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<td>When to perform the calibration</td>
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About This Guide

Purpose

This user guide provides reference information for the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems and describes how to prepare, maintain, and troubleshoot the systems.

WARNING! The protection provided by the equipment may be impaired if the instrument is operated outside the environment and use specifications, the user provides inadequate maintenance, or the equipment is used in a manner not specified by the manufacturer (Life Technologies).

Note: For information and instructions on performing experiments on the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems, refer to the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Getting Started Guide (Pub. no. 4489822) and the High Resolution Melt Module for QuantStudio™ 6 and 7 Flex Real-Time PCR System Software (Pub. no. 4489823).

Audience

This user guide is written for laboratory staff who operate and maintain the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems.

Assumptions

This guide assumes that your QuantStudio™ 6 and/or 7 Flex System has been installed by a Life Technologies service representative.

This guide also assumes that you have:

- Familiarity with Microsoft® Windows® operating system.
- Knowledge of techniques for handling and preparing nucleic acid samples for PCR.
- A general understanding of data storage, file transfers, and copying and pasting.
User attention words

Four user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

**Note:** Provides information that may be of interest or help but is not critical to the use of the product.

**IMPORTANT!** Provides information that is necessary for proper instrument operation or accurate chemistry kit use.

[⚠️] **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.

Except for IMPORTANTs, the safety alert words in user documentation appear with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instrument. See the “Safety” appendix for descriptions of the symbols.

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 symbol is used along with the user attention words to highlight important safety information. The following table gives the meaning of these symbols.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>English</th>
<th>Français</th>
</tr>
</thead>
<tbody>
<tr>
<td>⚠️</td>
<td>Caution, risk of danger</td>
<td>Attention, risque de danger</td>
</tr>
<tr>
<td></td>
<td>Consult the manual for further</td>
<td>Consulter le manuel pour d’autres</td>
</tr>
<tr>
<td></td>
<td>safety information.</td>
<td>renseignements de sécurité.</td>
</tr>
</tbody>
</table>
## Contents of this manual

This guide is divided into the following chapters and appendixes:

<table>
<thead>
<tr>
<th>Chapter/appendix</th>
<th>Content</th>
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</thead>
<tbody>
<tr>
<td>Chapter 1, Getting Started</td>
<td>Introduces the QuantStudio™ 6 and 7 Flex Systems, including descriptions of all related hardware and software.</td>
</tr>
<tr>
<td>Chapter 2, Calibrate the Instrument</td>
<td>Describes how to maintain a QuantStudio™ 6 or 7 Flex System, including calibration and performance verification.</td>
</tr>
<tr>
<td>Appendix A, Manual Instrument Operation</td>
<td>Describes how to operate the QuantStudio™ 6 and 7 Flex Systems manually using the touchscreen interface.</td>
</tr>
<tr>
<td>Appendix B, Maintenance</td>
<td>Describes how to replace the user-serviceable parts of the QuantStudio™ 6 and 7 Flex Systems and how to resolve infrequent problems that can occur during normal use.</td>
</tr>
<tr>
<td>Appendix C, Networking</td>
<td>Describes how to install the QuantStudio™ 6 and 7 Flex Systems to a local area network for remote monitoring and control.</td>
</tr>
<tr>
<td>Appendix D, Power On or Off, Store, and Move</td>
<td>Describes how to store, move, and reinstall the components of the QuantStudio™ 6 and 7 Flex Systems.</td>
</tr>
<tr>
<td>Appendix E, Security, Audit, and Electronic Signature</td>
<td>Describes how to configure the security, audit, and electronic signature functions of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.</td>
</tr>
<tr>
<td>Appendix F, Calibration Consumable Preparation</td>
<td>Describes how to prepare consumables for custom dye calibration.</td>
</tr>
<tr>
<td>Appendix G, Parts and Materials</td>
<td>Describes how to order parts, accessories, and consumables for the QuantStudio™ 6 and 7 Flex Systems.</td>
</tr>
<tr>
<td>Appendix H, Instrument Specification and Layout</td>
<td>Describes the QuantStudio™ 6 and 7 Flex System specifications, requirements, layout, and connections.</td>
</tr>
</tbody>
</table>
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- Instrument software .............................................................. 23
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- Interchangeable sample blocks, heated covers, and plate retainers .......... 26
Differences between the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems

The following table displays the differences between the QuantStudio™ 6 Flex System and the QuantStudio™ 7 Flex System.

<table>
<thead>
<tr>
<th>QuantStudio™ 6 Flex System</th>
<th>QuantStudio™ 7 Flex System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does not support the Twister® Robot</td>
<td>Supports the Twister® Robot</td>
</tr>
<tr>
<td>Does not support the Array Card block</td>
<td>Supports the Array Card block</td>
</tr>
<tr>
<td>Features a coupled five-color filter set</td>
<td>Features a decoupled six-by-six-color filter set</td>
</tr>
</tbody>
</table>

About the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems

The QuantStudio™ 6 and 7 Flex Systems use fluorescent-based polymerase chain reaction (PCR) reagents to provide:

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

Data collection

The QuantStudio™ 6 and 7 Flex Systems collect raw fluorescence data at different points during a PCR, depending on the type of run that the QuantStudio™ 6 and 7 Flex Systems perform:

<table>
<thead>
<tr>
<th>Run type</th>
<th>Data collection point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real-time runs</td>
<td>The QuantStudio™ 6 and 7 Flex Systems collect data following each extension step of the PCR.</td>
</tr>
<tr>
<td>Standard curve</td>
<td></td>
</tr>
<tr>
<td>Relative standard curve</td>
<td></td>
</tr>
<tr>
<td>Comparative C_T (ΔΔC_T)</td>
<td></td>
</tr>
<tr>
<td>Melting curve</td>
<td></td>
</tr>
</tbody>
</table>
The instrument collects raw fluorescence data following each extension step of the PCR. A data collection point, or read, on the QuantStudio™ 6 and 7 Flex System consists of three phases:

1. **Excitation:** The instrument illuminates all wells of the plate or array card, within the instrument, exciting the fluorophores in each reaction.

2. **Emission:** The instrument optics collect the residual fluorescence emitted from the wells of the plate or array card. 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 software stores the raw fluorescent image for analysis.

After a run, the software uses calibration data (ROI, background, uniformity, dye, and normalization) to determine the location and intensity of the fluorescent signals in each read, the dye associated with each fluorescent signal, and the significance of the signal.

### Instrument filters and supported dyes

The QuantStudio™ 6 Flex System features a coupled five-color filter set while the QuantStudio™ 7 Flex System features a decoupled six-by-six-color filter set that supports the dyes shown in the following table. For more information about the spectral dye calibration kits available for the QuantStudio™ 6 and 7 Flex Systems, please contact Life Technologies.

<table>
<thead>
<tr>
<th>Filter set</th>
<th>Color</th>
<th>Filter wavelength (nm)†</th>
<th>Supported dye</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Excitation</td>
<td>Emission</td>
</tr>
<tr>
<td>x1-m1</td>
<td>Blue</td>
<td>470 ± 15</td>
<td>520 ± 15</td>
</tr>
<tr>
<td>x2-m2</td>
<td>Green</td>
<td>520 ± 10</td>
<td>558 ± 12</td>
</tr>
<tr>
<td>x3-m3</td>
<td>Yellow</td>
<td>549.5 ± 10</td>
<td>586.5 ± 10</td>
</tr>
<tr>
<td>x4-m4</td>
<td>Orange</td>
<td>580 ± 10</td>
<td>623 ± 14</td>
</tr>
<tr>
<td>x5-m5</td>
<td>Red</td>
<td>640 ± 10</td>
<td>682 ± 14</td>
</tr>
<tr>
<td>x6-m6§</td>
<td>Deep red</td>
<td>662 ± 10</td>
<td>711 ± 12</td>
</tr>
</tbody>
</table>

† The central wavelengths are the optimized wavelengths.
Custom dyes

The QuantStudio™ 6 and 7 Flex Systems can run assays designed with custom dyes (dyes not supplied by Life Technologies) that are excited between 455 to 672 nm and read between 505 to 723 nm. To add a custom dye to the Dye Library, perform a custom dye calibration. For more information, see “Calibration Consumable Preparation” on page 149.

Instrument hardware

The QuantStudio™ 6 and 7 Flex Systems consist of the components shown in the following figure and described in the subsequent table.
## Instrument hardware

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
</table>
| A         | **USB ports** Provide USB communication with the instrument. Can be used to transfer data to and from the instrument.  
**Note:** If multiple USB drives are plugged into the instrument, the instrument mounts only the first drive that is installed, regardless of the USB port used. |
| B         | **Touchscreen** Provides access to the instrument functions. Can be used to run experiment, transfer data, and operate the instrument functions without the use of the computer. |
| C         | **Access door** Provides access to the instrument lamp, the heated cover, and the sample block. |
| D         | **Lamp** Illuminates the plate or array card during a run. |
| E         | **Heated cover** Covers the plate or array card during a run to prevent condensation and leakage through the cover. |
| F         | **Sample block** Heats the plate or array card during a run. |
| G         | **Side door** Opens to allow extension of the tray arm. |
| H         | **Plate adapter** Secures the plate or array card to the tray arm. |
| I         | **Tray arm** Conveys the plate or array card to and from the sample block in the interior of the instrument. |

### Rear

![Rear diagram](image)

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
</table>
| A         | **Ethernet port** An RJ45 port that provides Ethernet (gigabit) communication with the instrument.  
† |

*Note:* Numbers and labels in the diagram correspond to the descriptions in the table.
Barcode scanner

The QuantStudio™ 6 and 7 Flex Systems include an optional Handheld Barcode Scanner (Part no. 4453271) that you can purchase to assist you with data entry. The barcode scanner uses a 670 nm Class II laser to scan plates or array cards (for only the QuantStudio™ 7 Flex System) and it is capable of reading Code 128 (alphanumeric), which supports 128 ASCII character barcodes.

**WARNING! LASER.** Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam.

The optional hand-held barcode scanner functions as an extension of the keyboard. You can use the scanner to scan barcodes into the instrument software.

To scan a barcode using the hand-held barcode scanner:

1. Select the field in the instrument software where you want to enter the barcode.
2. Hold the hand-held barcode scanner 20–30 cm away from a plate or array card and aim at the center of the barcode, then press the trigger. Slowly move the scanning beam across the barcode until the scanner emits a high-pitched tone.

When the scanner scans a barcode, it automatically:

- Transmits the alphanumerical equivalent of the barcode to the instrument software. The software enters the barcode text wherever the cursor is active.
- Transmits a carriage-return character (the equivalent of pressing the Enter key).
For more information on the hand-held barcode scanner, see the user documentation shipped with the barcode scanner.

**IMPORTANT!** The Applied Biosystems Twister® Robot component is supported only by the QuantStudio™ 7 Flex System.

The QuantStudio™ 7 Flex System supports the use of the Applied Biosystems Twister® Robot, an optional QuantStudio™ 7 Flex System accessory that consists of the components shown below.

**Note:** See the *Applied Biosystems Twister® Robot Automation Accessory User Guide* (Pub. no. 4470693) for information on operating, calibrating, maintaining and integrating the Twister® Robot.

### Twister® Robot components

---

**Front view**

---

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Reach axis</td>
</tr>
<tr>
<td>B</td>
<td>Wrist mechanism</td>
</tr>
<tr>
<td>C</td>
<td>Grip</td>
</tr>
<tr>
<td>D</td>
<td>Robot tower/vertical axis</td>
</tr>
<tr>
<td>E</td>
<td>Rotary axis</td>
</tr>
<tr>
<td>F</td>
<td>Bar code reader</td>
</tr>
<tr>
<td>G</td>
<td>Base cover</td>
</tr>
<tr>
<td>H</td>
<td>Racks</td>
</tr>
<tr>
<td>I</td>
<td>Power LED</td>
</tr>
</tbody>
</table>
Instrument hardware

Chapter 1  Getting Started

Rack parts and functions

Racks are removable aluminum frames used as input and output locations for PCR consumables. Rack positions are numbered counter-clockwise, with position 1 closest to the front of the Twister® Robot (see below). Each rack is labeled for a specific position and cannot be exchanged with the other racks.

Component Description
A On/Off switch Power switch for the Twister® Robot, where the states are on (1) or off (0).
B Power port 100–240V port that provides power to the Twister® Robot.
C RS232 port Provides serial communication with the computer.
D Fuse cover Two T1.6A 250VAC, 5 x 20-mm electrical fuses that protect the Twister® Robot from excessive electrical current.

Component Description
A Handles For connecting or disconnecting racks from the pod.
B Rack locator notch Locks the rack onto the pod in the correct position.

Note: Do not drop the racks. If the rack is bent, the Twister® Robot cannot properly place the consumables.
**Electrical protective devices**

We recommend several protective devices to protect the instrument in environments with large voltage and power fluctuations.

**Power line regulator**

We recommend the use of a 1.5-kVA power line regulator in areas where the supplied power fluctuates in excess of ± 10% of the normal voltage. Power fluctuations can adversely affect the function of the instrument.

**Note:** A power line regulator monitors the input current and adjusts the power supplied to the instrument or computer. It does not protect against a power surge or failure.

**Uninterruptible power supply (UPS)**

We recommend the use of a 1.5-kVA uninterruptible power supply (UPS), especially in areas prone to power failure. Power failures and other events that abruptly terminate the function of the QuantStudio™ 12K Flex System can corrupt data and possibly damage the computer or the instrument.

**IMPORTANT!** UPSs provide power for a limited time. They are meant to delay the effects of a power outage, not to serve as replacement power sources. In the event of a power loss, power off the instrument and the computer, unless you expect to regain power within the battery life of the UPS.

**Surge protector**

We recommend the use of a 10-kVA surge protector (line conditioner) in areas with frequent electrical storms or near devices that are electrically noisy, such as refrigerators, air conditioners, or centrifuges. Short-duration, high-voltage power fluctuations can abruptly terminate the function of, and thereby damage the components of, the computer and the instrument.

**Note:** A dedicated line and ground between the instrument/computer and the building’s main electrical service can also prevent problems caused by power fluctuations.

**Instrument software**

The QuantStudio™ 6 and 7 Flex Systems include a suite of software applications that can be used to calibrate, run, and integrate the instrument into a laboratory workflow. The installation of the software contains the components described below.

The QuantStudio™ 6 and 7 Flex Systems include a suite of software applications that can be used to calibrate, run, automate, and integrate the instrument into a laboratory workflow. The installation of the software contains the components described below; however, additional software may be available for the QuantStudio™ 6 and 7 Flex Systems. Visit the QuantStudio™ 6 and 7 Flex Systems website for a complete list of compatible software, and for updates and patches for the QuantStudio™ 6 and 7 Flex System software and firmware:

Computer requirements

The requirements for the computer used to operate the QuantStudio™ 6 and 7 Flex Systems can vary depending on the version of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software that you are running. To determine the computer requirements for your QuantStudio™ 6 or 7 Flex System, check the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software release notes at the following location:

<drive>:\Program Files\Applied Biosystems\QuantStudio 6 and 7 Flex Software\QuantStudio 6 and 7 Software README.docx

Note: <drive> is the location where the software is installed.

IMPORTANT! Due to a known incompatibility, Life Technologies does not recommend installing the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to computers running the Windows Vista® operating system. If used with the operating system, the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software generates errors when saving experiment (.eds) files.

Software installation

The default installation of the instrument partitions the computer hard drive to create the logical drives as explained below.

<table>
<thead>
<tr>
<th>Drive</th>
<th>Software</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Microsoft® Windows® OS†</td>
<td>Operating system files.</td>
</tr>
<tr>
<td></td>
<td>QuantStudio™ 6 and 7 Flex Real-Time PCR System Software</td>
<td>Used to calibrate and perform experiments on the QuantStudio™ 6 and 7 Flex System. Stores the installation files, application data files, and user files (experiments, exports, and imports).</td>
</tr>
<tr>
<td></td>
<td>QuantStudio™ 6 and 7 Flex System Command Line Utility</td>
<td>Used to automate the creation of new experiments and the export of existing experiments.</td>
</tr>
<tr>
<td>C or D drive</td>
<td>Twister® Robot Software‡</td>
<td>Controls the Twister® Robot, stores all of the taught positions for the robot, and includes the VBA code required to operate the Twister® Robot with the automation control software.</td>
</tr>
</tbody>
</table>

† We recommend that you do not install programs to the C drive.
‡ QuantStudio™ 7 Flex System only.

Third-party software

Before you install third-party software to the computer running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, confirm that the software will not:

• Restrict Ethernet communication
• Interfere with QuantStudio™ 6 and 7 Flex Real-Time PCR System Software operation (see below)

To confirm that third-party software does not interfere with the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software:

1. Install the software to the computer that contains the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.
2. Perform several test experiments using “dummy” plates (plates that do not contain reagents).

Note: The goal of the test experiments is to run plates under conditions that match normal instrument operation. Therefore, the characteristics of the test experiments (plate layout and run method) must closely resemble your actual experiments.

3. Confirm that the QuantStudio™ 6 and 7 Flex Systems perform each test experiment without producing errors.

If the QuantStudio™ 6 and 7 Flex Systems perform the tests successfully, perform experiments normally. If the QuantStudio™ 6 and 7 Flex Systems encounter errors during the test runs, the software may not be compatible with the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

Instrument consumables

The QuantStudio™ 6 and 7 Flex Systems support a series of specialized consumables through interchangeable sample blocks. Use the consumables appropriate for the sample block of your QuantStudio™ 6 or 7 Flex Systems.

Note: The QuantStudio™ 6 Flex System does not support the Array Card block.

<table>
<thead>
<tr>
<th>Sample block</th>
<th>Consumable</th>
<th>Reaction volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well plate, 0.2 mL</td>
<td>• MicroAmp® Optical 8-Cap Strip</td>
<td>100 µL</td>
</tr>
<tr>
<td></td>
<td>• MicroAmp® 8-Tube Strips (0.2-mL)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• MicroAmp® Reaction Tubes without Caps (0.2-mL)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• MicroAmp® 96-Well Tray/Retainer Set</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• MicroAmp® Optical Adhesive Film</td>
<td>100 µL</td>
</tr>
<tr>
<td></td>
<td>• MicroAmp® Optical 96-Well Reaction Plate with Bar Code</td>
<td></td>
</tr>
<tr>
<td>96-well plate, 0.1 mL</td>
<td>• MicroAmp® Optical Adhesive Film</td>
<td>50 µL</td>
</tr>
<tr>
<td></td>
<td>• MicroAmp® Optical 96-Well Fast Reaction Plate with Bar Code</td>
<td></td>
</tr>
</tbody>
</table>
## Interchangeable sample blocks, heated covers, and plate retainers

The QuantStudio™ 6 and 7 Flex Systems feature a set of interchangeable sample blocks, heated covers, and plate retainers that allow the instruments to support multiple consumable formats. All of the hardware is user-installable. Replacement can be done without the need for recalibration of the instrument, provided that the calibrations for the new format have not expired. See Appendix B, “Maintenance” for more information about changing the sample block, heated cover, and plate retainer.

<table>
<thead>
<tr>
<th>Sample block</th>
<th>Consumable</th>
<th>Reaction volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>384-well plate</td>
<td>• MicroAmp® Optical Adhesive Film</td>
<td>20 µL</td>
</tr>
<tr>
<td></td>
<td>• MicroAmp® Optical 384-Well Reaction Plate with Bar Code</td>
<td></td>
</tr>
<tr>
<td>Array card†</td>
<td>Life Technologies Array Card</td>
<td>1 µL</td>
</tr>
</tbody>
</table>

† QuantStudio™ 7 Flex System only.
Calibrate the Instrument

- Calibration overview .................................................... 28
- Enable or change the calibration reminders ......................... 31
- Guidelines for preparing calibration plates and array cards ........ 35
- Preparing array cards for instrument calibration .................. 36
- ROI calibration ................................................................. 40
- Background calibration ..................................................... 44
- Uniformity calibration ....................................................... 48
- Dye calibration ................................................................. 52
- Normalization calibration .................................................. 57
- Verify the instrument performance .................................... 61
- Troubleshooting ............................................................... 69

IMPORTANT! Perform the procedures for preparing array cards only if you are verifying the performance of a QuantStudio™ 7 Flex System with an array card sample block. The QuantStudio™ 6 Flex System does not support the array card sample block.
Calibration overview

**Calibration and maintenance schedule**

The QuantStudio™ 6 and 7 Flex Systems require regular calibration and maintenance for proper operation. To ensure proper operation of your instrument, perform weekly, monthly, and semiannual maintenance as indicated in the following table.

**IMPORTANT!** Perform calibrations and run experiments under the environmental conditions specified in Appendix H, “Instrument Specification and Layout”. Exposure to extreme temperatures can have adverse effects on the run results, as well as shortening the life span of the instrument components.

**IMPORTANT!** Do not use organic solvents to clean the QuantStudio™ 6 and 7 Flex Systems.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>User-performed maintenance task</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weekly</td>
<td>Check the computer disk space. If necessary, archive or back up your experiment files and instrument settings.</td>
</tr>
<tr>
<td></td>
<td>Power off the computer that controls the instrument, then power on the computer after 30 seconds.</td>
</tr>
<tr>
<td></td>
<td>Clean the surface of the instrument with a lint-free cloth.</td>
</tr>
<tr>
<td>Monthly</td>
<td>Perform a background calibration.†</td>
</tr>
<tr>
<td></td>
<td>Run disk cleanup and disk defragmentation.</td>
</tr>
<tr>
<td></td>
<td>Perform an instrument self test.</td>
</tr>
<tr>
<td>Semi-annually (every 6 months)</td>
<td>Perform an ROI calibration.</td>
</tr>
<tr>
<td></td>
<td>Perform a background calibration.†</td>
</tr>
<tr>
<td></td>
<td>Perform a uniformity calibration.</td>
</tr>
<tr>
<td></td>
<td>Perform a dye calibration.</td>
</tr>
<tr>
<td></td>
<td>Perform a normalization calibration.</td>
</tr>
<tr>
<td>As needed</td>
<td>Perform an RNase P instrument verification run.</td>
</tr>
<tr>
<td></td>
<td>Replace the instrument lamp.‡</td>
</tr>
</tbody>
</table>

† You can perform a background calibration to check for contamination.
‡ After replacing the instrument lamp, perform all calibrations and an RNase P instrument verification run.

**Planned maintenance**

The instrument requires regular planned maintenance by Life Technologies service personnel to ensure that the instrument is operating within the installation specification. The planned maintenance service can help you maximize your productivity by minimizing downtime, lowering the cost of ownership by addressing potential problems before they manifest (lost data, samples, or reagents), and increasing the quality of your results by maintaining your instrument at optimal performance. For more information, contact your Life Technologies sales representative.
About maintenance reminders

The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software features an automated notification system that alerts you of required calibrations. The feature will also prevent you from operating an instrument that is out of calibration. See “Enable or change the calibration reminders” on page 31 for more information on setting the maintenance reminders.

Calibration workflow

The following figure shows the workflow for calibrating the QuantStudio™ 6 and 7 Flex Systems. Whether you are performing all calibrations or just a subset, perform them in the sequence shown below.

Start

Perform an ROI calibration

Perform a background calibration

Perform a uniformity calibration

Perform dye calibrations

Perform a normalization calibration

Perform an RNase P instrument verification test

Finish

View the calibration status of an instrument

You can use the Instrument Console of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to view the calibration status of a QuantStudio™ 6 or 7 Flex System.

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click 
   Instrument Console.

2. From the Instrument Console open the Instrument Manager:
Chapter 2 Calibrate the Instrument

Calibration overview

1. Select your instrument icon from the My Instruments group.

2. Click Manage Instrument.

   Note: If the Manage Instrument option is inactive, connect your QuantStudio™ 6 or 7 Flex System to the My Instruments group.

3. From the Instrument Manager, click Monitor, then click Information to open the instrument monitor.

   The instrument monitor screen displays the:
   a. The Instrument type (QuantStudio™ 6 Flex System or QuantStudio™ 7 Flex System).
   b. Status of all calibrations, including the date and time of each calibration.
   c. Maintenance status of the instrument.
   d. Properties of the instrument.
Chapter 2 Calibrate the Instrument

Enable or change the calibration reminders

The calibration reminder settings allow you to configure the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to alert you by email when the QuantStudio™ 6 or 7 Flex System requires calibration. The notifications settings feature is optional, and it does not affect instrument performance.

**IMPORTANT!** The calibration reminders feature requires a connection between the QuantStudio™ 6 or 7 Flex System computer and a computer network. If you choose to set up the feature as described below, ensure that your network has appropriate security measures in place to protect data from threats such as computer viruses.

**IMPORTANT!** The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software transmits emails only while the QuantStudio™ 6 or 7 Flex System is monitored. If the network connection is interrupted, the software will stop transmitting updates.

Collect the required information

The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software requires access to a Simple Mail Transfer Protocol (SMTP) server to email calibration reminders. Contact your systems administrator or information technology department for the following information:

- Network address of an SMTP server.
- A user name and password for the server, if required for access.
- The Secure Sockets Layer (SSL) setting of the server (on or off).

Define the mail server settings

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select Tools ➤ Preferences.
2. From the Preferences dialog box, select the **SMTP Settings** tab.
3. Define the settings for the SMTP server:
   a. From the **Outgoing Mail Server (SMTP)** field, enter the network address of a Simple Mail Transfer Protocol (SMTP) server.

   Optionally, you can specify the transmission control protocol (TCP) port for the server by appending the port number to the server name, separating the two using a colon (:).
Chapter 2 Calibrate the Instrument
Enable or change the calibration reminders

For example: smtp.mycompany.com:2023

Note: If a TCP port is not specified, the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software uses the default port number (25).

b. Select Encryption Required if the mail server has SSL enabled.

c. Select Authentication Required if the mail server requires a user name and password.

d. If the mail server requires authentication, enter the user name and password provided by your systems administrator.

4. Click OK.

5. Test the mail server settings:

   a. From the Home tab of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select any experiment in the Set Up panel, then click Set Up to create a new experiment.

   b. From the experiment document, click Run in the Experiment Menu, then click Notification Setting to view the notification settings for the experiment.

   c. From the Notification Settings of the experiment document, select Yes to enable the Enable Notifications setting.

   d. From the Enter e-mail addresses for notifications field, enter the email address that you want to receive the test emails.

   e. Click Test Configuration to test the mail server settings.
Chapter 2  Calibrate the Instrument
Enable or change the calibration reminders

Modify the notification settings for a monitored instrument

1. Open the Calibration Reminders screen for the QuantStudio™ 6 or 7 Flex System:
   a. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click Instrument Console.
   b. From the Instrument Console, select the icon for your instrument, then click Manage Instrument.
      Note: If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.
   c. From the Instrument Manager, click Maintenance, then click Calibration Reminders.

2. From the Calibration Reminders Setting table, configure the notification settings for the calibrations in interest. For each calibration that you want to monitor:
   a. From the Send a Reminder column, select the check box to configure the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to email a reminder to perform the calibration.
   b. From the Reminder Interval column, enter the number of days before the calibration expires that the software should send a reminder email.

3. From the Enter e-mail addresses for notifications field, enter the email address(es) that you want to receive notifications. Separate multiple email addresses with commas (, ).

Confirm that the email account you specified in step 5d received the test messages from the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software. If not, consult your network administrator to confirm that the mail server settings are defined correctly.
4. Click **Apply** to change the notification settings.
Guidelines for preparing calibration plates and array cards

Observe the following guidelines when preparing calibration plates and array cards:

- Wear appropriate protective eyewear, clothing, and gloves.
- Prepare and run calibration plates and array cards within the recommended time limits listed below.

<table>
<thead>
<tr>
<th>Consumable</th>
<th>Time to thaw</th>
<th>After thawed, run within...</th>
</tr>
</thead>
<tbody>
<tr>
<td>All 96-/384-well calibration consumables†</td>
<td>30 minutes</td>
<td>120 minutes</td>
</tr>
<tr>
<td>All array card calibration consumables‡</td>
<td>30 minutes</td>
<td>120 minutes</td>
</tr>
<tr>
<td>RNase P plate (96-/384-well)</td>
<td>5 minutes</td>
<td>30 minutes</td>
</tr>
<tr>
<td>RNase P array card§</td>
<td>15 minutes</td>
<td>60 minutes</td>
</tr>
</tbody>
</table>

† All 96-well plates and 384-well plates used in the calibration of the instrument [ROI, background, uniformity, dye, and normalization].
‡ All array cards used in the calibration of the QuantStudio™ 7 Flex System only [ROI, background, uniformity, dye, and normalization].
§ For use with QuantStudio™ 7 Flex System only.

- *(Plates only)* Do not discard the packaging for the calibration plates. Each plate can be used to calibrate the QuantStudio™ 6 and 7 Flex Systems 3 times for up to 6 months if it is stored in its packing sleeve.

- Store calibration plates or array cards in a dark place until you are ready to use them. The fluorescent dyes in the wells of calibration consumables are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dyes.

- Do not allow the bottoms of the plates or array cards to become dirty. Fluids and other contaminants that adhere to the bottoms of the consumables can contaminate the sample block and cause an abnormally high background signal.

- Confirm that your centrifuge is clean. Before centrifugation, wipe down the bucket(s) using a tissue.

- Vortex and centrifuge all calibration plates to ensure complete mixing and that all reagents are contained at the bottom of the wells. The calibration plates must be well mixed and centrifuged before use.
Preparing array cards for instrument calibration

IMPORTANT! Perform the following procedure only if you are verifying the performance of a QuantStudio™ 7 Flex System with an array card sample block. The QuantStudio™ 6 Flex System does not support the array card sample block.

Required materials

• QuantStudio™ 7 Flex System Array Card Spectral Calibration Dye Kit:
  – Array Cards, empty
  – Array Card Spectral Calibration Dye Kit, including: FAM™ dye mix, VIC® dye mix, ROX™ dye mix, ROI dye mix, Background Buffer, FAM™/ROX™ dye mix, and VIC®/ROX™ dye mix
• Array Card Staker/Sealer
• Centrifuge with array card buckets and array card carrier clips
• Permanent marker or pen
• Pipettor, 200-µL (with pipette tips)
• Powder-free gloves
• Safety glasses

Filling the calibration array cards

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

This procedure explains how to create all of the array cards required to calibrate the QuantStudio™ 7 Flex System, but not all of them are required for a monthly maintenance. Before preparing array cards for calibration, see “Calibration and maintenance schedule” on page 28 to determine which calibrations are required.

Note: For more information on the array card loading procedure, contact Life Technologies.

1. Remove the tubes of calibration solutions from the freezer, allow them to thaw, then vortex the tubes to mix the contents well.

2. Remove the array cards from their box and place them on a clean, dry surface.

3. Mark the side of the empty array cards with: Background, FAM, ROI, ROX, VIC, FAM/ROX, and VIC/ROX.

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

\[\text{Fill port}\]

\[\text{Vent port}\]

\[cREM\]

\[\text{Hold the pipette in an angled position (~45 degrees) and place the tip into the fill port. The fill port is the larger of the two holes on the left side of the fill reservoir.}\]

\[\text{DREM}\]

\[\text{Dispense the fluid so that it sweeps in and around the fill reservoir toward the vent port.}\]

\[\text{Pipet the entire 100 µL into the fill reservoir, but do not go past the first stop of pipettor plunger or you may blow the solution out of the port.}\]

\[\text{IMPORTANT!}\]

\[\text{Do not allow the tip to contact and possibly damage the coated foil beneath the fill port.}\]

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

6. Centrifuge the array card(s):

\[\text{a. Place the filled array card(s) into a centrifuge array card carrier clip and place empty array cards in the remaining slots. Confirm that the labels on the buckets and clips are oriented in the same direction.}\]

\[\text{b. Place the filled carrier clips into the centrifuge buckets. Make sure that the array card fill reservoirs and bucket and clip labels face outward when loaded into the centrifuge.}\]

\[\text{IMPORTANT!}\]

\[\text{You must run the centrifuge with all four buckets in place and each of the two carriers filled with array cards. Place empty array cards into unfilled slots.}\]

\[\text{IMPORTANT!}\]

\[\text{Balance the loads in opposite buckets in the centrifuge.}\]
c. Close the centrifuge cover, then spin the array card(s) for 1 minute at 1200 rpm.

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

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

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

<table>
<thead>
<tr>
<th>Correct fill</th>
<th>Incorrect/partial fill</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Filled array" /></td>
<td><img src="image2" alt="Empty array" /></td>
</tr>
<tr>
<td><img src="image3" alt="Correct fill" /></td>
<td><img src="image4" alt="Incorrect fill" /></td>
</tr>
</tbody>
</table>

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

8. Seal the array card(s):

a. With the carriage (roller assembly) of the Array Card Staker/Sealer in the Start position, place a filled array card into the fixture with the foil side up so that the fill reservoirs are the farthest away from the carriage.

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

d. Seal the array card by running the carriage slowly over it. Run the carriage over the array card in one direction only. Do not apply downward force on the carriage as you move it forward over the card.

![Image of array card being sealed]

9c. Remove the sealed array card from the fixture and trim the fill reservoirs from the array card assembly using scissors. Trim the foil array card so that the edge is even with the plastic carrier.

![Image of scissors trimming array card]

**IMPORTANT!** Completely remove the fill reservoirs from the array card so that the edge is free of residual plastic. The plastic from the fill reservoirs that extends beyond the edge of the card can prevent the array card from seating properly on the sample block and can affect amplification.

<table>
<thead>
<tr>
<th>Correct trim</th>
<th>Incorrect trim</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Correct trim image]</td>
<td>![Incorrect trim image]</td>
</tr>
</tbody>
</table>

9. Repeat step 8 to seal the remaining array cards.

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

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

A region of interest (ROI) calibration maps the positions of the wells on the sample block of the instrument. The software uses the ROI calibration data to associate increases in fluorescence during a run with the wells on the plate or array card. The instrument uses a set of optical filters to distinguish the fluorescence emissions gathered during runs. The software generates a calibration image for each filter to account for minor differences in the optical path.

When to perform the calibration

Perform the ROI calibration once every 6 months.

IMPORTANT! After every ROI calibration, you must perform (in order): a background calibration, uniformity calibration, dye calibration, and a normalization calibration.

About the calibration data

During the ROI calibration, the instrument software captures images of the ROI calibration plate or array card at each instrument filter. An ROI calibration passes if the collected image for each filter distinguishes all wells of the ROI plate or array card. Each well in the image must be distinct and visible at the same luminosity relative to the other wells in the image.

Note: You can review the ROI calibration image for each filter set by selecting the desired filter combination from the Filter Set menu of the ROI tab in the Instrument Manager.

<table>
<thead>
<tr>
<th>Passing image</th>
<th>Failing image</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Passing image" /></td>
<td><img src="image2.png" alt="Failing image" /></td>
</tr>
</tbody>
</table>

Green circles around all wells indicate that the wells calibrated successfully. Each green circle indicates that the region of interest for the well position is sufficiently bright.

Red circles around some of the wells indicate that the wells did not calibrate. The absence of a circle indicates that the region of interest for the well position is not sufficiently bright.
Prepare the calibration plate or array card

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare plates or array cards.

Prepare the calibration consumable appropriate for your QuantStudio™ 6 or 7 Flex System:

- Prepare the ROI calibration plate .................................................. 41
- Preparing array cards for instrument calibration .............................. 36

Prepare the ROI calibration plate

Materials required

- Region of Interest (ROI) Plate
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Prepare the plate

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare the plate.

1. Remove the ROI calibration plate from the freezer, then thaw it at room temperature for approximately 30 minutes.

   IMPORTANT! Use the calibration plate within 2 hours of defrosting it. Until you are ready to run the plate, store it in the dark and at ambient temperature (15–30°C). Do not remove the calibration plate from its packaging until you are ready to run it. The fluorescent dyes in the wells of the plate are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dyes.

2. Remove the ROI plate from its packaging. Do not remove the optical film.

3. Vortex and centrifuge the plate:
   a. Vortex the ROI calibration plate for 5 seconds.
   b. Centrifuge the plate for 2 minutes at < 1500 rpm.

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

   c. Confirm 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.

<table>
<thead>
<tr>
<th>Correct</th>
<th>Incorrect</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Correct" /></td>
<td><img src="image2" alt="Incorrect" /></td>
</tr>
</tbody>
</table>
| Liquid is at bottom of well. | • Not centrifuged with enough force, or  
• Not centrifuged for enough time. |
Perform the ROI calibration

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click Instrument Console.

2. From the Instrument Console, select the icon for your instrument, then click Manage Instrument.
   
   **Note:** If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.

3. From the Instrument Manager, start the calibration:
   
   a. Click Maintenance, then click ROI.
   
   b. From the ROI Calibration screen, click Start Calibration.

4. Click Next, then prepare for the calibration as instructed.

5. From the bottom of the Setup tab, enter the reagent information for the ROI plate or array card that you are using.

6. Load the calibration plate or array card into the instrument:
   
   a. From the instrument touchscreen, touch to eject the instrument tray.
   
   b. Load the plate or array card into the plate holder so that:
      
      - Well A1 of the plate or array card is in the top-left corner of the plate adapter.
      - The barcode faces the front of the instrument.

   c. From the instrument touchscreen, touch to close the instrument tray.

7. After loading the plate or array card, start the calibration:
   
   a. From the bottom of the Setup tab, select Check the box when the ROI calibration plate has been loaded, then click Next.
b. From the Run screen, click **START RUN**.

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

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

8. When the run is complete and the software displays the Analysis screen, select each filter from the Filter Set drop-down list, then confirm that the corresponding ROI Image displays a green circle around each well area.

9. After you inspect all ROI images, confirm the status of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or that the data it collected is unusable.

<table>
<thead>
<tr>
<th>Analysis status</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passed</td>
<td>Click <strong>Next</strong>.</td>
</tr>
<tr>
<td>Failed</td>
<td>Troubleshoot the failed calibration as described in “ROI calibration troubleshooting” on page 70.</td>
</tr>
</tbody>
</table>

10. Unload the calibration plate or array card:

a. After the calibration, touch ° on the touchscreen to eject the plate or array card.

**Note:** If the instrument does not eject the plate or array card, remove it as explained in “ROI calibration troubleshooting” on page 70.

b. Remove the ROI calibration plate or array card from the instrument tray and store it in the dark at room temperature until you are ready to perform the uniformity calibration.

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

c. Touch ° on the instrument touchscreen to close the instrument tray.

11. From the ROI Calibration screen, click **Finish** to complete the calibration, then click **Yes** when prompted to save the results.

**Note:** If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays the warning, “Saving ROI calibration will invalidate other existing calibrations except HRM Calibrations”, click **OK** to proceed.

**Note:** HRM (High Resolution Melt) is a separately licensed module. If your instrument requires this module, contact Life Technologies.

12. (Optional) Click **Print Report** in the upper right corner of the screen to print a summary of the calibration results for your records.
Background calibration

The software uses the background calibration data during subsequent experiments to remove background fluorescence from the experiment data.

When to perform the calibration

Perform the background calibration monthly.

About the calibration data

During the background calibration, the software captures a series of images of the background plate or array card using each instrument filter. The software compares the fluorescence from each well to the average for the plate or array card. A background calibration passes if the collected images for all filters are free of abnormal fluorescence.

Background fluorescence

Fluorescence data collected by the instrument includes a fluorescence signal inherent to the instrument, referred to as “background fluorescence.” Background fluorescence is a composite signal found in all spectral data that consists of fluorescence from several sources, including:

- Background electronic signal
- Contaminants in the sample block
- The plastic consumable (plate or array card)

Preparing the calibration plate or array card

Prepare the calibration consumable appropriate for your QuantStudio™ 6 or 7 Flex System:

- Prepare the background calibration plate ........................................ 45
- Preparing array cards for instrument calibration ................................. 36
Prepare the background calibration plate

Materials required
- Background Plate
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Prepare the plate

**IMPORTANT!** Wear powder-free gloves and safety glasses when you prepare the plate.

1. Remove the background calibration plate from the freezer, then thaw it at room temperature for approximately 30 minutes.

**IMPORTANT!** Use the calibration plate within 2 hours of defrosting it. Until you are ready to run the plate, store it in the dark and at ambient temperature (15–30°C). Do not remove the plate from its packaging until you are ready to run it.

2. Remove the background calibration plate from its packaging. Do not remove the optical film.

3. Vortex and centrifuge the plate:
   a. Vortex the background calibration plate for 5 seconds.
   b. Centrifuge the plate for 2 minutes at < 1500 rpm.

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

   c. Confirm that the liquid in each well of the background 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.

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

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<tr>
<th>Correct</th>
<th>Incorrect</th>
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<tbody>
<tr>
<td><img src="image1" alt="Correct" /></td>
<td><img src="image2" alt="Incorrect" /></td>
</tr>
</tbody>
</table>

- Liquid is at bottom of well.
- Not centrifuged with enough force, or
- Not centrifuged for enough time.
1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click Instrument Console.

2. From the Instrument Console, select the icon for your instrument, then click Manage Instrument.
   
   **Note:** If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.

3. From the Instrument Manager, start the calibration:
   
   a. Click Maintenance, then click Background.
   
   b. From the Background Calibration screen, click Start Calibration.

4. Click Next, then prepare for the calibration as instructed.

5. From the bottom of the Setup tab, enter the reagent information for the background plate or array card that you are using.

6. Load the calibration plate or array card into the instrument:
   
   a. From the instrument touchscreen, touch to eject the instrument tray.
   
   b. Load the calibration plate or array card into the plate holder so that:
      
      • Well A1 of the plate or array card is in the top-left corner of the plate adapter.
      
      • The barcode faces the front of the instrument.

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

   c. From the instrument touchscreen, touch to close the instrument tray.

7. After loading the plate or array card, start the calibration:

   a. From the Setup tab, select Check the box when the background calibration plate has been loaded, then click Next.
b. From the Run screen, click **START RUN**.

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

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

8. When the run is complete and the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays the Analysis screen, confirm the status of the calibration. The analysis status indicates the success of the calibration, where *passed* indicates that the run produced viable calibration data, and *failed* indicates that the run did not produce data or that the data it collected is unusable.

<table>
<thead>
<tr>
<th>Analysis status</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passed</td>
<td>Click <strong>Next</strong>.</td>
</tr>
<tr>
<td>Failed</td>
<td>Troubleshoot the failed calibration as described in “Background calibration troubleshooting” on page 71.</td>
</tr>
</tbody>
</table>

**Note:** Abnormal spectra or abnormally high background fluorescence can indicate the presence of contamination on the plate or array card or sample block, which can cause the calibration to fail.

9. Select the QC tab to review the quality check summary. The QC status indicates the quality of the calibration data, where “Passed” indicates that all wells produced data that passed the quality check, and “Failed” indicates that one or more wells produced spectra that deviate significantly from the other wells on the plate or array card.

**Note:** If the calibration failed, the QC tab can provide information regarding the cause of the failure.

10. Unload the calibration plate or array card:
   a. After the calibration, touch ![eject icon] on the touchscreen to eject the plate or array card.

   **Note:** If the instrument does not eject the plate or array card, remove it as explained in “Background calibration troubleshooting” on page 71.

   b. Remove the plate or array card from the instrument tray and discard it.

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

   c. Touch ![close icon] on the instrument touchscreen to close the instrument tray.

11. From the Background Calibration screen, click **Finish** to complete the calibration, then click **Yes** when prompted to save the results.

12. (Optional) Click **Print Report** in the upper right corner of the screen to print a summary of the calibration results for your records.
Uniformity calibration

The uniformity calibration generates data that allows the software to compensate for the physical effects of the instrument filters.

When to perform the calibration

Perform a uniformity calibration once every 6 months.

Preparing the calibration plate or array card

**IMPORTANT!** Wear powder-free gloves and safety glasses when you prepare plates or array cards.

Prepare the calibration consumable appropriate for your QuantStudio™ 6 or 7 Flex System:

- Prepare the ROI calibration plate ........................................... 48
- Preparing array cards for instrument calibration ....................... 36

Prepare the ROI calibration plate

**Materials required**

**Note:** The uniformity calibration requires the same materials used to perform the ROI calibration.

- Region of Interest (ROI) Plate
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

**Prepare the plate**

**IMPORTANT!** Wear powder-free gloves and safety glasses when you prepare the plate.

If you have a prepared ROI calibration plate from a recent ROI calibration, go to step 3b below; otherwise, prepare the ROI calibration plate as follows:

1. Remove the ROI calibration plate from the freezer, then thaw it at room temperature for approximately 30 minutes.

   **IMPORTANT!** Use the calibration plate within 2 hours of defrosting it. Until you are ready to run the plate, store it in the dark and at ambient temperature (15–30°C). Do not remove the plate from its packaging until you are ready to run it.

2. Remove the ROI plate from its packaging. Do not remove the optical film.

3. Vortex and centrifuge the plate:
   a. Vortex the ROI calibration plate for 5 seconds.
   b. Centrifuge the plate for 2 minutes at < 1500 rpm.

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

<table>
<thead>
<tr>
<th>Correct</th>
<th>Incorrect</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Correct" /></td>
<td><img src="image" alt="Incorrect" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Liquid is at bottom of well.</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Not centrifuged with enough force, or</td>
</tr>
<tr>
<td>• Not centrifuged for enough time.</td>
</tr>
</tbody>
</table>

Perform the uniformity calibration

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.
2. From the Instrument Console, select the icon for your instrument, then click **Manage Instrument**.
   **Note:** If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.
3. From the Instrument Manager, start the calibration:
   a. Click **Maintenance**, then click **Uniformity**.
   b. From the Uniformity Calibration screen, click **Start Calibration**.
4. Click **Next**, then prepare for the calibration as instructed.
5. From the bottom of the Setup tab, enter the reagent information for the ROI plate or array card that you are using.
6. Load the calibration plate or array card into the instrument:
   a. From the instrument touchscreen, touch to eject the instrument tray.
   b. Load the calibration plate or array card into the plate holder so that:
      • Well A1 of the plate or array card is in the top-left corner of the plate adapter.
      • The barcode faces the front of the instrument.

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

   c. From the instrument touchscreen, touch to close the instrument tray.

7. After loading the plate or array card, start the calibration:
   a. From the Setup tab, select Check the box when the ROI calibration plate has been loaded, then click Next.
   b. From the Run screen, click START RUN.

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

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

8. When the run is complete and the Analysis screen displays, confirm the status of the calibration. The analysis status indicates the success of the calibration, where passed indicates that the run produced viable calibration data, and failed indicates that the run did not produce data or that the data it collected is unusable.

<table>
<thead>
<tr>
<th>Analysis status</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passed</td>
<td>Click Next.</td>
</tr>
<tr>
<td>Failed</td>
<td>Troubleshoot the failed calibration as described in “Uniformity calibration troubleshooting” on page 72.</td>
</tr>
</tbody>
</table>

9. Select the QC tab to review the quality check summary.

   Note: The QC tab can provide information on the cause of a failed calibration.
10. Unload the calibration plate or array card:
   a. After the calibration, touch the touchscreen to eject the plate or array card.
      
      Note: If the instrument does not eject the plate or array card, remove it as explained in “Uniformity calibration troubleshooting” on page 72.

   b. Remove the calibration plate or array card from the instrument tray and discard it.

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

   c. Touch the instrument touchscreen to close the instrument tray.

11. From the Uniformity Calibration screen, click Finish to complete the calibration, then click Yes when prompted to save the results.

12. (Optional) Click Print Report in the upper right corner of the screen to print a summary of the calibration results for your records.
Dye calibration

The software uses the calibration data collected from the dye standards to characterize and distinguish the individual contribution of each dye in the total fluorescence collected by the instrument. After each run, the instrument software receives data in the form of a raw spectra signal for each reading. The software determines the contribution of the fluorescent dyes used in each reaction by comparing the raw spectra to the pure spectra calibration data. When you save an experiment after analysis, the instrument software stores the pure spectra with the collected fluorescence data for that experiment.

When to perform the calibration

Perform a dye calibration once every 6 months.

IMPORTANT! Perform a background calibration before every series of dye calibrations. Because the age and use of instrument components can affect spectra readings, you must perform a dye calibration at least every 6 months.

About the dye calibration data

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 calibration plate or array card. Following the calibration of each dye, the software “extracts” a spectral profile for each dye. The software plots the resulting data for each profile in a graph of fluorescence versus filter.

When the software extracts the dye calibration data, it evaluates the fluorescence signal generated by each well in terms of the collective spectra for the entire calibration plate or array card. Dye spectra are generally acceptable if they peak within the same filter as their group, but diverge slightly at other wavelengths (see below).

<table>
<thead>
<tr>
<th>Acceptable spectra</th>
<th>Unacceptable spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectra peak at the same wavelength and do not diverge significantly</td>
<td>Spectra peak at the different wavelengths</td>
</tr>
</tbody>
</table>

Note: Because the wells of a calibration plate or array card contain identical concentrations of a dye, the resulting signals for the wells should be similar. Variations in spectral position and peak position are caused by minor differences in the optical properties and excitation energy between the individual wells.
Preparation of the Calibration Plate or Array Card

**IMPORTANT!** Wear powder-free gloves and safety glasses when you prepare plates or array cards.

Prepare the calibration consumable appropriate for your QuantStudio™ 6 or 7 Flex System:
- Prepare the dye calibration plates .................................................. 53
- Preparing array cards for instrument calibration ............................ 36

**Preparation of the Dye Calibration Plates**

**Materials required**
- Spectral calibration plates
  - Spectral Calibration Plate with FAM™ Dye
  - Spectral Calibration Plate with VIC® Dye
  - Spectral Calibration Plate with ROX™ Dye
  - Spectral Calibration Plate with NED™ Dye
  - Spectral Calibration Plate with TAMRA™ Dye
  - Spectral Calibration Plates as required by the experiment that you intend to run
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

**Prepare the Plate**

**IMPORTANT!** Before performing a dye calibration, you must perform an ROI calibration, a background calibration, and a uniformity calibration.

**IMPORTANT!** Wear powder-free gloves and safety glasses when you prepare the plate.

1. Remove the dye calibration plate from the freezer, then thaw it at room temperature for approximately 30 minutes.

**IMPORTANT!** Use the calibration plates within 2 hours of defrosting them. Until you are ready to run the plates, store them in the dark and at ambient temperature (15–30°C). Do not remove the plates from their packaging until you are ready to run them.
2. Before using each dye plate, vortex the plate for 5 seconds, centrifuge it for 2 minutes at < 1500 rpm, then confirm that the liquid in each dye plate is at the bottom of the wells. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

<table>
<thead>
<tr>
<th>Correct</th>
<th>Incorrect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid is at bottom of well.</td>
<td>• Not centrifuged with enough force, or • Not centrifuged for enough time.</td>
</tr>
</tbody>
</table>

Perform the dye calibration

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

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click Instrument Console.

2. From the Instrument Console, select the icon for your instrument, then click Manage Instrument.

   **Note:** If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.

3. From the Instrument Manager, start the calibration:
   a. Click Maintenance, then click Dye.
   b. From the Dye Calibration screen, select System Dye Calibration, then click Start Calibration.

4. From the Dye Calibration screen, select the dye to calibrate from the Dye Name drop-down list, then perform the calibration as instructed.

5. From the bottom of the Setup tab, enter the reagent information for the dye plate or array card that you are using.

6. Load the dye plate or array card into the instrument:
   a. From the instrument touchscreen, touch to eject the instrument tray.
   b. Confirm that the dye plate or array card matches the dye selected in the software, then load it into the plate holder.

   Load the plate or array card so that:
   • Well A1 of the plate or array card is in the top-left corner of the plate adapter.
   • The barcode faces the front of the instrument.
Chapter 2 Calibrate the Instrument

Dye calibration

Note: The name of the dye contained by the plate or array card is next to the barcode on the front of it.

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

7. After loading the plate or array card, start the calibration:
   a. From the Dye Calibration screen, select **Check the box when the dye calibration plate has been loaded**, then click **Next**.
   b. From the Run screen, click **START RUN**.

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

8. When the run is complete and the software displays the Analysis screen, confirm the grouping of the dye spectra:
   a. Select the **Plate Layout** tab, then review the raw data. For each spectrum, confirm that the peak is:
      - Within the detectable range for the QuantStudio™ 6 and 7 Flex Systems.
      - Free of irregular spectral peaks.
      - Present at the correct filter for the dye (see “Instrument filters and supported dyes” on page 17).

   **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.

   b. Select the **QC** tab, then review the summary of wells that failed the quality check (QC).

9. After you inspect the dye spectra, confirm the analysis status of the calibration. The analysis status indicates the success of the calibration, where **passed** indicates that the run produced viable calibration data, and **failed** indicates that the run did not produce data or that the data it collected is unusable.
10. Select the QC tab to review the quality check summary. The QC status indicates the quality of the calibration data, where **passed** indicates that all wells produced data that passed the quality check, and **failed** indicates that one or more wells produced dye spectra that differ significantly from the other wells on the plate or array card.

**Note:** If the calibration failed, the QC tab can provide information regarding the cause of the failure.

11. Unload the calibration plate or array card:
   a. After the calibration, touch \(\text{ eject} \) on the touchscreen to eject the plate or array card.
      
      **Note:** If the instrument does not eject the plate or array card, remove it as explained in “Dye calibration troubleshooting” on page 73.
   
   b. Remove the calibration plate or array card from the instrument tray and discard it.
      
      **WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the plate or array card temperature can reach 100°C. Allow the consumable to cool to room temperature before removing.
      
   c. Touch \(\text{ close} \) on the instrument touchscreen to close the instrument tray.

12. Repeat the calibration to calibrate the instrument for the remaining dyes that are present in the experiments that you will be running.

13. (Optional) Click **Print Report** in the upper right corner of the screen to print a summary of the calibration results for your records.
Normalization calibration

During the normalization calibration, the instrument collects and stores the data from the normalization standards.

When to perform the calibration

Perform a normalization calibration once every 6 months.

About the calibration data

During the normalization calibration, the software captures a series of images of each normalization plate or array card using each instrument filter. The normalization calibration yields a “Pass” or “Fail” result for each normalization consumable used.

Preparing the calibration plate or array card

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare plates or array cards.

Prepare the calibration consumable appropriate for your QuantStudio™ 6 or 7 Flex System:

- Prepare the normalization calibration plates ................................................ 57
- Preparing array cards for instrument calibration ................................. 36

Prepare the normalization calibration plates

Materials required

- Normalization Plate with FAM™/ROX™ dyes
- Normalization Plate with VIC™/ROX™ dyes
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Prepare the plate

IMPORTANT! Before performing a normalization calibration, you must perform an ROI calibration, a background calibration, a uniformity calibration, and a dye calibration.

IMPORTANT! Wear powder-free gloves and safety glasses when you prepare the plate.

1. Remove the normalization calibration plates from the freezer, then allow them to warm to room temperature (approximately 30 minutes).

IMPORTANT! Use the calibration plates within 2 hours of defrosting them. Until you are ready to run the plates, store them in the dark and at ambient temperature (15–30°C). Do not remove the plates from their packaging until you are ready to run them.
2. Go to “Perform the normalization calibration” on page 58.
Before using each normalization plate, vortex the plate for 5 seconds, centrifuge it for 2 minutes at < 1500 rpm, then confirm that the liquid in each dye plate is at the bottom of the wells. If not, centrifuge the plate again at a higher rpm and for a longer period of time.

<table>
<thead>
<tr>
<th>Correct</th>
<th>Incorrect</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Correct Liquid at Bottom" /></td>
<td><img src="image2" alt="Incorrect Liquid at Bottom" /></td>
</tr>
</tbody>
</table>

- Not centrifuged with enough force, or
- Not centrifuged for enough time.

Perform the normalization calibration

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.
2. From the Instrument Console, select the icon for your instrument, then click **Manage Instrument**.
   **Note:** If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.
3. From the Instrument Manager, start the calibration:
   a. Click **Maintenance**, then click **Normalization**.
   b. From the Normalization Calibration screen, click **Start Calibration**.
4. From the Normalization Calibration screen, select the reporter/passive dye combination that you want to calibrate, then perform the calibration as instructed.
5. From the bottom of the Setup tab, enter the reagent information for the normalization plate or array card that you are using.
6. Load the calibration plate or array card into the instrument:
   a. From the instrument touchscreen, touch to eject the instrument tray.
   b. Confirm that the normalization plate or array card matches the selection in the software, then load it into the plate holder so that:
      - Well A1 of the plate or array card is in the top-left corner of the plate adapter.
      - The barcode faces the front of the instrument.

   Note: The dyes contained in each plate or array card appear next to the barcode on the front of it.

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

   c. From the instrument touchscreen, touch to close the instrument tray.

7. After loading the plate or array card, start the calibration:
   a. From the Normalization Calibration screen tab, select check the box when the normalization calibration plate has been loaded, then click Next.
   b. From the Run screen, click START RUN.

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

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

8. When the run is complete and the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays the Analysis screen, confirm the status of the calibration. The analysis status indicates the success of the calibration, where passed indicates that the run produced viable calibration data, and failed indicates that the run did not produce data or that the data it collected is unusable.

<table>
<thead>
<tr>
<th>Analysis status</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passed</td>
<td>Enter comments in the Comments field, click Next.</td>
</tr>
<tr>
<td>Failed</td>
<td>Troubleshoot the failed calibration as described in the &quot;Normalization calibration troubleshooting&quot; on page 74.</td>
</tr>
</tbody>
</table>
9. Select the QC tab to review the quality check summary. The QC status indicates the quality of the calibration data, where passed indicates that all wells produced data that passed the quality check, and failed indicates that one or more wells produced unusable data.

**Note:** If the calibration failed, the QC tab can provide information regarding the cause of the failure.

10. Unload the calibration plate or array card:
   
   a. After the calibration, touch on the touchscreen to eject the plate or array card.

   **Note:** If the instrument does not eject the plate or array card, remove it as explained in “Normalization calibration troubleshooting” on page 74.

   b. Remove the calibration plate or array card from the instrument tray and discard it.

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

   c. Touch on the instrument touchscreen to close the instrument tray.

11. From the Normalization Calibration screen, click Finish to complete the calibration, then click Yes when prompted to save the results.

12. Repeat the calibration procedure to perform the remaining normalization calibration.

13. (Optional) Click Print Report in the upper right corner of the screen to print a summary of the calibration results for your records.
Verify the instrument performance

When necessary, you can perform the RNase P instrument verification test to confirm the performance of the QuantStudio™ 6 and 7 Flex Systems.

When to perform the test

We recommend performing an RNase P instrument verification test:
- After moving the instrument to another location.
- As needed to confirm the function of the instrument.

About the RNase P kits

The RNase P instrument verification test uses instrument verification kits available from Life Technologies. The kits differ only in the consumable format for which they are designed: an RNase P Instrument Verification Plate for QuantStudio™ 6 and 7 Flex Systems with 96/384-well sample blocks and an Array Card RNase P Kit for the QuantStudio™ 7 Flex System with array card sample blocks.

RNase P Instrument Verification Plates

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: PCR master mix, RNase P primers, FAM™ dye-labeled probe, and a known concentration of human genomic DNA template.

The figure to the right illustrates the arrangement of the standard and unknown populations on a 96-well and Fast 96-well RNase P plate. The RNase P plate contains five replicate groups of standards (1250, 2500, 5000, 10000, and 20000 copies), two unknown populations (5,000 and 10,000 copies), and a no template control (NTC).

The figure to the right illustrates the arrangement of the standard and unknown populations on a 384-well RNase P plate. The RNase P plate contains five replicate groups of standards (1250, 2500, 5000, 10000, and 20000 copies), two unknown populations (5,000 and 10,000 copies), and a no template control (NTC).
Array Card RNase P Kits

The RNase P Kits include one empty array card and eight tubes of solution. Each tube contains reaction mix (PCR master mix, RNase P primers, and FAM™-MGB dye-labeled probe) and a known concentration of human genomic DNA template.

To perform an instrument verification run, each solution is loaded into the empty array card in the arrangement shown right. When complete, the array card contains five replicate groups of standards (200, 400, 800, 1600, and 3200 copies), two of unknown populations (800 and 1600 copies), and one that serves as a no template control (NTC).

Analytical performance

The software performs the same analysis of data from an instrument verification run regardless of the type of consumable used.

After the run, the software:

1. Generates a standard curve from the averaged threshold cycle (C₇) 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 QuantStudio™ 6 or 7 Flex System performance:

   \[|\text{CopyUnk}_2 - 3(\sigma_{\text{CopyUnk2}})| > |(\text{CopyUnk}_1) + 3(\sigma_{\text{CopyUnk1}})|\]

   where:
   - CopyUnk₁ = Average copy number of unknown population A
   - σ_{CopyUnk1} = Standard deviation of unknown population A
   - CopyUnk₂ = Average copy number of unknown population B
   - σ_{CopyUnk2} = Standard deviation of unknown population B

   **Note:** Unknown population A refers to the 5,000-copy population and unknown population B refers to the 10,000-copy population in the RNase P plate.
Installation specification

The QuantStudio™ 6 or 7 Flex System passes the installation specification if the inequality holds and the instrument successfully distinguishes between unknown populations A and B with a statistical confidence level of 99.7%.

As shown in the following table, you can omit a limited number of outlier wells from the unknown populations to meet the installation specification.

<table>
<thead>
<tr>
<th>Sample block</th>
<th>Maximum number of outlier wells that can be removed</th>
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<tbody>
<tr>
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<td>Unknown population A†</td>
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<td>96-well plate††</td>
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<td>384-well plate</td>
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<td>Array card‡‡</td>
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† 5,000-copy population for 384-well plates; 800-copy population for array cards.
†‡ 10,000-copy population for 384-well plates; 1,600-copy population for array cards.
§ Maximum number of wells that can be removed from each standard population.
†† Standard 96-well plates or Fast 96-well plates
‡‡ QuantStudio™ 7 Flex System only.

Prepare the RNase P plate

Materials required

- RNase P Instrument Verification Plate
- Centrifuge with plate adapter
- Powder-free gloves
- Safety goggles

Prepare the plate

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

1. Obtain the RNase P Instrument Verification Plate from the freezer, then allow the plate to warm to room temperature (for approximately 5 minutes).

**IMPORTANT!** Use the RNase P plate within 30 minutes of defrosting it. Until you are ready to run the plate, store it in the dark and at ambient temperature (15–30°C). Do not remove the plate from its packaging until you are ready to run it.

2. Remove the RNase P plate from its packaging.

3. Briefly vortex and centrifuge the RNase P plate:
   a. Vortex the plate for 5 seconds.
b. Centrifuge the reaction plate for 2 minutes at < 1500 rpm.

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

c. Confirm 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 period of time.

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

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**IMPORTANT!** Perform the following procedure only if you are verifying the performance of a QuantStudio™ 7 Flex System with an array card sample block.

**Required materials**
- Array Card Staker/Sealer
- Centrifuge with centrifuge array card carrier clips and buckets
- Powder-free gloves
- Safety goggles
- Pipettor, 200-µL (with pipette tips)
- RNase P Array Card Instrument Verification Reagents Kit:
  - Array Card
  - RNase P Array Card Instrument Verification Reagents Kit, including tubes with reagent mix for each port (8 tubes total)

**Preparing the RNase P Array Card**

**IMPORTANT!** Wear powder-free gloves while preparing the array card.

1. Remove the Array Card RNase P Kit from the freezer, then allow it to warm to room temperature (for approximately 15 minutes).

**IMPORTANT!** You must fill and run the RNase P array card within 60 minutes of thawing the Array Card RNase P Kit.

2. Remove an array card from its box and place it on a clean, dry surface.
3. Using a permanent marker, mark the side of the empty array card with RNase P.

4. Transfer 100 µL of each solution into the appropriate port of the array card:

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For each transfer:

a. Place the array card on a lab bench, with the foil side down.

b. Load 100 µL of fluid into a pipette.

c. Hold the pipette in an angled position (~45 degrees) and place the tip into the fill port. The fill port is the larger of the two holes on the left side of the fill reservoir.

d. Dispense the fluid so that it sweeps in and around the fill reservoir toward the vent port. Pipet the entire 100 µL into the reservoir, but do not go past the first stop of pipettor plunger or you may blow the solution out of the port.

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

5. Centrifuge and seal the array card as explained in steps a through 8 on page 37.
Run the RNase P plate or array card

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click Instrument Console.

2. From the Instrument Console, select your instrument icon, then click Manage Instrument.
   
   **Note:** If the Manage Instrument button is inactive, add your QuantStudio™ 6 and 7 Flex Instrument to the My Instruments group.

3. From the Instrument Manager, start the RNase P run:
   a. Click Maintenance, then click RNase P Run.
   b. From the RNase P Run screen, click Start RNase P Run.

4. Click Next, then prepare for the instrument verification test as instructed.

5. From the bottom of the Setup tab, enter the reagent information for the RNase P plate or array card that you are using.

6. Load the RNase P plate or array card into the instrument:
   a. From the instrument touchscreen, touch  to eject the instrument tray.
   b. Load the plate or array card into the plate holder so that:
      - Well A1 of the plate or array card is in the top-left corner of the adapter.
      - The barcode faces the front of the instrument.

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

   c. From the instrument touchscreen, touch  to close the instrument tray.

6. Run the prepared array card as soon as possible after filling it.

**IMPORTANT!** Store the array card in a dark place until you are ready to run it. The fluorescent dyes in the array card are photosensitive. Prolonged exposure to light can diminish the fluorescence of the dye.
Chapter 2  Calibrate the Instrument

Verify the instrument performance

Chapter 2  Calibrate the Instrument

Verify the instrument performance

7. After loading the plate or array card, start the instrument verification test:
   a. From the Overview screen, select **Check the box when the RNase P plate has been loaded**, then click **Next**.
   b. From the Run screen, click **START RUN** to start the test.

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

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

8. When the run is complete and the software displays the Analysis screen, confirm the status of the run.

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<tr>
<th>Analysis status</th>
<th>Action</th>
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<tr>
<td>Passed</td>
<td>The QuantStudio™ 6 or 7 Flex System passed the RNase P run.</td>
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<tr>
<td>Failed</td>
<td>The QuantStudio™ 6 or 7 Flex System failed the RNase P run. Review the data for outliers. If the run fails, the software may have included outliers that caused the initial analysis to fail. Error may cause some wells to be amplified insufficiently or not at all. These wells typically produce C&lt;sub&gt;T&lt;/sub&gt; 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.</td>
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9. From the Amplification Plot, select **Ct vs. Well** from the Plot Type menu.

10. Confirm the uniformity of each replicate population (controls, standards, and unknowns) on the plate or array card by comparing the groupings of C<sub>T</sub> values:
    a. From the Plate Layout, select the wells containing Unknown Population A:
    b. From the plot, confirm that the C<sub>T</sub> values of the replicate population are equivalent.

    **Note:** The numbers on the X-axis of the plot correspond to the wells of the plate or array card. Beginning with well A1, the wells are numbered from left-to-right, and top-to-bottom.
c. If an outlier is present in the selected population, select the corresponding well of the plate layout, then click **Omit** to remove the well from the analysis. If the total number of outliers for the replicate population exceeds the limit in the table below, repeat the test using another RNase P plate or array card.

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<thead>
<tr>
<th>Sample block</th>
<th>Maximum number of outlier wells that can be removed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unknown population A†</td>
</tr>
<tr>
<td>96-well plate‡‡</td>
<td>6</td>
</tr>
<tr>
<td>384-well plate</td>
<td>10</td>
</tr>
<tr>
<td>Array card‡‡</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Unknown population B‡</td>
</tr>
<tr>
<td>96-well plate‡‡</td>
<td>6</td>
</tr>
<tr>
<td>384-well plate</td>
<td>10</td>
</tr>
<tr>
<td>Array card‡‡</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Standards [STD]§</td>
</tr>
<tr>
<td>96-well plate‡‡</td>
<td>1</td>
</tr>
<tr>
<td>384-well plate</td>
<td>2</td>
</tr>
<tr>
<td>Array card‡‡</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>No template controls (NTC)</td>
</tr>
<tr>
<td>96-well plate‡‡</td>
<td>0</td>
</tr>
<tr>
<td>384-well plate</td>
<td>0</td>
</tr>
<tr>
<td>Array card‡‡</td>
<td>0</td>
</tr>
</tbody>
</table>

† 5,000-copy population for 384-well plates; 800-copy population for array cards.
‡ 10,000-copy population for 384-well plates; 1,600-copy population for array cards.
§ Maximum number of wells that can be removed from each standard population.
‡‡ Standard 96-well plates or Fast 96-well plates.
‡‡ QuantStudio™ 7 Flex System only.

11. Repeat step 10 for each replicate population (unknowns, standards, and no template controls) on the plate or array card.

12. Review the Results Table for quality flags generated by the test:
   a. Select the **Results Table** tab.
   b. Review the Flag column for wells that generated quality flags.
   c. Troubleshoot each well that generated a flag as explained in “RNase P test troubleshooting” on page 75.
      - AMPNC – Amplification in negative control
      - BADROX – Bad passive reference signal
      - BLFAIL – Baseline algorithm failed
      - CTFAIL – CT algorithm failed
      - EXPFAIL – Exponential algorithm failed
      - HIGHSD – High standard deviation in replicate group
      - NOAMP – No amplification
      - NOISE – Noise higher than others on the consumable
      - NOSIGNAL – No signal in well
      - OFFSCALE – Fluorescence is offscale
      - OUTLIERRG – Outlier in replicate group
      - SPIKE – Noise spikes
      - THOLDFAIL – Thresholding algorithm failed

13. If you omitted outliers, click **Reanalyze** to analyze the run.
    If the status of the RNase P Run is “Failed” after performing step 8–12, repeat the RNase P test using a different RNase P plate or array card. If the problem persists, contact Life Technologies.
14. 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. Confirm that the R² value is ≥ 0.990.

If the R² value is < 0.990, repeat the RNase P test using a different RNase P plate or array card. If the problem persists, contact Life Technologies.

15. In the Analysis screen, click **Next**, remove the plate or array card when the instrument ejects the tray arm, then discard the consumable.

**WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the plate or array card can reach 100°C. Before removing the consumable, be sure to wait until it reaches room temperature.

**Note:** If the instrument does not eject the plate or array card, remove it as explained in “RNase P test troubleshooting” on page 75.

16. Click **Finish**, then click **Yes** when prompted to save the test.

17. (Optional) Click **Print Report** in the upper right corner of the screen to print a summary of the RNase P test results for your records.

**Troubleshooting**

The tables in this section describe how to troubleshoot failed calibrations and RNase P instrument verification tests:

- ROI calibration troubleshooting .......................................................... 70
- Background calibration troubleshooting ............................................... 71
- Uniformity calibration troubleshooting .................................................. 72
- Dye calibration troubleshooting ............................................................ 73
- Normalization calibration troubleshooting ............................................. 74
- RNase P test troubleshooting ............................................................... 75
Table 1  ROI calibration troubleshooting

<table>
<thead>
<tr>
<th>Problem/symptom</th>
<th>Possible cause</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROI calibration failed.</td>
<td>• Plate or array card was centrifuged insufficiently.</td>
<td><strong>CAUTION! PHYSICAL INJURY HAZARD.</strong> During operation, the sample block can reach 100°C. Before removing the plate or array card, wait until the block reaches room temperature.</td>
</tr>
<tr>
<td>• ROI image is blurry</td>
<td>• Plate or array card contains old or insufficient reagents.</td>
<td>1. Unload the instrument and confirm that you loaded the correct plate or array card. If not, perform the calibration again using the correct type of consumable.</td>
</tr>
<tr>
<td>• ROI image is faint</td>
<td>• The sample block or heated cover may not be seated correctly.</td>
<td>2. View the wells of the plate or array card. If the liquid in the wells is not:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• At the bottom of the wells, centrifuge the plate or array card for a longer time, then repeat the calibration.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Equivalent in volume, the plate or array card is not sealed properly and the reagents have evaporated. Discard the consumable and run another.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. If the calibration plate or array card appears to be normal, discard it and run another.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. If the calibration fails again, check the sample block and heated cover positions:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a. Power off and unplug the instrument. Wait 15 minutes, then open the access door.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b. Firmly push the sample block and the heated cover toward the back of the instrument to confirm that they are seated correctly.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>IMPORTANT!</strong> Align the arrows on the front handle of the heated cover as shown. If the arrows are not aligned, push the heated cover into the instrument until the handle locks into place.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>IMPORTANT!</strong> Confirm that the indicator on the left side of the sample block is positioned behind the red line on the instrument rail. If not, push the sample block into the instrument until it is seated correctly.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c. Repeat the calibration.</td>
</tr>
<tr>
<td>Instrument does not eject the ROI pl ate</td>
<td>The adhesive cover may have adhered the plate or array card to the heated</td>
<td>Remove the plate or array card from the instrument as explained in “Remove a plate stuck within the instrument” on page 78.</td>
</tr>
<tr>
<td>or array card (it is stuck within the</td>
<td>cover.</td>
<td></td>
</tr>
<tr>
<td>instrument).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Instrument malfunction.</td>
<td>Multiple possible causes.</td>
<td>Contact your local Life Technologies Field Service Office.</td>
</tr>
</tbody>
</table>
### Table 2 Background calibration troubleshooting

<table>
<thead>
<tr>
<th>Problem/symptom</th>
<th>Possible cause</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background calibration failed.</td>
<td>• Plate or array card was centrifuged insufficiently.</td>
<td><strong>CAUTION! PHYSICAL INJURY HAZARD.</strong> During operation, the sample block can reach 100°C. Before removing the plate or array card, wait until the block reaches room temperature.</td>
</tr>
<tr>
<td></td>
<td>• Plate or array card contains old or insufficient reagents.</td>
<td>1. Unload the QuantStudio™ 6 or 7 Flex System and confirm that you loaded the correct plate or array card into the instrument. If not, perform the calibration again using the correct type of consumable.</td>
</tr>
<tr>
<td></td>
<td>• One or more wells of the background plate or array card produced spectra that exceed the maximum limit for the instrument.</td>
<td>2. View the wells of the plate or array card. If the liquid in the wells of the consumable is not:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• At the bottom of the wells, centrifuge the plate or array card for a longer time, then repeat the calibration.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Equivalent in volume, the plate or array card is not sealed and the reagents have evaporated. Discard the consumable and run another.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. If the calibration plate or array card appears to be normal, discard it and run another.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. If the calibration fails again, determine the source of the contamination, as explained in “How to identify contamination” on page 78.</td>
</tr>
<tr>
<td>Instrument does not eject the background plate or array card (it is stuck within the instrument).</td>
<td>The adhesive cover may have adhered the plate or array card to the heated cover within the instrument.</td>
<td>Remove the plate or array card from the instrument as explained in “Remove a plate stuck within the instrument” on page 78.</td>
</tr>
<tr>
<td>Instrument malfunction.</td>
<td>Multiple possible causes.</td>
<td>Contact your local Life Technologies Field Service Office.</td>
</tr>
</tbody>
</table>
### Table 3 Uniformity calibration troubleshooting

<table>
<thead>
<tr>
<th>Problem/symptom</th>
<th>Possible cause</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniformity calibration failed.</td>
<td>• Plate or array card was centrifuged insufficiently.</td>
<td><strong>CAUTION! PHYSICAL INJURY HAZARD.</strong> During operation, the sample block can reach 100°C. Before removing the plate or array card, wait until the block reaches room temperature.</td>
</tr>
<tr>
<td></td>
<td>• Plate or array card contains old or insufficient reagents.</td>
<td>1. Unload the QuantStudio™ 6 or 7 Flex System and confirm that you loaded the correct plate or array card into the instrument. If not, perform the calibration again using the correct type of consumable.</td>
</tr>
<tr>
<td></td>
<td>• Abnormally low spectra across all wells of the plate or array card.</td>
<td>2. Unload the QuantStudio™ 6 or 7 Flex System and view the wells of the plate or array card. If the liquid in the wells of the consumable is not:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• At the bottom of the wells, centrifuge the plate or array card for a longer time, then repeat the calibration.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Equivalent in volume, the plate or array card is not sealed and the reagents have evaporated. Discard the consumable and run another.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. If the calibration plate or array card appears to be normal, discard it and run another.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. If the calibration fails again, contact Life Technologies.</td>
</tr>
<tr>
<td>One or more wells produced spectra that deviate significantly from the rest of the plate or array card.</td>
<td>1. While viewing the calibration data in the Analysis screen, locate the well(s) with abnormal signal in the Plate Layout tab.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. Rotate the plate or array card 180°, then perform the calibration again.</td>
<td>3. Determine the location of the contaminated wells again. If the position(s) of the well(s) identified in the steps above are:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Identical – The sample block is contaminated. Decontaminate the sample block.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Reversed – The ROI plate or array card is contaminated. Discard the consumable, then perform the uniformity calibration using a new ROI plate or array card.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. If the calibration fails again, contact Life Technologies.</td>
</tr>
<tr>
<td>Instrument does not eject the ROI plate or array card (the consumable is stuck within the instrument).</td>
<td>The adhesive cover may have adhered the plate or array card to the heated cover within the instrument.</td>
<td>Remove the plate or array card from the instrument as explained in “Remove a plate stuck within the instrument” on page 78.</td>
</tr>
<tr>
<td>Instrument malfunction.</td>
<td>Multiple possible causes.</td>
<td>Contact your local Life Technologies Field Service Office.</td>
</tr>
</tbody>
</table>
## Table 4 Dye calibration troubleshooting

<table>
<thead>
<tr>
<th>Problem/symptom</th>
<th>Possible cause</th>
<th>Action</th>
</tr>
</thead>
</table>
| Dye calibration failed.                              | • Dye plate or array card was centrifuged insufficiently.  
• Dye plate or array card contains old or insufficient reagents. | CAUTION! PHYSICAL INJURY HAZARD. During operation, the sample block can reach 100°C. Before removing the plate or array card, wait until the block reaches room temperature.  
1. Unload the QuantStudio™ 6 or 7 Flex System and view the wells of the plate or array card. If the liquid in the wells of the consumable is not:  
• At the bottom of the wells, centrifuge the plate or array card for a longer time, then repeat the calibration.  
• Equivalent in volume, the plate or array card is not sealed and the reagents have evaporated. Discard the consumable and run another.  
2. If the dye calibration plate or array card appears to be normal, discard it and run another.  
3. If the problem persists, contact Life Technologies. |
|                                                      |                                                     |                                                                 |
| Spectra contain peaks in more than one filters.     | Fluorescent contaminants are present on the sample block or dye plate or array card. | Confirm that contaminants are not present by performing a background calibration. If the background calibration does not show sample block contamination, the dye plate or array card may be contaminated. |
| One or more raw spectra exceed the maximum limit for the instrument. |                                                     |                                                                 |
| Instrument does not eject the dye plate or array card (the consumable is stuck within the instrument). | The adhesive cover may have adhered the plate or array card to the heated cover within the instrument. | Remove the plate or array card from the instrument as explained in “Remove a plate stuck within the instrument” on page 78. |
| Instrument malfunction.                              | Multiple possible causes.                           | Contact your local Life Technologies Field Service Office.            |
### Table 5 Normalization calibration troubleshooting

<table>
<thead>
<tr>
<th>Problem/symptom</th>
<th>Possible cause</th>
<th>Action</th>
</tr>
</thead>
</table>
| Normalization calibration failed.                                              | Abnormally low spectra across all wells of the plate or array card.            | **CAUTION! PHYSICAL INJURY HAZARD.** During operation, the sample block can reach 100°C. Before removing the plate or array card, wait until the block reaches room temperature.  
1. Unload the QuantStudio™ 6 or 7 Flex System and confirm that you loaded the correct plate or array card into the instrument. If not, perform the calibration again using the correct consumable.  
2. If you are using the correct plate or array card, perform the calibration again using a different normalization consumable.  
3. If the calibration fails again, contact Life Technologies Field Service Office. |
| One or more wells produced spectra that deviate significantly from the rest of the plate or array card. |                                                                                  | 1. While viewing the calibration data in the Analysis screen, locate the well(s) with abnormal signal in the Plate Layout tab.  
2. Rotate the plate or array card 180°, then perform the calibration again.  
3. Determine the location of the contaminated wells again. If the position(s) of the well(s) identified in the steps above are:  
  • **Identical:** The sample block is contaminated. Decontaminate the sample block.  
  • **Reversed:** The normalization plate or array card is contaminated. Discard the consumable, then perform the normalization calibration using a new normalization plate or array card.  
4. If the calibration fails again, contact your local Life Technologies Field Service Office. |
| Instrument does not eject the normalization plate or array card (it is stuck within the instrument). | The adhesive cover may have adhered the plate or array card to the heated cover within the instrument. | Remove the plate or array card from the instrument as explained in “Remove a plate stuck within the instrument” on page 78. |
| Instrument malfunction.                                                        | Multiple possible causes.                                                        | Contact your local Life Technologies Field Service Office.                                                                      |
## Table 6  RNase P test troubleshooting

<table>
<thead>
<tr>
<th>Problem/symptom</th>
<th>Possible cause</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNase P plate or array card verification run failed.</td>
<td>• Contamination&lt;br&gt;• Defective seal&lt;br&gt;• Inaccurate fluid transfer during plate or array card manufacturing&lt;br&gt;• Insufficient centrifugation</td>
<td>CAUTION! PHYSICAL INJURY HAZARD. During operation, the sample block can reach 100°C. Before removing the plate or array card, wait until the block reaches room temperature.&lt;br&gt;1. Unload the RNase P plate or array card from the instrument.&lt;br&gt;2. Hold the plate or array card up to a light source, and confirm that all wells contain the same volume of fluid.&lt;br&gt;   - If there are differences in fluid volumes, check the heat seal of the wells with lower volumes for signs of damage or evaporation.&lt;br&gt;   - Compare the position of the wells that have lower volumes with the outliers that you have removed from the plate or array card. If the well positions coincide, the heat seal on the consumable may be defective, resulting in the evaporation of the associated samples.&lt;br&gt;3. Contact Life Technologies to order a replacement RNase P plate or array card. If the replacement consumable fails, contact Life Technologies for assistance.</td>
</tr>
<tr>
<td>Instrument does not eject the RNase P plate or array card (the consumable is stuck within the instrument).</td>
<td>Adhesive cover may have adhered the plate or array card to the heated cover within the instrument.</td>
<td>Remove the plate or array card from the instrument as explained in &quot;Remove a plate stuck within the instrument&quot; on page 78.</td>
</tr>
<tr>
<td>Negative control well displays the AMPNC flag, indicating that the well amplified.</td>
<td>Contamination in one or more PCR reaction components contained in the negative control well.</td>
<td>Contact Life Technologies to order a replacement RNase P plate or array card kit. If the replacement RNase P consumable fails, contact Life Technologies for assistance.</td>
</tr>
<tr>
<td>Well displays the BADROX flag, indicating that the passive reference signal is unacceptable for the normalization of the reporter dye signal.</td>
<td>• Droplets on the sides of the wells&lt;br&gt;• Improper sealing or seal leaks&lt;br&gt;• Condensation on the plate or array card</td>
<td>If a well is flagged, confirm the results:&lt;br&gt;1. Select the flagged well(s) in the plate layout or well table.&lt;br&gt;2. View the amplification plot ($R_n$ vs. Cycle), and review the data in the $C_t$ region for abnormalities.&lt;br&gt;3. Examine the plate or array card to check for condensation and/or inconsistent reaction volumes.&lt;br&gt;4. Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test and make sure to properly centrifuge the RNase P consumable. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance.</td>
</tr>
<tr>
<td>Problem/symptom</td>
<td>Possible cause</td>
<td>Action</td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
<td>-----------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| Well displays the BLFAIL flag, indicating that the software cannot calculate the best fit baseline for the data. | • Amplification too late  
• No amplification | If a well is flagged, confirm the results:  
1. Select the flagged well(s) in the plate layout or well table.  
2. View the amplification plot ($R_n$ vs. Cycle and $D_n$ vs. Cycle) and check for early, late, low, or no amplification.  
3. Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test, making sure to properly seal and centrifuge the RNase P plate or array card. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance. |
| Well displays the CTFAIL flag, indicating that the software cannot calculate the threshold cycle ($C_t$).  
• Well displays the EXPFAIL flag, indicating that the software cannot identify the exponential region of the amplification plot. | • Amplification too early  
• Amplification too late  
• Low amplification  
• No amplification | If a well is flagged, confirm the results:  
1. Select the flagged well(s) in the plate layout or well table.  
2. View the amplification plot ($R_n$ vs. Cycle), and review the data for abnormalities.  
3. Hold the plate or array card up to a light source, and check for condensation or evaporation.  
If there are differences in fluid volumes, check the heat seal of the wells with lower volumes for signs of damage or evaporation.  
4. Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test, and make sure to properly centrifuge the RNase P consumable. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance. |
| Well displays the HIGHSD flag, indicating that the $C_t$ standard deviation for the replicate group exceeds the current flag setting. | • Droplets on the sides of the wells  
• Improper sealing or seal leaks  
• Condensation on the plate or array card  
• Inconsistent volumes across the plate or array card | If a well is flagged, confirm the results:  
1. Select the flagged well[s] and the associated replication group[s] in the plate layout or well table.  
2. View the amplification plot ($R_n$ vs. Cycle) and the $C_t$ standard deviation of the replicate group(s) and check for abnormal or inconsistent values.  
3. Hold the plate or array card up to a light source, and check for condensation or evaporation.  
If there are differences in fluid volumes, check the heat seal of the wells with lower volumes for signs of damage or evaporation.  
4. Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test, and make sure to properly centrifuge the RNase P consumable. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance. |
| Well displays the NOAMP flag, indicating that the sample did not amplify. | • Missing template  
• Excitation source in the instrument stopped functioning | If a well is flagged, confirm the results:  
1. Select the flagged well(s) in the plate layout or well table.  
2. View the amplification plot ($R_n$ vs. Cycle), and check for low or no amplification.  
3. Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test, making sure to properly seal and centrifuge the RNase P plate or array card. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance. |
| Well displays the NOISE flag, indicating that the well produced more noise in the amplification plot than other wells on the plate or array card. | • Droplets on the sides of the wells  
• Improper sealing or seal leaks  
• Condensation on the plate or array card | If a well is flagged, confirm the results:  
1. Select the flagged well(s) in the plate layout or well table.  
2. View the amplification plot ($R_n$ vs. Cycle), and check for high noise levels.  
3. Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test, making sure to properly seal and centrifuge the RNase P plate or array card. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance. |
<table>
<thead>
<tr>
<th>Problem/symptom</th>
<th>Possible cause</th>
<th>Action</th>
</tr>
</thead>
</table>
| Well displays the NOSIGNAL flag, indicating that the well produced very low or no fluorescence signal. | Missing reaction mix resulting from pipetting error. | If a well is flagged, confirm the results:  
1. Consider omitting the well from the analysis.  
2. Note the location for each flagged well, and check each corresponding well in the plate or array card for evaporation or low reaction volume.  
3. Contact Life Technologies to order a replacement RNase P plate or array card. If the replacement RNase P consumable fails, contact Life Technologies for assistance. |
| Well displays the OFFSCALE flag, indicating that the fluorescence signal for one or more dyes in the well exceeds the instrument’s maximum detectable range for one or more cycles. | • Fluorescent contaminant on the plate or array card or sample block  
• Fluorescent contaminant in the reaction | 1. Perform a background calibration. If you detect fluorescent contamination, decontaminate the sample block.  
2. Contact Life Technologies to order a replacement RNase P plate or array card. If the replacement consumable fails, contact Life Technologies for assistance. |
| Well displays the OUTLIERRG flag, indicating that the \( C_T \) of the well deviates significantly from \( C_T \) values in the associated replicate group (only the outlier is flagged). | • Contamination  
• Improper sealing or seal leaks | 1. Decontaminate the work area and pipettors.  
2. Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test and make sure that the RNase P consumable is properly sealed. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance. |
| Well displays the SPIKE flag, indicating that the amplification curve contains one or more data points inconsistent with the other points in the curve. | • Bubbles in the reaction  
• Evaporation during the denaturation step because of improper sealing or seal leaks | Contact Life Technologies to order a replacement RNase P plate or array card. Repeat the test, and make sure to properly centrifuge the RNase P consumable. If the replacement RNase P plate or array card fails, contact Life Technologies for assistance. |
| Well displays the THOLDFAIL flag, indicating that the software cannot calculate the threshold. | • Amplification too early  
• Amplification too late  
• Low amplification  
• No amplification | If a well is flagged, confirm the results:  
1. Select the flagged well(s) in the plate layout or well table.  
2. View the amplification plot (\( R_n \) vs. Cycle and \( DR_n \) vs. Cycle), and check for early, late, low, or no amplification.  
3. Contact Life Technologies to order a replacement RNase P plate or array card. If the replacement RNase P consumable fails, contact Life Technologies for assistance. |
| Instrument malfunction.               | Multiple possible causes                                                      | Contact your local Life Technologies Field Service Office. |
How to identify contamination

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

To identify and resolve a possible contamination problem:

1. While viewing the background calibration data in the Analysis screen, select the QC tab to review the list of wells that failed the quality check.
2. Eject the background plate or array card from the instrument, rotate the plate 180°, then perform the background calibration again.
3. Determine the location of the contaminated wells again as in step 1.
   - If the position(s) of the contaminated well(s) in the steps above are:
     - **Identical** – The sample block is contaminated. Decontaminate the sample block as explained in “Decontaminate the sample block” on page 96.
     - **Reversed** – The background plate or array card is contaminated. Discard it, then perform the background calibration using a new background consumable.
4. If the calibration fails after you replace the background plate or array card and decontaminate the sample block, contact Life Technologies for further support.

Remove a plate stuck within the instrument

If the QuantStudio™ 6 or 7 Flex System does not eject a plate after you touch the touchscreen, the adhesive cover of the plate may have adhered it to the heated cover within the instrument. If so, you can remove the plate from the instrument as follows.

1. Power off the instrument. Wait 15 minutes, then power on the instrument and eject the plate.
2. If the plate does not eject, power off and unplug the instrument, then open the access door.
3. While wearing powder-free gloves, reach into the instrument and remove the plate from the heated cover, then close the access door.
4. Perform a background calibration to confirm that the sample block has not been contaminated.
Manual Instrument Operation

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- Operate the instrument from the touchscreen ............................ 81
- Maintain the instrument from the touchscreen ......................... 85
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Note: This appendix describes how to operate the QuantStudio™ 6 and 7 Flex Systems manually using the touchscreen interface. Although the QuantStudio™ 6 and 7 Flex Systems can be used without an Ethernet connection to a computer, the touchscreen allows you to perform only a subset of the total instrument functions.
Overview of instrument touchscreen functions

The QuantStudio™ 6 and 7 Flex Systems feature a touchscreen interface that you can use to transfer data, manage instrument settings, and configure the instrument for network use. The touchscreen does not provide access to all instrument functions. Features such as analyzing experiments, instrument calibration, and remote notification are available only through the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

The following table summarizes the functions that are available from the QuantStudio™ 6 or 7 Flex System touchscreen.

<table>
<thead>
<tr>
<th>Category</th>
<th>Function</th>
<th>Available in secure mode?†</th>
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<tr>
<td>Operational‡</td>
<td>Create experiments from templates</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Run experiments</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transfer and delete experiments, templates, and results to/from a USB drive</td>
<td></td>
</tr>
<tr>
<td>Maintenance§</td>
<td>Back up and restore the instrument settings</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Perform an instrument self test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Install and record a new lamp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Update the instrument firmware</td>
<td></td>
</tr>
<tr>
<td>Administration††</td>
<td>Define the date and time</td>
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<td>Define the instrument settings</td>
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</tbody>
</table>

† See "Enable/disable instrument security" on page 92.
‡ Tasks for users responsible for day-to-day use of the instrument.
§ Tasks for users who maintain the instrument.
†† Tasks for systems administrators or for information technology personnel.
Operate the instrument from the touchscreen

The touchscreen provides limited control of the QuantStudio™ 6 and 7 Flex Systems to run experiments and transfer data. You can perform the following functions from the touchscreen to operate the QuantStudio™ 6 and 7 Flex Systems without using the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software:

- Create an experiment from a template .................................................. 81
- Run an experiment ................................................................. 82
- Transfer experiments, templates, and results data ................................. 83

Note: If the QuantStudio™ 6 or 7 Flex System is operating in secure mode (see “Enable/disable instrument security” on page 92), users can only open and close the side door.

Create an experiment from a template

1. If necessary, download the experiment template to the QuantStudio™ 6 or 7 Flex System as described in “Transfer experiments from a USB drive” on page 83.
2. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press  .
3. In the Main Menu, touch View Templates .
4. In the View Templates screen, touch a template, then touch + New:
   To view the parameters of a template, select the desired template, then touch View. When finished, touch  to return to the View Templates screen.
   Note: You cannot modify the experiment parameters of a template.
5. In the Create New Experiment screen, touch each field to set the:
   • Touch the New Experiment Name field and use the keypad to enter a name (up to 100 characters) for the experiment.
   • Touch the Save to Folder field to open the Select Folder Screen.
   • Touch the Reaction Volume field to enter a reaction volume in µL.
   • (Optional) Touch the Barcode Number field to enter a barcode and touch the Notes field to enter notes (up to 200 characters) about the experiment.
6. When finished, either:
   • Touch Save & Exit.
   or
   • Touch Save & Start Run to proceed to the Start Run screen.
Run an experiment

1. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press .

2. In the Main Menu screen, then touch .

3. When the side door opens, load the appropriate plate or array card. Ensure that the consumable is properly aligned in the holder.
   - (A) Load 96/384-well plates with the A1 position at the top-left corner of the plate adapter.
   - (B) Load both plates and array cards with the barcode facing the front of the instrument.

4. In the Main Menu, touch Browse Experiments.

5. In the Experiments screen, touch the desired experiment, then touch either:
   - ✨ Start Run to start the run immediately, then go to step 10.
   - or
   - 🔍 View/Edit to view or edit the experiment before starting the run.

6. Modify the experiment parameters as needed. To:
   - Add a stage or step to the thermal profile, touch the stage or step to the left of where you want to add the stage or step, then touch Add.
   - Add a melt curve to the end of the thermal profile, touch Add Melt Curve.
   - Change the time or temperature of a stage or step, touch the time/temperature field of the stage or step, modify the settings as desired, then touch Close.
   - Change the cycle parameter of a stage, touch the cycle field, modify the setting as desired, then touch Close.
   - Delete a stage or step from the thermal profile, touch the stage or step you want to remove, then touch Delete.

7. When finished modifying the parameters, touch Save.

8. In the Save Experiment screen, touch each field to set the experiment, name, reaction volume, barcode, and any additional information to save to the experiment.

9. When finished, touch ✨ Save & Start Run to start the experiment.

10. In the Start Run screen, touch each field as needed to modify the associated parameter, then touch ✨ Start Run Now to start the experiment.

Note: When the run is complete, touch to unload the plate. You can download the experiment results to a computer if the QuantStudio™ 6 or 7 Flex System is connected to a network, or you can copy the data to a USB device (see “Transfer experiments, templates, and results data” on page 83).
Transfer experiments, templates, and results data

You can transfer experiments, templates, and results data to/from the QuantStudio™ 6 and 7 Flex Systems using a USB flash drive. Before transferring data, you must plug the drive into the USB port below the touchscreen.

IMPORTANT! Do not use the USB ports on the rear panel of the QuantStudio™ 6 and 7 Flex Systems. The rear USB ports are only for use by Life Technologies personnel to service the instrument.

Transfer templates from a USB drive

1. Plug a USB drive into the USB port below the touchscreen.

2. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press .

3. In the Main Menu, touch View Templates.

4. In the Browse Experiments screen, select the template:
   a. Touch , then touch USB.
   b. Touch the desired template, then touch Save.

5. In the Save Experiment As screen, set the name for the file.
   a. Touch the New Template Name field, then enter a name for the copied file.
   b. Touch the Save to Folder field, then select the folder to receive the file.
   c. Touch Save.

6. Touch to return to the Main Menu.

7. Unplug the USB drive.

Transfer experiments from a USB drive

1. Plug a USB drive into the USB port below the touchscreen.

2. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press .

3. In the Main Menu, touch Browse Experiments.

4. In the Browse Experiments screen, select the experiment:
   a. Touch , then touch USB.
   b. Touch the desired experiment, then touch Save.

5. In the Save Experiment As screen, touch the experiment that you want to transfer to the USB drive, then touch Save.

6. Touch to return to the Main Menu.

7. Unplug the USB drive.
Copy experiment results to a USB drive

1. Plug a USB drive into the USB port below the touchscreen.
2. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press .
3. From the Main Menu of the instrument touchscreen, touch Collect Results.
4. From the list of experiments, touch the experiment(s) of interest or touch Select All.
5. Touch Save to USB.
6. From the Copy Results To USB screen, check that the name of the USB drive is correct to ensure that it is mounted, then touch Save to USB.
7. Touch to return to the Main Menu.
8. Unplug the USB drive.

Note: After the results from a completed run have been collected, the corresponding experiment displays “Collected” and it can be deleted.

Delete results

Once you have collected results from the QuantStudio™ 6 or 7 Flex System, you can use the touchscreen to delete the files from the instrument hard drive.

1. From the Main Menu of the instrument touchscreen, touch Collect Results.
2. From the list of experiments, touch the experiment(s) of interest, then touch Delete.
3. When asked to confirm the deletion, touch OK.
4. Touch to return to the Main Menu.
Maintain the instrument from the touchscreen

The QuantStudio™ 6 and 7 Flex Systems touchscreen provides access to a few maintenance functions that cannot be accessed remotely from the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software. Unlike the maintenance performed using the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, some of the touchscreen functions are not part of the recommended maintenance schedule. The frequency that you perform them depends on your laboratory policies.

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**Note:** The touchscreen does not provide access to all instrument functions. Features such as instrument calibration and remote notification are available only through the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

Back up and restore the instrument settings

You can use the QuantStudio™ 6 or 7 Flex System touchscreen to back up the instrument settings (icon, standby time-out, and cover idle temperature), and some network settings (the Autodiscovery and Smart Monitoring options). If the QuantStudio™ 6 or 7 Flex Instrument settings are reset, you can restore the settings using the backup.

**Note:** The backup feature can be used as an administrative tool to manage QuantStudio™ 6 and 7 Flex Systems. You can use the feature to create a standard “image” for a QuantStudio™ 6 or 7 Flex Instrument that can then be restored on other instruments to bypass the manual setup process.

The QuantStudio™ 6 and 7 Flex Systems back up and restores instrument settings from a USB flash drive. Before backing up or restoring settings, you must plug the drive into one of the USB ports below the touchscreen.

**IMPORTANT!** Do not use the USB ports on the rear panel of the QuantStudio™ 6 and 7 Flex Systems. The rear USB ports are for use by Life Technologies personnel to service the instrument.

**Back up the instrument settings**

1. Plug a USB drive into the USB port below the touchscreen.
2. From the Main Menu of the instrument touchscreen, touch **Tools**, then touch **Backup Settings**.
3. From the Backup Settings screen, touch **Backup**.
4. Wait for the backup to complete, then touch **** to return to the Main Menu.
5. Unplug the USB drive.
Appendix A Manual Instrument Operation

Maintain the instrument from the touchscreen

Note: For administrative purposes, you can reuse the instrument settings saved to the USB drive to configure more than one QuantStudio™ 6 or 7 Flex System. Note that you must configure the network settings for each instrument individually.

Restore the instrument settings

1. Plug the USB drive that contains the instrument settings into the USB port below the touchscreen.

2. From the Main Menu of the instrument touchscreen, touch Tools, then touch Restore Settings.

3. From the Restore Settings screen, select the settings to restore:
   a. Touch the settings that you want to restore from the list.
   b. Touch Restore to upload the instrument settings from the USB drive.

IMPORTANT! Do not remove the USB drive from the QuantStudio™ 6 or 7 Flex System until instructed.

Note: Alternatively, touch Restore Default Settings to restore the QuantStudio™ 6 or 7 Flex System to the factory settings.

4. After the QuantStudio™ 6 or 7 Flex System restarts, unplug the USB drive.

Perform an instrument self test

You can use the QuantStudio™ 6 or 7 Flex System touchscreen to perform a comprehensive self test of the QuantStudio™ 6 or 7 Flex System subsystems. After the self test is complete, the QuantStudio™ 6 or 7 Flex System generates two files that provide a detailed summary of the instrument condition and function. In the event of a problem, you can save the results files to a USB drive and email them to Life Technologies technical support for a diagnosis.

Note: We recommend running the self test as part of regular maintenance to ensure optimal performance of the QuantStudio™ 6 or 7 Flex System.

1. From the Main Menu of the instrument touchscreen, touch Tools, then touch Run Self Test.

2. From the Self Test screen, touch Start Self Test, then wait for the test to complete.

3. (Optional) When the QuantStudio™ 6 or 7 Flex System completes the self test, save the results to a USB drive:
   a. Plug a USB drive into the USB port below the touchscreen.

IMPORTANT! Do not use the USB ports on the rear panel of the QuantStudio™ 6 and 7 Flex Systems. The rear USB ports are for use by Life Technologies personnel to service the instrument.
Appendix A  Manual Instrument Operation

Maintain the instrument from the touchscreen

b. Touch \(\text{Save}\).

**IMPORTANT!** Do not remove the USB drive from the QuantStudio™ 6 or 7 Flex System until instructed.

c. When the QuantStudio™ 6 or 7 Flex System finishes writing the results to the USB drive, touch \(\text{OK}\), then remove the USB drive.

4. Touch \(\text{M}\) to return to the Main Menu.

You can download QuantStudio™ 6 and 7 Flex Systems firmware updates directly from the service section of the Life Technologies website. After obtaining a firmware update, transfer the update to the QuantStudio™ 6 or 7 Flex System using a USB drive.

1. Download the firmware update:
   b. In the Software Downloads page, select Applied Biosystems QuantStudio™ 6 and 7 Flex Real-Time PCR Systems from the menu.
   c. In the Software Downloads page for your QuantStudio™ 6 or 7 Flex System, click Updates - Patches.
   d. Download the QuantStudio™ 6 and 7 Flex Systems firmware to a USB drive.

2. Plug a USB drive into the USB port below the touchscreen.

**IMPORTANT!** Do not use the USB ports on the rear panel of the QuantStudio™ 6 and 7 Flex Systems. The rear USB ports are for use by Life Technologies personnel to service the instrument.

3. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press \(\text{M}\):

4. In the Main Menu, touch \(\text{Tools}\), then touch \(\text{Upgrade Firmware}\).

5. In the Upgrade Firmware screen, select the update package, then touch \(\text{Upgrade Firmware}\). Allow the QuantStudio™ 6 or 7 Flex System to complete the upgrade.

**IMPORTANT!** Do not remove the USB drive from the QuantStudio™ 6 or 7 Flex System until you are instructed to do so.

6. After the upgrade is complete and the QuantStudio™ 6 or 7 Flex System reboots, confirm the upgrade success:
   a. Unplug the USB drive.
   b. Touch \(\text{Settings}\), then touch \(\text{About this instrument}\) to view the software version number to confirm that the firmware has been upgraded.
Administrate the instrument from the touchscreen

The touchscreen provides access to several administrative functions that you can use to integrate the QuantStudio™ 6 and 7 Flex Systems into a laboratory workflow. The following functions are available from the touchscreen and can be used after installation to customize the instrument settings and configure it for network use.

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Note: The touchscreen does not provide access to all instrument functions. Features such as instrument calibration and remote notification are available only through the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

Define the date and time

IMPORTANT! If your laboratory is subject to daylight savings time changes, you must manually adjust the time settings during daylight savings to ensure that the internal clocks of your computer and instrument are synchronized.

1. From the Main Menu of the instrument touchscreen, touch **Settings**, then touch **Set Date & Time**.

2. From the Set Date & Time screen:
   a. Touch the **Time zone** field, then touch the correct time zone from the list.
   b. Touch the **Date** field, enter the current date, then touch **Done**.
   c. Touch the **Date Format** drop-down list, then select the format for your region.
   d. Touch each Time field, enter the appropriate time units, then touch **Done**.
   e. Touch **12 Hour** or **24 Hour** to select the appropriate time format.
   f. Touch **Save** to save the settings, then touch **OK** when prompted.

3. Touch 🔄 to return to the Main Menu.

Define the instrument settings

1. From the Main Menu of the instrument touchscreen, touch **Settings**, then touch **Configure the Instrument**.

2. Touch the **Instrument Name** field, enter up to a 25-character name for the QuantStudio™ 6 or 7 Flex System, then touch **Done**.
The instrument name is the alphanumeric string used to identify the QuantStudio™ 6 or 7 Flex Instrument on the network.

**IMPORTANT!** To connect the QuantStudio™ 6 or 7 Flex System to a network, the name must be unique.

**IMPORTANT!** The instrument name cannot include spaces or special characters (such as; "<>*+=\|?,").

3. Upload the instrument icon:
The instrument icon is the graphic used to represent the QuantStudio™ 6 or 7 Flex System in the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Instrument Console:
   a. Save the replacement graphic to a USB drive, then plug the drive into the USB port below the touchscreen.

   **IMPORTANT!** Do not use the USB ports on the rear panel of the QuantStudio™ 6 and 7 Flex Systems. The rear USB ports are for use by Life Technologies personnel to service the instrument.

   b. Touch **Upload Icon**, select the desired graphic file, then touch **Done**.

   **Note:** The replacement graphic must be a maximum of 48 × 48 pixels and be stored in the portable net graphic (PNG) format.

4. Define the standby time-out setting:
   a. Select **Standby Time-out** to activate the feature.

   b. Touch the **Standby Time-out** field.

   c. Enter the number of minutes (1–300) that the QuantStudio™ 6 or 7 Flex System should remain idle until it enters standby mode, then touch **Done**.

   **Note:** When in standby mode, the QuantStudio™ 6 or 7 Flex System powers off the LCD screen backlight and enters low-power mode.

5. Define the heated cover temperature setting:
   a. Select **Cover Idle Temperature** to activate the feature.

   b. Touch the **Cover Idle Temperature** field.

   c. Enter the temperature (50.0–110.0°C) that the heated cover should maintain when the QuantStudio™ 6 or 7 Flex System is idle, then touch **Done**.

   **Note:** The recommended Cover Idle Temperature is 60.0°C for reaction plate formats and 85.0°C for the array card format.

6. Touch **Save** to save the settings, then touch **OK** when prompted.

7. Unplug the USB drive.

8. Touch ‼️ to return to the Main Menu.
Define the maintenance reminders

You can use the QuantStudio™ 6 or 7 Flex System touchscreen screen to:

- Set the expiration period for instrument calibrations and lamp replacement.
- Activate, deactivate, or change the frequency of the maintenance reminders displayed by the QuantStudio™ 6 or 7 Flex System.

To set the reminder:

1. From the Main Menu of the instrument touchscreen, touch **Settings**, then touch **Set Maintenance Reminders**.

2. For each reminder:
   
   a. Touch the **Calibration expires after** field.
   
   b. Use the keypad to enter the number of days (or, for the lamp, the number of hours) that should elapse before expiration, then touch **Done**.
   
   c. (Optional) Touch the checkbox to activate or deactivate an expiration reminder, then go to step 3.

3. (Optional) Configure the reminder:
   
   a. Touch the **Display reminders before** field.
   
   b. Enter the number of days (or, for the lamp, the number of hours from 1-2000) before which the instrument should display warnings of the impending expiration, then touch **Done**.

4. Touch **Save** to save the settings, then touch **OK** when prompted.

5. Touch 🌐 to return to the Main Menu.

Define the network settings

1. From the Main Menu of the instrument touchscreen, touch **Settings**, then touch **Set Network Information**.

   **Note:** The Set Network Information screen displays the Media Access Control (MAC) address of the QuantStudio™ 6 or 7 Flex System below the Autodiscovery and Smart Monitoring check boxes. The MAC address can be used to uniquely identify the QuantStudio™ 6 or 7 Flex System on the network.

2. Touch **Autodiscovery** to make the QuantStudio™ 6 or 7 Flex System discoverable by computers that are running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

3. Touch **Smart Monitoring** to enable the feature on the QuantStudio™ 6 or 7 Flex System.

   The Smart Monitoring feature allows Life Technologies service personnel to monitor the status of the QuantStudio™ 6 or 7 Flex System remotely through an internet connection. Smart Monitoring employs multiple layers of security, including a Secure Sockets Layer (SSL) and Lightweight Directory Access Protocol (LDAP) authentication, to provide real-time troubleshooting and problem resolution for the QuantStudio™ 6 or 7 Flex System. For a detailed description of the Smart Monitoring Service, see the **Smart Monitoring Service Product Bulletin: Leveraging the power of the Internet while maintaining system security** (Part no. 121PB07-03).

4. Set the Internet Protocol (TCP/IP) Properties for either DHCP or Static IP communication.
**Define the system shortcuts**

You can use the QuantStudio™ 6 or 7 Flex System touchscreen to map the shortcut buttons that appear in the Main Menu. You can configure shortcuts to automatically open specific files and folders so that you can access data quickly and easily without having to navigate to it.

1. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press .
2. In the Main Menu, configure the shortcuts as desired:
   - To add a shortcut:
     a. Touch the shortcut of interest, then touch Set Shortcut.
     b. Touch From Templates to link to a specific template file or touch From Folders to link to a folder.
     c. Touch the desired template file or folder to configure the shortcut.
   - To delete a shortcut, touch the shortcut of interest, then touch Remove Shortcut, or touch Remove All to delete all shortcuts.
3. When you are finished configuring the shortcuts, touch to return to the Main Menu.

**Review the instrument statistics**

You can use the QuantStudio™ 6 or 7 Flex System touchscreen to view usage statistics on the heated cover, lamp, and other instrument components.

1. From the Main Menu of the instrument touchscreen, touch Tools, then touch Show Statistics.
2. When you are finished, touch to return to the Main Menu.

**Review the instrument version information**

You can use the QuantStudio™ 6 or 7 Flex System touchscreen to view instrument version data, such as instrument serial number and version numbers for most instrument components and firmware.

1. From the Main Menu of the instrument touchscreen, touch Tools, then touch About the Instrument.
2. When you are finished, touch to return to the Main Menu.
Enable/disable instrument security

The QuantStudio™ 6 and 7 Flex Systems feature a secure mode that can be enabled to restrict local instrument functionality. When security is enabled, use of the touchscreen is restricted to administrative functions that change the instrument settings. After the QuantStudio™ 6 or 7 Flex System is secured, you must enter an administrator password to modify the instrument settings, use the firmware tools, or deactivate the secure mode.

IMPORTANT! If you enable or disable the QuantStudio™ 6 and 7 Flex Systems security, auditing, and electronic signature feature, you must similarly enable or disable the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software security (see page 131). The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software cannot connect to QuantStudio™ 6 and 7 Flex Systems that do not match security settings.

Note: Secure mode limits the number of features that are available from the QuantStudio™ 6 or 7 Flex System touchscreen; it does not provide user authentication functionality through the instrument touchscreen.

1. If the instrument is in standby, touch the QuantStudio™ 6 or 7 Flex System touchscreen to activate it, then press.
2. In the Main Menu, touch Settings, then touch Set Administrator Options.
3. In the Set Administrator Options screen, touch Secure Environment to enable (checked) or disable (unchecked) system security.
4. (Optional) To change the administrator password:
   a. Touch Change Password.
   b. Enter the current password, then touch Done.
   c. Enter the new password, then touch Done.
   d. Reenter the password when prompted.
   e. Touch OK when prompted.
   Note: The default password for the QuantStudio™ 6 or 7 Flex System touchscreen is password; however, the password can be changed during installation.
5. Touch Save.
6. Touch the Administrator Password field, enter the administrator password, then touch Done.
7. Touch to return to the Main Menu.

View the instrument log

You can use the QuantStudio™ 6 or 7 Flex System touchscreen to view a log that summarizes instrument activity from the last 6 months. For each recorded activity, the activity log provides a description of the activity and the date/time when it occurred.

1. From the Main Menu of the instrument touchscreen, touch Tools, then touch View Log.
2. From the View Log screen, configure the settings to display the records of interest:
   • Select an option from the drop-down menu to filter the log.
   • Select Earliest First or Latest First to determine the order to sort the records.
3. (Optional) Plug the USB drive into the port below the touchscreen, then touch Export to USB to save the log to the drive.

**IMPORTANT!** Do not use the USB ports on the rear panel of the QuantStudio™ 6 and 7 Flex Systems. The rear USB ports are for use by Life Technologies personnel to service the instrument.

4. Touch  to return to the Main Menu.
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IMPORTANT! This chapter contains all user service procedures for the QuantStudio™ 6 and 7 Flex Systems. Procedures other than those described in this document must be performed by a qualified Life Technologies Service Engineer.
Decontaminate the sample block

Perform this procedure to eliminate fluorescent contaminants from the QuantStudio™ 6 or 7 Flex System sample block. Contamination is generally evident in failed background calibrations where one or more wells consistently exhibit abnormally high signals.

CAUTION! PHYSICAL INJURY HAZARD. Do not remove the QuantStudio™ 6 or 7 Flex System cover. There are no components inside the instrument that you can safely service yourself. If you suspect a problem, contact a Life Technologies Service Representative.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the sample block can reach 100°C. Allow the sample block to cool to room temperature before performing the procedure.

CAUTION! Before using a cleaning or decontamination method other than those recommended by the Life Technologies, confirm with Life Technologies that the proposed method will not damage the equipment.

Materials required

- Bleach, 10% solution
- Tissue, lint-free
- Cotton or nylon swabs and lint-free cloths
- Ethanol, 95% solution
- Safety glasses
- Pipette (100-µL) with pipette tips
- Powder-free gloves
- Screwdriver
- Deionized water

How to handle the sample block

To prevent damaging or contaminating the sample block, handle the assembly as shown. Also, when the assembly has been removed from the QuantStudio™ 6 or 7 Flex System, place the sample block on a clean, dry surface or in its shipping container.
Clean the sample block

**WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the sample block can reach 100°C. Allow the sample block to cool to room temperature before performing the procedure.

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

1. Identify the contaminated wells of the sample block (see “How to identify contamination” on page 78).

2. Power off and unplug the QuantStudio™ 6 or 7 Flex System, then allow it to cool for 15 minutes.

3. Open the access door.

4. Firmly press down on the handle of the sample block, then remove it from the QuantStudio™ 6 or 7 Flex System. Place the sample block on a clean, dry surface.

5. Clean the contaminated wells of the sample block using deionized water:
   a. Pipet a small volume of deionized water into each contaminated well.
   b. In each well, pipet the water up and down several times to rinse the well.
   c. Pipet the water to a waste beaker.
   d. Use a cotton swab to scrub inside of each contaminated well.
   e. Use a lint-free cloth to absorb the excess deionized water.
6. Load the sample block into the QuantStudio™ 6 or 7 Flex System, then close the access door.

**IMPORTANT!** After installing the sample block, confirm that the indicator on the left side of the sample block is positioned behind the red line on the instrument rail. If the indicator is forward of the red line, push the sample block into the QuantStudio™ 6 or 7 Flex Instrument until it is seated correctly.

7. Close the access door.

**IMPORTANT!** Confirm that the access door is completely closed. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays an error message if the door is not completely closed and latched, or if the sample block is not seated correctly.

8. Plug in, then power on the QuantStudio™ 6 or 7 Flex System.

9. Perform a background calibration to confirm that you have eliminated the contamination.

10. If the contamination remains, repeat steps 2–5, then clean the contaminated wells of the sample block using a 95% ethanol solution:

    a. Pipet a small volume of 95% ethanol solution into each contaminated well.
    b. In each contaminated well, pipet the solution up and down several times to rinse the well.
    c. Pipet the ethanol solution to a waste beaker.

11. Repeat steps 5–9 to rinse the wells of the sample block and to ensure that you have eliminated the contamination.

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

12. If the contamination remains, repeat steps 2–5, then clean the contaminated wells of the sample block using 10% bleach solution:

    a. Pipet a small volume of 10% bleach solution into each contaminated well.
    b. In each contaminated well, pipet the solution up and down several times to rinse the well.
    c. Pipet the bleach solution to a waste beaker.

13. Repeat steps 5–9 to rinse the wells of the sample block and to confirm that you have eliminated the contamination.

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

14. If the contamination remains, contact Life Technologies.
Replace the halogen lamp

Replace the halogen lamp at or before 2000 hours of life.

**WARNING! PHYSICAL INJURY HAZARD.** The QuantStudio™ 6 or 7 Flex System and lamp are hot! The lamp can become very hot while in use. Before handling the lamp, allow it to cool for 15 minutes and put on protective, powder-free gloves.

**CAUTION! 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!** This QuantStudio™ 6 or 7 Flex System is designed for 12 V, 75 W halogen lamps only. Replace with halogen bulbs supplied by Life Technologies.

**Materials required**
- Safety glasses
- Powder-free gloves
- Halogen bulb (12 V, 75 W)

**Halogen lamp warnings**
The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can display the following warnings before or during an experiment:

- The lamp current is below the acceptable level at the start of the run. Replace the halogen bulb as explained in “Replace the lamp” on page 100.
- The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software stopped the run because the lamp current decreased below the acceptable level during the run. Replace the halogen bulb as explained in “Replace the lamp” on page 100. Click **OK** in the message box, then replace the lamp bulb.
- The lamp usage exceeds 2000 hours at the start of a run. Click **Cancel Run**, then replace the lamp before you perform another experiment.

**View the lamp status**
1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.
2. From the Instrument Console, select your QuantStudio™ 6 or 7 Flex System from the list of instruments, then review the Lamp Life and Last Serviced readings in the Maintenance Info pane.
   If the lamp usage has reached 2000 hours, replace the lamp (see “Replace the lamp” below).

   **Note:** The Lamp Life report displays the total number of hours that the halogen lamp has been illuminated. The Last Serviced report displays the date that the lamp was installed.
Replace the lamp

**IMPORTANT!** Always wear powder-free gloves when you handle the lamp. Fingerprints shorten the lamp life.

1. Record the lamp installation:
   a. From the Main Menu of the touchscreen, touch **Tools**, then touch **Record Lamp Installation**.
   b. Touch the **Name** field, enter any relevant information (such as the make, model, or serial number of the lamp), then touch **Done**.
   c. Touch the **Comments** field, enter any additional information that you want to record, then touch **Done**.
   d. Touch **Save**, then touch **OK**.
   e. When asked to confirm lamp replacement, touch **OK**.

   **Note:** The data that you enter for the new lamp appears in the instrument log.

2. Power off and unplug the QuantStudio™ 6 or 7 Flex System, then allow it to cool for 15 minutes.

3. Open the access door.

4. Remove the lamp from the QuantStudio™ 6 or 7 Flex System:
   a. Slide the lamp release lever downward.
   b. Firmly grasp the lamp and lift it up and out of the slotted mount.

5. Inspect the lamp for damage (carbon usually coats the inside of a failed lamp). If necessary, dispose of the lamp and obtain a replacement.

   **IMPORTANT!** Dispose of the lamp in accordance with your local municipal waste ordinances. Do not dispose of the lamp as unsorted municipal waste.

6. Install the new lamp into the QuantStudio™ 6 or 7 Flex System:
   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.

7. Close the access door.

8. Plug in and power on the QuantStudio™ 6 or 7 Flex System.

**IMPORTANT!** Confirm that the access door is completely closed. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays an error message if the door is not completely closed and latched, or if the lamp is not seated correctly.

9. Start the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, then perform an ROI calibration.

While the QuantStudio™ 6 or 7 Flex System is running, look through grating of the access door and confirm that the lamp is illuminated.

- If the lamp is illuminated, the lamp has been installed successfully.
- 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 QuantStudio™ 6 or 7 Flex System fuses for failure (see page 102).

10. After replacing the lamp, perform the following calibrations in the specified order:

   a. ROI calibration
   b. Background calibration
   c. Uniformity calibration
   d. Dye calibration
   e. Normalization calibration
   f. RNase P test
Replace the instrument fuses

Replace the QuantStudio™ 6 or 7 Flex System fuses when the fuses fail.

**CAUTION! 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 QuantStudio™ 6 or 7 Flex System.

**Materials required**
- Fuses, 12.5A, Time-Lag T, 250VAC, 5 × 20-mm (2)
- Fuses for AU/NZ only, 6.3A, Time-Lag T, 250VAC, 5 x 20-mm (2)
- Safety glasses
- Powder-free gloves
- Screwdriver, flathead

**Replace the fuses**

1. Power off, then unplug the QuantStudio™ 6 or 7 Flex System. Allow it to cool for 15 minutes.

2. Using a flat-head screwdriver, unscrew and remove the fuse holder.

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 12.5A, Time-Lag T, 250VAC, 5 × 20-mm Fuse.  
   **Note:** The voltage and amperage ratings are on the fuse holder.  
   **Note:** For AU/NZ only, replace each failed fuse with a 6.3A, Time-Lag T, 250VAC, 5 x 20-mm Fuse.

5. Install the fuse holder.

6. Plug in, then power on the QuantStudio™ 6 or 7 Flex System. The installation is successful if the instrument powers on.

**Note:** Fuse failure can result from fluctuations in the supplied power to the QuantStudio™ 6 or 7 Flex System. To prevent further failures, consider installing an electrical protective device, such as a UPS or a surge protector.
Install the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software

If you have extra software licenses, you can install the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to computers that meet the operating requirements (not recommend).

### Computer requirements

See “Computer requirements” on page 24 for a list of hardware and operating system requirements for the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

### Install the software

1. Load the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software CD into the computer.

2. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Installer, click Install QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

3. Complete the installation as instructed using the default settings:
   - From the Welcome window, enter your customer information.
   - From the License Agreement screen, accept the terms of the agreement.
   - From the Installation Location screen, accept the default location.
   - From the License screen, install the license key/file that you obtained from Life Technologies.

   The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software installation requires several minutes.

4. Eject the CD when the installation is complete.
Manage QuantStudio™ 6 and 7 Flex Real-Time PCR System Software licenses

You can use the License Central feature to activate, or install the licenses that control access to the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software base application and associated modules.

QuantStudio™ 6 and 7 Flex Real-Time PCR System Software license keys and files

The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software and associated modules require the installation and maintenance of valid license files for continued operation. The license files are generated by the Life Technologies website when a license key is activated. Each file pairs a software license key with the computer from which the key was activated. After a key is activated and a license file is generated, the file cannot be transferred to another computer. To transfer a license between computers, you must reactivate the license key using the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software on the target computer.

Note: License keys are found on the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software CD packaging, or they can be supplied by Life Technologies support.

Activate a license

If you have a valid license key for the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software or an associated module, you can activate the license as explained below.

**IMPORTANT!** An internet connection, a web browser, and a valid email account are required to activate a QuantStudio™ 6 and 7 Flex Real-Time PCR System Software license. If the computer that contains the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is not connected to the internet or it lacks a web browser application, contact Life Technologies support to request the license file.

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, select Tools > License Central.
2. From the License Central dialog box, select the license of interest from the table, click Activate License, then wait for the default web browser application to connect to the Life Technologies website.
3. From the Life Technologies Software License Activation website, click QuantStudio™ 6 and 7 Flex Real-Time PCR System Software from the list of products, then activate the license as instructed. After you successfully activate the license, the Life Technologies website emails you the activated license file (.lic) to install on your computer.

Install a license file

After you activate a license and receive an activated license file (.lic), install the file as explained below to unlock the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software or module.

**Note:** Each license file is generated specifically for the computer that was used to activate the license key.

1. Save the license (.lic) file to the computer that contains the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.
2. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, select Tools » License Central.

3. From the License Central dialog box, click Install License.

4. From the Open dialog box, navigate to and select the license file, then click Open.

5. Click OK to close the License Central dialog box.

Note: Contact Life Technologies support if the software fails to install the license.

Update the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software

Life Technologies may release updates to the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software that you can install without the aid of Life Technologies service personnel. You can obtain updates directly from the service section of the Life Technologies website.

For the latest services and support information for the QuantStudio™ 6 or 7 Flex System:


2. From the Software Downloads page, select Applied Biosystems QuantStudio™ 6 and 7 Flex Systems from the menu.

3. From the Applied Biosystems QuantStudio™ 6 and 7 Flex Systems Software Downloads page, click Updates - Patches.

The website opens the page describing the latest software updates for the QuantStudio™ 6 or 7 Flex System.

Update the software

Prepare for the upgrade

To prepare for the software update, back up all data files and experiments by creating a copy of the directory that you are using to store files. The default directories are:

- **Installation directory** – `<drive>:\Program Files\Applied Biosystems\QuantStudio 6 and 7 Flex Software`
- **Data directory** – `<drive>:\Applied Biosystems\AppData\QuantStudio 6 and 7 Flex Software`
- **User files directory** – `<drive>:\Applied Biosystems\QuantStudio 6 and 7 Flex Software\User Files\experiments`

Note: `<drive>` is the location where the software is installed.

Install the software

Install the software update according to the instructions that download with the software. If you are installing the update to a computer that already contains the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, the update automatically acquires the software license from the existing installation. If you are installing the
QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to a computer that does not contain a previous installation, you must have a license file supplied by Life Technologies. If you do not have a license file, obtain one as explained in “Manage QuantStudio™ 6 and 7 Flex Real-Time PCR System Software licenses” on page 104.

Update the Windows® operating system

Do not upgrade or update the Microsoft® Windows® operating system of the computer running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software without first consulting the software release notes or the Life Technologies website. Future versions and updates to the Windows® operating system may conflict with the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

To determine compatibility of an upgrade or update:

1. Open <drive>:\Program Files\Applied Biosystems\QuantStudio 6 and 7 Flex Software, double-click QuantStudio 6 and 7 Software README.docx, then read the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Release Notes for the compatibility of interest.

   Note: <drive> is the location where the software is installed.

2. If the website does not contain the information of interest, contact Life Technologies.

Change the sample block

⚠️ WARNING! PHYSICAL INJURY HAZARD. During instrument operation, the sample block and heated cover can reach 100°C. Allow the sample block to cool to room temperature before removing.

When to change the sample block

You can change the sample block:

- To change the consumable format of the QuantStudio™ 6 or 7 Flex System.
- In the event of a hardware failure.

Materials required

- Safety glasses
- Powder-free gloves
- Sample block

How to handle the sample block

To prevent damaging or contaminating the sample block, handle the assembly as shown below. After the assembly has been removed from the QuantStudio™ 6 or 7 Flex System, place the sample block on a clean, dry surface or in its shipping container.
Change the sample block

1. Power off and unplug the QuantStudio™ 6 or 7 Flex System, then allow it to cool for 15 minutes.

2. Open the access door.

3. Firmly press down on the handle of the sample block, then remove it from the QuantStudio™ 6 or 7 Flex System. Place the sample block on a clean, dry surface.

4. Install the new sample block into the QuantStudio™ 6 or 7 Flex System.

   **IMPORTANT!** After installing the sample block, confirm that the indicator on the left side of the sample block is positioned behind the red line on the instrument rail. If not, push the sample block into the QuantStudio™ 6 or 7 Flex System until it is seated correctly.

5. If you are installing a sample block of a different consumable format, replace the heated cover and plate adapter if necessary.

6. Close the access door.

   **IMPORTANT!** Confirm that the access door is completely closed. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays an error message if the door is not completely closed and latched, or if the sample block is not seated correctly.

7. Plug in and power on the QuantStudio™ 6 or 7 Flex System.

8. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.
9. From the Instrument Console, select your QuantStudio™ 6 or 7 Flex System from the list of instruments, then review the Block Type field in the Instrument Properties pane.

The installation is successful if the QuantStudio™ 6 or 7 Flex System powers on and if the Block Type field displays the correct type of sample block.

Note: The Block Type field displays the type of sample block installed to the QuantStudio™ 6 or 7 Flex System.

10. Check the calibration status of the QuantStudio™ 6 or 7 Flex System (see “View the calibration status of an instrument” on page 29).

If the calibrations for the new sample block/heated cover have expired, perform the following calibrations in the specified order:
   a. ROI calibration
   b. Background calibration
   c. Uniformity calibration
   d. Dye calibration
   e. Normalization calibration

IMPORTANT! If the calibrations for the new sample block/heated cover are valid, you do not need to recalibrate the QuantStudio™ 6 or 7 Flex System.

Change the heated cover

**WARNING! PHYSICAL INJURY HAZARD.** During instrument operation, the sample block and heated cover can reach 100°C. Allow the heated cover to cool to room temperature before removing.

When to change the heated cover

You can change the heated cover:

- To change the consumable format of the QuantStudio™ 6 or 7 Flex System.
- In the event of a hardware failure

Materials required

- Safety glasses
- Powder-free gloves
- Heated cover

How to handle the heated cover

To prevent damaging or contaminating the heated cover, handle the assembly as shown below. After the assembly has been removed from the QuantStudio™ 6 or 7 Flex System, place the heated cover on a clean, dry surface or in its shipping container.
Change the heated cover

Note: Confirm that the replacement heated cover supports the plate or array card format that you want to use. Some heated covers support more than one consumable type.

1. Power off and unplug the QuantStudio™ 6 or 7 Flex System, then allow it to cool for 15 minutes.

2. Open the access door.

3. Unlock the heated cover by pinching the handle together, then pull the assembly from the QuantStudio™ 6 or 7 Flex System and place it on a clean, dry surface.

4. Install the new heated cover into the QuantStudio™ 6 or 7 Flex System.

**IMPORTANT!** Align the arrows on the front handle as shown below. If the arrows are not aligned, push the heated cover into the QuantStudio™ 6 or 7 Flex System until the handle locks into place.

5. If you are installing a heated cover of a different consumable format, replace the sample block and plate adapter if necessary.
6. Close the access door and ensure it is completely closed.

   **Note:** The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays an error message if the door is not completely closed and latched, or if the sample block is not seated correctly.

7. Plug in and power on the QuantStudio™ 6 or 7 Flex System.

8. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.

9. From the Instrument Console, select your QuantStudio™ 6 or 7 Flex System from the list of instruments, then review the Heated Cover Firmware Version field in the Instrument Properties pane.
   
The installation is successful if the QuantStudio™ 6 or 7 Flex System powers on and if the Heated Cover Firmware Version field displays a version number.

10. Check the calibration status of the QuantStudio™ 6 or 7 Flex System (see “View the calibration status of an instrument” on page 29).
   
   If the calibrations for the new sample block/heated cover have expired, perform the following calibrations in the specified order:
   
   a. ROI calibration
   
   b. Background calibration
   
   c. Uniformity calibration
   
   d. Dye calibration
   
   e. Normalization calibration

   **IMPORTANT!** If the calibrations for the new sample block/heated cover are valid, you do not need to recalibrate the QuantStudio™ 6 or 7 Flex System.
Change the plate adapter

Change the plate adapter in the event of a hardware failure or if you want to change the consumable format of the QuantStudio™ 6 or 7 Flex System.

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

### When to change the plate adapter

You can change the plate adapter:
- To change the consumable format of the QuantStudio™ 6 or 7 Flex System.
- In the event of a hardware failure

### Materials required

- Safety glasses
- Powder-free gloves
- Plate adapter

### Change the plate adapter

1. Touch the instrument touchscreen to activate it, then press .
2. From the Main Menu, touch .
3. When the tray arm opens, pull the latch, then lift and remove the plate adapter.
4. Attach the new adapter to the tray arm, then pull the latch to allow the adapter to lower into place. If necessary, apply pressure as indicated until the adapter snaps into place.
5. From the Main Menu, touch .
6. If you are installing a tray adapter of a different consumable format, replace the sample block and heated cover if necessary.
Change the plate adapter
Networking

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**IMPORTANT!** This chapter does not provide adequate detail to integrate the QuantStudio™ 6 or 7 Flex Systems into all possible network architectures. Because your network may contain advanced features (such as a firewall or network domains), Life Technologies recommends that you consult a network administrator before connecting the QuantStudio™ 6 or 7 Flex Systems to your laboratory network.
Networking overview

After installing the QuantStudio™ 6 or 7 Flex System, you can connect the QuantStudio™ 6 or 7 Flex System to a local area network to enhance its functionality.

This appendix describes how to:

- Set up the QuantStudio™ 6 or 7 Flex System for use on a network.
- Set up a computer for remote monitoring.
- Test the network connection by engaging the remote monitoring feature.

Controlling and monitoring networked QuantStudio™ 6 and 7 Flex Systems

When the QuantStudio™ 6 or 7 Flex System is connected to a network, computers on the network that are running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can control or monitor it. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can control up to 4 instruments and monitor up to 10 instruments simultaneously. A networked QuantStudio™ 6 or 7 Flex System can be controlled by only one computer at a time. A networked computer running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can transfer experiments to and from an instrument, begin or stop a run, and perform some maintenance functions. During a run, the Remote Monitoring feature of the software can be used to view the run status, temperature, and amplification data in real-time.

Note: Remote monitoring does not allow you to control the QuantStudio™ 6 or 7 Flex System.

About the Ethernet port

The QuantStudio™ 6 or 7 Flex System features a Gigabit Ethernet port for direct communication with the QuantStudio™ 6 or 7 Flex System computer and for network communication. When the QuantStudio™ 6 or 7 Flex System is connected to a network, computers on the network that run the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can:

- Send and download experiments to and from the QuantStudio™ 6 or 7 Flex System.
- Run experiments on the QuantStudio™ 6 or 7 Flex System.
- Remote monitor the QuantStudio™ 6 or 7 Flex System as it performs runs.

The Ethernet port of the QuantStudio™ 6 or 7 Flex System supports:

- Static IP network service with subnet mask, primary and secondary data network service (DNS), and default gateway settings, or dynamic host configuration protocol (DHCP) network service
- mDNS/DNS for local domains
  
  Note: Because mDNS is limited to direct network connections, a QuantStudio™ 6 or 7 Flex System set for mDNS may not be visible to other nodes that are separated by a router, hub, or another network device.
- IPv4 link-local (IPV4LL) in the RFC (also known as Automatic Private IP Addressing [APIPA] or Internet Protocol Automatic Configuration [IPAC])
  
  Note: When the QuantStudio™ 6 or 7 Flex System is set for DHCP, APIPA is automatically enabled, and the QuantStudio™ 6 or 7 Flex System provides an IP address when no address is supplied by the DHCP server.
Example network layouts

Example 1

In the following example, one or more QuantStudio™ 6 or 7 Flex Systems, which have been configured for dynamic host configuration protocol (DHCP) operation, are connected to a network by their Ethernet ports. In this layout, any computer on the network can monitor and control the QuantStudio™ 6 or 7 Flex Systems.

**Note:** A networked computer running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can simultaneously control up to 4 instruments and monitor up to 10 instruments that have been connected to the network.

Example 2

In the example shown below, the QuantStudio™ 6 or 7 Flex System is connected directly to the QuantStudio™ 6 or 7 Flex System computer, which is also connected to the local area network. In this configuration, the computers on the network can exchange experiments with the QuantStudio™ 6 or 7 Flex System computer; however, the QuantStudio™ 6 or 7 Flex System can be neither monitored nor controlled remotely because it is physically isolated from the network.
Networking guidelines and best practices

- Consult a network administrator.
  - We recommend that you consult a network administrator before connecting the QuantStudio™ 6 or 7 Flex System to your laboratory network.
  - To enable the full functionality of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, the computer requires a network connection.

- Limit remote monitoring to 10 computers.
  Avoid using more than 10 computers to simultaneously monitor the QuantStudio™ 6 or 7 Flex System remotely. Although the QuantStudio™ 6 or 7 Flex System supports remote monitoring from multiple computers, each connection taxes the instrument microprocessor. Too many connections can overburden the QuantStudio™ 6 or 7 Flex System and result in instrument errors.
  Note: The effects of an overburdened QuantStudio™ 6 or 7 Flex System are evident in the Temperature Plot during a run. Symptoms can include extended hold times or brief, unexpected plateaus in the instrument Temperature Plot.

- Observe the restrictions to mDNS and Autodiscovery.
  The QuantStudio™ 6 or 7 Flex System supports mDNS but only when the QuantStudio™ 6 or 7 Flex System and computer share a direct network connection and are within the same subnet. Consequently, network computers that are separated from the QuantStudio™ 6 or 7 Flex System by a router, hub, or another network device may not be able to access the QuantStudio™ 6 or 7 Flex System by its host name.

- Confirm the uniqueness of the instrument name.
  The QuantStudio™ 6 or 7 Flex System does support name resolution but the instrument name must be unique within the subnet. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can automatically discover QuantStudio™ 6 or 7 Flex Systems on the link-local network that are configured for Autodiscovery.
  Note: The QuantStudio™ 6 or 7 Flex System does not test the uniqueness of the instrument name when it is set.

- Name QuantStudio™ 6 and 7 Flex Systems using lowercase letters.
  When you define the QuantStudio™ 6 and 7 Flex Systems settings, enter the instrument name using lowercase letters only.
Network setup workflow

The following figure illustrates the workflow for installing the QuantStudio™ 6 or 7 Flex System to a local area network.

Start

Collect the required network information.

Connect the QuantStudio™ 6 or 7 Flex System to the network.

Connect the computer to the network.

Monitor the QuantStudio™ 6 or 7 Flex System (to test the network connection).

Finish

Collect the required network information

Obtain the following information from your network administrator:

- Network policy for obtaining IP addresses (DHCP or static IP).

**IMPORTANT!** When the QuantStudio™ 6 or 7 Flex System is set for DHCP, APIPA is automatically enabled and the QuantStudio™ 6 or 7 Flex System self assigns an IP address when no address is supplied by a DHCP server.

- If the network requires static IP addresses, obtain the IP address, subnet mask, and gateway address for the QuantStudio™ 6 or 7 Flex System.
Appendix C
Networking
Connect the QuantStudio™ 6 or 7 Flex System to the network

Connect the QuantStudio™ 6 or 7 Flex System to the network

After deciding how to connect the QuantStudio™ 6 or 7 Flex System to a network, set up the QuantStudio™ 6 or 7 Flex System according to your network policies.

Materials required
Ethernet cable with RJ45 connectors (a CAT6 Ethernet cable for a 1000Mbit/s network connection or a CAT5 for 100Mbit/s connection)

Define the instrument internet protocol settings

1. Use the Ethernet cable to connect the Ethernet port of the QuantStudio™ 6 or 7 Flex System to the nearest network port.
2. Power on the QuantStudio™ 6 or 7 Flex System.
3. From the Main Menu of the touchscreen, touch Settings, then touch Set Network Information.

Note: The Set Network Information screen displays the Media Access Control (MAC) address of the QuantStudio™ 6 or 7 Flex System below the Autodiscovery and Smart Monitoring check boxes. The MAC address can be used to uniquely identify the QuantStudio™ 6 or 7 Flex System on the network.

4. Touch Autodiscovery to make the QuantStudio™ 6 or 7 Flex System discoverable by computers that are running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.
5. Touch Smart Monitoring to enable the feature on the QuantStudio™ 6 or 7 Flex System.

The Smart Monitoring feature allows Life Technologies service personnel to monitor the status of the QuantStudio™ 6 or 7 Flex System remotely through an internet connection. Smart Monitoring employs multiple layers of security, including a Secure Sockets Layer (SSL) and Lightweight Directory Access Protocol (LDAP) authentication, to provide real-time troubleshooting and problem resolution for the instrument. For a detailed description of the Smart Monitoring Service, see the Smart Monitoring Service Product Bulletin: Leveraging the power of the Internet while maintaining system security (Part no. 121PB07-03).

6. Set the Internet Protocol (TCP/IP) Properties for either DHCP or Static IP communication.

<table>
<thead>
<tr>
<th>Network service</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHCP</td>
<td>Touch Obtain an IP address automatically.</td>
</tr>
</tbody>
</table>
| Static IP       | 1. Touch Use the following IP address.  
|                 | 2. Touch the IP Address field, enter the IP address using the keypad, then touch Done.  
|                 | 3. Assign the:  
|                 | • IP addresses for the DNS Servers (primary and secondary)  
|                 | • Subnet Mask setting  
|                 | • Default Gateway setting |

7. Touch Save to save the settings, then touch OK when prompted.
8. Touch to return to the Main Menu.
Connect the computer to the network

Materials required
- Ethernet cable with RJ45 connectors

Computer requirement
If you are connecting a computer that you provided to a network, confirm that the computer contains a free network port.

Collect required information
Obtain the following information from your network administrator:
- Network policy for obtaining IP addresses (DHCP or static IP)
- If the network requires static IP addresses, obtain the IP address, subnet mask, and gateway address for the computer

Set up the computer

IMPORTANT! We recommend that you arrange for a network administrator to connect your computer to the network. The following procedure does not provide adequate detail for all network architectures.

Note: The following procedure is valid for the Microsoft® Windows® 7 operating system.

1. Use the Ethernet cable to connect the computer to the nearest network port.
2. Power on the computer, then log in using a user account that belongs to the Administrators user group.
3. From the desktop, right-click My Network Places, then select Properties.
4. Right-click Local Area Connection, then select Properties.
5. Select Internet Protocol (TCP/IP), then click Properties.
6. Set the Internet Protocol (TCP/IP) Properties for either DHCP or Static IP communication:

<table>
<thead>
<tr>
<th>Network configuration</th>
<th>Action</th>
</tr>
</thead>
</table>
| DHCP                 | 1. Select **Obtain an IP address automatically**.  
                          2. Set the DNS address. If the computer obtains DNS addresses: 
                            • Automatically – Select **Obtain DNS server address automatically**.  
                            • Statically – Select **Use the following DNS address**, enter the address of the preferred and alternate DNS servers if available. |
| Static IP             | 1. Select **Use the following IP address**.  
                          2. In the IP Address field, enter the static IP address.  
                          3. If necessary, enter a subnet mask.  
                          4. If necessary, enter a static gateway address in the Default Gateway field. |
Monitor the QuantStudio™ 6 or 7 Flex System

About remote monitoring

When the QuantStudio™ 6 or 7 Flex System is connected to the network, any computer on the network that is running the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can:

- Monitor the status of a run ......................................................... see below
- Download experiment files .................................................... 121
- Enable or change the calibration reminders ............................ 121

Guidelines for remote monitoring

To ensure optimal performance of the remote monitoring feature, observe the following guidelines:

- The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software can monitor up to 10 instruments.
- We do not recommend that a QuantStudio™ 6 or 7 Flex System be monitored by more than 10 computers simultaneously.
- Unless you are sure that your QuantStudio™ 6 or 7 Flex System and computer exist on the same subnet, we recommend that you use the IP address of the QuantStudio™ 6 or 7 Flex System to add it for remote monitoring.

Monitor the status of a run

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.
2. From the Instrument Console, select the icon for your instrument, then click **Manage Instrument**.
   
   **Note:** If the Manage Instrument button is inactive, add your QuantStudio™ 6 or 7 Flex System to the My Instruments group.

3. From the Instrument Manager, click **Monitor**, then click **Information**.

4. From the Monitor Instrument screen, click **Monitor Running Instrument**.
   
   The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays the status, attributes, calibration status, and plot data for the selected QuantStudio™ 6 or 7 Flex System. If a communications warning appears, contact your network administrator to troubleshoot the problem.

You can lose the software connection to the QuantStudio™ 6 or 7 Flex System if you:

- Change the QuantStudio™ 6 or 7 Flex System that is connected directly to your computer
- Use the touchscreen to change the instrument name or IP address

**Note:** To reestablish the connection, restart the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

---

**Download experiment files**

See “Transfer experiments, templates, and results data” on page 83 for more information on using QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to transfer experiment results from the QuantStudio™ 6 or 7 Flex System.

---

**Enable or change the calibration reminders**

The calibration reminder settings allow you to configure the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to alert you by email when the QuantStudio™ 6 or 7 Flex System requires calibration. The notifications settings feature is optional, and it does not affect performance.

**IMPORTANT!** The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software transmits email only while the QuantStudio™ 6 or 7 Flex System is monitored. If the network connection is interrupted, the software stops transmitting updates.

---

**Collect the required information**

The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software requires access to a Simple Mail Transfer Protocol (SMTP) server to email calibration reminders. Contact your systems administrator or information technology department for the following information:

- Network address of a SMTP server.
- A user name and password for the server, if required for access.
- The Secure Sockets Layer (SSL) setting of the server (on or off).

---

**Define the mail server settings**

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ➤ Preferences**.

2. From the Preferences dialog box, select the **SMTP Settings** tab.
Appendix C  Networking

Monitor the QuantStudio™ 6 or 7 Flex System

3. From the SMTP Settings tab, define the settings for the SMTP server:
   - **Outgoing Mail Server (SMTP) field** – Enter the network address of a Simple Mail Transfer Protocol (SMTP) server. Optionally, you can specify the transmission control protocol (TCP) port for the server by appending the port number to the server name, separating the two using a colon ( : ).
     For example: smtp.mycompany.com:2023
     **Note:** If a TCP port is not specified, the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software uses the default port number (25).
   - **Encryption Required** – Select if the mail server has SSL enabled.
   - **Authentication Required** – Select if the mail server requires a user name and password.
   - **User Name and Password fields** – If the mail server requires authentication, enter the user name provided by your systems administrator.

4. Click OK.

Modify the notification settings for a monitored QuantStudio™ 6 or 7 Flex System

1. Open the Calibration Reminders screen for the QuantStudio™ 6 or 7 Flex System:
   a. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Home tab, click **Instrument Console**.
   b. From the Instrument Console, select your QuantStudio™ 6 or 7 Flex System from the list of instruments on the network, then click **Add to My Instruments**.
      **Note:** You must add a QuantStudio™ 6 or 7 Flex System to your list before you can manage it.
   c. After the QuantStudio™ 6 or 7 Flex System is added to your list, select it, then click **Manage Instrument**.
   d. From the Instrument Manager, click **Maintenance**, then click **Calibration Reminders**.

2. From the Calibration Reminders Setting table, configure the notification settings for the calibrations in interest. For each calibration that you want to monitor:
   a. In the Expiry Interval column, enter the number of days that elapse before the type of calibration expires on the QuantStudio™ 6 or 7 Flex System.
   b. In the Send a Reminder column, select the check box to configure the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to email a reminder to perform the calibration.
   c. In the Reminder Interval column, enter the number of days that elapse before the software emails recipients a reminder to perform the calibration.

3. From the Enter e-mail addresses for notifications field, enter the email address(es) that you want to receive email notifications. Separate multiple email addresses with commas ( , ).

4. Click **Apply** to change the notification settings.
Power On or Off, Store, and Move

- Place the QuantStudio™ 6 or 7 Flex System on standby ................. 124
- Power on the QuantStudio™ 6 or 7 Flex System ......................... 124
- Power off the QuantStudio™ 6 or 7 Flex System .................... 125
- Store the QuantStudio™ 6 or 7 Flex System ......................... 126
- Move the QuantStudio™ 6 or 7 Flex System ......................... 127
Place the QuantStudio™ 6 or 7 Flex System on standby

If left unattended, the QuantStudio™ 6 or 7 Flex System automatically enters standby mode to conserve power. To enter standby mode manually, touch \( \text{PWR} \) on the QuantStudio™ 6 or 7 Flex System touchscreen.

Power on the QuantStudio™ 6 or 7 Flex System

To power on the QuantStudio™ 6 or 7 Flex System from a powered-off state:

1. Touch anywhere on the touchscreen to determine if the QuantStudio™ 6 or 7 Flex System is in standby mode.
   Does the touchscreen display the Standby screen after you touch it?
   • Yes – The instrument is ready for use. Go to step 4.
   • No – Go to step 2 to power on the instrument.

2. Power on the QuantStudio™ 6 or 7 Flex System. Toggle the power button on the rear of the QuantStudio™ 6 or 7 Flex System, then wait for it to start.

The QuantStudio™ 6 or 7 Flex System is ready to use when the touchscreen displays the Main Menu.

3. \( \text{QuantStudio™ 7 Flex System only} \) If you have an Applied Biosystems® Twister® Robot, toggle the power button on the rear of the Twister® Robot.
   \( \text{Note:} \) The Twister® Robot is ready to use when the power LED illuminates.

4. Power on the monitor.

5. Power on the computer:
   a. Press the computer power button, then wait for it to start.
   b. When the Login screen appears, enter your user name and password, then click OK.

6. Start the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software:
   a. From the desktop, double-click \textit{QuantStudio 6 and 7 Flex System Software}.
   \( \text{Note:} \) If the shortcut is not present on the desktop, select Start \( \rightarrow \) All Programs \( \rightarrow \) Applied Biosystems \( \rightarrow \) QuantStudio 6 and 7 Flex Software \( \rightarrow \) QuantStudio 6 and 7 Flex Software to start the software.
**IMPORTANT!** If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software does not start, confirm that no other instances of the instrument control software are open. If any instance of the software is open, close it before starting the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

b. If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Login appears, enter your user name and password, then click Log In.

![Login screen](image)

**Note:** Security is a separately licensed module. If your system requires this module, contact Life Technologies.

**Note:** If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays the License Central screen after you log into the software, your license file may have become corrupt. Contact Life Technologies support to obtain a replacement license file.

---

**Power off the QuantStudio™ 6 or 7 Flex System**

The QuantStudio™ 6 or 7 Flex Systems operate in low-power mode when not in use; however, the instrument can be powered off completely so that the components draw no power.

**Note:** If the QuantStudio™ 6 and 7 Flex Systems are inactive for extended periods of time, prepare them for storage as explained in “Store the QuantStudio™ 6 or 7 Flex System” on page 126.

1. Power off the QuantStudio™ 6 or 7 Flex System:
   a. If the touchscreen is not blank, touch to place the QuantStudio™ 6 or 7 Flex System into stand-by mode.
   b. Toggle the power button on the rear of the QuantStudio™ 6 or 7 Flex System.

2. Power off the QuantStudio™ 6 or 7 Flex System computer:
   a. From the desktop, select Start ➔ Shut Down.
   b. From the Shut Down Windows dialog box, select Shut Down, then click OK.
Store the QuantStudio™ 6 or 7 Flex System

The QuantStudio™ 6 or 7 Flex System 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 QuantStudio™ 6 or 7 Flex System.

Materials required

- Reaction plate or array card (unused)

Prepare the QuantStudio™ 6 or 7 Flex System

1. If you plan to store the QuantStudio™ 6 or 7 Flex System for more than a week or you plan to move it, load an unused plate or array card into the QuantStudio™ 6 or 7 Flex System:
   
   Note: The empty plate or array card protects the internal components of the QuantStudio™ 6 or 7 Flex System during transport or during periods of inactivity lasting more than a week.
   
   a. Touch the QuantStudio™ 6 or 7 Flex System touchscreen.
   
   b. Touch to eject the tray arm, place a plate or array card onto the plate adapter, then press again to load the plate.
   
   c. Touch to place the QuantStudio™ 6 or 7 Flex System into stand-by mode.

2. Toggle the power button on the rear of the QuantStudio™ 6 or 7 Flex System.

3. Power off the computer:
   
   a. Select Start > Shut Down.
   
   b. From the Shut Down Windows dialog box, select Shut Down, then click OK.

4. Power off the monitor.

5. (QuantStudio™ 7 Flex System only) If you have an Applied Biosystems® Twister® Robot, toggle the power button on the rear of the Twister® Robot.
Move the QuantStudio™ 6 or 7 Flex System

Perform this procedure to safely move the QuantStudio™ 6 or 7 Flex System short distances (for example, between laboratories of the same building).

**CAUTION! PHYSICAL INJURY HAZARD.** Do not attempt to lift the QuantStudio™ 6 or 7 Flex System 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 QuantStudio™ 6 or 7 Flex System. At least two people are required to lift it.

**IMPORTANT!** Moving your QuantStudio™ 6 or 7 Flex System can create subtle changes in the alignment of the instrument optics. Recalibrate the instrument if necessary.

**How to handle the sample block and heated cover**

To prevent damaging or contaminating the sample block or the heated cover, handle the assemblies as shown below. After you remove each assembly from the QuantStudio™ 6 or 7 Flex System, place them on a clean, dry surface or in its shipping container.

**Prepare the QuantStudio™ 6 or 7 Flex System components**

1. Power off the QuantStudio™ 6 or 7 Flex System and computer.
2. When the QuantStudio™ 6 or 7 Flex System and computer are powered off, disconnect all QuantStudio™ 6 or 7 Flex System components and package the cabling for the move.
3. Prepare the QuantStudio™ 6 or 7 Flex System for the move:
   a. Open the QuantStudio™ 6 or 7 Flex System access door.
   b. Firmly press down on the sample block handle, pull the sample block from the QuantStudio™ 6 or 7 Flex System, then place it on a clean, dry surface.
   c. Pinch the handle of the heated cover together, then pull the assembly from the QuantStudio™ 6 or 7 Flex System and place it on a clean, dry surface.
Move the QuantStudio™ 6 or 7 Flex System

Move the QuantStudio™ 6 or 7 Flex System according to the following guidelines:

- Ensure that the surface on which you will place the QuantStudio™ 6 or 7 Flex System can support at least 77.9 ± 0.6 kg (171.5 ± 0.13 lbs).
- Ensure that the path to transport the QuantStudio™ 6 or 7 Flex System is clear of obstructions.
- At least two people are needed to lift and carry the QuantStudio™ 6 or 7 Flex System.
- 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.

Reinstall the QuantStudio™ 6 or 7 Flex System

1. Reconnect the components of the QuantStudio™ 6 or 7 Flex System. Use the Ethernet cable supplied with the instrument to connect the QuantStudio™ 6 or 7 Flex System to the network interface card in the computer.

   **IMPORTANT!** Do not use a standard Ethernet cable to connect the QuantStudio™ 6 or 7 Flex System to the computer.

2. Install the sample block and heated cover assemblies.

3. Perform a RNase P instrument verification run. If the run:

   - **Passes** – Do not recalibrate the QuantStudio™ 6 or 7 Flex System. No further action is necessary.
   - **Fails** – Perform the following calibrations in the specified order:
     - a. ROI
     - b. Background
     - c. Uniformity
     - d. Dye
     - e. Normalization
Security, Audit, and Electronic Signature

- Administrator overview .................................................. 130
- Configure the security system ........................................... 131
- User account management ................................................ 133
- Audit trail management .................................................... 137
- Audit report generation ..................................................... 138
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- Generate e-signature reports ............................................ 144
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Administrator overview

IMPORTANT! Security, Audit, and Electronic Signature (SAE) is a separately licensed module available only on purchase. If your system requires this module, contact Life Technologies. The feature is disabled by default even after the installation of the license and needs to be enabled.

The Security, Audit, and Electronic Signature (SAE) module is a component of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software that allows you to configure the QuantStudio™ 6 and 7 Flex Systems to meet specific requirements. The module provides the following functionality:

- **Security** – Controls user access to the software. A default Administrator user account is provided, and additional user accounts and permissions can be user-defined.
  
  **Note:** You can enable or disable system security globally.

  **Note:** The default password for the Administrator user account is Administrator; however, the password can be changed during installation.

- **Auditing** – Tracks changes made to library items, actions performed by users, and changes to the Security, Audit, and Electronic Signature settings. The software automatically audits some actions silently. You can select other items for auditing and specify the audit mode. The Auditing function provides reports for audited library items, Security, Audit, and Electronic Signature changes, and actions.
  
  **Note:** You can enable or disable auditing globally and by record type.

- **Electronic signature (e-sig)** – Determines if users are required to provide a user name and password when performing certain functions. You can configure e-sig so that a user can print a report or start a run only if the associated data are signed. You can also configure each e-sig event to require multiple signatures and to require users with specific permissions to sign.
  
  **Note:** You can enable or disable electronic signature globally.

**Example applications**

You can configure the Security, Audit, and Electronic Signature module in a variety of ways. For example, you can:

- Require users to log in, and leave audit disabled.
- Allow only certain users to create or modify protocols.
- Allow only certain users to approve reviewed samples.
- Require experiments to be signed before users can run or print them.
Configure the security system

Access the Security screen and enable or disable security

Use the Security screen to disable and enable security, control restrictions and security policies for all user accounts, and set up notifications when certain security events occur.

**Note:** Security is disabled by default.

To enable or disable security:

1. In the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select Tools ➤ Security ➤ Settings.
2. In the Security Settings dialog box, select the System tab.
3. Select or deselect Enable Security. Note the following:
   - The software requires you to enter your user name and password when you enable security.
   - The enable commands are grayed when a run is in process.
   - When security is disabled, the ? is not active in lower parts of the screen.
   - Disabling Security inactivates Auditing and E-Signature.

**IMPORTANT!** If you disable security, you inactivate audit and electronic signature functions; however, no audit record is generated to indicate that audit and electronic signature functions are disabled.

**IMPORTANT!** If you enable or disable the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software security, auditing, and electronic signature feature, you must similarly enable or disable the QuantStudio™ 6 and 7 Flex Systems security (see “Enable/disable instrument security” on page 92). The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software cannot connect to QuantStudio™ 6 and 7 Flex Systems that do not match security settings.

4. Click Apply Settings.

Set account setup and security policies

**Note:** Security policies apply to all user accounts.

1. In the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select Tools ➤ Security ➤ Settings.
2. In Account Setup, specify User Name limits.

**IMPORTANT!** The software allows spaces in user names. Use spaces in user names with caution. For information, see “Spaces in user names and/or passwords” on page 132.

3. Specify User Password limits:
   a. Specify the passwords length limits.
   b. Specify password reuse. You cannot disable the password reuse restriction.
c. Specify the allowed characters in user passwords: spaces and alphabetical, numeric, uppercase, lowercase, and special characters (commas, periods, semicolons, dashes, underscores, and tildes).


Note: A session times out while a run is in progress if the time-out period is exceeded and there is no other user activity.

5. In the Open Non-Secure Data option, select Yes or No to determine whether users can open experiments and templates that were created without security settings.

6. Click **Set Up Messaging Notification Settings** to specify when and how the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software notifies the administrator of certain security events. For information, see “Set up messaging notifications” on page 132.

7. Click **Apply Settings**.

The new settings are applied to the user account the next time that the user logs in.

**Spaces in user names and/or passwords**

If you allow spaces in user names and/or passwords, be aware of the following issues:

- Leading and trailing spaces in user names are difficult to detect on the screen or in printed reports.
- The number of consecutive spaces in a user name is difficult to determine on the screen or in printed reports.

Spaces in user names may cause confusion when a user searches for an audit record associated with a user name. To find a record associated with a user name, specify the user name exactly, including leading, consecutive, and trailing spaces.

---

**Set up messaging notifications**

1. In the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ➔ Security ➔ Settings**.

2. In the Security screen, click **Set Up Messaging Notifications** to display the Setup Notifications dialog box.

3. Select the events for notification:

- **System security enabled or disabled** – Security has been enabled or disabled.
- **User did not enter correct password** – A user attempts to log in with an incorrect password. The message indicates the number of failed authentications.
- **User account suspended** – The user exceeds maximum number of allowed failed authentications (login attempts with an incorrect password).
- **User session timed out** – No activity occurred in a user account for the specified period of inactivity.
4. Select the notification method:
   - **Notify Admin at Login** – If an event triggers notification, the next time any user with an Administrator role logs in, the software lists those events, indicating the time each event occurred and the user who triggered the event.
     The Administrator has the option of acknowledging the event, which removes it from the notification list.
   - **Email Notification** – If an event triggers notification, the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software sends an email to the addresses in the adjoining Email Address column of the table. The email notification displays the triggered event and displays the time that the event occurred and the user who triggered the event.

5. Click OK.

**User account management**

**Create or edit user accounts**

The software includes a default Administrator user account with permissions (defined by the account user role) to perform all functions in the software. You cannot modify this account.

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select Tools > Security > Settings.

2. From the Security Settings dialog box, select the Users tab.

From the Users tab, you can perform the actions in the following table:

<table>
<thead>
<tr>
<th>To…</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Create a user account</td>
<td>1. Click Create to display the New User dialog box.</td>
</tr>
<tr>
<td></td>
<td>2. Enter user name, password, first name, middle initial (optional), and last name. Click a field to display the field limits, which are specified in Security settings.</td>
</tr>
<tr>
<td></td>
<td><strong>Note:</strong> First name, MI (middle initial), and last name are used to create User Full Name, which is displayed as the name of the logged-in user.</td>
</tr>
<tr>
<td></td>
<td><strong>Note:</strong> You cannot change the user name after you save the user account.</td>
</tr>
<tr>
<td></td>
<td>3. Select Password Expires at First Login to require the user account to specify a new password at first log in. The Password Expires On date is specified in Security settings.</td>
</tr>
<tr>
<td></td>
<td>4. Select the user role (described in “Create or edit a user role” on page 134) and the electronic signature state (determines if a user account has permission to electronically sign objects). Leave the status set to ACTIVE.</td>
</tr>
<tr>
<td></td>
<td>5. (Optional) Enter email (for information only), phone, and comments.</td>
</tr>
<tr>
<td></td>
<td>6. Click Save. A shaded Save button indicates an invalid entry in a field. Click a field to display the limits for the field, then enter a valid entry.</td>
</tr>
</tbody>
</table>
## Appendix E  Security, Audit, and Electronic Signature

### User account management

**To...** | **Action** |
---|---|
Edit a user account | 1. From the Users screen, select a user account, then click **Edit**.  
   **Note:** If you select multiple users, only Status and Role will be changed.  
2. Edit settings as needed. You cannot edit the user name of an existing user.  
3. Click **Save**. |
Activate a suspended user account | 1. From the Users screen, select the user.  
2. Click **Edit**.  
3. Change the Status from **SUSPENDED** to **ACTIVE**, then click **Save**. |
Disable (inactivate) a user account | **IMPORTANT!** You cannot delete a user, because user records are required for auditing. To disable a user account, inactivate it as follows.  
1. From the Users screen, select the user.  
2. Click **Edit**.  
3. Change the Status from **ACTIVE** to **INACTIVE**, then click **Save**. |
Reset a forgotten password | 1. From the Users screen, select the affected user account, then click **Edit**.  
2. Enter a replacement password for the user, then re-enter the password for confirmation.  
3. If you assigned the user a temporary password, then select **Password Expires at First Login** to require the user to specify a new password at log in.  
4. Click **Save**. |

---

**Determine the name of the logged-in user**

The title bar of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software window displays the name of the user.

**Create or edit a user role**

User roles determine the permissions associated with a user account. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software includes three default user roles:

- Administrator (cannot be edited or deleted)
- Lab Director
- Technologist

You can modify the Lab Director and Technologist roles, and you can create your own roles with customized settings as needed.

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools** ➤ **Security** ➤ **Settings**.  
2. From the Security Settings dialog box, select the **Roles** tab.

From the Roles tab, you can perform the actions in the following table:
### To... | Action
---|---
Create a user role | 1. Click **Create**.<br>2. Enter a role name and [optional] description.<br>3. Select permissions (see “Permissions and default user roles” on page 135). To select all permissions in a category, select the check box next to the category.<br><br>*Note:* Operations not shown in the following table are available to all user roles.<br><br>4. Click **Save Role**.

Edit a user role | 1. From the Roles screen, select a user role, then click **Edit**.<br>2. Edit settings as needed. You cannot edit the Administrator user role.<br>3. Click **Save Role**.

---

### Permissions and default user roles

*Note:* To determine the permissions for a default role or to edit it, select the role, then click **Edit**.

The following table shows all user-configurable permissions and the settings for the default user accounts.

<table>
<thead>
<tr>
<th>Category</th>
<th>Function</th>
<th>Lab Director</th>
<th>Technologist</th>
<th>Administrator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Setup</td>
<td>Create and edit experiments or experiment templates (includes running experiments)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Create, edit, and import sample attributes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Run</td>
<td>Perform a run using the Quickstart function</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Start a run</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Stop a run</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Targets (Library)</td>
<td>Create targets</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Edit targets</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Delete targets</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Analysis Settings (Library)</td>
<td>Create analysis settings (includes default settings)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Edit analysis settings (includes default settings)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Delete analysis settings</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Run Methods (Library)</td>
<td>Create a run method</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Delete a run method</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Dye (Library)</td>
<td>Create a custom dye</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Delete a dye</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
### View or print a user report

**Note:** The landscape and portrait functions are not available for the User or Roles reports. To print either report in landscape, save the report electronically (.pdf), then print the file in the desired orientation.

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select **Tools ▶ Security ▶ Settings**.
2. From the Security Settings dialog box, select the **Users** or **Roles** tab.
3. Click **View Report**.
4. From the Report screen, click tool bar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.
5. Click (Print) to print the report, or click (Save) to save the report electronically (.pdf). Close the report.

---

<table>
<thead>
<tr>
<th>Category</th>
<th>Function</th>
<th>Lab Director</th>
<th>Technologist</th>
<th>Administrator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>Create or edit a study (for example, edit a plate or analysis settings)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Add (transfer) experiments to a study</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Remove experiments from study</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Preferences</td>
<td>Edit the system preferences</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Export the system preferences</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Import the system preferences</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Calibrations</td>
<td>Perform calibrations</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>RNaseP</td>
<td>Perform an RNase P run</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Instrument</td>
<td>Add and remove instruments from monitoring</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Configuration</td>
<td>Upgrade and downgrade instrument firmware</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Security</td>
<td>Configure the security and audit feature</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Configuration</td>
<td>Log in to user sessions that have timed out</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Perform E-Signing</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Export</td>
<td>Export primary and secondary analysis results</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Audit trail management

Access the Audit screen and enable or disable auditing

Use the Audit screen to control the auditing state (disabled or enabled), the events that are audited, and the reasons available to users when audit mode is set to Prompt or Required.

Note: Auditing is disabled by default.

IMPORTANT! If you disable security, you inactivate audit and electronic signature functions; however, no audit record is generated to indicate that audit and electronic signature functions are disabled.

To enable auditing:

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select Tools > Security > Settings.
2. From the Security Settings dialog box, select the Audit tab.
3. Select or deselect Enable Audit.
4. Click Apply Settings.

Modify the auditing settings

<table>
<thead>
<tr>
<th>To…</th>
<th>Action</th>
</tr>
</thead>
</table>
| Select objects to audit | 1. In the Audit Settings group of the Audit tab, select the objects to audit and the mode for each enabled item [Experiments, Experiment Templates, or Study].
2. Set the Audit Mode for each item you enable for auditing:
   - **Optional** – The event is audited, a reason prompt is displayed, but the user can cancel and continue without entering a reason.
   - **Required** – The event is audited, a reason prompt is displayed, and the user must specify a reason.
   - **Silent** – The event is audited, no reason prompt is displayed.
3. Click Apply Settings. |
| Create audit reason settings | You can create, modify and delete the reasons that are available for selection in the Audit Reason dialog box (displayed when a user performs an audited action).
1. To require users to select a pre-defined reason in the Audit Reason dialog box (displayed when a user performs an audited action), enable **Require users to select a reason to change from the list**. Users are not permitted to enter a reason.
2. As needed, click **Create**, or select a reason from the list, then click **Edit** or **Delete**. |
Audit report generation

You can use the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to generate reports of audit history from both the Security Settings dialog box and from open experiments, templates, or studies.

■ Viewing audit histories from the Security Settings dialog box
■ Viewing audit histories from an experiment, template, or study

Viewing audit histories from the Security Settings dialog box

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select Tools ▶ Security ▶ Settings.
2. From the Security Settings dialog box, select the Audit tab, then click View Reports.

Note: To access the Audit Reports screen, the user role for an account must have the “Configure the security and audit feature” permission. Users without the permission can view object audit histories for individual entries in the libraries by selecting entries, then clicking View Audit History.

From the Audit Reports dialog box, you can perform the actions in the following table:

<table>
<thead>
<tr>
<th>To…</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Display audit histories</td>
<td>1. Select a tab to display:</td>
</tr>
<tr>
<td></td>
<td>• System Configuration History – Security, audit, and</td>
</tr>
<tr>
<td></td>
<td>electronic signature configuration records, including audit</td>
</tr>
<tr>
<td></td>
<td>history for each user account.</td>
</tr>
<tr>
<td></td>
<td>• Action Record – System-specified audit events.</td>
</tr>
<tr>
<td></td>
<td>2. (Optional) Select Filter by, then filter the table:</td>
</tr>
<tr>
<td></td>
<td>• Sort the table.</td>
</tr>
<tr>
<td></td>
<td>• Specify filters (date range, user name, action, object or</td>
</tr>
<tr>
<td></td>
<td>record type, object or record name, reason), then click</td>
</tr>
<tr>
<td></td>
<td>Refresh.</td>
</tr>
<tr>
<td></td>
<td>Note: The Reason field in System Configuration History is</td>
</tr>
<tr>
<td></td>
<td>not used.</td>
</tr>
<tr>
<td></td>
<td>• Select one or more records, then click View Report.</td>
</tr>
</tbody>
</table>

Review the action log

The Action Record log lists system-specified audit events.
All items in the action log are audited silently, except for the items noted as configurable. Configurable items may include comments in the action log.

• Audit Settings (Update)
• Auditing Event (Archive, Restore, Purge)
• Configuration (Import, Export)
• Data Audit (Archive, Restore, Purge)
• Login (Success, Failure)
• Logout (Success)
• Run (Start, Stop, Completed, Failed, Aborted, Error)
• User Account (Create, Update)
### View and print audit reports

1. Select the **System Configuration History** tab.
2. Display the records of interest.
3. Filter the list to decrease the time required to generate reports.

   **IMPORTANT!** You cannot cancel a report after you click a view button.

4. Click **View Report**.

   **Note:** From the audit report, the “Create” action value is listed as “Added”, “Delete” action value is displayed as “Removed”, and “Update” action value is displayed as “Updated”.

5. From the Report screen, click tool bar options to manipulate the report as needed. Place the mouse pointer over an item for a description of the item.
   - To print the report, click ![Print](button).
   - To save the report electronically (PDF), click ![Save](button).

6. Close the report.

### Archive, purge, and restore audit records

The audit archive function makes a copy of audit records. Purge makes a copy of audit records, and then deletes them. You can use the Restore function to restore purged audit records.

#### Archive and purge

To selectively archive or purge (delete) system configuration or action audit records:

1. Select the **System Configuration History** tab.
2. Select records in the appropriate screen.
3. Click **Archive** or **Purge**.
4. If you select Archive, specify a location and name for the archive file (.asz).

#### Restore

To restore system configuration or action audit records, click **Restore**, then select the .asz file to restore.

### Export audit records

You can export audit records to a .txt file for additional manipulation and reporting outside the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.

1. Display the records of interest as described above.
2. Click **Export**.
3. Specify a name and location for the export .txt file, then click **Save**.

   **Note:** If you export audit records for samples that have been deleted or moved, an error message is displayed. Return sample data files to their original location, then export again.

### Review the system configuration history

The System Configuration History lists security, audit, and electronic signature configuration records.
### Review the system configuration history

The following table summarizes the actions that can be audited using the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software. The System Configuration History lists security, audit, and electronic signature configuration records.

**Note:** If you have installed additional modules to the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, then the list can contain additional auditable records.

<table>
<thead>
<tr>
<th>Record type</th>
<th>Action</th>
<th>Corresponds to…</th>
</tr>
</thead>
<tbody>
<tr>
<td>Security Settings</td>
<td>Update</td>
<td>Disable, enable, or modify security policies and session time-out settings</td>
</tr>
<tr>
<td>Account Settings</td>
<td>Update</td>
<td>Modify password settings, security policies (password expiration and account suspension), or user name settings</td>
</tr>
<tr>
<td>User Group Manager</td>
<td>Update</td>
<td>Create, delete, or modify reason for change</td>
</tr>
<tr>
<td>User Role</td>
<td>Create</td>
<td>Create user role</td>
</tr>
<tr>
<td></td>
<td>Delete</td>
<td>Delete user role</td>
</tr>
<tr>
<td></td>
<td>Update</td>
<td>Modify user role</td>
</tr>
<tr>
<td>User Account</td>
<td>Create</td>
<td>Create new user account</td>
</tr>
<tr>
<td></td>
<td>Update</td>
<td>Edit or suspend a user account</td>
</tr>
<tr>
<td>Role Assignment</td>
<td>Delete</td>
<td>Assign a different user role to an existing user account</td>
</tr>
<tr>
<td></td>
<td>Update</td>
<td>Create a user account, or assign a different user role to an existing account</td>
</tr>
<tr>
<td>Audit Settings</td>
<td>Update</td>
<td>Enable or disable auditing</td>
</tr>
<tr>
<td>Audit Type</td>
<td>Update</td>
<td>Modify audit settings</td>
</tr>
<tr>
<td>Function Management Settings</td>
<td>Update</td>
<td>Update function management</td>
</tr>
<tr>
<td>Function Access Manager</td>
<td>Update</td>
<td>Update function access management</td>
</tr>
<tr>
<td>Function</td>
<td>Create</td>
<td>Create function</td>
</tr>
<tr>
<td></td>
<td>Delete</td>
<td>Delete function</td>
</tr>
<tr>
<td></td>
<td>Update</td>
<td>Update function</td>
</tr>
<tr>
<td>Role Permissions</td>
<td>Create</td>
<td>Create a user role†</td>
</tr>
<tr>
<td></td>
<td>Delete</td>
<td>Delete a user role</td>
</tr>
<tr>
<td></td>
<td>Update</td>
<td>Modify user role permissions</td>
</tr>
<tr>
<td>Audit Reason for Change</td>
<td>Delete</td>
<td>Create reason for change</td>
</tr>
<tr>
<td></td>
<td>Update</td>
<td>Delete or modify reason for change</td>
</tr>
<tr>
<td>Event Manager</td>
<td>Update</td>
<td>Update the event manager</td>
</tr>
<tr>
<td>E-signature Manager</td>
<td>Update</td>
<td>Enable or disable e-signature</td>
</tr>
<tr>
<td>E-signature Type</td>
<td>Create</td>
<td>Create an e-signature meaning</td>
</tr>
<tr>
<td></td>
<td>Delete</td>
<td>Delete an e-signature meaning</td>
</tr>
<tr>
<td></td>
<td>Update</td>
<td>Edit an e-signature meaning or an e-signature action</td>
</tr>
<tr>
<td>E-signature Function</td>
<td>Update</td>
<td>Edit an action requiring e-signature</td>
</tr>
<tr>
<td>File Name</td>
<td>Update</td>
<td>Update the file name</td>
</tr>
<tr>
<td>File Version</td>
<td>Update</td>
<td>Update the file version</td>
</tr>
</tbody>
</table>
1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, open an experiment (.eds), template (.edt), or study (.edm) file.

2. From the open file, click Audit, then click Audit Records.

3. (Studies only) Select the audit records of interest:
   - Study to view the audit records for the study.
   - The name of an experiment to view the audit records for the experiment.

From the Audit Records screen of the open file, you can perform the actions in the following table:

<table>
<thead>
<tr>
<th>To...</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Display the audit history</td>
<td>(Optional) Filter the table.</td>
</tr>
<tr>
<td></td>
<td>1. Check the Filter by check box.</td>
</tr>
<tr>
<td></td>
<td>2. Enter criteria for the records of interest, such as a date range, a user name, or a type of action.</td>
</tr>
<tr>
<td></td>
<td>3. Click Refresh.</td>
</tr>
<tr>
<td></td>
<td>To view details for a specific record:</td>
</tr>
<tr>
<td></td>
<td>1. Click the row in the list on the left to view the details of the record in the table on the upper right.</td>
</tr>
<tr>
<td></td>
<td>2. Click any row to view details for individual records in the table on the bottom right.</td>
</tr>
<tr>
<td>Export audit records</td>
<td>1. From the table on the left, select the records to be exported:</td>
</tr>
<tr>
<td></td>
<td>- Click in the table, then press Ctrl-A to select all the records in the table.</td>
</tr>
<tr>
<td></td>
<td>- Click and drag or press Shift to select continuous rows.</td>
</tr>
<tr>
<td></td>
<td>- Press Ctrl to select discontinuous rows.</td>
</tr>
<tr>
<td></td>
<td>2. Export the records:</td>
</tr>
<tr>
<td></td>
<td>- Click Export Summary to export only the records in the left table.</td>
</tr>
<tr>
<td></td>
<td>- Click Export Details to export the records in the left table and the associated details.</td>
</tr>
<tr>
<td></td>
<td>3. Select a location for the export file, enter a name for the file, then click Save.</td>
</tr>
<tr>
<td></td>
<td>4. Click OK in the confirmation message.</td>
</tr>
<tr>
<td>Print audit records</td>
<td>1. Click View Report to open the Print Preview dialog box.</td>
</tr>
<tr>
<td></td>
<td>2. Preview, save or print the report:</td>
</tr>
<tr>
<td></td>
<td>- Click [Save] to save the report as a .pdf or .html file. Enter the file name, select a location, select the file type, then click Save.</td>
</tr>
<tr>
<td></td>
<td>- Click [Print] to send the report to the printer. From the Print dialog box, select the printer and print options, then click OK.</td>
</tr>
<tr>
<td></td>
<td>3. Click [X] to close the Print Preview dialog box.</td>
</tr>
</tbody>
</table>
Manage electronic signature

Access the e-signature settings screen and enable or disable e-sig

Use the Security screen to disable and enable electronic signature.

**Note:** Electronic signature is disabled by default.

To enable or disable electronic signature:

1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, select Tools ➤ Security ➤ Settings.
2. From the Security Settings dialog box, select the **e-Signature** tab.
3. Select or deselect **Enable e-Signature**.

**IMPORTANT!** Enabling the electronic signature feature can substantially increase the size of experiment, template, and study files.

**IMPORTANT!** If you disable security, you inactivate audit and electronic signature functions. No audit record is generated for the disabling of audit and electronic signature functions when you disable security.

4. Click **Apply Settings**.

Configure the meanings of the electronic signatures

The e-signature meanings are the text that a user can select to describe a reason for an electronic signature. The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is installed with the following default meanings.

<table>
<thead>
<tr>
<th>E-Signature definition</th>
<th>Default data types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plate setup</td>
</tr>
<tr>
<td>Reviewed and Approved Plate Set Up</td>
<td>Yes</td>
</tr>
<tr>
<td>Reviewed and Approved Results</td>
<td>No</td>
</tr>
<tr>
<td>Reviewed and Approved Template</td>
<td>No</td>
</tr>
</tbody>
</table>

Use the Security Settings dialog box to add or remove electronic signature meanings and to determine the data types to which they apply.

<table>
<thead>
<tr>
<th>To...</th>
<th>Action</th>
</tr>
</thead>
</table>
| Add a meaning        | 1. From the e-Signature tab of the Security Settings dialog box, click **Add** in the e-Signature Meanings settings.  
2. From the Create Meaning dialog box, enter a description of the e-Signature meaning, then click **OK**.  
3. Select what data is signed for the selected meaning.  
4. Click **Apply Settings**. |
| Delete a meaning     | 1. Select the meaning from the e-Signature Meanings list, then click **Remove**.  
2. Click **Apply Settings**. |
To determine the user roles that can perform an electronic signature:

1. From the e-Signature tab of the Security Settings dialog box, select the check box next to the appropriate user roles in the User Role signature rights table.

2. Click Apply Settings.

**IMPORTANT!** Do not change electronic signature settings during calibration.

1. In the Signature Required column, select the check box next to each action for which you want to require electronic signatures (see below). This selection causes the software to present an e-sig prompt if a user performs the action on a data file that does not have the required signatures. The data must be signed before the user can perform the action.

<table>
<thead>
<tr>
<th>Action</th>
<th>The software requires e-signatures when a user...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Print Report</td>
<td>Prints a report from an experiment or study.</td>
</tr>
<tr>
<td>Start Run</td>
<td>Initiates a run from the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software.</td>
</tr>
</tbody>
</table>

2. For each meaning of each selected action, enter the number of e-signatures from each user role that are required before the software can execute the associated action. For example, in the following figure, at least two users from the Administrator user role must sign an experiment using the “Reviewed and Approved Plate Set Up” meaning before a user can start the associated run.

<table>
<thead>
<tr>
<th>Actions requiring signatures</th>
<th>Number of signatures required for the selected action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signature Required</td>
<td>Action</td>
</tr>
<tr>
<td>________________________</td>
<td>____</td>
</tr>
<tr>
<td>Yes</td>
<td>Print Report</td>
</tr>
<tr>
<td>Yes</td>
<td>Start Run</td>
</tr>
<tr>
<td>Yes</td>
<td>Reviewed and Approved Plate Set Up</td>
</tr>
<tr>
<td>Yes</td>
<td>Reviewed and Approved Results</td>
</tr>
<tr>
<td>Yes</td>
<td>Reviewed and Approved Template</td>
</tr>
</tbody>
</table>

3. Click Apply Settings.

If the system is configured to check for a signature before starting a run or printing a report and the data are not signed, the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software displays a message when the user clicks Start Run or Print Report.

**Example**

The e-signature system is configured to require signatures from two users from the user account named Administrator before a user can start a run. The experiment has not been signed.

A user attempts to begin the run. The following message is displayed:
Before the run can start, two administrators must sign. If a user with an incorrect user role signs, the message is displayed again.

## Generate e-signature reports

You can use the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software to generate reports of e-signature history from open experiment (.eds) or template (.edt) files.

<table>
<thead>
<tr>
<th>Task</th>
<th>Description</th>
</tr>
</thead>
</table>
| Display the e-sig records   | 1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, open an experiment or template file.  
2. From the open experiment, click [Audit], then click **E-Signatures**.  
3. (Optional) Click any row to view details for individual signatures. |
| Save or print e-sig records | 1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, open an experiment or template file.  
2. From the open experiment, click [Audit], then click **E-Signatures**.  
3. From the table, select the record to be saved or printed.  
4. Save or print the record:  
   • Click [Save], select a location for the export file, enter a name for the file, then click **Save**.  
   or  
   • Click [Print].  
5. Click **OK** in the confirmation message. |
| Save or print the table of e-signature events | 1. From the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, open an experiment or template file.  
2. From the open file, click [Audit], then click **Print E-Signatures**.  
3. Save or print the record:  
   • Click [Save], select a location for the export file, enter a name for the file, then click **Save**.  
   or  
   • Click [Print].  
4. Click **OK** in the confirmation message. |
Export and import user, security, audit, and e-signature settings

**Note:** The export/import feature can be used to replicate identical security, audit, and e-signature settings across multiple computers. The feature allows you to create a standard security, audit, and e-signature settings “image” for the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software that can then be imported by other copies of the software to bypass manual setup.

<table>
<thead>
<tr>
<th>Task</th>
<th>Description</th>
</tr>
</thead>
</table>
| **Export** | 1. In any screen of the Security Settings dialog box, click **Export**.  
2. Select the items to export: **All** or **Custom**.  
Custom contains select settings:  
   - **Users & Roles** – All user accounts with “Active” status and all user roles and associated permissions (in case a user account specifies a user role that does not exist on the system into which you import the profiles).  
   - **System & Roles** – All system settings and all user roles and associated permissions.  
3. Click **Export** or **OK**.  
4. When prompted, specify the name and location for the exported file (.dat), then click **Save**. A message is displayed when the export completes. |
| **Import** | 1. In any screen in the Security Settings dialog box, click **Import** in the Experiment Menu.  
2. Select the .dat file to import, then click **Open**. A message is displayed asking if you want to overwrite the current system configuration. Click **Yes**.  
If any imported user accounts already exist on the system, you are prompted to overwrite or skip each account. |
User overview

The Security, Audit, and Electronic Signature (SAE) module is a component of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software that provides the following functionality:

- **System security** – Controls user access to the software.
- **Auditing** – Tracks changes made to library items, actions performed by users, and changes to the Security, Audit, and Electronic Signature settings.
- **Electronic signature** – Requires users to provide a user name and password when performing certain functions.

Depending on the way that your administrator configures these features, you may see the following dialog boxes and prompts when you use the software.

**User account permissions**

If your user account does not have permission to perform any function in the software, menu commands are shaded.

**Account suspension**

If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is configured to suspend a user account for failed logins, and if you repeatedly enter an incorrect user name and/or password, your user account is suspended, and the Log In dialog box indicates that your account is inactive.

There are two ways to activate a suspended account:

- You can wait until the suspension period ends.
- An administrator can change the account status from Suspended to Active.

**Note:** While a user is suspended, another user can click Reset, then log in and replace the suspended user.

**Session time-out**

If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is configured to time-out and there is no user activity for the specified time, the Log In dialog box indicates that your user session has timed out. You must enter your user name and password to access the software.

The administrator or another user with permission to log in to timed-out sessions can click Reset, then log in.
## User security tasks

<table>
<thead>
<tr>
<th>Task</th>
<th>Description</th>
</tr>
</thead>
</table>
| Log in | Your access to functions in the software is based on the permissions associated with your user account. Functions for which you do not have permissions are shaded.  
**Note:** If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is configured for password expiration, you are periodically prompted to change your password.  
**Note:** If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is configured to monitor failed log in attempts, you will be locked out of the software if you repeatedly enter an incorrect user name and/or password. |
| Change your password when it expires | When your password is about to expire, a message is displayed when you log in.  
To change your password, select **Tools ▶ Change Password**. Enter your current password, then enter the new password two times, then click **OK**. |
| Enter a reason for change | If the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software is configured for auditing, you may be prompted to specify a reason when you make certain changes in the software.  
Depending on your QuantStudio™ 6 and 7 Flex Real-Time PCR System Software configuration, you can either select a reason from the list or enter a reason for change. |
| Perform an electronic signature | If your system is configured for electronic signature, you may be required to have the experiment signed by other users before you can print a report or start a run. If an item is set to require multiple signatures, all approvers must sign the associated data before the action can be completed.  
If electronic signature is enabled for experiments, any of the following may apply:  
- The **Tools ▶ Security ▶ Sign Data** menu option is enabled.  
- You are prompted to sign. |
Appendix E  Security, Audit, and Electronic Signature
User overview
Calibration Consumable Preparation

- Create a background plate or array card ........................................... 150
- Create a custom dye plate for calibration ............................................ 152
Create a background plate or array card

Whenever possible, use a Background Plate or the TaqMan® Array Background Buffer that is included with the spectral calibration kit. The plates/array cards supplied in the kit contain a buffer that accurately simulates the reagents used for PCR, and, therefore, produces high-quality calibration data. If a background plate or array card from a spectral calibration kit is not available, you can create one as described below.

Required materials

96/384-Well Plate Sample Block
- MicroAmp® Optical 96/384-Well Reaction Plate
- Safety glasses
- Optical Adhesive Cover or Optical Flat Caps
- Pipettor, 200-µL (with pipette tips)
- Powder-free gloves
- Deionized water

Array Card Sample Block (QuantStudio™ 7 Flex System only)
- Array Cards
- Applied Biosystems® Array Card Staker/Sealer
- Centrifuge with array card buckets and array card carrier clips
- Permanent marker or pen
- Pipettor, 200-µL (with pipette tips)
- Powder-free gloves
- Safety glasses
- Deionized water

Create a background plate

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

1. Remove a MicroAmp®96/384-Well Optical Reaction Plate from its box and place it on a clean, dry surface.
2. Aliquot 20 µL deionized water to each well of the reaction plate.
3. Seal the plate using an optical adhesive cover or optical flat caps.
4. Use the plate for background calibration as you would a background plate from the spectral calibration kit.
Create a background array card (QuantStudio™ 7 Flex System only)

1. Remove an array card from its box and place it on a clean, dry surface.
2. Using a permanent marker, write “Background” on the side of the empty card.

3. Pipet 100 µL of deionized water into each of the eight reservoirs in the card:
   a. Place the array card on a lab bench, with the foil side down.
   b. Load 100 µL of the solution into a pipette.
   c. Hold the pipette in an angled position (~45°) and place the tip into the fill port.
      There is a fill port on the left arm of each fill reservoir – the larger of the two holes.
   d. Dispense the fluid so that it sweeps in and around the fill reservoir toward the vent port.
      When pipetting the reagents into the array card, pipet the entire 100-µL volume into the fill reservoir, but do not go past the first stop of pipettor plunger or you may blow the solution out of the port.

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

4. Centrifuge and seal the array card as explained in “Filling the calibration array cards” on page 36.
Create a custom dye plate for calibration

The QuantStudio™ 6 or 7 Flex System can be used to run assays designed with custom dyes (dyes not manufactured by Life Technologies). Custom dyes must excite between 455 and 672 nm and read between 505 and 723 nm.

Before you use custom dyes

Before using custom dyes with the QuantStudio™ 6 or 7 Flex System, you must:

- Determine optimum dye concentration.
- Create a custom dye plate.
- Add the custom dye to the software.
- Perform a dye calibration.

Required materials

- Centrifuge with plate adapter
- Custom dye(s)
- Safety glasses
- Powder-free gloves
- MicroAmp® Optical 96/384-Well Reaction Plate
- Optical Adhesive Cover
- Pipettors and pipette tips (200-µL and 1000-µL)
- Tubes (2-mL and 10-mL)
- Deionized water

Note: Wear powder-free gloves while creating the dye plate.

1. Prepare and load the custom dye plate:
   a. In the center of a 96/384-well plate, prepare a dilution series of the custom dye (for example, 25, 50, 100, 200, 400, 800, 1600, and 3200 nM) using 20 µL volumes for a 96/384-well plate.
   b. Seal the reaction plate using an optical adhesive cover.
   c. Load the prepared reaction plate.

2. Start the calibration wizard:
   a. In the Home screen of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, click Instrument Console.
   b. In the Instrument Console, select your QuantStudio™ 6 or 7 Flex System, then click Add to My Instruments.
   c. Select the QuantStudio™ 6 or 7 Flex System, then click Manage Instrument.
   d. In the Instrument Manager, click Maintenance, then click ROI.
   e. In the ROI Calibration screen, click Start Calibration.
   f. In the ROI dialog box, click Next until prompted to load the QuantStudio™ 6 or 7 Flex System. When the side door opens, load the sealed plate. Ensure that the plate/array card is properly aligned in the holder.
g. In the ROI dialog box, select Check the box when the ROI calibration plate has been loaded, click Next twice, then click START RUN to start the calibration.

3. When the run is complete, inspect the ROI images:
   a. Select the first filter from the Filter drop-down list.
   b. Record the coordinate of the well that contains the lowest concentration of dye and that is encircled by a ring. This well contains the optimal concentration of the custom dye at the given filter.
   c. Repeat steps a and b for the remaining filters.
   d. After you determine the optimum concentration for each filter, determine the optimum concentration for the custom dye. Compare the results from all filters, then select the concentration that yields the highest possible signal in all filters.

4. Discard the plate.

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

5. In the ROI dialog box, click Finish to complete the calibration, then click No when prompted to save the results.

---

**Create a custom dye plate**

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

1. Prepare 2 mL of the custom dye at the concentration determined in “Determine optimum dye concentration” on page 152.
2. Pipet 20 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 minutes at < 1500 rpm.
   **Note:** The custom dye calibration plate must be well mixed and centrifuged.
5. 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.

<table>
<thead>
<tr>
<th>Correct</th>
<th>Incorrect</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Correct" /> Liquid is at bottom of well.</td>
<td><img src="image" alt="Incorrect" /> - Not centrifuged with enough force, or - Not centrifuged for enough time</td>
</tr>
</tbody>
</table>
Add the custom dye to the software

1. Start the dye calibration:
   a. In the Home screen of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, click **Instrument Console**.
   b. In the Instrument Console, select your QuantStudio™ 6 or 7 Flex System from the list of instruments on the network, then click **Add to My Instruments**.
   c. Select the QuantStudio™ 6 or 7 Flex System, then click **Manage Instrument**.
   d. In the Instrument Manager, click **Maintenance**, then click **Dye**.
   e. In the Background Calibration screen, click **Start Calibration**.

2. In the Dye window, select a custom dye from the list or create the custom dye:
   a. Click **New Dye**.
   b. In the Dye Library dialog box, click **New**.
   c. Complete the New Dye dialog box, then click **OK**.
   d. Click **Close**.

3. In the Dye window, enter a temperature setting for the calibration. Set the temperature to match the temperature at which you intend to collect data. For example, the temperature for all Life Technologies system dyes is 60°C because data collection for TaqMan® reagents occurs during the 60°C extension step of the PCR.

4. Load the appropriate dye plate into the plate adapter, select **Please check the box when the dye calibration plate has been loaded**, click Next twice, then click **START RUN** to start the calibration.

5. When the run is complete and the QuantStudio™ 6 or 7 Flex System ejects the plate, remove and discard the plate or array card.

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

6. In the Dye dialog box of the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, click **Next**.

7. 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 QuantStudio™ 6 and 7 Flex Systems.
   • Free of irregular spectral peaks.
   • Present at the correct filter for the dye.

**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.

8. Verify the status of the calibration. If the calibration:

   **Passed** – If all spectra are acceptable, finish the calibration:
   a. Click Next.
   b. Enter any comments you have in the Comments field, click Finish, then click Yes when prompted to save the calibration results.

   **Failed** – Create another custom dye plate using the next dye concentration greater than the concentration determined in “Determine optimum dye concentration” on page 152, then perform the calibration again.
Appendix F Calibration Consumable Preparation

Create a custom dye plate for calibration
Parts and Materials

- How to order ................................................. 158
- Kits, consumables, and accessories ............................. 160
- General-use materials and consumables ...................... 165
How to order

You can order materials and accessories directly from the Life Technologies store over the internet.

Note: Product availability and pricing may vary according to your region or country. Online ordering through the Life Technologies Store is not available in all countries. Contact your local Life Technologies representative for help.

To order through the website or the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software:

- Confirm that your computer has an Internet connection.
- We recommend the following browsers and Adobe® Acrobat® Reader versions to use the Life Technologies website:

<table>
<thead>
<tr>
<th>Operating system</th>
<th>Microsoft® Internet Explorer®</th>
<th>Apple® Safari®</th>
<th>Mozilla® Firefox®</th>
<th>Adobe® Acrobat® Reader</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsoft® Windows®</td>
<td>v6.x or later</td>
<td>None†</td>
<td>v2.x or later</td>
<td>v4.0 or later</td>
</tr>
<tr>
<td>Macintosh®</td>
<td>None†</td>
<td>v2.0.4 or later</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Browser not available for this platform.

Note: Confirm that cookies and Javascript are turned on for the website to function correctly.

How to order from the Life Technologies website

<table>
<thead>
<tr>
<th>To order...</th>
<th>Procedure</th>
</tr>
</thead>
</table>
| Assays and reagents | 1. Go to www.lifetechnologies.com  
2. Under “I Want to Buy,” select the product of interest. |
| Instrument parts and accessories | 1. Go to www.lifetechnologies.com/quantstudio6flex or www.lifetechnologies.com/quantstudio7flex  
2. Click Parts and accessories.  
3. Select the desired components, the complete the order as instructed. See "Kits, consumables, and accessories” on page 160 for a complete list of compatible instrument parts, accessories, and kits. |
| Calibration kits | |

How to order from the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software

1. To find your assay on the Life Technologies Store, complete the Find Assay pane in the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software:
   a. Enter a gene name in the Enter Gene Name field, then click Find Assay.
   b. In the Find Assay Results dialog box, select your assay.
   c. Click Apply Assay Selection. The selected assay gets added to your shopping list.

2. Check that the Experiment Shopping List contains the desired materials, other than the assay selected in the previous step, and that the quantities are correct, then click Order Materials in List.
3. In the Order Materials - Login dialog box, enter your user name and password for the Life Technologies Store, then click **Log In and Submit**.

![Image of Order Materials - Login dialog box](image)

**Note:** If you do not have an account with the Life Technologies Store, click **Register Now** to create an account.

When you are connected to the Life Technologies Store, follow the prompts to complete your order.
Kits, consumables, and accessories

The following kits, consumables, and reagents are to be used with the QuantStudio™ 6 and 7 Flex Systems.

- Fast 96-well consumables ................................................................. 160
- 96-well consumables ...................................................................... 161
- 384-well consumables .................................................................... 162
- Array card consumables ................................................................. 163
- QuantStudio™ 6 and 7 Flex Systems accessories ......................... 164

Note: For reagent or consumable shelf-life expiration date, see the package label.

Note: The QuantStudio™ 6 Flex System does not support the Array Card block.

### Fast 96-well consumables

<table>
<thead>
<tr>
<th>Consumable</th>
<th>Part no.</th>
<th>Storage (°C)</th>
<th>Shelf life at storage temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast 96-Well Spectral Calibration Plate with FAM™ Dye</td>
<td>4432600</td>
<td>≤–20</td>
<td>Use the consumable by the expiration date on the packaging</td>
</tr>
<tr>
<td>Fast 96-Well Spectral Calibration Plate with VIC® Dye</td>
<td>4432607</td>
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<tr>
<td>Fast 96-Well Spectral Calibration Plate with ROX™ Dye</td>
<td>4432614</td>
<td></td>
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<tr>
<td>Fast 96-Well Spectral Calibration Plate with TAMRA™ Dye</td>
<td>4432632</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast 96-Well Region of Interest (ROI) and Background Plates</td>
<td>4432638</td>
<td>≤–20</td>
<td></td>
</tr>
<tr>
<td>• Fast 96-Well Region of Interest (ROI) Calibration Plate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Fast 96-Well Background Plate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fast 96-Well Normalization Plates with FAM™/ROX™ and VIC®/ROX™ Dyes</td>
<td>4443774</td>
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<tr>
<td>• Fast 96-Well Normalization Plate with FAM™/ROX™ Dye</td>
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<tr>
<td>• Fast 96-Well Normalization Plate with VIC®/ROX™ Dye</td>
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<tr>
<td>RNase P Fast 96-Well Instrument Verification Plate</td>
<td>4432656</td>
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<tr>
<td>Fast 96-Well Background Plate</td>
<td>4481308</td>
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</table>
# 96-well consumables

<table>
<thead>
<tr>
<th>Consumable</th>
<th>Part no.</th>
<th>Storage (°C)</th>
<th>Shelf life at storage temperature</th>
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</thead>
<tbody>
<tr>
<td>96-Well Spectral Calibration Plate with FAM™ Dye</td>
<td>4432538</td>
<td>≤–20</td>
<td>Use the consumable by the expiration date on the packaging.</td>
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<tr>
<td>96-Well Spectral Calibration Plate with VIC® Dye</td>
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<td>96-Well Spectral Calibration Plate with ROX™ Dye</td>
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<tr>
<td>96-Well Spectral Calibration Plate with TAMRA™ Dye</td>
<td>4442918</td>
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<tr>
<td>96-Well Region of Interest (ROI) and Background Plates</td>
<td>4432626</td>
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<td>• 96-Well Region of Interest (ROI) Calibration Plate</td>
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<tr>
<td>• 96-Well Background Plate</td>
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<tr>
<td>96-Well Normalization Plates with FAM™/ROX™ and VIC®/ROX™ Dyes</td>
<td>4443661</td>
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<tr>
<td>• 96-Well Normalization Plate with FAM™/ROX™ Dye</td>
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<td>• 96-Well Normalization Plate with VIC®/ROX™ Dye</td>
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<td>96-Well Background Plate</td>
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</table>
## Kits, consumables, and accessories

### 384-well consumables

<table>
<thead>
<tr>
<th>Consumable</th>
<th>Part no.</th>
<th>Storage (°C)</th>
<th>Shelf life at storage temperature</th>
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</thead>
<tbody>
<tr>
<td>384-Well Spectral Calibration Plate with FAM™ Dye</td>
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<tr>
<td>384-Well Spectral Calibration Plate with VIC® Dye</td>
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<td>384-Well Spectral Calibration Plate with ROX™ Dye</td>
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<tr>
<td>384-Well Spectral Calibration Plate with TAMRA™ Dye</td>
<td>4443619</td>
<td>≤-20</td>
<td>Use the consumable by the expiration date on the packaging.</td>
</tr>
<tr>
<td>384-Well Region of Interest (ROI) and Background Plates</td>
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<td>• 384-Well Region of Interest (ROI) Calibration Plate</td>
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<td>• 384-Well Background Plate</td>
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<tr>
<td>384-Well Normalization Plates with FAM™/ROX™ and VIC®/ROX™ Dyes</td>
<td>4432620</td>
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<tr>
<td>• 384-Well Normalization Plate with FAM™/ROX™ Dye</td>
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<td>• 384-Well Normalization Plate with VIC®/ROX™ Dye</td>
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<td>RNase P 384-Well Instrument Verification Plate</td>
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<td>384-Well Background Plate</td>
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### Array card consumables

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<th>Consumable</th>
<th>Part no.</th>
<th>Storage (°C)</th>
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</thead>
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<tr>
<td>Array Card Spectral Calibration Dye Kit</td>
<td>4432644</td>
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<td>• Array Calibration with FAM™ Dye</td>
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<tr>
<td>• Array Calibration with VIC® Dye</td>
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<td></td>
</tr>
<tr>
<td>• Array Calibration with ROX™ Dye</td>
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</tr>
<tr>
<td>• Array Calibration with ROI Dye</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Array Background Buffer</td>
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<td></td>
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</tr>
<tr>
<td>• Array Calibration with FAM™/ROX™ Dye</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Array Calibration with VIC®/ROX™ Dye</td>
<td></td>
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</tr>
<tr>
<td>Kit, RNase P Array Card Instrument Verification Reagents</td>
<td>4432650</td>
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<td>Use the consumable by the expiration date on the packaging.</td>
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<td>• Port 2 Unknown A</td>
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<td>• Port 3 Unknown B</td>
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<td>• Port 4 Standard 200 Copies</td>
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<td>• Port 5 Standard 400 Copies</td>
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<tr>
<td>Array Card Background Buffer Kit</td>
<td>4481307</td>
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<tr>
<td>• Background Buffer, 6 tubes</td>
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<tr>
<td>• Array Card Kit [empty], 6-pack</td>
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</tr>
</tbody>
</table>

**Note:** The Array Card block consumables are applicable only to the QuantStudio™ 7 Flex System.
### QuantStudio™ 6 and 7 Flex Systems accessories

<table>
<thead>
<tr>
<th>Accessory</th>
<th>Part no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>384-Well Plate/Array Card Heated Cover</td>
<td>4454163</td>
</tr>
<tr>
<td>384-Well Plate Sample Block</td>
<td>4454165</td>
</tr>
<tr>
<td>96-Well Plate Heated Cover</td>
<td>4459841</td>
</tr>
<tr>
<td>96-Well Plate Sample Block</td>
<td>4459839</td>
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<tr>
<td>Array Card Bucket/Clip Set</td>
<td>4452205</td>
</tr>
<tr>
<td>Array Card Sample Block</td>
<td>4454164</td>
</tr>
<tr>
<td>Fast 96-Well Plate Heated Cover</td>
<td>4459842</td>
</tr>
<tr>
<td>Fast 96-Well Plate Sample Block</td>
<td>4459840</td>
</tr>
<tr>
<td>Halogen lamp [12 V, 75 W]</td>
<td>4480914</td>
</tr>
<tr>
<td>Handheld Barcode Scanner</td>
<td>4453271</td>
</tr>
<tr>
<td>Life Technologies Array Card Staker/Sealer</td>
<td>4456102</td>
</tr>
</tbody>
</table>

### QuantStudio™ 6 and 7 Flex Systems empty plates and array cards

<table>
<thead>
<tr>
<th>Consumable</th>
<th>Part no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-Well Fast Plate (empty), 10-pack</td>
<td>4366907</td>
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<tr>
<td>96-Well Fast Plate with Barcode (empty), 10-pack</td>
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<td>96-Well Plate (empty), 10-pack</td>
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<td>96-Well Plate with Barcode (empty), 10-pack</td>
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<tr>
<td>Adhesive Seal Applicator Kit, 5-pack</td>
<td>4481196</td>
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<tr>
<td>Array Card Kit (empty), 1-pack†</td>
<td>4445702</td>
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<tr>
<td>Array Card Kit (empty), 4-pack†</td>
<td>4445701</td>
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<tr>
<td>Optical Adhesive Covers, 25-pack</td>
<td>4481193</td>
</tr>
</tbody>
</table>

† QuantStudio™ 7 Flex System only.
General-use materials and consumables

The following general-use materials and consumables are required to calibrate, maintain, and operate the QuantStudio™ 6 and 7 Flex Systems. Unless indicated otherwise, all materials shown below are available from major laboratory suppliers (MLS).

<table>
<thead>
<tr>
<th>Material/consumable</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleach, 10% solution</td>
<td>MLS</td>
</tr>
<tr>
<td>Centrifuge with 96-well plate buckets</td>
<td>MLS</td>
</tr>
<tr>
<td>Cotton or nylon swabs and lint-free cloths</td>
<td>MLS</td>
</tr>
<tr>
<td>Ethanol, 95% solution</td>
<td>MLS</td>
</tr>
<tr>
<td>Optical clear adhesive film for PCR</td>
<td>MLS</td>
</tr>
<tr>
<td>Pipettors, 100-µL and 200-µL (with pipette tips)</td>
<td>MLS</td>
</tr>
<tr>
<td>Powder-free gloves</td>
<td>MLS</td>
</tr>
<tr>
<td>Safety glasses</td>
<td>MLS</td>
</tr>
<tr>
<td>Screwdriver, flathead</td>
<td>MLS</td>
</tr>
<tr>
<td>Tissue, lint-free</td>
<td>MLS</td>
</tr>
<tr>
<td>Deionized water</td>
<td>MLS</td>
</tr>
</tbody>
</table>
Instrument Specification and Layout

- QuantStudio™ 6 and 7 Flex Systems specifications .......................... 168
- QuantStudio™ 6 and 7 Flex Systems layout and connections ............. 171
QuantStudio™ 6 and 7 Flex Systems specifications

The figure below summarizes the specifications and requirements for the QuantStudio™ 6 and 7 Flex Systems. For more information, refer to the QuantStudio™ 6 and 7 Flex Systems Site Preparation Guide.

<table>
<thead>
<tr>
<th>Component</th>
<th>Height</th>
<th>Depth</th>
<th>Width</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>75.0 cm</td>
<td>70.0 cm</td>
<td>53.0 cm</td>
<td>70 kg</td>
</tr>
<tr>
<td></td>
<td>[29.5 in]</td>
<td>[27.6 in]</td>
<td>[20.9 in]</td>
<td>[154.3 lbs]</td>
</tr>
<tr>
<td>Computer</td>
<td>56.5 cm</td>
<td>54.7 cm</td>
<td>21.6 cm</td>
<td>10 kg</td>
</tr>
<tr>
<td></td>
<td>[22.3 in]</td>
<td>[22.4 in]</td>
<td>[8.5 in]</td>
<td>[22.0 lbs]</td>
</tr>
<tr>
<td>Monitor</td>
<td>38.0 cm</td>
<td>13.7 cm</td>
<td>37.4 cm</td>
<td>3.0 kg</td>
</tr>
<tr>
<td></td>
<td>[15.0 in]</td>
<td>[5.4 in]</td>
<td>[14.7 in]</td>
<td>[6.7 lbs]</td>
</tr>
<tr>
<td>Keyboard</td>
<td>2.75 cm</td>
<td>44.5 cm</td>
<td>13.75 cm</td>
<td>0.75 kg</td>
</tr>
<tr>
<td></td>
<td>[1.1 in]</td>
<td>[17.8 in]</td>
<td>[5.5 in]</td>
<td>[1.65 lbs]</td>
</tr>
</tbody>
</table>

† Weight varies depending on the sample block installed.

Required clearances

The QuantStudio™ 6 and 7 Flex Systems require the following additional clearances:

<table>
<thead>
<tr>
<th>Component</th>
<th>Top</th>
<th>Front</th>
<th>Sides</th>
<th>Back</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>30.5 cm</td>
<td>122 cm</td>
<td>51.0 cm</td>
<td>15.2 cm</td>
</tr>
<tr>
<td></td>
<td>[12.0 in]</td>
<td>[48.0 in]</td>
<td>[20.0 in]</td>
<td>[6.0 in]</td>
</tr>
<tr>
<td>Computer</td>
<td>—</td>
<td>30.5 cm</td>
<td>—</td>
<td>15.2 cm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[12.0 in]</td>
<td></td>
<td>[6.0 in]</td>
</tr>
</tbody>
</table>

Appendix H  Instrument Specification and Layout
QuantStudio™ 6 and 7 Flex Systems specifications
Electrical requirements

**WARNING!** For safety, the power outlet used for powering the instrument must be accessible at all times. See “Required clearances” on page 168 for information about the space needed between the wall and the instrument. In case of emergency, you must be able to immediately disconnect the main power supply to all the equipment. Allow adequate space between the wall and the equipment so that the power cords can be disconnected in case of emergency.

**Note:** Place the QuantStudio™ 6 or 7 Flex System and computer power receptacle on an electrical circuit that is not shared with electrically noisy devices or devices that can cause power surges, such as refrigeration units.

The following table provides electrical specifications for the instrument and associated devices. For all indicated input voltages, a 15 A circuit is required.

<table>
<thead>
<tr>
<th>Device</th>
<th>Rated current</th>
<th>Rated power</th>
<th>Rated voltage</th>
<th>Rated frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>12.5 A</td>
<td>950 VA</td>
<td>100–240 V ± 10% VAC</td>
<td>50/60 Hz</td>
</tr>
<tr>
<td>Computer</td>
<td>2.1 A</td>
<td>125 VA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monitor</td>
<td>1.5 A</td>
<td>65 VA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** The instrument, monitor, and computer self-adjust for 100–240 V input voltages of 50/60 Hz.

Environmental requirements

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude</td>
<td>Located between sea level and 6000 ft (~1800 m) above sea level</td>
</tr>
<tr>
<td>Electromagnetic</td>
<td>Do not use this device in close proximity to sources of strong electromagnetic radiation (for example, unshielded intentional RF sources). Strong electromagnetic radiation may interfere with the proper operation of the device.</td>
</tr>
<tr>
<td>interference</td>
<td></td>
</tr>
<tr>
<td>Humidity</td>
<td>15–80% (noncondensing)</td>
</tr>
<tr>
<td>Installation site</td>
<td>For indoor use only</td>
</tr>
<tr>
<td>Pollution</td>
<td>The instrument has a Pollution Degree rating of II. The instrument may only be installed in an environment that has nonconductive pollutants such as dust particles or wood chips. Typical environments with a Pollution Degree II rating are laboratories and sales and commercial areas. The noise output of the instrument is &lt;60 dB at idle.</td>
</tr>
<tr>
<td>Temperature</td>
<td>15–30°C (60–85°F)</td>
</tr>
<tr>
<td>Thermal output</td>
<td>During operation the net thermal output, based on the actual current draw of the QuantStudio™ 6 and 7 Flex Instrument and computer, is expected to be approximately 2731 Btu/h [800 W].</td>
</tr>
<tr>
<td>Vibration</td>
<td>Ensure that the instrument is not adjacent to strong vibration sources, such as a centrifuge, pump, or compressor. Excessive vibration will affect instrument performance.</td>
</tr>
<tr>
<td>Other conditions</td>
<td>Ensure the room is away from any vents that could expel particulate material on the instrument components. Avoid placing the instrument and computer adjacent to heaters, cooling ducts, or in direct sunlight.</td>
</tr>
</tbody>
</table>
Network requirements

The computer is factory-configured for the TCP/IP protocol. The product includes a fast Ethernet adapter (10/100 Mbps) with an RJ45-type connector and one 3-m (9.8-ft) crossover Ethernet cable that connects the computer and the instrument.

If the instrument will be connected to a LAN, an active, tested LAN connection must be in place before the scheduled installation date. Due to differences in network connections, the Life Technologies service representative cannot configure the instrument to access a specific network.

You must supply a standard Category 5 Ethernet cable of the required length to connect the computer to your LAN.
QuantStudio™ 6 and 7 Flex Systems layout and connections

The QuantStudio™ 6 and 7 Flex Systems consist of the components in the following figure.

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuantStudio™ 6 or 7 Flex System</td>
<td>Performs fluorescence detection and data collection of experiment and calibration plates or array cards.</td>
</tr>
<tr>
<td>Computer</td>
<td>Run the QuantStudio™ 6 and 7 Flex Real-Time PCR System Software that is used to:</td>
</tr>
<tr>
<td>Monitor</td>
<td>• Calibrate the QuantStudio™ 6 and 7 Flex Systems.</td>
</tr>
<tr>
<td>Keyboard</td>
<td>• Set up experiments.</td>
</tr>
<tr>
<td>Mouse</td>
<td>• (Optional) Run experiments.</td>
</tr>
<tr>
<td>Barcode scanner</td>
<td>• Analyze experiments.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Connection</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Power cables Supply power to the computer and the QuantStudio™ 6 and 7 Flex Systems.†</td>
</tr>
<tr>
<td>B</td>
<td>LAN connection or Ethernet cable‡ Connects the QuantStudio™ 6 and 7 Flex Systems [Ethernet port] to the Ethernet port on the network interface card in the computer. The components can be connected over a local area network or a via a direct physical connection.</td>
</tr>
<tr>
<td>C</td>
<td>DVI cable Connects the monitor to the computer (DVI port).</td>
</tr>
<tr>
<td>D</td>
<td>Barcode scanner cable Connects the optional barcode scanner to the computer (USB port).</td>
</tr>
<tr>
<td>E</td>
<td>Keyboard cable Connects the keyboard to the computer (USB port).</td>
</tr>
<tr>
<td>F</td>
<td>Mouse cable Connects the mouse to the computer (USB port).</td>
</tr>
</tbody>
</table>

† Supplies 115/230 V depending on the geographic location of the installation.
‡ Supplied with the QuantStudio™ 6 and 7 Flex Systems.
Symbols on this instrument ................................................. 174
Locations of safety labels on instrument ............................. 177
Safety alerts on this instrument ........................................... 178
Instrument safety .............................................................. 178
Barcode scanner laser safety ............................................. 180
Workstation safety ............................................................ 180
Safety and electromagnetic compatibility (EMC) standards ........ 181
Chemical safety ............................................................... 182
Biological hazard safety .................................................... 183

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 symbol is used along with user attention words described in the “About This Guide” section to highlight important safety information. The following table gives the meaning of these symbols.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>English</th>
<th>Français</th>
</tr>
</thead>
</table>
| ![Caution, risk of danger](image) | Caution, risk of danger  
Consult the manual for further safety information. | Attention, risque de danger  
Consulter le manuel pour d'autres renseignements de sécurité. |
<p>| <img src="image" alt="Caution, hot surface" /> | Caution, hot surface | Attention, surface chaude |
| <img src="image" alt="Caution, risk of electrical shock" /> | Caution, risk of electrical shock | Attention, risque de choc électrique |
| <img src="image" alt="Laser radiation" /> | Laser radiation | Rayonnement laser |
| <img src="image" alt="Moving parts" /> | Moving parts | Parties mobiles |
| <img src="image" alt="Potential biohazard" /> | Potential biohazard | Danger biologique potentiel |
| <img src="image" alt="Ultraviolet light" /> | Ultraviolet light | Rayonnement ultraviolet |
| <img src="image" alt="Potential slipping hazard" /> | Potential slipping hazard | Danger de glisser potentiel |
| <img src="image" alt="On" /> | On | On [marche] |
| <img src="image" alt="Off" /> | Off | Off [arrêt] |
| <img src="image" alt="On/Off" /> | On/Off | On/Off [marche/arrêt] |
| <img src="image" alt="Standby" /> | Standby | En attente |
| <img src="image" alt="Earth (ground) terminal" /> | Earth [ground] terminal | Borne de [mise à la] terre |
| <img src="image" alt="Protective conductor terminal (main ground)" /> | Protective conductor terminal (main ground) | Borne de conducteur de protection [mise à la terre principale] |
| <img src="image" alt="Terminal that can receive or supply alternating current or voltage" /> | Terminal that can receive or supply alternating current or voltage | Borne pouvant recevoir ou envoyer une tension ou un courant de type alternatif |</p>
<table>
<thead>
<tr>
<th>Symbol</th>
<th>English</th>
<th>Français</th>
</tr>
</thead>
<tbody>
<tr>
<td>![symbol]</td>
<td>Terminal that can receive or supply alternating or direct current or voltage</td>
<td>Borne pouvant recevoir ou envoyer une tension ou un courant continu ou alternatif</td>
</tr>
<tr>
<td>![symbol]</td>
<td>Do not dispose of this product in unsorted municipal waste <strong>CAUTION!</strong> 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.</td>
<td>Ne pas éliminer ce produit avec les déchets usuels non soumis au tri sélectif. <strong>CAUTION!</strong> 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.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Conformity mark</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>![CE]</td>
<td>Indicates conformity with European Union Directives (see “Safety and electromagnetic compatibility (EMC) standards” on page 181).</td>
</tr>
<tr>
<td>![UL US]</td>
<td>Indicates conformity with safety requirements for Canada and U.S.A.</td>
</tr>
<tr>
<td>![C]</td>
<td>Indicates conformity with Australian standards for electromagnetic compatibility.</td>
</tr>
</tbody>
</table>
The following table describes symbols that may be displayed on the Life Technologies QuantStudio™ 6 and 7 Flex Systems, consumables, or reagents.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="LOT" /></td>
<td>BATCH CODE&lt;br&gt;&lt;br&gt;This symbol is accompanied by the manufacturer’s batch code. It indicates the lot number.</td>
</tr>
<tr>
<td><img src="image" alt="REF" /></td>
<td>CATALOGUE NUMBER&lt;br&gt;&lt;br&gt;The manufacturer’s catalogue number will appear after or below this symbol.</td>
</tr>
<tr>
<td><img src="image" alt="i" /></td>
<td>CONSULT INSTRUCTIONS FOR USE&lt;br&gt;&lt;br&gt;This symbol indicates that you should consult the product insert for further information. If a product insert does not exist, or if the product insert does not contain the symbol or the required information, consult the user manual.</td>
</tr>
<tr>
<td><img src="image" alt="CONT" /></td>
<td>CONTAINS</td>
</tr>
<tr>
<td><img src="image" alt="x" /></td>
<td>DO NOT REUSE&lt;br&gt;&lt;br&gt;For one time use only.</td>
</tr>
<tr>
<td><img src="image" alt="" /></td>
<td>LOWER LIMIT OF TEMPERATURE&lt;br&gt;&lt;br&gt;The lower limit of temperature is indicated adjacent to the symbol.</td>
</tr>
<tr>
<td><img src="image" alt="" /></td>
<td>MANUFACTURER&lt;br&gt;&lt;br&gt;The name and the address of the manufacturer will appear next to this symbol.</td>
</tr>
<tr>
<td><img src="image" alt="SN" /></td>
<td>SERIAL NUMBER&lt;br&gt;&lt;br&gt;The serial number will appear after or below this symbol.</td>
</tr>
<tr>
<td><img src="image" alt="" /></td>
<td>TEMPERATURE LIMITATION&lt;br&gt;&lt;br&gt;The upper and lower limits of temperature are indicated adjacent to the upper and lower horizontal lines.</td>
</tr>
<tr>
<td><img src="image" alt="" /></td>
<td>UPPER LIMIT OF TEMPERATURE&lt;br&gt;&lt;br&gt;The upper limit of temperature is indicated adjacent to the symbol.</td>
</tr>
<tr>
<td><img src="image" alt="" /></td>
<td>USE BY&lt;br&gt;&lt;br&gt;This symbol is accompanied by a date to indicate that the device should not be used after the end of the date shown.</td>
</tr>
</tbody>
</table>
Locations of safety labels on instrument

The QuantStudio™ 6 and 7 Flex Systems contain warnings at the following locations:
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.

<table>
<thead>
<tr>
<th>English</th>
<th>French translation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CAUTION! Hazardous chemicals.</strong> Read the Safety Data Sheets [SDSs]</td>
<td><strong>ATTENTION! Produits chimiques dangereux. Lire les fiches signalétiques (FS) avant de manipuler les produits.</strong></td>
</tr>
<tr>
<td>before handling.</td>
<td></td>
</tr>
<tr>
<td>**CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for</td>
<td><strong>ATTENTION! Déchets dangereux. Lire les fiches signalétiques (FS) et la réglementation locale associées à la manipulation et à l’élimination des déchets.</strong></td>
</tr>
<tr>
<td>handling and disposal.</td>
<td></td>
</tr>
<tr>
<td>**CAUTION! Class 2(II) visible and/or invisible laser radiation present</td>
<td><strong>ATTENTION! Rayonnement laser visible ou invisible de classe 2 (II) présent en position ouverte. Ne pas regarder directement dans le faisceau ou avec des instruments optiques.</strong></td>
</tr>
<tr>
<td>when open Do not stare directly into the beam or view directly with</td>
<td></td>
</tr>
<tr>
<td>optical instruments.</td>
<td></td>
</tr>
</tbody>
</table>

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.
- Ensure 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.
**Electrical**

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

**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! Ensure appropriate electrical supply.** For safe operation of the instrument:
- Plug the instrument 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.**
- If hazardous materials are spilled onto the instrument, the instrument should be appropriately decontaminated.
- Using cleaning or decontamination methods other than those recommended by the manufacturer may compromise the safety or quality of the instrument.
- Care should be taken to not use decontamination or cleaning agents which would cause a hazard as a result of a reaction with parts of the equipment or with material contained in it.
- For the protection of others, ensure the instrument is properly decontaminated prior to having the instrument serviced at your facility or before sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan. Decontamination forms may be requested from customer service.

**Instrument, component, and accessory disposal**

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.
Barcode scanner laser safety

Laser classification

The optional barcode scanners included with the QuantStudio™ 6 and 7 Flex Systems are categorized as Class 2 (II) lasers.

Laser safety requirements

Class 2 (II) lasers are low-power, visible-light lasers that can damage the eyes. Never look directly into the laser beam. The scanner is designed to prevent human access to harmful levels of laser light during normal operation, user maintenance, or during prescribed service operations.

WARNING! LASER HAZARD. Class 2 (II) lasers can cause damage to eyes. Avoid looking into a Class 2 (II) laser beam or pointing a Class 2 (II) laser beam into another person’s eyes.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.

CAUTION! MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.
## 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:

<table>
<thead>
<tr>
<th>Safety</th>
<th>Reference</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IEC 61010-1</td>
<td>Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements</td>
<td></td>
</tr>
<tr>
<td>EN 61010-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UL 61010-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSA C22.2 No. 61010-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IEC 61010-2-010</td>
<td>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials</td>
<td></td>
</tr>
<tr>
<td>EN 61010-2-010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IEC 61010-2-081</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>EN 61010-2-081</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EMC</th>
<th>Reference</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EN 61326-1</td>
<td>Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements</td>
<td></td>
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<tr>
<td>FCC Part 18 (47 CFR)</td>
<td>U.S. Standard “Industrial, Scientific, and Medical Equipment”</td>
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<tr>
<td>AS/NZS 2064</td>
<td>Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment</td>
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<tr>
<td>ICES-001, Issue 3</td>
<td>Industrial, Scientific and Medical (ISM) Radio Frequency Generators</td>
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<tr>
<th>Environmental design</th>
<th>Reference</th>
<th>Description</th>
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</table>
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.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.
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. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:
- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: [www.cdc.gov/biosafety](http://www.cdc.gov/biosafety)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: [www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html](http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html)
- Your company’s/institution’s Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: [www.cdc.gov](http://www.cdc.gov)

In the EU:
Documentation and Support

Obtaining information from the Help system

The QuantStudio™ 6 and 7 Flex Systems have 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 QuantStudio™ 6 and 7 Flex Real-Time PCR System Software window.
- Select Help ▶ QuantStudio™ 6 and 7 Flex Real-Time PCR System Software Help.
- 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.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com

At the Life Technologies website, you can:

- Access worldwide telephone and fax numbers to contact Life Technologies Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Life Technologies user documents, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

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.
<table>
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<tr>
<th>Glossary Term</th>
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<tr>
<td>aligned melt curve plot</td>
<td>Plot of the re-scaled melt curve.</td>
</tr>
<tr>
<td>allele</td>
<td>In a diploid organism, one of two DNA sequences found at the same locus (for example, a particular gene), but located on homologous chromosomes. Two corresponding alleles may have the identical sequence, or they may differ somewhat, often at one or more single-base sites (SNPs).</td>
</tr>
<tr>
<td>amplicon</td>
<td>A segment of DNA amplified during PCR.</td>
</tr>
<tr>
<td>amplification</td>
<td>Part of the instrument run in which PCR amplifies the target. Fluorescence data collected during amplification are displayed in an Amplification Plot, and the data are used to calculate results.</td>
</tr>
<tr>
<td></td>
<td><strong>Note:</strong> Only quantitative real-time PCR experiments, not end-point experiments, take amplification data into account.</td>
</tr>
<tr>
<td>amplification efficiency [EFF%]</td>
<td>Calculation of the efficiency of the PCR amplification in an experiment. EFF% is calculated using the slope of the regression line in the standard curve. A slope close to -3.32 indicates optimal, 100% PCR amplification efficiency.</td>
</tr>
<tr>
<td>Amplification Plot</td>
<td>Display of data collected during the cycling stage of PCR amplification. The Amplification Plot can be viewed as:</td>
</tr>
<tr>
<td></td>
<td>• Baseline-corrected normalized reporter (ΔRₙ) vs. cycle</td>
</tr>
<tr>
<td></td>
<td>• Normalized reporter (Rₙ) vs. cycle</td>
</tr>
<tr>
<td></td>
<td>• Threshold cycle (Cₜ) vs. well</td>
</tr>
<tr>
<td>amplification stage</td>
<td>Part of the instrument run in which PCR amplifies the target. The amplification stage, called a cycling stage in the thermal profile, consists of denaturing, primer annealing, and extension steps that are repeated. Fluorescence data collected during the extension stage are displayed in an amplification plot, and the data are used to calculate results. With chemistry, the last two steps of a PCR stage are typically combined. See also cycling stage.</td>
</tr>
<tr>
<td>assay</td>
<td>In a PCR reaction mix, two target-specific primers or two primers and a probe used to amplify a target.</td>
</tr>
<tr>
<td>assay mix</td>
<td>PCR reaction component in assays. The assay mix contains primers designed to amplify a target and a probe designed to detect amplification of the target.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>------</td>
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</tr>
<tr>
<td>automatic baseline</td>
<td>An analysis setting in which the software calculates the baseline start and end cycles for the amplification plot. See also baseline.</td>
</tr>
<tr>
<td>automatic threshold</td>
<td>An analysis setting in which the software calculates the baseline start and end cycles and the threshold in the amplification plot. The software uses the baseline and threshold to calculate the threshold cycle ($C_T$). See also threshold cycle (CT).</td>
</tr>
<tr>
<td>background calibration</td>
<td>Type of calibration in which the instrument performs reads of a background plate or array card, averages the spectra recorded during the run, and extracts the resulting spectral component to a calibration file. The software then uses the calibration file during subsequent runs to remove the background fluorescence from the run data.</td>
</tr>
<tr>
<td>baseline</td>
<td>From the Amplification Plot, a cycle-to-cycle range that defines background fluorescence.</td>
</tr>
<tr>
<td>baseline-corrected normalized reporter ($\Delta R_n$)</td>
<td>The magnitude of normalized fluorescence signal generated by the reporter. In experiments that contain data from real-time PCR, the magnitude of normalized fluorescence signal generated by the reporter at each cycle during the PCR amplification. From the $\Delta R_n$ vs. Cycle Amplification Plot, $\Delta R_n$ is calculated at each cycle as: $\Delta R_n (cycle) = R_n (cycle) - R_n (baseline)$ where $R_n =$ normalized reporter. See also normalized reporter (Rn).</td>
</tr>
<tr>
<td>blocked IPC</td>
<td>In presence/absence experiments, a reaction that contains IPC blocking agent, which blocks amplification of the internal positive control (IPC). In QuantStudio™ 6 and 7 Flex Real-Time PCR System Software, also the name of the task for the IPC target in wells that contain IPC blocking agent. See also negative control-blocked IPC wells.</td>
</tr>
<tr>
<td>CE mark</td>
<td>The CE Mark (an acronym for the French “Conformite Europeene”) of conformity certifies that a product has met the essential requirements of the applicable European Directive.</td>
</tr>
<tr>
<td>chemistry</td>
<td>See reagents.</td>
</tr>
<tr>
<td>control</td>
<td>Sample of known genotype or melt-curve shape.</td>
</tr>
<tr>
<td>$C_T$</td>
<td>See threshold cycle (CT).</td>
</tr>
<tr>
<td>$C_T$ algorithm</td>
<td>The QuantStudio™ 6 and 7 Flex Real-Time PCR System Software uses the Baseline Threshold algorithm.</td>
</tr>
<tr>
<td>cycle threshold</td>
<td>See threshold cycle (CT).</td>
</tr>
<tr>
<td>cycling stage</td>
<td>In the thermal profile, a stage that is repeated. A cycling stage is also called an amplification stage. See also amplification stage.</td>
</tr>
<tr>
<td>Glossary Term</td>
<td>Definition</td>
</tr>
<tr>
<td>---------------</td>
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</tr>
<tr>
<td>data collection</td>
<td>During the instrument run, a process in which an instrument detects fluorescence data from each well of the plate or array card. The instrument transforms the signal to electronic data and saves the data in the experiment file.</td>
</tr>
<tr>
<td>delta $R_n$ ($\Delta R_n$)</td>
<td>See baseline-corrected normalized reporter (DRn).</td>
</tr>
</tbody>
</table>
| derivative melt curve plot | The default view of the melting curve. It plots the negative derivative data ($-R_n'$) vs. temperature.  
*Note:* An alternative view is the raw data view, which plots the normalized fluorescence from reporter dye vs. temperature. |
<p>| derivative reporter ($-R_n'$) | The rate of change in fluorescence as a function of temperature. $R_n'$ is used to plot a melting curve. Significant decrease of the fluorescent signal generates a positive peak on the derivative view of the melting curve. |
| dissociation curve | See melt curve. |
| dye calibration | Type of calibration in which the software collects spectral data from a series of dye standards and stores the spectral information for the dye standards in a pure spectra calibration file. This file is used during experiment runs to characterize and distinguish the individual contribution of each dye in the total fluorescence collected by the instrument. |
| EFF% | See amplification efficiency (EFF%). |
| error | The standard error of the slope of the regression line in the standard curve. The error can be used to calculate a confidence interval (CI) for the slope. Because the amplification efficiency (EFF%) is calculated from the slope, knowing the error allows a CI for the amplification efficiency to be calculated. |
| filter | Dye excitation and emission filter combination that is set for an experiment. The QuantStudio™ 7 Flex System includes a decoupled, six by six-color filter set. The QuantStudio™ 6 Flex System includes a coupled five-color filter set. The excitation and emission filter combinations are preset by the experiment document (.eds) file for your experiment. |
| flag | A quality control (QC) indicator which, when applied by the software to a well during analysis, indicates a possible issue with that reaction. For example, a flag may be issued if no amplification is detected in a well. Flags indicating potential problems are displayed in the Quality Control tab of the Plate Layout, Well Table, and QC Summary screens. |
| forward primer | Oligonucleotide that flanks the 5’ end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target. |
| heterozygote | Samples having both allele 1 and allele 2. |
| holding stage | In the thermal profile, the stage that holds the temperature constant for a defined period of time. |</p>
<table>
<thead>
<tr>
<th>Glossary Term</th>
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</tr>
</thead>
<tbody>
<tr>
<td>homozygote</td>
<td>Samples having only allele 1 or only allele 2.</td>
</tr>
<tr>
<td>Instrument Console</td>
<td>A software feature that allows you to monitor the status of any instrument on the network; view calibration, maintenance, and instrument properties for a selected instrument; and open and close the instrument drawer.</td>
</tr>
<tr>
<td>Instrument Manager</td>
<td>A software feature that allows you to monitor the status of an instrument; monitor Amplification Plots and temperature plots in real time; view the calibration status, perform calibrations and manage files on the instrument, including downloading completed experiments to your computer.</td>
</tr>
<tr>
<td>internal positive control (IPC)</td>
<td>In presence/absence experiments, a short synthetic DNA template that is added to PCR reactions. The IPC can be used to distinguish between true negative results (the target is absent in the samples) and negative results caused by PCR inhibitors, incorrect assay setup, or reagent or instrument failure.</td>
</tr>
<tr>
<td>IPC</td>
<td>See internal positive control (IPC).</td>
</tr>
<tr>
<td>IPC+</td>
<td>See negative control-IPC wells.</td>
</tr>
<tr>
<td>melt curve</td>
<td>A plot of data collected during the melt curve stage. Peaks in the melt curve can indicate the melting temperature ($T_m$) of the target, or they can identify nonspecific PCR amplification. In the software, you can view the melt curve as normalized reporter ($R_n$) vs. temperature or as derivative reporter ($-R_n'$) vs. temperature.</td>
</tr>
<tr>
<td>melt curve characteristics</td>
<td>The melt curve shape and the difference in melting temperature ($T_m$) values.</td>
</tr>
<tr>
<td>melt curve stage</td>
<td>In the thermal profile, a stage with a temperature increment to generate a melt curve.</td>
</tr>
<tr>
<td>melting temperature ($T_m$)</td>
<td>The temperature at which 50% of the DNA is double-stranded and 50% of the DNA is dissociated into single-stranded DNA. In a melt curve experiment, the melt curve plot displays the melting temperature.</td>
</tr>
<tr>
<td>melting transition region</td>
<td>In Melt Curve experiments, the region before and after the melting temperature ($T_m$).</td>
</tr>
<tr>
<td>negative control (NC)</td>
<td>The task for targets or SNP assays in wells that contain water or buffer instead of sample. No amplification of the target should occur in negative control wells. Previously called no template control (NTC).</td>
</tr>
<tr>
<td>negative control-blocked IPC wells</td>
<td>In presence/absence experiments, wells that contain IPC blocking agent instead of sample in the PCR reaction. No amplification should occur in negative control-blocked IPC wells because the reaction contains no sample and amplification of the IPC is blocked. Previously called no amplification control (NAC).</td>
</tr>
<tr>
<td>negative control-IPC wells</td>
<td>In presence/absence experiments, wells that contain IPC template and buffer or water instead of sample. Only the IPC template should amplify in negative control-IPC wells because the reaction contains no sample. Previously called IPC+.</td>
</tr>
</tbody>
</table>
no template control (NTC)  
See negative control (NC).

nonfluorescent quencher-minor groove binder (NFQ-MGB)  
Molecules that are attached to the 3′ end of probes. When the probe is intact, the nonfluorescent quencher (NFQ) prevents the reporter dye from emitting fluorescence signal. Because the NFQ does not fluoresce, it produces lower background signals, resulting in improved precision in quantification. The minor groove binder (MGB) increases the melting temperature ($T_m$) of the probe without increasing its length, allowing for the design of shorter probes.

normalization calibration  
Type of calibration in which the software collects data from the normalization standards, then stores it in a normalization calibration file.

normalized quantity  
Either the $C_T$ Avg. of the target gene minus the $C_T$ Avg. of the endogenous control (Comparative $C_T$ experiments), or the $Q$ Avg. of the target divided by the $Q$ Avg. of the endogenous control (Relative Standard Curve experiments).

normalized quantity mean  
The relative standard curve equivalent of the $\Delta C_T$ mean value found in Comparative $C_T$ experiments (computed as the geometric mean).

normalized quantity SE  
The relative standard curve equivalent of the $\Delta C_T$ SE value found in Comparative $C_T$ experiments (computed as the geometric standard error of the mean).

normalized reporter ($R_n$)  
Fluorescence signal from the reporter dye normalized to the fluorescence signal of the passive reference dye (usually ROX™ dye on Life Technologies instruments).

outlier  
A measurement (such as a $C_T$) that deviates significantly from the measurement of the other replicates for that same sample.

passive reference  
A dye that produces fluorescence signal independent of PCR amplification, and that is added to each reaction at a constant concentration. Because the passive reference signal should be consistent across all wells, it is used to normalize the reporter dye signal to account for non-PCR related fluorescence fluctuations caused by minor well-to-well differences in volume. Normalization to the passive reference signal generally results in data with noticeably high precision among technical replicates.

plate layout  
An illustration of the grid of wells and assigned content in the plate or array card. The number of rows and columns in the grid depends on the sample block that you use. From the software, you can use the plate layout as a selection tool to assign well contents, to view well assignments, and to view results. The plate layout can be printed, included in a report, exported, and saved as a slide for a presentation.

point  
One standard in a standard curve. The standard quantity for each point in a standard curve is calculated based on the starting quantity and serial factor.

positive control  
In genotyping and presence/absence experiments, a DNA sample with a known genotype, homozygous or heterozygous.

In the software, the task for the SNP assay in wells that contain a sample with a known genotype.
Glossary

**pure dye**  
Fluorescent compound used to calibrate the instrument.  
See system dye.

**quantity**  
In quantification experiments, the amount of target in the samples. Absolute quantity can refer to copy number, mass, molarity, or viral load. Relative quantity refers to the fold-difference between normalized quantity of target in the sample and normalized quantity of target in the reference sample.

**quencher**  
A molecule attached to the 3’ end of probes to prevent the reporter from emitting fluorescence signal while the probe is intact. With reagents, a nonfluorescent quencher-minor groove binder (NFQ-MGB) can be used as the quencher.

**R² value**  
Regression coefficient calculated from the regression line in the standard curve. An important quality value, the R² value indicates the closeness of fit between the standard curve regression line and the individual Cₜ data points from the standard reactions. A value of 1.00 indicates a perfect fit between the regression line and the data points.

**Raw Data Plot**  
A plot of raw fluorescent signal as detected through each emission filter, used to view raw data for individual wells and at individual cycles.

**reaction mix**  
A solution that contains all components to run the PCR reaction, except for the template (sample, standard, or control). Also called a “PCR cocktail”.

**reagents**  
The PCR reaction components used to amplify the target and to detect amplification.

**real-time PCR**  
Process of collecting fluorescence data during PCR. Data from the real-time PCR are used to calculate results for quantification experiments or to troubleshoot results for genotyping or presence/absence experiments.

**reference sample**  
In relative standard curve and Comparative Cₜ (ΔΔCₜ) experiments, the sample used as the basis for relative quantification results. Also called the calibrator.

**region of interest (ROI) calibration**  
Type of calibration in which the software maps the positions of the wells on the sample block of the instrument. The software uses the ROI calibration data to associate increases in fluorescence during a run with specific wells of the plate or array card. A calibration image for each individual filter must be generated to account for minor differences in the optical path.

**regression coefficients**  
Values calculated from the regression line in standard curves, including the R² value, slope, and y-intercept. You can use the regression coefficients to evaluate the quality of results from the standards. See also standard curve.

**regression line**  
In standard curve and relative standard curve experiments, the best-fit line from the standard curve. Regression line formula:

\[ C_T = m \log (\text{Qty}) + b \]

where m is the slope, b is the y-intercept, and Qty is the standard quantity.  
See also regression coefficients.
reject well  An action that the software performs during analysis to remove one or more wells from further analysis if a specific flag is applied to the well.

relative standard curve method  A method to determine relative quantities. This method compensates for target and endogenous control efficiency differences within each run. In all experiments, unknown samples and dilution series of template (such as cDNA) are amplified. Following a run, the instrument software interpolates relative quantities for each unknown sample from the appropriate dilution curve, then normalizes the data for each sample (or set of replicates) as follows: target QAvg. ÷ endogenous control QAvg.

replicate group  A user-defined biological grouping. A replicate group may be a set of identical reactions in a experiment.

replicates  Total number of identical reactions containing identical components and identical volumes.

reporter  A fluorescent dye used to detect amplification. With reagents, the reporter dye is attached to the 5′ end.

reverse primer  An oligonucleotide that flanks the 3′ end of the amplicon. The reverse primer and the forward primer are used together in PCR reactions to amplify the target.

reverse transcriptase  An enzyme that converts RNA to cDNA.

Rn  See normalized reporter (Rn).

ROX™ dye  A dye supplied by Life Technologies, which is used as a passive reference in some experiments.

run method  Definition of the reaction volume and the thermal profile for the instrument run. The run method specifies the temperature, time, ramp, and data collection points for all steps and stages of the instrument run.

sample  The biological tissue or specimen that you are testing for a target gene.

sample definition file  A tab-delimited file (.txt) or Microsoft® Excel® file (.xls or .xlsx) that contains the sample name and custom sample properties for a single experiment.
Glossary

security, auditing
and e-signature

A separately licensed software module that provides:

- **System Security** – Controls user access to the software. Provides a default Administrator user account. You can define additional user accounts and permissions.

- **Auditing** – Tracks changes made to library items, actions performed by users, and changes to the Security and Audit settings. The software automatically audits some actions silently. You can select other items for auditing and specify the audit mode. Provides reports for audited library items, Security and Audit changes, and actions.

- **Electronic Signature (e-signature)** – Controls whether users are permitted, prompted, or required to provide a user name and password when accessing certain software features. You can select which features are controlled and the number of signatures required for access. When authorized persons use this feature, they are creating a legally binding signature.

serial factor

In the software, a numeric value that defines the sequence of quantities in the standard curve. The serial factor and the starting quantity are used to calculate the standard quantity for each point in the standard curve. For example, if the standard curve is defined with a serial factor of 1:10 or 10X, the difference between any two adjacent points in the curve is 10-fold.

slope

Regression coefficient calculated from the regression line in the standard curve. The slope indicates the PCR amplification efficiency for the assay. A slope of \(-3.32\) indicates 100% amplification efficiency.

See also amplification efficiency (EFF%) and regression line.

stage

In the thermal profile, a group of one or more steps. Examples: PCR stage, cycling stage (also called amplification stage), and hold stage.

standard

A sample that you dilute and amplify along with unknown samples. This dilution series can contain known starting quantities of the target of interest (absolute standard curve) or it can be of known dilution factor (relative standard curve). Following the run, the software interpolates the C\(_T\) values of the unknowns to this curve, yielding either specific quantities of the target (for absolute curves) or relative quantities (for relative dilution curves).

See also standard curve.

standard curve

In standard curve and relative standard curve experiments:

- The best-fit line in a plot of the C\(_T\) values from the standard reactions plotted against standard quantities. See also regression line.

- A set of standards containing a range of known quantities. Results from the standard curve reactions are used to generate the standard curve. The standard curve is defined by the number of points in the dilution series, the number of standard replicates, the starting quantity, and the serial factor.

standard curve method

Method for determining absolute target quantity in samples. With the standard curve method, the software measures amplification of the target in samples and in a standard dilution series. Data from the standard dilution series are used to generate the standard curve. Using the standard curve, the software interpolates the absolute quantity of target in the samples.
### Glossary

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<th>Definition</th>
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<tr>
<td><strong>standard dilution series</strong></td>
<td>In standard curve and relative standard curve experiments, a set of standards containing a range of known quantities. The standard dilution series is prepared by serially diluting standards. For example, the standard stock is used to prepare the first dilution point, the first dilution point is used to prepare the second dilution point, and so on. In the software, the volumes needed to prepare a standard dilution series are calculated by the number of dilution points, the number of standard replicates, the starting quantity, the serial factor, and the standard concentration in the stock. See also standard curve.</td>
</tr>
<tr>
<td><strong>standard quantity</strong></td>
<td>In the PCR reaction, a known quantity. In standard curve experiments, the quantity of target in the standard. From the software, the units for standard quantity can be for mass, copy number, viral load, or other units for measuring the quantity of target. Standard quantity can also refer to dilution factor.</td>
</tr>
<tr>
<td><strong>starting quantity</strong></td>
<td>When defining a standard curve in the software, the highest quantity.</td>
</tr>
<tr>
<td><strong>step</strong></td>
<td>A component of the thermal profile. For each step in the thermal profile, you can set the ramp rate (ramp increment for melt curve steps), hold temperature, and hold time (duration) are set as part of the .tdd file. Data collection can also be turned on or off for the ramp or the hold parts of the step in each .tdd file. For cycling stages, a step is also defined by the AutoDelta status.</td>
</tr>
<tr>
<td><strong>system dye</strong></td>
<td>Dye supplied by Life Technologies and calibrated on the QuantStudio™ 6 and 7 Flex Systems. Before you use system dyes in your experiments, make sure the dye calibration is current in the Instrument Console.</td>
</tr>
<tr>
<td><strong>target</strong></td>
<td>The nucleic acid sequence to amplify and detect.</td>
</tr>
<tr>
<td><strong>target color</strong></td>
<td>From the software, a color assigned to a target to identify the target in the plate layout and analysis plots.</td>
</tr>
<tr>
<td><strong>task</strong></td>
<td>From the software, the type of reaction performed in the well for the target or SNP assay.</td>
</tr>
<tr>
<td><strong>technical replicates</strong></td>
<td>Wells containing identical reaction components, including sample; important for evaluating precision.</td>
</tr>
<tr>
<td><strong>temperature plot</strong></td>
<td>From the software, a display of temperatures for the instrument cover and instrument block during the instrument run.</td>
</tr>
<tr>
<td><strong>template</strong></td>
<td>The type of nucleic acid to add to the PCR reaction.</td>
</tr>
<tr>
<td><strong>experiment</strong></td>
<td>Refers to the entire process of performing a run, including setup, run, and analysis.</td>
</tr>
<tr>
<td><strong>thermal profile</strong></td>
<td>Part of the run method that specifies the temperature, time, ramp, and data collection points for all steps and stages of the instrument run.</td>
</tr>
</tbody>
</table>
### Glossary

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<tbody>
<tr>
<td>experiment document file (.eds)</td>
<td>The Life Technologies name for the electronic records that comprise all information about a particular plate or array card, including metadata (name, barcode, comments), plate setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, analysis results, audit records, and other consumable-specific data. Experiment document files have the .eds suffix.</td>
</tr>
<tr>
<td>threshold</td>
<td>In Amplification Plots, the level of fluorescence above the baseline and within the exponential growth region.</td>
</tr>
<tr>
<td>threshold cycle (Cₜ)</td>
<td>The PCR cycle number at which the fluorescence meets the threshold in the Amplification Plot.</td>
</tr>
<tr>
<td>Tₘ</td>
<td>See melting temperature (Tm).</td>
</tr>
<tr>
<td>touchscreen</td>
<td>Instrument display that you touch to control the instrument.</td>
</tr>
<tr>
<td>uniformity calibration</td>
<td>Type of calibration in which the software measures sample block uniformity. The calibration generates data that compensate for the physical effects of the QuantStudio™ 6 and 7 Flex Systems filters on data collected during a experiment.</td>
</tr>
<tr>
<td>unknown</td>
<td>From the software, the task for the target in wells that contain the sample being tested. In quantification experiments, the task for the target in wells that contain a sample with unknown target quantities. In genotyping experiments, the task for the SNP assay in wells that contain a sample with an unknown genotype. In presence/absence experiments, the task for the target in wells that contain a sample in which the presence of the target is not known. In melt curve experiments, the task for the target in wells that contain a sample with an unknown melt curve profile.</td>
</tr>
<tr>
<td>variant</td>
<td>A sample (or group of samples) with a unique melt curve (that is, the melt curve is different from the melt curves of other samples or controls used in the experiment). The software determines melt curve differences by the melting temperature (Tₘ) and the shape of the melt curve.</td>
</tr>
<tr>
<td>y-intercept</td>
<td>From the standard curve, the value of y where the regression line crosses the y-axis. The y-intercept indicates the expected threshold cycle (Cₜ) for a sample with quantity equal to 1.</td>
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