QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Plate

Pub. No. 4478673 Rev. D

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 v1.6 or later Maintenance and Administration Guide	MAN0018832
QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide	4470935
QuantStudio™ 12K Flex AccuFill™ System User Guide	MAN0025669

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
OpenArray™ Plate		OpenArray™ Plate	33 nL

Guidelines for handling consumables

- Wear gloves that are one size smaller than the size you typically wear. This helps prevent excess glove material from contacting the OpenArray™ Plate while loading.
- Hold the plate by the edges of the cases. Do not touch the through-holes.
- Load and seal the plate within one hour after opening the plate packaging.
- If you drop a loaded plate, discard it in the appropriate waste container.

Types of calibration for an OpenArray™ Plate block

IMPORTANT! The following calibration types must be performed in the order shown.

1. Background calibration
2. Uniformity calibration
3. Dye calibration
4. RNase P instrument verification

Required materials for calibration

Calibration	Materials	Cat. No.	Contents
Background calibration	QuantStudio™ 12K Flex OpenArray™ Calibration Kit	4478601	• FAM™ dye • OpenArray™ Calibration Plaque • OpenArray™ Calibration Case (4)
Uniformity calibration			
Dye calibration			
RNase P instrument verification	QuantStudio™ 12K Flex OpenArray™ Block RNase P Kit	4469602	• TaqMan™ RNase P QuantStudio™ 12K Flex OpenArray™ Instrument Verification Kit (Cat. No. 4469594) • QuantStudio™ 12K Flex OpenArray™ Accessories Starter Kit (Cat. No. 4469586)

About the OpenArray™ Calibration Plaque

The OpenArray™ Calibration Plaque is a specialized tool that is used to perform background and uniformity calibrations of the QuantStudio™ 12K Flex Instrument with an OpenArray™ sample block. The plaque consists of a thin sheet of black plastic that has two distinct sides shown below.

Black side	Orange side
• Dull, matte black in color. • Completely smooth. • Performs the background calibration.	• Glossy, dark orange in color. • Textured with a faint lattice pattern. • Performs the uniformity calibration.
	

Caring for the OpenArray™ Calibration Plaque

The OpenArray™ Calibration Plaque is sensitive to light and must be kept clean at all times. Adhere to the following handling, storage, and cleaning guidelines when using the tool.

Action	Guidelines
Handling	• Always wear powder-free gloves. • Grasp the tool by the edges. • Ensure that the tool does not become dirty or dusty.
Storing	When not in use, store the OpenArray™ Calibration Plaque under the following conditions: • At room temperature. • In the original packaging sleeve or in a clean plastic bag. • In a dark, clean place, such as a drawer or cabinet.
Cleaning	If the OpenArray™ Calibration Plaque becomes dirty, clean the tool as follows: 1. Place the calibration plaque on a clean, dry surface. 2. Pipet a small volume of 95% ethanol or 95% isopropanol solution onto a lint-free wipe, then thoroughly swab the surface of the tool. 3. Use a lint-free wipe to absorb the excess solution.

Background calibration

IMPORTANT! Perform the following procedure only if you are calibrating a QuantStudio™ 12K Flex Instrument with an OpenArray™ sample block.

During a background calibration, the QuantStudio™ 12K Flex System performs the following functions:

- Performs two reads of the OpenArray™ Calibration Plaque for 10 minutes at 60°C.
- Averages the spectra recorded during the run and extracts the resulting spectral component to a calibration file.

The QuantStudio™ 12K Flex Software then uses the calibration file during subsequent runs to remove background fluorescence from the run data.

Required materials

- OpenArray™ Calibration Plaque
- Powder-free gloves
- Safety goggles

When to perform the calibration

Perform the background calibration monthly or as often as necessary, depending on instrument use.

About the background calibration data

During the background calibration, the QuantStudio™ 12K Flex Software captures a series of images of the *black* side of the OpenArray™ Calibration Plaque using each instrument filter. The software measures the fluorescence across the image. A background calibration passes if the collected images for all filters have signals that are within normal range.

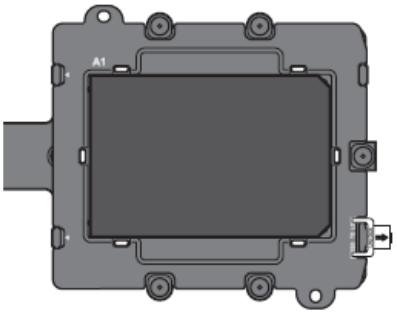
IMPORTANT! A user must be present throughout the duration of the calibration. Following the first read, the OpenArray™ Calibration Plaque must be rotated 180° before the instrument can complete the calibration.

Load the OpenArray™ Calibration Plaque

1. When the instrument door opens, load the OpenArray™ Calibration Plaque *(black* side up) into the plate retainer.

IMPORTANT! Ensure that the plaque is loaded into the plate retainer so that the *black* side of the tool is facing up.

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



2. Start the calibration.
   a. Select **Check the box when the calibration plaque has been loaded**, then click **Next**.
   b. In the **Run** screen, click **START RUN**.

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

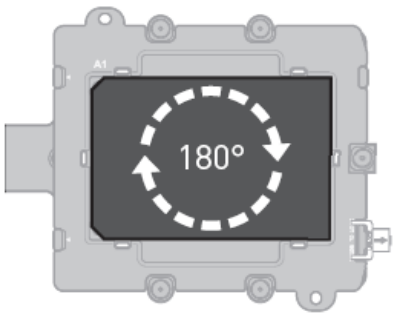
Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach temperature.

Rotate the OpenArray™ Calibration Plaque

The instrument door opens and you are prompted to rotate the OpenArray™ Calibration Plaque.

1. Rotate the calibration plaque 180°, then place it back into the plate retainer (*black* side up).

IMPORTANT! Do not flip the calibration plaque over. The *black* side of the tool must face up.



2. Click **OK** to close this dialog box, then click **START RUN** in the **Run** screen to perform the second reading.

Complete the calibration

IMPORTANT! Wear powder-free gloves and safety glasses when you handle the OpenArray™ Calibration Plaque.

1. Verify the status of the calibration.

   The **Analysis Status** displayed by the QuantStudio™ 12K Flex Software indicates the success of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or the data it collected is unusable.

Analysis status	Action
Passed	1. Click **Next**. 2. Enter any comments you have in the **Comments** field, click **Finish**, then click **Yes** when prompted to save the results.
Failed	1. Repeat the calibration. If necessary, clean the calibration plaque before you repeat the calibration (see "Caring for the OpenArray™ Calibration Plaque" on page 3). 2. If the calibration fails again, contact Support.

2. When the instrument door opens, remove the calibration plaque from the instrument tray.

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

3. Return the calibration plaque to its original packaging or a clean plastic bag.

IMPORTANT! Do not expose the calibration plaque to sunlight for extended periods of time. When not in use, store the plaque at room temperature within the original packaging in a clean, dark location.

IMPORTANT! If the instrument does not eject the calibration plaque, see *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

Uniformity calibration

IMPORTANT! Perform the following procedure only if you are calibrating a QuantStudio™ 12K Flex System with an OpenArray™ plate sample block.

The uniformity calibration generates data that allow the QuantStudio™ 12K Flex Software to compensate for the physical effects of the QuantStudio™ 12K Flex System filters.

Required materials

- OpenArray™ Calibration Plaque
- Powder-free gloves
- Safety goggles

When to perform the calibration

Perform a uniformity calibration at least once per year or more often, depending on use.

IMPORTANT! You must perform a uniformity calibration before a dye calibration.

About the uniformity calibration

During the uniformity calibration, the QuantStudio™ 12K Flex Software captures a series of images of the *orange* side of the OpenArray™ Calibration Plaque using each instrument filter. The software uses the captured images to calibrate the optical uniformity of the QuantStudio™ 12K Flex Instrument.

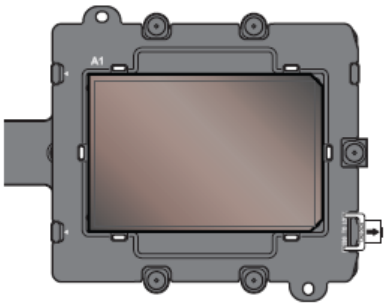
IMPORTANT! A user must be present throughout the duration of the calibration. Following the first read, the calibration plaque must be rotated 180 degrees before the instrument can complete the calibration.

Load the OpenArray™ Calibration Plaque

1. When the instrument door opens, load the OpenArray™ Calibration Plaque (*orange* side up) into the plate retainer.

IMPORTANT! Ensure that the calibration plaque is loaded into the plate retainer so that the *orange* side of the tool is facing up.

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



2. Start the calibration.
   a. Select **Check the box when the calibration plaque has been loaded**, then click **Next**.
   b. In the **Run** screen, click **START RUN**.

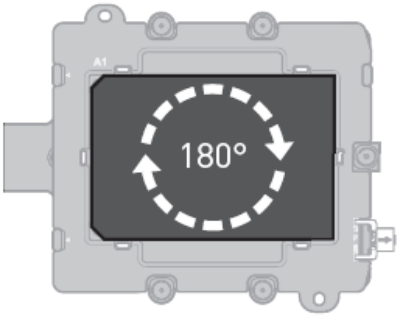
IMPORTANT! Do not attempt to open the access door during the run. The door is locked while the instrument is in operation.

Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach temperature.

Rotate the OpenArray™ Calibration Plaque

The instrument door opens and you are prompted to rotate the OpenArray™ Calibration Plaque.

1. Rotate the calibration plaque 180°, then place it back into the plate retainer *(orange* side up).



IMPORTANT! Do not flip the calibration plaque over. The *orange* side of the tool must be facing up.

2. Click **OK** to close this dialog box, then click **START RUN** in the **Run** screen to perform the second reading.

Complete the calibration

IMPORTANT! Wear powder-free gloves and safety glasses when you handle the OpenArray™ Calibration Plaque.

1. Verify the status of the calibration.

   The **Analysis Status** displayed by the QuantStudio™ 12K Flex Software indicates the success of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or the data it collected is unusable.

Analysis status	Action
Passed	1. Click **Next**. 2. Enter any comments you have in the **Comments** field, click **Finish**, then click **Yes** when prompted to save the results.
Failed	1. Repeat the calibration. If necessary, clean the calibration plaque before you repeat the calibration (see "Caring for the OpenArray™ Calibration Plaque" on page 3). 2. If the calibration fails again, contact Support.

2. When the instrument door opens, remove the calibration plaque from the instrument tray.

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

3. Return the calibration plaque to its original packaging or a clean plastic bag.

IMPORTANT! Do not expose the calibration plaque to sunlight for extended periods of time. When not in use, store the plaque at room temperature, in the original packaging, in a clean, dark location.

IMPORTANT! If the instrument does not eject the calibration plaque, see *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

Dye calibration

IMPORTANT! Perform the following procedure only if you are calibrating a QuantStudio™ 12K Flex System with an OpenArray™ plate sample block.

During a dye calibration, the system performs the following functions:

- Collects spectral data from the FAM™ dye standard.
- Stores the spectral information for the dye standard in a dye calibration file.

The QuantStudio™ 12K Flex Software uses the pure spectra data during experiment runs to characterize and distinguish the individual contribution of dyes in the total fluorescence collected by the instrument. After each run, the software receives data in the form of a raw spectra signal for each reading. It determines the contribution of each fluorescent dye used in the sample by comparing the raw spectra to the pure spectra calibration data. When you save an experiment after analysis, the software stores the pure spectra with the collected fluorescence data for that experiment.

IMPORTANT! Calibrate only those dyes that are present in the chemistries that you intend to run on your system.

Required materials

- QuantStudio™ 12K Flex OpenArray™ Calibration Kit
  - FAM™ dye
  - OpenArray™ Calibration Cases (4)
  - Plugs (4)
  - Calibration syringe and tip
- Powder-free gloves
- Safety glasses

When to perform the dye calibrations

Perform a dye calibration at least once per year or more often, depending on use.

IMPORTANT! You must perform a background calibration before every dye calibration. Because the age and use of instrument components can affect spectra readings, we recommend performing a dye calibration at least every year.

About the dye calibration

The dye calibration is a two-part procedure in which the QuantStudio™ 12K Flex Instrument performs two readings of the OpenArray™ Calibration Cases.

- A preread of the empty OpenArray™ Calibration Cases
- A postread of the OpenArray™ Calibration Cases filled with FAM™ dye

About the dye calibration data

The product of the dye calibration is a spectral profile that represents the fluorescence signature of the FAM™ dye standard. The profile consists of a set of spectra that correspond to the fluorescence collected from the OpenArray™ Calibration Cases. The QuantStudio™ 12K Flex Software plots the resulting data for the spectral profile in a graph of fluorescence versus filter.

When the software extracts the dye calibration data, it evaluates the fluorescence signal generated by each calibration case in terms of the collective spectra for the entire tool. Dye spectra are generally acceptable if they peak within the same filter as their group but diverge slightly at other wavelengths.

The software can compensate for some differences in a spectral profile by replacing the spectra of unacceptable wells with the spectra of other regions of the calibration cases (auto-repairing). The software allows only a few replacements, and it may reject the calibration if the spectra between neighboring wells vary significantly.

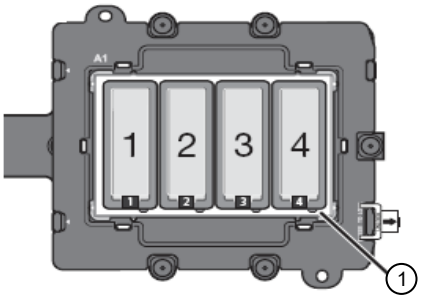
Guidelines for handling the OpenArray™ Calibration Cases

- Wear gloves that are one size smaller than the size you typically wear, to help prevent excess glove material from contacting the OpenArray™ Calibration Cases while loading.
- Hold the calibration cases by the edges.
- If you drop a loaded calibration case, discard it in the appropriate waste container.

Perform the empty reading of the OpenArray™ Calibration Cases

IMPORTANT! Wear powder-free gloves while preparing the OpenArray™ Calibration Cases.

1. Load the empty OpenArray™ Calibration Cases into the OpenArray™ Calibration Carrier according to the labels on the cases.
   a. Remove the calibration cases from their packaging.
   b. Remove the protective film from all of the OpenArray™ Calibration Cases.
   c. Load case 1 into the position closest to the instrument followed by the remaining cases in sequence as shown in the following figure.



① Plugs

IMPORTANT! Confirm that the calibration cases are positioned so that the plugs are oriented away from the A1 position as shown.

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

2. After loading the calibration cases, start the calibration.
   a. In the **Dye Calibration** screen, select **Check the box when the dye calibration cases have been loaded**, then click **Next**.
   b. In the **Run** screen, click **START RUN**.

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

Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach temperature.

Perform the filled reading of the OpenArray™ Calibration Cases

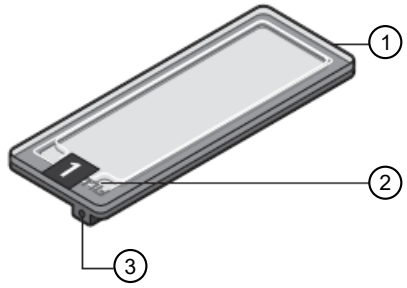
IMPORTANT! Wear powder-free gloves while preparing the OpenArray™ Calibration Cases.

When the instrument door opens and you are prompted to perform the filled reading, load the OpenArray™ Calibration Cases with FAM™ dye.

1. Attach a syringe tip to the syringe, then place the assembly on a clean surface.

   IMPORTANT! The application of the syringe tip requires force. Confirm that the tip is locked firmly in place before proceeding.

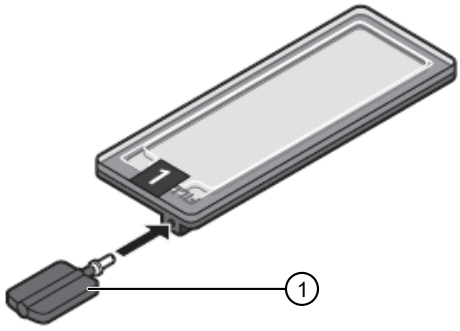
2. Carefully draw approximately 2 mL of FAM™ dye into the syringe.
3. Grasp the OpenArray™ Calibration Case in position 1 by the edges, then remove it from the OpenArray™ Calibration Carrier.
4. Remove the "RUN EMPTY FIRST" label that covers the fill port of the calibration case.
5. While holding the calibration case vertically, insert the syringe tip into the fill port at end of the case, then dispense the fluid completely in one gentle continuous motion.



① Empty OpenArray™ Calibration Case
② Fill label
③ Fill port

Note: Try to minimize creating air bubbles when you dispense the fluid. You can leave one small air bubble at the fill port to prevent overfilling.

6. Seal the loading port by inserting an OpenArray™ Plug into the port and twisting it clockwise until hand-tight, then remove the handle from the plug.



① OpenArray™ Plug (insert and twist)

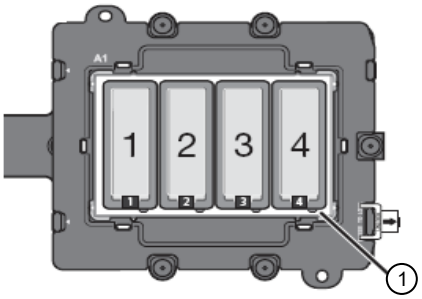
7. Load the sealed calibration case into the *same* position on the calibration carrier that it previously occupied (position 1).

   IMPORTANT! You *must* load the filled calibration cases into the same positions on the calibration carrier.

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

8. Repeat step 1 to step 7 to fill the remaining three calibration cases.

   IMPORTANT! Confirm that the calibration cases are in their original positions and that their plugs are oriented away from the A1 position as shown.



① Plugs

9. Click **OK** to close this dialog box, then click **START RUN** in the **Run** screen to start the filled reading.

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

Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach temperature.

Complete the calibration

IMPORTANT! Wear powder-free gloves while preparing the OpenArray™ Calibration Cases.

1. Verify the status of the calibration.
   - **Analysis Status**—Indicates the success of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or the data it collected is unusable.
   - **QC Status**—Indicates the quality of the calibration data, where *passed* indicates that all the OpenArray™ Calibration Cases produced data that passed the quality check, and *failed* indicates that one or more cases produced dye spectra that vary significantly.

Analysis status	Action
Passed	1. Click **Next**. 2. Enter any comments you have in the **Comments** field, click **Finish**, then click **Yes** when prompted to save the results.
Failed	Discard the OpenArray™ Calibration Cases, then prepare and run replacement cases. If the calibration fails again, contact Support for further assistance.

2. When the instrument door opens, remove the OpenArray™ Calibration Carrier from the instrument tray.

WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the cases can reach 100°C. Ensure the cases are at room temperature before removing.

3. Discard the OpenArray™ Calibration Cases.

IMPORTANT! If the instrument does not eject the plate, (see *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832)).

Verify the instrument performance

IMPORTANT! Perform the following procedure only if you are performing a verification experiment for a QuantStudio™ 12K Flex System with an OpenArray™ plate sample block.

IMPORTANT! When performing the RNase P instrument verification experiment:

- Perform all calibrations first.
- Run the OpenArray™ plate soon after you allow the plate or reagents to thaw. Minimizing the time between thaw and run ensures optimal performance.
- Wear powder-free gloves and safety glasses when you prepare OpenArray™ plates.

Perform the RNase P instrument verification experiment to verify the performance of the OpenArray™ Calibration Cases.

When to perform the RNase P experiment

- After moving the instrument to another location.
- As needed to verify the function of the system.

About the QuantStudio™ 12K Flex OpenArray™ Block RNase P Kit

The kit includes the following components:

- Empty OpenArray™ Plate (1)
- OpenArray™ RNase P Reaction Mix, contains the following components in a single tube:
  - TaqMan™ OpenArray™ Real-Time PCR Master Mix
  - RNase P primers
  - FAM™-MGB dye-labeled probe
- A known concentration of human genomic DNA template

Installation specification

The QuantStudio™ 12K Flex System passes the installation specification if the standard deviation of the C_t values for all through-holes on the OpenArray™ Plate is ≤0.25. The data from up to 48 through-holes can be omitted from the population to meet the installation specification.

Guidelines for handling the OpenArray™ Plate

- Hold the OpenArray™ case by the edges.
- Do not touch the through-holes of the OpenArray™ Plate.
- Load and seal an OpenArray™ Plate within *one hour* after opening the packaging.
- If you drop a loaded OpenArray™ Plate, discard it in the appropriate waste container.

Required materials

- QuantStudio™ 12K Flex OpenArray™ Block RNase P Kit, including the following components:
  - OpenArray™ RNase P Reaction Mix
  - QuantStudio™ 12K Flex System OpenArray™ Lid
  - QuantStudio™ 12K Flex System OpenArray™ Plug
  - QuantStudio™ 12K Flex System OpenArray™ Immersion Fluid
  - QuantStudio™ 12K Flex System OpenArray™ Immersion Fluid Tip
  - OpenArray™ Digital PCR Plate
  - OpenArray™ 384-well Sample Plate
- QuantStudio™ 12K Flex AccuFill™ System
- QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0
- Bleach (10%)
- Ethanol
- OpenArray™ 384-well Sample Plates
- OpenArray™ AccuFill™ System Tips
- Pipettes
- Powder-free gloves
- Safety glasses

Prepare for the verification experiment

IMPORTANT! Wear powder-free gloves while preparing the OpenArray™ Plate.

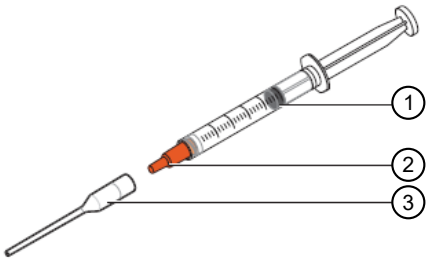
1. Confirm that the OpenArray™ 384-well Sample Plate, the OpenArray™ AccuFill™ System Tips, and the plate holder are completely clean and dry.

2. Remove an OpenArray™ Plate from the freezer, but do not open the packaging. Allow the plate to thaw at room temperature (approximately 15 minutes).

   Note: Unopened OpenArray™ Plates can remain at room temperature for up to 24 hours.

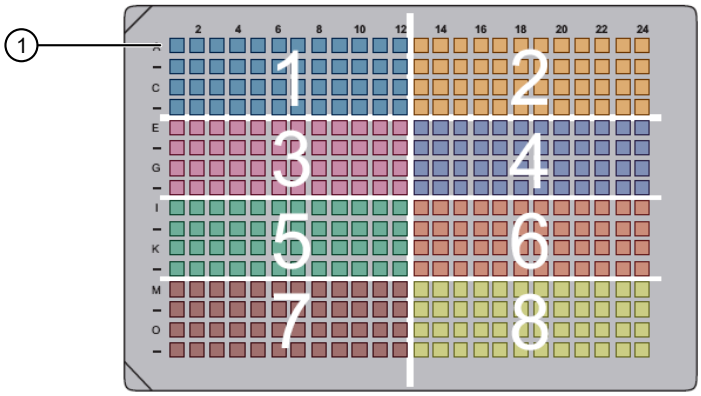
3. Prepare a syringe containing OpenArray™ Immersion Fluid. Attach the syringe tip to the syringe, then set the assembly on a clean surface.

   IMPORTANT! The application of the syringe tip requires force. Confirm that the tip is locked firmly in place before proceeding.



① OpenArray™ Immersion Fluid
② Cap (remove)
③ Syringe tip (attach)

4. Pipet 5.0 µL of the RNase P solution into loading position 1 of the OpenArray™ 384-well Sample Plate.



① Loading position 1 of the OpenArray™ 384-well Sample Plate

5. Cover the sample plate with a foil seal, then score or cut the foil into the 8 sections shown above.

6. Centrifuge the plate for 1 minute at 1500 rpm, then place the plate on ice to keep the samples cold.

Load the OpenArray™ Plate (OpenArray™ AccuFill™ Software v2.0)

There are multiple workflows available with OpenArray™ AccuFill™ Software v2.0. The quick run workflow without sample information is recommended to prepare an OpenArray™ Plate for RNase P instrument verification.

For other workflow options and more information, see *QuantStudio™ 12K Flex AccuFill™ System User Guide* (Pub. No. MAN0025669).

Initialize the system

1. Ensure that the instrument door is closed.

2. Power on the instrument, if it is off.

3. Start the software ( ).

   The software checks the computer and connections as the system starts.

Proceed to set up the system (see “Set up the system” on page 13).

Set up the system

IMPORTANT! To safely operate the instrument, keep the deck clear and have enough room in the waste bin to eject the used pipette tips.

1. Open the instrument door, empty the waste bin, then place the waste bin back on the instrument deck.

   CAUTION! Wear appropriate personal protective equipment while handling the waste bin.

2. Ensure that the sample plate holder and the OpenArray™ Plate holders are empty.
3. Replace the tip boxes, if necessary.

   Each tip box contains 384 tips, divided into 8 sections.

   When setting up a run, the status of the tip boxes is confirmed in the software. A full tip box is recommended when starting a run.

   Do not reuse tips.
4. Remove the cover from each tip box.

   Note: Ensure that the tip box covers are removed from the instrument deck.
5. Close the instrument door.

The system is ready to start a run. A self-test is initiated the first time that one of the following items is clicked after starting the software:

- **Full Run**
- **Quick Run**
- **Service ▸ Diagnostics**

Configure the run

Navigate to the **Quick Run** screen. Ensure that the **Load without sample plate information** radio button is selected.

1. *(Optional)* In the **Quick Run** screen, in the **Sample plate - optional** field, enter information to identify the sample plate.
   - Enter the information about the sample plate, for example, ***RNase P***.
   - Use a barcode scanner to scan the sample plate or manually enter the barcode text string.
2. Select one sample per subarray.
3. In the **OpenArray Plate name and position** field, enter information to identify the OpenArray™ Plate.

   We recommend using the serial number of the OpenArray™ Plate as the identifying information.

   The name and position are recorded in the loading history log.
4. Click a section of the sample plate to change the corresponding OpenArray™ Plate.

   Note: The first section of the sample plate is selected.

   The OpenArray™ Plate position displays the color that corresponds to the section of the sample plate.

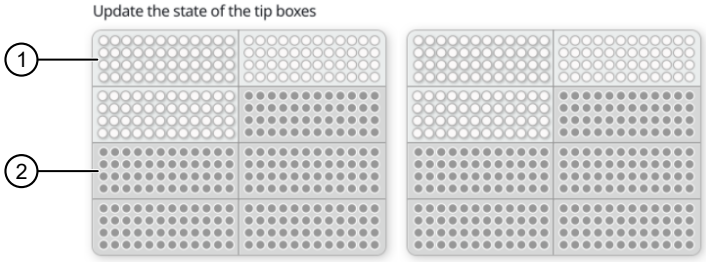
Proceed to “Verify the run setup and start the run” on page 13.

Verify the run setup and start the run

1. Click each tip box so that the status on the **Verify the run setup and start the run** section matches the physical tip box in the instrument.

   We recommend starting the run with full tip boxes.

   The instrument does not start the run if there are not enough tips on the deck.



① Section of the tip box is full ② Section of the tip box is empty

2. Select the section of the sample plate that will be used to fill the OpenArray™ Plate.
3. Remove the foil from the appropriate section of the sample plate, then click the checkbox to confirm.
4. Close the instrument door.
5. Click **Start Run**.

   The run does not begin under any of the following conditions:
   - The waste bin is not in position
   - The sample plate is not in position
   - The OpenArray™ Plates are not in position
   - There are more OpenArray™ Plates on the instrument deck than are defined in the experiment setup

The **Deck** screen is displayed.

IMPORTANT! Each OpenArray™ Plate must be prepared for PCR immediately after it is filled (see "Remove the OpenArray™ Plate from the instrument" on page 14).

Remove the OpenArray™ Plate from the instrument

After the OpenArray™ Plate in the run is filled, the **Remove plate** dialog box is displayed.

Remove the OpenArray™ Plate *immediately* after it has been filled.

1. Open the instrument door and remove the OpenArray™ Plate that is indicated by the blue box in the dialog box.

   IMPORTANT! Remove the OpenArray™ Plate within 30 seconds, to avoid evaporation within the plate.

2. Seal the case and fill the OpenArray™ Plate with immersion fluid.

   See "Seal the OpenArray™ Plate" on page 15.

3. Close the instrument door.

After the OpenArray™ Plate has been loaded, the **Deck** screen displays **Run completed successfully. Empty the waste bin before performing another run.**

Seal the OpenArray™ Plate

IMPORTANT! Throughout this procedure, handle the OpenArray™ Plate and the OpenArray™ Case only by the edges.

Wear snug-fitting gloves when working with OpenArray™ Plates.

Note: The OpenArray™ Case consists of the sealed OpenArray™ Plate and the OpenArray™ Case Lid.

1. Place the newly loaded OpenArray™ Plate in the QuantStudio™ 12K Flex OpenArray™ Plate Press 2.0.

   Ensure that the barcode is facing left and the serial number is facing right.

2. From the OpenArray™ Lid, remove the clear protective film from the *inside* of the lid ① and the red adhesive-protective strip ② from around the edge of the lid.

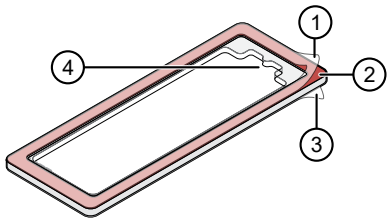
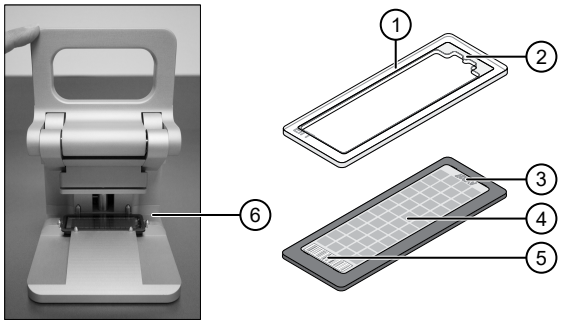


Figure 1 OpenArray™ Lid

① Protective film on inside of the lid (remove before *sealing*)
② Red adhesive-protective strip (remove before *sealing*)
③ Protective film on the outside of the lid (remove before *running*)
④ Notched end (align with serial number on plate)

3. Place the lid in the Plate Press using the alignment pins of the Plate Press for orientation.

IMPORTANT! The notched end of the case lid must be oriented towards the furthest back right-side of the Plate Press.



① OpenArray™ case lid
② Notched end of lid
③ Serial number of plate
④ OpenArray™ Plate
⑤ Barcode of plate
⑥ Alignment pins

4. Seat the lid on the OpenArray™ Plate with the lid adhesive against the plate.

5. Engage the press mechanism until the green flashing light changes to a steady green light (after 20 seconds).

   The status light turns solid green, indicating that the case is sealed.

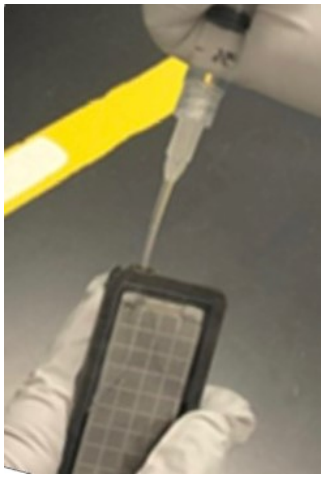
   Note: Do not apply additional pressure onto the Plate Press during its actuation.

6. Disengage the press and carefully remove the OpenArray™ Case.

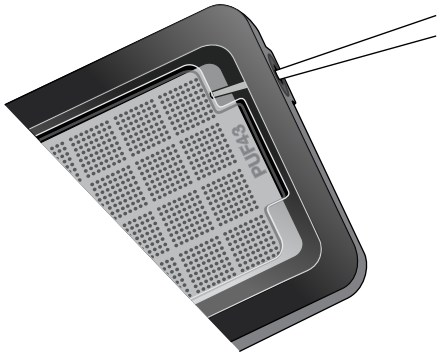
7. Prepare the immersion fluid. Remove the cap, insert the accompanying syringe tip, and prime the syringe by ejecting a small amount of immersion fluid onto a paper towel to ensure no air gap remains in the newly attached pipette tip.

IMPORTANT! If the syringe is not primed, the direct burst of air and fluid can negatively affect the assay(s) at the end of the array.

8. While holding the case upright by its edges at a 15–30 degree angle so that the port is at the highest point of the array, insert the prepared syringe tip into the port in the case.



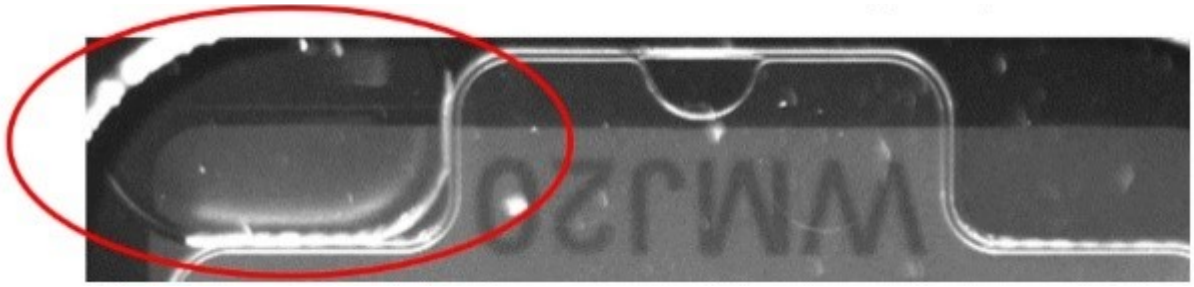
The syringe tip must be in front of the array when filling the case with immersion fluid.



9. Slowly inject the OpenArray™ Immersion Fluid until the case is filled, which should take about 10 seconds to fill. Minimize the creation of additional air bubbles when you dispense the fluid. Leave a small air bubble as shown below.

IMPORTANT! If injected too quickly, the fluid can flush out the samples that are suspended in the through-holes.

Overfilling the array and/or not leaving a small bubble may cause a leak during the PCR run.



10. While holding the case *vertically*, remove the syringe tip, insert the screw end of the OpenArray™ plug into the port of the case, then rotate clockwise until the black handle breaks off.

Note: Ensure that you are screwing the plug in at the same angle the case base is at. If it is off, it can cause the plug to break off prematurely.

IMPORTANT! To avoid leaking of immersion fluid, hold the case *vertically* and rotate the plug slowly to avoid cross-threading.

If the plug handle breaks off prematurely, use a Phillips #0 screwdriver to complete this step. Do not overtighten. If plastic or adhesive remains attached to the screw due to premature breakout of the plug handle, remove it with forceps prior to loading it into the instrument.

11. If needed, clean the case with the lint-free cloth included with the OpenArray™ Plate or a laboratory wipe that has been thoroughly sprayed with ethanol, then dry the case with a clean laboratory wipe.

The plate is ready for PCR.

IMPORTANT! Run the prepared verification plates within one hour after loading them (see “Run the experiment” on page 17).

Discard the filled plate after a successful instrument verification.

Run the experiment

1. In the QuantStudio™ 12K Flex Software home screen, click **Instrument Console**.
2. In the **Instrument Console**, select your QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.

   Note: You must add an instrument to your list before you can manage it.
3. After the QuantStudio™ 12K Flex Instrument is added to your list, select it, then click **Manage Instrument**.
4. In the **Instrument Manager**, start the RNase P wizard.
   a. Click **Maintenance**, then click **RNase P Run**.
   b. In the **RNase P Run** screen, click **Start RNase P Run**.
5. Complete the calibration as instructed by the wizard. When the instrument door opens, load the OpenArray™ Plate into *any* position on the plate carrier. Confirm that the OpenArray™ Plate is positioned so that the barcode is closest to the A1 position on the plate retainer and that the plug is oriented toward the front of the instrument.

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

   Note: The OpenArray™ Plate can be loaded into *any* position on the plate carrier.
6. Remove the clear protective film from the outside of the OpenArray™ Case (sealed plate + lid).

   See Figure 1 on page 15.
7. After loading the OpenArray™ Plate, start the calibration.
   a. In the **Overview** screen, select **Check the box when the RNase P calibration plate has been loaded**, then click **Next**.
   b. In the **Run** screen, click **START RUN** to start the calibration.

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

   Note: Before starting the calibration, the instrument may pause (up to 10 minutes) to allow the heated cover to reach temperature.
8. When the run is complete and the software displays the **Analysis** screen, verify the status of the run.

Analysis status	Action
Passed	Go to step 13.
Failed	Go to step 9 to review the data for outliers. If the run fails, the software may have included outliers that caused the initial analysis to fail. Experimental error may cause some through-holes to be amplified insufficiently or not at all. These through-holes typically produce C_t values that differ significantly from the average for the associated replicate through-holes. If included in the calculations, these outlying data (outliers) can result in erroneous measurements.

9. In the **Amplification Plot**, select **C_{RT} vs. Well** from the **Plot Type** menu, then verify the uniformity of the C_t values for the replicate population.
   a. In the plate layout, select all through-holes.
   b. In the plot, verify that the C_t values of the replicate population are equivalent.
   c. If an outlier is present in the population, select the corresponding through-hole of the plate layout, then click **Omit** to remove the through-hole from the analysis. If the total number of outliers for the replicate population exceeds 48 through-holes, repeat the experiment using another OpenArray™ Plate.
10. Review the **Results Table** for quality flags generated by the experiment.
    a. Select the **Results Table** tab.

b. Review the **Flag** column for through-holes that generated quality flags.

c. Troubleshoot each through-hole that generated a flag (see *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832)).

- AMPNC—Amplification in negative control
- EXPFAIL—Exponential algorithm failed
- HIGHSD—High standard deviation in replicate group
- NOAMP—No amplification
- NOISE—Noise higher than others in plate
- NOSIGNAL—No signal in through-hole
- OFFSCALE—Fluorescence is offscale
- OUTLIERRG—Outlier in replicate group
- SPIKE—Noise spikes
- THOLDFAIL—Thresholding algorithm failed

11. If you omitted outliers, click **Reanalyze** to analyze the run.

    If the status of the RNase P Run is "Failed" after performing step 9 to step 11, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Support.

12. Complete the calibration as instructed. When the instrument ejects the tray arm, discard the OpenArray™ Plate.

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

IMPORTANT! If the instrument does not eject the OpenArray™ Plate, see *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832).

13. Click **Finish**, then click **Yes** when prompted to save the experiment.

Perform an experiment

Overview of gene expression experiments

This section describes the procedure for preparing the 384-well plate for a gene expression experiment.

For detailed procedures, see *QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide* (Pub. No. 4470935).

Overview of the OpenArray™ 384-well Sample Plate

Use a 8- or 12-channel pipette to transfer nucleic acid samples from the 96-well reaction plates to a OpenArray™ 384-well Sample Plate.

You need to track the sample locations from the 96-well reaction plates to the appropriate locations in the OpenArray™ 384-well Sample Plate

The workflow for preparing the OpenArray™ 384-well Sample Plate varies. The workflow depends on the starter kit or the type of experiment.

Required materials

IMPORTANT! For the SDS of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
96-well reaction plates, containing prepared cDNA samples	User-supplied
TaqMan™ OpenArray™ Real-Time PCR Master Mix (2X), 1.5 mL	4462159[1]
OpenArray™ 384-well Sample Plates	4406947[1]
OpenArray™ 384-Well Plate Seals	4469876[1]
RT-PCR Grade Water	AM9935[2]
Fine-tip marker	MLS

[1] Provided in the starter kit.
[2] Not provided in the starter kit.

Track the samples

Track the samples from the 96-well reaction plates to the 384-well sample plates. For OpenArray™ AccuFill™ Software v2.0, samples are tracked in the OpenArray™ AccuFill™ Software. The samples are tracked in the **Map Plates** tab.

For more information about OpenArray™ AccuFill™ Software v2.0, see *QuantStudio™ 12K Flex AccuFill™ System User Guide* (Pub. No. MAN0025669).

Navigate to the **Full Run** screen.

1. In the **Configure design** pane, in the **Experiment type** section, select **Gene expression**.
2. In the **Plate format** section, select a format for your experiment.

Experiment type	Format
Starter kit experiments	Gene Expression—56
Your own experiments	• Gene Expression—18 • Gene Expression—56 • Gene Expression—112 • Gene Expression—168 • Gene Expression—224

3. In the **Pipettor** section, select a type of pipette.
   - **Fixed**
   - **Adjustable**

   The number of OpenArray™ Plate formats displayed in the **Add your OpenArray plate serial numbers** section depends on the selections made in the previous steps.
4. In the **Add your OpenArray Plate serial numbers** section, click **Choose File**, navigate to the location of the TPF file, then select the file.

   Repeat for each TPF file.
5. In the **Add your sample plates - optional** section, click **Choose File**, navigate to the location of the CSV file, then select the file.

   If the sample plate file is not imported, the samples must be added manually.

   The file must be a CSV file for a 96-well format.

   The format of the sample plate file is validated. For information about the required format, see *QuantStudio™ 12K Flex AccuFill™ System User Guide* (Pub. No. MAN0025669).

   The name of the file is displayed in the **Select file** field.
6. Repeat step 5 for each CSV file.
7. Click **Next**.

   The **Map plates** pane is displayed.
8. *(Optional)* Add or edit the sample name.
9. Label the OpenArray™ 384-well Sample Plate with a fine-tip marker.

   Based on the tracking information on the **Map plates** pane, mark the sections of the OpenArray™ 384-well Sample Plate to transfer samples from the 96-well reaction plates.
10. Click **Next**.

The **Start run** pane is displayed.

Set up the samples in the OpenArray™ 384-well Sample Plate, then prepare to load the OpenArray™ Plate formats in the QuantStudio™ 12K Flex AccuFill™ System.

Prepare the PCR Reaction Mix

1. Gently invert the tube of TaqMan™ OpenArray™ Real-Time PCR Master Mix several times.
2. Combine the following components to prepare the PCR Reaction Mix.

Component	Volume for 1 area of the 384-well sample plate[1]
TaqMan™ OpenArray™ Real-Time PCR Master Mix (2X)	132.0 µL
RT-PCR Grade Water	68.6 µL
Total volume of PCR Reaction Mix	**200.6 µL**

[1] One area of a 384-well sample plate corresponds to a single OpenArray™ Plate.

3. Mix well by pipetting up and down.

Transfer the samples to the OpenArray™ 384-well Sample Plate and add the PCR mix

Thaw the cDNA samples at room temperature.

1. Vortex the cDNA samples to mix, then centrifuge for 1 minute at 1,000 *x* g to collect the contents at the bottom of the tube.
2. Review the concentration of the normalized samples. The recommended starting concentration for human cDNA, gDNA, and plasmid DNA samples is approximately 100 ng/µL.

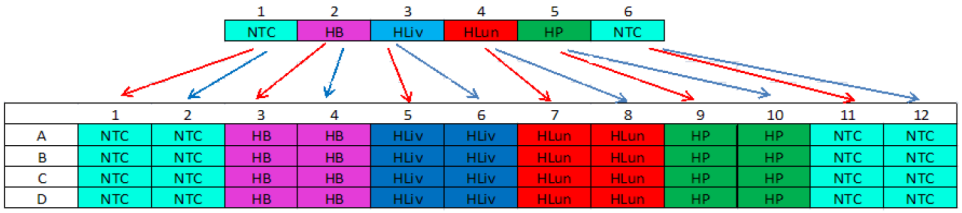
   Note: For optimal results, normalize all cDNA, gDNA, and plasmid DNA samples in an experiment. For example, if you use 200 ng/µL total RNA starting material and assume 100% efficiency in the reverse transcription reaction, you should obtain a human cDNA concentration of approximately 100 ng/µL equivalent to the total RNA.

3. Load the OpenArray™ 384-well Sample Plate based on the plate layout.

   See “Track the samples” on page 19.

   a. Add 5 µL of each PCR sample to the 384-well sample plate (see “Prepare the PCR Reaction Mix” on page 20).

   b. Using 6 tips from an 8- or 12-channel pipette, transfer the normalized cDNA, gDNA, or plasmid DNA samples from the 96-well reaction plate to the OpenArray™ 384-well Sample Plate.



Component	Volume per 384-well sample plate well[1]	
	Format 56 (starter kit experiment)	Format 18 (in triplicate) and remaining formats
Prepared PCR mix	3.8 µL	3.8 µL
Normalized human cDNA, gDNA, or plasmid DNA samples	1.2 µL	1.2 µL
Total volume	**5.0 µL**	**5.0 µL**

[1] One well of a 384-well sample plate corresponds to one subarray of an TrueMark™ OpenArray™ Plate. The number of subarrays required depends on the format of the TrueMark™ OpenArray™ Plate.

4. Seal the sample plate, vortex gently to mix, then centrifuge for 1 minute at 2,000 *x* g to eliminate bubbles and to collect the contents at the bottom of the wells.
5. Place the sample plate on ice for up to 1 hour.

Prepare the TrueMark™ OpenArray™ Plate. See *QuantStudio™ 12K Flex Real–Time PCR System: OpenArray™ Experiments User Guide* (Pub. No. 4470935).

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 TrueMark™ 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 TrueMark™ 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.

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

Revision history: Pub. No. 4478673 D

Revision	Date	Description
D	23 September 2024	• Sealing instructions were updated ("Seal the OpenArray™ Plate" on page 15). • Minor verbiage updates throughout document.
C	1 July 2024	• The reference to the instrument user guide was updated to *QuantStudio™ 12K Flex Real–Time PCR System v1.6 or later Maintenance and Administration Guide* (Pub. No. MAN0018832). • The materials required for calibration were updated. • The instructions to load the OpenArray™ Plate were updated for OpenArray™ AccuFill™ Software v2.0. • The instructions to seal the OpenArray™ Plate for RNase P instrument verification were updated. • Instructions were added to perform the RNase P instrument verification on the QuantStudio™ 12K Flex Instrument.
B	22 April 2014	Baseline for this revision history.