QuantStudio™ 12K Flex Real–Time PCR System

Multi-well plates and TaqMan™ Array Cards with QuantStudio™ 12K Flex Software v1.6 or later

Pub. No. MAN0018833 Rev. B.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

About this quick reference

This document provides the following information:

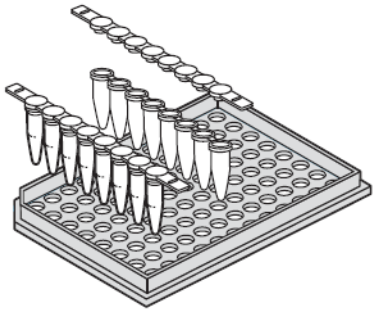
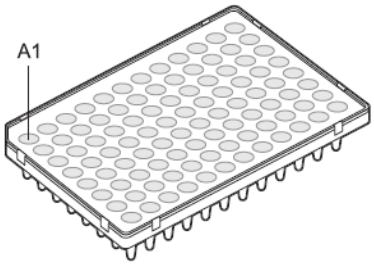
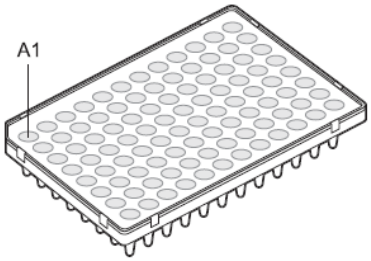
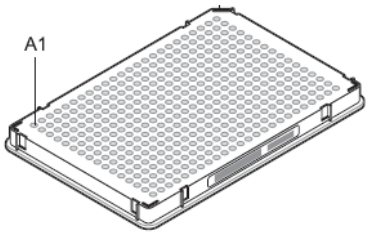
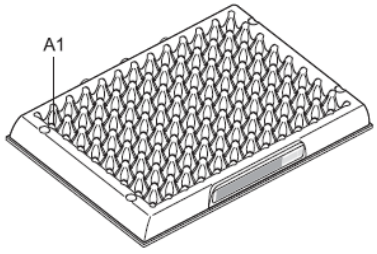
See the following documents for more information:

Document	Pub. No.
QuantStudio™ 12K Flex Real–Time PCR System: Multi-Well Plates and Array Card Experiments User Guide	4470050
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
QuantStudio™ 12K Flex Software v1.6 or later	
QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide	MAN0018832
QuantStudio™ 12K Flex Software v1.4 or earlier	
QuantStudio™ 12K Flex Real–Time PCR System v1.4 Maintenance and Administration Guide	4470689
QuantStudio™ 12K Flex Real–Time PCR System: Multi-Well Plates and Array Card Quick Reference	4470688

Consumables for the QuantStudio™ 12K Flex Instrument

Compatible consumables

The instrument supports a series of specialized consumables through interchangeable sample blocks. Use the consumables appropriate for the sample block on your instrument.

Sample block	Consumable		Reaction volume
96-well plate, 0.2 mL		• MicroAmp™ Optical 8-Cap Strip • MicroAmp™ 8-Tube Strips (0.2-mL) • MicroAmp™ Reaction Tubes without Caps (0.2-mL) • MicroAmp™ 96-Well Tray/Retainer Set	50 µL
		• MicroAmp™ Optical Adhesive Film • MicroAmp™ Optical 96-Well Reaction Plate with Bar Code	50 µL
96-well plate, 0.1mL		• MicroAmp™ Optical Adhesive Film • MicroAmp™ Optical 96-Well Fast Reaction Plate with Bar Code	30 µL
384-well plate		• MicroAmp™ Optical Adhesive Film • MicroAmp™ Optical 384-Well Reaction Plate with Bar Code	20 µL
TaqMan™ Array Card		TaqMan™ Array Card	1 µL

Sample block	Consumable		Reaction volume
TaqMan™ OpenArray™ Plate		TaqMan™ OpenArray™ Plate	33 nL

Guidelines for handling consumables

- 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 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.
- *(TaqMan™ OpenArray™ Plates only)* Hold the plate by the edges of the cases. Do not touch the through-holes.
- *(TaqMan™ OpenArray™ Plates only)* Load and seal the plate within one hour after opening the plate packaging.
- *(TaqMan™ OpenArray™ Plates only)* If you drop a loaded plate, discard it in the appropriate waste container.

Types of calibration

Types of calibration for a multi-well plate block and a TaqMan™ Array Card block

IMPORTANT! 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

   Note: Normalization calibration is only required for the TaqMan™ Array Card block with QuantStudio™ 12K Flex Software v1.6 or later.

   It is not required for the 96-well and 384-well plates with QuantStudio™ 12K Flex Software v1.6 or later.
6. Instrument verification

Calibration with QuantStudio™ 12K Flex Software v1.6 or later

IMPORTANT! The spectral calibration plates for the QuantStudio™ 3 and 5 Instruments are used for the calibration procedure with QuantStudio™ 12K Flex Software v1.6 or later. For more information, see the *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

QuantStudio™ 12K Flex Software v1.6 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™ 12K Flex Software v1.6 or later.
- A data file generated with QuantStudio™ 12K Flex Software v1.6 or later cannot have the calibration overridden if it is opened using a previous version of the software.

Types of calibration for a TaqMan™ OpenArray™ Plate

See the following documents for more information:

- *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832)
- *QuantStudio™ 12K Flex Real–Time PCR System v1.4 Maintenance and Administration Guide* (Pub. No. 4470689)
- *QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Plate Quick Reference* (Pub. No. 4478673)

Materials for calibration

Calibration kits for the 384-well plate block

The kits in the following table are for QuantStudio™ 12K Flex Software v1.6 or later.

Note: Spectral calibration plates for the QuantStudio™ 5 Instrument are used for the calibration procedure with QuantStudio™ 12K Flex Software v1.6 or later.

For QuantStudio™ 12K Flex Software v1.4 or earlier, see the *QuantStudio™ 12K Flex Real–Time PCR System v1.4 Maintenance and Administration Guide* (Pub. No. 4470689).

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

[1] Catalog numbers that appear as links open the web pages for those products.

Calibration kits for the 96-well 0.2-mL plate block

The kits in the following table are for QuantStudio™ 12K Flex Software v1.6 or later.

Note: Spectral calibration plates for the QuantStudio™ 3 and 5 Instruments are used for the calibration procedure with QuantStudio™ 12K Flex Software v1.6 or later.

For QuantStudio™ 12K Flex Software v1.4 or earlier, see the *QuantStudio™ 12K Flex Real–Time PCR System v1.4 Maintenance and Administration Guide* (Pub. No. 4470689).

Consumable	Cat. No.[1]	Storage
QuantStudio™ 3/5 10-Dye Spectral Calibration Kit, 96-Well 0.2-mL (Contains all 3 spectral calibration plates listed below)	A26343	–25°C to –15°C
QuantStudio™ 3/5 Spectral Calibration Plate 1, 96-Well 0.2-mL (FAM™, VIC™, ROX™, SYBR™ dyes)	A26331	
QuantStudio™ 3/5 Spectral Calibration Plate 2, 96-Well 0.2-mL (ABY™, JUN™, MUSTANG PURPLE™ dyes)	A26332	
QuantStudio™ 3/5 Spectral Calibration Plate 3, 96-Well 0.2-mL (TAMRA™, NED™, Cy®5 dyes)	A26333	
Region of Interest (ROI) and Background Plates, 96-Well 0.2-mL (2 plates)	4432364	
TaqMan™ RNase P Instrument Verification Plate, 96-Well 0.2-mL	4432382	

[1] Catalog numbers that appear as links open the web pages for those products.

Calibration kits for the 96-well 0.1-mL plate block

The kits in the following table are for QuantStudio™ 12K Flex Software v1.6 or later.

Note: Spectral calibration plates for the QuantStudio™ 3 and 5 Instruments are used for the calibration procedure with QuantStudio™ 12K Flex Software v1.6 or later.

For QuantStudio™ 12K Flex Software v1.4 or earlier, see the *QuantStudio™ 12K Flex Real–Time PCR System v1.4 Maintenance and Administration Guide* (Pub. No. 4470689).

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

[1] Catalog numbers that appear as links open the web pages for those products.

Calibration kits for the TaqMan™ Array Card block

Note: The calibration procedure for the TaqMan™ Array Card block is the same for the QuantStudio™ 12K Flex Software v1.4 or earlier and the QuantStudio™ 12K Flex Software v1.6 or later.

Kit	Cat. No.	Storage
ViiA™ 7 Array Card Spectral Calibration Kit The kit includes the following items: • Empty TaqMan™ Array Cards • FAM™ dye • VIC™ dye • ROX™ dye • ROI dye mix • Background buffer • FAM™/ROX™ dye • VIC™/ROX™ dye	4432314	–25°C to –15°C
ViiA™ 7 Array Card RNaseP Verification Kit The kit includes the following items: • 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	4432265	

Materials required for calibrating the TaqMan™ OpenArray™ Plate block

See the following documents for more information:

- *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832)
- *QuantStudio™ 12K Flex Real–Time PCR System v1.4 Maintenance and Administration Guide* (Pub. No. 4470689)
- *QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Plate Quick Reference* (Pub. No. 4478673)

Prepare TaqMan™ Array Cards for instrument calibration

IMPORTANT! Perform the following procedure only if you are verifying the performance of a QuantStudio™ 12K Flex System with a TaqMan™ Array Card sample block.

Required materials

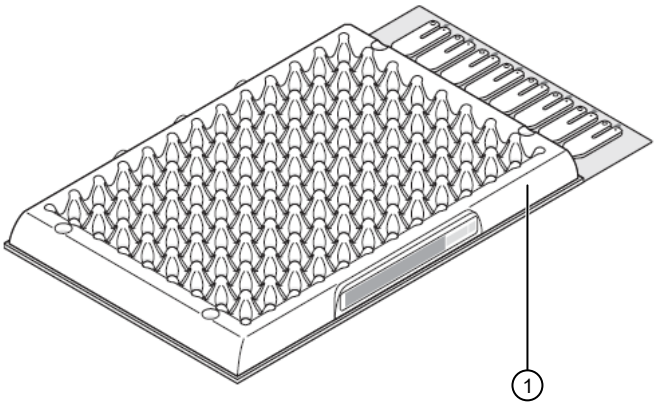
- ViiA™ 7 Array Card Spectral Calibration Kit
  The kit includes the following items:
  - Empty TaqMan™ Array Cards
  - FAM™ dye
  - VIC™ dye
  - ROX™ dye
  - ROI dye mix
  - Background buffer
  - FAM™/ROX™ dye
  - VIC™/ROX™ dye
- TaqMan™ Array Card Sealer
- Centrifuge with array card buckets and array card carrier clips
- Permanent marker or pen
- Pipettor, 200-µL (with pipette tips)
- Powder-free gloves
- Safety glasses

Fill the calibration TaqMan™ 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 System, but not all of them are required for a monthly maintenance.

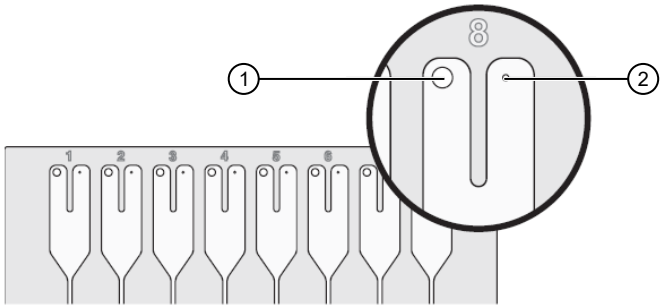
1. Remove the tubes of calibration solutions from the freezer, allow them to thaw, then vortex the tubes to mix the contents well.
2. Remove the array cards from their box and place them on a clean, dry surface.
3. Mark the side of the empty array cards with the following information.
   - Background
   - FAM™ dye
   - ROI
   - ROX™ dye
   - VIC™ dye
   - FAM™/ROX™ dye
   - VIC™/ROX™ dye



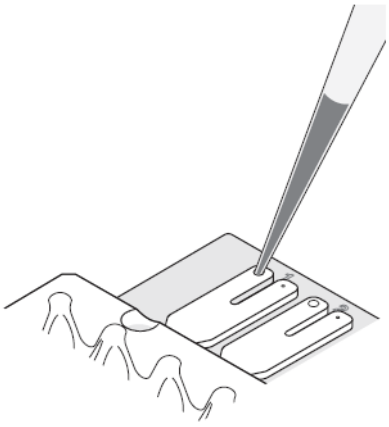
① Location to mark the information

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

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

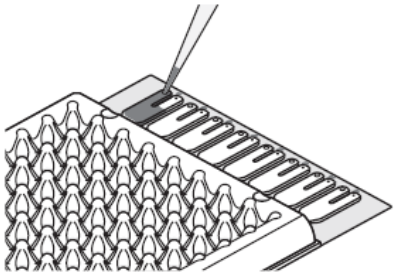


① Fill port ② Vent port



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

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

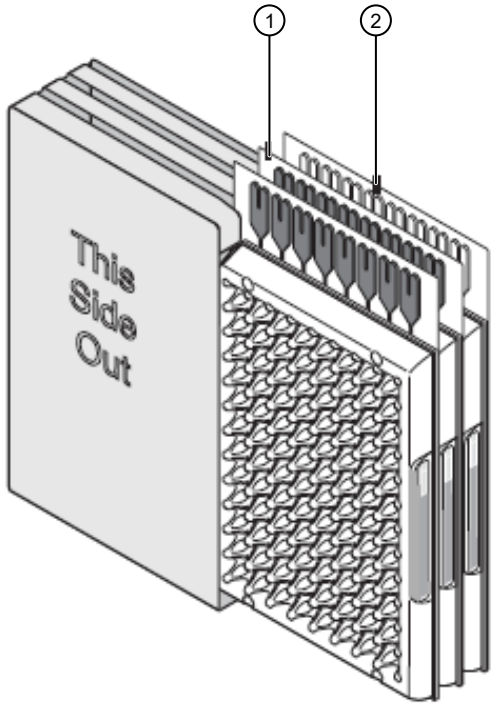


IMPORTANT! Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.

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

6. Place the filled array card(s) into a centrifuge array card carrier clip and place empty array cards in the remaining slots.

Confirm that the labels on the buckets and clips face the same way.

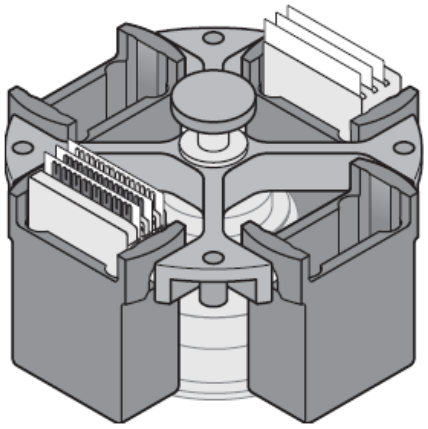


① Filled TaqMan™ Array Cards ② Empty TaqMan™ Array Cards

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.



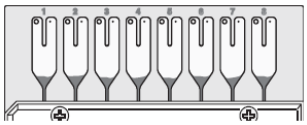
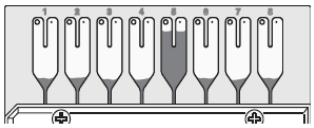
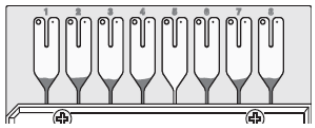
IMPORTANT! Balance the loads in opposite buckets in the centrifuge.

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.

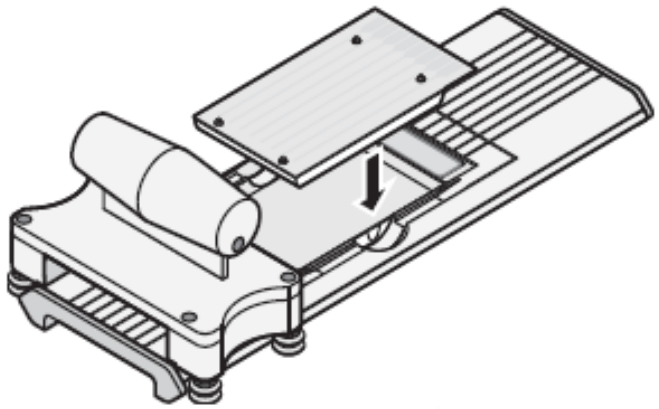
10. When the second run is finished, open the centrifuge and check that the fluid levels in the reservoirs of each array card have decreased by the same amount. Check for the formation of bubbles in all wells and note possible problems.

Correct fill	Incorrect/partial fill
	 

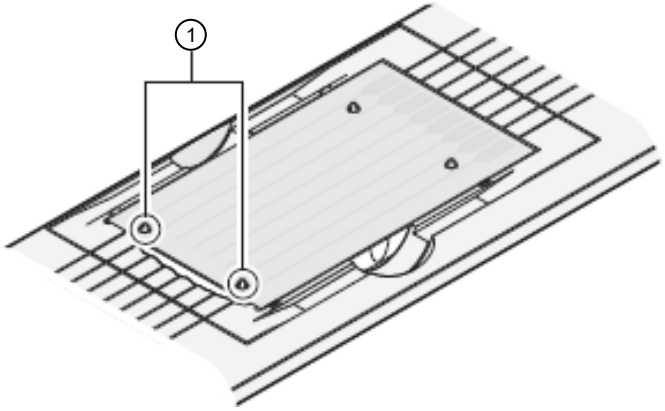
If necessary, centrifuge the array cards for an additional minute to fill any unfilled wells. Do not exceed three 1-minute runs or centrifuge the array card for longer than 1 minute at a time.

11. Seal the array card(s).

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.

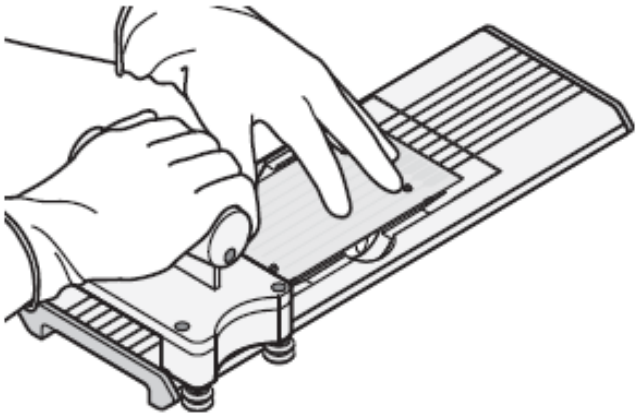


b. Press down on all four corners of the array card to ensure that it is fully seated within the fixture.

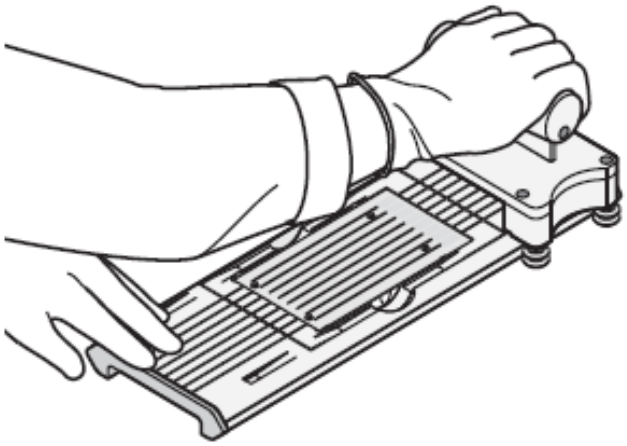


① Alignment pins

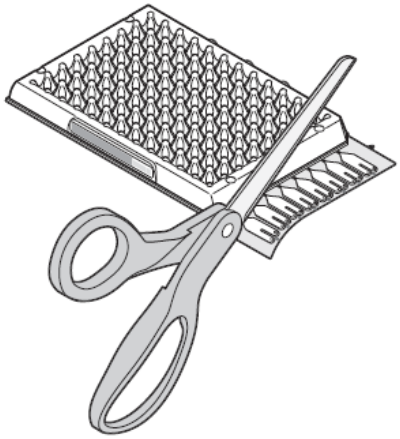
c. Use the two alignment pins in the fixture to position the array card correctly.



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
	

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.

Perform an experiment

Workflow

Design the experiment (page 11)

▼

Guidelines to prepare the reactions with plates (page 13) | Prepare the reactions using the TaqMan™ Array Card (page 16)

▼

Start the experiment (page 20)

▼

Unload the plate or the TaqMan™ Array Card (page 21)

Design the experiment

1. In the **Experiment Properties** screen, define the following parameters.
   - 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)
     - Fast 96-well (0.1 mL)
     - 384-well
     - TaqMan™ Array Card
   - Experiment type
     - Standard Curve
     - Relative Standard Curve
     - Comparative C_t ($\Delta\Delta C_t$)
     - Melt Curve
     - Genotyping
     - Presence/Absence

2. In the **Experiment Properties** screen, select the reagents.

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™ reagents, then you have the option of including a melt curve for that experiment.

3. In the **Experiment Properties** screen, define the instrument run properties.
   - Ramp speed
     - Standard
     - Fast
   - *(Genotyping and Presence/Absence)* Data collection point
     - Pre-PCR read
     - Amplification
     - Post-PCR read
   - *(SYBR GREEN™ reagents)* Select whether or not to include a melt curve
   - *(Melt curve)* Select whether or not to include PCR

4. In the **Define** screen, define the targets or SNPs, the samples, the biological replicates, and the dye.
   - *(All experiments except Genotyping)* Define the targets.
   - (All experiments) Define the samples.
   - *(Optional for Standard Curve, Relative Standard Curve, and Comparative C_t)* Define the 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. In the **Assign** screen, assign the targets and the samples.
   - *(All experiments except Genotyping)* Assign the targets, the tasks, and the samples to the wells.
     Tasks include the following items and can vary, depending on the experiment type:
     – Unknown
     – Standard
     – Positive control
     – Negative control
   - *(Genotyping)* Assign the SNP assays, the tasks, and the samples to the wells.
   - *(Standard Curve and Relative Standard Curve)* Define and set up the standards.
     a. Click **Define and Set Up Standards**.
     b. Select a target.
     c. Define the standard curve.
     d. Select and arrange the wells for the standards.
   - *(Optional for Standard Curve, Relative Standard Curve, and Comparative C_t)* Assign the biological replicate groups to the wells.

6. In the **Run Method** screen, define the run method.
   a. Reaction volume:
   - 96-well plate: **1-200 µL**
   - Fast 96-well plate: **1-100 µL**
   - 384-well plate: **1-30 µL**
   - TaqMan™ Array Card: **1 µL**

   b. Edit the thermal profile.

   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 data collection or click  to disable data collection.

   c. Edit the 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  (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 save the experiment as a template, then create experiments from the template using the **QuickStart** feature.

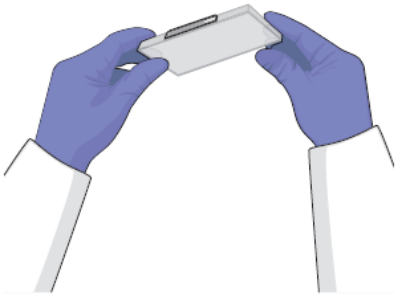
Guidelines to prepare the reactions with plates

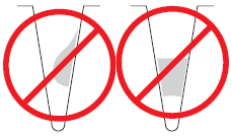
To prepare the reactions with TaqMan™ Array Cards, see page 16.

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

- Use TE buffer or water to dilute the standards and samples.
- Include excess volume in your calculations to provide for loss during reagent transfers.
- Prepare the reagents according to the manufacturer's instructions.
- 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.
- Mix the master mix thoroughly by swirling the bottle.
- Resuspend the assay mix by vortexing, then centrifuge the tube briefly.
- Thaw any frozen samples, resuspend them by vortexing, then centrifuge the tubes briefly.
- *(Genotyping)* Prepare the reactions for each SNP separately.
- Place the reaction plate at 4°C in the dark until you are ready to load it into the instrument.
- Ensure 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 and cause an abnormally high background signal.



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

Apply the optical adhesive film to seal the plate

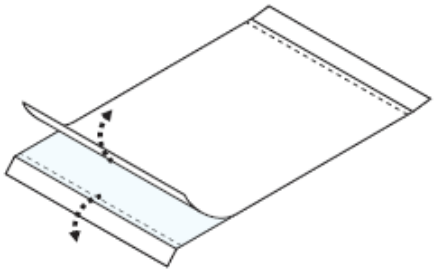
Load the plate with the prepared reactions.

1. Remove a single optical adhesive film from the box and bend both end-tabs upward. Hold the film backing side up.

2. In one swift movement, peel back the white protective backing from the center sealing surface.

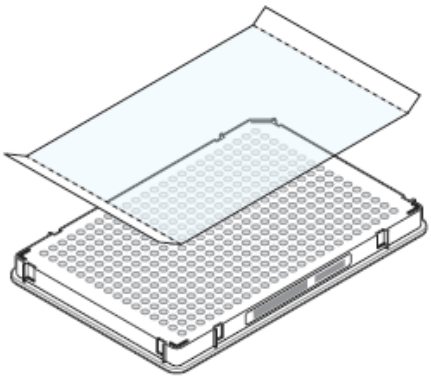
   Do not touch the center sealing surface.

   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.

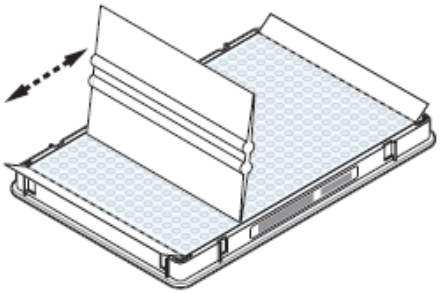


3. Holding the film by the end-tabs, lower the film onto the reaction plate (adhesive side facing the reaction plate).

   Ensure that the film completely covers all wells of the reaction plate.



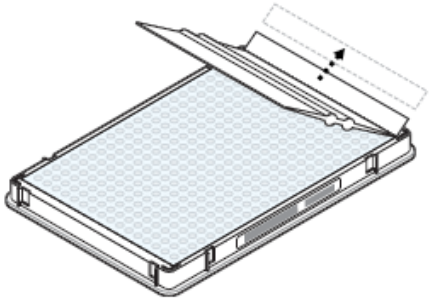
4. While applying firm pressure, move the applicator slowly across the film, horizontally and vertically, to ensure good contact between the film and the entire surface of the reaction plate.



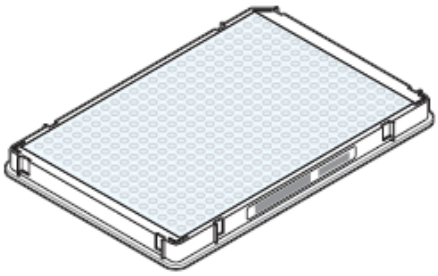
5. While using the applicator to hold the edge of the film in place, grasp one end of the end-tab and pull up and away sharply. Repeat this step for the other end-tab.

   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.



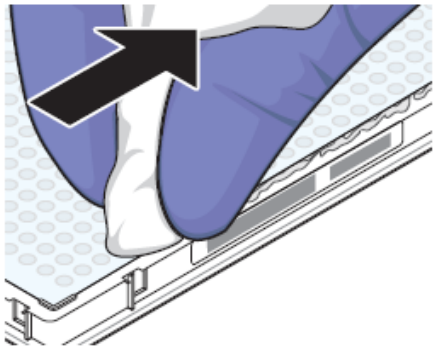
6. Inspect the reaction plate to ensure that all wells are sealed.



   You should see an imprint of all wells on the surface of the film.

7. Check that the perforated tab is completely torn off to avoid plates sticking to the instrument after a run.

8. Remove all excess adhesive from the perimeter of the optical adhesive cover.



   IMPORTANT! 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™ Robot or to the instrument sample block.

Start the experiment (see “Start the experiment” on page 20).

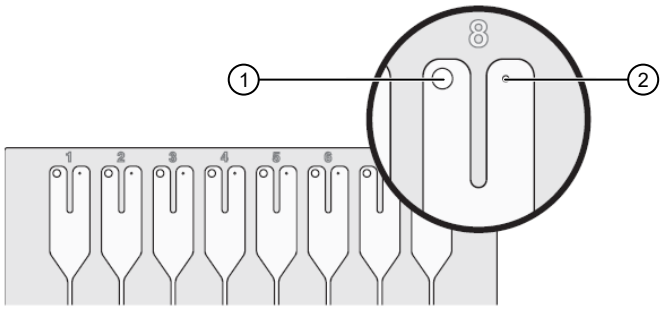
Prepare the reactions using the TaqMan™ Array Card

To prepare the reactions using the plates, see “Guidelines to prepare the reactions with plates” on page 13.

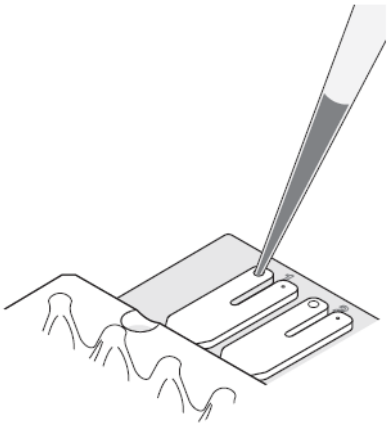
IMPORTANT! Wear powder-free gloves when you handle the plate or array card.

1. Remove the TaqMan™ Array Card from the box and place it on a clean, dry surface.
2. Load the reagents into the array card port.
   a. Place the array card on a lab bench, with the foil side down.
   b. Load 100 µL of fluid into a pipette.
   c. Hold the pipette at an angle (approximately 45 degrees), then and place the tip into the fill port of the card.

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

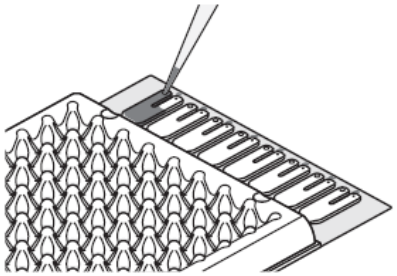


① Fill port ② Vent port



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

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

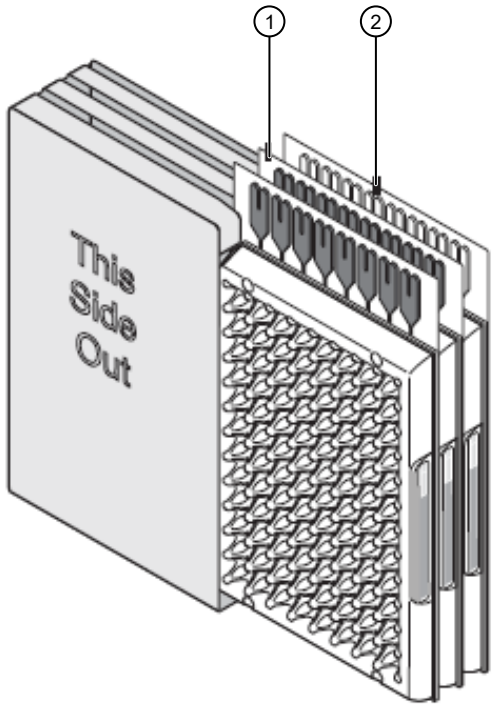


IMPORTANT! Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.

3. Repeat step 2 to fill the remaining array card with the appropriate reagents.

4. Place the filled array card(s) into a centrifuge array card carrier clip and place empty array cards in the remaining slots.

   Confirm that the labels on the buckets and clips face the same way.

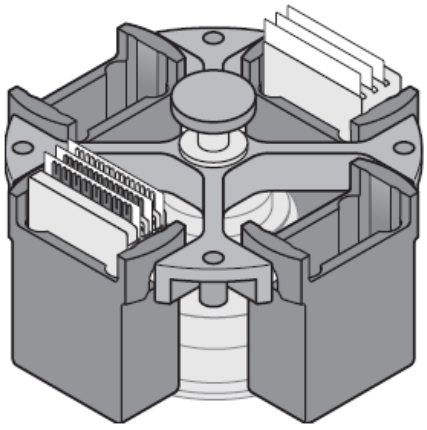


① Filled TaqMan™ Array Cards ② Empty TaqMan™ Array Cards

5. 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.



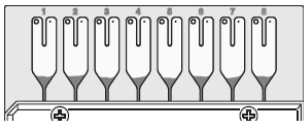
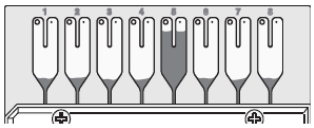
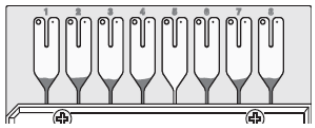
IMPORTANT! Balance the loads in opposite buckets in the centrifuge.

6. Close the centrifuge cover, then spin the array card(s) for 1 minute at 1200 rpm.

7. 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.

8. 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. Check for the formation of bubbles in all wells and note possible problems.

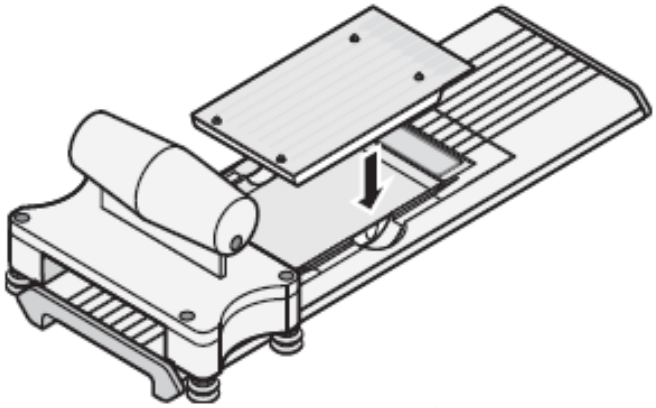
Correct fill	Incorrect/partial fill
	 

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.

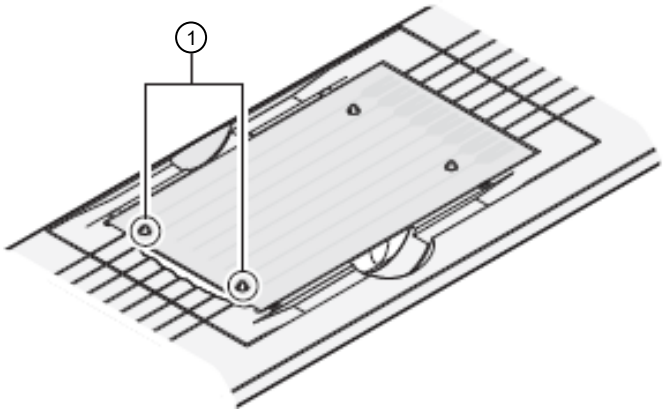
Proceed immediately to “Seal the TaqMan™ Array Card” on page 18.

Seal the TaqMan™ Array Card

1. 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.

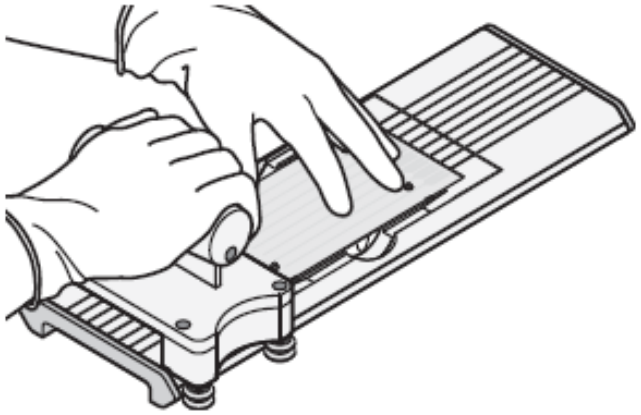


2. Press down on all four corners of the array card to ensure that it is fully seated within the fixture.

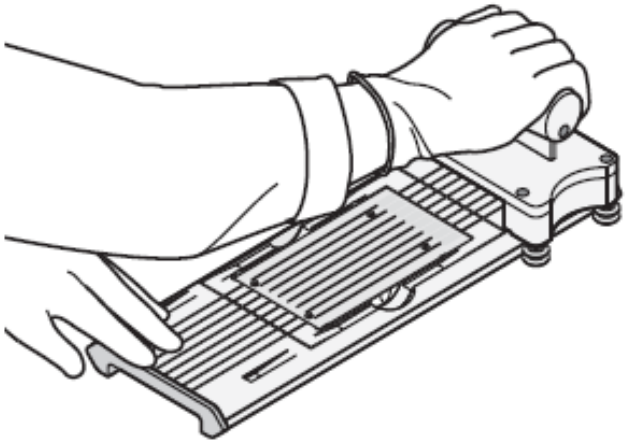


① Alignment pins

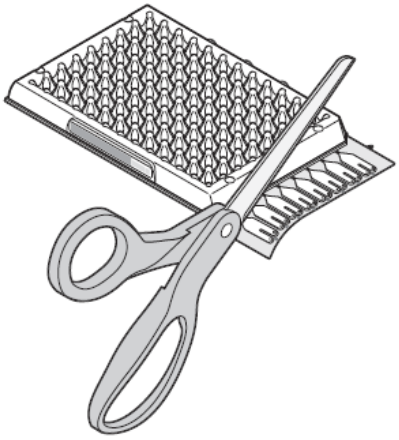
3. Use the two alignment pins in the fixture to position the array card correctly.



4. 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.



5. 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
	

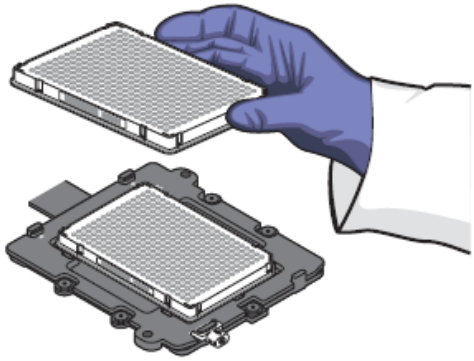
IMPORTANT! If you seal multiple array cards, store them in a dark place. Do not expose the array cards to light until you are ready to use them. The dyes in the array cards are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dye.

Proceed to “Start the experiment” on page 20.

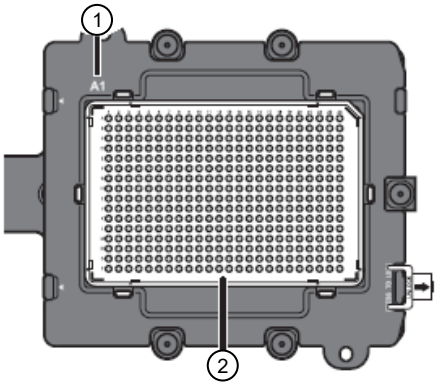
Start the experiment

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

1. Load the plate or array card into the instrument.
   a. On the instrument touchscreen, touch to eject the plate adapter.
   b. Place the plate or array card on the plate adapter.



   c. Ensure that the plate or array card is properly aligned in the holder.



① Load 96- and 384-well plates with the A1 position at the top-left corner of the plate adapter.

② Load both plates and array cards with the barcode facing the front of the instrument.

   d. Touch to load the plate.

2. In the **Experiment** menu of the QuantStudio™ 12K Flex Software, click **Run**.

3. Click **START RUN** and select the instrument from the drop-down list.

You can monitor a run while it is in progress.

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

Unload the plate or the TaqMan™ Array Card

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

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

2. If the instrument does not eject the plate, remove the plate as follows.
   a. Power off and unplug the instrument.
   b. Wait for 15 minutes, then power on the instrument and eject the plate.
   c. If the plate does not eject, power off the instrument, then open the instrument door.
   d. Wear powder-free gloves to reach into the 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 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 instrument and, in extreme cases, influence experimental results.

IMPORTANT! Do not use organic solvents to clean the 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 instrument, then after 30 seconds, power on the computer.
	Clean the surface of the instrument with a lint-free cloth.
	Perform an instrument self test.
Monthly	Perform a background calibration. **Note:** You can perform a background calibration to check for contamination. If any parts of the optics are replaced or moved, you must perform all of the calibrations, including an RNase P instrument verification run.
	Run disk cleanup and disk defragmentation.
Annually	Perform a regions of interest (ROI) calibration. **Note:** ROI calibration is not required for the TaqMan™ OpenArray™ Plate block.
	Perform a background calibration.
	Perform a uniformity calibration.
	Perform a dye calibration.
	Perform a normalization calibration. **Note:** Normalization calibration is not required for the TaqMan™ OpenArray™ Plate block. Normalization calibration is not required for 96- and 384-well plates with QuantStudio™ 12K Flex Software v1.6 or later.
	Perform an instrument verification run.
As needed	Decontaminate the instrument.
	Replace the instrument fuses.
	Update the Windows™ operating system.
	Update the QuantStudio™ 12K Flex Software and the firmware.

Power off the instrument

The QuantStudio™ 12K Flex Instrument operates in low-power mode when not in use. The instrument can be powered off completely so that the components draw no power.

1. Power off the instrument.
   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 computer.

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.

Revision history: Pub. No. MAN0018833

Revision	Date	Description
B.0	26 July 2023	• The software version was updated to v1.6 or later. • The volume for 96-well plate, 0.1mL was corrected to 30 µL.
A.0	1 February 2021	Initial document release for the QuantStudio™ 12K Flex Real–Time PCR System with the QuantStudio™ 12K Flex Software v1.5. Based on *QuantStudio™ 12K Flex Real–Time PCR System: Multi-Well Plates and Array Card Quick Reference* (Pub. No. 4470688, Rev. B), with the following changes: • Normalization calibration is not required for the 96-well plate blocks (0.2 mL and 0.1 mL) and the 384-well plate block. • Updated the 96-well and 384-well spectral calibration plates.