

QuantStudio™ 6 and 7 Flex Real-Time PCR Systems

QuantStudio™ Real-Time PCR Software v1.6.1 or later

Pub. No. MAN0018829 Rev. C.0

Note: For safety and biohazard guidelines, see the “Safety” appendix in the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.6.1 or later) Maintenance and Administration Guide* (Pub. No. MAN0018828). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Power on the QuantStudio™ 6 or 7 Flex Real-Time PCR System

To power on the QuantStudio™ 6 or 7 Flex System from a powered-off state:

1. Tap anywhere on the touchscreen to determine if the QuantStudio™ 6 or 7 Flex Real-Time PCR System is in standby mode.
2. Power on the system. Toggle the power button on the rear of the system, then wait for it to start.

The system is ready to use when the touchscreen displays the main menu.

3. Power on the monitor.
4. Power on the computer:
 - a. Press the computer power button, then wait for it to start.
 - b. When the **Login** screen appears, enter your user name and password, then click **OK**.
5. Start the QuantStudio™ Real-Time PCR Software:
 - a. On the desktop, double-click **QuantStudio™ Real-Time PCR Software**.

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 instrument control 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**.

Calibrate the instrument

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

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

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 or later. For more information, see the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.6.1 or later) Maintenance and Administration Guide* (Pub. No. MAN0018828).

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

Calibration and maintenance schedule

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

IMPORTANT! Perform calibrations and run experiments under the environmental conditions specified in the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.6.1 or later) Maintenance and Administration Guide* (Pub. No. MAN0018828). Exposure to extreme temperatures can have adverse effects on the run results, as well as shortening the life span of the instrument components.

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

Frequency	User-performed maintenance task
Weekly	Check the computer disk space. If necessary, archive or back up your experiment files and instrument settings.
	Power off the computer that controls the instrument, then power on the computer after 30 seconds.
	Clean the surface of the instrument with a lint-free cloth.
Monthly	Perform a background calibration. ^[1]
	Run disk cleanup and disk defragmentation.
	Perform an instrument self test.
Semi-annually (every 6 months)	Perform an ROI calibration.
	Perform a background calibration. ^[1]
	Perform a uniformity calibration.
	Perform a dye calibration.
	(<i>TaqMan™ Array Card block only</i>) Perform a normalization calibration. ^[2]
As needed	Perform an RNase P instrument verification run.
	Replace the instrument lamp. ^[3]

^[1] You can perform a background calibration to check for contamination.

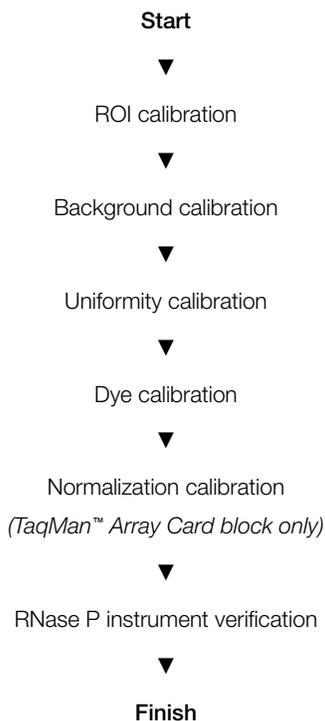
^[2] A normalization calibration is not required for the 96-well plate blocks (0.1-mL or 0.2-mL) or the 384-well plate block.

^[3] After replacing the instrument lamp, perform all calibrations and an RNase P instrument verification run.

Calibration workflow

Whether you are performing all calibrations or just a subset, perform calibrations for the QuantStudio™ 6 and 7 Flex Systems in the following sequence. See the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.6.1 or later) Maintenance and Administration Guide* (Pub. No. MAN0018828).

Note: A normalization calibration is not required for the 96-well plate blocks (0.1-mL or 0.2-mL) or the 384-well plate block.



Guidelines for preparing calibration plates and array cards

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

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

Consumable	Time to thaw	After thawed, run within...
All 96-/384-well calibration consumables ^[1]	30 minutes	120 minutes
All array card calibration consumables ^[2]	30 minutes	120 minutes
RNase P plate (96-/384-well)	5 minutes	30 minutes
RNase P array card ^[3]	15 minutes	60 minutes

^[1] All 96-well plates and 384-well plates used in the calibration of the instrument (ROI, background, uniformity, and dye).

^[2] All array cards used in the calibration of the QuantStudio™ 7 Flex System only (ROI, background, uniformity, dye, and normalization).

^[3] For use with the QuantStudio™ 7 Flex System only.

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

Filling the calibration array cards (*QuantStudio™ 7 Flex System only*)

For detailed instructions to create the array cards required to calibrate the QuantStudio™ 7 Flex System, see *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.6.1 or later) Maintenance and Administration Guide* (Pub. No. MAN0018828).

For the general procedure to fill and seal array cards, see “Fill the TaqMan™ Array Card (QuantStudio™ 7 Flex System only)” on page 12.

Note: Not all array cards are required for a monthly maintenance. Before preparing array cards for calibration, determine which calibrations are required (see “Calibration and maintenance schedule” on page 2).

Prepare the calibration plates

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

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

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

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

3. Vortex and centrifuge the plate:

- a. Vortex the calibration plate for 5 seconds.
- b. Centrifuge the plate for 2 minutes at < 1500 rpm.

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

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

Correct	Incorrect
 <p>Liquid is at bottom of well.</p>	 <ul style="list-style-type: none">• Not centrifuged with enough force, or• Not centrifuged for enough time.

Perform the calibration

The following procedures are general instructions for performing an instrument calibration. For specific instructions, see the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.6.1 or later) Maintenance and Administration Guide* (Pub. No. MAN0018828).

A normalization calibration is not required for the 96-well plate block or for the 384-well plate block. A normalization calibration is only required for the TaqMan™ Array Card block. For more information, see “Calibration with QuantStudio™ Real-Time PCR Software v1.6.1 or later” on page 1.

1. In the **Home** tab, click **Instrument Console**.
2. In the **Instrument Console**, select the icon for your instrument, then click **Manage Instrument**.
3. From the **Instrument Manager**, start the calibration:
 - a. Click  **Maintenance**, then select the calibration.
 - b. From the **Calibration** screen, click **Start Calibration**.
4. Click **Next**, then prepare for the calibration as instructed.
5. In the bottom of the **Setup** tab, enter the reagent information for the plate or array card that you are using.
6. Load the calibration plate or array card into the instrument:
 - a. From the instrument touchscreen, tap  to eject the instrument tray.

- b. Load the plate or card into the plate holder so that:
 - Well A1 of the plate or array card is in the top-left corner of the plate adapter.
 - The barcode faces the front of the instrument.

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

- c. From the instrument touchscreen, tap  to close the instrument tray.

7. 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.

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

Analysis status	Action
Passed	Click Next .
Failed	Troubleshoot the failed calibration (see <i>QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.6.1 or later) Maintenance and Administration Guide</i> (Pub. No. MAN0018828).

9. Unload the calibration plate or array card:

- a. After the calibration, tap  on the touchscreen to eject the plate or array card.
- b. Remove the calibration plate or array card from the instrument tray.



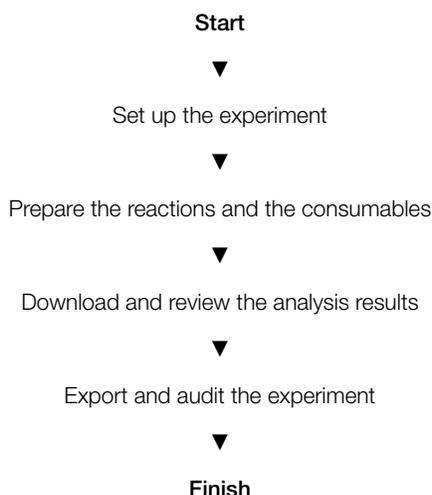
WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the plate or array card temperature can reach 100°C. Allow the consumable to cool to room temperature before removing.

- c. Tap  on the instrument touchscreen to close the instrument tray.

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

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

Experiment workflow



Note: Instructions for the setup, run, and analysis of an experiment vary depending on the specific experiment that you perform. For more information, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).

Set up the experiment

In the **Home** tab, select **Experiment Setup**, then complete the setup screens.

Define the experiment properties

1. In the **Experiment Properties** screen, enter the information identifying your experiment:
 - a. In the **Experiment Name** field, enter up to 100 characters to uniquely identify the experiment.
 - b. *(Optional)* In the **Barcode** field, enter or scan the barcode of the plate or array card you are using to run the experiment.
 - c. *(Optional)* In the **User Name** field, enter up to 100 characters to identify the owner of the experiment.
 - d. *(Optional)* In the **Comments** field, enter up to 2000 characters to associate with the experiment.

2. Select the instrument type you are using to run the experiment:

Option	Description
QuantStudio™ 6 Flex System	Includes a coupled five-color filter set. Supports the 384-well, 96-well Standard, and 96-well Fast sample blocks.
QuantStudio™ 7 Flex System	Includes a decoupled, six by six-color filter set. Supports the 384-well, 96-well Standard, 96-well Fast, and TaqMan™ Array Card sample blocks.

3. Select the block type you are using to run the experiment:

- **96-Well (0.2mL)**
- **Fast 96-Well (0.1mL)**
- **384-Well**
- **Array Card** (*QuantStudio™ 7 Flex System only*)

4. Select the type of experiment to set up:

- **Standard Curve**
- **Relative Standard Curve**
- **Comparative C_t ($\Delta\Delta C_t$)**
- **Melt Curve**
- **High Resolution Melt**
- **Genotyping**
- **Presence/Absence**

Note: High Resolution Melt (HRM) is a separately licensed module. If your experiment requires this module, contact Thermo Fisher Scientific.

5. Select the reagent you are using to detect the target sequence:

- **TaqMan™ Reagents**
- **SYBR™ Green Reagents**
- **MeltDoctor™ HRM Reagents** (*High Resolution Melt experiments only*)
- **Other**

6. Select the run properties:

- Select the ramp speed for the experiment: **Standard** or **Fast**.
- *(Optional)* If you selected:
 - **Melt Curve** or **High Resolution Melt** as the experiment type, then you have the option of including a PCR stage for that experiment.
 - **Genotyping** or **Presence/Absence** as the experiment type, then you have the option of including a Pre-PCR Read and Amplification stage for that experiment.
 - **SYBR™ Green** as the reagent, then you have the option of including a melt curve for that experiment.

7. (Optional) In the reagent information panel, click **New** to add a row for data entry, then enter or scan the detailed information for the reagents that you will use (**Part Number**, **Lot Number**, and **Expiration Date**).

Note: The expiration dates you enter must occur after the current date.

Experiment: **QS6_QuantStudio_384-Well...** Type: **Standard Curve** Reagents: **TaqMan® Reagents**

How do you want to identify this experiment?

* Experiment Name: QS6_QuantStudio_384-Well_Standard_Curve_Example **1a** Comments: Standard Curve Example
 Barcode:
 User Name: Example User

Which instrument type are you using to run the experiment? **2**

QuantStudio™ 6 Flex System **QuantStudio™ 7 Flex System**

Which block are you using to run the experiment? **3**

384-Well **96-Well (0.2mL)** **Fast 96-Well (0.1mL)**

What type of experiment do you want to set up? **4**

Standard Curve **Relative Standard Curve** **Comparative Ct (ΔΔCt)** **Melt Curve**
 High Resolution Melt **Genotyping** **Presence/Absence**

Which reagents do you want to use to detect the target sequence? **5**

TaqMan® Reagents **SYBR® Green Reagents** **Other**

What properties do you want for the instrument run? **6**

Standard **Fast**

What is the reagent information? **7**

New Delete

Type	Name	Part Number	Lot Number	Expiration Date
Master Mix	TaqMan Fast Universal PCR Master Mix	4984571	1206155	12-31-2013

Define the targets, samples, biological replicates, passive reference dye, and controls

- In the **Experiment Menu**, select **Define** in the **Setup** group.
- Define the targets to detect in the experiment:
 - Click **New** to add a new target to the experiment.
 - Define the target properties:
 - In the **Target Name** field, enter a target name.
 - Select a reporter dye and a quencher dye from the **Reporter** and **Quencher** drop-down lists.

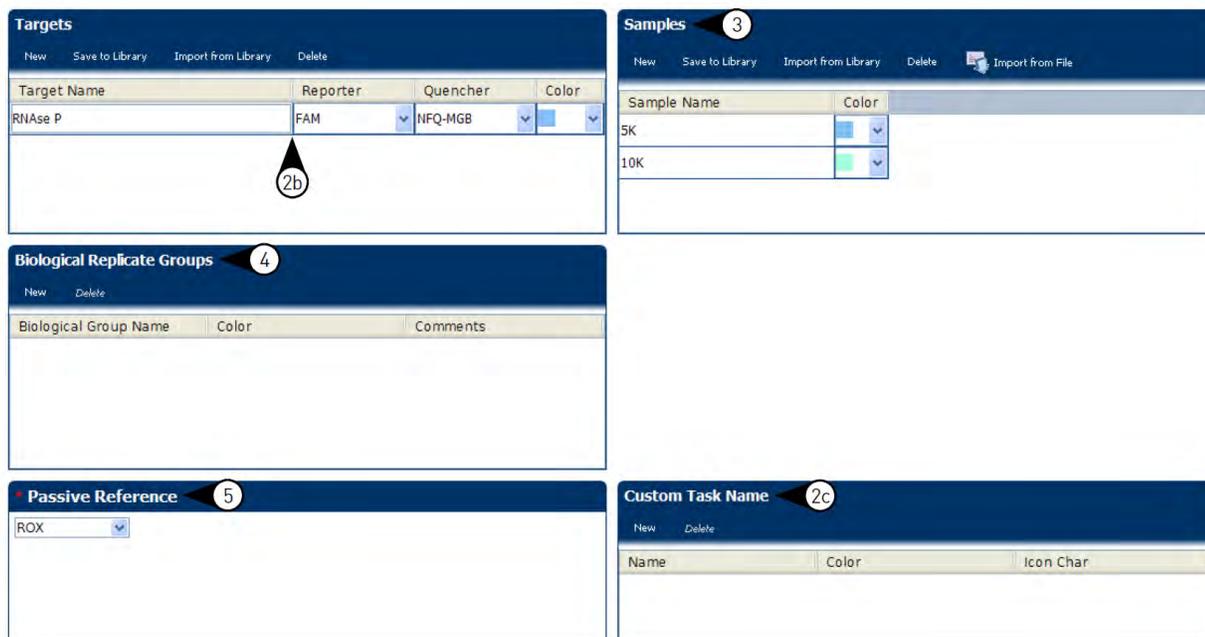
Note: The default reporter and quencher dyes used depend on the reagent selected during experiment setup. For example, if **TaqMan™ Reagents** is the selected reagent, the default reporter is FAM™ dye and default quencher is NFQ-MGB.
 - Select a target color from the **Color** drop-down list.

Note: For information on defining SNP assays to detect in Genotyping experiments, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).
 - (Optional, if selected in **Tools ▶ Preferences ▶ Setup**) Add and define the custom tasks to assign to targets (see *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822)).
- In the **Define** screen, enter or import the names of the samples loaded into the plate or array card.

Do either of the following:

- Click **New** to add a new sample and manually define the sample properties:
 - Enter a sample name in the **Sample Name** field.

- Select a target color from the **Color** drop-down list.
 - (Optional) Add and define a custom attribute for the sample in the **Custom Attribute** field (see *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822)).
- Import the sample data from a sample definition file. Click **Import from File**, select the sample definition file (TXT, XLS, or XLSX) from which you want to import sample data, then click **Open**.
4. (Optional for Standard Curve, Relative Standard Curve, and Comparative C_t ($\Delta\Delta C_t$) experiments) Define the biological replicate groups to use in the experiment:
 - a. Click **New** to add a new biological replicate group to the experiment.
 - b. Define the biological replicate group properties:
 - Enter a biological group name in the **Biological Group Name** field.
 - Select a sample color from the **Color** drop-down list.
 - (Optional) Enter comments about the biological replicate group in the **Comments** field.
 5. (For all experiments except for Presence/Absence) Select a dye from the **Passive Reference** drop-down list. **Passive Reference** drop-down menu.
ROX™ dye is the default selection.



6. (Relative Standard Curve, and Comparative C_t ($\Delta\Delta C_t$) experiments) Select a reference sample and an endogenous control from the **Reference Sample** drop-down list and the **Endogenous Control** drop-down list.
7. (High Resolution Melt experiments) Add and define the controls to use in the experiment (see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822)).

Assign the targets, samples, biological replicates, and controls

1. In the **Experiment** menu, select **Assign** in the **Setup** group.
2. (Standard Curve and Relative Standard Curve experiments) Define and set up standards in the plate or array card:
 - a. Select well(s) using the **Plate Layout** view or the **Well Table** view.
 - b. Click **Define and Set Up Standards**, select a target, define the standard curve, then select and arrange wells for the standards.
For more information, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489823).
3. Assign targets to wells in the plate or array card:
 - a. Select well(s) using the **Plate Layout** view or the **Well Table** view.
 - b. Click a checkbox in the **Targets** list to assign a target to the selected well(s).

- c. Select the detection task for the target from the **Task** drop-down list.

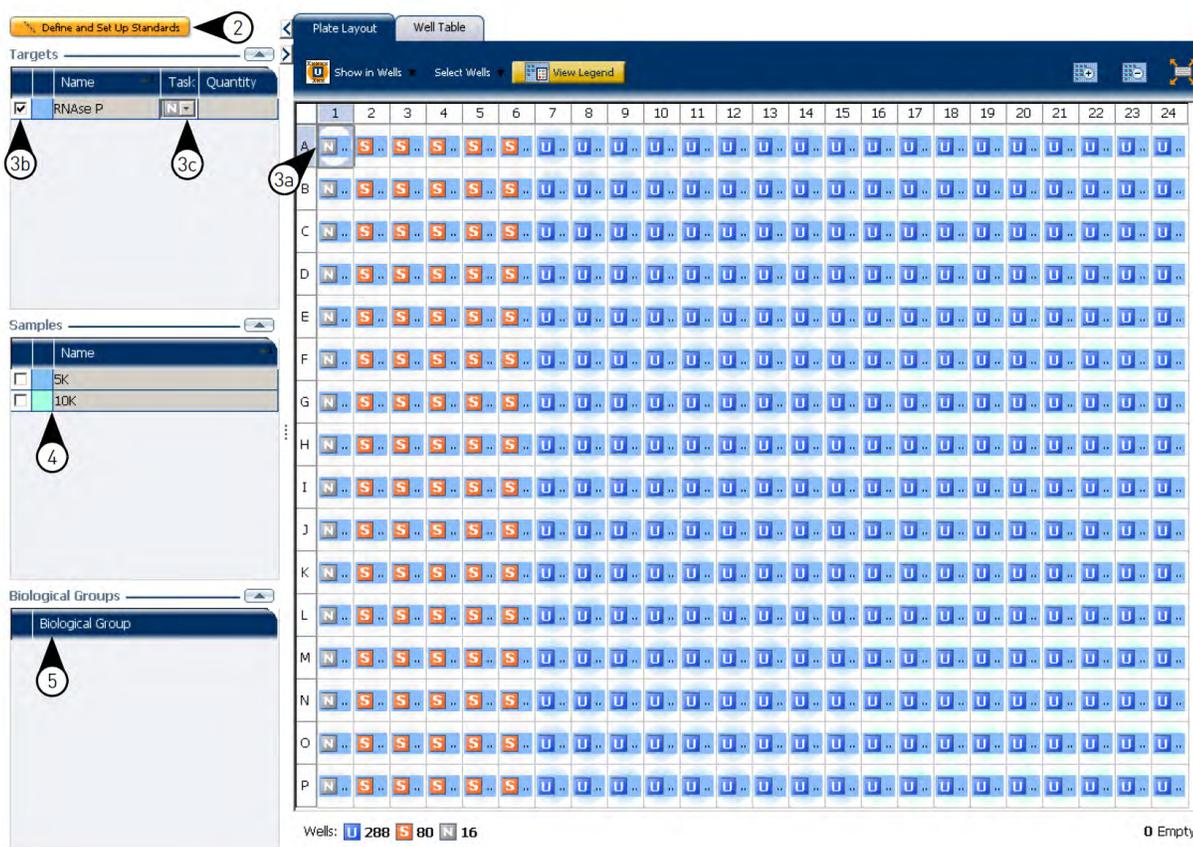
The available tasks include **Unknown**, **Standard**, **Negative Control**, **Positive Control**, and **Custom**. The available tasks depend on the experiment type.

For more information about target tasks, and for information on assigning SNP assays to Genotyping experiments, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489823).

- Assign samples to wells in the plate or array card:
 - Select well(s) using the **Plate Layout** view or the **Well Table** view.
 - Click a checkbox in the **Samples** list to assign a sample to the selected well(s).

IMPORTANT! Do not apply more than one sample to a well.

- (Optional for Standard Curve, Relative Standard Curve, and Comparative C_t ($\Delta\Delta C_t$) experiments) Assign biological replicate groups to wells in the plate or array card:
 - Select well(s) using the **Plate Layout** view or the **Well Table** view.
 - Click a checkbox in the **Biological Groups** list to assign a biological replicate group to the selected well(s).



Define the run method

- In the **Experiment** menu, select **Run**.
- In the **Reaction Volume per Well** field, enter the reaction volume.

Option	Description
96-well Standard plate	1–200 μL
96-well Fast plate	1–100 μL
384-well plate	1–30 μL
TaqMan™ Array Card (QuantStudio™ 7 Flex System only)	1 μL

3. Review the information in the **Graphical View** tab and edit the run method as needed:
 - Add and delete steps or stages
 - Edit the time, temperature, or ramp rate for a step
 - Enable or disable data collection
 - (Cycling stage) Edit the number of cycles and select AutoDelta settings (enable or disable and enter the Starting Cycle)
 - (Melt curve stage) Select the ramp increment (Step and Hold or Continuous)
4. (Optional, if selected in **Tools > Preferences > Defaults**) Select the **Optical Filters** tab and select the PCR (cycling stages only) and Melt Curve (melt stages only) filter set which matches the profile of the dye(s) you have added to the plate or array card.

Save the experiment

Click  **Save** to save the experiment.

You can also save the experiment as a template, then create experiments from the template. For more information, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).

Prepare the reactions

Instructions for the preparation of reactions vary depending on the specific experiment that you perform. For more information, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).

Prepare the plate or the TaqMan™ Array Card

Guidelines to prepare the plate or the TaqMan™ Array Card

- Wear appropriate protective eyewear, clothing, and gloves.
- Use only plates or array cards, reagents, and kits that are approved for use with the QuantStudio™ 6 and 7 Flex Systems.
- Do not allow the consumable bottoms to become dirty.
- Store prepared plates or array cards in the dark until they can be loaded into the instrument.
- (Plates only) Confirm that the liquid in each well of the plate is at the bottom of the well. If not, centrifuge the plate briefly for 2 minutes at <1500 rpm.

Correct	Incorrect
	
Liquid is at bottom of well.	<ul style="list-style-type: none"> – Not centrifuged with enough force, or – Not centrifuged for enough time.

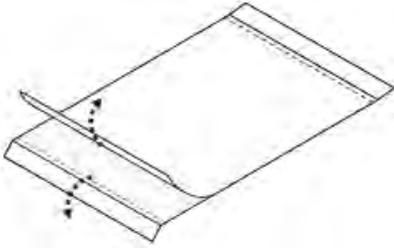
Note: For more information about preparing reaction tubes and tube strips, see *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).

Seal the reaction plate

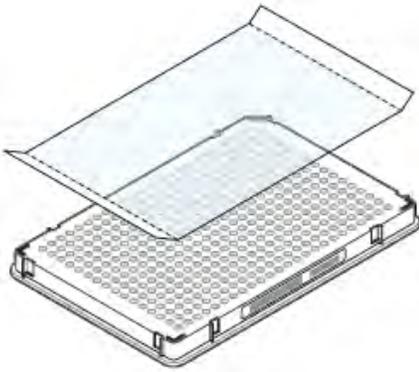
IMPORTANT! Wear powder-free gloves while sealing plates.

1. Load the plate with prepared reactions.
2. Remove a single optical adhesive film from the box. While holding the film backing-side up, bend both tabs upward.

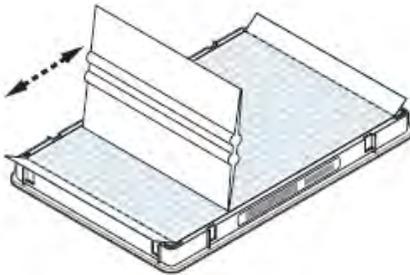
3. In one swift movement, peel back the white protective backing from the center sealing surface. Do not touch the center sealing surface.



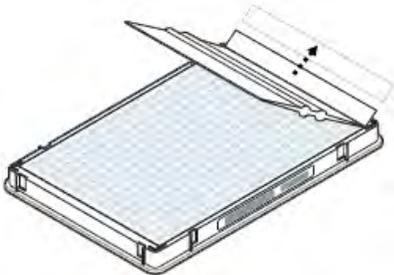
4. While holding the film by the tabs, lower the film onto the reaction plate (adhesive side facing the plate). Make sure that the film completely covers all wells of the reaction plate.



5. While applying firm downward pressure, move the applicator slowly across the film, both horizontally and vertically, to ensure good contact between the film and the entire surface of the reaction plate.



6. Use the applicator to hold the edge of the film in place, then grasp one end of the tab and sharply pull up and away. Repeat the action to remove the other tab.



IMPORTANT! You must cleanly remove each tab along the precut dotted line.

7. Repeat step 5 on page 11 again to ensure a tight, evaporation-free seal. While applying pressure, run the edge of the applicator along all four sides of the outer border of the film.

IMPORTANT! You must apply pressure to the optical film during application to ensure a tight, evaporation-free seal.

8. Inspect the reaction plate to confirm that all wells are sealed. The plate is properly sealed when an imprint of each well is visible on the surface of the film.

9. Use a lint-free wipe to remove all excess glue from around the perimeter of the adhesive film.



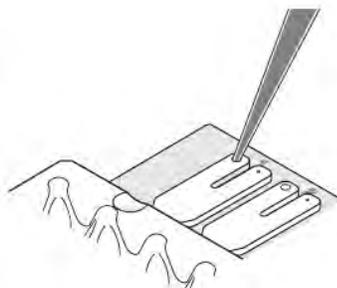
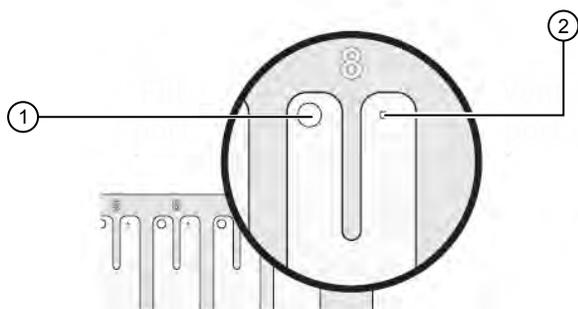
IMPORTANT! You must remove any excess glue from the plate.

Fill the TaqMan™ Array Card (*QuantStudio™ 7 Flex System only*)

IMPORTANT! Wear powder-free gloves while filling cards.

Note: The instructions below describe only the array card loading procedure. For more information, contact Thermo Fisher Scientific.

1. Remove an array card from its box and place it on a clean, dry surface.
2. Pipet 100 μ L of the appropriate 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 μ L of the calibration solution into a pipette.
 - c. Hold the pipette in an angled position (approximately 45 degrees) and place the tip into the fill port. The fill port is the larger of the two holes on the left side of the fill reservoir.



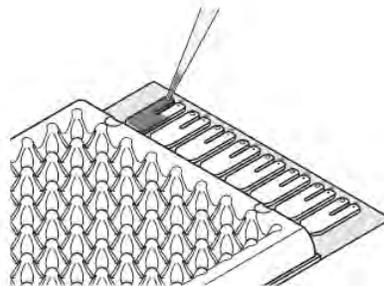
① Fill port

② Vent port

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

Pipet the entire 100 μ L 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.



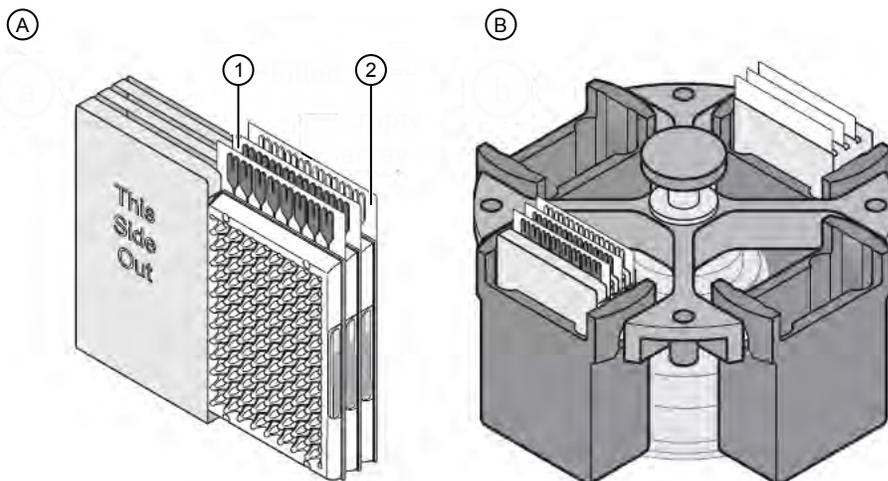
3. Centrifuge the array card:

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

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

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

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



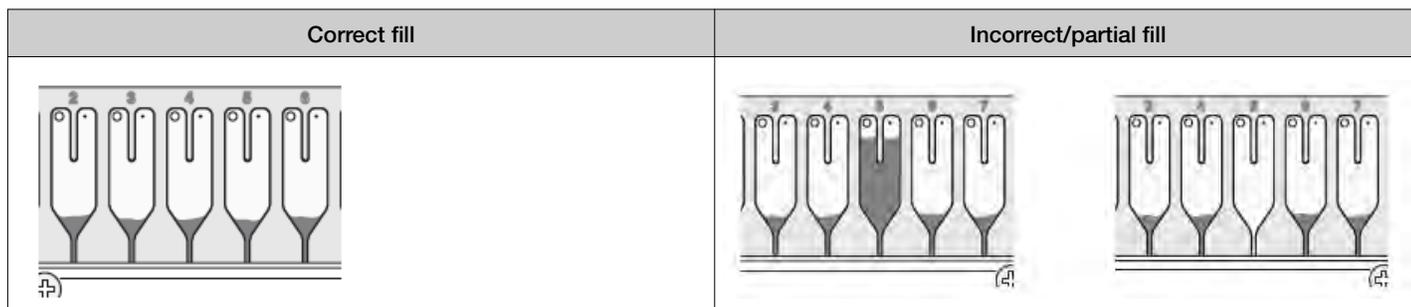
① Filled array card

② Empty array card

- c. Close the centrifuge cover, then spin the array card for 1 minute at 1200 rpm.
 d. When the run is finished, stop the centrifuge, then spin the array card again for 1 minute at 1200 rpm.

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

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

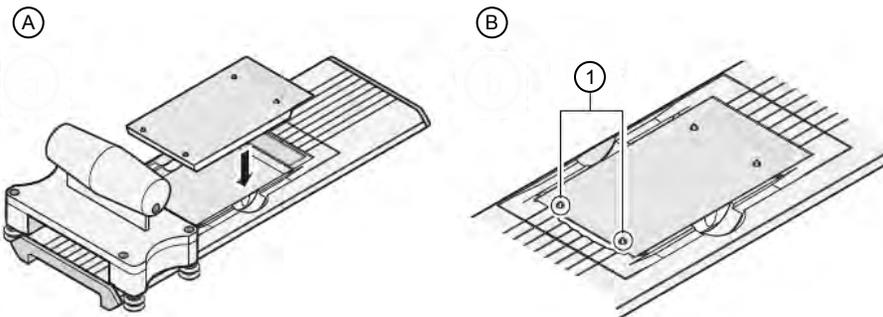


If necessary, centrifuge the cards for an additional minute to fill any unfilled wells. Do not exceed three 1 minute runs or centrifuge the card for longer than 1 minute at a time.

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

Note: In some documents, the TaqMan™ Array Card Sealer is referred to as the Array Card Staker/Sealer.

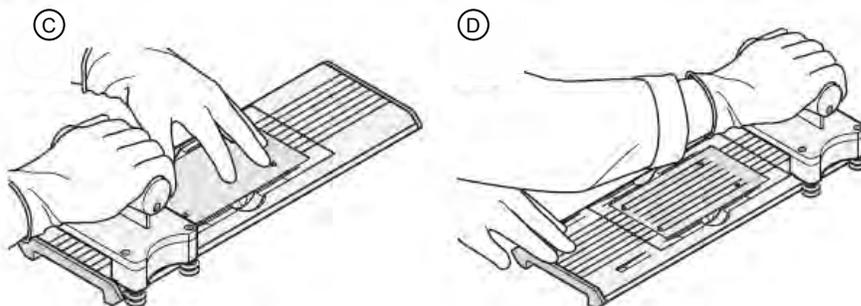
b. Press down on all four corners of the array card to ensure that it is fully seated within the fixture.



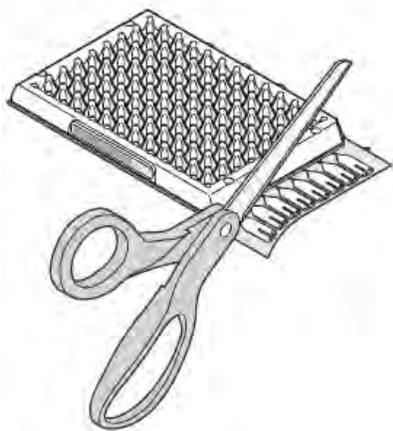
① Pins

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

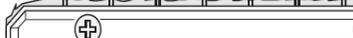
d. Seal the array card by running the carriage slowly over it. Run the carriage over the array card in one direction only. Do not apply downward force on the carriage as you move it forward over the card.



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



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

Correct trim	Incorrect trim
	

IMPORTANT! Store the array card in a dark place until you are ready to load it. Do not expose the array card to light until you are ready to use it. The dyes in the array card is photosensitive. Prolonged exposure to light can diminish the fluorescence of the dye.

Run the experiment

Load the plate or the TaqMan™ Array Card into the instrument

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

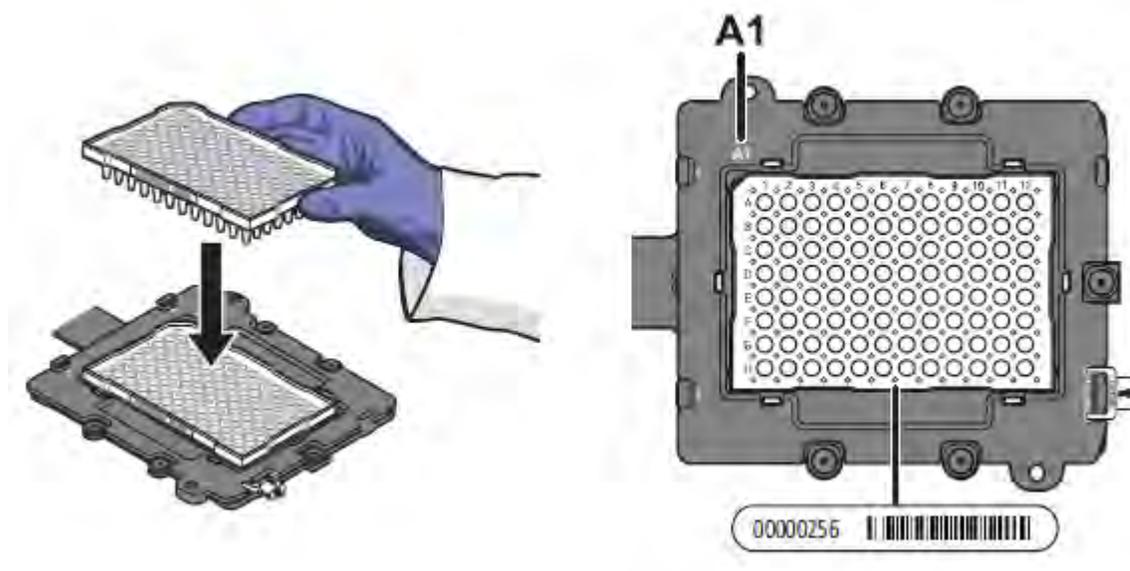
1. Eject the instrument tray.

Option	Description
From the instrument touchscreen	Tap  .
From the QuantStudio™ Real-Time PCR Software	Select Tools ▶ Instrument Console , select the icon for your instrument, then click Open Door .

2. Load the plate or the array card into the plate adapter.

When you load the plate or the array card, ensure that:

- Well A1 is positioned at the top-left corner of the instrument tray.
- The barcode is facing the front of the instrument.



3. Close the instrument tray.

Option	Description
From the instrument touchscreen	Tap  .
From the QuantStudio™ Real-Time PCR Software	Click Close Door .

Start the experiment

IMPORTANT! Perform calibrations and run experiments under the environmental conditions specified in the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems (v1.6.1 or later) Maintenance and Administration Guide* (Pub. No. MAN0018828). Exposure to extreme temperatures can have adverse effects on the run results, as well as shortening the life span of the components.

1. In the QuantStudio™ Real-Time PCR Software **Experiment** menu, click **Run**.

2. Click **START RUN**, then select the instrument to run the experiment from the **My Instruments** drop-down list.

If your instrument is not available, the instrument name will not be displayed in the **My Instruments** drop-down list after you 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.

During the experiment

IMPORTANT! The information in this section provides general guidelines for reviewing the real-time data of experiments as they are being run on the instrument. For detailed instructions to review the data, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).

Monitor the experiment from the instrument

After you start the experiment, you can view the time remaining, time elapsed, and the event log from the instrument touchscreen.

- Tap **Elapsed Time** to display the time elapsed for the run.
- Tap **Remaining Time** to display the time remaining for the run.
- Tap  to close the event list.

Monitor the experiment from the software

1. In the **Home** tab, click **Instrument Console**.
2. In the **Instrument Console** screen, select the instrument icon, then click **Manage Instrument**.
3. In the **Manage Instrument** screen, click **Monitor Running Experiment** to view the **Run** screen.

For instructions on viewing the Amplification Plot and Temperature Plot, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).

After the experiment is complete

Unload the instrument

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

When the instrument displays the **Main Menu** screen, you can unload the plate or the TaqMan™ Array Card.

1. On the instrument touchscreen, tap .
2. Remove the plate or the array card from the instrument tray and dispose of it according to your laboratory regulations.
3. Tap  to close the instrument tray.

Download the completed experiment

If the QuantStudio™ Real-Time PCR Software was closed during the run or if the computer-instrument connection was disrupted, then you can transfer the experiment data to the computer using the software or a USB drive.

Download experiments using the QuantStudio™ Real-Time PCR Software

1. In the home screen, click **Instrument Console**.
2. Select your instrument, then click **Manage Instrument**.
3. Click **Manage Files**, then click **File Manager**.
4. In the **File Manager** screen, download the files:
 - a. In the **Folders** field, select the folder that contains the files that you want to download.
 - b. In the **Experiments** field, select the files to download.
Control + click or shift + click to select multiple files.
 - c. When you have selected the files that you want to download, click **Download**.
 - d. From the **Save** dialog box, select the folder to hold the experiment results and click **Save**.

Download experiments using a USB drive

For more information on using a USB drive to transfer experiment results, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).

Review the results

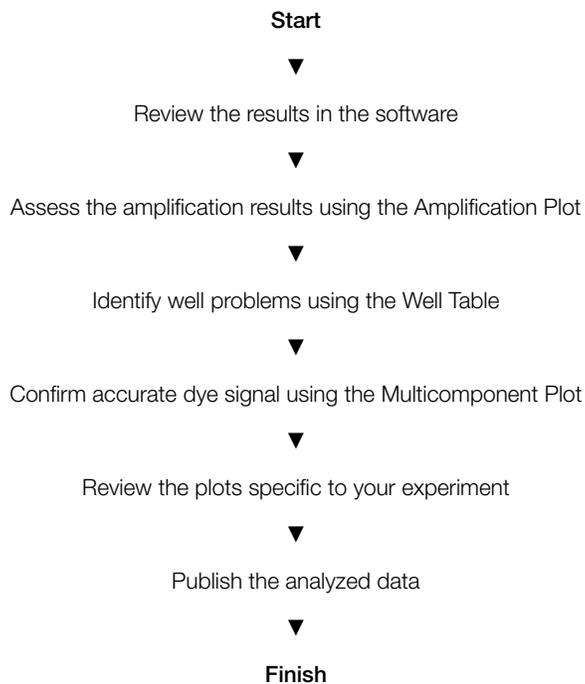
The following information provides general guidelines for reviewing an experiment run on the QuantStudio™ 6 and 7 Flex Systems.

For specific instructions on reviewing experiment data, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).

General analysis workflow

The process for reviewing analysis results can vary significantly and depends on the type of experiment that you perform.

For the workflow specific to your experiment, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).



Review the results in the software

The QuantStudio™ Real-Time PCR Software allows you to control how the plots display the analyzed experiment data. When reviewing the analyzed data, you can perform the actions in the following table.

To...	Action
Display data from specific wells	<ul style="list-style-type: none"> To select wells of a specific type, select Sample, Target, or Task from the Select Wells With drop-down lists, then select the sample, target, or task name. To select a single well, click the well in the plate layout. To select multiple wells, ctrl+click or shift+click the desired wells in the Plate Layout. To select all wells, click the upper left corner of the Plate Layout.
Display multiple plots	<p>You can use the Multiple Plots View screen to display up to four plots simultaneously. To navigate within the Multiple Plots View screen, in the Experiment Menu pane, select Analysis ▶ Multiple Plots View.</p> <ul style="list-style-type: none"> To display four plots, click Show plots in a 2 x 2 matrix. Similarly, click to display two plots in rows, or click to display two plots vertically. To display a specific plot, select the plot from the drop-down list above each plot display.
Display an expanded view of a plot or wells	<ul style="list-style-type: none"> Click to expand the view of a plot, displayed on the left side of the screen. Click to expand the view of the Plate Layout or Well Table displayed on the right side of the screen.
Edit the plot properties	<ol style="list-style-type: none"> Click on the Analysis screen (the icon appears above the plot) to open the Plot Properties dialog box. Edit the settings under the General, X Axis, and Y Axis tabs. <ul style="list-style-type: none"> Select the General tab to edit the plot title text, font, or color. You can also select whether to show the plot title. Select the X Axis tab to: edit the X axis label text, font, or color; select the tick marks and tick mark labels to display; and select the range to display. Select the Y Axis tab to: edit the Y axis label text, font, or color; select the tick marks and tick mark labels to display; and select the range to display. Click OK.
Save the current settings as default	To save a plot setting, select the Save current settings as the default check box on the respective plot screens under Experiment Menu and Analysis .

Assess amplification results using the amplification plot

For more information about reviewing the plot, see *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).

- In the **Experiment Menu** pane, select **Analysis ▶ Amplification Plot**.
- In the **Amplification Plot** screen, select the plot type and well colors.
- View the baseline values:
 - In the **Graph Type** drop-down list, select **Linear**.
 - Select **Baseline** to show the start cycle and end cycle.
 - Review the plot.
- View the threshold values:
 - From the **Graph Type** menu, select **Log**.
 - Select **Threshold** to show the threshold.
 - Review the plot.
- Review the uniformity of the replicate populations:
 - From the **Plot Type** drop-down list, select **Ct vs. Well**.
 - Review the plot.

Identify well problems using the well table

1. In the **Experiment Menu** pane, select **Analysis**, then select the **Well Table** tab.
2. Use the **Group By** drop-down list to group wells by a specific category.

Note: You can select only one category at a time.

To group by...	Action
Replicate	In the Group By drop-down list, select Replicate to group the data by replicate wells (negative controls, standards, and samples).
To group by C _t value	From the Group By drop-down list, select C_t to group the wells by C _t value (low, medium, high, and undetermined).

Confirm accurate dye signal using the multicomponent plot

1. In the **Experiment Menu** pane, select **Analysis** ▶ **Multicomponent Plot**.
2. Display the unknown and standard wells one at a time in the **Multicomponent Plot** screen:
 - a. Click the **Plate Layout** tab.
 - b. Select one or more wells in the plate layout to show the data in the **Multicomponent Plot** screen.

Note: If you select multiple wells, the **Multicomponent Plot** displays the data for all selected wells simultaneously.
3. From the **Plot Color** drop-down list, select **Dye**.
4. Click  to display the plot legend.
5. For each replicate population of unknowns and controls, select the wells in the **Plate Layout**, then confirm that all dye signals behave as expected.

In general, when viewing the **Multicomponent Plot**, review the:

- **Passive reference plots**—The passive reference dye fluorescence level should remain relatively constant throughout the PCR process.
- **Reporter dye plots**—The reporter dye fluorescence level should display a flat region corresponding to the baseline, followed by a rapid rise in fluorescence as the amplification proceeds.
- **Signal irregularities**—The plot should not be any spikes, dips, and/or sudden changes in the fluorescent signal.
- **Negative control wells**—There should not contain any amplification in the negative control wells.

Determine signal accuracy using the raw data plot

1. In the **Experiment Menu** pane, select **Analysis** ▶ **Raw Data Plot**.
2. Click the upper-left corner of the **Plate Layout** to display all wells in the **Raw Data Plot** screen.
3. Click  to display the legend for the plot.

The legend displays the color code for each row of the reaction plate.
4. Click and drag the **Show Cycle** pointer from cycle 1–40 and review the changes in fluorescence during the PCR.

In general, when viewing the raw data, look for:

- Characteristic signal growth
- No abrupt changes or dips in signal

Review plots specific to your experiment

The QuantStudio™ Real-Time PCR Software may allow you to review your analyzed experiment data using additional plots defined by the experiment type. For instructions on using the QuantStudio™ Real-Time PCR Software plots to analyze data specific to the experiment you are running, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).

Publish the analyzed data

You can publish the experiment data from the **Analysis** screen in several ways.

To...	Action
Save a plot as an image file	Click  , then save the image as instructed.
Print a plot	Click  , then print the image as instructed.
Copy a plot to the clipboard	Click  , then paste the image into a compatible application.
Export data	Click  , then export the experiment report as instructed. For more information about the export feature, see the <i>QuantStudio™ Real-Time PCR Software Getting Started Guide</i> (Pub. No. 4489822). IMPORTANT! If you choose the save the results as an export set, you must enter a file name <240 characters.
Print a report	<ol style="list-style-type: none"> 1. Select File ▶ Print Report or click  Print Report. 2. Select data for the report, then click Print Report. <p>The printed report summarizes the data and results for each sample in your experiment. The report can contain a variety of data, depending on your selection. All components of the report are optional.</p>

Export and audit the experiment

Set an experiment for Auto Export

You can configure an experiment to automatically export (Auto Export) the analyzed data to a default location defined in the QuantStudio™ Real-Time PCR Software preferences. You can activate the feature at any time, both before and after an experiment has been run.

1. Open the experiment file that contains the data to export.
2. In the **Experiment Menu**, click  **Export**.
3. In the **Export Experiment** screen, select **Auto Export**.
4. Set up the export file location:
 - a. Select **Tools ▶ Preferences**.
 - b. From the **Preferences** dialog box, select the **Export** tab.
 - c. Select either **Use Last File Location** or **Use Default Folder** and enter a path for the export directory.
 - d. Click **OK**.
5. Click **Start Export**.

The experiment is now configured for automatic export.

After the experiment is run, the software will automatically export the results to the directory defined in the software preferences.

Export an experiment

1. In the **Experiment Menu** of the open experiment document file, click  **Export**.
2. In the **Export Experiment** screen, select to export all data in one file or in separate files for each data type.

Option	Description
One File	All data types are exported in one file. If you select the XLS file format, a worksheet is created for each data type. If you select the TXT file format, the data are grouped by data type.
Separate Files	Each data type is exported in a separate file.

3. (Optional) Select **Open file(s) when export is complete** to automatically open the file when export is complete.

4. Enter a file name and location:
 - Enter a name for the export file in the **Export File Name** field.

IMPORTANT! If you choose to save your experiment results as an export set, you must enter a file name <240 characters.

 - Enter the **Export File Location**. Click **Browse** if you do not want to save the export file in the default export folder.
5. Select the file format for exported data.
 - TXT
 - XLS
 - XLSX
6. Select the data to export.
7. (Optional) After you define the export properties or after you change the table heading order, click **Save Export Set As** to save the settings as an export set. Later, you can import the heading order into another file by clicking **Load Export Set**.
8. Click **Start Export**.

About viewing the audit records for an experiment

Note: Auditing is a separately licensed module. If your system requires this module, contact Thermo Fisher Scientific.

By default, auditing is enabled in the QuantStudio™ Real-Time PCR Software.

For information on selecting actions to audit and creating and editing audit reasons, see the *QuantStudio™ Real-Time PCR Software Getting Started Guide* (Pub. No. 4489822).

View the audit information

1. In the **Experiment Menu**, click  **Audit**, then click **Audit Records**.
2. Select **Filter by** to view specific records.
3. Enter criteria for the records of interest, such as a date range, a user name, record type and name, a reason for audit, and the action required.
4. Click **Refresh**.
5. In the **Audit** screen, click **View Report** to view a report of the audit records.

Power off the QuantStudio™ 6 and 7 Flex Systems

Place the QuantStudio™ 6 and 7 Flex Systems on standby

If left unattended, the system automatically enters standby mode to conserve power. To enter standby mode manually, tap  on the instrument touchscreen.

Power off the QuantStudio™ 6 or 7 Flex Real-Time PCR System

The systems operate in low-power mode when not in use. The system can be powered off completely so that the components draw no power.

1. Power off the QuantStudio™ 6 or 7 Flex Real-Time PCR System:
 - a. If the touchscreen is not blank, tap  to place the system into stand-by mode.
 - b. Toggle the power button on the rear of the QuantStudio™ 6 or 7 Flex Real-Time PCR System.
2. Power off the computer:
 - a. From the desktop, select **Start ▶ Shut Down**.
 - b. From the Shut Down Windows™ dialog box, select **Shut Down**, then click **OK**.
3. Power off the monitor.

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.



Life Technologies Holdings Pte Ltd | Block 33 | Marsiling Industrial Estate Road 3 | #07-06, Singapore 739256
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.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0018829

Revision	Date	Description
C.0	9 July 2020	Updated to QuantStudio™ Real-Time PCR Software to v1.6.1 or later. No changes to workflow or user interface covered in this document. Removed Twister™ Robot.
B.0	14 January 2020	Updated QuantStudio™ Real-Time PCR Software to v1.6.1. No changes to workflow or user interface compared to v1.6.
A.0	4 October 2019	Initial document release for the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems with the QuantStudio™ Real-Time PCR Software v1.6. Based on <i>QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Quick Reference</i> (Pub. No. 4489826, Rev. A) with a new calibrator workflow for plates.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

©2020 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Windows is a registered trademark of Microsoft Corporation. Twister is a registered trademark of Caliper Life Sciences, Inc. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license.