USER GUIDE

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# Applied Biosystems® 7300Plus Real-Time PCR Instrument

## Calibration and Maintenance

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life technologies

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# Contents

# About this guide

**CAUTION! ABBREVIATED SAFETY ALERTS.** Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

## Revision history

| Revision | Date | Description |
|---|---|---|
| A | January 2015 | Initial release of document, applicable to 7300Plus Real-Time PCR Instruments running Real-Time PCR Software v2.4 or later. |

## Purpose

The Applied Biosystems® 7300Plus Real-Time PCR Instrument User Guide provides information about calibrating and maintaining the Applied Biosystems® 7300Plus Real-Time PCR Instrument.

## Intended use

The Applied Biosystems® 7300Plus Real-Time PCR Instrument is a real-time nucleic acid amplification and detection instrument that measures nucleic acid signals from DNA or reverse transcribed RNA and converts them to comparative quantitative readouts using fluorescent detection of dual-labeled hydrolysis probes.

The Applied Biosystems® 7300Plus Real-Time PCR Instrument is to be used only by technologists trained in laboratory techniques, procedures, and on use of the analyzer.

Note: The manufacturer is not liable for damage or injury that results from use of this manual by unauthorized or untrained parties.

# 1 Overview



**Note:** For more information about any of the topics discussed in this guide, access the Help from within Real-Time PCR Software by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ Real-Time PCR Software Help**.

**Note:** The software information in this document is applicable to Real-Time PCR Software v2.4 and later versions.

## About the 7300Plus Instrument

The Applied Biosystems® 7300Plus Real-Time PCR Instrument is a 96-well, four-color platform that uses fluorescence-based polymerase chain reaction (PCR) reagents to provide:

- Quantitative detection of target nucleic acid sequences (targets) using real-time analysis.
- Qualitative detection of targets using post-PCR (endpoint) analysis.
- Qualitative analysis of the PCR product (achieved by melt curve analysis that occurs post-PCR).

### About data collection

The 7300Plus Instrument collects raw fluorescence data at different points during a PCR, depending on the type of run that the instrument performs:

| Run type | Analysis | Data collection point |
|---|---|---|
| Real-time runs | Standard curve | The instrument collects data after each extension step of the PCR. |
| | Relative standard curve | |
| | Comparative $C_T$ ($\Delta\Delta C_T$) | |

| Run type | Analysis | Data collection point |
|---|---|---|
| Post-PCR (endpoint) runs | Genotyping | The instrument collects data:<br>• Before the PCR. (For presence/absence experiments, data collection before the PCR is optional but recommended.)<br>• (Optional) During the PCR. The instrument can collect data during the run (real-time); collecting data during the run can be helpful for troubleshooting.<br>• After the PCR. |
| | Presence/absence | |

Regardless of the run type, a data collection point or *read* on the 7300Plus Instrument consists of three phases:

1. **Excitation**– The instrument illuminates all wells of the reaction plate within the instrument, exciting the fluorophores in each reaction.
2. **Emission**– The instrument optics collect the residual fluorescence emitted from the wells of the reaction plate. The resulting image collected by the device consists only of light that corresponds to the range of emission wavelengths.
3. **Collection**– The instrument assembles a digital representation of the residual fluorescence collected over a fixed time interval. The Real-Time PCR Software stores the raw fluorescence image for analysis.

After a run, the Real-Time PCR Software uses region of interest (ROI), optical, dye, and background calibrations to determine the location and intensity of the fluorescence in each read, the dye associated with each fluorescent signal, and the significance of the signals.

## About the filters

The 7300Plus Instrument uses the following filters:

| Filter | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **Dye** | • FAM™ dye<br>• SYBR® dye | • JOE™ dye<br>• VIC® dye | • TAMRA™ dye<br>• NED™ dye<br>• Cy™3 dye | • ROX™ dye<br>• Texas Red ® dye |

## For more information

For information on the 7300Plus Instrument and experiments performed using the Real-Time PCR Software, refer to the *Real-Time PCR Software Help*.

**Note:** To open the Help, select **Help ▸ Real-Time PCR Software Help** in the Real-Time PCR Software.

# How to use this guide

This guide describes how to calibrate and maintain the Applied Biosystems® 7300Plus Real-Time PCR Instrument. Chapter 2, "Perform the Regions of Interest (ROI) Calibration" through Chapter 5, "Verify Instrument Performance" of this manual describe calibrations that you must perform as regular maintenance of the 7300Plus Instrument. Chapter 6, "User-Performed Maintenance" and the appendices contain maintenance procedures that you may need to resolve infrequent problems.

| Chapter/ Appendix | Description |
|---|---|
| Chapter 2, "Perform the Regions of Interest (ROI) Calibration" | How to perform an ROI calibration, which allows the Real-Time PCR Software to map the positions of the wells on the sample block so that it can associate the fluorescence collected during a run with specific wells of the plate. |
| Chapter 3, "Perform the Background Calibration and Optical Calibration" | How to perform background and optical calibrations which allows the Real-Time PCR Software to remove background fluorescence from experiment data. |
| Chapter 4, "Perform the Dye Calibration" | How to perform dye calibrations, which allow the software to distinguish the individual contribution of each dye in the total fluorescence collected by the instrument. |
| Chapter 5, "Verify Instrument Performance" | How to perform a TaqMan® RNase P Instrument Verification Plate run that can be used to verify the performance of a 7300Plus Instrument. |
| Chapter 6, "User-Performed Maintenance" | How to:<br>• Replace the user-serviceable parts of the 7300Plus Instrument.<br>• Resolve infrequent problems that can occur during instrument use. |
| Appendix A, "Store, Move, and Install the 7300Plus Instrument" | How to store, move, and reinstall the components of the 7300Plus Instrument. |
| Appendix B, "Create a Custom Dye Plate" | How to create a dye plate that can be used to calibrate the 7300Plus Instrument for a dye not manufactured by Thermo Fisher Scientific. |
| Appendix C, "Create a Background Plate" | How to create a background plate. |

**IMPORTANT!** If you use and/or install the 7300Plus Instrument in an unspecified manner, you may impair the protection provided by the equipment.

# Recommended maintenance schedule

The following table displays the recommended maintenance schedule for the 7300Plus Instrument and computer. The procedures listed in the table are intended for the user(s) of the 7300Plus Instrument. To ensure proper operation of your instrument, perform the regular weekly, monthly, and semiannual maintenance indicated below.

**IMPORTANT!** The numbered lists in the table below indicate that the tasks must be performed in sequence.

| Perform... | User-performed maintenance task |
|---|---|
| Weekly | • Check the computer disk space. If necessary, archive or back up your experiment files.<br>• Power off the computer controlling the 7300Plus Instrument, then after 30 sec, power on the computer.<br>• Clean the surface of the 7300Plus Instrument with a lint-free cloth.<br><br>**IMPORTANT!** Do not use organic solvents to clean the 7300Plus Instrument. |
| Monthly | • Check the lamp status. If necessary, replace the halogen lamp.<br>• Perform a background calibration.[1]<br>• Run disk cleanup and disk defragmentation. |
| Semiannually (6 Months) | • Check the lamp status. If necessary, replace the halogen lamp.<br>• Perform a regions of interest (ROI) calibration.<br>• Perform a background calibration.<br>• Perform an optical calibration.<br>• Perform a dye calibration.<br>• Perform an RNase P instrument verification run. |
| As needed | • Decontaminate the 7300Plus Instrument.<br>• Replace the Halogen Lamp.<br>• Replace the 7300Plus Instrument fuses.<br>• Update the Real-Time PCR Software. |

[1] You can perform a background calibration to check for contamination. If any parts of the optics are replaced or moved, you must run an ROI calibration, a background calibration, an optical calibration, a dye calibration, and an RNase P instrument verification run.

# Maintain the computer hard drive

### When to defragment the hard drive

- At least once every month
- When a message is displayed by the Windows® operating system instructing you to defragment

### For more information

In the desktop, select **Start ▸ Help and Support** to access the Help for the Windows® operating system. Use the search function of the Help to find information on the "Disk Cleanup" and "Disk Defragment" utilities.

**Note:** Do not run the disk management utilities and Real-Time PCR Software at the same time.

## Archive and back up EDS files

### Archive EDS files regularly

To conserve space on the computer hard drive, older EDS files can be archived using a data compression utility. Several commercially available compression utilities are available. PKZIP and *.arc are archive formats common to the Microsoft® Windows® operating system.

### Back up EDS files

We strongly recommend that you back up your experiments.

Backing up data:

- Protects against potential loss of data caused by an unforeseen failure of the computer or its hard drive.
- Conserves space on the hard drive and optimizes performance, if you remove old data after backing up.

### Develop a data management strategy

We recommend developing a strategy for managing the files produced by the Real-Time PCR Software.

**Note:** Real-time runs generate significantly more data than genotyping or presence/absence experiments. During one day of real-time operation, the 7300Plus Instrument can generate more than 10 MB of data.

### Check disk space

If you perform real-time experiments on your 7300Plus Instrument, check the amount of available space on your hard drive weekly. When the hard drive is within 20% of maximum capacity, transfer the older data to a backup storage device.

**Note:** You can perform a background calibration to check for contamination. If any parts of the optics are replaced or moved, you must run an ROI calibration, a background calibration, an optical calibration, a dye calibration, and an RNase P instrument verification run.

# 2 Perform the Regions of Interest (ROI) Calibration



**Note:** For more information about any of the topics discussed in this guide, access the Help from within Real-Time PCR Software by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ Real-Time PCR Software Help**.

## Overview

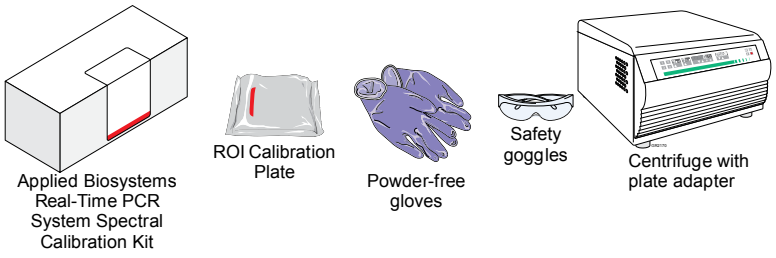
A regions of interest (ROI) calibration maps the positions of the wells on the sample block of the Applied Biosystems® 7300Plus Real-Time PCR Instrument. The Real-Time PCR Software uses the ROI calibration data to associate increases in fluorescence during a run with specific wells of the plate. The instrument uses a set of optic filters to distinguish the fluorescence emissions gathered during runs. You must generate a calibration image for each individual filter to account for minor differences in the optical path.

**Note:** The ROI calibration is a user-performed maintenance procedure.

### Time required

Around five minutes.

### Materials required




### When to perform the calibration

Perform an ROI calibration:

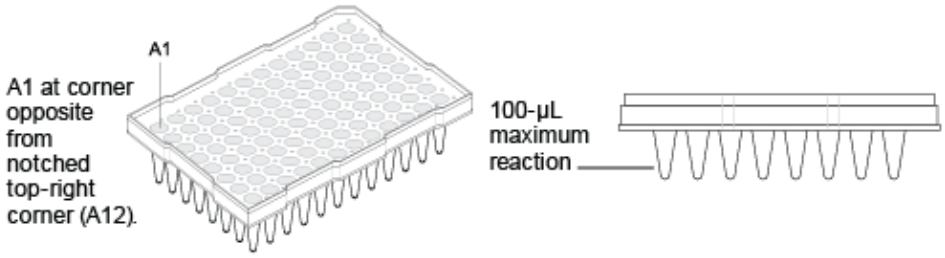
- When installing the 7300Plus Instrument. You must perform in sequence the ROI, background, optical, and dye calibrations and the instrument verification run.
- Every 6 months, or as often as necessary, depending on instrument use.
- After replacing the lamp.

**IMPORTANT!** After every ROI calibration, you must perform a background calibration, optical calibration, dye calibration, and instrument verification.

# Prepare the ROI calibration plate

## Standard plates

Use the Standard plates on your 7300Plus Instrument.

| Standard Plates – 7300Plus Instruments |
|---|
| Notched corner at top right<br>  <br><br>Vortex standard plates to ensure complete mixing, then centrifuge to ensure that all reagents are contained in the bottom of the well. |

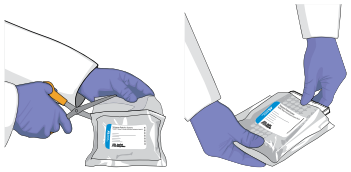
## Prepare the plate

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

1. Obtain the ROI calibration plate from the spectral calibration kit in the freezer.
2. Allow the ROI calibration plate to warm to room temperature (approximately 5 min).

   **IMPORTANT!** Do not remove an ROI calibration plate from its packaging until you are ready to run it. The fluorescent dye in the wells of the plate is photosensitive. Prolonged exposure to light can diminish the fluorescence from the plate.
3. Remove the ROI calibration plate from its packaging. Leave the optical film on the plate.

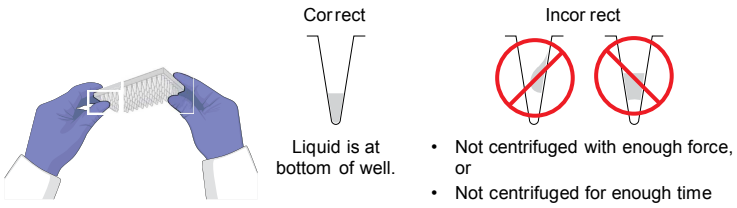
   **IMPORTANT!** Do not discard the packaging for the ROI calibration plate. The plate can be used up to three times if it is stored in its original packaging sleeve.

   
4. Vortex the ROI calibration plate for 5 sec.

5. Centrifuge the plate for 2 min at 750 - 1000 g.

**IMPORTANT!** The ROI calibration plate must be well mixed and centrifuged.

6. Verify that the liquid in each well of the ROI calibration plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

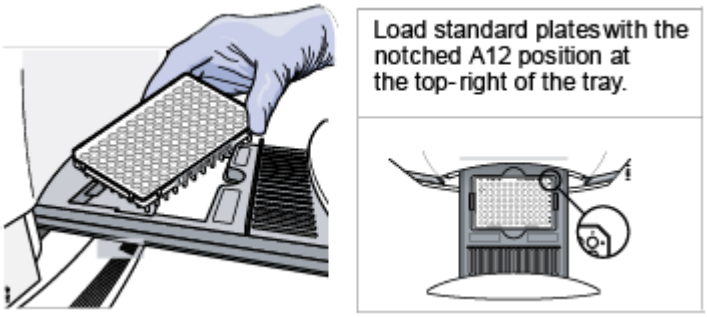



## Load the plate

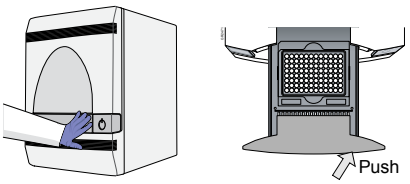
**WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Push the tray door to open it.

2. Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.




3. Close the tray door. Apply pressure to the right side of the tray door at an angle.



# Perform the ROI calibration

## Start the Calibration

1. In the Real-Time PCR Software, select **Instrument ▸ Instrument Maintenance Manager**.
2. In the ROI screen of the Instrument Maintenance Manager, click **Start Calibration**.
3. Complete the calibration as instructed by the wizard.

   The ROI Calibration dialog box displays three tabs:

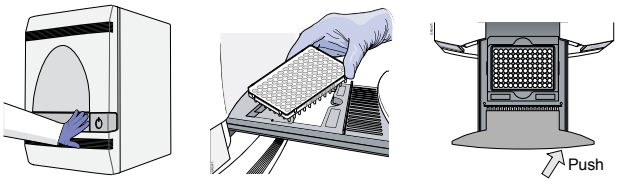
   - **Setup** – Displays instructions for setting up the ROI calibration. Clicking **Next** opens the Run tab.
   - **Run** – Clicking **START RUN** starts the calibration process and displays the processing messages. Clicking Next opens the Analysis tab.
   - **Analysis** – Indicates the calibration status (Passed/Failed).

   If you cannot obtain a passing calibration, see "Troubleshoot the ROI calibration" on page 18.

# Unload the plate

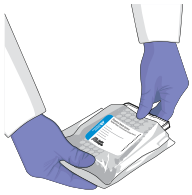
**WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Remove the calibration plate:
   a. Push the tray door to open it.
   b. Remove the calibration plate.
   c. Push the tray door to close it.




2. Place the calibration plate inside its packaging sleeve. If you plan to perform background and optical calibrations:
   - Within the next 8 hr, keep the ROI calibration plate at room temperature. The optical calibration uses the ROI calibration plate.
   - On another day, return the packaged plate to the spectral calibration kit in the freezer.

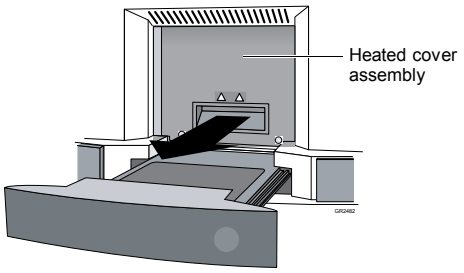
**IMPORTANT!** Do not discard the calibration plate. If the plate is stored in its original packaging sleeve, you can use it up to three times after you open it.



Continue with "Perform the Background Calibration and Optical Calibration".

**IMPORTANT!** After you perform an ROI calibration, you must also perform a background calibration (see page 20), an optical calibration (see page 25), dye calibrations (see page 32), and instrument verification (see page 46).

## Troubleshoot the ROI calibration

| Observation | Possible cause | Recommended action |
|---|---|---|
| ROI calibration failed | The sample block may be in its lowered position. | 1. Check that the heated cover assembly is pulled all the way forward to ensure that the tray can be pushed in properly. If the 7300Plus Instrument has a heated cover latch installed, check that the latch is in a locked position.<br>  <br>2. If the ROI calibration continues to fail, check the status of the halogen lamp within the 7300Plus Instrument (see "Monitor the Lamp Status"), then replace the lamp if necessary (see "Replace the Halogen Lamp"). |

# 3 Perform the Background Calibration and Optical Calibration



**Note:** For more information about any of the topics discussed in this guide, access the Help from within Real-Time PCR Software by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ Real-Time PCR Software Help**.

## Perform the background calibration

During a background calibration, the 7300Plus Instrument:

- Performs reads of a background plate containing PCR buffer for 10 min at 60°C.
- Averages the spectra recorded during the run and extracts the resulting spectral component to a calibration file.

The Real-Time PCR Software then uses the calibration file during subsequent runs to remove the background fluorescence from the run data.

**Note:** The background calibration is a user-performed maintenance procedure.

### Time required

10 min

### Materials required

### When to perform the calibration

Perform a background calibration:

- When installing the 7300Plus Instrument. You must perform in sequence the ROI, background, optical, and dye calibrations and the instrument verification run.
- Monthly, or as often as necessary, depending on instrument use.
- After replacing the lamp.

### Background fluorescence

Fluorescence data collected by the 7300Plus Instrument includes a fluorescence signal inherent to the system, referred to as background fluorescence. Background fluorescence is a composite signal found in all spectral data. This signal consists of fluorescence from several sources, including:

- Background electronic signal
- Contaminants in the sample block
- The plastic consumable (plates and caps)

### Guidelines for calibration

- Make sure the centrifuge you use is clean. Before centrifuging, wipe down the bucket using a tissue.
- Handle the calibration plates with care to prevent contamination. Do not place plates on a lab bench, which may contaminate the plate. Always put calibration plates back into their original bags.

## Perform the background calibration

### Prepare the plate

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

1. Obtain the prepared background plate from the spectral calibration kit in the freezer.
2. Allow the background plate to warm to room temperature (at least 5 min).
3. Remove the background plate from its packaging.

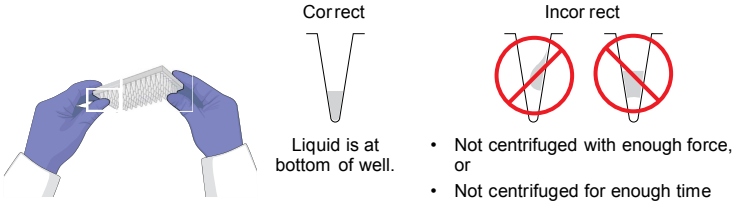
   **IMPORTANT!** Do not discard the packaging for the plate. The background plate can be used up to three times if it is stored in its original packaging sleeve.

4. Vortex the plate for 5 sec.
5. Centrifuge the plate for 2 min at 750 - 1000 g.

   **IMPORTANT!** The plate must be well mixed and centrifuged.

6. Verify that the liquid in each well of the background plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

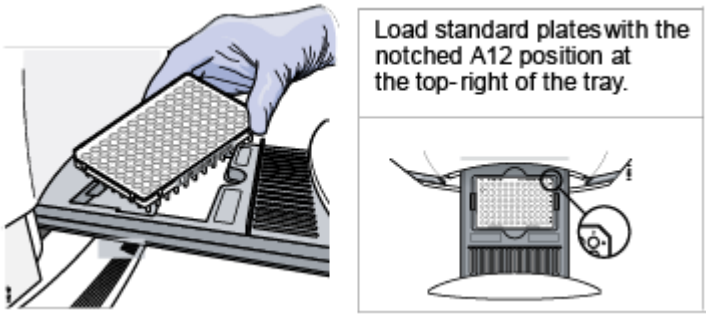
**IMPORTANT!** Do not allow the bottom of the background plate to become dirty. Fluids and other contaminants that adhere to the bottom of the plate can contaminate the sample block and cause an abnormally high background signal.



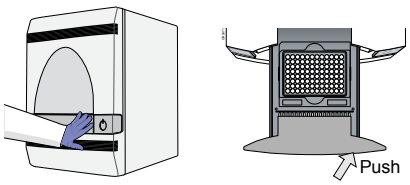

## Load the plate

**WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Push the tray door to open it.
2. Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.




3. Close the tray door. Apply pressure to the right side of the tray door at an angle.



# Prepare the background calibration plate

## Perform the calibration

1. In the Real-Time PCR Software, select **Instrument ▶ Instrument Maintenance Manager**.
2. In the Instrument Maintenance Manager, select the Background tab.
3. In the Background tab, click **Start Calibration**.
4. Complete the calibration as instructed by the wizard.

   The Background Calibration dialog box displays four tabs:

   - **Overview** – Displays information describing the calibration.
   - **Setup** – Displays instructions for setting up the background calibration. Clicking Next opens the Run tab.
   - **Run** – Clicking START RUN starts the calibration process and displays the processing messages. Clicking Next opens the Analysis tab.
   - **Analysis** – Indicates the calibration status (Passed/Failed).

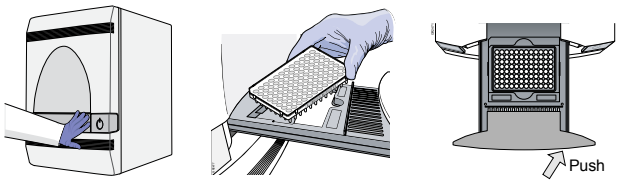
   If you cannot obtain a passing calibration, see "Troubleshoot the background calibration" on page 27.

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

## Unload the plate

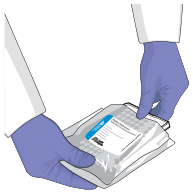
**WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Remove the calibration plate:
   a. Push the tray door to open it.
   b. Remove the calibration plate.
   c. Push the tray door to move it into the instrument.



2. Place the calibration plate inside its packaging sleeve, then return the packaged plate to the spectral calibration kit in the freezer.

   **IMPORTANT!** Do not discard the calibration plate. If the plate is stored in its original packaging sleeve, you can use the plate up to three times after you open it.



If necessary, continue with"Perform the optical calibration" on page 25 .

You must perform an optical calibration (see "Perform the optical calibration" on page 25), dye calibrations (see "Perform the dye calibration" on page 31), and instrument verification (see Chapter 5, "Verify Instrument Performance") if you are performing the background calibration:

- As part of your semiannual maintenance
- After replacing or moving any parts of the optics

For more information, see "Recommended maintenance schedule" on page 12.

# Perform the optical calibration

The optical calibration compensates for the physical effects of the optical system in the 7300Plus Instrument.

**Note:** The optical calibration is a user-performed maintenance procedure.

## Time required

10 min

## Materials required

ROI calibration plate

## When to perform the calibration

Perform an optical calibration:

- When installing the 7300Plus Instrument. You must perform in sequence the ROI, background, optical, and dye calibrations and the instrument verification run.
- Every 6 months, or as often as necessary, depending on instrument use.

# Prepare the calibration plate

## Prepare the plate

If you kept your ROI calibration plate at room temperature after performing an ROI calibration (see Chapter 2, "Perform the Regions of Interest (ROI) Calibration"), skip to step 5 to spin down any condensation that may have formed when the plate was at room temperature. If the ROI calibration plate is in the freezer, go to step 1.

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

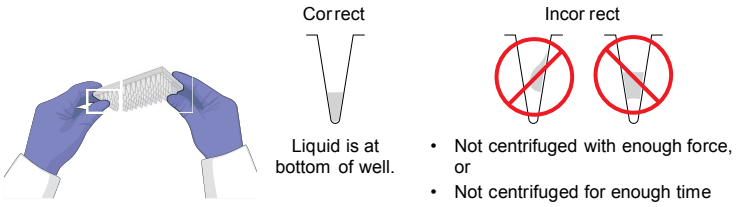
1. Obtain the ROI calibration plate from the spectral calibration kit in the freezer.
2. Allow the ROI calibration plate to warm to room temperature (at least 5 min).
3. Remove the ROI calibration plate from its packaging.

   **IMPORTANT!** Do not discard the packaging for the plate. The ROI calibration plate can be used up to three times if it is stored in its original packaging sleeve.

4. Vortex the plate for 5 sec.
5. Centrifuge the plate for 2 min at 750 - 1000 g.

   **IMPORTANT!** The ROI calibration plate must be well mixed and centrifuged.

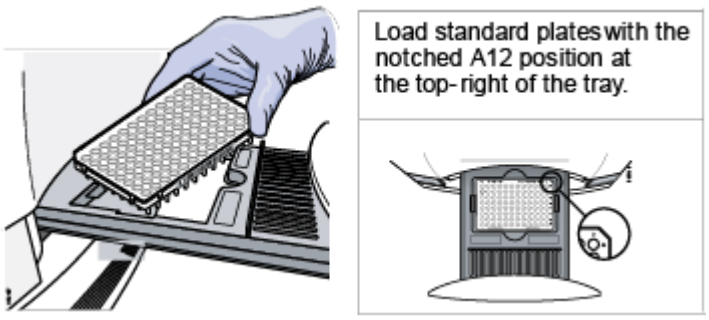
6. Verify that the liquid in each well of the ROI calibration plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.



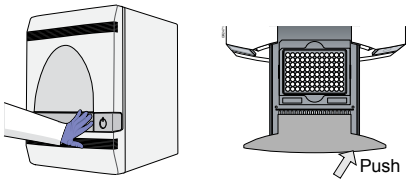
### Load the plate

**WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Push the tray door to open it.
2. Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.




3. Close the tray door. Apply pressure to the right side of the tray door at an angle.




## Perform the optical calibration

### Perform the calibration

1. In the Real-Time PCR Software, select **Instrument ▶ Instrument Maintenance Manager**.
2. In the Instrument Maintenance Manager, select the Optical tab.
3. In the Optical screen, click **Start Calibration**.

4. Complete the calibration as instructed by the wizard.

   The Optical Calibration dialog box displays four tabs:

   - **Overview** – Displays information describing the calibration.
   - **Setup** – Displays instructions for setting up the optical calibration. Clicking Next opens the Run tab.
   - **Run** – Clicking START RUN starts the calibration process and displays the processing messages. Clicking Next opens the Analysis tab.
   - **Analysis** – Indicates the calibration status (Passed/Failed).

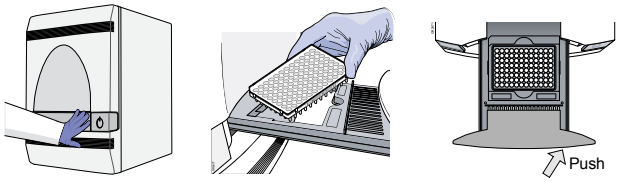
   If you cannot obtain a passing calibration, see "Troubleshoot the background calibration" on page 27.

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

## Unload the plate

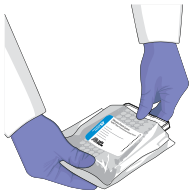
**WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Remove the calibration plate:
   a. Push the tray door to open it.
   b. Remove the calibration plate.
   c. Push the tray door to move it into the instrument.




2. Place the calibration plate inside its packaging sleeve. Return the packaged plate to the spectral calibration kit in the freezer.

   **IMPORTANT!** Do not discard the calibration plate. If the plate is stored in its original packaging sleeve, you can use it up to three times after you open it.



Continue with Chapter 4, "Perform the Dye Calibration".

# Troubleshoot the background calibration

| Observation | Possible cause | Recommended action |
|---|---|---|
| Background calibration failed | One or more wells of the background plate produced spectra that exceed the maximum limit for the instrument. | 1. Repeat the calibration using the same background plate.<br>2. If the calibration fails again, repeat the calibration using a different background plate.<br>3. If the calibration fails again, determine the source of the contamination, as explained in "How to identify contamination" on page 27. |

## How to identify contamination

Signals that exceed the limit of normal background fluorescence may indicate fluorescent contaminants on the calibration plate or the sample block. Common contaminants include ink residue from permanent pens, powder from disposable gloves, and dust.

To determine the source and location of the contamination:

1. While viewing the raw spectra, locate the contaminated well position(s) by selecting successively smaller regions of the plate layout.
2. Rotate the background plate 180°, then perform the background calibration again.
3. Repeat step 1 to locate the contamination. If the well position(s) of the contamination in steps 1 and 3 are:
   - **Identical** – The sample block is contaminated. Decontaminate the sample block as explained in "Decontaminate the sample block" on page 50.
   - **Reversed** – The background plate is contaminated. Discard the background plate and perform the background run using a new background plate.

   If the calibration fails after you replace the background plate and decontaminate the sample block:
   - Load a plate covered by a piece of black paper into the 7300Plus Instrument.
   - Perform the background run as explained in this chapter.
   - After the run is complete, select all wells of the plate layout.
   - View the Spectral plot for the peak(s). If a peak is:
     - **Visible** – The optics of your 7300Plus Instrument may be contaminated. Contact Technical Support as explained in "Customer and technical support" on page 83.
     - **Absent** – The sample block is contaminated. Decontaminate the sample block as explained in "Decontaminate the sample block" on page 50.

# 4 Perform the Dye Calibration



**Note:** For more information about any of the topics discussed in this guide, access the Help from within Real-Time PCR Software by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ Real-Time PCR Software Help**.

## Overview

During a dye calibration, the Applied Biosystems® 7300Plus Real-Time PCR Instrument:

- Collects spectral data from a series of dye standards.
- Stores the spectral information for the dye standards in a pure spectra calibration file.

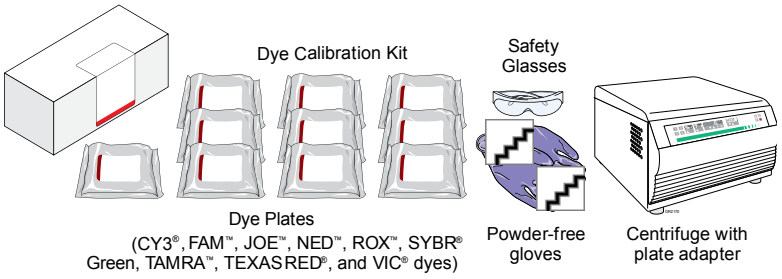
The software uses the pure spectra data during experiment runs to characterize and distinguish the individual contribution of each dye in the total fluorescence collected by the instrument. After each run, the Real-Time PCR Software receives data in the form of a raw spectra signal for each reading. 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.

**Note:** The dye calibration is a user-performed maintenance procedure.

### Time required

1 hr

### Materials required




**Note:** If you store the dye calibration plates in their original packaging in the freezer, you can use them to calibrate a 7300Plus Instrument up to 3 times for 6 months after opening them.

## When to perform dye calibration

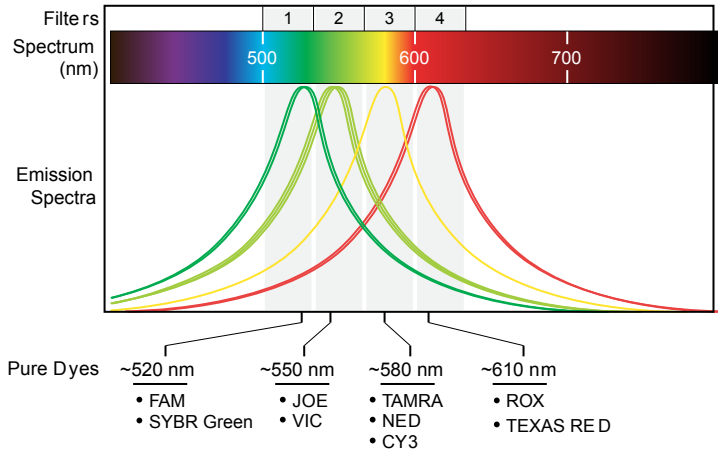
Perform a dye calibration:

- When installing the 7300Plus Instrument. You must perform in sequence the ROI, background, optical, and dye calibrations and the instrument verification run.
- Every 6 months, or as often as necessary, depending on instrument use.

**IMPORTANT!** You must perform a background run before every series of dye calibrations. Because the age and use of instrument components can affect pure spectra readings, we recommend performing a dye calibration at least every 6 months.

## Dye sets

The Applied Biosystems® 7300Plus Real-Time PCR Instruments use the following dye sets for calibration: Cy™3 dye, FAM™ dye, JOE™ dye, NED™ dye, ROX™ dye, SYBR® Green dye, TAMRA™ dye, TEXAS RED ™dye, and VIC® dye. The following figure shows the emission spectrum for each dye, and the filters and wavelengths at which each dye is read.




## Custom dye

The 7300Plus Instrument can be used to run assays designed with custom dyes (dyes not supplied by Thermo Fisher Scientific). However, before using custom dyes with the 7300Plus Instrument, you must create and run a custom calibration plate. The Real-Time PCR Software uses the custom calibration plate to create a spectral standard to distinguish the custom dye in the fluorescence data collected during the run. See Appendix B, "Create a Custom Dye Plate" for information on custom dye calibrations.

**IMPORTANT!** To use a custom dye on your 7300Plus Instrument, it must fluoresce within the 520 to 650 nm spectral range measured by the 7300Plus Instrument.

## About the analysis

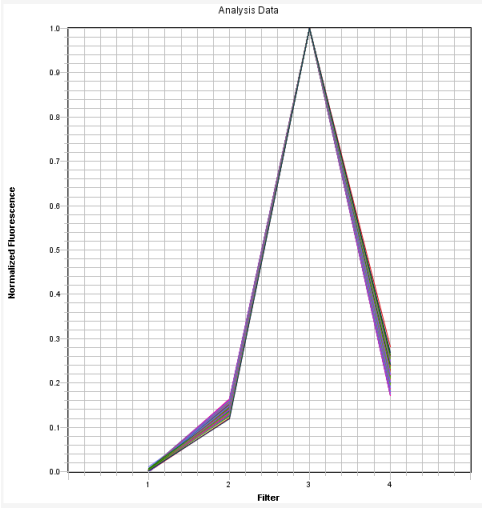
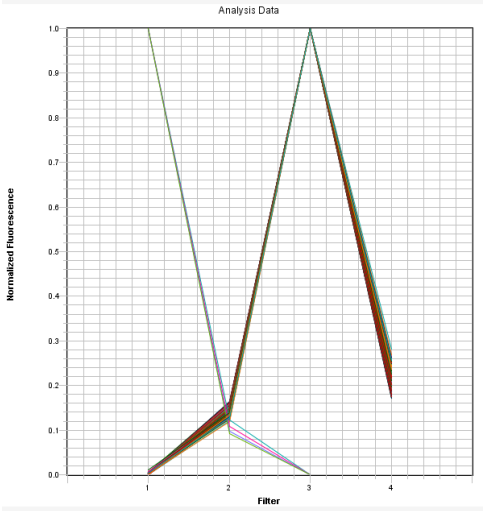
The product of a dye calibration is a collection of spectral profiles that represent the fluorescence signature of each dye standard. Each profile consists of a set of spectra that correspond to the fluorescence collected from the wells of the spectral calibration plate. The Real-Time PCR Software plots the resulting data for each spectral profile in a graph of fluorescence versus filter.

When the Real-Time PCR Software extracts the calibration data from a dye run, it evaluates the fluorescence signal generated by each well in terms of the collective spectra for the entire calibration plate. Dye spectra are generally acceptable if they

peak within the same filter as their group but diverge slightly at other wavelengths (see below).

The Real-Time PCR Software can compensate for some differences in a spectral profile by replacing (auto-repairing) the spectra of unacceptable wells with the spectra of other wells on the reaction plate. However, the software allows only a few replacements and may reject the calibration if the spectra between neighboring wells vary significantly.

**Note:** Because the wells in a calibration plate contain dyes at identical concentrations, the resulting signals for the wells containing each dye should be similar. Among wells containing the same dye, variations in spectral position and peak position are caused by minor differences in the optical properties and excitation energy between the individual wells.

| Acceptable Spectra | Unacceptable Spectra |
|---|---|
|  |  |
| Spectra peak at the same wavelength and do not diverge significantly | Spectra peak at the different wavelengths |

# Prepare the dye calibration plates

**IMPORTANT!** Before performing a dye calibration, you must perform an ROI calibration (see Chapter 2, “Perform the Regions of Interest (ROI) Calibration“), a background calibration (see “Perform the background calibration“ on page 20), and an optical calibration (see “Perform the optical calibration“ on page 25).

### Prepare the plates

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

1. Obtain the spectral calibration kit from the freezer, then remove all of the dye plates.
2. Return the spectral calibration kit to the freezer.
3. Allow the dye plates to warm to room temperature (approximately 5 min).

   **IMPORTANT!** Do not remove a dye plate from its packaging until you are ready to run it. The fluorescent dye in the wells of each dye plate is photosensitive. Prolonged exposure to light can diminish the fluorescence signal strength of the plate.

   **Note:** If you store the dye calibration plates in their original packaging in the freezer, you can use them to calibrate a 7300Plus Instrument up to 3 times for 6 months after opening them.

Continue with "Perform the calibration" on page 31.

## Perform the dye calibration

### Perform the calibration

1. In the Real-Time PCR Software, select **Instrument ▶ Instrument Maintenance Manager**.
2. In the Instrument Maintenance Manager, select the Dye tab.
3. In the Dye screen, select **System Dye Calibration**.
4. Click **Start Calibration**.
5. Complete the calibration for each plate as instructed by the wizard.

   **IMPORTANT!** The wizard guides you through the calibration of each dye separately. You must set up, run, and analyze each dye plate independently.

   The Dye Calibration dialog box displays four tabs:

   - **Overview** – Displays information describing the calibration.
     When the software prompts you to obtain the required materials, select the dyes that you want to calibrate.
   - **Setup** – Displays instructions for setting up the dye calibration. Clicking Next opens the Run tab.
     When the software prompts you to load each dye plate, prepare and load the plates as described in "Load a dye plate" on page 32.
   - **Run** – Clicking START RUN starts the calibration process and displays the processing messages. Clicking Next opens the Analysis tab.
   - **Analysis** – Indicates the calibration status (Passed/Failed).

When the software prompts you to analyze the spectra collected from each dye plate, verify the status of the calibration:

- **Passed** – The 7300Plus Instrument passed the calibration. Go to "Analyze the calibration data" on page 33.
- **Failed** – The 7300Plus Instrument failed the calibration. Troubleshoot the error as described in "Troubleshoot the dye calibration" on page 36.

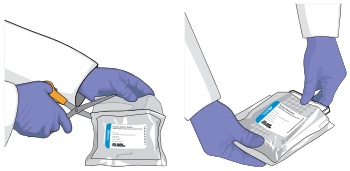
**Note:** Before starting the calibration, the instrument may pause (up to 10 min) to allow the heated cover to reach temperature.

## Load a dye plate

**Note:** Because the wizard guides you through the calibration of each dye separately, perform the following procedure for each dye that you calibrate.

1. Remove the dye plate that is specified by the software from its packaging.

   **IMPORTANT!** Do not discard the packaging for the plate. The plate can be used up to three times if it is stored in its original packaging sleeve.

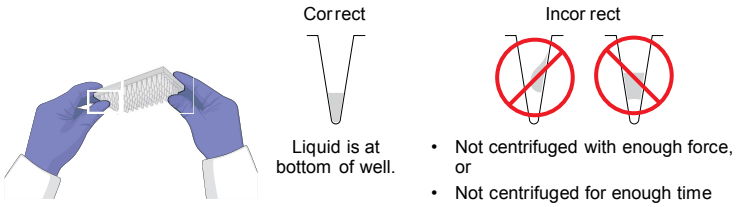


2. Vortex the plate for 5 sec.

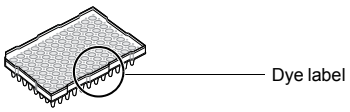
3. Centrifuge the plate for 2 min at 750 -1000 g.

   **IMPORTANT!** The plate must be well mixed and centrifuged.

4. Verify that the liquid in each well of the plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

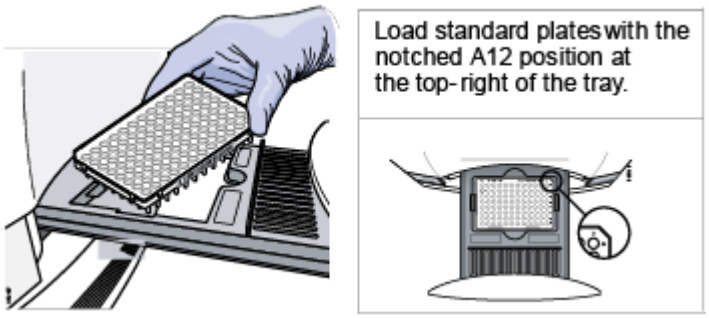



5. Verify that the dye plate that you are about to load matches the dye selected in the Real-Time PCR Software.




6. Push the tray door to open it.

7. Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.




8. Close the tray door. Apply pressure to the right side of the tray door at an angle.

   **Note:** If you cannot open the tray, the sample block may be in its raised position, locking the tray position. To lower the block, select InstrumentCalibrate, then exit the ROI Inspector.

## Analyze the calibration data

**Note:** Because the wizard guides you through the calibration of each dye separately, perform the following procedure for each dye that you calibrate.

1. Verify the status of the calibration:
   - **Passed** – The 7300Plus Instrument passed the calibration. Go to Analyze the Calibration Data on page 33.
   - **Failed** – The 7300Plus Instrument failed the calibration. Troubleshoot the error as described in "Troubleshoot the dye calibration" on page 36.

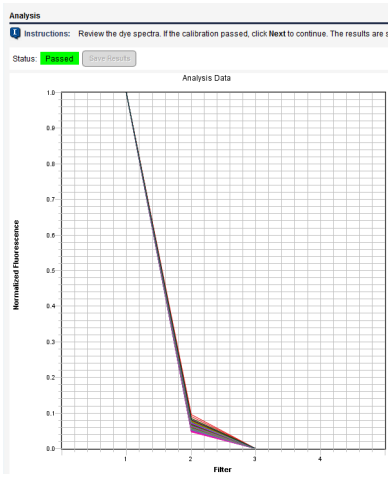
2. Verify the grouping of the dye spectra:
   a. In the plate layout, select the wells of the plate.

   b. Inspect the raw data. For each spectrum, verify that the peak is:
      - Within the detectable range for the 7300Plus Instrument.
      - Free of irregular spectral peaks.
      - Present in the correct channel for the dye (see Table 1).

      If a spectrum does not match the criteria above, troubleshoot the problem as described in "Troubleshoot the dye calibration" on page 36.

      **Note:** Among wells containing the same dye, variations in spectral position and peak position are caused by minor differences in the optical properties and excitation energy between the individual wells.

3. If all spectra are acceptable, then click **Next**.



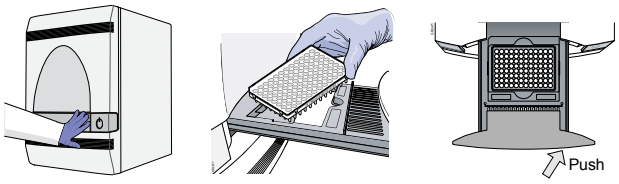

4. Remove the calibration plate:

**WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

a. Push the tray door to open it.

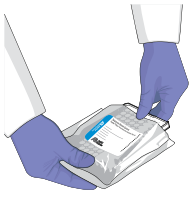
b. Remove the calibration plate.

c. Push the tray door to close it.




d. Place the calibration plate inside its packaging sleeve. Return the packaged plate to the spectral calibration kit in the freezer.

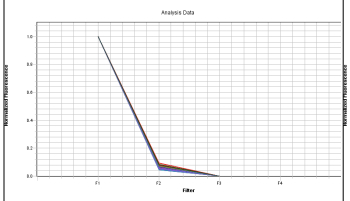
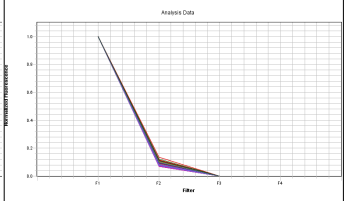
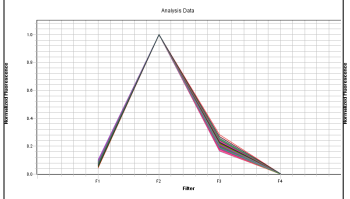
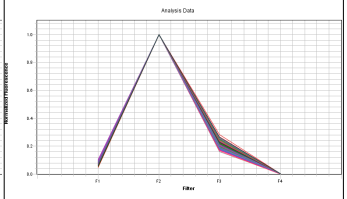
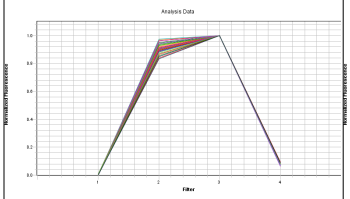
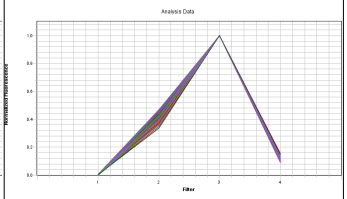
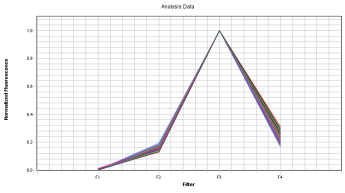
**Note:** If you store the dye calibration plates in their original packaging in the freezer, you can use them to calibrate 7300Plus Instruments up to 3 times for 6 months after opening them.

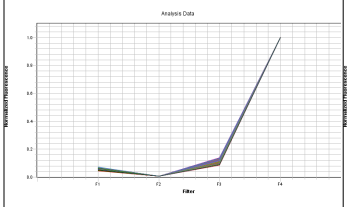
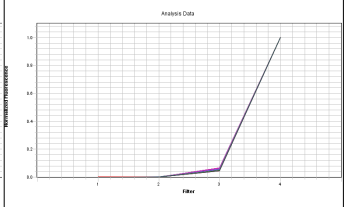


5. After you remove the dye plate as instructed, click **Finish**.

6. Prepare and run the next plate as explained in "Prepare the plates" on page 31.

**Table 1** 7300Plus Instrument dye spectra

| Filter | Peak (nm) | Dye/Spectra | |
|---|---|---|---|
| 1 | ~520 | FAM™ dye  | SYBR® Green dye  |
| 2 | ~550 | JOE™ dye  | VIC® dye  |
| 3 | ~580 | Cy®3 dye  | NED™ dye  |
| | | TAMRA™ dye  | |

| Filter | Peak (nm) | Dye/Spectra | |
|---|---|---|---|
| | | ROX™ dye | TEXAS RED™ dye |
| 4 | ~610 |  |  |

# Troubleshoot the dye calibration

| Observation | Possible cause | Recommended action |
|---|---|---|
| One or more raw spectra are at or below the detectable threshold | • The spectral calibration plate was centrifuged insufficiently.<br>• The spectral calibration plate contains old or insufficient reagents.<br>• If you are running a custom spectral calibration plate, the dye may not be present at a sufficient concentration. | 1. Unload the 7300Plus Instrument and view the wells of the spectral calibration plate. If the liquid in the wells is not:<br>a. At the bottom of the wells, centrifuge the plate for a longer time, then repeat the calibration.<br>b. Equivalent in volume, the plate is not sealed and the reagents have evaporated. Discard it and run another.<br>2. If the spectral calibration plate appears to be normal, discard the plate and run another.<br>3. If the problem persists, contact Support.<br>**Note:** If you are running a custom spectral calibration plate, create another plate but increase the concentration of the dye that produced insufficient signal. |
| One or more raw spectra exceed the maximum limit<br>or<br>The spectra contain peaks in more than one filter. | • Fluorescent contaminants are on the sample block(s) or spectral calibration plate.<br>• If you are running a custom spectral calibration plate, the dye may be too concentrated. | Verify that contaminants are not present by performing a background calibration as explained in Chapter 3, "Perform the Background Calibration and Optical Calibration" If the background calibration does not show sample block contamination, the spectral calibration plate may be contaminated.<br>**Note:** If you are running a custom spectral calibration plate, create another plate but decrease the concentration of the dye that exceeds the detectable limit. |

# 5 Verify Instrument Performance



**Note:** For more information about any of the topics discussed in this guide, access the Help from within Real-Time PCR Software by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ Real-Time PCR Software Help**.

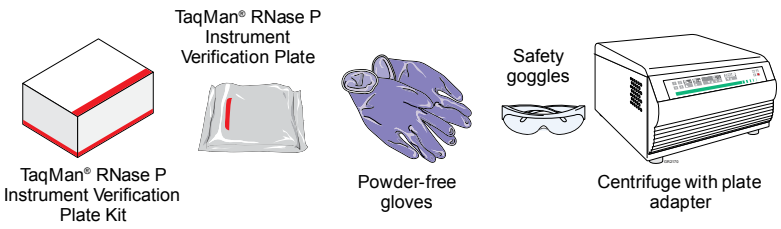
## Overview

Perform the TaqMan® RNase P Instrument Verification Plate run to verify the performance of an Applied Biosystems® 7300Plus Real-Time PCR Instrument.

### Time required

≈1.5 hours

### Materials required




### When to perform the RNase P experiment

We recommend performing an RNase P experiment:

- When installing the 7300Plus Instrument. You must perform in sequence the ROI, background, optical, and dye calibrations and the instrument verification run.
- After moving the instrument to another location.
- As needed to verify the function of the 7300Plus Instrument.

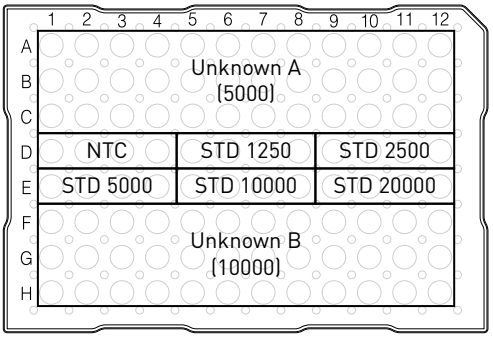
## About the RNase P plate

The RNase P plate is preloaded with the reagents necessary for the detection and quantitation of genomic copies of the human RNase P gene (a single-copy gene encoding the RNase moiety of the RNase P enzyme).

Each well contains:

- 1X TaqMan® Universal PCR Master Mix
- RNase P primers
- FAM™ dye-labeled probe
- Known concentration of human genomic DNA template

The figure below illustrates the arrangement of the standard and unknown populations on the RNase P plate. The RNase P plate contains five replicate groups of standards (1250, 2500, 5000, 10,000, and 20,000 copies), two unknown populations (5000 and 10,000 copies), and negative control wells (NC).




After the run, the Real-Time PCR Software:

1. Generates a standard curve from the averaged threshold cycle ($C_T$) values of the replicate groups of standards.
2. Calculates the concentration of the two unknown populations using the standard curve.
3. Calculates the following to assess the 7300Plus Instrument performance:
   $[(CopyUnk_2) - 3(\sigma_{CopyUnk2})] > [(CopyUnk_1) + 3(\sigma_{CopyUnk1})]$
   where:
   - $CopyUnk_1$ = Average copy number of unknown #1 (5,000-copy population)
   - $\sigma_{CopyUnk1}$ = Standard deviation of unknown #1 (5,000-copy population)
   - $CopyUnk_2$ = Average copy number of unknown #2 (10,000-copy population)
   - $\sigma_{CopyUnk2}$ = Standard deviation of unknown #2 (10,000-copy population)

### Installation specification

The 7300Plus Instrument passes the installation specification if the inequality holds and the instrument successfully distinguishes between 5,000 and 10,000 copies with a statistical confidence level of 99.7%.

To meet the installation specification, you can omit a limited number of outlier wells from the 5,000- and 10,000-copy unknown populations.

| | Unknown population | | Standard (STD) | Negative controls (NC) |
|---|---|---|---|---|
| | 5,000-copy | 10,000-copy | | |
| Maximum outliers that can be removed | 6 | 6 | 0 | 0 |

## Set up the experiment

Prepare the TaqMan® RNase P Instrument Verification Plate for the run.

### Prepare the RNase P plate

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

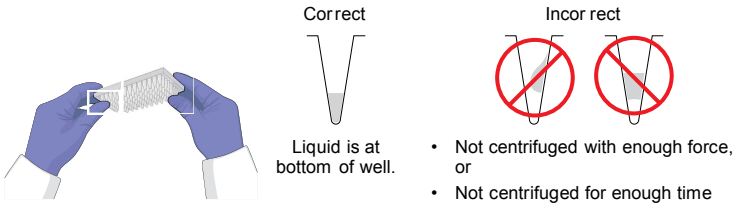
1. Obtain the TaqMan® RNase P Instrument Verification Plate from the freezer, then allow the reaction plate to warm to room temperature (for approximately 5 min).
2. Remove the RNase P plate from its packaging.

3. Vortex the plate for 5 sec.
4. Centrifuge the reaction plate for 2 min at 750 - 1000 g.

   **IMPORTANT!** The reaction plate must be well mixed and centrifuged.

5. Verify that the liquid is at the bottom of each well of the reaction plate. If not, centrifuge the reaction plate again at a greater rpm and for a longer time.

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



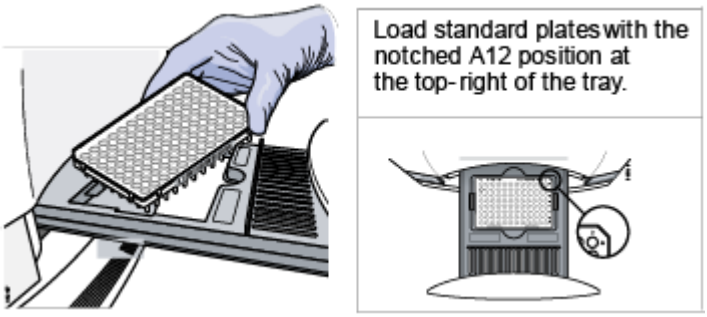

# Run the experiment

After preparing the TaqMan® RNase P Instrument Verification Plate, load the plate into the 7300Plus Instrument and start the run.

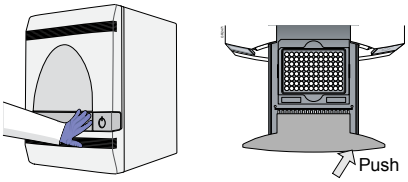
## Load the plate

**WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

1. Push the tray door to open it.
2. Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.




3. Close the tray door. Apply pressure to the right side of the tray door at an angle.



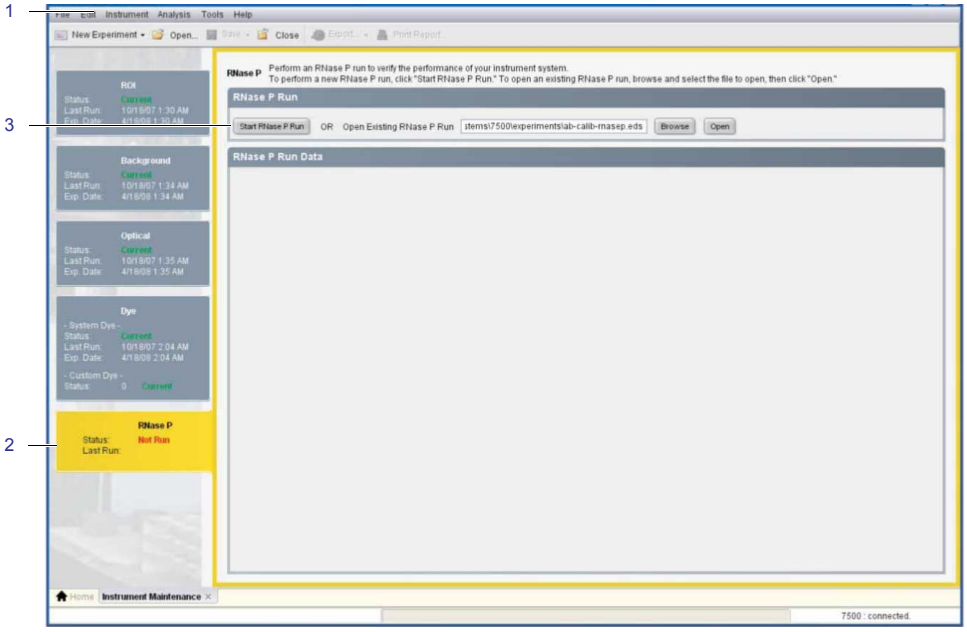
### Start the run

1. In the Real-Time PCR Software, select **Instrument ▸ Instrument Maintenance Manager**.
2. In the Instrument Maintenance Manager, select the **RNase P** tab.
3. In the RNase P screen, click **Start RNase P Run**.
4. Complete the calibration as instructed by the wizard.

   The RNase P dialog box displays four tabs:

   - **Overview** – Displays information describing the calibration.
   - **Setup** – Displays instructions for setting up the RNase P run. Clicking Next opens the Run tab.
   - **Run** – Clicking START RUN starts the run process and displays the processing messages. Clicking Next opens the Analysis tab.
   - **Analysis** – Indicates the run status (Passed/Failed).

   **Note:** Before starting the run, the instrument may pause (up to 10 min) to allow the heated cover to reach the correct temperature.




## Analyze the experiment

Review the data to verify the results of the experiment.

## Verify the results of the analysis

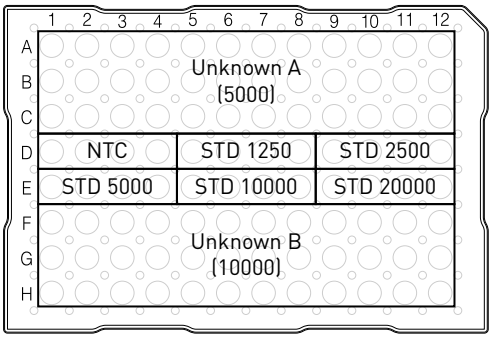
**Note:** After the Real-Time PCR Software completes the RNase P run, it automatically analyzes the run and displays the results in the Analysis screen.

1. In the Analysis screen of the RNase P Run wizard, verify the status of the run:
   - **Passed** – The 7300Plus Instrument passed the RNase P run. Go to Verify the Results of the Analysis on page 43.
   - **Failed** – The 7300Plus Instrument failed the RNase P run. Go to step 2 to review the data for outliers.

   If the run fails, the automated analysis may have included outliers that caused the initial analysis to fail. Experimental error may cause some wells to be amplified insufficiently or not at all. These wells typically produce $C_T$ values that differ significantly from the average for the associated replicate wells. If included in the calculations, these outlying data (outliers) can result in erroneous measurements.

2. In the Amplification Plot, select **Ct vs. Well** from the Plot Type drop-down list.

3. Verify the uniformity of each replicate population on the reaction plate (controls, standards, and unknowns) by comparing the groupings of $C_T$values:
   a. In the plate layout, select the wells containing the 10,000-copy unknown population (wells rows F, G, and H).




   b. In the plot, verify that the $C_T$s of the replicate population are equivalent.

      **Note:** The numbers on the X-axis of the plot correspond to the wells of the reaction plate. Beginning with well A1, the wells are numbered from left-to-right, and top-to-bottom.

   c. If an outlier is in the selected population, select the corresponding well in the plate layout, then click Omit to remove the well from the analysis.

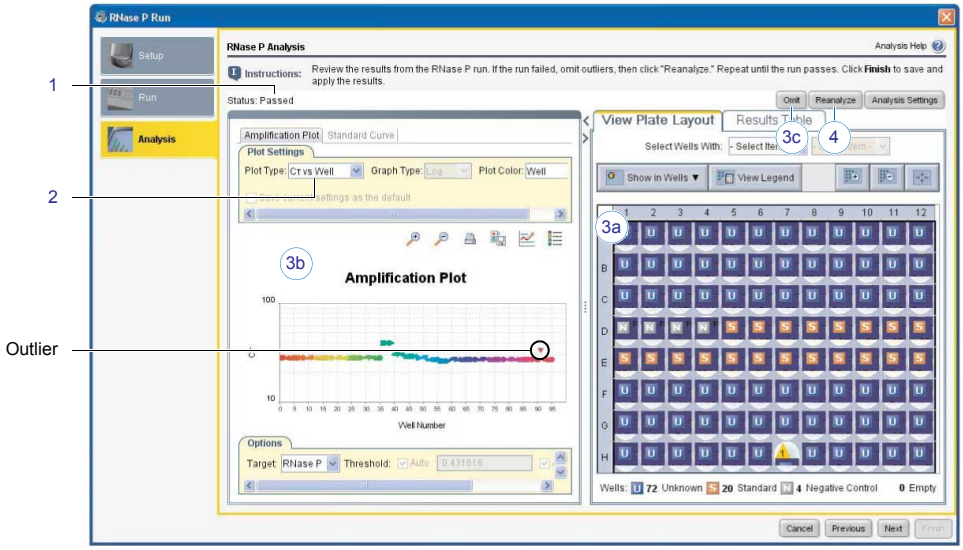
| | Unknown population | | Standard (STD) | Negative control (NC) |
|---|---|---|---|---|
| | 5,000-copy | 10,000-copy | | |
| **Maximum outliers that can be removed** | 6 | 6 | 0 | 0 |

**IMPORTANT!** If the number of outliers exceeds the limit in the table above, order another RNase P plate and repeat the experiment.

d. Repeat steps 3a through 3c for each replicate population (unknowns, standards, and negative controls) on the reaction plate.

4. Click **Reanalyze** to analyze the run without the outliers.

   If the status of the RNase P Run is "**Failed**" after performing steps 2 through 4, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Support.



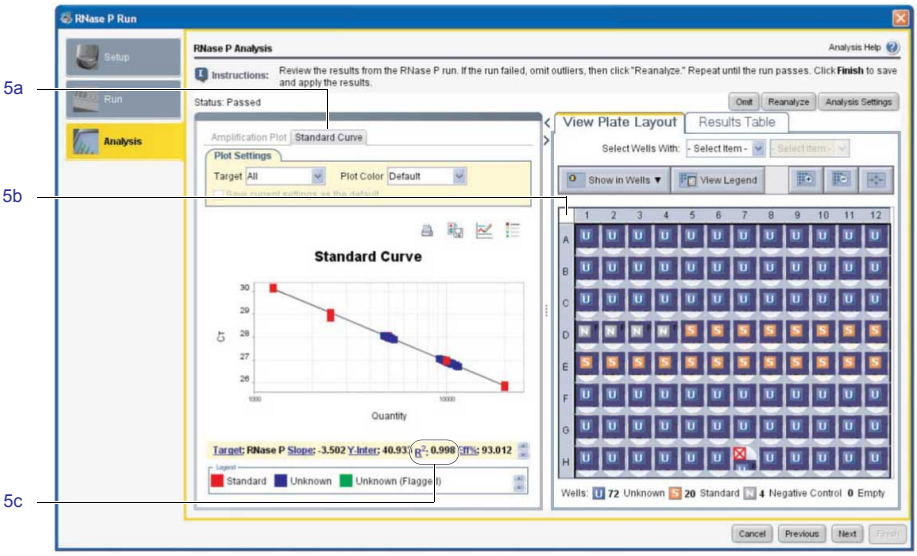

5. Review the standard curve:

   a. Select the **Standard Curve** tab.

   b. Click the upper-left corner of the Plate Layout to select all wells.

   c. Verify that the $R^2$ value is greater than or equal to 0.990.

   If the $R^2$ value is less than 0.990, repeat the RNase P experiment using a different RNase P plate. If the problem persists, contact Support.



6. Click **Next**, then remove the calibration plate.

   **WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

   a. Push the tray door to open it.

   b. Remove the calibration plate.

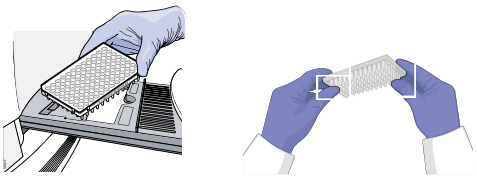
   c. Push the tray door to move it into the instrument.

7. Discard the plate.

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

## Troubleshoot the RNase P experiment

| Observation | Possible cause | Recommended action |
|---|---|---|
| More than the maximum number of outliers are present in RNase P data | • Possible contamination<br>• Pipetting inaccuracy | Contact the service and sales representative to order a replacement TaqMan® RNase P Instrument Verification Plate. If the replacement RNase P plate fails, contact Support or your service representative for further assistance. |

| Observation | Possible cause | Recommended action |
|---|---|---|
| Verification run failed | • Insufficient centrifugation<br>• Defective plate seal | **WARNING!** PHYSICAL INJURY HAZARD. During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.<br><br>1. Unload the RNase P plate from the instrument:<br>a. Push the tray door to open it.<br>b. Remove the RNase P plate from the tray.<br>c. Push the tray back into the instrument.<br>  <br>2. Hold the plate up to a light source, and verify that all wells contain the same volume of fluid.<br>If there are differences in fluid volumes, check the heat seal of the wells with lower volumes for signs of damage or evaporation.<br>Also, compare the position of the wells that have lower volumes with the outliers that you have removed from the plate. If the well positions coincide, the heat seal on the plate may be defective and resulted in the evaporation of the associated samples.<br>3. Contact the service and sales representative to order a replacement TaqMan® RNase P Instrument Verification Plate. If the replacement RNase P plate fails, contact Support or your service representative for further assistance. |

# 6 User-Performed Maintenance



**Note:** For more information about any of the topics discussed in this guide, access the Help from within Real-Time PCR Software by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ Real-Time PCR Software Help**.

## Monitor the 7300Plus Instrument

You can monitor the state of the Applied Biosystems® 7300Plus Real-Time PCR Instrument using the Function Test, Lamp Status/Replacement, and Instrument Log tools of the Real-Time PCR Software. The tools enable you to assess the health of the 7300Plus Instrument, check the replacement status of the instrument lamp, and view a recent history of instrument activity.

### View the Instrument log

Use the Instrument Log to view the recent event history of the 7300Plus Instrument. The log displays the major instrument activity for either the most recent 25 runs (including calibrations), or the events that pertain only to a specific EDS file.

#### Display the Instrument Log

1. In the Real-Time PCR Software, select **Instrument ▸ Instrument Events Log**.
2. In the Instrument Events Log dialog box, select either:
   - **System Log** – To view events that occurred during the 25 most recent runs (experiments) or calibrations.
   - **Document Log** – To view events that pertain only to the experiment currently open in the Real-Time PCR Software.

3. If necessary, modify the data displayed in the table by filtering the data and adding or removing columns.

| To... | Action |
|---|---|
| Filter the data in the events table | 1. In the Filter drop-down list, select a property.<br>2. Enter the appropriate conditions into the drop-down lists and fields that appear automatically.<br>3. Click **Apply** to filter the data.<br>**Note:** To reset the log, select **Filter ▸ Show All Records**. |
| Add or remove columns to/from the events table | Click **Show Columns**, then select the column desired column in the drop-down list. |
| Sort the data in the events table | Click the heading of the column of interest once to sort the data in ascending order. Click the column heading again to sort the data in reverse order. |
| Export the contents of the instrument event log | 1. Select the rows of interest in the event table,<br>2. Press **Ctrl**+**C** to copy the data.<br>3. Paste the data into a spreadsheet application or a text file.<br>**Note:** The software exports the data in tab-delimited format. |

4. When you finish viewing the events, click **Close**.

## Monitor the instrument status

Use the Function Test dialog box to perform a high-level diagnostic of the major 7300Plus Instrument components. In general, you need not perform the function tests unless you experience a suspected hardware failure, or you are instructed to do so by an Thermo Fisher Scientific representative.

### Perform function tests of system components

1. In the Real-Time PCR Software, select **Instrument ▸ Function-Test**.
2. Perform function tests as needed.

   To test:

   - All system components – Click **All Tests**, then wait for the software to perform all of the function tests.

- One or more specific components – Click one or more of the following.

  **USB** – Tests the universal serial bus (USB) connection between the 7300Plus Instrument and computer. The test passes if the Real-Time PCR Software can establish communication with the 7300Plus Instrument.

  **CCD** – Tests the CCD camera in the 7300Plus Instrument. The test passes if the camera can capture an image.

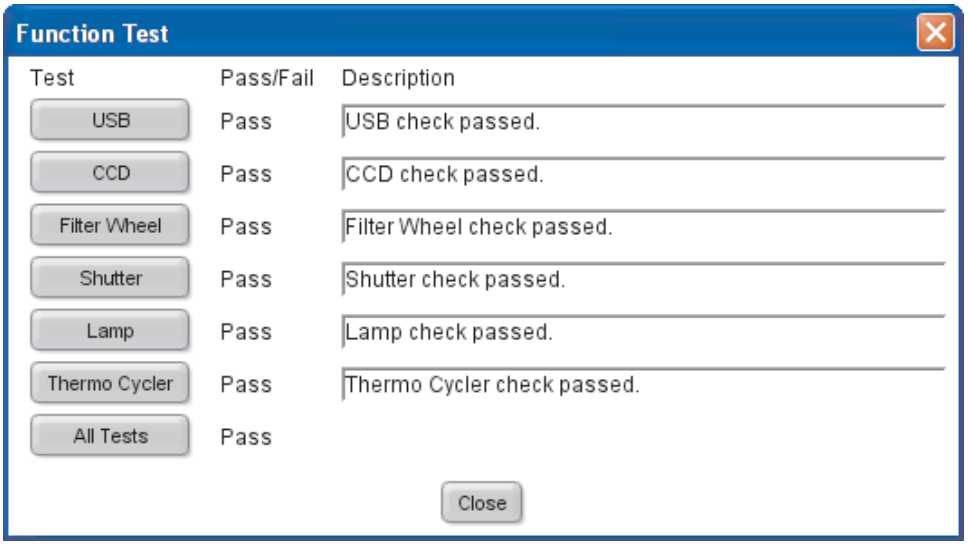
  **Filter Wheel** – Tests the filter wheel in the 7300Plus Instrument. The test passes if the filter wheel controller is running the correct version of firmware.

  **Shutter** – Tests the optic shutter of the 7300Plus Instrument. The test passes if the shutter controller is running the correct version of firmware.

  **Lamp** – Tests the halogen lamp of the 7300Plus Instrument. The test passes if the lamp controller is running the correct version of firmware.

  **Thermal Cycler** – Tests the thermal cycler sample block in the 7300Plus Instrument. The test passes if the thermal cycler controller is running the correct version of firmware.

When the Real-Time PCR Software completes a test, the software reports the pass/fail status of the test and provides a description of the outcome.




3. When you finish testing, click **Close**.

## Monitor the lamp status

Use the Lamp Status/Replacement dialog box to monitor the status of the halogen lamp that the 7300Plus Instrument uses to illuminate samples during runs.

### Check the lamp status

In the Real-Time PCR Software, select **Instrument ▸ Lamp Status/Replacement** to determine the status of the halogen lamp.


The Lamp Status/Replacement dialog box displays:

- **Condition** – Indicates one of the following conditions:
  - **Good** – The lamp is functioning well and does not need to be replaced. Click Close.
  - **Failed** – The lamp bulb must be replaced. Click **Close**, then replace the lamp as explained in "Replace the halogen lamp" on page 54.
  - **Change Soon** – The lamp usage is above 2000 hrs; Thermo Fisher Scientific recommends that you replace it. Click Close, then decide whether or not to replace the lamp. If you choose to replace the lamp, see "Replace the halogen lamp" on page 54.
- **Usage (Hours)** – The total number of hours that the lamp has been illuminated.
- **Lamp Current** – The output current of the lamp in amperes (A). Low current can indicate a potential future failure of the lamp.
- **Date Last Replaced** – The date of the last lamp replacement.

### Warnings

The Real-Time PCR Software can display the following warnings before or during a run:

| Message | Description |
|---|---|
| **WARNING! Warning** – Cannot detect sufficient current from lamp. Either lamp is not installed properly or needs to be replaced. | The lamp current is below the acceptable level at the start of the run. You cannot proceed with the run until you replace the halogen bulb as explained in "Replace the halogen lamp" on page 54. |
| **WARNING! Warning** – Cannot detect sufficient current from lamp. Either lamp is not installed properly or needs to be replaced. | The Real-Time PCR Software stopped the run because the lamp current decreased below the acceptable level during the run. You cannot proceed with the run until you replace the halogen bulb as explained in "Replace the halogen lamp" on page 54. Click **OK** in the message box, inspect the Instrument Log, then replace the lamp bulb. |
| **WARNING! Warning** - The lamp usage has exceeded 2000 hr. We recommend replacing the lamp soon to ensure optimal assay performance. | The lamp usage exceeds 2000 hr at the start of a run. Click **Cancel Run**, then replace the lamp, or click **Continue Run**.<br>Rerun ROI, Background, Optical and Dye calibrations. |

## Decontaminate the sample block

Perform the following procedure to eliminate fluorescent contaminants from the sample block of the 7300Plus Instrument. Fluorescent contamination is generally evident in failed background runs where one or more wells consistently exhibit abnormally high signals.

**WARNING! PHYSICAL INJURY HAZARD.** Do not remove the instrument cover. There are no components inside the 7300Plus Instrument that you can safely service yourself. If you suspect a problem, contact an Thermo Fisher Scientific Service Representative.

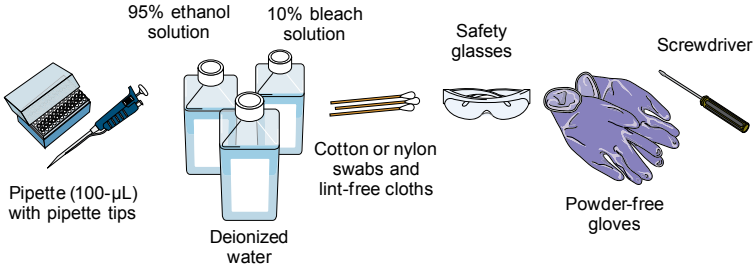
**WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can be heated to 100°C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

**WARNING!** Before using a cleaning or decontamination method other than those recommended by the Thermo Fisher Scientific, verify with Thermo Fisher Scientific that the proposed method will not damage the equipment.

## Time required

30 min

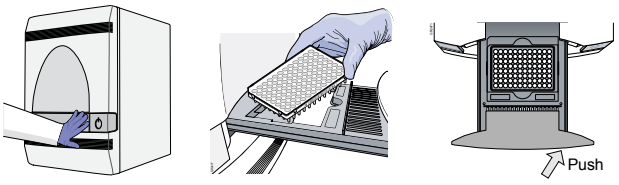
## Materials required




## Clean the sampleblock

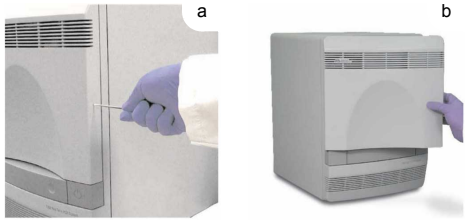
**IMPORTANT!** Wear powder-free gloves when you perform this procedure.

1. Identify the contaminated wells of the sample block (see "How to Identify Contamination").
2. Remove the plate and the tray holder from the 7300Plus Instrument:
   a. Push the tray door to open it.
   b. Remove the plate and the tray holder.
   c. Close the tray door. Apply pressure to the right side of the tray door at an angle.



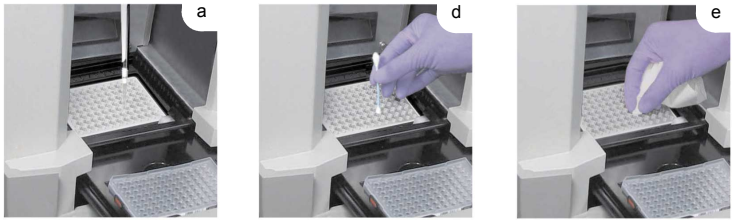

3. Manually raise the block:
   a. In the Real-Time PCR Software, select **Instrument ▸ Instrument Maintenance Manager**.
   b. In the ROI tab of the Instrument Maintenance Manager, click **Start Manual Calibration**.
   c. In the ROI Inspector dialog box, click **Move Block**.
   d. When the ROI Inspector dialog box displays "Block Down," click **Done**.
4. Power off, then unplug the 7300Plus Instrument. Allow it to cool for 15 min.
5. Open the access door to the 7300Plus Instrument.
   a. Insert a thin screwdriver into the keyhole on the edge of the access door, then push to unlatch the door.

b. Open the access door.

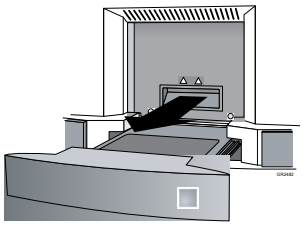



6. Lift the latch, then push the heated cover door to the back of the instrument.

7. Clean the contaminated wells of the sample block using a small volume of deionized water:
   a. Pipette a small volume of deionized water into each contaminated well.
   b. Pipette the water up and down several times to rinse the well.
   c. Pipette the water to a waste beaker.
   d. Using a cotton swab, scrub inside of each contaminated well.
   e. Using a lint-free cloth, absorb the excess deionized water.




8. Pull the heated cover door to the front of the 7300Plus Instrument. Lift the latch, then secure the heated cover door to the cross bar.



9. Close the access door to the 7300Plus Instrument.

10. Plug in, then power on the 7300Plus Instrument.

11. Verify that you have eliminated the contamination by performing a background calibration run (see "Perform the background calibration" on page 20).

12. If the contamination remains, repeat Clean the Sample Block on page 51 through Clean the Sample Block on page 52, then clean the contaminated wells of the sample block using 95% ethanol solution:
    a. Pipette a small volume of 95% ethanol solution into each contaminated well.
    b. In each contaminated well, pipette the solution up and down several times to rinse the well.
    c. Pipette the ethanol solution to a waste beaker.

    **IMPORTANT!** Always use deionized water to rinse wells after cleaning with bleach or ethanol solution.

13. Repeat Clean the Sample Block on page 52 through "Clean the sampleblock" on page 51to rinse the wells of the sample block and to verify that you have eliminated the contamination.

14. If the contamination remains, repeat steps 1 through 6, then clean the contaminated wells of the sample block using 10% bleach solution:
    a. Pipette a small volume of 10% bleach solution into each contaminated well.
    b. In each contaminated well, pipette the solution up and down several times to rinse the well.
    c. Pipette the bleach solution to a waste beaker.

    **IMPORTANT!** Always use deionized water to rinse wells after cleaning with bleach or ethanol solution.

15. Repeat steps 7 through11 to rinse the wells of the sample block and to verify that you have eliminated the contamination.

    If the contamination remains, contact Thermo Fisher Scientific support (see "How to Obtain Support").

16. Ensure that the heated cover door is completely closed and latched. If it is not, the Real-Time PCR Software displays an error message.

# Replace the halogen lamp

Replace the halogen lamp after approximately 2000 hr of life.

**WARNING! PHYSICAL INJURY HAZARD.** The 7300Plus Instrument and lamp are hot! The lamp can become very hot while in use. Allow the lamp to cool for 15 min and put on protective, powder-free gloves before handling it.

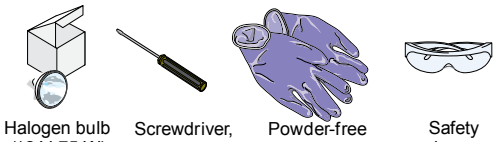
**WARNING! PHYSICAL INJURY HAZARD.** Wear disposable, powder-free gloves when handling the lamp to prevent burns and to prevent shortening the life of the replacement lamp.

**WARNING! WARNING.** This instrument is designed for 12 V, 75 W halogen lamps only.

## Time required

30 min

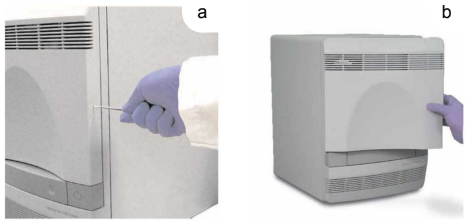
## Materials required




## Replace the lamp

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

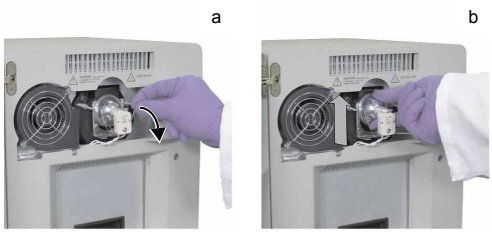
1. Power off, then unplug the 7300Plus Instrument. Allow the instrument to cool for 15 min.
2. Open the access door to the 7300Plus Instrument:
   a. Insert a thin screwdriver into the keyhole on the edge of the access door, then push to unlatch the door.
   b. Open the access door.



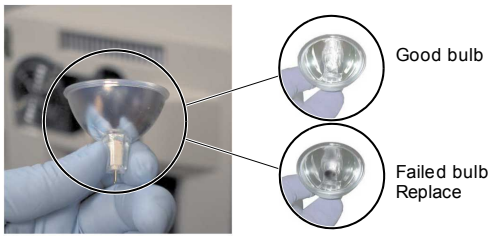

3. Remove the lamp from the instrument:
   a. Slide the lamp release lever downward.

b. Firmly grasp the lamp and lift it up and out of the slotted mount.

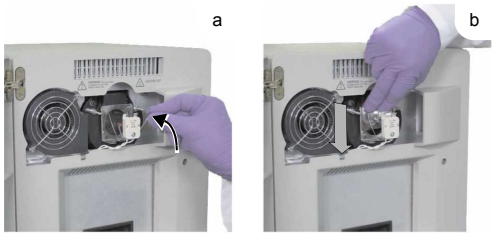
**IMPORTANT!** Do not touch the lamp without powder-free gloves. Finger prints shorten the lamp life.




4. Inspect the lamp for signs of failure (carbon typically coats the inside of failed lamps).



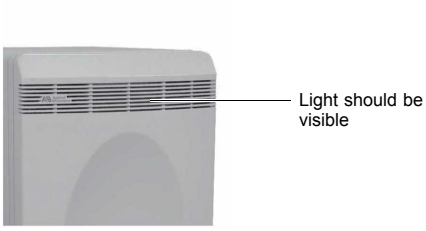

5. Install the new lamp into the instrument:
   a. Slide the lamp release lever upward.
   b. Firmly grasp the lamp, place it into the slotted mount, then carefully slide the lamp downward into place.




6. Close the access door.

7. Plug in and power on the 7300Plus Instrument.

8. Open the ROI Inspector dialog box:
   a. In the Real-Time PCR Software, select **Instrument ▸ Instrument Maintenance Manager**.
   b. In the ROI tab of the Instrument Maintenance Manager, click **Start Manual Calibration**.
9. In the ROI Inspector dialog box, select **Lamp Control ▸ Idle**.
10. While the instrument is running, look through grating of the access door and verify that the lamp is illuminated, then click **Done**.




11. If the lamp is illuminated, select **Instrument ▸ Lamp Status/Replacement** in the Real-Time PCR Software, click **Reset Lamp Timer**, then click **OK**.
    If the lamp is not illuminated, the replacement halogen lamp may be defective. Replace the lamp again. If the second lamp does not illuminate, check the instrument fuses for failure (see page 66).
12. Perform the following calibrations after replacing the lamp. See:
    - Chapter 2, “Perform the Regions of Interest (ROI) Calibration“
    - Chapter 3, “Perform the Background Calibration and Optical Calibration“
    - Chapter 4, “Perform the Dye Calibration“
    - Chapter 5, “Verify Instrument Performance“

## Replace the instrument fuses

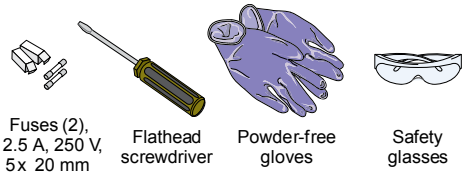
Replace the 7300Plus Instrument fuses when the fuses fail.

**WARNING!** FIRE **HAZARD.** For continued protection against the risk of fire, replace fuses only with listed and certified fuses of the same type and rating as those currently in the instrument.

### Time required

30 min

### Materials required



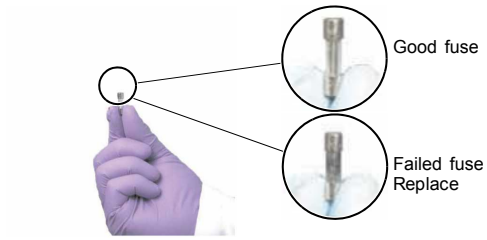
## Replace the fuses

1. Power off the instrument, then unplug it.

> **DANGER! ELECTRICAL SHOCK HAZARD.** Severe electrical shock, which could cause physical injury or death, can result from working on an instrument when the high voltage power supply is operating. To avoid electrical shock, disconnect the power supply to the instrument, unplug the power cord, and wait at least 1 minute before working on the instrument.

2. Using a flat-head screwdriver, unscrew and remove the fuse holders from the instrument.

3. Remove each fuse from its fuse holder and inspect it for damage. Carbon typically coats the inside of failed fuses.




4. Replace each failed fuse with a 12.5 A, 250 V, 5 · 20-mm fuse.

   **Note:** The voltage and amperage ratings are on the fuse holder.

5. Install the fuse holder.

6. Plug in, then power on the instrument.

   The installation is successful if the instrument powers on.

   **Note:** Fuse failure can result from fluctuations in the supplied power to the instrument. To prevent further failures, consider installing an electrical protective device, such as a UPS or other surge protector. For more information about fuses, see the Applied Biosystems® 7300Plus Real-Time PCR Instrument Site Preparation Guide.

## Update the Windows Operating System

Do not upgrade or update the Microsoft Windows operating system of the computer running the Real-Time PCR Software without first consulting the software release notes or the Thermo Fisher Scientific website. Future versions of the Windows operating system and updates to the operating system can conflict with the Real-Time PCR Software.

### Determine compatibility of an upgrade or update

1. Open **D:\Applied Biosystems\ Real-Time PCR Software** , double-click **release-notes.html**, then read the *N/A Release Notes* for the compatibility of interest.
2. If the release notes do not mention the compatibility, use an internet browser to visit **www.lifetechnologies.com**, then search the website for the compatibility of interest.
3. If the website does not contain the information of interest, contact Support.

## Update the Real-Time PCR Software

If you want to update the Real-Time PCR Software, prepare your computer by exporting the application libraries and backing up your experiment files.

### Visit the website

You can obtain Real-Time PCR Software updates directly from the service section of the Applied Biosystems website. For the latest services and support information for the 7300Plus Instrument:

1. Go to **www.lifetechnologies.com/support**
2. In the Software Downloads page, select the appropriate instrument from the drop-down list.
3. In the Software Downloads page for your instrument, click **Updates - Patches**.

The website opens the page describing the latest software updates.

### Prepare for the upgrade

Before updating the Real-Time PCR Software:

1. Back up the application libraries:
   a. In the main menu of the Real-Time PCR Software, select **Tools<desired library>**.

b. When the library dialog box opens, select the element(s) that you want to export, then click **Export**.

c. In the Export dialog box, click **Save** to archive the selected records.

d. Repeat steps 1a through 1c for the remaining libraries to archive them.

2. Back up all experiment files by creating a copy of the directory that you are using to store files.

   The default directory for experiments is:
   D:\Applied Biosystems\Real-Time PCR Software\experiments

# Store, Move, and Install the 7300Plus Instrument



**Note:** For more information about any of the topics discussed in this guide, access the Help from within Real-Time PCR Software by pressing **F1**, clicking in the toolbar, or selecting **Help ▸ Real-Time PCR Software Help**.

## Store the 7300Plus Instrument

The Applied Biosystems® 7300Plus Real-Time PCR Instrument can be powered off and stored for extended periods of time. The length of the period of inactivity determines the method you use to power off the instrument.

**Time required**

5 min

**Materials required**

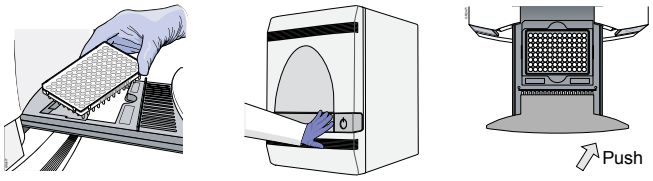
MicroAmp® Optical 96-Well Reaction Plate (unused)

**Prepare the instrument**

1. Open the instrument tray door.
2. If the tray contains a plate, remove it.
3. If you plan to store the 7300Plus Instrument for more than a week or you plan to move the instrument, load an unused plate into the tray.

   **Note:** The empty plate protects the internal components of the 7300Plus Instrument during transport or during periods of inactivity lasting more than a week.

4. Push the tray door to move it into the instrument.




5. Press the instrument power button.

6. Power off the computer and monitor:
   a. Select **Start ▸ Shut Down**.
   b. In the Shut Down Windows dialog box, select **Shut Down**.
   c. Power off the monitor.

## Move the 7300Plus Instrument

Perform this procedure to safely move the 7300Plus Instrument short distances (for example, between laboratories of the same building).

**WARNING! PHYSICAL INJURY HAZARD.** Do not attempt to lift the instrument or any other heavy objects unless you have received related training. Incorrect lifting can cause painful and sometimes permanent back injury. Use proper lifting techniques when lifting or moving the instrument. At least 2 people are required to lift the 7300Plus Instrument.

**IMPORTANT!** Moving your Applied Biosystems® 7300Plus Real-Time PCR Instrument can create subtle changes in the alignment of the instrument optics.

### Materials required

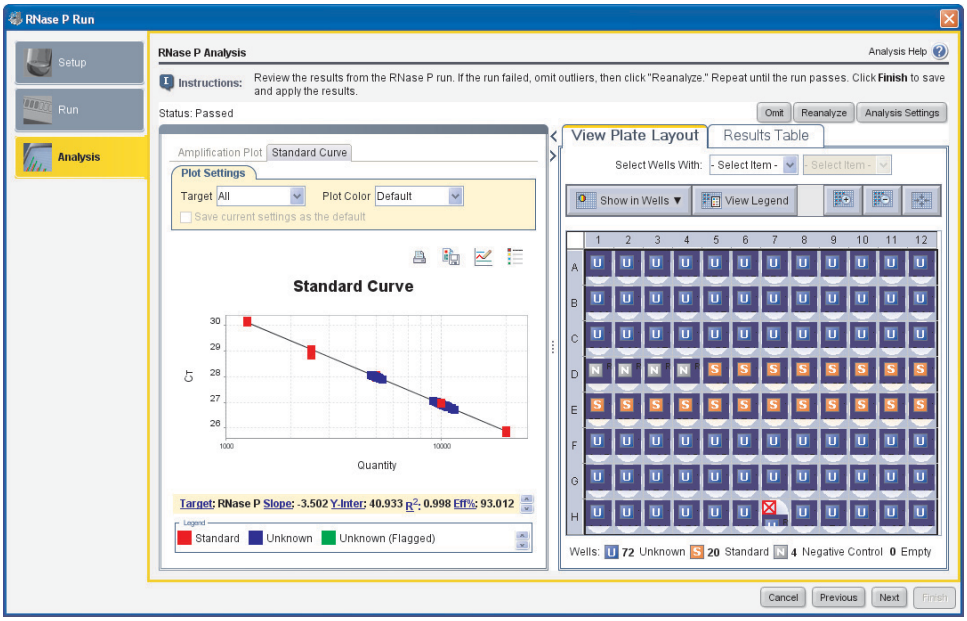
MicroAmp® Optical 96-Well Reaction Plate (unused)

### Prepare the instrument

1. Load the empty reaction plate into the 7300Plus Instrument.
2. Using the ROI Inspector, manually raise the sample block:
   a. In the Real-Time PCR Software, select InstrumentInstrument Maintenance Manager.
   b. In the ROI tab of the Instrument Maintenance Manager, click Start Manual Calibration.
   c. In the ROI Inspector dialog box, click Block Up.
3. Power off the 7300Plus Instrument and computer.
4. Disconnect all 7300Plus Instrument components.
5. Move the 7300Plus Instrument according to the following guidelines:
   - Verify that the surface on which you will place the instrument can support at least 54.5 kg (120 lbs).
   - Verify that the pathway to the final position of the instrument is clear of obstructions.
   - Keep your spine in a good neutral position.
   - Bend at the knees and lift with your legs.
   - Do not lift an object and twist your torso at the same time.
   - Coordinate your intentions with your assistant before lifting and carrying.

6. Reconnect the components of the 7300Plus Instrument (see "Set Up the 7300Plus Instrument" on page 62).

7. Run a TaqMan® RNase P Instrument Verification Plate (see "Run the experiment" on page 40).
   a. If the run passes, recalibrations are not necessary.
   b. If the run fails, perform steps 8 through 12 to recalibrate the instrument.




8. Perform an ROI calibration (see page 13).

9. Perform a background calibration (see page 23).

10. Perform an optical calibration (see page 25).

11. Perform a dye calibration (see page 32).

12. Perform an instrument verification run (see page 45).

# Set Up the 7300Plus Instrument

## Set up the computer

Refer to the Applied Biosystems Real-Time System Computer Setup Guide for information on setting up a computer for use with the 7300Plus Instrument.

## Set up the 7300Plus Instrument

**IMPORTANT!** Do not connect the USB cable to the 7300Plus Instrument until you are instructed to do so by this guide.

### Materials required

- Phillips screwdriver (small and thin)
- Power cord

### Set Up the 7300Plus Instrument

1. Prepare the installation site as described in the *7300Plus Real-Time PCR Instrument Site Preparation Guide*.
2. Open the access door to the 7300Plus Instrument.
   a. Insert a thin screwdriver into the keyhole on the edge of the access door, then push to unlatch the door.
   b. Open the access door.
3. Verify that the heated cover assembly is pulled fully toward the front of the instrument. If the 7300Plus Instrument has a heated cover latch installed, check that the latch is in a locked position.
4. Inspect the instrument for damage caused by the transportation of the 7300Plus Instrument.

   If the instrument is damaged, record the location and appearance of the damage, then contact Support or your service representative for assistance.
5. Close the access door.
6. Connect the power cord to the 7300Plus Instrument, then to the wall receptacle.
7. Press the power button at the lower right front panel, then wait for the 7300Plus Instrument to start up (about 30 sec).
8. When the Power status light on the lower left front panel is lit, push the tray door to open it.
9. Remove the packaging plate from the tray and set it aside.
10. Close the tray door, then press the power button again to power off the instrument.

    **Note:** Install any additional hardware.

    **IMPORTANT!** Do not connect the USB cable to the 7300Plus Instrument at this time.

11. Verify that the Real-Time PCR Software is installed to the computer.

    If the computer does not have the Real-Time PCR Software, use the Real-Time PCR Software CD to install the software.

12. Once you verify that the computer contains the Real-Time PCR Software, connect the USB cable to the 7300Plus Instrument.

B

# Create a Custom Dye Plate



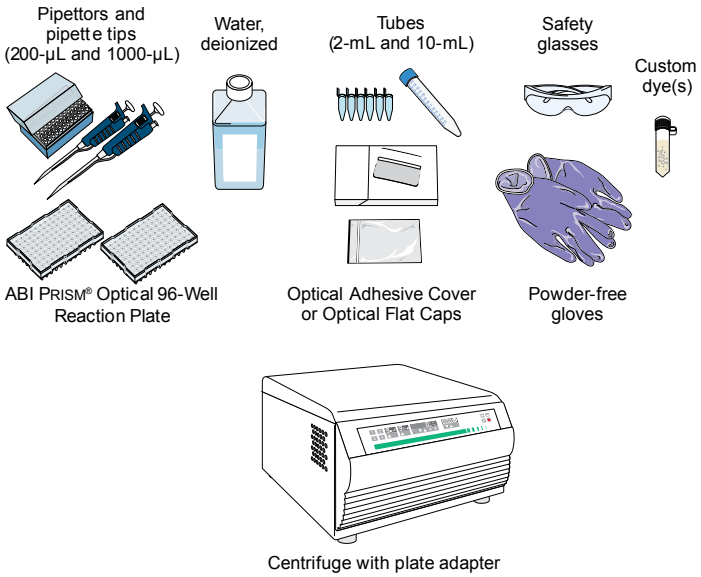
The Applied Biosystems® 7300Plus Real-Time PCR Instrument can be used to run assays designed with custom dyes (dyes not manufactured by Thermo Fisher Scientific). Custom dyes must fluoresce within the 520 to 650 nm spectral range measured by the 7300Plus Instrument.

## Before using custom dyes

Before using custom dyes with the 7300Plus Instrument, you must:

- Determine optimum dye concentration
- Create a custom dye plate
- Add the custom dye to the software
- Perform a dye calibration (see Chapter 4, “Perform the Dye Calibration“)

# Materials required




# Dilute the custom dye to an optimal concentration

## Custom dye dilution guidelines

Use the following guidelines to prepare a dilution series for each custom dye:

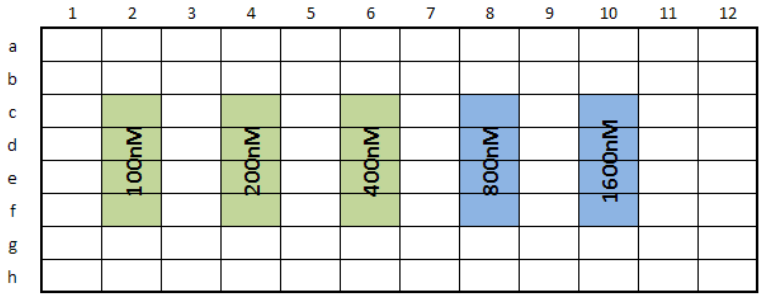
- Target several dye concentrations within a range of 100–2000 nM.
- Choose a 2- or 3-fold difference in dilution points.
- Use a recommended volume of 50 µL/well.
- Dilute the dye in buffer that is compatible with your master mix. See Appendix C, "Create a Background Plate"for detailed instructions for preparing a background plate using the buffer provided with your custom dye.
- *(Intercalating dyes only)* Add the appropriate amount of amplified PCR product to generate fluorescence.

## Prepare a custom dye dilution plate

**IMPORTANT!** Wear powder-free gloves while creating the dye plate.

Prepare and load the custom dye dilution plate:

1. Prepare a dilution series of the custom dye following the dilution guidelines.
2. Pipet the appropriate volume of the dilution series to the center of a 96-well plate, then seal the plate.



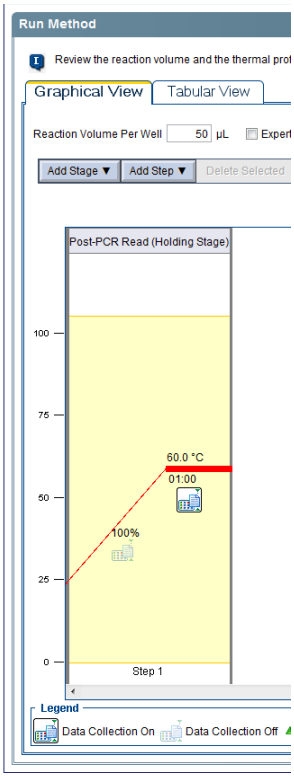

3. Vortex the plate for 5 seconds, then centrifuge it for 2 minutes at 750 - 1000g. Confirm that the liquid in each well of the plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.
4. Load the plate into the instrument.

## Run the dilution plate as an experiment

Run the custom dye dilution plate as a new experiment:

1. On the Home screen of your system software, create a new **Genotyping** experiment and uncheck **Pre-PCR Read** and **Amplification**.
2. Enter the required experiment properties, then add the dilution series information to the appropriate wells in the plate layout.

3. Edit the run method to include a Post-PCR Read with a 1 minute hold. Make sure to enter the appropriate reaction volume and select the filters of interest.
   For example:



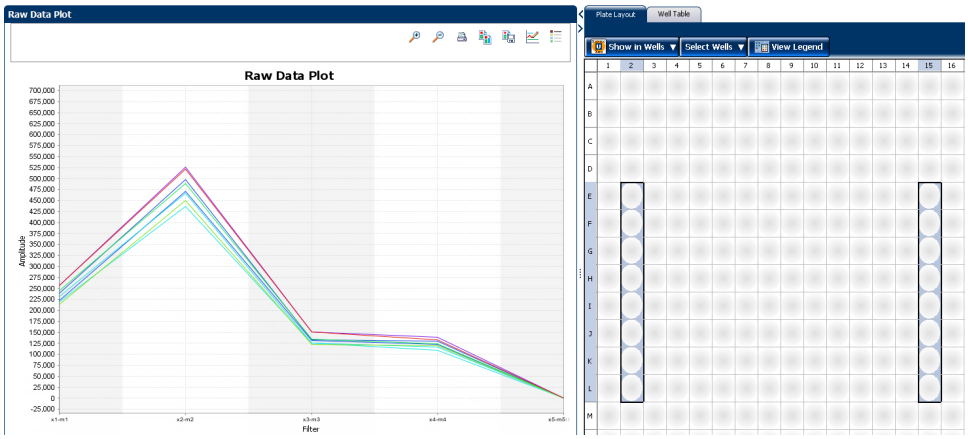

4. Save the experiment and start the run.

## Determine the optimal dye concentration

Review the run results and select the dilution to calibrate:

1. When the run is complete, review the dye signal data:
   a. In the Analysis area of your system software, select the **Raw Data Plot**.
      This plot displays the raw fluorescent signal of each dye as detected through each emission filter, for individual wells during the Post-PCR read.

b. For each replicate population of dilutions, select the wells in the Plate Layout tab to view in the plot. For example:




c. Examine the raw data and identify the well(s) yielding signals according the ranges shown in the following table, then select the lowest (optimal) dye concentration that falls within the acceptable signal range:

| Plate type | Acceptable signal range |
|---|---|
| 96-well | 1,000,000–12,000,000 |

**Note:** You can also export the raw data and average for the various concentrations.

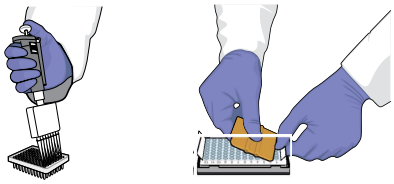
2. Unload the plate from the instrument and discard the plate.

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

# Create a custom dye plate

IMPORTANT! Wear powder-free gloves while creating the dye plate.

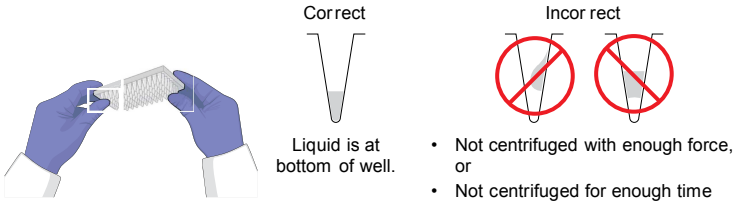
1. Prepare at least 5 mL of the custom dye at the concentration determined in step 8.
2. Pipette 50 µL of the diluted custom dye to all wells of an optical reaction plate.
3. Seal the wells of the reaction plate using an optical adhesive cover.



4. Centrifuge the plate for 2 min at 750 - 1000 g.

   **IMPORTANT!** The custom dye calibration plate must be well mixed and centrifuged.

5. Verify that the liquid in each well of the custom dye plate is at the bottom of the well. If not, centrifuge the plate again at a higher rpm and for a longer period of time.




## Add the custom dye to the software

1. In the main screen of the Real-Time PCR Software, select **Instrument> Instrument Maintenance Manager**.

2. In the Instrument Maintenance Manager:
   a. In the navigation pane, click **Dye**.
   b. In the Dye screen, select **Custom Dye Calibration**.
   c. Click **Start Calibration**.

3. In the Setup screen of the Dye Calibration dialog box, select a custom dye from the list or create the custom dye as follows:
   a. Click **New Dye**.
   b. In the Dye Manager dialog box, click **New**.
   c. Complete the New Dye dialog box, then click **OK**.

| Field/Option | Action |
|---|---|
| Name | Enter a name for the custom dye. |
| Wavelength | Enter the wavelength at which the dye fluoresces. |
| Type | Select:<br>• **Reporter** if the dye works in conjunction with a quencher dye to report an increase of PCR product.<br>• **Quencher** if the dye suppresses the fluorescence of a reporter dye until amplification of PCR product.<br>• **Both** if the dye reports an increase of PCR product without the aid of a quencher dye. |

   d. Click **Close**.

4. In the Setup screen of the Dye Calibration dialog box, enter a temperature setting for the calibration.

   **Note:** Set the temperature to match the temperature at which you intend to collect data. For example, the temperature for all Applied Biosystems system dyes is 60°C because data collection for TaqMan® reagents occurs during the 60°C extension step of the PCR.

5. Select **The custom dye plate is loaded in the instrument**, then click **Next**.

6. In the Run screen, click **Start Run**, then wait for the 7300Plus Instrument to complete the dye calibration.

   **Note:** If the Real-Time PCR Software displays messages during the run, troubleshoot the errors as described in "Troubleshoot the dye calibration" on page 36.

7. When the 7300Plus Instrument displays the Main Menu, unload the custom calibration plate.

8. Analyze the custom spectral calibration as explained in "Analyze the calibration data" on page 33.

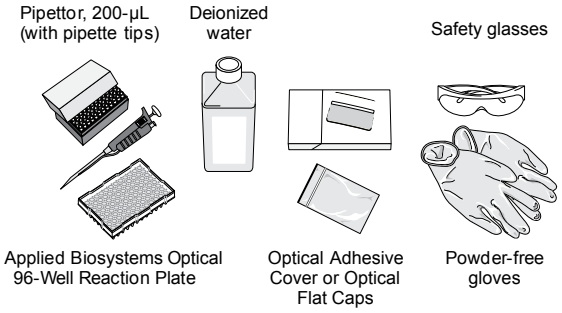
C

# Create a Background Plate



Whenever possible, use a background plate that is included with the spectral calibration kit. The plates supplied in the kit contain a buffer that accurately simulates the reagents used for PCR, and, therefore, produces high-quality calibration data. However, if a background plate from a spectral calibration kit is not available, you can create one as described below.

## Materials required




## Create a background plate

**IMPORTANT!** Wear powder-free gloves while creating the background plate.

1. Remove an Applied Biosystems 96-Well Optical Reaction Plate from its box and place it on a clean, dry surface.
2. Aliquot 50 µL of deionized water to each well of the reaction plate.
3. Seal the plate using an optical adhesive cover or optical flat caps.

   Use the plate for background calibration in the same way you use a background plate from the spectral calibration kit. See Chapter 3, "Perform the Background Calibration and Optical Calibration"

D

# Safety



**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 this document.

- 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, see the "Documentation and Support" section in this document.

# Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words:

- **CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

| Symbol | English | Français |
|---|---|---|
|  | Caution, risk of danger<br>Consult the manual for further safety information. | Attention, risque de danger<br>Consulter le manuel pour d'autres renseignements de sécurité. |
|  | Caution, risk of electrical shock | Attention, risque de choc électrique |
|  | Moving parts | Parties mobiles |
|  | Caution, hot surface | Attention, surface chaude |
|  | On/Off | On/Off (marche/arrêt) |
|  | Protective conductor terminal (main ground) | Borne de conducteur de protection (mise à la terre principale) |

| Symbol | English | Français |
|---|---|---|
|  | Do not dispose of this product in unsorted municipal waste<br><br>**CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options. | Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif.<br><br>**CAUTION!** Pour minimiser les conséquences négatives sur l'environnement à la suite de l'élimination de déchets électroniques, ne pas éliminer ce déchet électronique avec les déchets usuels non soumis au tri sélectif. Se conformer aux ordonnances locales sur les déchets municipaux pour les dispositions d'élimination et communiquer avec le service à la clientèle pour des renseignements sur les options d'élimination responsable. |

## Safety alerts on this instrument

Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

| English |
|---|
| **CAUTION! Hazardous chemicals.** Read the Safety Data Sheets (SDSs) before handling. |
| **CAUTION! Hazardous waste.** Refer to SDS(s) and local regulations for handling and disposal. |
| **DANGER! High voltage.** |
| **WARNING!** To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Thermo Fisher Scientific qualified service personnel. |

### Location of safety labels on this instrument

The Applied Biosystems® 7300Plus Real-Time PCR Instrument contains warnings at the following locations.


## Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

# Instrument safety

## General

**CAUTION! Do not remove instrument protective covers.** If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

## Physical injury

**CAUTION! Moving and Lifting Injury.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

**CAUTION! Moving Parts.** Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

### Electrical

**WARNING! Fuse Installation.** Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

**WARNING! Voltage Selector Switch.** Before installing the instrument, verify that the voltage selector switch is set for the supply voltage. This will prevent damage to the instrument, reduce risk of fire, and enable proper operation.

**WARNING! Ensure appropriate electrical supply.** For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

**WARNING! Power Supply Line Cords.** Use properly configured and approved line cords for the power supply in your facility.

**WARNING! Disconnecting Power.** To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

### Cleaning and decontamination

**CAUTION! Cleaning and Decontamination.** Use only the cleaning and decontamination methods specified in the manufacturer's user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that could cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be requested from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment

## Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

## Safety

| Reference | Description |
|---|---|
| IEC 61010-1<br>UL 61010-1<br>CSA C22.2 No. 61010-1 | *Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements* |
| IEC 61010-2-010 | *Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials* |
| IEC 61010-2-081 | *Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes* |

## EMC

| Reference | Description |
|---|---|
| IEC 61326-1 | *Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements* |
| AS/NZS 2064 | *Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment* |

## Environmental design

| Reference | Description |
|---|---|
| Directive 2012/19/EU | European Union "WEEE Directive" – Waste electrical and electronic equipment |

# Chemical safety

**WARNING! GENERAL CHEMICAL HANDLING.** To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

**WARNING! HAZARDOUS WASTE (from instruments).** Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

# Biological hazard safety

**WARNING! Potential Biohazard.** Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  **www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf**
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  **www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf**

# Documentation and support

## Related documentation

The following related documents are shipped with the system:

| Document | Publication number | Description |
|---|---|---|
| 7300Plus Real-Time PCR Instrument Documentation | 100027704 | Provides the reference for accessing user documentation from the product website and contact details for service and technical support. |
| *Applied Biosystems® Real-Time PCR Software Help* | N/A | Explains how to use the Real-Time PCR Software to:<br>• Set up, run, analyze, export, and print experiments.<br>• Monitor 7300Plus Instruments.<br>• Calibrate and verify the performance of the 7300Plus Instruments.<br>Intended for laboratory staff and principal investigators who perform experiments using the Real-Time PCR Software. |
| *7300Plus Real-Time PCR Instrument Site Preparation Guide* | 100027692 | Explains how to prepare your site to receive and install the 7300Plus Instruments. Intended for personnel who schedule, manage, and perform the tasks required to prepare your site for installation of the 7300Plus Instruments. |

**Note:** For additional documentation, see "Customer and technical support" on page 83.

## Obtaining information from the Help system

The Applied Biosystems® 7300Plus Real-Time PCR Instrument has a Help system that describes how to use each feature of the user interface. Access the Help system by doing one of the following:

- Click in the toolbar of the Real-Time PCR Software window.
- Select **Help ▸ Contents and Index**.
- Press **F1**.

You can use the Help system to find topics of interest by:

- Reviewing the table of contents
- Searching for a specific topic
- Searching an alphabetized index

You can also access PDF versions of all documents in the 7300Plus Instrument document set from the Help system.

## Customer and technical support

Visit **www.lifetechnologies.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    **Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

## Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

# Index

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