


Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Multi-Well Plates and Array Card

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In this guide

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-  **WARNING! GENERAL SAFETY.** Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Maintenance and Administration Guide* (Part no. 4470689).
- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
 - Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs and Documentation and Support information refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Maintenance and Administration Guide* (Part no. 4470689).
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For details on all the subjects indicated in this guide, refer to the following documents:

- *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689)
- *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Multi-Well Plates and Array Card Experiments User Guide* (Part no. 4470050)
- *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: OpenArray® Experiments User Guide* (Part no. 4470935)

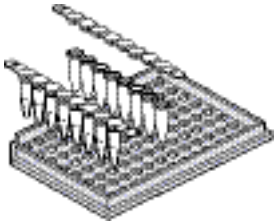
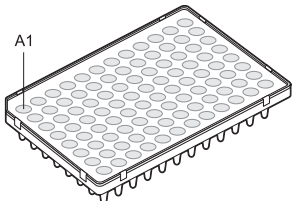
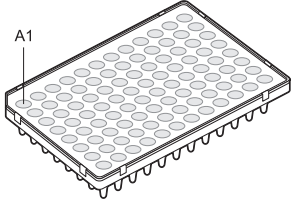
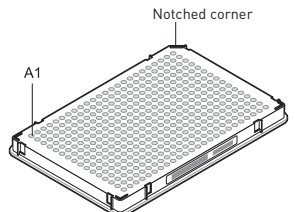
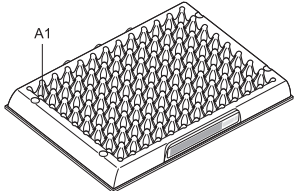
The following topics are covered in this quick reference guide:

■ QuantStudio™ 12K Flex Real-Time PCR System consumables.	2
■ Calibration types.	3
■ Calibration materials	4
■ Preparing the array cards for instrument calibration	6
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QuantStudio™ 12K Flex Real-Time PCR System consumables

Compatible consumables

The QuantStudio™ 12K Flex Real-Time PCR System supports a series of specialized consumables through interchangeable sample blocks. Use the consumables appropriate for the sample block of your QuantStudio™ 12K Flex Real-Time PCR instrument.

Sample block	Consumable	Reaction volume
96-well plate, 0.2 mL	 <ul style="list-style-type: none"> • MicroAmp® Optical 8-Cap Strip • MicroAmp® 8-Tube Strips (0.2-mL) • MicroAmp® Reaction Tubes without Caps (0.2-mL) • MicroAmp® 96-Well Tray/ Retainer Set 	50 µL
	 <ul style="list-style-type: none"> • MicroAmp® Optical Adhesive Film • MicroAmp® Optical 96-Well Reaction Plate with Barcode 	50 µL
96-well plate, 0.1 mL	 <ul style="list-style-type: none"> • MicroAmp® Optical Adhesive Film • MicroAmp® Optical 96-Well Fast Reaction Plate with Barcode 	50 µL
384-well plate	 <ul style="list-style-type: none"> • MicroAmp® Optical Adhesive Film • MicroAmp® Optical 384-Well Reaction Plate with Barcode 	20 µL
Array card	 <p>Applied Biosystems Array Card</p>	1 µL

Guidelines for handling consumables

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

- Store the calibration plates or array cards in a dark place until you are ready to use them. The fluorescent dyes in the wells of calibration consumables are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dyes.
- Do not allow the bottoms of tubes or plates to become dirty. Fluids and other contaminants that adhere to the bottoms of the consumables can contaminate the sample block and cause an abnormally high background signal.
- Confirm that the centrifuge you use is clean. Before centrifugation, wipe down the bucket using a tissue.
- *(Plates only)* Vortex all calibration plates to ensure complete mixing, then centrifuge them to ensure that all reagents are contained in the bottom of the wells. The calibration plates must be well mixed and centrifuged before use.
- *(Plates only)* Do not discard the packaging for the calibration plates. Each plate can be used to calibrate the QuantStudio™ 12K Flex Real-Time PCR Instrument 3 times for up to 6 months if it is stored in its packing sleeve.
- *(Plates only)* Handle the calibration plates with care to prevent contamination. Do not place the plates on a lab bench, to avoid contaminating them. Always put calibration plates back into their packaging sleeves.
- *(96-well plates only)* If you are using cap strips to seal your plates, firmly seal all wells before running the plate. Partially seated caps can leak during the experiment, causing evaporation.
- *(Tubes only)* Firmly seal all individual tubes and tube strips. Partially seated caps can leak during the experiment, causing evaporation.
- *(OpenArray® plates only)* Wear gloves that are one size smaller than the size you typically wear, to help prevent excess glove material from contacting the OpenArray® plates while loading.
- *(OpenArray® plates only)* Hold OpenArray® plates by the edges of the cases. Do not touch the through-holes.
- *(OpenArray® plates only)* Within one hour after opening the plate packaging, load and seal the TaqMan® OpenArray® plates.
- *(OpenArray® plates only)* If you drop a loaded OpenArray® plate, discard it in the appropriate waste container.

Calibration types

Calibration types using the multi-well or array card sample blocks

Note: The following calibration types must be performed in the order shown.

1. Regions of interest (ROI)
2. Background
3. Uniformity
4. Dye (only for dyes used in your experiments)
5. Normalization
6. Instrument verification

Calibration types using the TaqMan® OpenArray® plate

Note: For procedures for performing calibration with the TaqMan® OpenArray® plate, see *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: OpenArray® Plate Quick Reference* (Part no. 4478673) or *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470692).

Calibration materials

Materials required for calibration using the multi-well or array card sample blocks

384-well sample block kits

QuantStudio™ 12K Flex Real-Time PCR system consumable	Part number	Storage (°C)
384-Well Spectral Calibration Plate with FAM™ Dye	4432271	-15 to -25
384-Well Spectral Calibration Plate with VIC® Dye	4432278	
384-Well Spectral Calibration Plate with ROX™ Dye	4432284	
384-Well Spectral Calibration Plate with NED™ Dye	4432302	
384-Well Spectral Calibration Plate with SYBR® Green Dye	4432290	
384-Well Spectral Calibration Plate with TAMRA™ Dye	4432296	
384-Well Region of Interest (ROI) and Background Plates <ul style="list-style-type: none"> 384-Well Region of Interest (ROI) Calibration Plate 384-Well Background Plate 	4432320	
384-Well Normalization Plates with FAM™/ROX™ and VIC®/ROX™ Dyes <ul style="list-style-type: none"> 384-Well Normalization Plate with FAM™/ROX™ Dye 384-Well Normalization Plate with VIC®/ROX™ Dye 	4432308	
Kit, TaqMan® RNase P Fast 384-Well Instrument Verification Plate <ul style="list-style-type: none"> 384-Well TaqMan® RNase P Fast Instrument Verification Plate 	4455280	

96-well sample block kits

QuantStudio™ 12K Flex Real-Time PCR system consumable	Part number	Storage (°C)
96-Well Spectral Calibration Plate with FAM™ Dye	4432327	-15 to -25
96-Well Spectral Calibration Plate with VIC® Dye	4432334	
96-Well Spectral Calibration Plate with ROX™ Dye	4432340	
96-Well Spectral Calibration Plate with SYBR® Green Dye	4432346	
96-Well Spectral Calibration Plate with TAMRA™ Dye	4432352	
96-Well Spectral Calibration Plate with NED™ Dye	4432358	
96-Well Region of Interest (ROI) and Background Plates <ul style="list-style-type: none"> 96-Well Region of Interest (ROI) Calibration Plate 96-Well Background Plate 	4432364	
96-Well Normalization Plates with FAM™/ROX™ and VIC®/ROX™ Dyes <ul style="list-style-type: none"> 96-Well Normalization Plate with FAM™/ROX™ Dye 96-Well Normalization Plate with VIC®/ROX™ Dye 	4432370	
Kit, TaqMan® RNase P 96-Well Instrument Verification Plate <ul style="list-style-type: none"> TaqMan® RNase P 96-Well Instrument Verification Plate 	4432382	

Fast 96-well sample block kits

QuantStudio™ 12K Flex Real-Time PCR system consumable	Part number	Storage (°C)
Fast 96-Well Spectral Calibration Plate with FAM™ Dye	4432389	-15 to -25
Fast 96-Well Spectral Calibration Plate with VIC® Dye	4432396	
Fast 96-Well Spectral Calibration Plate with ROX™ Dye	4432402	
Fast 96-Well Spectral Calibration Plate with SYBR® Green Dye	4432408	
Fast 96-Well Spectral Calibration Plate with TAMRA™ Dye	4432414	
Fast 96-Well Spectral Calibration Plate with NED™ Dye	4432420	
Fast 96-Well Region of Interest (ROI) and Background Plates <ul style="list-style-type: none"> Fast 96-Well Region of Interest (ROI) Calibration Plate Fast 96-Well Background Plate 	4432426	
Fast 96-Well Normalization Plates with FAM™/ROX™ and VIC®/ROX™ Dyes <ul style="list-style-type: none"> Fast 96-Well Normalization Plate with FAM™/ROX™ Dye Fast 96-Well Normalization Plate with VIC®/ROX™ Dye 	4432432	
Kit, TaqMan® RNase P Fast 96-Well Instrument Verification Plate <ul style="list-style-type: none"> TaqMan® RNase P Fast 96-Well Instrument Verification Plate 	4351979	

Array card sample block kits

QuantStudio™ 12K Flex Real-Time PCR system consumable	Part number	Storage (°C)
Array Card Spectral Calibration Dye Kit <ul style="list-style-type: none"> TaqMan® Array Calibration with FAM™ Dye TaqMan® Array Calibration with VIC® Dye TaqMan® Array Calibration with ROX™ Dye TaqMan® Array Calibration with ROI Dye TaqMan® Array Calibration with FAM™/ROX™ Dye TaqMan® Array Calibration with VIC®/ROX™ Dye TaqMan® Array Background Buffer 	4432376	-15 to -25
Kit, TaqMan® RNase P Array Card Instrument Verification Reagents <ul style="list-style-type: none"> Port 1 NTC Port 2 Unknown A Port 3 Unknown B Port 4 Standard 200 Copies Port 5 Standard 400 Copies Port 6 Standard 800 Copies Port 7 Standard 1600 Copies Port 8 Standard 3200 Copies 	4432464	

Materials required for calibration using the TaqMan® OpenArray® plate

Note: For information on the materials required for performing calibration with the TaqMan® OpenArray® plate, see *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: OpenArray® Plate Quick Reference* (Part no. 4478673) or *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470692).

Preparing the array cards for instrument calibration

IMPORTANT! Perform the following procedure only if you are verifying the performance of a QuantStudio™ 12K Flex Real-Time PCR instrument with an array card sample block.

Required materials

- QuantStudio™ 12K Flex Real-Time PCR Array Card Spectral Calibration Dye Kit:
 - Applied Biosystems Array Cards, empty
 - Array Card Spectral Calibration Dye Kit, including:
 - FAM™ dye mix
 - VIC® dye mix
 - ROX™ dye mix
 - ROI dye mix
 - Background Buffer
 - FAM™/ROX™ dye mix
 - VIC®/ROX™ dye mix
- Applied Biosystems Array Card Staker/Sealer
- Centrifuge with array card buckets and array card carrier clips
- Permanent marker or pen
- Pipettor, 200- μ L (with pipette tips)
- Powder-free gloves
- Safety glasses

Filling the calibration array cards

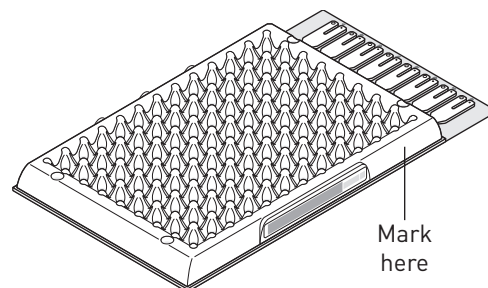
IMPORTANT! Wear powder-free gloves while creating the calibration array cards.

Note: This procedure explains how to create *all* of the array cards required to calibrate the QuantStudio™ 12K Flex Real-Time PCR instrument, but not all of them are required for a monthly maintenance. Before preparing array cards for calibration, see “Recommended calibration and maintenance” in the *Applied Biosystems QuantStudio 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689) to determine which calibrations are required.

Note: You can view a video of the array card loading procedure on the Life Technologies website. To view the demonstration, go to:

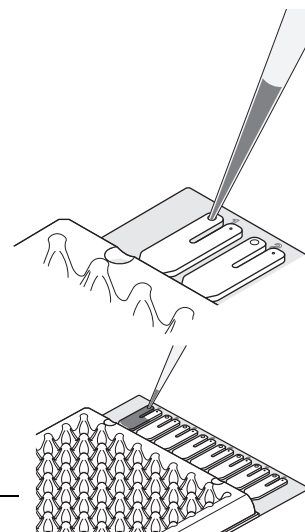
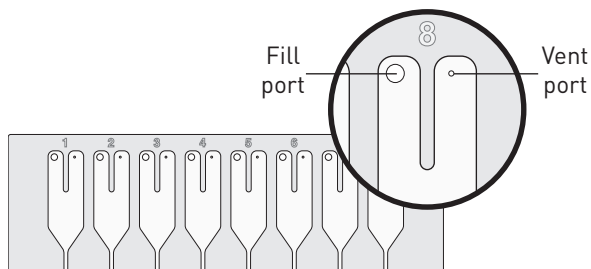
www2.appliedbiosystems.com/lib/multimedia/taqman_tlda/tlda_1.cfm

1. Remove the tubes of calibration solutions from the freezer, allow them to thaw, then vortex the tubes to mix the contents well.
2. Remove the Applied Biosystems Array Cards from their box and place them on a clean, dry surface.
3. Using a permanent marker, mark the side of the empty array cards with:
 - Background
 - FAM
 - ROI
 - ROX
 - VIC
 - FAM/ROX
 - VIC/ROX



4. For each array card, pipet 100 μ L of the appropriate calibration solution into each of the eight reservoirs in the array card:
 - a. Place the array card on a lab bench, with the foil side down.
 - b. Load 100 μ L of the calibration solution into a pipette.
 - c. Hold the pipette in an angled position ($\sim 45^\circ$) and place the tip into the fill port.

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

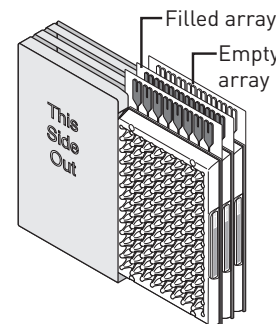


- d. Dispense the fluid so that it sweeps in and around the fill reservoir toward the vent port.

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

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

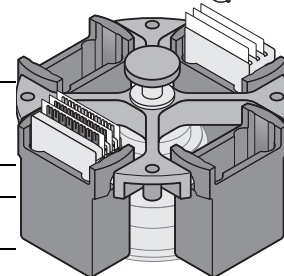
5. Repeat step 4 to fill the remaining array card with the appropriate calibration reagents.
6. Place the filled array card(s) into a centrifuge array card carrier clip and place empty array cards in the remaining slots. Confirm that the labels on the buckets and clips face the same direction.



7. Place the filled carrier clips into the centrifuge buckets. Ensure that the array card fill reservoirs and bucket and clip labels face outward when loaded into the centrifuge.

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

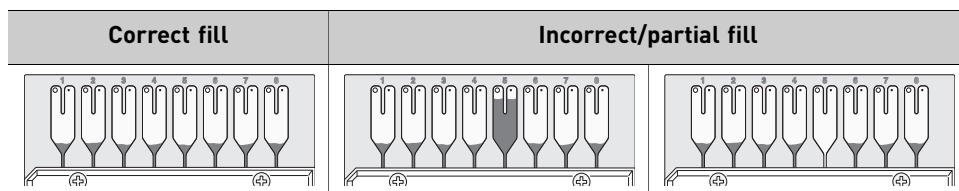
IMPORTANT! Balance the loads in opposite buckets in the centrifuge.



8. Close the centrifuge cover, then spin the array card(s) for 1 minute at 1200 rpm.
9. When the run is finished, stop the centrifuge, then spin the array card(s) again for 1 minute at 1200 rpm.

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

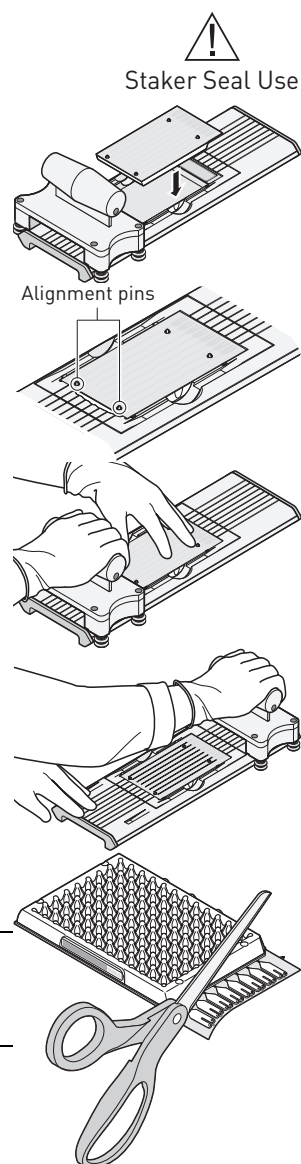
10. When the second run is finished, open the centrifuge and check that the fluid levels in the reservoirs of each array card have decreased by the same amount. Also, check for the formation of bubbles in all wells and note possible problems.



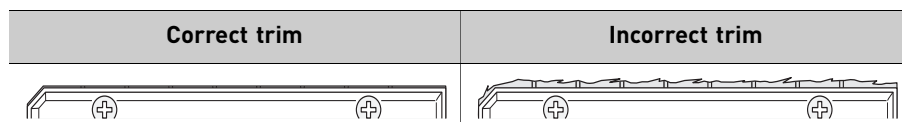
If necessary, centrifuge the array cards for an additional minute to fill any unfilled wells. Do not exceed three 1-minute runs or centrifuge the array card for longer than 1 minute at a time.

11. Seal the array card(s):

- 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.
- Press down on all four corners of the array card to ensure that it is fully seated within the fixture.
- Use the two alignment pins in the fixture to position the array card correctly.
- 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.
- 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.



12. Repeat step 11 to seal the remaining array cards.

IMPORTANT! As you seal the remaining filled array cards, store them in a dark place. Do not expose the array cards to light until you are ready to use them. The dyes in the array cards are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dye.

IMPORTANT! If an array card is sealed improperly, the card may leak and contaminate the sample block and/or it can cause the associated calibration or RNase P experiment to fail.

Performing the ROI calibration

The ROI calibration needs to be performed while using the QuantStudio™ 12K Flex Real-Time PCR software. Follow the on-screen instructions in the software.

Note: To conduct other calibration types, refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: OpenArray® Plate Quick Reference* (Part no. 4478673) or *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: OpenArray® Experiments User Guide* (Part no. 4470935).

Perform an experiment using a multi-well or array card sample block

Workflow

[Design experiment](#) ▶ [Prepare the reactions using the plates](#) ▶ [Start the experiment using a plate or an array card](#)



Design experiment

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

Experiment type	Reagent options
Standard Curve, Relative Standard Curve, and Comparative C _T	TaqMan® Reagents, SYBR® Green Reagents, or Other
Melt Curve	SYBR® Green Reagents, or Other
Genotyping and Presence/Absence	TaqMan® Reagents, or Other

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

- Define the instrument run properties (Experiment Properties screen).
 - Ramp speed: Standard or Fast
 - (Genotyping and Presence/Absence) Collect data during Pre-PCR Read, Amplification, or Post-PCR Read
 - (Experiments using SYBR® Green) Select whether or not to include a melt curve.
 - (Melt Curve) Select whether or not to include PCR.
- Define targets or SNPs, samples, biological replicates, and dye (Define screen)
 - (All experiments except Genotyping) Define Targets.
 - (All experiments) Define Samples.

- (Optional) (Standard Curve, Relative Standard Curve, and Comparative C_T) Define Biological Replicate Groups.
 - (All experiments except Presence/Absence) Select the Passive Reference dye.
 - Relative Standard Curve and Comparative C_T) Select the Reference Sample and the Endogenous Control.
5. Assign targets and samples (Assign screen)
- (All experiments except Genotyping) Assign targets, tasks, and samples to wells. Tasks include Unknown, Standard, Positive, and Negative controls, depending on experiment type.
 - (Genotyping) Assign SNP assays, tasks, and samples to wells.
 - (Standard Curve and Relative Standard Curve) Define and set up standards: Click **Define and Set Up Standards**, select a target, define the standard curve, then select and arrange wells for the standards.
 - (Optional) (Standard Curve, Relative Standard Curve, and Comparative C_T) Assign Biological Replicate Groups to wells.
6. Define the run method (Run Method screen)
- a. Reaction volume:
- 96-well plate: **1-200 μL**
 - Fast 96-well plate: **1-100 μL**
 - 384-well plate: **1-30 μL**
 - Array card: **1 μL**
- b. Edit the thermal profile as needed:
- Add and delete steps or stages.
- Edit the time, temperature, or ramp rate for a step.
- Click to enable or click to disable data collection.
- Note:** For real-time data collection during amplification, change the default analysis settings (Start Cycle and End Cycle) in Preferences.
- Add and delete steps or stages.
 - Edit the time, temperature, or ramp rate for a step.
 - Click  to enable or click  to disable data collection.
- c. For cycling stages:
- Edit the number of cycles.
- Enable or disable AutoDelta. For an AutoDelta step, enter the Starting Cycle.
- d. For a melt curve stage, select the ramp increment:
- Step and Hold:** Click the Step and Hold field, select the minutes or seconds, then use the up or down arrow keys or click the up or down buttons in the field until you reach the desired time.
- Continuous** (default): Click **1.6 $^{\circ}\text{C}/\text{s}$** (the ramp rate), select the value in the field, then enter the desired ramp rate.
7. Save the file
- After you design an experiment, you can also save the experiment as a template, then create experiments from the template using QuickStart.

Prepare the reactions using the plates

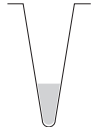

Note: To prepare the reactions using the array card see , [“Prepare the reactions using the array card” on page 12.](#)

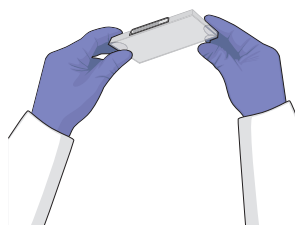
IMPORTANT! Wear powder-free gloves when you handle the plate or array card.

1. Include excess volume in your calculations to provide for loss during reagent transfers.
2. Use TE buffer or water to dilute the standards and samples.
3. Prepare the reagents according to the manufacturer’s instructions.

4. Keep the dilutions and assay mix protected from light and in the freezer, until you are ready to use them. Excessive exposure to light may affect the fluorescent probes or dyes.
5. Mix the master mix thoroughly by swirling the bottle.
6. Resuspend the assay mix by vortexing, then centrifuge the tube briefly.
7. Thaw any frozen samples, resuspend them by vortexing, then centrifuge the tubes briefly.
8. Verify that the liquid is at the bottom of each well of the plate. If not, centrifuge the plate again at a greater rpm and for a longer time.

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

Correct	Incorrect
 <p data-bbox="159 800 310 852">Liquid is at bottom of well</p>	 <ul style="list-style-type: none"> • Not centrifuged with enough force, <i>or</i> • Not centrifuged for enough time

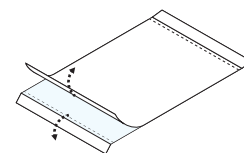


9. (Genotyping) Prepare the reactions for each SNP separately.
10. Place the reaction plate or array card at 4°C in the dark until you are ready to load it into the instrument.

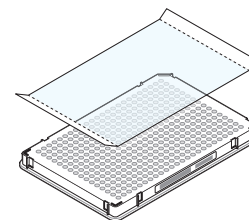
Seal the plate

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

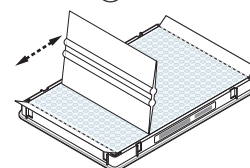
Note: Improper peeling of the optical adhesive film may result in haziness, but it will not affect results. Haziness disappears when the film comes into contact with the heated cover in the instrument.



- c. Holding the film by the end-tabs, lower the film onto the reaction plate (adhesive side facing the reaction plate). Be sure that the film completely covers all wells of the reaction plate.

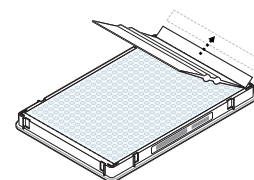


- d. While applying firm pressure, move the applicator slowly across the film, horizontally and vertically, to ensure good contact between the film and the entire surface of the reaction plate.



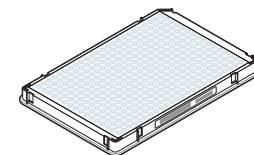
- e. While using the applicator to hold the edge of the film in place, grasp one end of the end-tab and pull up and away sharply. Repeat this step for the other end-tab.

To ensure a tight, evaporation-free seal, while applying firm pressure, move the applicator slowly across the film, horizontally and vertically, to ensure good contact between the film and the entire surface of the reaction plate. While applying firm pressure, run the edge of the applicator along all four sides of the outside border of the film.

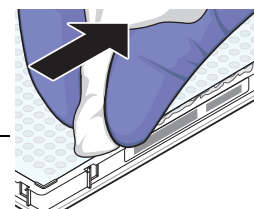


Note: Optical adhesive films do not adhere on contact. The films require the application of pressure to ensure a tight, evaporation-free seal.

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



IMPORTANT! Remove all excess adhesive from the perimeter of the optical adhesive cover. When the film is applied, the glue from the optical adhesive cover can adhere to the edges of the plate. If the excess glue is not removed, the plate may adhere to the gripper of the Twister® II Robot or to the sample block of the QuantStudio™ 12K Flex Real-Time PCR instrument.



- g. Start the experiment. See [“Start the experiment using a plate or an array card” on page 14.](#)

Prepare the reactions using the array card

Note: To prepare the reactions using the plates see [“Prepare the reactions using the plates” on page 10.](#)

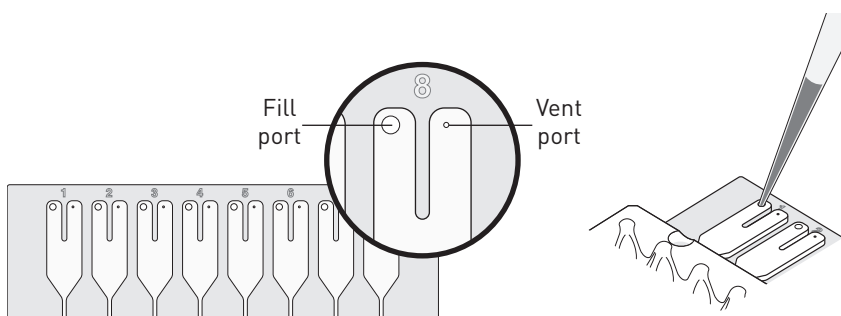
IMPORTANT! Wear powder-free gloves when you handle the plate or array card.

Note: Visit the Life Technologies website, log in to store, and view an online video of loading, centrifuging, and sealing an array card.

1. Remove the Applied Biosystems Array Card from the box and place it on a clean, dry surface.
2. Load chemicals and solutions into the array card port. For each chemical and solution transferred:
 - a. Place the array card on a lab bench, with the foil side down.
 - b. Load 100 μ L of fluid into a pipette.
 - c. Hold the pipette at an angle ($\sim 45^\circ$) and place the tip into the fill port.

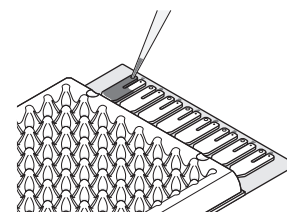
Note: The fill port is the larger of the two holes on the left arm of each fill reservoir.

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



3. Pipet fluid into the fill reservoir

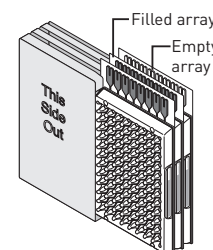
Dispense the fluid so that it sweeps in and around the fill reservoir toward the vent port. Pipette fluid into the fill reservoir, but do not go past the first stop of pipettor plunger when pipetting the reagents into the array card, or you may blow the solution out of the port.



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

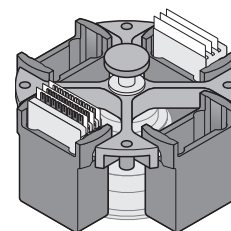
4. Centrifuge the array card

- a. Place the filled array card into a centrifuge array-card carrier clip and place empty array cards in the remaining slots. Ensure that the labels on the buckets and clips face the same way.



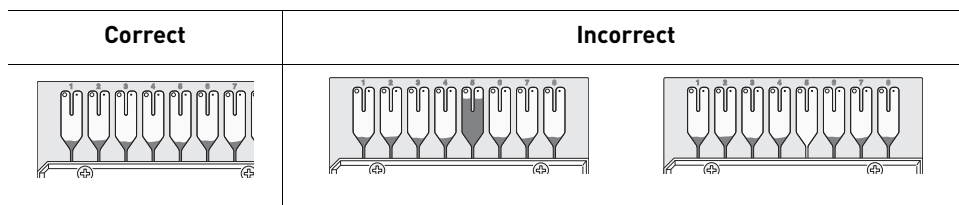
IMPORTANT! Balance the loads in opposite buckets in the centrifuge.

- b. Place the filled carrier clips into the centrifuge buckets. Ensure that the array-card fill reservoirs and bucket and clip labels face outward when loaded into the centrifuge.
- c. 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.
- d. Close the centrifuge cover, then centrifuge the array card for 1 minute at 1200 rpm. When the run is finished, stop the centrifuge, then centrifuge the array card again for 1 minute at 1200 rpm.



IMPORTANT! Do not try to save time by doing one spin for 2 minutes. Centrifuging twice is important for a good fill into the array card.


- e. Open the centrifuge and check that the fluid levels in the reservoirs of the array card have decreased by the same amount. Also, check for bubbles in all wells.

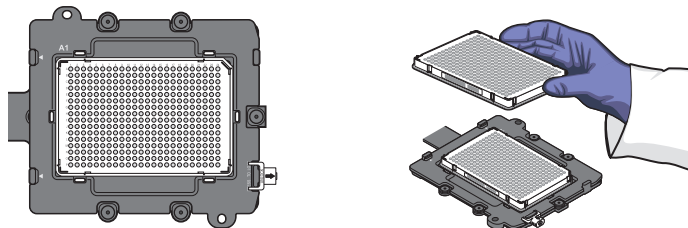


If necessary, centrifuge the array card for an additional minute to fill unfilled wells. Do not exceed three 1-minute runs or centrifuge the array card for longer than 1 minute at a time.



5. Seal the array card. See [“Seal the array card\(s\):”](#) on page 8.

Start the experiment using a plate or an array card

1. Load the plate or array card
 - a. Touch  on the instrument touchscreen to eject the plate adapter.
 - b. Place the plate or array card on the plate adapter. Ensure that the plate or array card is properly aligned in the holder:
 - Load plates and array cards with the A1 position aligned with the A1 position of the tray.
 - Load plates and array cards with the barcode facing the front of the instrument.





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

- c. Touch  to load the plate.
2. From the QuantStudio™ 12K Flex software, click  **Run** in the Experiment menu.
3. Click **START RUN** and select the instrument from the drop-down menu.

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

4. (Optional) Monitor the experiment.

How to unload the plate or array card

1. To unload the plate or array card, touch , remove the plate or array card from the plate adapter, then touch  to retract the plate adapter.



WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the plates or plaques can reach 100°C. Ensure the plate or plaque is at room temperature before removing.

If the QuantStudio™ 12K Flex Real-Time PCR instrument does not eject the plate, remove the plate as follows:

- a. Power off and unplug the QuantStudio™ 12K Flex Real-Time PCR instrument.
- b. Wait for 15 minutes, then power on the QuantStudio™ 12K Flex Real-Time PCR instrument and eject the plate.
- c. If the plate does not eject, power off the QuantStudio™ 12K Flex Real-Time PCR instrument, then open the instrument door.
- d. Wear powder-free gloves to reach into the QuantStudio™ 12K Flex Real-Time PCR instrument and remove the plate from the heated cover, then close the instrument door.
- e. Perform a background calibration to confirm that the sample block has not been contaminated.

Maintain the instrument

IMPORTANT! Calibrate the QuantStudio™ 12K Flex Real-Time PCR instrument at the same ambient temperature at which you will run experiments. Extreme variations in ambient temperature can affect the heating and cooling of the QuantStudio™ 12K Flex Real-Time PCR instrument and, in extreme cases, influence experimental results.

IMPORTANT! Do not use organic solvents to clean the QuantStudio™ 12K Flex Real-Time PCR instrument.


Frequency	Maintenance task
Weekly	Check the computer disk space. If necessary, archive or back up your experiment files and instrument settings.
	Power off the computer that controls the QuantStudio™ 12K Flex Real-Time PCR instrument, then after 30 seconds, power on the computer.
	Clean the surface of the QuantStudio™ 12K Flex Real-Time PCR instrument with a lint-free cloth.
	Perform a QuantStudio™ 12K Flex Real-Time PCR instrument self test.
Monthly	Perform a background calibration. [†]
	Run disk cleanup and disk defragmentation.
Annually	Perform a regions of interest (ROI) calibration. [‡]
	Perform a background calibration.
	Perform a uniformity calibration.
	Perform a dye calibration.
	Perform a normalization calibration. [‡]
	Perform an instrument verification run.
As needed	Decontaminate the QuantStudio™ 12K Flex Real-Time PCR instrument.
	Replace the QuantStudio™ 12K Flex Real-Time PCR instrument fuses.
	Update the Windows® operating system.
	Update the QuantStudio™ 12K Flex software and firmware.

[†] You can perform a background calibration to check for contamination. If any parts of the optics are replaced or moved, you must perform all calibrations, including an RNase P instrument verification run.

[‡] ROI and normalization calibrations are not required for QuantStudio™ 12K Flex Real-Time PCR instrument with OpenArray® plate sample blocks.

Power off the instrument

The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR operates 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 Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR
 - a. If the instrument 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 Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR computer
 - a. In the desktop, select **Start ▶ Shut Down**.
 - b. In the Shut Down Windows dialog box, select **Shut Down**, then click **OK**.
 - c. (If necessary) Power off the monitor.

For Research Use Only. Not for human or animal therapeutic or diagnostic use.

NOTICE TO PURCHASER: PLEASE REFER TO THE APPLIED BIOSYSTEMS QUANTSTUDIO™ 12K FLEX REAL-TIME PCR INSTRUMENT PRODUCT INSERT AND PROTOCOL FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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