203

Setup file

When setup file is selected as an export option, the 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 ViiA™ 7 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 bar code printed on the plate.	100-character string

```
*** Setup File Version <version number>
*** Output Plate Size <number of wells>
*** Output Plate ID <plate id>
```

Assay (detector) data

The assay data describes the qualities of the target assays present on the consumable. (In the context of the 7900HT System, target assays are referred to as “detectors.”) The section consists of multiple lines that define the total target assays followed by a column header and tab-separated data. The first line defines the total number of target assays on the consumable formatted as follows:

```
*** Number of Detectors <number of assays>
```

The column header defines the columns of exported data followed by one or more lines, where each row defines the properties of a single assay separated by tab characters.

Column	Description	Output
Detector	The name of one target in the well, if applicable. If a well contains multiple targets one row is used per target.	100-character string
Reporter	The reporter dye that labels the probe for the target assay.	100-character string
Quencher	The quencher dye that labels the probe for the target assay.	100-character string
Description	The standard.	1024-character string
Comments	The additional text that describes the well.	1024-character string

Well data

After the assay data, the software exports the well data that describes the configuration of samples and assays on the experiment consumable. The table below describes the well data that can be exported from absolute quantification, relative quantification, or presence/absence experiments. If a well contains more than one assay, the 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 to 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 software can export the data used to generate the multicomponent plot of a real-time PCR experiment. The multicomponent data tracks the raw fluorescence of all reporter dyes present on the reaction consumable throughout the duration of the experiment run.

The file begins with a line that names the export format (SDS 2.3) and the type of data contained by the file (multicomponent). A column header occurs next followed by the multicomponent data, where each row contains the data for a single well separated by tab characters. The multicomponent data contains a dye column for each dye present on the reaction consumable, including reporter dyes, quencher dyes (except non-fluorescent dyes), and the passive reference.

Column	Description	Output
Well	The number of the well on the consumable.	Integer (1 to 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 ViiA™ 7 Instrument recorded the fluorescence data.	Integer
Cycle	The cycle of the run during which the ViiA™ 7 Instrument recorded the fluorescence data.	Integer
<Dye name>	The raw fluorescence for the designated dye measured by the ViiA™ 7 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 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 ViiA™ 7 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 bar code 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 Quantificatio n`
Run Datetime	The date and time that the ViiA™ 7 Instrument finished running the experiment.	< date and time>
Operator	The user logged into the 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 software can export the results data from an analyzed experiment file. The format and content of the results data depends on the experiment type and the analysis settings.

The section begins with a column header followed by the results data, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the 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 Ct experiments" on page 204
- "Genotyping experiments" on page 205

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 to 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`
Ct	The calculated threshold cycle (C_t) for the target at the specified well.	Float
Quantity	• Unknown wells—The calculated quantity for the sample at the well. • Standard wells—The quantity assigned to the standard at the well.	Float
Quantity Mean	• Unknown wells—The average quantity of the replicate wells for the target/sample. • Standard wells—The quantity assigned to the replicate wells for the target/sample.	Float
Quantity SD	The standard deviation of the average quantity of the replicate wells for the target/sample combination.	Float
Ct Median	The median C_t of the replicate wells for the specified target/sample combination.	Float
Ct Mean	The average C_t of the replicate wells for the specified target/sample combination.	Float

(continued)

Column	Description	Output
Ct SD	The standard deviation of the average C_t of the replicate wells for the specified target.	Float
Automatic Ct Baseline	Indicates whether the baseline was determined automatically (true) or manually (false).	`TRUE` or `FALSE`
Baseline Start	The first cycle used to calculate the baseline.	Integer
Baseline End	The last cycle used to calculate the baseline.	Integer
Automatic Ct Threshold	Indicates whether the threshold was determined automatically (true) or manually (false).	`TRUE` or `FALSE`
Ct Threshold	The threshold cycle (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 to 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

(continued)

Column	Description	Output
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 to 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 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 software and third-party applications.

IMPORTANT! The RDML export format is available only for standard curve, gene expression, and relative standard curve experiments.

For more information

The RDML standard is maintained by the RDML consortium, an organization that consists of key developer groups and a member community. For more information on the RDML format, visit the RDM organization website **(www.rdml.org)**. The website features free data management tools, including an on-line RDML file generator and RDML software libraries.

F

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.

(continued)

Symbol and description
CAUTION! Hot surface.
CAUTION! Potential biohazard.
CAUTION! Ultraviolet light.

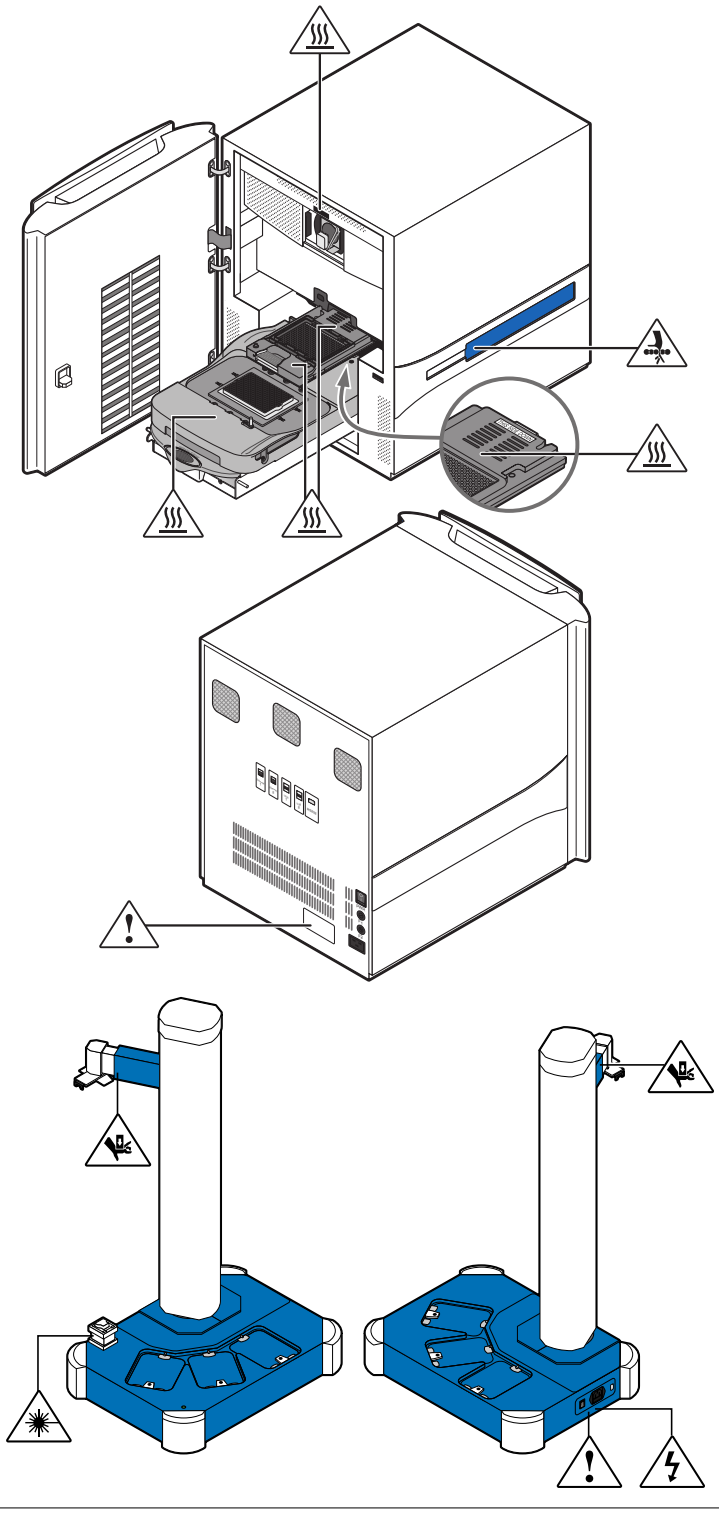
Symbole et description
MISE EN GARDE ! Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
MISE EN GARDE ! Risque de choc électrique.
MISE EN GARDE ! Surface chaude.
MISE EN GARDE ! Danger biologique potentiel.
MISE EN GARDE ! Rayonnement ultraviolet.

Additional safety symbols

Symbol and description
 **CAUTION!** Moving parts.
 **CAUTION!** Moving parts.
 **CAUTION!** Laser radiation.

Symbole et description
 **MISE EN GARDE !** Parties mobiles.
 **MISE EN GARDE !** Parties mobiles.
 **MISE EN GARDE !** Laser radiation.

Location of safety labels

Label and location


Control and connection symbols

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

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.
	Indicates conformity with Australian standards for electromagnetic compatibility.
	Indicates conformity with China RoHS, Environmental Friendly Use Period 25 Years.
	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, you may be exposed to serious hazards including, but not limited to, severe electrical shock.

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.

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—barcode scanner

The optional barcode scanners included with the ViiA™ 7 Real-Time PCR System 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	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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at:
  osha.gov/laws-regs/regulations/standardnumber/1910/1910.1030
- Your company's or institution's Biosafety Program protocols for working with or handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **cdc.gov**
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Pub. No.
Applied Biosystems™ ViiA™ 7 Real-Time PCR System Site Preparation Guide	4445302
Applied Biosystems™ ViiA™ 7 Real-Time PCR System Getting Started Guide	4441434
QuantStudio™ Real-Time PCR Software v1.6.1	
Applied Biosystems™ ViiA™ 7 Real-Time PCR System Quick Reference Guide	MAN0018831
QuantStudio™ Real-Time PCR Software v1.4	
Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security	4442661
Applied Biosystems™ ViiA™ 7 Real-Time PCR System Quick Reference Guide	4448987

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - 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

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Glossary

AIF	See “assay information file (AIF)” on page 224.
AIX	XML version of the assay information file. See also “assay information file (AIF)” on page 224.
allele	In a diploid organism, one of two DNA sequences found at the same locus (for example, a particular gene), but located on homologous chromosomes. Two corresponding alleles may have the identical sequence, or they may differ somewhat, often at one or more single-base sites (SNPs).
amplicon	A segment of DNA amplified during PCR.
amplification	Part of the instrument run in which PCR amplifies the target. Fluorescence data collected during amplification are displayed in an amplification plot, and the data are used to calculate results. **Note:** Only quantitative real-time PCR experiments, not end-point experiments, take amplification data into account.
amplification efficiency (EFF %)	Calculation of the efficiency of the PCR amplification in an experiment. EFF% is calculated using the slope of the regression line in the standard curve. A slope close to –3.32 indicates optimal, 100% PCR amplification efficiency.
amplification plot	Display of data collected during the cycling stage of PCR amplification. The amplification plot can be viewed as: • Baseline-corrected normalized reporter (ΔRn) vs. cycle • Normalized reporter (Rn) 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 225.

assay

In a PCR reaction mix, two target-specific primers or two primers and a probe used to amplify a target.

Assay ID

Identifier assigned by Thermo Fisher Scientific to TaqMan™ assays.

assay information file (AIF)

Tab-delimited data file on a CD shipped with each assay order. The AIF contains technical details about all assays in the shipment. It includes information about assay concentrations, reporters and quenchers used,, part and lot numbers, and assay, vial, and plate ID numbers. The file name includes the number from the bar code on the plate.

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

See also “normalized reporter (Rn)” on page 228.

baseline threshold algorithm

Expression estimation algorithm (C_t) which subtracts a baseline component and sets a fluorescent threshold in the exponential region for gene quantification.

biological replicates

Reactions that contain identical components and volumes, but evaluate separate samples of the same biological source (for example, samples from three different mice of the same strain, or separate extractions of the same cell line or tissue sample).

When an experiments uses biological replicate groups in a gene expression study, the values displayed in the Biological Replicates tab are calculated by combining the results of the separate biological samples and treating this collection as a single population (that is, as one sample). For ΔC_t computations (normalizing by the endogenous control) in a singleplex experiment, the software treats separate biological samples as unpaired data when computing variability estimates of the single biological replicate. Individual contributions of the separate biological samples to the single biological replicate results are observed in the Technical Replicates tab.

See also “technical replicates” on page 234.

blocked IPC In presence/absence experiments, a reaction that contains IPC blocking agent, which blocks amplification of the internal positive control (IPC). In the software, also the name of the task for the IPC target in wells that contain IPC blocking agent. See also “negative control (NC)” on page 228.

calibrator See “reference sample” on page 230.

chemistry See “reagents” on page 230.

C_t See “threshold cycle (Ct)” on page 235.

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 ViiA™ 7 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 235.

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 223.

delta Rn (ΔRn) See “baseline-corrected normalized reporter (ΔRn)” on page 224.

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 223.

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%)” on page 223 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 metadata (name, bar code, comments), plate setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, analysis results, audit records, and other plate-specific data. Experiment documents have the suffixes EDS (experiment document single), EDT (template), and EDM (multiple).

experiment name

Entered during experiment setup, the name that is used to identify the experiment.

Experiment Setup

A software feature that allows you to set up an experiment according to your experiment design. Experiment Setup provides you with maximum flexibility in the design and setup of your experiment.

experiment type

The type of experiment to perform:

- Standard curve
- Comparative C_t ($\Delta\Delta C_t$)
- Relative standard curve
- Genotyping
- Presence/absence
- Melt curve

The experiment type that you select affects setup, run, and analysis.

export

A software feature that allows you to export experiment setup files, experiment results, instrument information, and security and auditing settings to spreadsheet, presentation, or text files. You can edit the default location of the exported file.

filter

Dye excitation and emission filter combination that you select for an experiment. The ViiA™ 7 System includes a six-color filter set that supports FAM™, NED™, ROX™, SYBR™ Green, TAMRA™, and VIC™ dyes.

flag	A quality control (QC) indicator which, when applied by the software to a well during analysis, indicates a possible issue with that reaction. For example, a flag may be issued if no amplification is detected in a well. Flags indicating potential problems are displayed in the Quality Control tab of the plate layout, well table, and QC Summary screens.
forward primer	Oligonucleotide that flanks the 5' end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.
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.
housekeeping gene	A gene that is involved in basic cellular functions and that may be constitutively expressed. Housekeeping genes may be candidates for use as endogenous controls; however, their constancy should always be validated experimentally.
import	A software feature that allows you to import plate setup information or security settings before an experiment run. You can also import information into some libraries in the system.
Instrument Console	A software feature that allows you to view information about instruments on the network. In the Instrument Console, you can monitor the status of any instrument on the network; view calibration, maintenance, and instrument properties for a selected instrument; and open and close the instrument drawer.
Instrument Manager	A software feature that allows you to view information about instrument available on the network. In the Instrument Manager, you can monitor the status of an instrument; monitor amplification plots and temperature plots in real time; view the calibration status, perform calibrations and manage files on the instrument, including downloading completed experiments to your computer.
internal positive control (IPC)	In presence/absence experiments, a short synthetic DNA template that is added to PCR reactions. The IPC can be used to distinguish between true negative results (the target is absent in the samples) and negative results caused by PCR inhibitors, incorrect assay setup, or reagent or instrument failure.
IPC	See "internal positive control (IPC)" on page 227.
IPC+	See "negative control (NC)" on page 228
melt curve stage	In the thermal profile, a stage with a temperature increment to generate a melt curve.

melting temperature (Tm)	The temperature at which 50% of the DNA is double-stranded and 50% of the DNA is dissociated into single-stranded DNA. In a melt curve experiment, the melt curve plot displays the melting temperature.
melting transition region	In Melt Curve experiments, the region before and after the “melting temperature (Tm)” on page 227.
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).
no template control (NTC)	See “negative control (NC)” on page 228.
nonfluorescent quencher-minor groove binder (NFQ-MGB)	Molecules that are attached to the 3’ end of TaqMan™ probes. When the probe is intact, the nonfluorescent quencher (NFQ) prevents the reporter dye from emitting fluorescence signal. Because the NFQ does not fluoresce, it produces lower background signals, resulting in improved precision in quantification. The minor groove binder (MGB) increases the melting temperature (T_m) of the probe without increasing its length, allowing for the design of shorter probes.
normalization calibration	Type of calibration in which the software collects data from the normalization standards, then stores it in a normalization calibration file. This file is used in comparisons of data from multiple instruments within a study.
normalized quantity	Either the C_t Avg. of the target gene minus the C_t Avg. of the endogenous control (Comparative C_t experiments), or the Q Avg. of the target divided by the Q Avg. of the endogenous control (Relative Standard Curve experiments).
normalized quantity mean	The relative standard curve equivalent of the ΔC_t mean value found in Comparative C_t experiments (computed as the geometric mean).
normalized quantity SE	The relative standard curve equivalent of the ΔC_t SE value found in Comparative C_t experiments (computed as the geometric standard error of the mean).
normalized reporter (Rn)	Fluorescence signal from the reporter dye normalized to the fluorescence signal of the passive reference dye (usually ROX™ dye on Thermo Fisher Scientific 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.
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 234.
quantification cycle (Cq)	The fractional PCR cycle used for quantification, according to the “Real-time PCR Data Markup Language (RDML)” on page 230 data standard. C_t and C_{rt} are the algorithm-specific calculations of C_q.
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.
R^2 value	Regression coefficient calculated from the regression line in the standard curve. An important quality value, the R^2 value indicates the closeness of fit between the standard curve regression line and the individual C_t data points from the standard reactions. A value of 1.00 indicates a perfect fit between the regression line and the data points.
raw data plot	A plot of raw fluorescent signal as detected through each emission filter, used to view raw data for individual wells and at individual cycles.
reaction mix	A solution that contains all components to run the PCR reaction, except for the template (sample, standard, or control). Also called a "PCR cocktail".
reagents	The PCR reaction components used to amplify the target and to detect amplification.
real-time PCR	Process of collecting fluorescence data during PCR. Data from the real-time PCR are used to calculate results for quantification experiments or to troubleshoot results for genotyping or presence/absence experiments.
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 **thermofisher.com** for an 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.

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 231.

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.

replicate group A user-defined biological grouping. A replicate group may be a set of identical reactions in an experiment.

replicates Total number of identical reactions containing identical components and identical volumes.

reporter A fluorescent dye used to detect amplification. With TaqMan™ reagents, the reporter dye is attached to the 5’ end. With SYBR™ Green reagents, the reporter dye is SYBR™ Green dye. SYBR™ and HRM-specific dyes are DNA-binding dyes.

reverse primer An oligonucleotide that flanks the 3’ end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.

reverse transcriptase An enzyme that converts RNA to cDNA.

Rn See “normalized reporter (Rn)” on page 228.

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 230.

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.

security, auditing and eSignature
An optional software module that provides:

- **System Security** – Controls user access to the software. A default Administrator user account is provided, and you can define additional user accounts and permissions.
- **Auditing** – Tracks changes made to library items, actions performed by users, and changes to the Security and Audit settings. The software automatically audits some actions silently. You can select other items for auditing and specify the audit mode. Provides reports for audited library items, Security and Audit changes, and actions.
- **Electronic Signature (eSignature)** – Controls whether users are permitted, prompted, or required to provide a user name and password when accessing certain software features. You can select which features are controlled and the number of signatures required for access. When authorized persons use this feature, they are creating a legally binding signature.

serial factor
In the software, a numeric value that defines the sequence of quantities in the standard curve. The serial factor and the starting quantity are used to calculate the standard quantity for each point in the standard curve. For example, if the standard curve is defined with a serial factor of 1:10 or 10×, the difference between any 2 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 223 and “regression line” on page 231.

stage
In the thermal profile, a group of one or more steps. Examples: PCR stage, cycling stage (also called amplification stage), and hold stage.

standard A sample that you dilute and amplify along with unknown samples. This dilution series can contain known starting quantities of the target of interest (absolute standard curve) or it can be of known dilution factor (relative standard curve). Following the run, the software interpolates the C_t values of the unknowns to this curve, yielding either specific quantities of the target (for absolute curves) or relative quantities (for relative dilution curves).

See also “standard curve” on page 233.

standard curve In standard curve and relative standard curve experiments:

- The best-fit line in a plot of the C_t values from the standard reactions plotted against standard quantities.
- 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” on page 232 and “standard curve” on page 233.

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 233.

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 ViiA™ 7 System. Before you use system dyes in your experiments, make sure the system dye calibration is current in the Instrument Console.

The system dyes are:

- FAM™ dye
- JOE™ dye
- ROX™ dye
- NED™ dye
- SYBR™ Green dye
- TAMRA™ dye
- VIC™ dye

TaqMan™ reagents

PCR reaction components that consist of primers designed to amplify the target and a TaqMan™ probe designed to detect amplification of the target.

target

The nucleic acid sequence to amplify and detect.

target color

In the software, a color assigned to a target to identify the target in the plate layout and analysis plots.

task

In the software, the type of reaction performed in the well for the target or SNP assay. Available tasks:

- Unknown
- Negative Control
- Standard (standard curve and relative standard curve experiments)
- Positive control (genotyping experiments)
- IPC (presence/absence experiments)
- Blocked IPC (presence/absence experiments)

technical replicates

Wells containing identical reaction components, including sample; important for evaluating precision.

temperature plot

In the software, a display of temperatures for the instrument cover and instrument block during the instrument run.

template

The type of nucleic acid to add to the PCR reaction.

template file

A user-created file that contains experiment setup information (experiment type, sample names, target name, and thermal conditions) to be used as a starting point for new experiment setup. Template files have an EDT extension.

thermal profile	Part of the run method that specifies the temperature, time, ramp, and data collection points for all steps and stages of the instrument run.
threshold	• In amplification plots, the level of fluorescence above the baseline and within the exponential growth region. For the Baseline Threshold algorithm, the threshold can be determined automatically or can be set manually. • 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" on page 235 in the "amplification plot" on page 223.
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 ViiA™ 7 System filters on data collected during an experiment.
unknown	In the software, the task for the target or SNP assay in wells that contain the sample being tested. In quantification experiments, the task for the target in wells that contain a sample with unknown target quantities. In genotyping experiments, the task for the SNP assay in wells that contain a sample with an unknown genotype. In presence/absence experiments, the task for the target in wells that contain a sample in which the presence of the target is not known. In melt curve experiments, the task for the target in wells that contain a sample with an unknown melt curve profile.
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.