In this guide

**WARNING!** GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Maintenance and Administration Guide* (Part no. 4470689).

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs and Documentation and Support information refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Maintenance and Administration Guide* (Part no. 4470689).

For details on all the subjects indicated in this guide, refer to the following documents:

- *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470692)

The following topics are covered in this quick reference guide:

- QuantStudio™ 12K Flex Real-Time PCR System consumables ................................................................. 2
- Calibration types ........................................................................................................................................ 2
- Calibration materials ............................................................................................................................ 2
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**QuantStudio™ 12K Flex Real-Time PCR System consumables**

**Compatible consumables**

The QuantStudio™ 12K Flex Real-Time PCR System supports a series of specialized consumables through interchangeable sample blocks. Use the consumables appropriate for the sample block of your QuantStudio™ 12K Flex Real-Time PCR Instrument. This quick reference guide covers the OpenArray® plate.

<table>
<thead>
<tr>
<th>Sample block</th>
<th>Consumable</th>
<th>Reaction volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>OpenArray® plate</td>
<td>OpenArray® plate</td>
<td>33 nL</td>
</tr>
</tbody>
</table>

**Guidelines for handling consumables**

Observe the following guidelines when using OpenArray® plates:

- Wear gloves that are one size smaller than the size you typically wear, to help prevent excess glove material from contacting the OpenArray® plates while loading.
- Hold OpenArray® plates by the edges of the cases. Do not touch the through-holes.
- **Within one hour after opening the plate packaging**, load and seal the TaqMan® OpenArray® plates.
- If you drop a loaded OpenArray® plate, discard it in the appropriate waste container.

**Calibration types**

**Calibration types using the TaqMan® OpenArray® plate**

- Background calibration
- Uniformity calibration
- Dye calibration
- RNase P instrument verification

**Calibration materials**

**Materials required for background and uniformity calibration**

The QuantStudio™ 12K Flex OpenArray® Calibration Starter Kit (Part no. 4478650) contains accessories for assembling calibration materials for the QuantStudio™ 12K Flex OpenArray® sample block. Background calibration and uniformity calibration require only this kit, which uses the OpenArray® Calibration Plaque.

For information on instrument setup, the calibration process, and data analysis, please refer to the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide* (Part no. 4470689).

For more information, visit our website: [www.lifetechnologies.com](http://www.lifetechnologies.com).

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Part</th>
<th>Storage Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>4478650</td>
<td>QuantStudio™ 12K Flex OpenArray® Calibration Starter Kit</td>
<td>Ambient temperature [15 to 30°C]</td>
</tr>
<tr>
<td>4478586</td>
<td>QuantStudio™ 12K Flex OpenArray® Calibration Plaque</td>
<td></td>
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<tr>
<td>4469893</td>
<td>QuantStudio™ 12K Flex Plug Assembly [2]</td>
<td></td>
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<td>4478588</td>
<td>QuantStudio™ 12K Flex Calibration Syringe</td>
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<tr>
<td>4478587</td>
<td>QuantStudio™ 12K Flex Calibration Carrier</td>
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</tr>
<tr>
<td>4469912</td>
<td>QuantStudio™ 12K Flex Calibration Case Assemblies</td>
<td></td>
</tr>
</tbody>
</table>
Materials required for dye calibration

The QuantStudio™ 12K Flex OpenArray® FAM™ Dye (-20°C) (Part no. 4469963) is used to perform the dye calibration for the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR Instrument.

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Part</th>
<th>Storage Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>4469963</td>
<td>Applied Biosystems QuantStudio™ 12K Flex OpenArray® FAM™ Dye (-20°C)</td>
<td>-15 to -25°C</td>
</tr>
</tbody>
</table>

Materials for RNase P instrument verification

The QuantStudio™ 12K Flex OpenArray® Block RNase P Kit (Part no. 4469602) is made up of two QuantStudio™ OpenArray® kits. These kits are used to verify the performance of the QuantStudio™ 12K Flex Real-Time PCR System.

- TaqMan® RNase P QuantStudio™ 12K Flex OpenArray® Instrument Verification Kit (Part no. 4469594).
- The QuantStudio™ OpenArray® Accessories Kit (Part no. 446986).

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Part</th>
<th>Storage Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>4469594</td>
<td>TaqMan® RNase P QuantStudio™ 12K Flex OpenArray® Instrument Verification Kit</td>
<td>Freezer (-30 to -10°C)</td>
</tr>
<tr>
<td>4463465</td>
<td>OpenArray® RNase P Reaction Mix</td>
<td></td>
</tr>
<tr>
<td>4470186</td>
<td>TaqMan® OpenArray® Digital PCR Kit 1 PAK</td>
<td></td>
</tr>
<tr>
<td>4469586</td>
<td>QuantStudio™ OpenArray® Accessories Kit</td>
<td>Ambient temperature (15 to 30°C)</td>
</tr>
<tr>
<td>4469879</td>
<td>QuantStudio™ 12K Flex OpenArray® Lid</td>
<td></td>
</tr>
<tr>
<td>4469893</td>
<td>QuantStudio™ 12K Flex OpenArray® Plug</td>
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<tr>
<td>4469573</td>
<td>QuantStudio™ 12K Flex OpenArray® Carrier</td>
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<tr>
<td>4472095</td>
<td>QuantStudio™ 12K Flex OpenArray® Immersion Fluid</td>
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<tr>
<td>4406947</td>
<td>OpenArray® 384-Well Sample Plates</td>
<td></td>
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<tr>
<td>4457246</td>
<td>OpenArray® AccuFill™ System Tips</td>
<td></td>
</tr>
</tbody>
</table>

Note: For safety and biohazard guidelines, refer to the “Safety” section in the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide (Part no. 4470689). For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Perform the background calibration

**IMPORTANT!** Perform the following procedure only if you are calibrating a QuantStudio™ 12K Flex Real-Time PCR with an OpenArray® plate sample block.

During a background calibration, the QuantStudio™ 12K Flex Real-Time PCR:

- Performs reads of the QuantStudio™ 12K Flex 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**

- QuantStudio™ 12K Flex 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 not abnormally high.

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.

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 Real-Time PCR with an OpenArray® sample block. The plaque consists of a thin sheet of black plastic that has two distinct sides shown below.

<table>
<thead>
<tr>
<th>Black side</th>
<th>Orange side</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Dull, matte black in color.</td>
<td></td>
</tr>
<tr>
<td>• Completely smooth.</td>
<td></td>
</tr>
<tr>
<td>• Performs the background calibration.</td>
<td></td>
</tr>
<tr>
<td>• Glossy, dark orange in color.</td>
<td></td>
</tr>
<tr>
<td>• Textured with a faint lattice pattern.</td>
<td></td>
</tr>
<tr>
<td>• Performs the uniformity calibration.</td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Action</th>
<th>Guidelines</th>
</tr>
</thead>
</table>
| Handling | When handling the OpenArray® Calibration Plaque:  
• Always wear powder-free gloves.  
• Grasp the tool by the edges.  
• Ensure the tool remains clean. |
| Storage | When not in use, store the OpenArray® Calibration Plaque:  
• 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 | Clean the OpenArray® Calibration Plaque as follows:  
a. Place the OpenArray® Calibration Plaque on a clean, dry surface.  
b. Pipet a small volume of 95% ethanol or 95% isopropanol solution onto a lint-free wipe, then thoroughly swab the surface of the tool.  
c. Use a lint-free wipe to absorb the excess solution. |
**Load the plaque**

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

   **IMPORTANT!** Ensure that the OpenArray® Calibration Plaque is loaded into the plate retainer so that the *black* side of the tool is facing up.

![Image of OpenArray® Calibration Plaque]

**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 OpenArray® Calibration Plaque, 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 QuantStudio™ 12K Flex Real-Time PCR instrument is in operation.

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

**Rotate the plaque**

When the instrument door opens and you are prompted to rotate the OpenArray® Calibration Plaque:

1. Rotate the OpenArray® Calibration Plaque 180°, then place it back into the plate retainer (black side up).

   **IMPORTANT!** Do not flip the OpenArray® Calibration Plaque over. The *black* side of the tool must face up.

![Image of OpenArray® Calibration Plaque rotated 180°]

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 that the data it did collect is unusable.

<table>
<thead>
<tr>
<th>Analysis status</th>
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</tr>
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</table>
| 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 OpenArray® Calibration Plaque before you repeat the calibration.  
                      2. If the calibration fails again, contact Life Technologies for further assistance. |

2. When the instrument door opens, remove the OpenArray® 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 OpenArray® Calibration Plaque to its original packaging or a clean plastic bag.

**IMPORTANT!** Do not expose the OpenArray® 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 QuantStudio™ 12K Flex Real-Time PCR instrument does not eject the OpenArray® Calibration Plaque, remove the plate as explained in “Troubleshooting” in the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Maintenance and Administration Guide* (Part no. 4470689).

Perform the uniformity calibration

**IMPORTANT!** Perform the following procedure only if you are calibrating a QuantStudio™ 12K Flex Real-Time PCR with an OpenArray® plate sample block.

The uniformity calibration generates data that allows the QuantStudio™ 12K Flex software to compensate for the physical effects of the QuantStudio™ 12K Flex Real-Time PCR filters.

**Required materials**
- QuantStudio™ 12K Flex OpenArray® Calibration Plaque
- Powder-free gloves
- Safety goggles
When to perform the calibration

Perform a uniformity calibration every year, or as often as necessary, depending on instrument 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 using each instrument filter. The QuantStudio™ 12K Flex software uses the captured images to calibrate the optical uniformity of the QuantStudio™ 12K Flex Real-Time PCR instrument.

**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.

About the OpenArray® Calibration Plaque

See “About the OpenArray® Calibration Plaque” on page 4 for information on the use and maintenance of the OpenArray® Calibration Plaque.

Load the plaque

1. When the instrument door opens, load the OpenArray® Calibration Plaque into the plate retainer with the orange side of the tool facing up.

   ![OpenArray® Calibration Plaque](image)

   **IMPORTANT!** Ensure that the OpenArray® 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 QuantStudio™ 12K Flex Real-Time PCR instrument is in operation.

   **Note:** Before starting the calibration, the instrument may pause (≤ 10 minutes) to allow the heated cover to reach temperature.
Rotate the plaque
When the instrument door opens and you are prompted to rotate the OpenArray® Calibration Plaque:

1. Rotate the OpenArray® Calibration Plaque 180°, then place it back into the plate retainer with the orange side of the tool facing up.

**IMPORTANT!** Do not flip the OpenArray® 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 that the data it did collect is unusable.

<table>
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| 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 OpenArray® Calibration Plaque before you repeat the calibration.  
                  2. If the calibration fails again, contact Life Technologies for further assistance. |

2. When the instrument door opens, remove the OpenArray® 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 OpenArray® Calibration Plaque to its original packaging or a clean plastic bag.

**IMPORTANT!** Do not expose the OpenArray® 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 QuantStudio™ 12K Flex Real-Time PCR instrument does not eject the OpenArray® Calibration Plaque, remove the plate as explained in “Troubleshooting” in the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Maintenance and Administration Guide* (Part no. 4470689).
Perform the dye calibration

**IMPORTANT!** Perform the following procedure only if you are calibrating a QuantStudio™ 12K Flex Real-Time PCR with an OpenArray® plate sample block.

During a dye calibration, the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR:
- 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 the FAM™ dye in the total fluorescence collected by the QuantStudio™ 12K Flex Real-Time PCR instrument. After each run, the QuantStudio™ 12K Flex 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 QuantStudio™ 12K Flex 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 QuantStudio™ 12K Flex Real-Time PCR.

**Required materials**
- QuantStudio™ 12K Flex Real-Time PCR Installation/Calibration Kit:
  - OpenArray® FAM™ Dye Solution
  - QuantStudio™ 12K Flex Real-Time PCR OpenArray® Calibration Cases (4)
  - QuantStudio™ 12K Flex Real-Time PCR OpenArray® Plugs (4)
  - QuantStudio™ 12K Flex Real-Time PCR OpenArray® Calibration Syringe and Tip
- OpenArray® Plate Press
- Pipettes
- 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 instrument use. Perform a background calibration before every dye calibration.

**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 Real-Time PCR 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 OpenArray® FAM™ Dye Solution

**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 QuantStudio™ 12K Flex software extracts the dye calibration data, it evaluates the fluorescence signal generated by each OpenArray™ 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 QuantStudio™ 12K Flex 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 OpenArray™ calibration case (auto-repairing). The QuantStudio™ 12K Flex 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 OpenArray® Calibration Cases by the edges.
- If you drop a loaded OpenArray® Calibration Case, discard it in the appropriate waste container.

**Perform the empty reading**

**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 protective film from all of the OpenArray® Calibration Cases.
   b. Load case 1 into the position closest to the QuantStudio™ 12K Flex Real-Time PCR instrument followed by the remaining cases in sequence as shown in the following figure.

   ![Plug Diagram]

   **IMPORTANT!** Ensure that the OpenArray® Calibration Cases are positioned so that the plugs are oriented away from the A1 position as shown.

2. After loading the OpenArray® 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 QuantStudio™ 12K Flex Real-Time PCR instrument is in operation.

**Note:** Before starting the calibration, the instrument may pause (≤ 10 minutes) to allow the heated cover to reach temperature.
Perform the filled reading

**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 OpenArray® FAM™ Dye Solution:

1. Attach a syringe tip to a syringe, then place the assembly on a clean surface.
2. Carefully draw approximately 2 mL of OpenArray® FAM™ Dye Solution 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 OpenArray® Calibration Case.
5. While holding the OpenArray® 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.

**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.

7. Load the sealed OpenArray® calibration case into the *same* position on the OpenArray® Plate Carrier that it previously occupied (position 1).

**IMPORTANT!** You *must* load the filled OpenArray® Calibration Cases into the same positions on the OpenArray® 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 steps 1-7 to fill the remaining three OpenArray® Calibration Cases.
IMPORTANT! Confirm that the OpenArray® Calibration Cases are in their original positions and that their plugs are oriented away from the A1 position as shown.

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 QuantStudio™ 12K Flex Real-Time PCR instrument is in operation.

**Note:** Before starting the calibration, the instrument may pause (≤ 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 that the data it did collect is unusable.
   - **QC Status** – Indicates the quality of the calibration data, where *passed* indicates that all OpenArray® calibration cases produced data that passed the quality check, and *failed* indicates that one or more cases produced dye spectra that vary significantly.

<table>
<thead>
<tr>
<th>Analysis status</th>
<th>Action</th>
</tr>
</thead>
</table>
| 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 Life Technologies for further assistance. |

2. When the instrument door opens, remove the OpenArray® Plate Carrier 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. Discard the OpenArray® Calibration Cases.

**IMPORTANT!** If the QuantStudio™ 12K Flex Real-Time PCR instrument does not eject the plate, remove the plate as explained in "Troubleshooting" in the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Maintenance and Administration Guide* (Part no. 4470689).
Verify the instrument performance

Perform the RNase P instrument verification experiment to verify the performance of an Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR.

**IMPORTANT!** When performing the RNase P instrument verification experiment:
- Perform all calibrations beforehand.
- Run the OpenArray® plate soon after you allow the plate or reagents to thaw. Minimizing the time that the prepared consumable sits on the bench ensures optimal performance.
- Wear powder-free gloves and safety glasses when you prepare OpenArray® plates.

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 a OpenArray® plate within *one hour* after opening its packaging.
- If you drop a loaded OpenArray® plate, discard it in the appropriate waste container.

Required materials

- QuantStudio™ 12K Flex Real-Time PCR RNase P Kit, including:
  - OpenArray® RNase P Reaction Mix
  - QuantStudio™ 12K Flex Real-Time PCR OpenArray® Lid
  - QuantStudio™ 12K Flex Real-Time PCR OpenArray® Plug
  - QuantStudio™ 12K Flex Real-Time PCR OpenArray® Immersion Fluid
  - QuantStudio™ 12K Flex Real-Time PCR OpenArray® Immersion Fluid Tip
  - OpenArray® Digital PCR Plate
  - OpenArray® 384-Well Sample Plate
- OpenArray® AccuFill™ System
- OpenArray® Plate Press
- Bleach (10%)
- Ethanol
- OpenArray® AccuFill™ System Loader Tips
- Pipettes
- Powder-free gloves
- Safety glasses

Preparing for the verification experiment

**IMPORTANT!** Wear powder-free gloves while preparing the OpenArray® plates.

1. Confirm that the OpenArray® 384-well sample plate, OpenArray® AccuFill™ Loader Tips, and 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:
   - a. Remove the cap from the syringe containing OpenArray® Immersion Fluid.
   - b. Attach the syringe tip to the syringe, then set the assembly on a clean surface.
4. Pipette 5.0 µL of the RNase P solution into rows A to D of the 384-well sample plate (loading positions 1 and 2).

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.

**Initializing the system**

1. Close the enclosure door, then start the OpenArray® Accufill™ software. The software checks the computer and connections as the system starts.

   When prompted, clear the deck and empty the waste bin of used tips:

   a. Open the instrument by grasping the enclosure door handle and gently, but firmly, pulling the enclosure door up.

      **Note:** To safely operate the instrument, it is important to keep the deck clear and have enough room in the waste bin to eject the used pipette tips.

   b. Empty the waste bin and place it back on the deck.

2. If there is a plate holder on the deck, check if there are any OpenArray® plates on it. If necessary, remove them.
3. If necessary, replace the tip boxes.

   Note: Tip boxes contain 384 tips, divided into 8 sections. When you click Load, the OpenArray® Accufill™ instrument loads as though a new, full box of tips is on the deck. OpenArray® Accufill™ software prompts you to verify that tips are in the locations shown in the Setup Deck screen. Clicking a section in the Setup Deck window confirms that tips are in that section of the tip box.

   a. Place tip boxes into the assigned locations.
   b. Place tip boxes on the deck in the two side-by-side recessed rectangular platforms. See the illustration for the location of the tip boxes (purple and white).
   c. Remove the cover before using the tips for loading.

4. Close the door on the instrument.

5. Click Proceed to begin the System Self Test. The application performs a number of self tests and is then ready for you to continue.

   Note: System Self Test runs only at start up. The test does not run again unless the system is restarted or a self test is intentionally run. The System Self Test utility is in the Instrument drop-down menu in the OpenArray® Accufill™ application.

Preparing for loading

1. Click Setup & Load.

2. Open the enclosure door of the OpenArray® Accufill™ instrument by grasping the door handle and lifting the door up.

3. In the Setup Load Information window, enter or scan the barcode of your TaqMan® RNase P OpenArray® Plate Instrument Verification Reagents Kit into the Sample Plate field.

4. Insert the 384-well sample plate with the foil cover still in place. Press on the plate until it snaps into place.

   Note: Do not remove the foil from the 384-well sample plate at this stage.

5. Enter the data for the OpenArray® plate:

   a. Select 1 from the Samples Per Subarray drop-down list.
   b. In the plate holder Position 1 text field, enter RNase P (the sample loaded into first position of the plate holder).
   c. Place a thawed OpenArray® Digital PCR Plate into the plate holder.

      Hold the OpenArray® plate by the edges and place it into the plate holder with the barcode face up and to the left.
6. Click Next.

**Loading the OpenArray® plate**

1. Verify that the Tip Status window in the software matches the state of the tips on the deck. Ensure that:
   - Gray areas in the Tip Status window indicate that tips are not present.
   - White areas indicate that tips are present.
   
   If the software and the tips on the deck do not match, click the appropriate section in the Tip Status window.

2. Verify each of the following conditions and then select each check box:
   - Tips are configured as shown in step 1.
   - Waste bin is empty.
   - OpenArray® plate is in the plate holder.

   ![Tip Status window](image)

   ![Setup Deck](image)

   **Note:** The software will not continue until you select all the check boxes.

3. With forceps, peel off the foil covering the area of the sample plate containing the samples to be loaded on the OpenArray® plate.

4. Select check box: **Remove foil from the highlighted section of the Sample Plate.**

5. Close the instrument door.
6. Click Load.
   **Note:** If the number of OpenArray® plates in the instrument differs from the number that is entered in the Setup Load Information window, an error message instructs you to remove any extra plates. Correct the error and continue.

7. When the Remove OpenArray® Plate window appears, open the instrument door, carefully remove the indicated OpenArray® plate, then immediately seal the plate as explained in “Sealing the OpenArray® plate”.
   **IMPORTANT!** Once an OpenArray® plate has been filled, you must seal it within 90 seconds to prevent excessive evaporation.

8. Close the instrument door.
   **Note:** After you run the plate, clean the OpenArray® Accufill™ instrument. For instructions, see “Maintenance” in the *Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Maintenance and Administration Guide* (Part no. 4470689)

**Sealing the OpenArray® plate**

1. Remove the protective film from the top and bottom of an OpenArray® Case Lid.
   **IMPORTANT!** Remove the protective film from both sides of the lid.

2. Grasp the OpenArray® case by the top (nearest the barcode) using the thumb and index finger of your left hand. Then, gently lift the case from the plate holder and load it into the OpenArray® Plate Press.

3. Place the OpenArray® Case Lid with protective film removed (both top and bottom) onto the OpenArray® plate using the alignment pins of the OpenArray® Plate Press for orientation.

4. Actuate the OpenArray® Plate Press for 10 seconds.
5. Load the OpenArray® case with OpenArray® Immersion Fluid:

**IMPORTANT!** Do not expose the Immersion fluid in the OpenArray® cases to air for more than 60 minutes.

a. Remove the sealed OpenArray® case from the press, grasping the case by the edges.

b. Insert the syringe tip into the loading port at end of the sealed OpenArray® case, then dispense the fluid completely in one gentle continuous motion.

**IMPORTANT!** Expel the OpenArray® Immersion Fluid slowly. If injected too quickly, the fluid can flush out the samples suspended in the through-holes.

Note: Try to minimize creating air bubbles when you dispense the fluid: one small air bubble is acceptable.

c. While holding the OpenArray® plate vertically, seal the loading port with OpenArray® Plug by inserting the plug into the port and twisting the plug clockwise until hand-tight. Once secure, remove the knob from the plug.

d. Clean the case with a laboratory wipe that has been thoroughly sprayed with ethanol. To dry the case, wipe the case downward with a clean laboratory wipe. Gently handle the case; be sure to not apply pressure on the OpenArray® plate within the case.

The sealed OpenArray® plate can be loaded into the QuantStudio™ 12K Flex Real-Time PCR instrument.

**Note:** Dust or excess sample on the case may interfere with thermal uniformity and can fluoresce. Make sure you thoroughly clean each case.
IMPORTANT! Run the prepared calibration OpenArray® plates within one hour after loading them. Discard the filled plate after a successful RNase P verification.

For Troubleshooting procedures, see “Calibrating OpenArray® Plate Sample Blocks” in the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide (Part no. 4470692)

Run the experiment
Run the experiment from the QuantStudio™ 12K Flex software. See “Running the experiment” in the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: Maintenance and Administration Guide (Part no. 4470689).

Perform a Gene Expression Experiment
This section describes the procedure for preparing the 384-well plate for a gene expression experiment. For a detailed explanation of the complete procedure, see the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System: OpenArray® Experiments User Guide (Part no. 4470935).

Prepare the 384-well plate
Use a 8- or 12-channel pipette to transfer the nucleic acid samples from the 96-well reaction plates to TaqMan® OpenArray® 384-Well Sample Plates (see Appendix B in Booklet 4, QuantStudio™ 12K Flex System TaqMan® OpenArray® Experiments). You will also track the sample locations from the 96-well reaction plates to the appropriate locations in the 384-well sample plates. The workflow for preparing the 384-well sample plate varies, depending on the starter kit (or experiment type)

Required materials

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
<th>Part no.‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well reaction plates, containing prepared cDNA samples</td>
<td>User-supplied</td>
<td>—</td>
</tr>
<tr>
<td>2X TaqMan® TaqMan® OpenArray® Real-Time PCR Master Mix, 1.5 mL</td>
<td>Life Technologies</td>
<td>4462159</td>
</tr>
<tr>
<td>TaqMan® OpenArray® 384-Well Sample Plates</td>
<td>Life Technologies</td>
<td>4406947</td>
</tr>
<tr>
<td>QuantStudio™ 12K Flex Real-Time PCR TaqMan® OpenArray® 384-Well Plate Seals</td>
<td>Life Technologies</td>
<td>4469876</td>
</tr>
<tr>
<td>RT-PCR Grade Water</td>
<td>Major Laboratory Suppliers (MLS)</td>
<td>AM9935§N</td>
</tr>
<tr>
<td>Fine-tip marker</td>
<td>MLS</td>
<td>—</td>
</tr>
</tbody>
</table>

† For the SDS of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.
‡ Provided in starter kit.
§ Not provided in starter kit.

Track the samples
Track the samples from the 96-well reaction plates to the 384-well sample plates. For gene expression experiments, we recommend that you use the OpenArray® Sample Tracker Software to track your samples.

Note: This section provides brief procedures for using the OpenArray® Sample Tracker Software. For detailed procedures, refer to the OpenArray® Sample Tracker Software Quick Reference.

1. In the OpenArray® Sample Tracker Software Properties window, enter general information about the gene expression experiment:
   a. From the Experiment Type drop-down list, select Gene Expression
   b. From the TaqMan® OpenArray® Plate drop-down list, select the appropriate TaqMan® OpenArray® plate format:
      • (For the starter kit experiment) Gene Expression – 56
c. From the Pipettor drop-down list, select Fixed or Adjustable.

d. If you have added a serial number or barcode to the TaqMan® OpenArray® 384-Well Sample Plate, enter the serial number.

2. Enter the sample information:

   (For the starter kit experiment) Navigate to and import sample information from the gene expression starter kit *.csv file into the OpenArray® Sample Tracker Software. The sample information file is located at:
   
   <drive>:\Program Files\Applied Biosystems\OpenArray Sample Tracker\examples\Endogenous Control Plate 96 well.csv - where <drive> is the computer hard drive on which the OpenArray® Sample Tracker Software is installed. The default installation drive for the software is the C: drive.

   (Recommended for your own experiments) Navigate to and import sample information from a sample information *.csv file into the OpenArray® Sample Tracker Software, if available.

   (Optional) Manually enter sample information from the 96-well reaction plates into the OpenArray® Sample Tracker Software.

   The OpenArray® Sample Tracker Software automatically maps the sample locations from the 96-well reaction plates to the appropriate locations in the 384-well sample plates and TaqMan® OpenArray® plates.

3. Export the sample information in table format (*.csv):

   a. In the Sample Mapping window, select the 384-well tab, then click Export → Export *.csv.

   b. Select the plates to export as *.csv files:
      
      (Recommended, and for the starter kit experiment) 384-well Plate – Use this file with the QuantStudio™ TaqMan® OpenArray® AccuFill™ Software to create a loaded transcript plate file (*.tpf).
      
      (Optional) OpenArray Plate → Use this *.csv file to import setup information into the QuantStudio™ Software.

   All plates are saved to individual *.csv files in the export directory. The OpenArray® Sample Tracker Software automatically assigns the file names.

4. Using a fine-tip marker:

   a. Label the 384-well sample plate with a unique identifier.

   b. Based on the tracking information obtained in steps 1 to 3 above, mark the sections of the 384-well sample plate that you will transfer the samples to from the 96-well reaction plates.

Prepare the PCR mix

1. Mix the 2X TaqMan® TaqMan® OpenArray® Real-Time PCR Master Mix by gently inverting the tube a few times.

2. Combine the following components:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL) for 1 area of the 384-well sample plate†</th>
<th>Stock concentration</th>
<th>Final concentration</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>2X TaqMan® TaqMan® OpenArray® Real-Time PCR Master Mix</td>
<td>132.0</td>
<td>2</td>
<td>1</td>
<td>X</td>
</tr>
<tr>
<td>RNase-free water</td>
<td>68.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Final volume of PCR mix</td>
<td>200.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

† One area of a 384-well sample plate corresponds to a single TaqMan® TaqMan® OpenArray® plate (see Appendix B in Booklet 4, QuantStudio™ 12K Flex System TaqMan® OpenArray® Experiments - Appendices).

3. Mix well by pipetting up and down.
Transfer the samples

1. Thaw the cDNA samples at room temperature. Mix the samples by vortexing, then centrifuge for 1 minute @ 1000 rpm.

2. Review the concentration of the normalized samples. The recommended starting concentration for human cDNA, gDNA, and plasmid DNA samples is ~100 ng/µL.

   Note: For optimal results, it is important to 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 ~100 ng/µL equivalent to the total RNA.

3. Based on the Assay Layout you are using, load the 384-well sample plate:
   a. Add 5 µL of each PCR sample from the microfuge tubes to the 384-well sample plate.
   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 TaqMan® OpenArray® 384-well sample plate.

4. Seal the sample plate, vortex gently to mix, then centrifuge for 1 minute @ 2000 rpm to eliminate bubbles.

5. Place the sample plate on ice for up to 1 hour.

Troubleshooting

For Troubleshooting procedures, see Calibrating OpenArray® Plate Sample Blocks” in the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR System Maintenance and Administration Guide (Part no. 4470692)

Maintain the instrument

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

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

† One well of a 384-well sample plate corresponds to one subarray of an TaqMan® OpenArray® plate. The number of subarrays required depends on the format of the TaqMan® OpenArray® plate. For detailed information about the TaqMan® OpenArray® plates, see Appendix B in Booklet 4, QuantStudio™ 12K Flex System TaqMan® OpenArray® Experiments Appendixes.
IMPORTANT! Do not use organic solvents to clean the QuantStudio™ 12K Flex Real-Time PCR instrument.

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

† You can perform a background calibration to check for contamination. If any parts of the optics are replaced or moved, you must perform all calibrations, including an RNase P instrument verification run.
‡ ROI and normalization calibrations are not required for QuantStudio™ 12K Flex Real-Time PCR instrument with OpenArray® plate sample blocks.

**Power off the instrument**

The Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR operates in low-power mode when not in use; however, the instrument can be powered off completely so that the components draw no power.

1. Power off the Applied Biosystems QuantStudio™ 12K Flex Real-Time PCR
   a. If the instrument touchscreen is not blank, touch \( \mathbf{0} \) to place the instrument into stand-by mode.
   b. Toggle the power button on the rear of the instrument.

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

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

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

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