applied biosystems by life technologies

QuantStudio™ 6 and 7 Flex Real-Time PCR Systems

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Note: For safety and biohazard guidelines, refer to the "Safety" section in the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide*. For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

The following topics are covered in this quick reference guide:

Start the QuantStudio™ 6 and 7 Flex Systems

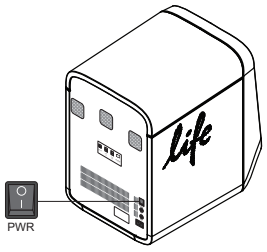
1. Touch anywhere on the touchscreen to determine if the instrument is in standby mode.

   Does the touchscreen display the Standby screen after you touch it?

   - **Yes** – The instrument is ready for use. Go to step 3.
   - **No** – Go to step 2 to power on the instrument.

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

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



3. *(QuantStudio™ 7 Flex System only)* If you have an Applied Biosystems® Twister® Robot, toggle the power button on the rear of the Twister® Robot.

   Note: The Twister® Robot is ready to use when the power LED illuminates.

4. Power on the monitor.

5. 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**.

6. Start the QuantStudio™ 6 and 7 Flex System Software:

   a. From the desktop, double-click **QuantStudio 6 and 7 Flex System Software**.

   b. If the Login dialog box appears, enter your user name and password, then click **Log In**.

   Note: Security is a separately licensed module. If your system requires this module, contact Life Technologies.

7. Add the instrument to the My Instruments group:

   a. From the Home tab, click **Instrument Console**.

   b. From the Instrument Console, select the icon for your instrument, then click **Add to My Instruments**.

Maintain the QuantStudio™ 6 and 7 Flex Systems

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.

Frequency	User-performed maintenance task
Weekly	Check the computer disk space. If necessary, back up your experiment files and instrument settings.
	Power off the computer, then power on the computer after 30 seconds.
	Clean the instrument with a lint-free cloth.
Monthly	Perform a background calibration.†
	Run disk cleanup and disk defragmentation.
	Perform an instrument self test.
Semi-annually (every 6 months)	Perform an ROI calibration.
	Perform a background calibration.†
	Perform a uniformity calibration.
	Perform a dye calibration.
	Perform a normalization calibration.
As needed	Perform an RNase P instrument verification run.
	Replace the instrument lamp.‡

† You can perform a background calibration to check for contamination.

‡ After replacing the instrument lamp, perform all calibrations and an RNAse P instrument verification run.

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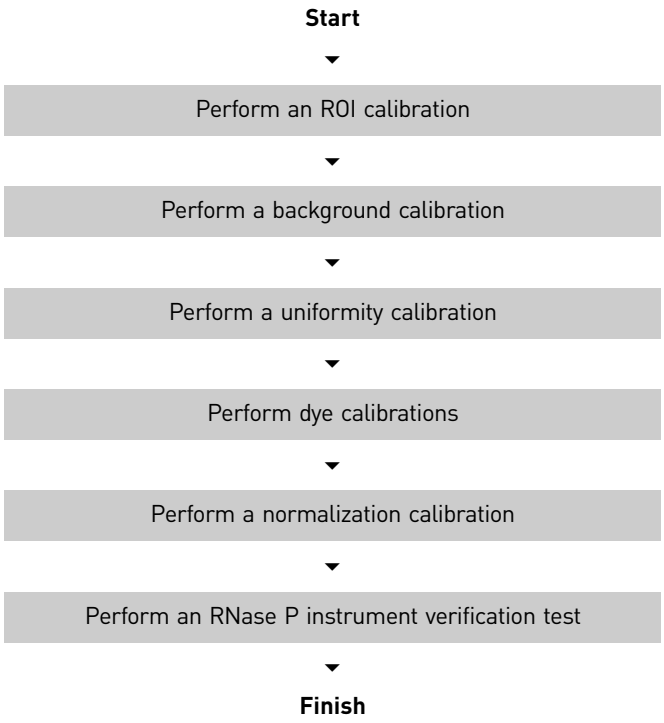
Calibration workflow

The following figure shows the workflow for calibrating the QuantStudio™ 6 and 7 Flex Systems. Whether you are performing all calibrations or just a subset, perform them in the sequence shown below.



Guidelines for handling calibration consumables

- 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†	30 minutes	120 minutes
All array card calibration consumables‡	30 minutes	120 minutes
RNase P plate (96-/384-well)	5 minutes	30 minutes
RNase P array card§	15 minutes	60 minutes

† All 96-well plates and 384-well plates used in the calibration of the instrument (ROI, background, uniformity, dye, and normalization).

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

§ For use with QuantStudio™ 7 Flex System only.

- 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)*

Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide* for detailed instructions on creating the array cards required to calibrate the QuantStudio™ 7 Flex System. See "Fill an array card (QuantStudio™ 7 Flex System only)" on page 8 for the general procedure for filling and sealing array cards.

Note: Not all array cards are required for a monthly maintenance. Before preparing array cards for calibration, see "Maintain the QuantStudio™ 6 and 7 Flex Systems" on page 1 to determine which calibrations are required.

Prepare the calibration plates

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

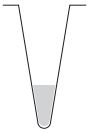
1. Remove the calibration plate from the freezer, then thaw it at room temperature for approximately 30 minutes.

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

Perform the calibration

Note: The following procedures are general instructions for performing an instrument calibration. For specific instructions, refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide*.

1. From the Home tab, click **Instrument Console**.
2. From 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 click the calibration.
   b. From the calibration screen, click **Start Calibration**.
4. Click **Next**, then prepare for the calibration as instructed.
5. From 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, touch 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, touch to close the instrument tray.
7. After loading the plate or array card, start the calibration:
   a. From the Setup tab, select **Check the box when the calibration plate has been loaded**, then click **Next**.
   b. From 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 as described in the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide*.

9. Unload the calibration plate or array card:
   a. After the calibration, touch 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. Touch on the instrument touchscreen to close the instrument tray.
10. From 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

Start

Set up the experiment

Prepare the reactions and consumables

Run and monitor the experiment

Download and review the analysis results

Export and audit the experiment

Finish

Note: Instructions for the setup, run, and analysis of an experiment vary depending on the specific experiment that you perform. Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide* for more information.

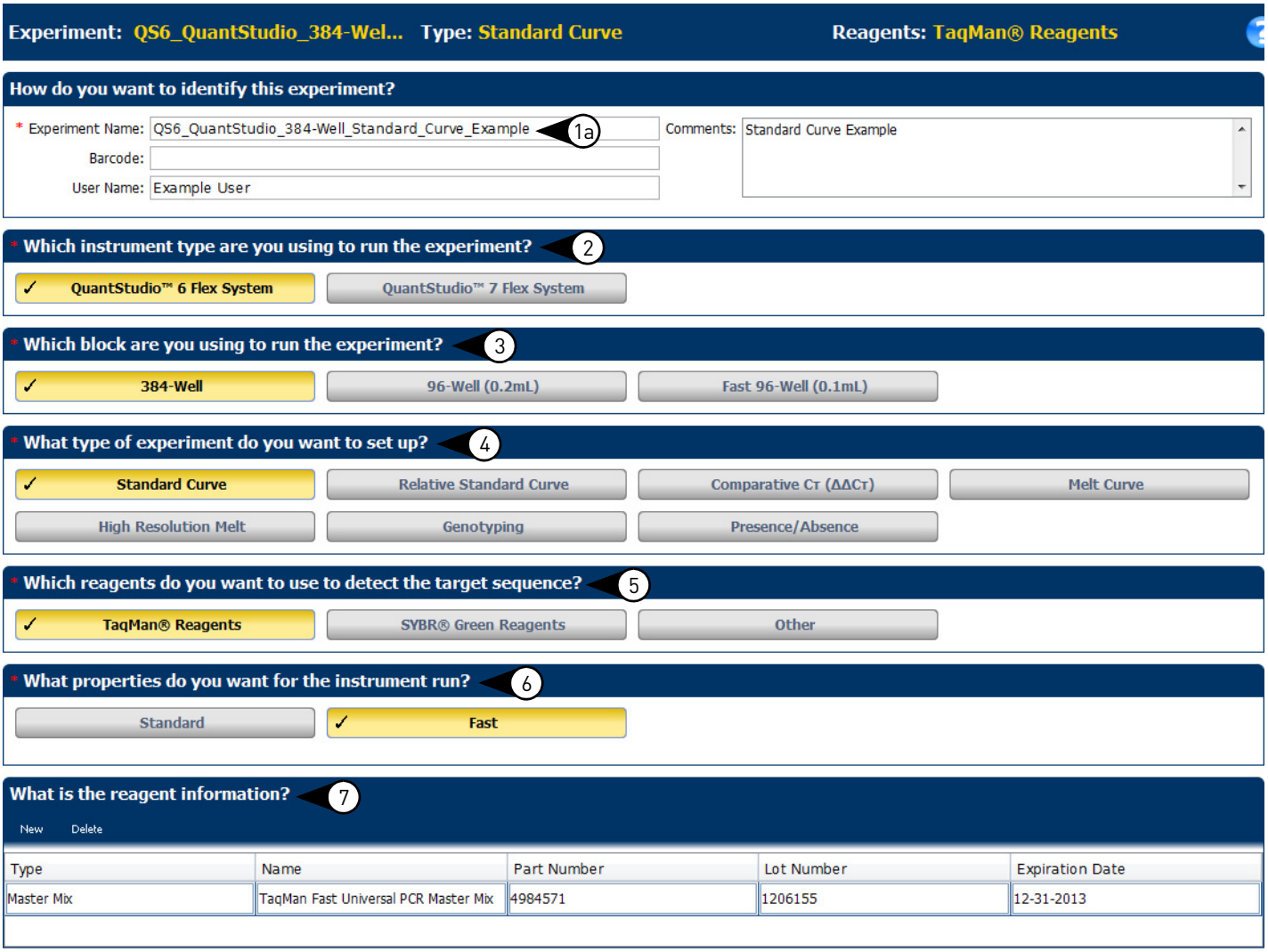
Set up the experiment

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

Define the experiment properties

1. From the Experiment Properties screen, enter 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:
   - **QuantStudio™ 6 Flex System** - includes a coupled five-color filter set and supports the 384-Well, 96-Well, and Fast 96-Well sample blocks
   - **QuantStudio™ 7 Flex System** - includes a decoupled, six by six-color filter set and supports the 384-Well, 96-Well, Fast 96-Well, and Array Card sample blocks
3. Select the block type you are using to run the experiment: **384-Well**, **Array Card** *(QuantStudio™ 7 Flex System only)*, **96-Well (0.2mL)**, or **Fast 96-Well (0.1mL)**.
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**, or **Presence/Absence**.

   Note: High Resolution Melt (HRM) is a separately licensed module. If your experiment requires this module, contact Life Technologies.
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)*, or **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 (including the **Part Number**, **Lot Number**, and **Expiration Date**) of the reagents you will use in your experiment.

IMPORTANT! The expiration dates you enter must occur after the current date.

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

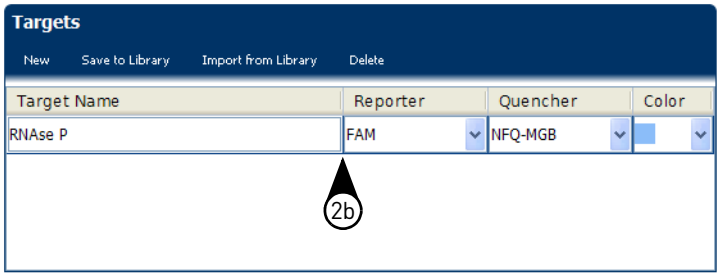
1. From the Experiment Menu, select **Define** in the Setup group.
2. Define the targets to detect in the experiment:
   a. Click **New** to add a new target to the experiment.
   b. Define the target properties:
      - Enter a **Target Name**.
      - Select a **Reporter** and **Quencher** dye.

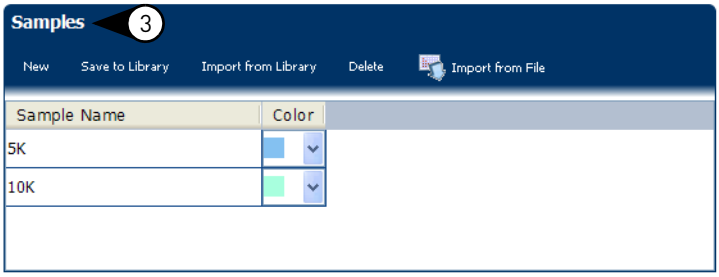
        Note: The default reporter and quencher dyes used depend on the reagent selected during experiment setup. For example, if TaqMan® is the selected reagent, the default reporter is **FAM** and default quencher is **NFQ-MGB**.
      - Select a target **Color**.

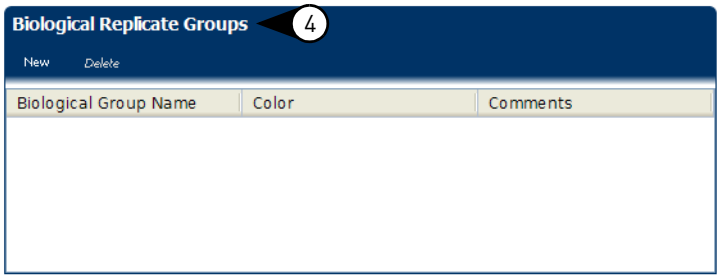
      Note: Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide* for information on defining SNP assays to detect in Genotyping experiments.

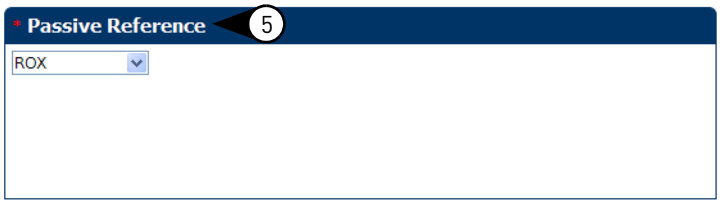
   c. (Optional, if selected in **Tools** ▸ **Preferences** ▸ **Setup**) Add and define the custom tasks to assign to targets. Refer to the *QuantStudio™ 6 and 7 Flex System Software Help* for more information.
3. From the Define screen, enter or import the names of the samples loaded into the plate or array card.

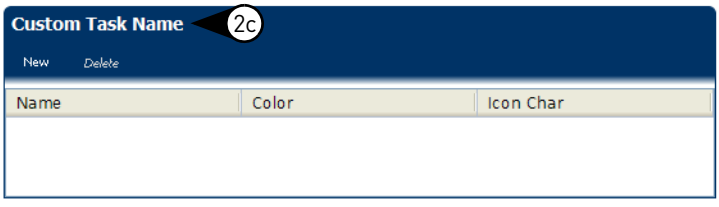
   Do either of the following:
   - Click **New** to add a new sample and manually define the sample properties:
     - Enter a **Sample Name**.
     - Select a sample **Color**.
     - (Optional) Add and define a **Custom Attribute** for the sample. Refer to the *QuantStudio™ 6 and 7 Flex System Software Help* for more information.
   - 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**.







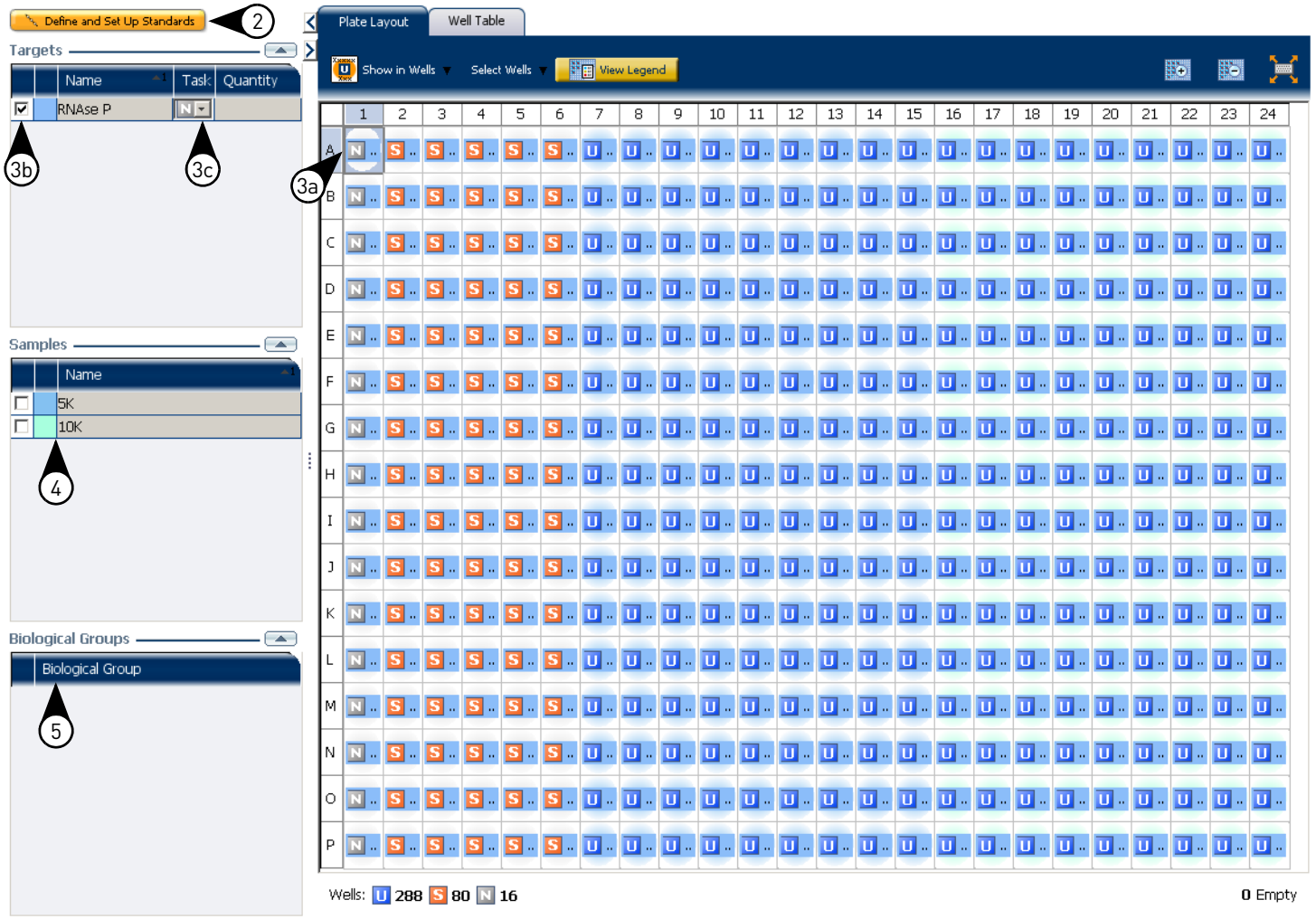




4. (Optional for Standard Curve, Relative Standard Curve, and Comparative C_T ($\Delta\Delta C_T$) experiments) Define the biological replicate groups to use in the experiment:
   a. Click **New** to add a new biological replicate group to the experiment.
   b. Define the biological replicate group properties:
      - Enter a **Biological GroupName**.
      - Select a sample **Color**.
      - (Optional) Click in the **Comments** field to enter comments about the biological replicate group.
5. (All experiments except Presence/Absence) Select a dye from the **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 **Endogenous Control** target to use in the experiment from the appropriate drop-down menu.
7. (High Resolution Melt experiments) Add and define the controls to use in the experiment. Refer to the *QuantStudio™ 6 and 7 Flex System Software Help* for more information.

Assign the targets, samples, biological replicates, and controls

1. From 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 or the Well Table.
   b. Click **Define and Set Up Standards**, select a target, define the standard curve, then select and arrange wells for the standards. Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide* for more information.
3. Assign targets to wells in the plate or array card:
   a. Select well(s) using the Plate Layout or the Well Table.
   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 menu. Available tasks include **Unknown**, **Standard**, **Negative Control**, **Positive Control**, **Custom**, and others, depending on the experiment type.



Note: Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide* for more information about target tasks, and for information on assigning SNP assays to Genotyping experiments.

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

IMPORTANT! Apply no more than one sample to each well.

5. (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:
   a. Select well(s) using the Plate Layout or the Well Table.
   b. Click a checkbox in the **Biological Groups** list to assign a biological replicate group to the selected well(s).

Define the run method

1. From the Experiment Menu, select **Run**.
2. Enter the **Reaction Volume per Well** to use to run the experiment:
   - 96-Well plate: **1-200 µL**
   - Fast 96-Well plate: **1-100 µL**
   - 384-Well plate: **1-30 µL**
   - 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.

Note: You can also save the experiment as a template, then create experiments from the template. Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide* for more information.

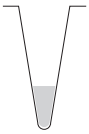
Prepare the reactions

Instructions for the preparation of reactions vary depending on the specific experiment that you perform Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide* for more information.

Prepare the plate or array card

Guidelines for plate or array card preparation

- 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.

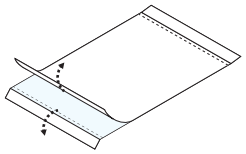
Correct	Incorrect
	
Liquid is at bottom of well.	• Not centrifuged with enough force, *or* • Not centrifuged for enough time.

Note: Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide* for information on preparing 96-well reaction tubes and tube strips.

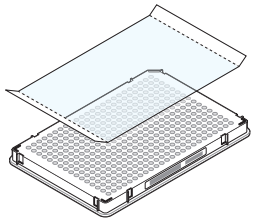
Seal the reaction plate

IMPORTANT! Wear powder-free gloves while sealing plates.

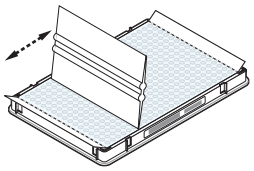
1. Load the plate with prepared reactions.
2. Remove a single optical adhesive film from the box. While holding the film backing-side up, bend both tabs upward.
3. In one swift movement, peel back the white protective backing from the center sealing surface. Do not touch the center sealing surface.



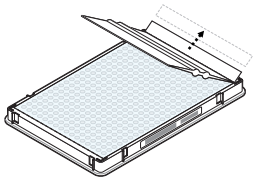
4. While holding the film by the tabs, lower the film onto the reaction plate (adhesive side facing the plate). Make sure that the film completely covers all wells of the reaction plate.



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.



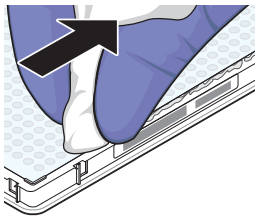
IMPORTANT! You must cleanly remove each tab along the precut dotted line.

7. Repeat step 5 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.



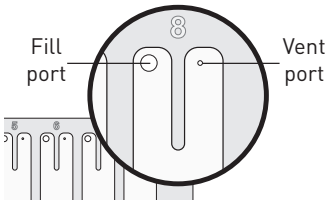
IMPORTANT! You must remove any excess glue from the plate.

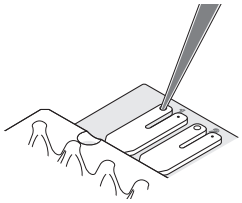
Fill an array card *(QuantStudio™ 7 Flex System only)*

IMPORTANT! Wear powder-free gloves while filling cards.

Note: The instructions below describe only the array card loading procedure. For more information, contact Life Technologies.

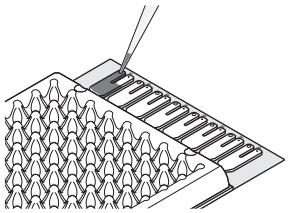
1. Remove an array card from its box and place it on a clean, dry surface.
2. Pipet 100 µL of the appropriate solution into each of the eight reservoirs in the array card:
   a. Place the array card on a lab bench, with the foil side down.
   b. Load 100 µL of the calibration solution into a pipette.
   c. Hold the pipette in an angled position (~45 degrees) and place the tip into the fill port. The fill port is the larger of the two holes on the left side of the fill reservoir.





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

   Pipet the entire 100 µL 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.

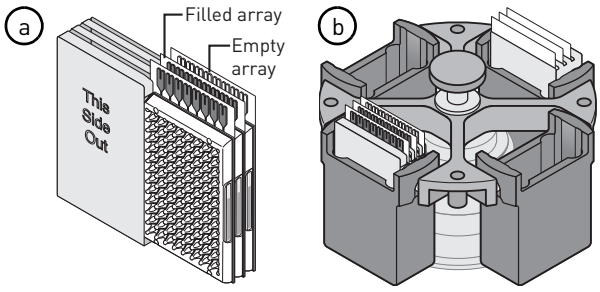


IMPORTANT! Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.

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.

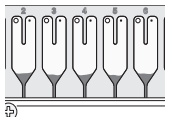
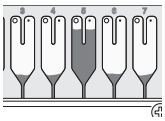
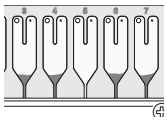


c. Close the centrifuge cover, then spin the array card for 1 minute at 1200 rpm.

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.

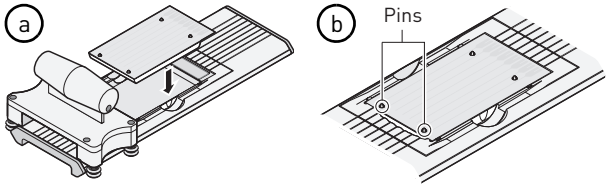
Correct fill	Incorrect/partial fill	
		

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:

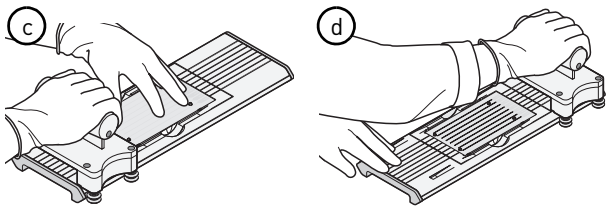
a. With the carriage (roller assembly) of the Array Card Staker/Sealer in the Start position, place a filled array card into the fixture with the foil side up so that the fill reservoirs are the farthest away from the carriage.

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

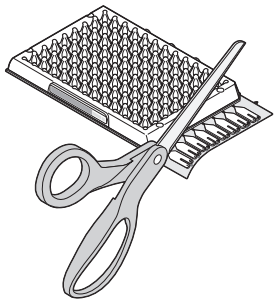


c. Use the two alignment pins in the fixture to position the array card correctly.

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



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



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

Correct trim	Incorrect trim
	

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

Note: *(QuantStudio™ 7 Flex System only)* If you have an Applied Biosystems® Twister® Robot, refer to the *Applied Biosystems® Twister® Robot Automation Accessory Quick Reference* for instructions on running an experiment using the Automation Controller Software.

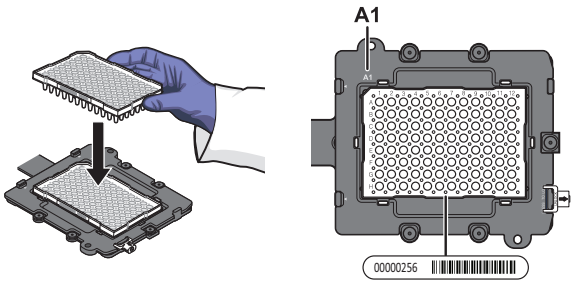
Load the plate or array card into the instrument

IMPORTANT! Plates and 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 by doing either of the following:
   - From the instrument touchscreen, touch  .
   - From the QuantStudio™ 6 and 7 Flex System Software, select **Tools** ▸ **Instrument Console**, select your instrument icon, then click **Open Door**.
2. Load the plate or array card into the plate adapter.

When you load the plate or 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 by doing either of the following:
   - From the instrument touchscreen, touch .
   - From the Instrument Console screen, 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 Maintenance and Administration Guide*. Exposure to extreme temperatures can have adverse effects on the run results, as well as shortening the life span of the components.

1. From the QuantStudio™ 6 and 7 Flex System Software, click **Run** in the Experiment Menu.
2. Click **START RUN**. Select the instrument to run the experiment from the My Instruments drop-down menu.

IMPORTANT! If your instrument is unavailable, clicking START RUN does not display instrument names in the drop-down menu.

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. Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide* for specific instructions on reviewing your data.

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.

Touch...	To...
Elapsed Time	Display the time elapsed for the run.
Remaining Time	Display the time remaining for the run.
	View the run events that occurred during the run. Touch again to close the event list.

Monitor the experiment from the software

To open the Run screen for an instrument running an experiment:

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

For instructions on viewing the Amplification Plot and Temperature Plot, refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide*.

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 array card as follows:

1. After the experiment, touch on the touchscreen to eject the plate or array card.
2. Remove the plate or array card from the instrument tray and dispose of it according to your laboratory regulations.
3. Touch on the instrument touchscreen to close the instrument tray.

Download the completed experiment

If the QuantStudio™ 6 and 7 Flex System 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™ 6 and 7 Flex System Software

1. From the Home screen, click **Instrument Console**.
2. Select your instrument, then click **Manage Instrument**.
3. Click **Manage Files**, then click **File Manager**.

4. From the File Manager screen, download the file(s):
   a. From the Folders field, select the folder that contains the files that you want to download.
   b. From the Experiments field, select the files to download. To select multiple files, **Ctrl**- or **Shift**-click files in the list.
   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

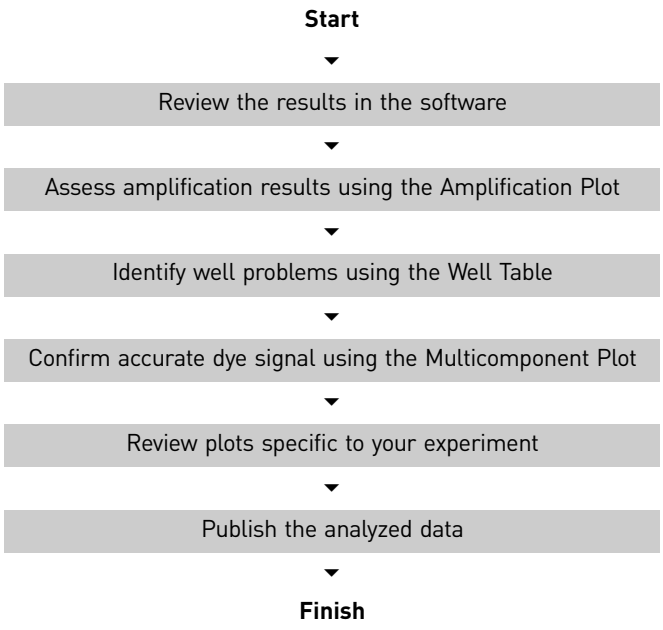
Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide* for more information on using a USB drive to transfer experiment results from the QuantStudio™ 6 and 7 Flex Systems.

Review the results

IMPORTANT! The following information provides general guidelines for reviewing an experiment run on the QuantStudio™ 6 and 7 Flex Systems. Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide* for specific instructions on reviewing experiment data.

General analysis workflow

The process for reviewing analysis results can vary significantly and depends on the type of experiment that you perform. The following figure illustrates a generalized workflow for reviewing experiments run on the QuantStudio™ 6 and 7 Flex Systems. For the workflow specific to your experiment, refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide*.



Review the results in the software

The QuantStudio™ 6 and 7 Flex System 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	• To select wells of a specific type, select **Sample**, **Target**, or **Task** from the Select Wells With drop-down menus, 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	You can use the Multiple Plots View screen to display up to four plots simultaneously. To navigate within the Multiple Plots View screen, from the Experiment Menu pane, select **Analysis ▸ Multiple Plots View**. • To display four plots, click **Show plots in a 2 × 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 menu above each plot display.
Display an expanded view of a plot or wells	• 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	1. Click on the Analysis screen (the icon appears above the plot) to open the Plot Properties dialog box. 2. Edit the settings under the General, X Axis, and Y Axis tabs. • 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. 3. 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 ▸ Analysis**.

Assess amplification results using the Amplification Plot

1. From the Experiment Menu pane, select **Analysis ▸ Amplification Plot.**
2. From the Amplification Plot screen, select the plot type and well colors.
3. View the baseline values:
   a. From the Graph Type drop-down menu, select **Linear**.
   b. Select **Baseline** to show the start cycle and end cycle.
   c. Review the plot as explained in the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide*.
4. View the threshold values:
   a. From the Graph Type menu, select **Log**.
   b. Select **Threshold** to show the threshold.
   c. Review the plot as explained in the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide.*
5. Review the uniformity of the replicate populations:
   a. From the Plot Type drop-down menu, select **Ct vs. Well**.
   b. Review the plot as explained in the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide*.

Identify well problems using the Well Table

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

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

To group by...	Action
Replicate	From the Group By drop-down menu, 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 menu, 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. From 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 menu, 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. From 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™ 6 and 7 Flex System Software may allow you to review your analyzed experiment data using additional plots defined by the experiment type. For instructions on using the QuantStudio 6 and 7 Flex System Software plots to analyze data specific to the experiment you are running, refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide*.

For general information about any QuantStudio 6 and 7 Flex System Software plot or feature, refer to the *QuantStudio™ 6 and 7 Flex System Software Help*.

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.

To...	Action
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. Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Getting Started Guide* for more information about using the export feature. **IMPORTANT!** If you choose the save the results as an export set, you must enter a file name <240 characters.
Print a report	1. Select **File ▸ Print Report** or click **Print Report**. 2. Select data for the report, and click **Print Report**. 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.

Export and audit the experiment

Setting 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™ 6 and 7 Flex System 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. From the Experiment Menu, click **Export**.
3. From 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 QuantStudio 6 and 7 Flex System Software will automatically export the results to the directory defined in the Preferences settings.

Export an experiment

1. From the Experiment Menu of the open experiment document file, click **Export**.
2. From the Export Experiment screen, select to export all data in one file or in separate files for each data type.
   - **One File** – All data types are exported in one file. If you select the .xls format, a worksheet is created for each data type. If you select the .txt 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 the 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, and .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 Life Technologies.

By default, auditing is enabled in the QuantStudio™ 6 and 7 Flex System Software. Refer to the *QuantStudio™ 6 and 7 Flex Real-Time PCR Systems Maintenance and Administration Guide* for information on selecting actions to audit and creating and editing audit reasons.

View the audit information

1. From 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. From the Audit screen, click **View Report** to view a report of the audit records.

Power off the QuantStudio™ 6 and 7 Flex Systems

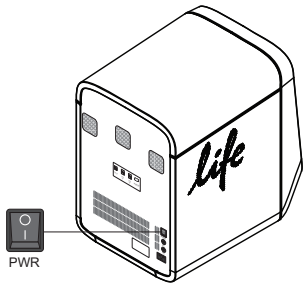
Place the QuantStudio™ 6 and 7 Flex Systems on standby

If left unattended, the QuantStudio™ 6 and 7 Flex Systems automatically enter standby mode to conserve power. To enter standby mode manually, touch on the instrument touchscreen.

Power off the QuantStudio™ 6 and 7 Flex Systems

The QuantStudio™ 6 and 7 Flex Systems operate in low-power mode when not in use; however, the instrument can be powered off completely so that the components draw no power.

1. Power off the instrument:
   a. If the touchscreen is not blank, touch to place the instrument into stand-by mode.
   b. Toggle the power button on the rear of the instrument.



2. Power off the instrument 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.
4. *(QuantStudio™ 7 Flex System only)* If you have an Applied Biosystems® Twister® Robot, toggle the power button on the rear of the Twister® Robot.

Limited product warranty

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