ViiA™ 7 Real-Time PCR System

QuantStudio™ Real-Time PCR Software v1.6.1

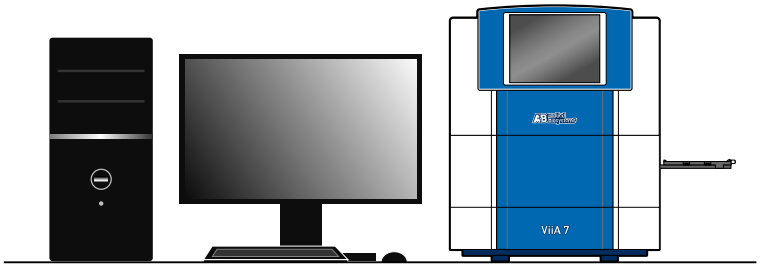
Pub. No. MAN0018831 Rev. C.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security* (Pub. No. MAN0018830). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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).



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.

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

IMPORTANT! The 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. For more information, see the *Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security* (Pub. No. MAN0018830).

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.

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.
	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.

Calibrate the ViiA™ 7 Real-Time PCR System

The ViiA™ 7 Real-Time PCR System uses the following calibration types:

- Regions of interest (ROI)
- Background
- Uniformity
- Dye
- Instrument verification
- *(TaqMan™ Array Card block only)* Normalization

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		
Region of Interest (ROI) and Background Plates, 96-Well 0.2-mL (2 plates)	4432364		
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		

Prepare the calibration plate or array card

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

1. Remove the calibration plate or the calibration solution from the freezer, then allow it to thaw to room temperature in the dark (approximately 5 minutes).

   IMPORTANT! Protect the calibration plate and the calibration solution from light. The fluorescent dyes in the calibration plate wells and calibration solutions are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dye.

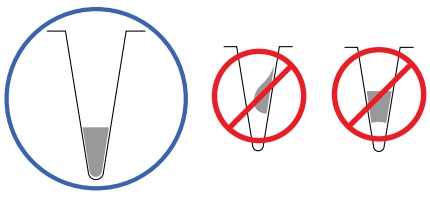
   Do not remove the calibration plate from its packaging until you are ready to run it.

2. Prepare the calibration plate or array card.
   - To prepare an array card, fill it with calibration solution. For a normalization calibration, fill two cards with separate normalization solutions.
   - To prepare a plate:
     a. Remove the plate from its packaging.

        IMPORTANT! Do not discard the packaging. The plate can be used up to three times if it is stored in its original packaging sleeve.

     b. Vortex the plate for 5 seconds, then centrifuge it for 2 minutes.
     c. Verify that the liquid is at the bottom of each well of the plate. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

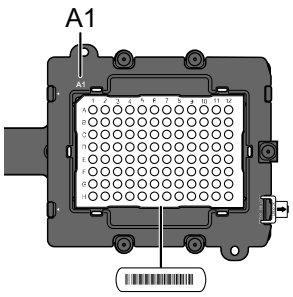
        IMPORTANT! Do not allow the bottom of the plate to become dirty. Fluids and other contaminants that adhere to the bottom of the plate can contaminate the sample block(s) and cause an abnormally high background signal.



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 of instruments, select it, then click **Manage Instrument**.
4. In the Instrument Manager, select the calibration, then click **Start Calibration**.
5. Click **Next,** then perform the calibration as instructed. When the side door opens, load the 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 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.

Review the results and complete the calibration

1. When the run is complete and the software displays the Analysis screen, confirm the analysis status of the calibration, then select the **QC** tab and review the quality check summary. If the run failed, troubleshoot the failed calibration as described in the *Applied Biosystems™ ViiA™ 7 Real-Time PCR System User Guide: Calibration, Maintenance, Networking, and Security* (MAN0018830).

   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.
2. After you inspect results, click **Next**, then remove the plate or array card when the instrument ejects the plate adapter.

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.

3. Click **Finish** to complete the calibration.
4. Discard or store the plate or array card.

Consumable	Action
Array card	Discard the array card.
Plate	Discard the plate, unless it can be reused. If you can store the plate for reuse, return the calibration plate to its packaging sleeve, then return the packaged plate to the freezer.

Maintain the ViiA™ 7 system

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.

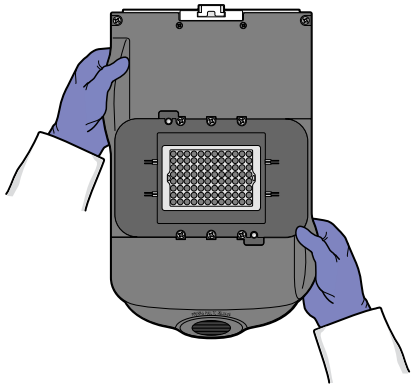
CAUTION! Before using a cleaning or decontamination method other than those recommended in this guide, verify with Thermo Fisher Scientific that the proposed method will not damage the equipment.

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.



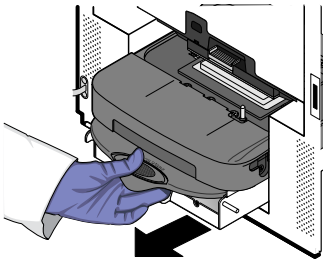
Clean the sample block

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.

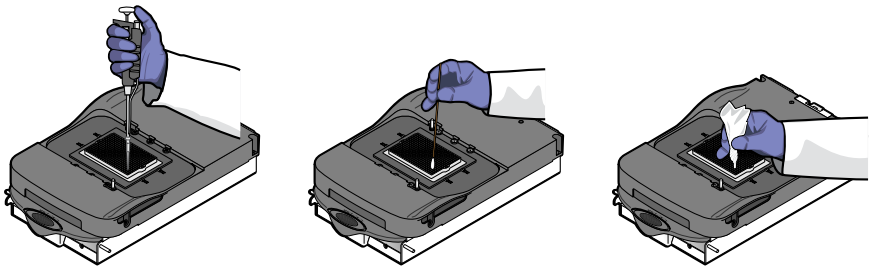
IMPORTANT! Wear powder-free gloves when you perform this procedure.

1. Identify the contaminated wells of the sample block.
2. Power off and unplug the instrument, then allow it to cool for 15 minutes.
3. Open the access door.

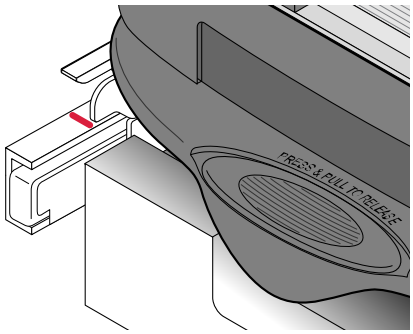
4. Firmly press down on the handle of the sample block, then remove it from the instrument. Place the sample block on a clean, dry surface.



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 sample block

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

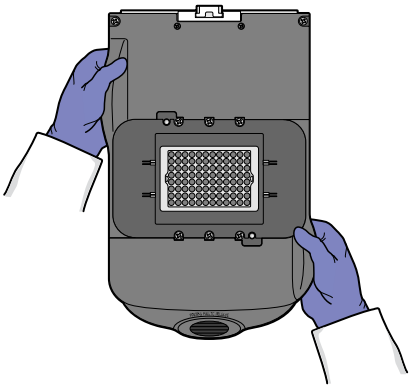
WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the sample block and heated cover can be heated to 100°C. Before removing the sample block, be sure to wait until it reaches room temperature.

Materials required

- Safety glasses
- Powder-free gloves
- Sample block

How to handle the sample block

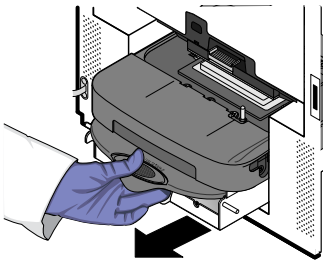
To prevent damaging or contaminating the sample block, handle the assembly as shown below. After the assembly has been removed from the ViiA™ 7 Instrument, place the sample block on a clean, dry surface or in its shipping container.



Replace the sample block

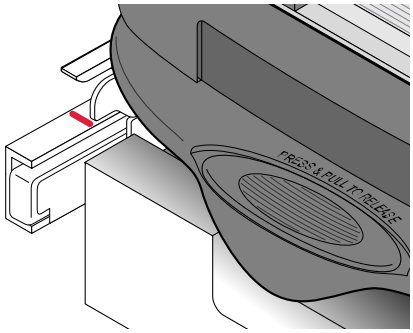
IMPORTANT! If you are installing a sample block of a different format (for example, 96/384-well plate to array card), you must also change the plate adapter to match the new consumable format.

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 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.

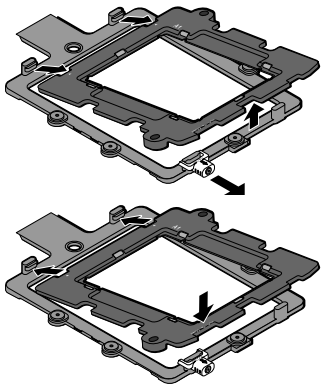
1. Touch the instrument touchscreen to awaken it, then press .
2. In the Main Menu, touch .

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.



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.

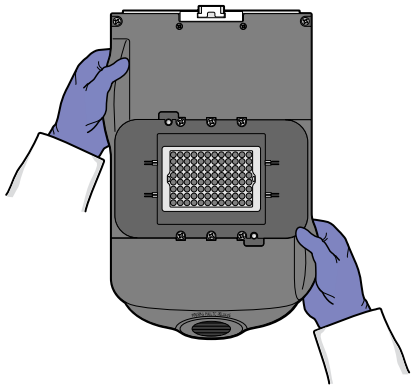
WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the sample block and heated cover can be heated to 100°C. Before removing the heated cover, be sure to wait until it reaches room temperature.

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.



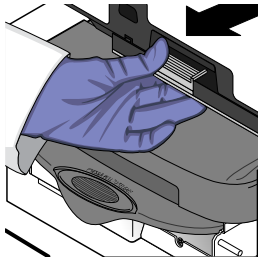
Replace the heated cover

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.

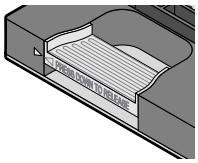
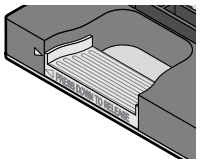
2. Open the access door.

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

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**.

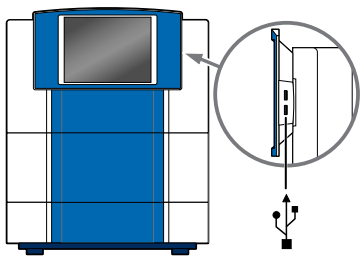
3. In the Self Test screen, touch **Start Self Test**, then wait for the test to complete.

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.

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 the instrument user guide.

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.

Perform an experiment

Materials required

- For all sample blocks:
  - DI water/DEPC water
  - Microcentrifuge tubes
  - Pipettors and pipette tips
  - Vortex mixer
  - Safety goggles
  - Powder-free gloves
  - Sample stock
  - Standard stock
  - Reaction mix components
- For 384-well, 96-well, or Fast 96-well sample blocks:
  - Centrifuge with plate adapter
  - Plate or (96-well and Fast 96-well blocks only) tube strip, or single tube
  - Optical adhesive film
- For array card sample blocks:
  - Centrifuge with centrifuge array-card carrier clips and buckets
  - Array-card staker/sealer fixture
  - Array card

Workflow

Design the experiment (page 13)

▼

"Prepare the reaction plate or array card" on page 14

▼

"Start and monitor the experiment" on page 18

▼

"Unload the plate or array card" on page 19

▼

"View analysis reports" on page 19

▼

"Export results" on page 19

Design the experiment

Define the experiment properties

1. In the Experiment properties screen, define the experiment name and type.
   - Experiment name
   - User name of the experiment owner
   - *(Optional)* Barcode for the plate or array card
   - *(Optional)* Comments
   - Block type: **384-well, Array Card, 96-well (0.2mL),** or **Fast 96-well (0.1mL)**
   - Experiment type: **Standard Curve, Relative Standard Curve, Comparative C_t ($\Delta\Delta C_t$), Melt Curve, Genotyping,** or **Presence/Absence**
2. Select the reagents.

Experiment type	Reagent options
Standard Curve	**TaqMan™ Reagents, SYBR™ Green Reagents,** or **Other**
Relative Standard Curve	**TaqMan™ Reagents, SYBR™ Green Reagents,** or **Other**
Comparative C_t	**TaqMan™ Reagents, SYBR™ Green Reagents,** or **Other**
Melt Curve	**SYBR™ Green Reagents** or **Other**
Genotyping	**TaqMan™ Reagents** or **Other**
Presence/Absence	**TaqMan™ Reagents** or **Other**

   Note: If you select SYBR™ Green as the reagent, then you have the option of including a melt curve for that experiment.

3. Define the instrument run properties
   - Ramp speed: **Standard** or **Fast**
   - (Genotyping and Presence/Absence) Collect data during **Pre-PCR Read, Amplification,** or **Post-PCR Read**
   - (Experiments using SYBR™ Green) Select whether or not to include a melt curve.
   - (Melt Curve) Select whether or not to include PCR.

Define targets, samples, biological replicates, and dye

In the Define screen, define targets, samples, biological replicates, and dye

- (All experiments except Genotyping) Define Targets.
- (Genotyping) Define SNPs.

- (All experiments) Define Samples.
- *(Optional)* (Standard Curve, Relative Standard Curve, and Comparative C_t) Define Biological Replicate Groups.
- (All experiments except Presence/Absence) Select the Passive Reference dye.
- (Relative Standard Curve and Comparative C_t) Select the Reference Sample and the Endogenous Control.

Assign targets and samples

In the Assign screen, assign targets and samples.

- (All experiments except Genotyping) Assign targets, tasks, and samples to wells. Tasks include Unknown, Standard, Positive, and Negative controls, depending on experiment type.
- (Genotyping) Assign SNP assays, tasks, and samples to wells.
- (Standard Curve and Relative Standard Curve) Define and set up standards: Click **Define and Set Up Standards,** select a target, define the standard curve, then select and arrange wells for the standards.
- *(Optional)* (Standard Curve, Relative Standard Curve, and Comparative C_t) Assign Biological Replicate Groups to wells.

Define the run method

1. In the Run method screen, enter the reaction volume:
   - 384-well plate: **1 - 30 µL**
   - Array card: **1µL**
   - 96-well plate: **1 - 200 µL**
   - Fast 96-well plate: **1 - 100 µL**
2. Edit the thermal profile as needed.
   - Add and delete steps or stages.
   - Edit the time, temperature, or ramp rate for a step.
   - Click  to enable or click  to disable data collection.

     Note: For real-time data collection during amplification, change the default analysis settings (Start Cycle and End Cycle) in Preferences.
3. Edit the cycling stages of the thermal protocol as needed.
   - Edit the number of cycles.
   - Enable or disable AutoDelta. For an AutoDelta step, enter the Starting Cycle.
4. For a melt curve stage, select the ramp increment.
   - **Step and Hold:** Click the Step and Hold field, select the minutes or seconds, then use the up or down arrow keys or click the up or down buttons in the field until you reach the desired time.
   - **Continuous** (default): Click 1.6 °C/s (the ramp rate), select the value in the field, then enter the desired ramp rate.

Save the file

After you design an experiment, you can also save the experiment as a template, then create experiments from the template using QuickStart.

Prepare the reaction plate or array card

IMPORTANT! Wear powder-free gloves when you handle the plate or array card.

Guidelines for preparing the reaction plate or array card

- Include excess volume in your calculations to provide for loss during reagent transfers.
- Use TE buffer or water to dilute the standards and samples.
- Prepare the reagents according to the manufacturer's instructions.
- Keep the dilutions and assay mix protected from light, in the freezer, until you are ready to use it. Excessive exposure to light may affect the fluorescent probes or dyes.
- To prepare the reactions:
  - Mix the master mix thoroughly by swirling the bottle.

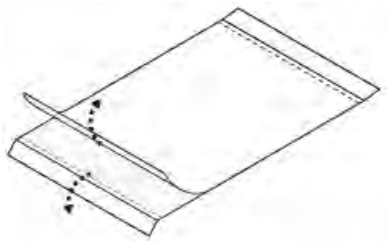
- Resuspend the assay mix by vortexing, then centrifuge the tube briefly.
- Thaw any frozen samples, resuspend them by vortexing, then centrifuge the tubes briefly.

- (*Genotyping only*) Prepare the reactions for each SNP separately
- Place the reaction plate or array card at 4°C in the dark until you are ready to load it into the instrument.

Prepare the reaction plate

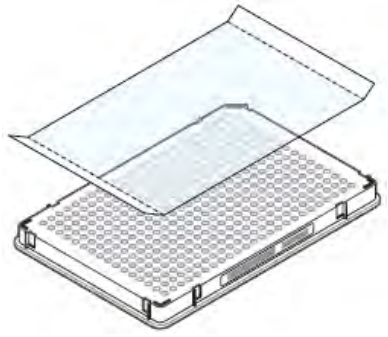
1. Load the plate with the prepared reactions.
2. Remove a single optical adhesive film from the box and bend both end-tabs upward. Hold the film backing side up.
3. In one swift movement, peel back the white protective backing from the center sealing surface. Do not touch the center sealing surface.

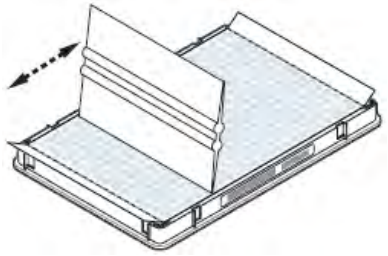
   IMPORTANT! Improper peeling of the optical adhesive film may result in haziness, but it will not affect results. Haziness disappears when the film comes into contact with the heated cover in the instrument.



4. Holding the film by the end-tabs, lower the film onto the reaction plate (adhesive side facing the reaction plate). Be sure that the film completely covers all wells of the reaction plate.
5. While applying firm pressure, move the applicator slowly across the film, horizontally and vertically, to ensure good contact between the film and the entire surface of the reaction plate.
6. While using the applicator to hold the edge of the film in place, grasp one end of the end-tab and pull up and away sharply. Repeat this step for the other end-tab.
7. To ensure a tight, evaporation-free seal, repeat step d on page 15: While applying firm pressure, run the edge of the applicator along all four sides of the outside border of the film.

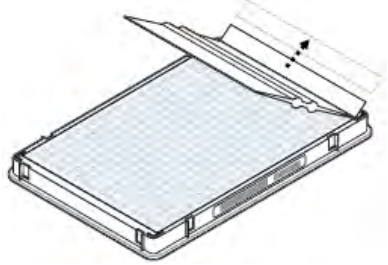
   Note: Optical adhesive films do not adhere on contact. The films require the application of pressure to ensure a tight, evaporation-free seal.

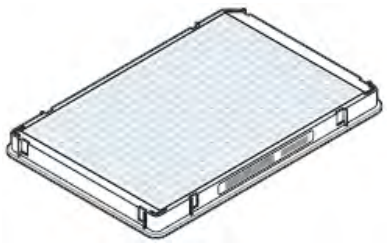




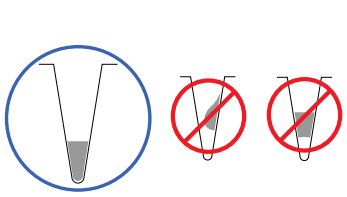
8. Inspect the reaction plate to be sure that all wells are sealed. You should see an imprint of all wells on the surface of the film. Check for the perforated tab to be completely torn off to avoid plates sticking to the instrument after a run.

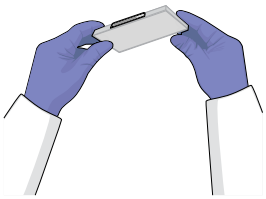
   IMPORTANT! Remove all excess adhesive from the perimeter of the optical adhesive cover. When the film is applied, the glue from the optical adhesive cover can adhere to the edges of the plate. If the excess glue is not removed, the plate may adhere to the sample block of the ViiA™ 7 System.

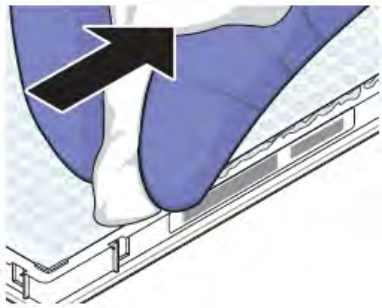




9. Centrifuge the plate for 2 minutes, then verify that the liquid is at the bottom of each well of the plate. If not, centrifuge the plate again at a higher rpm and for a longer period of time.







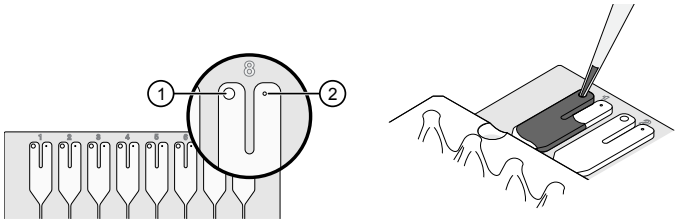
IMPORTANT! Do not allow the bottom of the plate to become dirty. Fluids and other contaminants that adhere to the bottom of the plate can contaminate the sample block(s) and cause an abnormally high background signal.

Prepare the array card

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

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

2. For each chemical and solution transferred, pipette 100 µL 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.

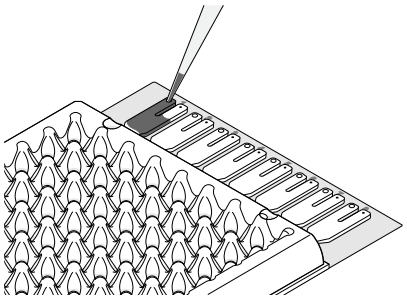


① Fill port ② Vent port

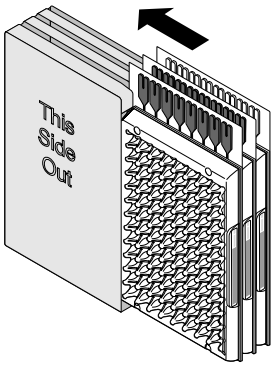
   d. Dispense the fluid so that it sweeps in and around the fill reservoir toward the vent port.

   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.

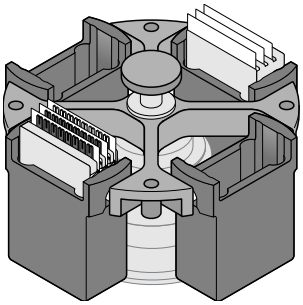
   IMPORTANT! Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.



3. 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.



4. 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.

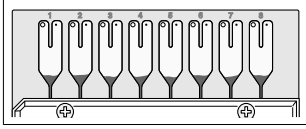
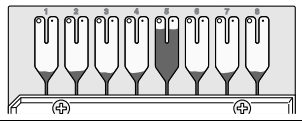
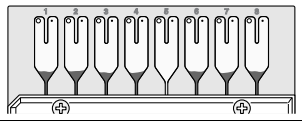
IMPORTANT! Balance the loads in opposite buckets in the centrifuge.

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

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

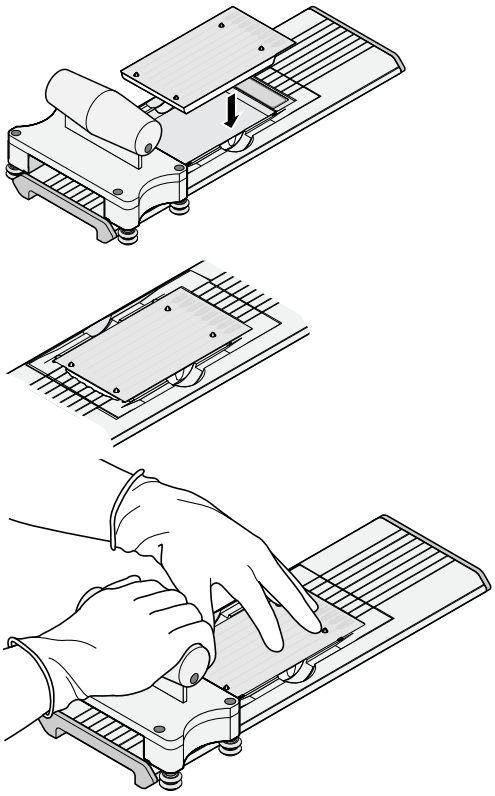
IMPORTANT! Do not try to save time by doing one spin for 2 minutes. The two sets of ramps are important for a good fill into the array card.

7. 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.

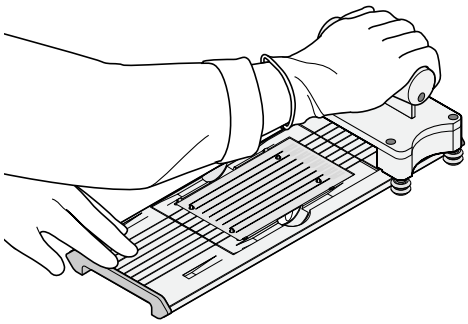
8. Seal the array card(s):
   a. With the carriage (roller assembly) of the Array Card Staker/Sealer in the Start position, place a filled array card into the fixture with the foil side up so that the fill reservoirs are the farthest away from the carriage.
   b. Press down on all four corners of the array card to ensure that it is fully seated within the fixture.
   c. Use the two alignment pins in the fixture to position the array card correctly.

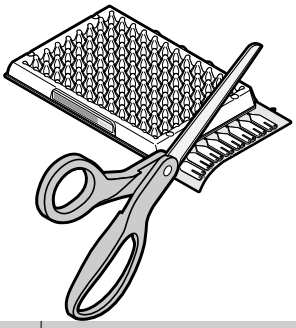


d. Seal the array card by running the carriage slowly over it. Run the carriage over the array card in one direction only. Do not apply downward force on the carriage as you move it forward over the card.

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.





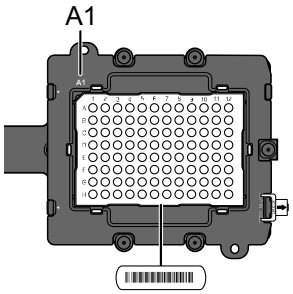
Correct trim	Incorrect trim
	

IMPORTANT! Keep the filled array card our of the light. The fluorescent dye in the array card is photosensitive. Prolonged exposure to light can diminish the fluorescence of the dye.

Start and monitor the experiment

1. Touch on the instrument touchscreen to eject the plate adapter.

2. Place the plate or array card on 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.

3. Touch to load the plate.

4. Start the experiment in the QuantStudio™ Real-Time PCR Software:
   a. In the Experiment menu, click **Run** in the Experiment menu.
   b. Click **START RUN**, then select the instrument from the drop-down menu.

IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the ViiA™ 7 System is in operation.

5. *(Optional)* Monitor the experiment.

Unload the plate or array card

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.

Touch , remove the plate or array card from the plate adapter, then touch to retract the plate adapter.

If the ViiA™ 7 System does not eject the plate, remove the plate as follows:

1. Power off and unplug the ViiA™ 7 System.
2. Wait for 15 minutes, then power on the ViiA™ 7 System and eject the plate.
3. If the plate does not eject, power off the ViiA™ 7 System, then open the instrument door.
4. While wearing powder-free gloves, reach into the ViiA™ 7 System and remove the plate from the heated cover, then close the instrument door.
5. Perform a background calibration to confirm that the sample block has not been contaminated.

View analysis reports

Plot	Applicable experiment type	Purpose
Amplification	All experiment types (if the experiment includes amplification)	• To set baseline and threshold • To compare the data on a per well basis
Multicomponent	All experiment types (if the experiment includes amplification)	To review data for anomalies
Raw Data	All experiment types	To review data for anomalies
QC Summary	All experiment types	To view flags triggered by experiment data
Well Table	All experiment types	To identify well problems
Gene Expression	Comparative C_t and Genotyping	To view the genotypes identified in the experiment
QC Plot	Comparative C_t, Relative Standard Curve	To view the Endogenous Control Profile for an experiment
Presence/Absence	Presence/Absence	To confirm the presence or absence of a target gene
Standard Curve	Standard Curve, Relative Standard Curve	To confirm that the r2 value is acceptable
Derivative Melt Curve	Melt Curve and any other type, when the experiment includes a melt curve	To review melt curve data

Export results

1. From the experiment file, click **Export.**
2. Select the type and format of the file to which data will be exported:
   - ViiA™ 7
   - 7900
   - RDML
3. Select to export all data in one file or in separate files for each data type.
4. Select the contents of the file. Include any of the following information in your export file:
   - Sample setup data
   - Raw data
   - Amplification data
   - Multicomponent data
   - Results

5. Depending on the file type, enter or select export file properties (name, type, and location).

6. *(Optional)* Save export settings. Select the **Save Export Set As** check box and name the settings file. You can select the settings file later when you perform a similar experiment.

7. Click **Start Export.**

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**.

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

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

Revision history: Pub. No. MAN0018831

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	Initial document release for the ViiA™ 7 Real-Time PCR System with QuantStudio™ Real-Time PCR Software v1.6. Based on *Applied Biosystems™ ViiA™ 7 Real-Time PCR System Quick Reference Guide* (Pub. No. 4448987, Rev. C) with a new calibration workflow for plates.