applied biosystems

# QuantStudio™ Design and Analysis Software v2

## USER GUIDE

QuantStudio™ Design and Analysis Software v2.7

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ThermoFisher SCIENTIFIC

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**Revision history: MAN0018200 J.0 (English)**

| Revision | Date | Description |
|---|---|---|
| J.0 | 3 July 2023 | The document was updated for QuantStudio™ Design and Analysis Software v2.7.<br>• The list of compatible instruments was updated.<br>• The list of compatible data files was updated.<br>• Information was added about pre-run files for the OpenArray™ Plate format ("Overview of pre-run files for the OpenArray™ Plate format" on page 10).<br>• Information was added to specify that template files and data files are checksum protected. This helps to ensure that they are not edited outside of the system.<br>• Information was added about application profiles for the SAE Administrator Console.<br>• Additional information was added in order connect the SAE Administrator Console.<br>• The icons for data collection in the run method were updated.<br>• Instructions were added to adjust the view during the plate setup ("Edit the view" on page 31).<br>• The instructions to edit the plate setup were updated to include the OpenArray™ Plate.<br>• The instructions to manage the target dyes were updated to include adding a dye from the dye library.<br>• The instructions to edit the plate information were updated to indicate that the experiment name can be edited.<br>• The instructions to set up a standard curve were updated to use a starting quantity of greater than 0.<br>• The option to select by subarray for the OpenArray™ Plate format was added to the instructions to review and analyze the data.<br>• The instructions to review and edit the baseline and threshold values were updated to indicate that these values can be updated only if the baseline threshold algorithm setting is used.<br>• The instructions to edit the QC alerts in the primary analysis settings were updated to indicate that a rule that is set up for a sample type takes precedence over a rule that is set up for all samples.<br>• The instructions to view the plots in the **Quality Check** tab were updated to include the option to show or hide the background grid.<br>• The instructions to edit the advanced primary analysis settings were updated to include an algorithm to reduce dye signal cross-talk.<br>• The advanced primary analysis settings for the $\Delta R_n$ were updated for QuantStudio™ Design and Analysis Software v2.7 and later. Any curves with the $\Delta R_n$ below the threshold are set to non-amplified. This is regardless of whether there is a $C_q$ value.<br>• The instructions to edit the melt analysis settings were updated to include a melt peak parity factor.<br>• Descriptions of the possible curve quality flags were added.<br>• The instructions to generate a report were updated to remove the selection of the paper size. This is set in the system preferences.<br>• The information that is included in a report was updated.<br>• The instructions to generate a report were updated to allow selection by subarray for the OpenArray™ Plate format.<br>• Instructions were added to view and adjust the images of the OpenArray™ Plate format.<br>• The information about the analysis modules was updated to include a primary analysis module.<br>• The instructions to manage the preferences were updated to include adding custom dyes to a dye library.<br>• A change in passive reference was added as a QC acceptance criteria.<br>• The instructions to select an analysis module were updated to indicate that the genotyping analysis module is automatically applied to data files from OpenArray™ Plate genotyping runs.<br>• Instructions were added to use different calibrations in order to analyze a file.<br>• The instructions to edit the export settings were updated, including updating the text for the plate information fields and the column headers. |

| Revision | Date | Description |
|---|---|---|
| J.0 *(contin-ued)* | 3 July 2023 | • Instructions were added to create a new plate file from a data file.<br>• Instructions were added to remove a data file.<br>• Additional information was added about the raw data plot. |
| H.0 | 24 June 2021 | Changes for v2.6: Added language packs |
| G.0 | 4 November 2020 | Changes for v2.5: Added QC alerts overview and acceptance criteria; added review results in the plate layout; added batch generate plate files; added export/import/scan/TaqMan™ Array Cards in plate setup; added export/import/refresh/calibration status in instruments; added pause cycle in a PCR step; added advanced settings, preferences, and filter results; included minor UI changes |
| F.0 | 15 April 2020 | Changes for v2.4: Added e-signature functions for SAE; added analysis module plots in results report; added custom sample attributes; added restricted editing in plate files and data files; added use analysis settings from a different file |
| E.0 | 17 March 2020 | Changes for v2.3.3: Updated options to edit $C_q$ settings |
| D.0 | 12 December 2019 | Changes for v2.3: Added results report; added recommended master mix run method information; added plugin management |
| C.0 | 14 October 2019 | Changes for v2.2: Added SAE information; added biogroup information; added 7900 file type; documented minor UI changes |
| B.0 | 26 August 2019 | Changes for v2.1: Removed standard curve analysis; included instrument calibration override; embedded user guide and sample plate setup files; documented minor UI changes; included compatible instruments, computer requirements, and analysis module information |
| A.0 | 16 April 2019 | New document for v2.0 |

The information in this guide is subject to change without notice.

# Contents

# 1 About the software

QuantStudio™ Design and Analysis Software v2 is used to set up instrument runs on and analyze data generated with QuantStudio™ real-time PCR systems (see "Compatible instruments" on page 9).

The compatibility in the following sections is specific to QuantStudio™ Design and Analysis Software v2.7.

## Compatible instruments

Use the software to create plate files to run on the following instruments:

- QuantStudio™ 7 Pro Real-Time PCR System
- QuantStudio™ 6 Pro Real-Time PCR System
- QuantStudio™ 12K Flex Real–Time PCR System (except the OpenArray™ Plate format)
- QuantStudio™ 7 Flex Real-Time PCR System
- QuantStudio™ 6 Flex Real-Time PCR System
- QuantStudio™ 5 Real-Time PCR System
- QuantStudio™ 3 Real-Time PCR System
- QuantStudio™ 1 Real-Time PCR System

Pre-run files for the OpenArray™ Plate cannot be created in the QuantStudio™ Design and Analysis Software v2. For more information, see "Overview of pre-run files for the OpenArray™ Plate format" on page 10.

## Compatible data files

### Compatible data files

The software is compatible with data files from the following real-time PCR instruments, if the plate file for the run was created with QuantStudio™ Design and Analysis Software v2:

- QuantStudio™ 7 Pro Real-Time PCR System (including TaqMan™ Array Card format)
- QuantStudio™ 6 Pro Real-Time PCR System
- QuantStudio™ 12K Flex Real–Time PCR System (including TaqMan™ Array Card format, but not including the OpenArray™ Plate format)
- QuantStudio™ 7 Flex Real-Time PCR System (including TaqMan™ Array Card format)
- QuantStudio™ 6 Flex Real-Time PCR System
- QuantStudio™ 5 Real-Time PCR System

- QuantStudio™ 3 Real-Time PCR System
- QuantStudio™ 1 Real-Time PCR System

Pre-run files for the OpenArray™ Plate format cannot be created with the QuantStudio™ Design and Analysis Software v2.

Data files for the OpenArray™ Plate are in the legacy file format. For information about a workflow for the OpenArray™ Plate, see "Overview of pre-run files for the OpenArray™ Plate format" on page 10.

## Compatible data files (legacy file format)

Data files for the following instruments are a legacy file format that can be opened and analyzed in the software, but can only be saved as the updated file format:

- QuantStudio™ 7 Flex Real-Time PCR System (including the TaqMan™ Array Card format)
- QuantStudio™ 6 Flex Real-Time PCR System
- QuantStudio™ 12K Flex Real–Time PCR System (including the TaqMan™ Array Card and the OpenArray™ Plate format)
- QuantStudio™ 5 Real-Time PCR System
- QuantStudio™ 3 Real-Time PCR System
- QuantStudio™ 1 Real-Time PCR System
- StepOnePlus™ Real-Time PCR System
- ViiA™ 7 Real-Time PCR System
- 7500/7500 Fast Real-Time PCR System
- 7900HT Real-Time PCR System

**Note:** To convert a legacy data file into the updated file format, open the data file, then click **Actions ▸ Save As**.

## Overview of pre-run files for the OpenArray™ Plate format

Pre-run files for the OpenArray™ Plate format cannot be created with the QuantStudio™ Design and Analysis Software v2.

A data file from the OpenArray™ Plate format can be opened in the QuantStudio™ Design and Analysis Software v2. The plate setup can be saved as a CSV file. This file can be used as an OA.csv file format in the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System. This file can also be used in the QuantStudio™ Design and Analysis Software v2 to edit a plate setup.

For information about saving the plate setup as a CSV file, see "Export a plate setup file" on page 48.

## Computer requirements for the desktop software

The desktop software can be installed on a customer-provided computer. The following list contains the minimum software requirements for a customer-provided computer.

- Operating system—Windows™ 10 (64-bit) or Macintosh™ OS 10.01
- Processor—Pentium® 4 processor or comparable
- Memory—4 GB RAM
- Hard drive—10 GB
- Monitor—1280 × 1024 resolution

# 2 Workflow

## ① Set up a plate file



## ② Review and analyze data

# 3 Use the software with the Security, Auditing, and E-signature Administrator Console

The following chapter covers the SAE Administrator Console v2.0 and later. This is compatible with the QuantStudio™ 7 Pro Real-Time PCR System (see "System components with the SAE functions enabled" on page 14).

For information about the different features of the SAE Administrator Console v2.0 and later, see *SAE Administrator Console v2.0 or later User Guide for PCR systems* (Pub. No. MAN0017468).

The SAE Administrator Console is compatible with other systems that are not covered in this document, for example, the QuantStudio™ Absolute Q™ Digital PCR System.

Some of the instruments have security, auditing, and e-signature functions included in the instrument software. These functions are not integrated with the SAE Administrator Console and the QuantStudio™ Design and Analysis Software v2.

## Overview of the SAE functions

The SAE is a client-server configuration that includes three components:

- SAE Administrator Console that an administrator uses to configure the module.
- SAE server that stores settings, user accounts, and audit records. By default, the SAE server is installed on the same computer as the SAE Administrator Console.
- SAE screens in an application (sign in and audit) that a user interacts with. QuantStudio™ Design and Analysis Software v2 is an application.

The SAE Administrator Console provides the following SAE functionality in the QuantStudio™ Design and Analysis Software v2:

- **System security**—Controls user sign in and access to functions.
- **Auditing**—Tracks changes and actions performed by users.
- **E-signature**—Allows users to provide an electronic signature (user name and password) when performing certain functions.

Depending on the way that your SAE administrator configures these features:

- Some features and functions that are described in this guide might not be accessible to you.
- You might see dialog boxes and prompts when you use the software.

## System components with the SAE functions enabled

The following system components can be used with the SAE functions enabled:

- QuantStudio™ 7 Pro Real-Time PCR Instrument
- QuantStudio™ Design and Analysis Software v2
- Plate files for the QuantStudio™ 7 Pro Real-Time PCR Instrument—SAE-enabled plate files are created in the QuantStudio™ Design and Analysis Software v2 with SAE functions enabled.
  - If the SAE functions are enabled in the QuantStudio™ Design and Analysis Software v2, you can only create plate files for the QuantStudio™ 7 Pro Real-Time PCR Instrument.
  - If the SAE functions are enabled on the QuantStudio™ 7 Pro Real-Time PCR Instrument, you cannot create or edit a plate file from the instrument touchscreen.
- QuantStudio™ 7 Pro Real-Time PCR Instrument data file—SAE-enabled data files are created on the QuantStudio™ 7 Pro Real-Time PCR Instrument with SAE functions enabled.

We recommend enabling the SAE functions for all system components (see “Enable the SAE functions” on page 16). If one or more of the components have a conflicting SAE status, some features might not be available (see “Compatibility between SAE-enabled and SAE-disabled components” on page 14).

**Note:** Template and data files are checksum protected.

- Checksum protection helps to ensure that files produced by the instruments are not edited outside of the system.
- Files produced by the software applications are checksum protected by the software, regardless of whether the SAE functions are enabled.

### Compatibility between SAE-enabled and SAE-disabled components

We recommend enabling the SAE functions for all system components (for more information, see “Enable the SAE functions” on page 16). If one or more of the components have a conflicting SAE status, some features might not be available. See the following table for more information.

| Component | Functionality with an SAE-enabled plate or data file | Functionality with an SAE-disabled plate or data file |
|---|---|---|
| QuantStudio™ Design and Analysis Software v2 with SAE functions enabled | • The file can be edited, depending on the SAE configuration.<br>• The audit trail is continued. | • The file is opened in read-only mode.<br>• The file cannot be edited or saved. |
| QuantStudio™ Design and Analysis Software v2 with SAE functions disabled | • SAE-disabled files allowed—The file is opened and can be edited. The file can be saved as an invalid SAE file only.[1]<br>• QuantStudio™ 7 Pro Real-Time PCR Instrument forbidden—The file cannot be opened. | The file can be opened, edited, and saved. |

*(continued)*

| Component | Functionality with an SAE-enabled plate or data file | Functionality with an SAE-disabled plate or data file |
|---|---|---|
| QuantStudio™ 7 Pro Real-Time PCR Instrument with SAE functions enabled | • The file can be opened from the run queue, a USB drive, or other sources.[2]<br>• The file cannot be edited.<br>• The audit record is continued. | The file cannot be opened. |
| QuantStudio™ 7 Pro Real-Time PCR Instrument with SAE functions disabled | • The plate file can be opened and edited.<br>• The file can be saved as an invalid SAE file only.[1]<br>• The file can be used to start a run, but the data file will be an invalid SAE file.[1] | The file can be opened, edited, and saved. |

[1] Invalid SAE files contain incomplete audit records.
[2] You cannot import plate files from the Thermo Fisher™ Connect Platform when the instrument has the SAE functions enabled.

## QuantStudio™ Design and Analysis Software v2 functionality when SAE is enabled or disabled

The following occur when SAE functions are enabled in the QuantStudio™ Design and Analysis Software v2:

- Users must sign in with an SAE user account to use QuantStudio™ Design and Analysis Software v2.
- Auditing functions are active (if they are enabled in the SAE Administrator Console).
- Plate setup and software functions for a user are determined by the SAE application profile and user account settings.
- Plate files can only be created for the QuantStudio™ 7 Pro Real-Time PCR System. SAE functions are not compatible with the QuantStudio™ 6 Pro Real-Time PCR System.
- Plate files and data files with SAE disabled can only be opened in read-only mode (see "Compatibility between SAE-enabled and SAE-disabled components" on page 14).

# Enable the SAE functions

## Workflow

**Enable SAE functions**

**Install the application profiles**

See "Application profiles" on page 17.

**Enable the SAE functions on the QuantStudio™ 7 Pro Real-Time PCR Instrument** (page 17)

**Connect the QuantStudio™ Design and Analysis Software v2 to the SAE Administrator Console** (page 18)

**Enable the SAE functions in QuantStudio™ Design and Analysis Software v2** (page 19)

## Firewall ports that must be open

The following ports must be open for the operating system on the computer that is running the SAE Administrator Console.

| SAE Administrator Console version | Port | Condition |
|---|---|---|
| v2.0 | 8201 | • Instrument-to-SAE Administrator Console server connection<br>• Computer-to-SAE Administrator Console server connection[1] |
| v2.1 and later | 8443 | • Instrument-to-SAE Administrator Console server connection<br>• Computer-to-SAE Administrator Console server connection[1] |

[1] If the software is installed on a different computer than the SAE Administrator Console.

# Application profiles

For detailed instructions to install application profiles, see *SAE Administrator Console v2.0 or later User Guide for PCR systems* (Pub. No. MAN0017468).

The application profiles must be installed before the application can be connected to the SAE Administrator Console.

The following application profiles are available:

| Application (instrument or software) | Application profile[1] |
| --- | --- |
| QuantStudio™ 7 Pro Real-Time PCR Instrument | `QuantStudio 7 Pro Instrument (<…>).dat` |
| QuantStudio™ Design and Analysis Software v2 | `Design and Analysis Software (<…>).dat` |

[1] <…> is the version of the application profile. For more information, see “Application profile versions” on page 17.

**IMPORTANT!** The application profile for the QuantStudio™ 7 Pro Real-Time PCR Instrument must be installed before the application profile for the QuantStudio™ Design and Analysis Software v2.

## Application profile versions

The SAE Administrator Console requires an application profile to be installed for each application. For example, in order to use the QuantStudio™ 7 Pro Real-Time PCR Instrument with the SAE Administrator Console, the application profile specific to the instrument must be installed.

Each application profile has a version. For compatibility between the versions of the SAE Administrator Console, the application, and the application profile, see the chapter specific to your application.

Application profiles have the following naming convention:

```
<Application name> (<Application profile version number>).dat
```

The file format for an application profile is DAT.

The following file name is an example of the application profile for the QuantStudio™ 7 Pro Real-Time PCR Instrument. It is version 1.3.0.

```
QuantStudio 7 Pro Instrument (1.3.0).dat
```

# Enable the SAE functions on the QuantStudio™ 7 Pro Real-Time PCR Instrument

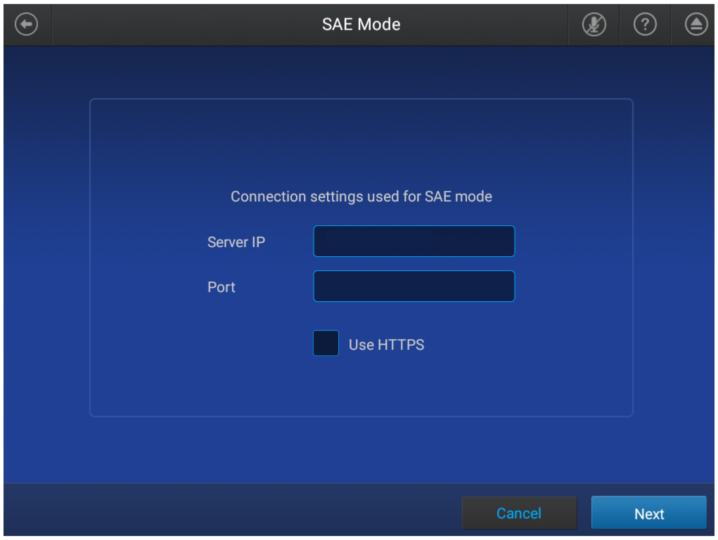
This procedure requires a local administrator profile on the instrument and an SAE administrator user account in the SAE Administrator Console.

1. In the home screen, tap ⚙ **(Settings)** ▸ **SAE**.
   The **SAE Mode** screen is displayed.

2. In the **SAE Mode** screen, set the **SAE Mode** slider to **Enable**.

3. Tap the **Server IP** field, then enter the IP address of the SAE server.

   The server IP is the IP address of the computer on which the SAE Administrator Console is installed.




4. Tap the **Port** field, then enter the port.

   The port that is entered is the firewall port. It is dependent on the version of the SAE Administrator Console. See "Firewall ports that must be open" on page 16.

5. Tap **Next**.

6. Enter the SAE administrator user name and password when prompted, then tap **Enable**.

The home screen is displayed. The SAE administrator is signed in.

## Connect the QuantStudio™ Design and Analysis Software v2 to the SAE Administrator Console

Close all plate files and data files before connecting to the SAE Administrator Console.

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**Note:** Connect the software and instruments to the same instance of the SAE Administrator Console to ensure that audit records are maintained across system components.

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1. In the menu bar, click **System ▸ SAE Connection Settings**.

2. Enter the server and port number of the SAE Administrator Console.

   If the SAE Administrator Console is installed on the same computer as the QuantStudio™ Design and Analysis Software v2, enter ***localhost***.

   If the SAE Administrator Console is installed on a different computer from the QuantStudio™ Design and Analysis Software v2, enter the IP address of the computer on which the SAE Administrator Console is installed.

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   **Note:** If using a dynamic IP address, enter the hostname instead of the IP address to prevent the loss of a connection (see "Determine the hostname" on page 19).

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   The port number is the firewall port. It is dependent on the version of the SAE Administrator Console. See "Firewall ports that must be open" on page 16.

3. (*Optional*) Click **Test Connection** to confirm that the connection information is correct.

4. Click **Save**.

### Determine the hostname

If the SAE Administrator Console is on a separate computer from the application and a dynamic IP address is used, the hostname is recommended instead of the IP address. This helps to prevent the loss of a connection between the SAE Administrator Console and the application

1. In the Windows™ search bar, enter ***cmd*** to open the **Command Prompt**.

2. Enter ***hostname***, then press **Enter**.

The hostname of the computer is displayed in the **Command Prompt**.

## Enable the SAE functions in QuantStudio™ Design and Analysis Software v2

This procedure requires an SAE administrator user account.

Complete the following tasks before you enable the SAE functions in the QuantStudio™ Design and Analysis Software v2:

- Connect to the SAE Administrator Console (see "Connect the QuantStudio™ Design and Analysis Software v2 to the SAE Administrator Console" on page 18).
- Close all plate files and data files.

1. In the QuantStudio™ Design and Analysis Software v2, select **System ▸ Enable Security**.

2. Enter your SAE administrator account user name and password, then click **Sign In**.

The SAE administrator account is automatically signed into the software after the SAE functions are enabled. The SAE user account name is displayed in the upper-right corner of the software menu bar.

# Sign into QuantStudio™ Design and Analysis Software v2 using an SAE account

Sign in for the QuantStudio™ Design and Analysis Software v2 is only required if SAE functions are enabled by an SAE administrator (see "Enable the SAE functions in QuantStudio™ Design and Analysis Software v2" on page 19).

1. In the QuantStudio™ Design and Analysis Software v2 sign in screen, enter your SAE username and password.

2. Click **Sign In**.

The username of the SAE account that is signed in to the software appears in the menu bar.

# Sign out of the software using an SAE account

1. In the upper-right corner of the software menu bar, click the SAE account username.
2. Click **Sign Out**.

# Change your SAE account password

**Note:** External user accounts (External/Federated LDAP repository accounts) cannot change their password in the software.

1. In the upper-right corner of the QuantStudio™ Design and Analysis Software v2 menu bar, click the SAE account user name.
2. Click **Change Password**.
3. Enter the password information, then click **OK**.

# Specify audit reason

Depending on the way that your SAE administrator configures audit settings in the SAE Administrator Console, the **Enter Audit Reason** screen may be displayed when you make changes to a plate file or a data file in the QuantStudio™ Design and Analysis Software v2.

Select a reason from the dropdown list, or add a custom reason.

**Note:** **Custom Reason** is not displayed if audit settings are configured to require users to select a reason.

# View audit records for a plate file or data file

1. In QuantStudio™ Design and Analysis Software v2, in an open plate file or data file, select the **Audit** tab.
   - The **Audit Summary** pane contains a list of all the audit records created each time the plate file or data file was saved.
   - The **Change Records** pane displays all events in a selected audit record.
2. (*Optional*) Enter a date range to filter the displayed records.
3. (*Optional*) Click 🔍 to search the audit records.
4. Select an audit record in the **Audit Summary** pane to view audit record details in the **Change Records** pane.

# Export audit records

1. In QuantStudio™ Design and Analysis Software v2, in an open plate file or data file, select the **Audit** tab.
2. In the upper-right corner of the **Audit** tab, click ⋯ **(Actions)** ▸ **Generate Full Audit Report**.
3. Enter a file name, select a download folder, then click **Export**.

The exported PDF file contains the information displayed in the **Audit Summary** and **Change Records** panes of the **Audit** tab.

# Sign data in the software

1. Save any new changes to an open plate file or data file.
2. Click **Actions**, then select **Sign Data**.
3. Select an option from the dropdown list to indicate the meaning of the e-signature.
   - Reviewed and Approved Template (includes plate setup and run method)
   - Reviewed and Approved Plate Results
4. Enter your user name and password.
5. (*Optional*) To preview the e-signature report for the plate file or date file, click **Preview**.

   To generate an e-signature report for the plate file or data file, see “Generate an E-Signature Report” on page 22.
6. Click **Sign**.

A record of the e-signature is available in the **e-Signature** tab (see “View e-signatures in the software” on page 22).

# View e-signatures in the software

1. In an open SAE-enabled plate file or data file, select the **e-Signature** tab.
   All of the e-signatures for the file display in the table. The table cannot be modified.

2. Review all of the e-signatures for the file in the table. The **Status** column indicates if the e-signature is **Current** or **Obsolete**.

| Column | Description |
|---|---|
| **Date** | Indicates the date and time that the e-signature was added to the plate file or data file |
| **User Name** | Indicates the user name of the person that added the e-signature to the plate file or data file |
| **User Role** | Indicates the role assigned to the user in the SAE Administrator Console |
| **Meaning** | Indicates the meaning of the e-signature:<br>• Reviewed and Approved Template<br>• Reviewed and Approved Plate Results |
| **Status** | Indicates whether the e-signature is **Current** or **Obsolete** |

# Generate an E-Signature Report

1. In an open SAE-enabled plate file or data file, in the **e-Signature** tab, select an e-signature record from the list.

2. In the upper-right corner of the **e-Signature** tab, click ⋯ **(Actions)** ▸ **Generate E-signature Report**.
   The E-Signature Report opens in a new window.

3. Use the icons in the tool bar to review, print, or download the PDF.

# Use QuantStudio™ Design and Analysis Software v2 when the SAE server is offline

If your SAE administrator has configured the QuantStudio™ Design and Analysis Software v2 to allow use when the SAE server is offline (**Client offline login** System setting in the SAE Administrator Console), you can use the software for the period of time specified by the SAE administrator for **Client offline login**.

**Note:** If you have not previously signed in to QuantStudio™ Design and Analysis Software v2 with your SAE account, you cannot sign in when the SAE server is offline.

All SAE records are retained if QuantStudio™ Design and Analysis Software v2 is disconnected from an SAE server. When QuantStudio™ Design and Analysis Software v2 is reconnected to the SAE server, SAE records are uploaded to the server.

The following functions are not available when the SAE server is offline:

- Account lockout, password reminder, mandatory password change
- Disable SAE
- Change Password

## Disable the SAE functions in the QuantStudio™ Design and Analysis Software v2

This procedure requires an SAE administrator account.

Close all plate files and data files.

1. In QuantStudio™ Design and Analysis Software v2, select **System** ▸ **Disable Security**.
2. Enter the password of the SAE administrator account, then click **Sign In**.

# 4 Set up a plate file

## Select a system template or existing plate file to set up a new plate file

Plate files cannot be created for the OpenArray™ Plate format with the QuantStudio™ Design and Analysis Software v2. For information about a workflow for the OpenArray™ Plate format, see "Overview of pre-run files for the OpenArray™ Plate format" on page 10.

A limited number of items in the data files for the OpenArray™ Plate format can be edited. It is noted in each section if this format can be edited.

A new plate file must be created from a system template or a previously created plate file. For more information about system templates and plate files, see "Overview of system templates and plate files" on page 77.

**IMPORTANT!** You must select a system template or a plate file that corresponds to your instrument, block, and run mode. These properties are not editable once the plate file has been created.

Not all of the variations of the EDT files and EDS files are provided in the galleries with the software installer.

1. In the home screen, click **Set Up Plate**.
   The **Plate Gallery** opens to the **System Templates** tab.

2. In the left pane, select the appropriate options to filter the system template and plate file lists.

   - **Instrument**
   - **Block**
   - **Run Mode**
   - **Analysis**

   **Note:** Thermal protocol, plate setup, and post-run analysis options are independent of analysis module selection. Analysis module selection can be changed at any point during plate file set or post-run analysis (see "Select an analysis module" on page 84).

3. Navigate to appropriate **Plate Gallery** tab.

| Tab | Description |
|---|---|
| **System Templates** | Contains system templates, non-editable plate files that are included with the software.<br>Click a system template to automatically generate a new plate file that can be edited, then saved. |
| **My Plate Files** | Contains plate files that were previously saved to **My Plate Files**.<br>Click an existing plate file to automatically generate a new plate file that can be edited, then saved.<br>To edit the existing plate file, hover over the plate file, then click ⋯ **(Actions)** ▸ **Edit**. |
| **Recents** | Contains plate files that were recently opened. Recently opened plate files from **System Templates** and **My Plate Files** do not populate this tab.<br>Click a plate file to open it. The plate file can be edited, then saved, or saved as a new plate file. |

**Note:** Click 🔍 to search for a plate file based on a tag (see "Edit plate file or data file information" on page 47).

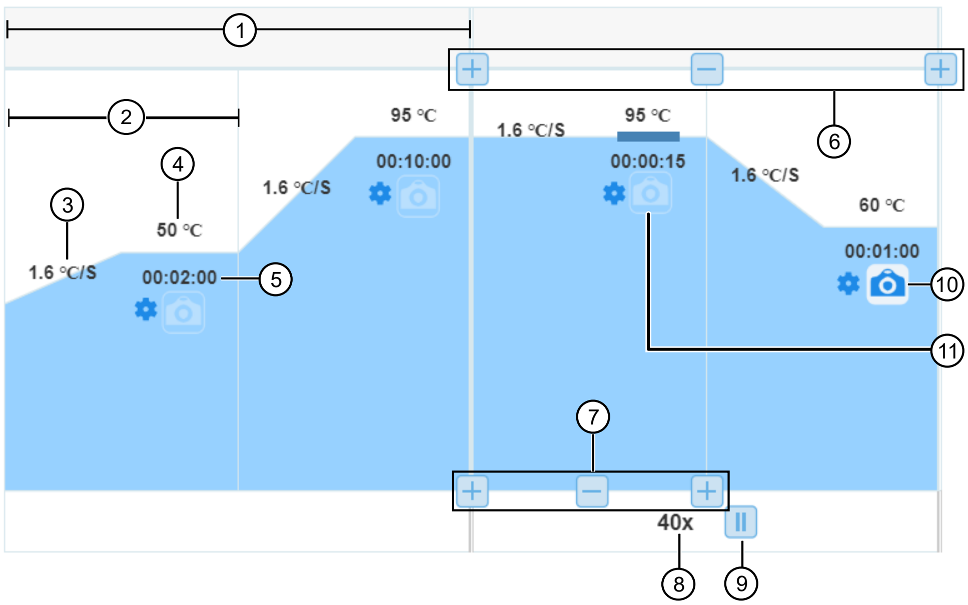
4. Select a system template or plate file to open. To view all options for opening the plate file, hover over the plate file, then click ⋯ **(Actions)**.

The plate file opens in the **Run Method** tab.

# Confirm or edit run method

## Run method elements

Edit run method elements in the **Run Method** tab.




(1) Stage
(2) Step within a stage
(3) Temperature ramp rate of a step
(4) Temperature of a step
(5) Time length of a step
(6) Add/remove stage
(7) Add/remove step
(8) Number of PCR cycles
(9) PCR stage pause cycle
(10) Data collection on
(11) Data collection off

## Apply the recommended run method for your master mix

Override the current run method with the run method that we recommend for use with your master mix, instrument, block, and run mode. The recommended run method can differ depending on the application being used. We recommend that you confirm the run method in the master mix user guide.

1. On the right side of the **Run Method** tab, click ⋯ **(Actions)** ▸ **Select Master Mix**.


2. Search for your master mix by name or catalog number, or select a master mix from the list.

3. (*Optional*) To add the master mix to the reagents table in the **Plate Setup** tab, select **Append selected master mix to reagent table**.
   The master mix is added to the Reagent Table in the **Plate Setup** tab (see "Edit reagent information" on page 45).

4. Click **Apply**.

The run method is updated to the recommended run method for the selected master mix.

## Edit temperature ramp rate, temperature, and time length for a step

1. In the **Run Method** tab, in the step of interest, click the temperature ramp rate, temperature, or time length element.
   For more information about elements in the run method, see "Run method elements" on page 26.

2. Enter the value, then click outside the element to stop editing.
   Each filter set has a minimum exposure time. A warning is displayed if the time is shorter than the minimum exposure time.

## Add or remove a step

1. In the **Run Method** tab, hover over the stage for which you want to edit steps to view the + and − buttons.
   - The buttons at the top of the stage control adding and removing stages.
   - The buttons at the bottom of the stage control adding and removing steps.
   - For more information about elements in the run method, see "Run method elements" on page 26.

2. At the insert location, click + at the bottom of the stage.

3. (*Optional*) Edit the temperature ramp rate, temperature, or time length of the new step (see "Edit temperature ramp rate, temperature, and time length for a step" on page 27).

4. To remove a step, click − at the bottom of the step.

## Add or remove a stage

1. In the **Run Method** tab, hover over where you want to insert the stage to view the + and − buttons.
   - The buttons at the top of the stage control adding and removing stages.
   - The buttons at the bottom of the stage control adding and removing steps.
   - For more information about elements in the run method, see "Run method elements" on page 26.

2. At the insert location, click + at the top of the stages.

3. Select the type of stage from the list.

| Option | Description |
| --- | --- |
| **Hold** | • Multiple hold stages can be added. |
| **Pre-Read** | • Only one pre-read stage can be added.<br>• A pre-read stage can only be added to the beginning of the run method. |
| **Post-Read** | • Only one post-read stage can be added.<br>• A post-read stage can only be added after the final PCR stage of the run method. |
| **Infinite** | • Only one infinite hold stage can be added.<br>• An infinite hold stage can only be added to the end of the run method. |
| **PCR** | • Multiple PCR stages can be added. |
| **Melt Curve** | • Multiple melt curve stages can be added. |

4. (*Optional*) Edit the temperature ramp rate, temperature, or time length for steps of the new stage (see "Edit temperature ramp rate, temperature, and time length for a step" on page 27).

5. To remove a stage, hover of the stage of interest, then click ⊟ at the top of the stage.

## Add, edit, or remove a pause cycle in a PCR stage

1. In a legacy template, in the **Run Method** tab, click ⏸ at the bottom of the PCR stage.
   For more information about elements in the run method, see "Run method elements" on page 26.

2. To add a pause to the PCR stage, select **Pause Cycle**.

3. To edit a pause, enter a pause temperature between 4°C and 99.9°C.

> **CAUTION! PHYSICAL INJURY HAZARD.** During instrument operation, the plate temperature can reach 100°C. To access the plate during a run pause, enter room temperature as the pause temperature and allow the plate to cool to room temperature before handling.

4. Enter the appropriate cycle after which the pause will occur.

5. Click **Save**.

6. (*Optional*) To remove a pause, click ⏸, then deselect **Pause Cycle**.

## Add, edit, or remove a pause cycle in a PCR step

1. In a QuantStudio™ 6 Pro or 7 Pro Real-Time PCR System template, in the **Run Method** tab, click ⚙ **(Advanced Setting)** in a step.

   **Note:** Any changes apply only to the step in which you clicked.

2. In the **Pause Setting** tab, select **Pause Cycle**.

   **Note:** **Pause Setting** is not available for the melt curve dissociation step.

3. In the **Pause Temperature** field, enter a pause temperature between 4°C and 99.9°C.

**CAUTION!** **PHYSICAL INJURY HAZARD.** During instrument operation, the plate temperature can reach 100°C. To access the plate during a run pause, enter room temperature as the pause temperature and allow the plate to cool to room temperature before handling.

4. In the **Pause After Cycle** field, enter the appropriate cycle after which the pause will occur.

5. Click **Save**.
   A PCR step with a pause cycle applied to it is denoted with ⏸ in the top-left corner of the step.

6. (*Optional*) To remove a pause cycle, deselect **Pause Cycle**.

## Edit the ramp increment for the melt curve dissociation step

1. In the **Run Method** tab, in the **Melt** stage, click the name of the ramp increment method, then select one of the following options:

| Option | Description |
|---|---|
| **Continuous** (default) | Continuously increases the temperature by the ramp increment (°C/sec). |
| **Step and Hold** | Increases the temperature by the ramp increment (°C), then holds at that temperature for the specified time. |
| **No. of Data Points per Degree** | Increases the temperature by the ramp increment (°C) and collects the specified number of data points per degree increased. |

2. Edit the temperature ramp increment.
   a. Click the ramp increment element in the **Dissociation** step.
   b. Enter a value or use the up/down arrows (default is 0.15°C/s).

3. (*Step and Hold only*) Edit the hold time after each temperature increase.
   a. Click the time field next to **Step and Hold**.
   b. Enter a value or use the up/down arrows (default is 5 seconds).

4. (*No. of Data Points per Degree only*) Edit the number of data points to be collected with each degree increase.
   a. Click the number of data points element in the **Dissociation** step.
   b. Enter a value or use the up/down arrows (default is 10 data points).

## Enable, edit, or disable Auto Delta

Auto Delta enables the incremental increase or decrease of the temperature or hold time for a step in a PCR stage.

Not all instruments support Auto Delta. For specific information about Auto Delta, see the instrument documentation.

1. In the **Run Method** tab, click **(Advanced Setting)** in a step.

   **Note:** Any changes apply only to the step in which you clicked.

2. In the **Auto Delta Settings** tab, select **Enable Auto delta**.
3. In the **Temperature Delta** field, enter the numerical difference in the temperature. The software indicates the appropriate range.
4. In the **Time Delta** field, enter the numerical difference in the time. The software indicates the appropriate range.
5. In the **Starting Cycle** field, enter the first cycle to which you want Auto Delta settings to apply.
6. Click **Save**.
   A PCR step with an Auto Delta setting applied to it is denoted with A.
7. (*Optional*) To remove Auto Delta, deselect **Enable Auto delta**.

## Enable, edit, or disable VeriFlex™ zones

**VeriFlex™ Zones** enable independent temperature zones ≤5°C of adjacent zones.

- The number of VeriFlex™ zones depends on the instrument. For specific information about VeriFlex™ zones, see the instrument documentation.
- VeriFlex™ zones are only available for 96-well blocks.

1. In the **Run Method** tab, click **(Advanced Setting)** in a step.

   **Note:** Any changes apply only to the step in which you clicked.

2. In the **VeriFlex™ Zones** tab, select **Enable VeriFlex™**.
3. In the **Adjusted Temperature (°C)** fields, enter the adjusted temperature.
4. Click **Save**.
   A step with VeriFlex™ Zones applied to it is denoted with V.
5. (*Optional*) To remove VeriFlex™ Zones, deselect **Enable VeriFlex™**.

## Confirm or edit filter settings

The need to edit optical filter settings is rare, and it is for advanced or custom uses only. For more information about instrument supported dyes and their calibration and optical filter selection, see the instrument documentation.

Use the optical filters settings to select a filter set to match the profile of a custom dye.

1. On the right side of the **Run Method** tab, click ··· **(Actions)** ▸ **Filter Settings**.

   The excitation (x) and emission (m) wavelengths that correspond to each filter are shown on the screen.

2. Select the check boxes to enable or disable filters.

   **IMPORTANT!** If you select the wrong filters, you cannot correct the selection and retrieve data after a run has been completed.

3. Click **Save**.

# Confirm or edit plate setup

**Note:** The table view is not available for the OpenArray™ Plate format. The table view is noted by the icon.

## Edit the view

### Edit the grid view

The grid view is displayed in the **Plate Setup** tab. It matches the plate layout of a physical plate.

This is not available for the OpenArray™ Plate format.

In the plate layout pane, click **(Grid View)**.

1. In the top-right corner of the plate layout pane, click **(Zoom In)** and **(Zoom Out)**.
2. Click the percentage value, then use the slider to select a scaling based on a numerical value.
3. Click **(Reset)** to reset the zoom to fit the screen width.

4. Click **(Settings)**, the select the following options from the dialog box.

| Section | Option |
| --- | --- |
| **Show** | • **Sample Color**<br>• **Target Color** or **SNP Assay Color**<br><br>Select one, none, or all of the options. |
| **Tab/Auto Fill Direction** | • **Horizontal**<br>• **Vertical**<br><br>Select one option.<br>This option selects the direction of the next well that is selected when **Tab** or **Enter** is pressed. |
| **Dock Table** | • **To Right**<br>• **To Bottom**<br><br>Select one option.<br>This option selects whether the tables are displayed on the right of the screen or on the bottom of the screen.<br>The tables include the **Samples** table, the **Targets** table, and the **SNP Assays** table. |

5. If any of the wells display an invalid setup, hover over the warning icon to view the reason.

## Edit the table view

The table view is displayed in the **Plate Setup** tab.

This is not available for the OpenArray™ Plate format.

In the plate layout pane, click ☰ **(Table View)**.

1. In the top-right corner, click **View**.
2. Select or deselect the checkbox associated with each item to display in the table view.

| Table | Options |
|---|---|
| Target | • **Well** checkbox<br>• **Sample Name** checkbox<br>• **Sample Type** checkbox<br>• **Target Name** checkbox<br>• **Target Reporter** checkbox<br>• **Target Quencher** checkbox<br>• **Task** checkbox<br>• **Quantity** checkbox |
| SNP | • **Well** checkbox<br>• **Sample Name** checkbox<br>• **Sample Type** checkbox<br>• **Assay Name** checkbox<br>• **Allele 1 Name**<br>• **Allele 1 Reporter** checkbox<br>• **Allele 1 Quencher** checkbox<br>• **Allele 2 Name**<br>• **Allele 2 Reporter** checkbox<br>• **Allele 2 Quencher** checkbox<br>• **Task** checkbox |

3. Click ⚙ **(Settings)**, the select the following options from the **Dock Table** dialog box.
   - **To Right** radio button
   - **To Bottom** radio button

   This setting selects whether the tables are displayed on the right of the screen or on the bottom of the screen.
   The tables include the **Samples** table, the **Targets** table, and the **SNP Assays** table.

## Select plate wells or ports

- Select plate wells or ports in the **(Grid View)**.

**Note:** Selecting ports is only available for TaqMan™ Array Card plate setup.

| To | Action |
|---|---|
| Select a single well | Click a well in the plate |
| Select multiple wells | Click-drag in the plate |
| Select contiguous wells | Shift-click wells in the plate |
| Select non-contiguous wells | PC: Ctrl-click wells in the plate<br>Mac: Cmd-click wells in the plate |
| Select a column of wells | Click a column header |
| Select all wells | Click the top-left corner of the plate grid |
| Select a block of wells | Click a well to define a corner, then shift-click another well on the opposite corner |
| Select a single port[1] | Click a cell in the **Port** column |
| Select multiple ports[1] | Click-drag in the **Port** column |
| Select all ports[1] | Click the **Port** column header |

[1] For TaqMan™ Array Cards only.

- Select plate wells in the **(Table View)**.
  This view is not available for the OpenArray™ Plate format.

| To | Action |
|---|---|
| Select a single well | Click a row in the table |
| Select contiguous wells | Shift-click rows in the table |
| Select non-contiguous wells | PC: Ctrl-click rows in the table<br>Mac: Cmd-click rows in the table |
| Deselect a single well | PC: Ctrl-click the selected row<br>Mac: Cmd-click the selected row |

## Add samples and assign to plate wells

### Import a plate setup file (samples)

Import a plate setup file that was previously exported from the software (see “Export a plate setup file” on page 48 ), or a user-created plate setup file. The following file types can be imported as a plate setup file:

- TXT—text format
- CSV—comma-separated values format

A sample layout can be imported for the OpenArray™ Plate format. Targets or SNP assays cannot be imported for an OpenArray™ Plate format.

For all of the other formats, the plate setup file can include both samples and targets or SNP assays.

1. In the **Plate Setup** tab, in the plate layout pane, click one of the following options.

| Format | Procedure |
|---|---|
| 96-well, 0.2-mL plate<br>96-well, 0.1-mL plate<br>384-well plate<br>TaqMan™ Array Card | Click ⋯ **(Actions)** ▸ **Import Plate Setup**. |
| OpenArray™ Plate | Click ⋯ **(Actions)** ▸ **Import Sample Layout**. |

2. Navigate to, then select the file.

3. Click **OK** to confirm that the plate setup is overwritten.

## Copy samples from the plate layout

Copy is available for all formats.

For the TaqMan™ Array Card plate setup and the OpenArray™ Plate format, this feature copies the information to the clipboard. It can be copied into another program for reference.

Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Navigate to the **Plate Setup** tab.

2. In the **Samples** table, click ⋯ **(Actions)** ▸ **Copy all samples**.

The information is available in the clipboard. It can be pasted into another program for reference.

## Paste samples into the plate layout

You can copy samples from an Excel™ spreadsheet, then paste them into the **Plate Setup** tab.

**Note:** Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Create an Excel™ spreadsheet with the plate setup information.

2. In the spreadsheet, select, then copy the cells of interest.

3. Navigate to the **Plate Setup** tab.

4. In the **Samples** table, click ⋯ **(Actions)** ▸ **Paste samples**.

The samples are copied to the **Samples** table. They must be assigned to the plate layout (see “Manually add or assign a sample to a well or port” on page 36.

## Manually add samples to the Samples table

Each sample name in the **Samples** table must be unique. Do not add multiple entries for technical replicates.

Adding a single sample is not available for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, select an option in the upper right corner of the **Samples** table.

| Option | Description |
|---|---|
| Add a single sample | Click + **(Add)**. |
| Copy/paste multiple samples | a. Copy the sample information from one of the following sources:<br>• Previously created plate file or data file—Click ⋯ **(Actions)** ▸ **Copy all Samples** in the upper-right corner of **Samples** table.<br>• Excel file—Select, then copy data, including column headers.[1]<br>• Plate setup file—Select, then copy data, including column headers.[1]<br>b. Click ⋯ **(Actions)** ▸ **Paste Samples**. |

[1] Column headers must match the column headers in the **Samples** table.

**Note:**
- Click ⋯ **(Actions)** ▸ **Export Samples** to export samples.
- Click ⋯ **(Actions)** ▸ **Import Samples** to import samples.

2. (*Optional*) Edit the sample color and sample type (see "Edit sample name, color, and type" on page 37).

**Note:** The software automatically assigns a task to the target or SNP assay based on the sample type in a well (see "(Optional) Edit the task assigned to a target or SNP assay in one or more wells" on page 43).

3. To remove a sample from the table, click ✕ **(Remove)** in the last column.

## Manually add or assign a sample to a well or port

Sample assignment by port is available only for TaqMan™ Array Card plate setup.

This is not available for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the plate layout pane, perform one of the following actions.
   - Select one or more wells in the **(Grid View)** or in the **(Table View)**.
   - Select one or more ports in the **(Grid View)**.

2. Assign a sample to the selected well or port.

**Note:** The user can only assign up to eight samples by port in the **(Grid View)** for TaqMan™ Array Card plate setup.

| Option | Description |
|---|---|
| (Grid View) | |
| Sample not defined | Enter the sample name in the text field. |
| Sample previously defined | • Start typing the sample name, then select the sample name from the autocomplete list.<br>• Select the checkbox of the sample in the **Samples** table. |
| (Table View) | |
| Sample previously defined | Select the checkbox of the sample in the **Samples** table. |

3. (*Optional*) Edit the sample color and sample type (see "Edit sample name, color, and type" on page 37).

**Note:** The software automatically assigns a task to the target or SNP assay based on the sample type in a well (see "(Optional) Edit the task assigned to a target or SNP assay in one or more wells" on page 43).

## Edit sample name, color, and type

The software automatically assigns a task to the target or SNP assay based on the sample type in a well (see "(Optional) Edit the task assigned to a target or SNP assay in one or more wells" on page 43).

The sample name, color, and type can be edited for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the **Samples** table, click the sample name in the **Name** column.
2. Enter a new name, then press **Enter**.
3. Click the sample color in the **Color** column, then select a color from the color picker.
4. Select a sample type from the **Type** column dropdown list.
   - **Unknown** (default)
   - **Standard**

   **Note:** A standard sample requires that you enter a value in the **Quantity** column of the **Samples** table. The software uses this value to populate the **Quantity** field for standard target tasks in the **Targets** table.

   - **Negative Control**
   - **Positive Control**
   - **Positive 1/1**—A sample that is homozygous for allele 1.
   - **Positive 2/2**—A sample that is homozygous for allele 2.
   - **Positive 1/2**—A sample that is heterozygous for allele 1 and 2.

**IMPORTANT!** Editing the sample type after a run can affect the validity of the plate setup.

## Add a custom attribute to samples

Custom attributes can be added for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the **Samples** table, click ⋯ **(Actions)** ▶ **Add Custom Attribute**.
2. In the **Add Custom Attribute** window, enter the custom attribute name, then click **Done**.
   A column for the custom attribute is added to **Samples** table, and a new tab for the custom attribute is created.
3. Select an option to define the choices for the custom attribute:

   | Option | Description |
   | --- | --- |
   | Define in the **Samples** table | In the **Samples** table, define the custom attribute in the appropriate field in the custom attribute column. |
   | Define in the custom attribute tab | a. In the custom attribute tab, click + **(Add)**.<br>b. In the table, click in the field to edit the custom attribute name. |

   The custom attribute options are added to the following locations:
   - **Samples** table, in the dropdown list in the custom attribute column
   - Table in the custom attribute tab
4. In the **Samples** table, for each sample, select the custom attribute from the dropdown list.
5. (*Optional*) Edit the custom attribute name or color.
   a. Select the custom attribute tab.
   b. In the table, click a field to edit.

## Add targets or SNP assays and assign to plate wells

### Import plate setup from TaqMan™ files

Import assay information using your TaqMan™ assay order details. The plate setup information extracted from TaqMan™ files is the same as the information in the Assay Information File (AIF), and does not include sample information.

Importing the TaqMan™ files is not available for the OpenArray™ Plate format.

**Note:** Using this feature requires an internet connection.

1. In the **Plate Setup** tab, in the plate layout pane, click ⋯ **(Actions)** ▸ **Import TaqMan™ assay/plates & card files**.
2. Select a product from the dropdown list, then enter the required information.

| Product | Required Information |
|---|---|
| TaqMan™ Assays | • Sales Order Number<br>• Rack/Plate ID |
| Fixed TaqMan™ Array Cards | • Part Number<br>• Lot Number |
| Custom Gene Expression TaqMan™ Array Cards | • Sales Order Number<br>• Lot Number |
| Custom Advanced miRNA TaqMan™ Array Cards | • Sales Order Number<br>• Lot Number |
| Fixed TaqMan™ Array Plates | • Part Number<br>• Batch Number |
| Custom TaqMan™ Array Plates | • Sales Order Number<br>• Batch Number |

3. Click **Import Plate Setup**.

## Import an Assay Information File (AIF)

An Assay Information File (AIF) is provided with every TaqMan™ assay order. An AIF does not include sample information.

Importing an AIF is not available for the OpenArray™ Plate format.

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**Note:** TaqMan™ Array Card plate setup requires AIF import, as targets cannot be added, assigned, or edited manually for TaqMan™ Array Cards.

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Prior to plate setup, download the AIF for your order at **thermofisher.com/taqmanfiles**.

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**Note:** To directly import AIF information into the **Plate Setup** without having to first download the file, see "Import plate setup from TaqMan™ files" on page 39.

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1. In the **Plate Setup** tab, in the plate layout pane, click ⋯ **(Actions)** ▸ **Import AIF**.
2. Navigate to, then select the previously downloaded AIF file.
3. Click **Open**.

## Import a plate setup file

Import a plate setup file that was previously exported from the software (see "Export a plate setup file" on page 48 ), or a user-created plate setup file. The following file types can be imported as a plate setup file:

- TXT—text format
- CSV—comma-separated values format

This is not available for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the plate layout pane, click ⋯ **(Actions)** ▸ **Import Plate Setup**.
2. Navigate to, then select the file.
3. Click **OK** to confirm that the plate setup is overwritten.

## Copy targets or SNP assays from the plate layout

Copy is available for all formats.

For the TaqMan™ Array Card plate setup and the OpenArray™ Plate format, this feature copies the information to the clipboard. It can be copied into another program for reference.

Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Navigate to the **Plate Setup** tab.
2. In the **Targets** table or the **SNP assays** table, click one of the following items.
   - ⋯ **(Actions)** ▸ **Copy all targets**
   - ⋯ **(Actions)** ▸ **Copy all SNP assays**

The information is available in the clipboard. It can be pasted into another program for reference.

## Paste targets or SNP assays in the plate layout

You can copy targets or SNP assays from an Excel™ spreadsheet, then paste them into the **Plate Setup** tab.

**Note:** Paste is not available for TaqMan™ Array Card plate setup or the OpenArray™ Plate format.

1. Create an Excel™ spreadsheet with the plate setup information.
2. In the spreadsheet, select, then copy the cells of interest.
3. Navigate to the **Plate Setup** tab.
4. In the **Targets** table or the **SNP assays** table, click one of the following items.
   - ⋯ **(Actions)** ▸ **Paste targets**
   - ⋯ **(Actions)** ▸ **Paste SNP assays**

The targets or SNP assays are copied to the respective table. They must be added to the plate layout (see "Manually add or assign a target or SNP assay to a well" on page 42).

## Manually add targets or SNP assays to the Targets or SNP Assays table

Targets cannot be added manually for TaqMan™ Array Card plate setup. To add targets for TaqMan™ Array Cards, see "Import an Assay Information File (AIF)" on page 40.

Targets cannot be added manually for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the plate layout pane, select **Target** or **SNP** to display the appropriate table in the right pane.
2. Select an option in the upper right corner of the **Targets** or **SNP Assays** table.

| Option | Description |
|---|---|
| Add a single target or SNP assay | Click + **(Add)**. |
| Copy/paste multiple targets or SNP assays | a. Copy the information from one of the following sources:<br>• **Targets** or **SNP Assays** table from plate file or data file—Click ⋯ **(Actions)** ▸ **Copy all Targets/SNP Assays** in the upper-right corner of **Targets** or **SNP Assay** table.<br>• Excel file—Select, then copy data, including column headers.[1]<br>• Plate setup file—Select, then copy data, including column headers.[1]<br>b. Click ⋯ **(Actions)** ▸ **Paste Targets/SNP Assays**. |

[1] Column headers must match the column headers in the **Targets** or **SNP Assays** table.

**Note:**
- Click ··· **(Actions)** ▸ **Export Targets/SNP Assays** to export targets or SNP assays.
- Click ··· **(Actions)** ▸ **Import Targets/SNP Assays** to import targets or SNP assays.

3. Click in a cell in the table to edit the attributes for the target or SNP assay.

4. To remove a target or SNP assay, click ✕ **(Remove)**.

## Manually add or assign a target or SNP assay to a well

Targets cannot be added or assigned manually for TaqMan™ Array Card plate setup. To add or assign targets for TaqMan™ Array Cards, see "Import an Assay Information File (AIF)" on page 40.

This is not available for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the plate layout pane, select one or more wells in the **(Grid View)** or the **(Table View)**.

2. Assign the target or SNP assay to the selected well.

| Option | Description |
|---|---|
| (Grid View) | |
| Target or SNP assay not defined | Enter the target or SNP assay in the text field. |
| Target or SNP assay previously defined | • Select the target or SNP assay from the dropdown list.<br>• Select the checkbox of the target or SNP assay in the **Targets** table or **SNP Assays** table. |
| (Table View) | |
| Target or SNP assay previously defined | Select the checkbox of the target or SNP assay in the **Targets** table or **SNP Assays** table. |

**Note:** In **Targets** table or **SNP Assays** table, change the default selections for the reporter and quencher dyes and for tasks (see "(Optional) Edit the task assigned to a target or SNP assay in one or more wells" on page 43).

## (*Optional*) Edit the task assigned to a target or SNP assay in one or more wells

The software automatically assigns a task to the target or SNP assay in a well based on the sample type in that well. The automatic task assignment can be edited, if needed (except for TaqMan™ Array Card plate setup and the OpenArray™ Plate format).

1. In the **Plate Setup** tab, in the plate layout pane, select plate wells in the **(Grid View)** or the **(Table View)** (see "Select plate wells or ports" on page 34).

   If selecting multiple wells, only select well that have the same target or SNP assay, and the same sample type.

2. In the **Targets** or **SNP Assays** table, confirm that the checkbox of the target or SNP assay is selected.

3. Select a detection task from the **Task** column dropdown list.

   The available task options depend on the sample type in the selected well (see "Edit sample name, color, and type" on page 37).

| Task | Description |
|---|---|
| Unknown (default) | The well contains an unknown sample. |
| Standard[1] | The well contains a sample with known standard quantities.<br>**Note:** The quantity for the standard sample should be entered in the **Samples** table. For each target, the value entered in **Quantity** column in the **Targets** table must be the same for every well. |
| Negative Control | The well contains water or buffer instead of sample. |
| Positive Control | The well contains a positive control. |
| Internal positive control (IPC) [2] | The well contains a short synthetic DNA template. The IPC is used to distinguish between true negative results and negative results caused by PCR inhibitors, incorrect assay setup, or reagent or instrument failure. |
| Blocked IPC | The well contains an IPC blocking agent, which blocks amplification of the IPC. |
| Positive 1/1 | The well contains a sample homozygous for allele 1. |
| Positive 2/2 | The well contains a sample homozygous for allele 2. |
| Positive 1/2 | The well contains a sample heterozygous for allele 1 and 2. |

[1] For standard curve and relative standard curve analysis only.
[2] For presence/absence analysis only.

## Edit the target or SNP assay name and color

The sample name and color can be edited for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the **Targets** table or the **SNP Assays** table, click the name in the **Target** column or the **SNP Assays** column.

2. Enter a new name, then press **Enter**.

3. Click the sample color in the **Color** column, then select a color from the color picker.

## Manage target dyes

Managing the target dyes is not available for the OpenArray™ Plate format.

To add a custom dye from the library, ensure that the custom dye has been added (see Chapter 13, "Manage preferences").

1. In the **Plate Setup** tab, in the plate layout pane, click ⋯ **(Actions)** ▸ **Manage Dyes**.
   The **Manage Dyes** dialog box is displayed.
2. View system dyes in the **System Dyes** tab.
   All of the system dyes are available to set up the plate file.
3. Add a custom dye.

| Option | Instructions |
|---|---|
| Add a custom dye from the library. | a. Click the **Custom Dyes** tab.<br>b. Select the **Show custom dyes from the library, and click to import** checkbox.<br>c. In the **Library Dye Name** column click the dye to add.<br>The dye and the details of the dye are displayed in the table on the right.<br>d. Click **Close**. |
| Add a new custom dye. | a. Click the **Custom Dyes** tab.<br>b. Deselect the **Show custom dyes from the library, and click to import** checkbox.<br>c. Click + **(Add)**.<br>d. Edit the following fields:<br>• Enter a name in the **Dye Name** field.<br>• Select a color from the color picker.<br>• Select a type from the **Type** dropdown list.<br>• Enter a wavelength in the **Wavelength** field.<br>e. Click **Close**. |

   If a new custom dye is added when setting up a plate file, it is not applied to the system. A custom dye must be added in the **Preferences** page in order to apply to the system.

4. (*Optional*) Click ✖ **(Remove)** ▸ **OK** to remove a dye from the table.
5. Click **Close**.

## Edit reagent information

Reagents can be edited for the OpenArray™ Plate format.

1. In the **Plate Setup** tab, in the **Targets/SNP Assays** table pane, click **Reagents**.
2. In the **Reagents** table, click + **(Add)**.

   **Note:**
   - Click ··· **(Actions)** ▸ **Export Reagents** to export reagents.
   - Click ··· **(Actions)** ▸ **Import Reagents** to import reagents.
   - Click ··· **(Actions)** ▸ **Scan Reagents** to scan reagents.

3. Enter the following information for each reagent.
   - Name
   - Type
   - Barcode
   - Part Number
   - Lot Number
   - Expiration Date

   **Note:** If the master mix that you enter is not compatible with the current run method, you have the option to apply the recommended run method for your master mix, instrument, block, and run mode.
   For more information about setting up the recommended run method for your master mix, see "Apply the recommended run method for your master mix" on page 26.

4. (*Optional*) Click ✕ **(Remove)** in the row of a reagent to delete it from the table.

## Select a passive reference

1. In the upper-left corner of the **Plate Setup** tab, select a passive reference from the dropdown list.
2. (*Optional*) Save the plate file or data file.

## Set up the standard curve

A standard curve is only required for standard curve or relative standard curve analysis. See the appropriate analysis module for more information.

**Note:**
- Multiple targets can be assayed using standard curve analysis, but each target requires its own standard curve.
- You can also set up the standard curve during sample setup (see "Add samples and assign to plate wells" on page 34).

1. In the **Plate Setup** tab, in the plate setup pane, click ··· **(Actions)** ▸ **Standard Curve Setup**.
   The **Standard Curve Wizard** opens.
2. In the **Standard Curve Wizard** pane, enter the sample name prefix.

3. Select the target for the standard curve.

| Option | Instructions |
| --- | --- |
| Target previously defined | Select the target from the dropdown list. |
| Target not previously defined | **1.** Type the target name, then press **Enter**.<br>**2.** Select a reporter from the dropdown list.<br>**3.** Select a quencher from the dropdown list. |

4. Adjust the parameters for the dilution series if needed.
    - **Number of points**—5 recommended
    - **Number of replicates**—3 recommended
    - **Starting Quantity**—The highest or lowest standard quantity, without units.

      **Note:** The quantity must be greater than 0.
    - **Serial Factor**

      **Note:** The serial factor calculates quantities for all standard curve points.

      – Starting quantity is the highest value—Select 1:10 to 1:2.
      – Starting quantity is the lowest value—Select 2× to 10×.

5. Select an option to select the wells for the standard.
    - Select **Automatically**.
    - Select **Manually**, then select wells using the displayed plate layout.

6. Select to arrange the standards in **Rows** or **Columns**.

7. Click **Apply Standard Curve**, then click **Close** to return to the **Plate Setup** tab.

## Add biogroups and assign samples

Biogroups, or Biological Replicate Groups, are reactions that contain identical components and volumes, but evaluate separate samples of the same biological source. Biogroups can be used in relative quantification analysis.

1. In the **Plate Setup** tab, in the upper right pane, select an option to add biogroups:

| Option | Description |
|---|---|
| Add biogroups in the **Samples** table | In the **Samples** table, enter the new biogroup name in the **Biogroup** field.<br>The biogroup is added to the following locations:<br>• **Samples** table **Biogroup** dropdown list<br>• **Biogroup** table |
| Add biogroups in the **Biogroup** table | a. Click **Biogroup**.<br>b. In the **Biogroup** table, click + **(Add)**. |

2. In the **Samples** table, for each sample in a biogroup, select a biogroup from the dropdown list.

3. (*Optional*) Edit the biogroup name or color.
   a. Click **Biogroup**.
   b. In the **Biogroup** table, click a field to edit.

# Edit plate file or data file information

The plate file can be edited for the OpenArray™ Plate format.

1. Click **Actions ▸ Plate Information**.

2. In the **Plate Information** dialog box, edit the **Experiment Name** field.

3. (*Optional*) Click in the **Barcode** field, then perform one of the following steps.
   - Scan the plate barcode with a barcode scanner.
   - Manually enter the plate barcode.

4. (*Optional*) Add a tag.

5. (*Optional*) Enter a comment in the **Comment** field.

6. Click **Save**.

Save the updated plate file or data file.

# Review the plate file and send to the instrument run queue

The instrument run queue is available only for the QuantStudio™ 6 Pro Real-Time PCR Instrument and the QuantStudio™ 7 Pro Real-Time PCR Instrument.

1. In the **Run Summary** tab, review the run method selections, then edit if needed (see "Confirm or edit run method" on page 26).
2. Review the plate setup, then edit if needed (see "Confirm or edit plate setup" on page 31).
3. (*Optional*) Click the barcode field, then scan the plate barcode or manually enter the barcode.
4. (*Optional*) Select **Add to My Plates Gallery**.
5. Select an instrument from the list.

   If the instrument does not appear on the list, click **System ▸ Instruments** to add a new instrument (see "Add an instrument" on page 81).
6. Save the plate file (see "Save a plate file or data file" on page 78).
7. Click **Send to Run Queue**.
8. Click **Done** to close **Run Sent** dialog box.

Start the run on an instrument. For specifics on starting an instrument run, see the instrument documentation.

# Export a plate setup file

Export a plate setup file to use during future plate setups (see "Import a plate setup file (samples)" on page 34).

The following files types can be exported:

- TXT—text format
- CSV—comma-separated values format

Samples are exported for the OpenArray™ Plate format. The targets are not exported.

Export the sample layout to use for sample integration in the QuantStudio™ 12K Flex OpenArray™ AccuFill™ System.

1. In the **Plate Setup** tab, in the plate layout pane, click one of the following options.

| Format | Procedure |
|---|---|
| 96-well, 0.2-mL plate<br>96-well, 0.1-mL plate<br>384-well plate<br>TaqMan™ Array Card | Click ⋯ **(Actions)** ▸ **Export Plate Setup**. |
| OpenArray™ Plate | Click ⋯ **(Actions)** ▸ **Export Sample Layout**. |

2. Name the file, navigate to the desired folder location, select the file format, then click **Save**.

# Print the layout

Printing the layout saves the information in one of the following file formats:

- XLSX
- PDF

For the OpenArray™ Plate format, each cell in an XLSX file format represents a subarray.

Samples are represented for the OpenArray™ Plate format. The targets are not represented.

These files are for reference. To export in a format that can be imported, see "Export a plate setup file" on page 48.

1. In the **Plate Setup** tab, in the plate layout pane, click one of the following options.

| Format | Procedure |
|---|---|
| 96-well, 0.2-mL plate<br>96-well, 0.1-mL plate<br>384-well plate<br>TaqMan™ Array Card | Click ⋯ **(Actions)** ▸ **Print Layout**. |
| OpenArray™ Plate | Click ⋯ **(Actions)** ▸ **Print Sample Layout**. |

2. Name the file, then navigate to the desired folder location.

3. Select the file format.
   - Select the **XLSX** radio button
   - Select the **PDF** radio button

4. If PDF file format was selected, select the paper size.
   - Select the **A4** radio button
   - Select the **Letter** radio button

5. Click **Save**.

# 5 Review and analyze data

We recommend that you analyze data using the default analysis settings. If the default analysis settings are not suitable for the data, modify the analysis settings, then reanalyze the data.

For information about general procedures to analyze data in the **Quality Check** tab, see Chapter 7, "General procedures to analyze data in the Quality Check tab".

## Workflow: General procedures to review analysis results

The software automatically analyzes run data using the analysis settings that are specified during plate file set up. The software then displays analysis results in the **Quality Check** tab.

Review results in the Amplification Plot to confirm or correct threshold and baseline settings (Review results in the Amplification Plot (page 51))

▼

Review data for outliers and (*optional*) omit wells (Identify and omit outliers from analysis (page 57))

▼

(*Optional*) View the Multicomponent Plot to review the dye signal profile (Review results in the multicomponent plot (page 57))

▼

(*Optional*) View the Raw Data Plot to review the signal profile (Review results in the raw data plot (page 58))

▼

(*Optional*) Review flags and QC alerts (Review QC alerts in the well table (page 60))

▼

(*Optional*) Edit analysis settings (Edit primary analysis settings (page 61))

▼

(*Optional*) Perform additional analysis (Perform additional analysis (page 67))

**IMPORTANT!** If you omit wells, click **Analyze** to reanalyze the data.

# Open a data file

1. In the home screen, click **Open File**.

2. Navigate to, then open a data file.

| Option | Description |
|---|---|
| Open data files that were automatically transferred to the software from the instrument. | The data file is saved to the same location as the plate file that was used for the instrument run. |
| Open data files that were manually transferred from the instrument. | Navigate to the location that was selected when the data files were transferred from the instrument. |

- The data file opens and the analysis results are displayed in the **Quality Check** tab.
- The data file is added to the **Data Gallery**, and appears in the **Recents** tab.

# Select by subarray

When viewing the **Quality Check** tab for data from the OpenArray™ Plate format, the option to view by subarray is provided.

1. In the **Quality Check** tab, in the plate layout pane, toggle **Select by Subarray** on.

2. Click the subarray of interest.

3. Select multiple subarrays, if required.
   - Use the control + click function to select multiple subarrays.
   - Use the click + drag function to select multiple subarrays.

4. To select subarrays and single cells, select the subarrays, toggle **Select by Subarray** off, then select single cells.

The items within the selected subarray or subarrays are highlighted in blue in the well table view. The plots display the results from the selected subarray or subarrays.

# Review results in the Amplification Plot

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

For more information about the **Amplification Plot**, see "Amplification Plot overview" on page 98.

## Evaluate the overall shape of the curves in the amplification plot

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.
2. Click ⚙ **(Settings)**, then make the following selections:
   - **Color By**: **Target**, **Sample**, or **Well**
   - **Y Value**: **ΔRn**
   - **Y Scale**: **Log**
3. *(Optional)* To show or hide the background grid in the amplification plot, click ⚙ **(Settings)**, then select or deselect the **Grid** checkbox.
4. Review the overall shape of the curves in the amplification plot.

   For more information about the amplification plot, see "Amplification Plot overview" on page 98.

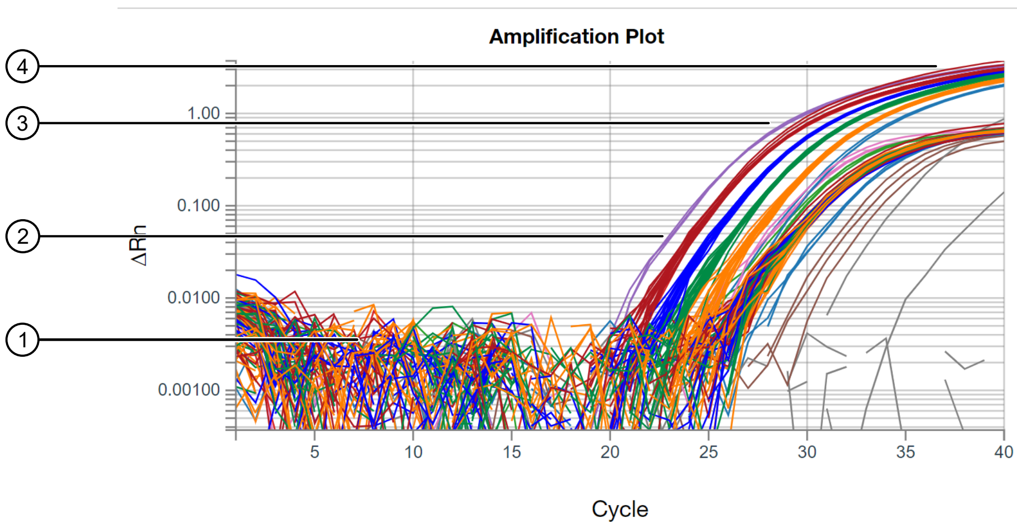



**Figure 1 Typical amplification plot**

A typical amplification curve has four distinct sections:

① Baseline
② Exponential (geometric) phase
③ Linear phase
④ Plateau phase

## Review the amplification status for each well

**Note:** **Amp Status** is only applicable for analysis that includes a PCR stage.

In the **Quality Check** tab, in the **Well Table**, review the amplification status of each well.

The **Amp Status** column displays one of four values:

| Amplification status value | Description |
|---|---|
| Amp | Target amplified. |
| No Amp | Target did not amplify. |
| Inconclusive | Unable to determine if amplification occurred. Review run data. |
| N/A | One of the following occurred.<br>• The well was omitted from analysis.<br>• Insufficient cycle number to determine if amplification occurred. |

## Review or edit threshold settings in the amplification plot

The default analysis setting is for automatic threshold. To set the threshold manually, see "View or edit Cq settings" on page 61.

The threshold values can be edited only if the baseline threshold is selected as the algorithm (see "View or edit Cq settings" on page 61).

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.

2. Click **(Settings)**, then make the following selections:

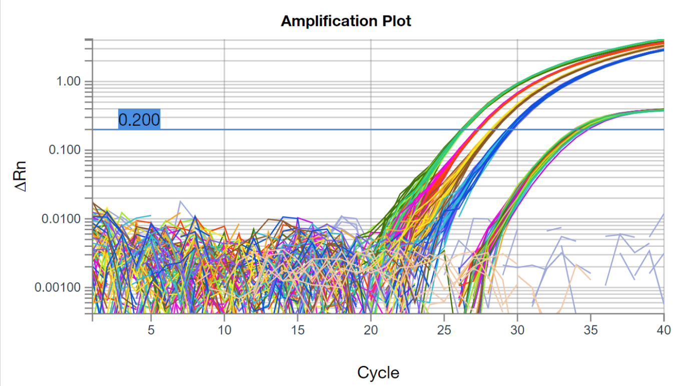
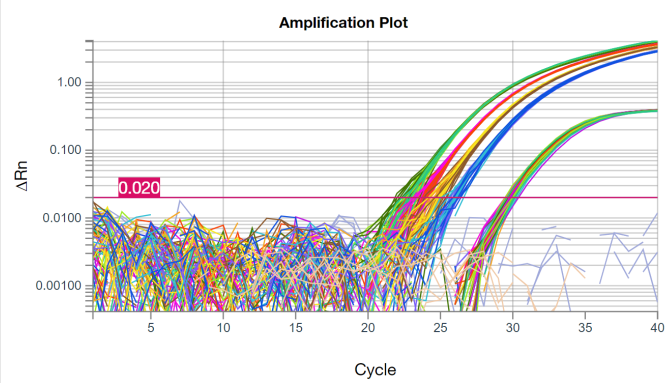
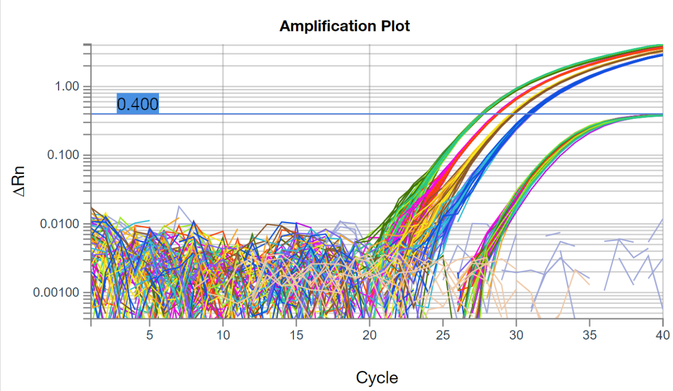
   - **Plot Color**: **Target**, **Sample**, or **Well**
   - **Y Value**: **ΔRn**
   - **Y Scale**: **Log**

   The amplification plot is displayed for all wells.

3. *(Optional)* To show or hide the background grid in the amplification plot, click **(Settings)**, then select or deselect the **Grid** checkbox.

4. Review the threshold values to determine if editing is necessary. A threshold set above or below the optimum can increase the standard deviation of the replicate groups.

**Table 1 Examples of threshold settings**

| Threshold setting evaluation | Example |
|---|---|
| Threshold set correctly. |  |
| Threshold set too low. |  |
| Threshold set too high. |  |

5. (*Optional*) Adjust the threshold in the exponential phase of the amplification curve.

**Note:** For easier viewing, ensure that the **Y Scale** is set to log (default), not linear.

- Click-drag the threshold bar into the exponential phase of the curve.
- Edit the $C_q$ analysis settings (see "View or edit Cq settings" on page 61).

## Review or edit baseline settings in the amplification plot

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.

2. In the plot pane, click **(Settings)**, then make the following selections:

   - **Y Value**: **Rn**
   - **Y Scale**: **Linear**
   - **Color By**: **Well**
   - **Baseline**

   The baseline can be selected only if the baseline threshold is selected as the algorithm (see "View or edit Cq settings" on page 61).

   **Note:** The start and end cycles are used to calculate the baseline.

   The amplification plot is displayed for the selected wells in the **Plate Layout** pane.
   The start and end cycles display for each well.

3. *(Optional)* To show or hide the background grid in the amplification plot, click **(Settings)**, then select or deselect the **Grid** checkbox.

4. (*Optional*) Adjust the start and end cycle values for the baseline (see "View or edit Cq settings" on page 61).

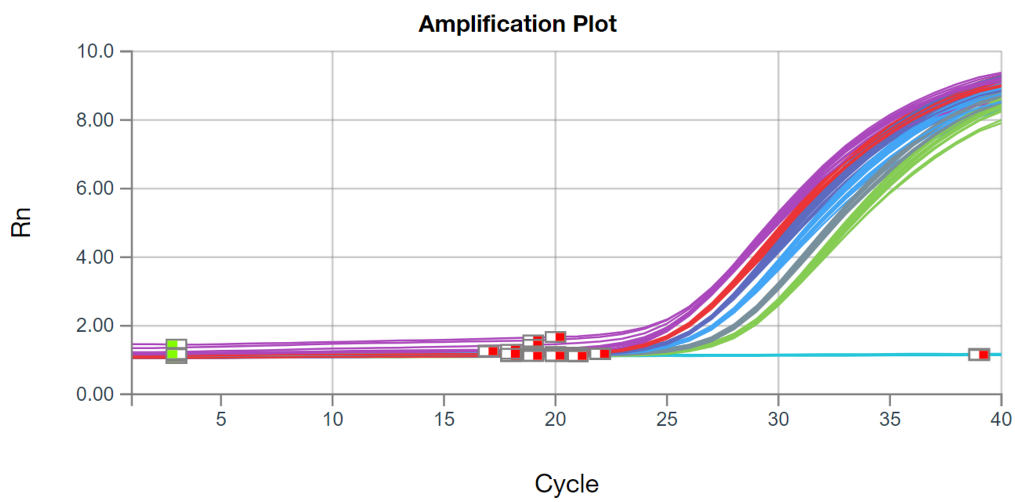



**Figure 2 Example of correct baseline**

Set the end cycle a few cycles before the cycle number where significant fluorescence signal is detected.

## Optimize display of negative controls in the amplification plot

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.
2. In the plot pane, click ⚙ **(Settings)**, then make the following selections:
   - **Y Value**: **ΔRn**
   - **Y Scale**: **Linear**
   - **Color By**: **Target**
   - Deselect **Show: Threshold**
   - Deselect **Show: Baseline**
3. *(Optional)* To show or hide the background grid in the amplification plot, click ⚙ **(Settings)**, then select or deselect the **Grid** checkbox.
4. In either the **Plate Layout** or **Well Table**, select the negative control wells (wells that should not have amplification for a particular target).
5. In the plot pane, click ⚙ **(Settings)**, then make the following selections in the **Y Axis** tab.
   a. Deselect **Auto-adjust range**.
   b. Enter **Minimum value** of –1.
   c. Enter **Maximum value** of 2.

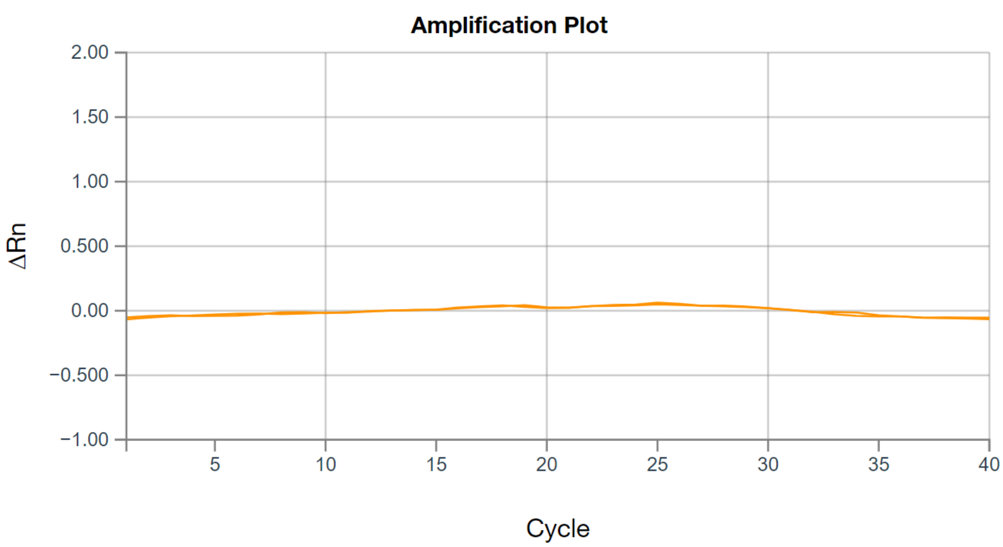



**Figure 3 Example amplification plot of negative controls**

The linear plot displays the amplification plot for negative controls as smooth lines. The expanded y-axis displays low levels of amplification.

# Identify and omit outliers from analysis

Outlier wells have $C_q$ values that differ significantly from the average for the associated replicate wells. To ensure $C_q$ precision, consider omitting the outliers from analysis.

1. In the **Quality Check** tab, select **Amplification Plot** from the dropdown list.
2. In the plot pane, click **(Settings)**, then make the following selections to configure the plot:
   - **Y Value**: **ΔRn**
   - **Y Scale**: **Linear**
   - **Color By**: **Well**
3. *(Optional)* To show or hide the background grid in the amplification plot, click **(Settings)**, then select or deselect the **Grid** checkbox.
4. To identify outliers in the **Plate Layout**, select **Cq** from the dropdown list.
   The $C_q$ values for each well are color-coded according to the value.
5. Omit outliers in either the **Well Table** or **Plate Layout**.
   - In the **Well Table**, select **Omit** in the row of the outlier well.
   - In the **Plate Layout**, select a well or multiple wells, then select ··· **(Actions)** ▸ **Omit Wells**.
6. Click **Analyze** to reanalyze the run data with any outliers removed.

# Review results in the multicomponent plot

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Multicomponent Plot** from the dropdown list.
2. Click **(Settings)**, then select **Dye** from the **Color By** dropdown list.
   The **Multicomponent Plot** is displayed for all wells.
3. (*Optional*) To edit the dyes that are displayed in the plot, click **Dyes**, then select dyes from the dropdown list.
4. *(Optional)* To show or hide the background grid in the multicomponent plot, click **(Settings)**, then select or deselect the **Grid** checkbox.

5. In the **Plate Layout**, select wells one at a time, then examine the **Multicomponent Plot** for the following plot characteristics.

| Plot characteristic | Description |
|---|---|
| Passive reference dye | The passive reference dye fluorescence signal should remain relatively constant throughout the PCR process. |
| Reporter dye | The reporter dye fluorescence signal should display a flat region corresponding to the baseline, followed by a rapid rise in fluorescence as the amplification proceeds. |
| Irregularities in the signal | Spikes, dips, and/or sudden changes in the fluorescence signal may have an impact on the data. |
| Negative control wells | The negative control wells should show no significant increase in fluorescence signal. |

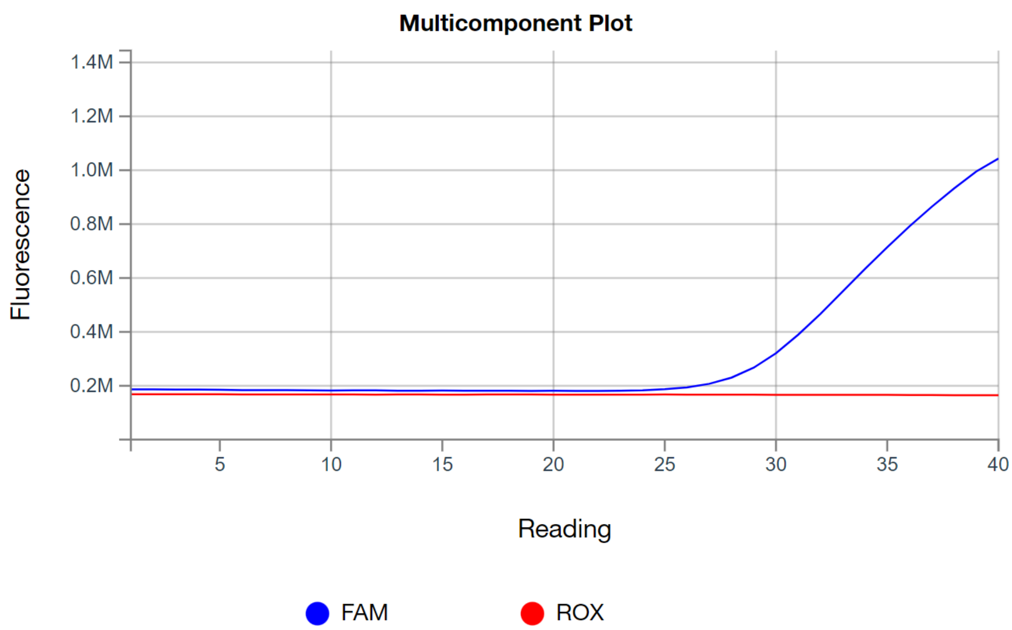



**Figure 4 Example multicomponent plot (single well)**

# Review results in the raw data plot

For more information about the raw data plot, see “Raw Data Plot overview” on page 99.

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Raw Data Plot** from the dropdown list.

2. *(Optional)* To show or hide the background grid in the raw data plot, click ⚙ **(Settings)**, then select or deselect the **Grid** checkbox.

3. Click-drag the **Cycle Number** slider from cycle 1 to cycle 40, then confirm that each filter displays the characteristic signal increase.

   For more information on each filter set, see the instrument documentation.

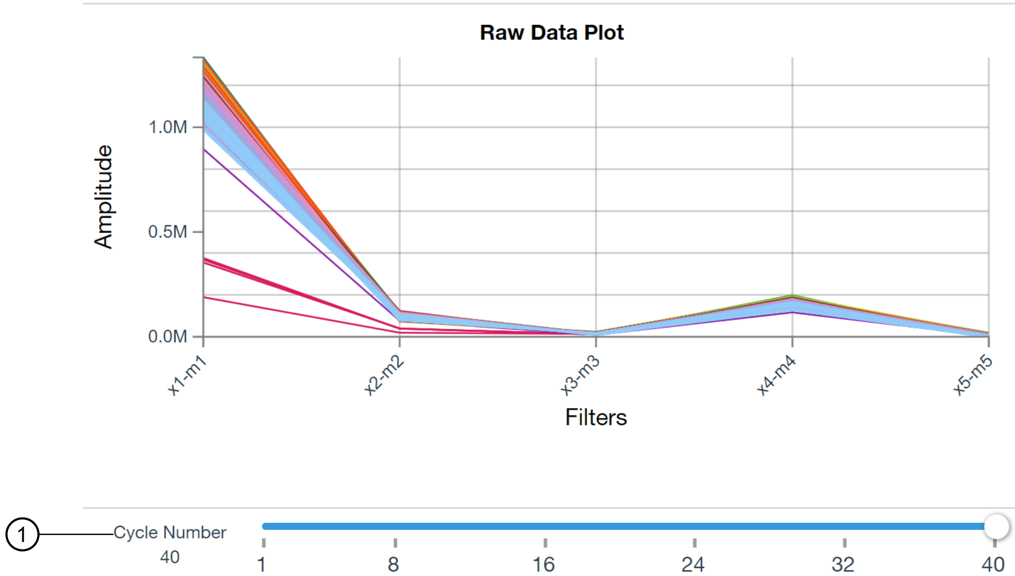



**Figure 5 Example Raw Data Plot**

① Slider to select the cycle

# Review results in the melt curve plot

For custom experiments with more than one melt curve stage. For more information about the melt curve plot and the melt peak parity factor, see "Melt Curve Plot overview" on page 100.

For analysis with more than one melt curve stage, select the melt curve stage to analyze in the analysis settings (see "View or edit melt analysis settings" on page 64).

1. In the **Quality Check** tab, in the plot pane, select **Melt Curve Plot** from the dropdown list.
2. In the plot pane, click ⚙ **(Settings)**, then make the following selections:
   - **Color By**: **Sample**, **Target**, or **Well**
   - **Plot Type**: **Derivative**
3. *(Optional)* To show or hide the background grid in the melt curve plot, click ⚙ **(Settings)**, then select or deselect the **Grid** checkbox.
4. Review the plot for evidence of unexpected multiple peaks, which can indicate non-specific amplification or formation of primer-dimers.
5. Review the **Well Table** for the calculated $T_m$ in each well.

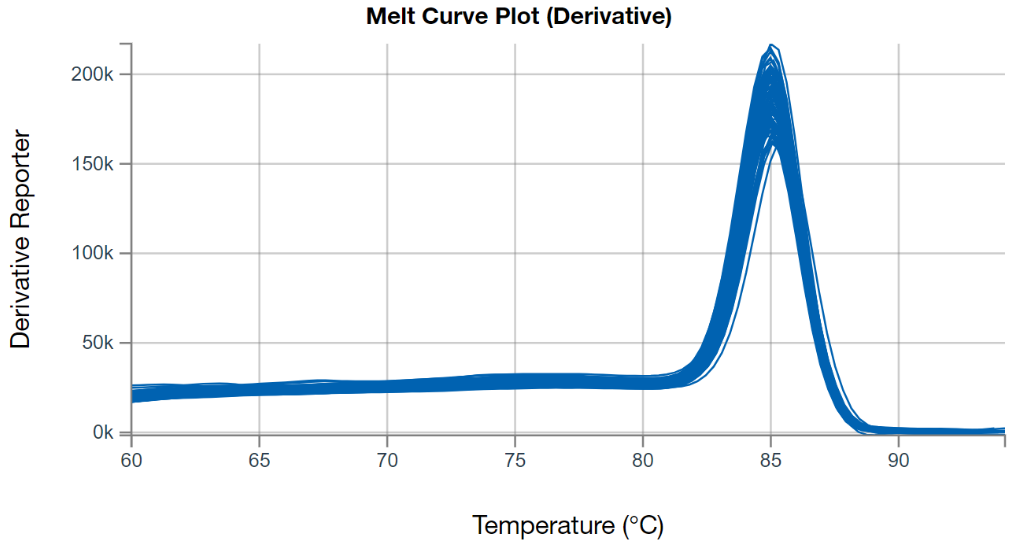



**Figure 6 Example Melt Curve Plot**

## Review QC alerts in the well table

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, review the **Well Table** for alerts in the following columns.
   - **Curve Quality** column
   - **Result Quality Issues** column

   For more information about the QC alerts, see "Overview of the result quality checks" on page 95.

2. Adjust the QC alert settings as needed (see "View or edit QC alerts settings" on page 62), then reanalyze.

## Review results in the plate layout

1. In the **Quality Check** tab, in the **Plate Layout** pane, select one of the following from the **Color By** dropdown list:
   - **Sample**
   - **Target**
   - **$C_q$**
   - **$C_q$ Confidence**
   - **Amp Score**
   - **Amp Status**

2. Review the results for each well (see "Acceptance criteria for result quality checks" on page 96).

# Edit primary analysis settings

Primary analysis settings include:

- $C_q$ settings
- Melt settings
- QC settings

We recommend that you analyze data with the default analysis settings. If the default analysis settings are not appropriate for the data, modify the analysis settings, then reanalyze the data.

## View or edit $C_q$ settings

- The default $C_q$ settings are appropriate for most applications. Edit the threshold and baseline settings for analysis of atypical or unexpected run data.
- For information about $C_q$ analysis, see "About the quantification cycle (Cq)" on page 92.
- For information about $C_q$ settings, see "Cq settings overview" on page 93.

1. Open a plate file or data file, then click **Actions ▸ Primary Analysis Setting**.
2. In the **General** tab, select an option from the **PCR Stage/Step** dropdown list.
3. Select an option from the **Algorithm Settings** dropdown list.
   - **Relative Threshold**
   - **Baseline Threshold**
4. *(For relative threshold algorithm settings)* Select the PCR stage and step from the **PCR Stage/Step** dropdown list.
5. *(For relative threshold algorithm settings)* Enter a start cycle in the **Default $C_{RT}$ Start Cycle** field.
6. *(For baseline threshold algorithm settings)* To select the default threshold and baselines settings for a target, select the checkbox in the **Use Default** column.

7. *(For baseline threshold algorithm settings)* To edit the settings, make the edits in the appropriate table row.

| Option | Action |
|---|---|
| Use Auto Threshold | Select the checkbox in the **Auto Threshold** column. |
| Manually set the Threshold | Deselect the checkbox in the **Auto Threshold** column, then edit the value in the **Threshold** column. |
| Use Auto Baseline | Select the checkbox in the **Auto Baseline** column.<br>• To specify the Baseline Start cycle, Click **AUTO** in the **Baseline Start** column, then enter the cycle number. The software will automatically determine the Baseline end cycle.<br>• To remove the specified Baseline Start cycle, click the cycle number, then delete it. The **Baseline Start** will revert back to **AUTO**. |
| Manually set the Baseline | Deselect the checkbox in the **Auto Baseline** column, then edit the values in the **Baseline Start** field and the **Baseline End** field. |

8. (*Optional*) In the **Well Cq** tab, make the edits in the appropriate table row to apply custom $C_q$ settings to a specific well.

9. Click **Save**.

10. (*Optional*) To reset to the default settings, click **Reset to Default**.

## View or edit QC alerts settings

1. Open a plate file or data file, then click **Actions ▸ Primary Analysis Setting**.

2. In the **QC Alerts** tab, review selections:
   - Curve Quality—(*default*) enabled
   - Results Quality—(*default*) disabled

3. (*Optional*) Deselect **Curve Quality**.

4. (*Optional*) Enable **Results Quality**.
   a. Select **Results Quality**.

   b. Click + **(Add)**.

   c. Select the **Sample Type** from the dropdown list, then select the **Target** from the dropdown list.

      If a rule is set up for a specific sample type, it takes precedence over a rule that is set for all samples.

   d. In the right panel, select the acceptance criteria for each sample type and target combination (see "Acceptance criteria for result quality checks" on page 96).

e. (*Optional*) Click ✕ **(Remove)** to remove a sample type and target combination from the table.

5. Click **Save**.

6. (*Optional*) To reset to the default settings, click **Reset to Default**.

In the **Quality Check** tab, click **Analyze**, then review the QC alerts in the **Well Table**.

## View or edit advanced settings

1. Open a plate file or data file, then click **Actions ▸ Primary Analysis Setting**.

2. In the **Advanced** tab, select and/or enter the following, then click **Save**
   - **Use a variant of primary analysis algorithm via plugin**
   - **Set the Delta-Rn below which curves will be considered Non-Amplified**

   For QuantStudio™ Design and Analysis Software v2.7 and later, any curves with the $\Delta R_n$ below the threshold are set to non-amplified. This is regardless of whether there is a $C_q$ value.
   For QuantStudio™ Design and Analysis Software v2.6 and earlier, if the $\Delta R_n$ is below the threshold but there is a $C_q$ value, the curves are set to inconclusive

3. Select the **Reduce dye signal crosstalk by algorithm**.

   **Note:** Selecting this option might slow down the analysis.

   For a description of this setting, see "Overview of the algorithm reduce dye signal cross-talk" on page 64.

4. Click **Update** to upload a custom configuration file for the algorithm to reduce dye signal crosstalk.

   **Note:** A custom configuration file must be obtained from Thermo Fisher Scientific.

   A default configuration file is included in the primary analysis plugin of the software.
   A configuration file is in the CFG file format.
   If a custom configuration file is uploaded, the name of the configuration file is displayed in the dialog box.

5. If a custom configuration file was uploaded, click **Reset** to return to the default configuration file.
   The custom configuration file is removed.

6. Click **Save**.

7. (*Optional*) To reset to the default settings, click **Reset to Default**.

### Overview of the algorithm reduce dye signal cross-talk

Dye signal cross-talk can be observed when the dye calibration does not exactly match the spectrum of the assay. This appears as false dye signal that rises or drops under a true dye amplification.

An algorithm is available to reduce the dye signal cross-talk. The default setting is that it is not used. It can be applied in the primary analysis settings, in the **Advanced** tab.

The use of the algorithm slows down the analysis.

The impact and effectiveness of this algorithm must be assessed for individual assays.

This algorithm cannot be used for the OpenArray™ Plate format.

The algorithm is not available if any of the following conditions apply:

- The algorithm in the primary analysis plugin is different than what was used for the primary analysis of the data file.
- The configuration file was updated between the time of data analysis and the that the algorithm would be applied.

If the algorithm was applied, the data must be reanalyzed when there are changes to the dyes in the plate setup. The reanalysis applies to the wells that were edited.

If the algorithm was applied, the data must be reanalyzed if calibrations from another file are applied.

## View or edit melt analysis settings

For descriptions of the melt analysis settings, see “Melt analysis settings overview” on page 94.

1. Open a plate file or data file, then click **Actions ▸ Primary Analysis Setting**.
2. In the **Melt** tab, select an option from the **Melt Stage/Step** dropdown list.
3. In the **Multi-Peak Calling** column, select the checkbox.
   The threshold type, peak level, and peak height settings are available only when multi-peak calling is enabled.
4. In the **Threshold Type** column, select one of the following options.
   - **Percentage**
   - **Height**
5. In the **Peak Level (%)** column, enter a value.
   A value can be entered only if **Percentage** was selected in step 4.
6. In the **Peak Height** column, enter a value.
   A value can be entered only if **Height** was selected in step 4.
7. *(Optional)* In the **Melt Peak Parity Factor** field, enter a factor for the applicable targets.

   **Note:** If the melt peak parity factor is applied to wells without a reaction mix, this can lead to unexpected $T_m$ results.

A factor of ***0*** turns off the feature. Negative values are not permitted.

The melt peak parity factor does not affect the High Resolution Melt Analysis Module if this module is enabled.

---

8. Click **Save**.

9. (*Optional*) To reset to the default settings, click **Reset to Default**.

## Use the analysis settings from another file

Apply analysis settings from a plate file or data file to an open data file. If you are analyzing a legacy data file (see "Compatible data files" on page 9), you can use this feature to apply updated analysis settings to the legacy data file before analysis.

The following analysis settings are applied to the data file:

- Primary analysis settings
- Analysis module analysis settings

Applying analysis settings from another file is not available for the OpenArray™ Plate format.

---

**Note:** You can only apply analysis settings from another file if the analysis settings are compatible with the new file. Confirm the following before applying analysis settings to the new file:

- The run method consists of the same stages in both files (PCR stage, Melt stage, Pre-Read stage and Post-Read stage).
- The selected analysis module is the same in both files.
- The sample and target/SNP assay information is the same in both files. Because some analysis settings are specific to samples and target/SNP assay setup, remove any sample and target/SNP assay information that is not applicable to the new data file.

---

1. Open a data file, then click **Actions ▸ Use Settings from Another File**.

2. Navigate to the plate file or data file that contains the desired analysis settings.

3. Select the file, then click **Open**.
   The data is reanalyzed using the new analysis settings.

4. Click **Actions ▸ Save** to save the new analysis settings to the data file.

# View instrument calibration results

Transfer calibration data files from the instrument. For more information about instrument calibration, see the instrument documentation.

Calibration results from the OpenArray™ Plate format are not available.

1. In the home screen, click **View Data**.
2. In the **Data Gallery**, click **Actions ▸ Open File**.
3. Navigate to the location that was selected when the calibration data files were transferred from the instrument, then select the calibration data file.
   1. The calibration data file is opened, and calibration results are displayed.
   2. The calibration data file is added to the **Data Gallery**, and appears in the **Recents** tab.

## Review ROI/Uniformity calibration results

1. In the **ROI** tab, select a **Filter Set** from the dropdown list to see the corresponding results.
2. In the **Uniformity** tab, review results in the plot, the  **Well Table**, or the **Plate Layout**.

## Review Background calibration results

1. Review the calibration properties, including calibration status, in the menu bar.
2. Select the plate wells in the **Plate Layout** or the **Well Table** to view the corresponding curves.
3. Review data in the **Well Table**.
   a. Review the results for each well in tabular format.
   b. Sort the wells according to well or normalized fluorescence with each filter.
   c. Select wells to review data in the analysis plot.

## Review Dye calibration results

1. Review the calibration properties, including calibration status, in the menu bar.
2. Select a Dye row in the **Calibration** table to view the corresponding analysis data plot.
3. Select the plate wells in the **Plate Layout** or the **Well Table** to view the corresponding curves in the plot .
4. Review data in the **Well Table**.
   a. Review the results for each well in tabular format.
   b. Sort the wells according to well or normalized fluorescence with each filter.
   c. Select wells to review data in the analysis plot.

## Use the calibrations from another file

The use of calibrations from another file is not available for the OpenArray™ Plate format.

The calibrations must be from a run on the same instrument type and the same block type. The calibrations must contain all of the applicable types of calibration for the instrument and block type.

The original calibrations are retained in the data file. You can revert back to the original calibrations.

Only one additional set of calibrations can be retained in the data file, excluding the original calibrations.

If the calibrations are reverted back to the original ones, the different calibrations are not retained in the data file.

1. Open a data file, then click **Actions ▸ Use Calibrations From Another File**.
2. Navigate to the location of the calibration file.
3. Select the file, then click **Open**.
   The data are reanalyzed with the new calibrations.
4. *(Optional)* Click **Actions ▸ Revert to Original Calibrations**.
   The data are reanalyzed with the original calibrations.

# Perform additional analysis

Perform additional analysis using one of the following options:

| Option | Description |
|---|---|
| QuantStudio™ Design and Analysis Software v2 analysis module | To perform additional analysis using the QuantStudio™ Design and Analysis Software v2, select an analysis module (see "Select an analysis module" on page 84).<br>For more information about analysis modules, see "About analysis modules" on page 83. |
| Application on the Thermo Fisher™ Connect Platform | To perform additional analysis using an application on the Thermo Fisher™ Connect Platform, go to **apps.thermofisher.com**. Select the appropriate application for your analysis. |

# 6 Export results

For information about export settings, see Chapter 12, "Manage export settings".

## Export the Well Table

1. In the **Quality Check** tab, in the **Well Table**, click ⋯ **(Actions)** ▸ **Export**.
2. Name the file, navigate to the desired folder location, then click **Save**.

## Export plate layout as an Excel™ spreadsheet

To save an image of the plate layout, see "Export plate layout image" on page 68.

1. In the **Quality Check** tab, in the plate layout pane, click ⋯ **(Actions)** ▸ **Export Plate View**.
2. Name the file, navigate to the desired folder location, select the values to include, then click **Save**.

## Export plate layout image

To save as an Excel™ spreadsheet, see "Export plate layout as an Excel™ spreadsheet" on page 68.

1. In the **Quality Check** tab, in the plate layout pane, click ⋯ **(Actions)** ▸ **Save Image**.
2. Name the file, navigate to the desired folder location, then click **Save**.

## Export images of plots

1. In the **Quality Check** tab, in the plot pane, select a plot from the dropdown list.
2. Click ⋯ **(Actions)** ▸ **Save Image**.
3. Name the file, navigate to the desired folder location, select the file format and size, then click **Save**.

# Export data

Export analyzed data for further analysis.

Save the analyzed data file before exporting the results (see "Save a plate file or data file" on page 78).

1. In a data file, click **Actions ▸ Export**.

2. Enter or select the following, then click **Save**.
   - **Export Name**
   - **File Format**
   - **Destination**—Click browse to navigate to the location.
   - **Export Settings**—Select an export setting from the dropdown list, or edit the export settings (see "View or edit export settings" on page 87).

3. Click **Export**.

# Export data in the RDML format

Export analyzed data in RDML (Real-Time PCR Data Markup Language) format for standard curve, relative standard curve, and comparative $C_t$ analysis.

Save the analyzed data file before exporting the results (see "Save a plate file or data file" on page 78).

1. In a data file, click **Actions ▸ Export to RDML**.

2. Name the file, navigate to the desired folder location, then click **Save**.

# Generate a report

Generate a customizable results report.

1. In a data file, click **Actions ▸ Generate report**.

2. (*Optional*) Edit the file name.

3. (*Optional*) To edit the file destination, click **Browse**, then navigate to the desired location.

4. In the **Report Content** pane, select the content to include in the report:

| Report Content | Description |
|---|---|
| Summary | Displays a summary of the experiment, including the following information:<br>• Bar Code<br>• File Name<br>• Run Start Date and Time<br>• Run End Date and Time<br>• Run Duration<br>• Operator<br>• Instrument Name<br>• Instrument Type<br>• Block Type<br>• Block Serial Number<br>• Heated Cover Serial Number<br>• PCR Stage/Step Number<br>• Quantification Cycle Method<br>• Comment<br>• Software Name and Version<br>• Plugin Name and Version<br>• Analysis Date and Time |
| Well Table | Displays the following information from **Well Table** in the **Quality Check** tab:<br>• Well<br>• Sample<br>• Target<br>• Task<br>• $C_q$<br>• $C_q$ Confidence<br>• Amplification Score<br>• Amplification Status<br>• $C_q$ Threshold (not applicable if the relative threshold algorithm is selected to calculate the $C_q$ values)<br>• Baseline Start and End (not applicable if the relative threshold algorithm is selected to calculate the $C_q$ values) |
| Replicate Group Results | Displays the following information from **Replicate Table** in the **Quality Check** tab:<br>• Sample<br>• Target<br>• Number of Replicates<br>• $C_q$ Mean<br>• $C_q$ Standard Deviation |

*(continued)*

| Report Content | Description |
|---|---|
| Plate Layout | Displays the sample name, sample color, target or targets, and target $C_q$ value or values for each well.<br>The subarrays are displayed for the OpenArray™ Plate format. The sample name is displayed on each subarray. |
| Amplification Plot (dRn) | Displays the Amplification Plot (dRn vs Cycle)[1] |
| Amplification Plot (Rn) | Displays the Amplification Plot (Rn vs Cycle)[1] |
| Melt Curve Plot | Displays the Melt Curve Plot (Derivative Reporter vs Temperature)[2]<br>This option only displays if the Run Method includes a Melt Curve stage. |
| Run Method | Displays the thermal cycling protocol used in the Run Method.<br>(Not applicable for the OpenArray™ Plate format.) |
| Primary Analysis Settings | Displays the primary analysis information.<br>This includes the PCR state and step, the qualification cycle method, the baseline information, and the threshold information.<br>The QC alerts and advanced settings are displayed. See the following sections:<br>• "View or edit QC alerts settings" on page 62<br>• "Overview of the result quality checks" on page 95<br>• "View or edit advanced settings" on page 63 |
| Analysis Module | Displays the plots and analysis results for the selected analysis modules.<br>This option only displays if an analysis module is selected. |

[1] For more information about the Amplification Plot, see "Amplification Plot overview" on page 98.
[2] For more information about the Melt Curve Plot, see "Melt Curve Plot overview" on page 100.

5. Select the wells or the subarrays to include in the report.
   - (*Default*) **All Wells** checkbox.
   - **Customize** checkbox—Select one or more wells or subarrays in the plate layout. The wells or subarrays are colored by sample.

| Option | Action |
|---|---|
| Select one well or subarray | Click the well or subarray |
| Select multiple contiguous wells or subarrays | Click-drag over the wells or subarrays |
| Select non-contiguous wells or subarrays | PC: Ctrl-click each well or subarray<br>Mac: Cmd-click each well or subarray |

# 7 General procedures to analyze data in the **Quality Check** tab

For detailed procedures, see Chapter 5, “Review and analyze data”.

## View the post-run summary

1. Open the data file.
2. In the **Run Summary** tab, view a summary of the run, including the following information:
   - **Run Start** and **Run End**
   - **Operator** and **Block Type**
   - **Heated Cover S/N** and **Block Serial S/N**
   - **Instrument Software** and **Instrument Name**
   - **Run Events** and **Calibration**

## Configure the layout of the Quality Check tab

1. In the **Quality Check** tab, click **Actions ▸ Page Layout Setting**.
2. Drag up to four options to the display on the right.
   Each option will display in a pane in the **Quality Check** tab.
3. (*Optional*) Drag the panes to rearrange the display in the **Quality Check** tab.
4. Click **Save**.
5. (*Optional*) To reset to the default settings, click **Reset to Default**.

## Filter results in the Quality Check tab

1. In the left pane of the **Quality Check** tab, select the following to filter the results.
   - **Sample Type**
   - **QC Alerts**
     Filtering by QC alerts is available only if there is at least one QC alert in the results.
   - **Samples**

- **Targets**
- **Biogroups**
- *(OpenArray™ Plate only)* **View**

2. *(Optional)* To clear the selections, click **Clear all**.

# Review the OpenArray™ Plate images

1. In the **View** dropdown list, select **Plate Image**.
   The image is displayed.
2. In the **Image type** dropdown list, select one of the following options:
   - **ROX Images**
   - **Spotfind Images**
   - **Quant Images**
3. Click the file to view.
   The file is highlighted in blue. The file name is also displayed at the bottom of the image.
4. Use the magnification tools at the top-right corner of the image to zoom in, to zoom out, and to reset the magnification.
5. Click on a subarray to view a magnified image of the subarray.
   The selected subarray is displayed below the image of the full OpenArray™ Plate.
6. Use the sliders to adjust the image brightness and contrast.
   - **Brightness** slider
   - **Contrast** slider
7. Click the **Comments** field to add a comment for an image.

## Overview of OpenArray™ Plate images

OpenArray™ Plate images can be viewed in the software. The images can be used to troubleshoot problems.

Three categories of images are available:

- ROX™ images
- Spotfind images
- Quantification images

The ROX™ images should display uniform fluorescence throughout the OpenArray™ Plate. The following patterns indicate an issue.

**Table 2 ROX™ image patterns and possible causes**

| Pattern | Possible cause |
|---|---|
| Fluorescence is not displayed in sections of the subarray where the OpenArray™ AccuFill™ Instrument turns when loading the OpenArray™ Plate. | The OpenArray™ AccuFill™ Instrument is misaligned. |
| Fluorescence is not displayed in large sections of a subarray near the fill port. | The immersion fluid is injected too quickly or injected without purging the syringe. This causes the sample to be knocked out of the through-holes near the fill port. |
| Fluorescence is not displayed in large sections of a subarray at the end of the fill path. | There is an insufficient volume of reagents in the sample plate. The tips run out of reagents before they reach the end of the fill path. |
| There are obscured areas at the edge of the OpenArray™ Plate. | The case lid was not aligned correctly in the plate press. |
| There are very bright spots in a well. | These bright spots are caused by dust or other contaminants. |
| The wells display a dark center. | There is evaporation. This is caused by low humidity levels and excessive time passing before the immersion fluid is added to the OpenArray™ Plate. |

The spotfind images can indicate whether there are leaks. The spotfind images should appear with a uniform pattern. Dark spots indicate leaks. Leaks should be confirmed by reviewing the quantification images.

Quantification images display the fluorescent signals. They can indicate the following issues.

**Table 3 Quantification image patterns and possible causes**

| Pattern | Possible cause |
|---|---|
| There are very bright spots in a well. | These bright spots are caused by dust or other contaminants. |
| The wells display a dark center. | There is evaporation. This is caused by low humidity levels and excessive time passing before the immersion fluid is added to the OpenArray™ Plate. |
| There is variability in the brightness of the signal within the well. | There are leaks. |

## Workflow to review the OpenArray™ Plate images

## Review the images

### Check for loading issues

View the ROX™ image `POST-READ_CHANNEL_4`.

### Check for leaks or displaced samples

View the following spotfind images:

- Genotyping runs: `S01_C001_T0<...>_P0001_M2_X3_E1_CP<...>_spotfind`, where <…> is variable
- Gene expression runs: `S00_C001_T01_P0001_M2_X3_E1_CP<...>_spotfind`, where <…> is variable

### Check for fluorescent abnormalities and confirm any issues detected in the spotfind images

View the quantification images:

- Genotyping runs: `STAGE3_CYCLE1_CHANNEL_1` and `STAGE3_CYCLE1_CHANNEL_2`
- Gene expression runs: `STAGE2_CYCLE40_CHANNEL_1`

# Review individual well results in the Well Table

In the **Quality Check** tab, view results for individual wells in the **Well Table**.

- Click **View** to select the columns that are displayed.
- Click on any column header to sort the table by that value.
- Select a well from the table to highlight the well in the **Plate Layout** or plot.

**Note:** Well selections are retained when viewing different plots.

# Review Replicate Group results

1. In the **Quality Check** tab, click **Replicate Group**.
2. Examine the $C_q$ mean and standard deviation for each replicate group to assess the precision of $C_q$ values.

# Configure general plot settings

1. In the **Quality Check** tab, in the plot pane, click ⚙ **(Settings)**.
2. Edit the following settings in the **General** tab.
   - **Plot Title**
   - **Color By**
   - **Y Value**
   - **Y Scale**
   - **Thickness**
   - **Max Curves**
   - **Show**
3. Edit the following **X Axis** and **Y Axis** settings in their respective tabs.
   - **Label**
   - **Auto-adjust range**—If deselected, select a **Minimum value** and **Maximum value**.
4. Click outside of the dialog box to close.

# 8 Manage plate files and data files

## Overview of system templates and plate files

A plate file contains the information that is necessary to perform an instrument run. A system template is a non-editable plate file that is included with the software. Opening a system template automatically generates a new plate file that can be edited, then saved (see "Select a system template or existing plate file to set up a new plate file" on page 24).

A plate file can contain the following information:

| Information type | Properties |
|---|---|
| Instrument setup | • Instrument type<br>• Block<br>• Run mode |
| Run Method | • Thermal protocol<br>• Filter settings |
| Plate Setup | • Sample definitions and well-assignments<br>• Target or SNP assay definitions and well-assignments<br>• Reagent information<br>**Note:** Plate setup information is not included in the system template and must be defined by the user. |
| Primary Analysis Settings | • $C_q$ settings<br>• Melt settings<br>• QC settings<br>• Advanced settings |
| Analysis Module | Analysis modules are plugins that enable additional data analysis in the software (see "About analysis modules" on page 83).<br>**Note:** The user can select an analysis module pre- or post-instrument run ("Select an analysis module" on page 84). |
| Additional Information | Plate information — plate barcode and user-defined description |

# Overview of data files

A data file contains the information from the plate file that was used to perform the instrument run. A data file can also contain the following information:

| Information type | Properties |
|---|---|
| Run summary | • **Run Start** and **Run End**<br>• **Operator** and **Block Type**<br>• **Heated Cover S/N** and **Block Serial S/N**<br>• **Instrument Software** and **Instrument Name**<br>• **Run Events** and **Calibration** |
| Analysis results | • Data plots<br>• $C_q$ and $C_q$ confidence<br>• Amplification score and status<br>• Melting temperature<br>• Flags |
| Analysis module results (if applicable) | Data analysis completed using an analysis module.[1] |

[1] For more information about analysis modules, see "About analysis modules" on page 83.

# Save a plate file or data file

- To save a plate file for the first time, or to save a plate file or data file with a new name, click **Actions ▸ Save As**.
- To save the plate file or data file with the same name, click **Actions ▸ Save**.

# Restrict editing of a plate file or data file

**IMPORTANT!** If you enable restricted editing in a plate file or data file, then you cannot save the file with unrestricted editing. We recommend that you save a backup version of the file before you restrict editing.

1. In an open plate file or data file, click **Actions ▸ Restrict Editing**.
2. In the **Restrict Editing** window, select the features for which you want to restrict editing.
    - Edit analysis settings
    - Edit target/assay
    - Assign target/assay
    - Edit run method
      The run method is never editable in a data file.

**Note:** A blue checkbox (☑) indicates that the function cannot be edited.

3. Enter and confirm a password, then click **Restrict**.

   **Note:** Record the password because lost passwords cannot be recovered.

   A lock icon ( ) appears next to the file name in the software menu bar to indicate that restricted editing is enabled.

4. (*Optional*) To update editing restrictions for a file, enter your password, modify the selections, then click **Update**.

5. (*Optional*) To remove editing restrictions from a file, enter your password, then click **Remove**.

# Add a plate file to My Plate Files

Save a plate file before adding it to **My Plate Files** (see "Save a plate file or data file" on page 78).

In an open plate file, click **Actions ▸ Add to My Plates**.
The plate file appears in the in the **Plate Gallery**, in the **My Plate Files** tab.

# Search for a plate file or data file

Add a tag to your plate file or data file to enable searching by that tag (see "Edit plate file or data file information" on page 47).

1. Open the **Plate Gallery** or the **Data Gallery**.

2. Click , then enter the tag or tags.
   Plate files or data files with the tag are displayed.

# Set up new plate file from a data file

1. In the home screen, click **View Data**.

2. *(Optional)* Use the filter tools on the left pane to filter the data files.

3. Hover over the data file, then click one of the following options.

   - ··· **(Actions) ▸ Rerun**.
   - ··· **(Actions) ▸ Rerun in new window**.

   A new plate file is generated.

4. Click **Actions ▸ Save As**.

5. In the **Save As** dialog box, enter a file name in the **File Name** field, then select a location to save the file.

6. Click **Save**.

A new plate file is saved.

## Remove a data file

1. In the home screen, click **View Data**.

2. *(Optional)* Use the filter tools on the left pane to filter the data files.

3. Hover over the data file, then click ⋯ **(Actions)** ▸ **Remove**.

The data file is removed from the software. It is not deleted from the system. The file is still available in the folder where it is saved.

## Batch generate plate files

Batch generate multiple EDT files with different barcodes and sample assignments.

Save a plate file before generating multiple EDT files (see “Save a plate file or data file” on page 78).

1. In an open plate file in the **Plate Gallery**, click **Actions** ▸ **Generate Plate Files**.
   The **Generate Plate Files** dialog box is displayed.

2. In the **Plate file naming** pane, enter or select the following, if needed.
   - **Use Sample Assignment File name as the barcode (only if Sample Assignment File is added)**
   - **Plate File Name prefix**

3. In the **Plate batch generation** pane, in the table:
   - Click + **(Add)** to add the following.
     – **Add Barcode File**
     – **Add Sample Sheet**
   - Click ✕ **(Remove)** to delete a row in the table.

4. To edit the file destination in the **Plate batch generation** pane, click **Browse**, then navigate to the desired location.

5. Click **Generate**.

# 9 Manage instruments

## Add an instrument

The QuantStudio™ 6 Pro Real-Time PCR Instrument and the QuantStudio™ 7 Pro Real-Time PCR Instrument can be added to the software.

- Instrument access must be enabled from the instrument touchscreen before it can be added in the software. For more information, see *QuantStudio™ 6 Pro Real-Time PCR System and QuantStudio™ 7 Pro Real-Time PCR System User Guide* (Pub. No. MAN0018045).
- Obtain the instrument remote access key from the instrument touchscreen or the instrument administrator.
- The firewall port `7443` must be open.

1. In the home screen, click **Manage Instruments**.

   To access instruments from a different screen, click ⚙ **System ▸ Instruments**.

2. Click **Actions ▸ Add Instrument**.

   ---

   **Note:**
   - Click **Actions ▸ Export Instruments** to export instruments in CSV file format.
   - Click **Actions ▸ Import Instruments** to import instruments in CSV file format.
   - Click **Actions ▸ Refresh** to refresh the instruments.

   ---

3. Select an instrument to add using one of the following options:

| Option | Description |
| --- | --- |
| **By discovery** | Select an instrument from the list of instruments that are connected to the network. |
| **By IP address**[1] | Enter the instrument IP address in the field.[2] |

[1] If using the IP address option, we recommend that you configure the instrument to use a static IP address. If the instrument is not configured to use a static IP address, the IP address may change after instrument reboot. See the instrument documentation for more information.

[2] Contact the instrument administrator if you do not know the instrument IP address.

4. Enter the instrument **Remote Access Key**.

5. Click **Add Instrument**.

---

**Note:** If the instrument remote access key is removed, or if instrument access is disabled, the instrument will be removed from the software.

---

# Review instrument status

For more information about adding an instrument, see "Add an instrument" on page 81.

1. In the home screen, click **Manage Instruments**.

   To access instruments from a different screen, click **System ▸ Instruments**.

2. The status for the instrument is displayed:
   - Offline
   - Idle
   - Running

     If running, the remaining time of the run is also displayed.
   - Standby
   - Error
   - Diagnostics

# Review calibration status

The calibration status can be reviewed in the QuantStudio™ Design and Analysis Software v2. The instrument must be added to the **Manage Instruments** list.

For more information about adding an instrument, see "Add an instrument" on page 81.

1. In the home screen, click **Manage Instruments**.

   To access instruments from a different screen, click **System ▸ Instruments**.

2. In the **Summary** tab for the instrument, the calibration status is displayed in the **Calibration Status** column.

# Remove an instrument

1. In the home screen, click **Manage Instruments**.

   To access instruments from a different screen, click **System ▸ Instruments**.

2. Hover over the instrument, then click ⋯ **(Actions) ▸ Remove Instrument**.

# 10 Manage analysis modules

To see all of the analysis modules that are installed, click **System ▸ Plugins**.

## About analysis modules

Analysis modules are plugins that enable additional data analysis using QuantStudio™ Design and Analysis Software v2.

- To see all of the analysis modules that are installed, click **System ▸ Plugins**.
- To select an analysis module, see "Select an analysis module" on page 84.

There are two types of analysis modules:

| Analysis Module type | Description |
|---|---|
| Built-in | Analysis modules that pre-installed in the software. These analysis modules cannot be uninstalled.<br>The latest versions of the analysis modules are included with the software installer. When a new version of the QuantStudio™ Design and Analysis Software v2 is installed, the latest versions of the analysis modules are installed. Previous versions of the analysis modules are not retained. |
| User-installed | Analysis modules that are installed by the user. These analysis modules can be uninstalled. |

The following analysis modules are available to use with the software:

| Analysis Module | Type | Description |
|---|---|---|
| Primary analysis | Built-in | Calculates dye signals, Cq values, and other primary results from the filter signals. |
| Standard curve | Built-in | Use to determine absolute target quantity in test samples. |
| Genotyping | Built-in | Use to detect single nucleotide polymorphism (SNP) variants of a target nucleic acid sequence. |
| Presence absence | Built-in | Use to determine the presence or absence of a target nucleic acid sequence in a sample. |

*(continued)*

| Analysis Module | Type | Description |
|---|---|---|
| Relative quantification | Built-in | Use to determine the relative quantity of a target of interest in a test sample relative to a reference sample. The analysis module supports relative quantification using either comparative CT (ΔΔCT) analysis or relative standard curve analysis. |
| High resolution melt | User-installed | Use to detect single nucleotide polymorphism (SNP) variants of a target nucleic acid sequence.<br>This analysis module must be reinstalled when a new version of QuantStudio™ Design and Analysis Software v2 is installed.<br>**Note:** You must purchase a license key to perform analysis using this module. |

For detailed information about the analysis modules, see one of the following sources:

- Analysis Module User Guide—See Appendix B, "Documentation and support".
- Analysis Module Help—With an analysis module selected, click ⓘ **Help**, then select the Analysis Module Help.

# Select an analysis module

The analysis module selection can be selected in either the plate file or the data file.

The genotyping analysis module and the presence absence analysis module are the only ones that are available for OpenArray™ Plate data files.

The genotyping analysis module is automatically applied to data files from OpenArray™ Plate genotyping runs.

1. In a plate file or data file, click **Actions ▸ Analysis Modules**.
2. In the **Analysis Modules** window, select the analysis module, then click **OK**.

| File type | Result |
|---|---|
| Plate file | The Help for the selected analysis module is displayed in the Help menu (ⓘ **Help ▸ <Analysis Module> Help Contents**). |
| Data file | • The analysis module Help is displayed in the Help menu (ⓘ **Help ▸ <Analysis Module> Help Contents**).<br>• The analysis module tab opens. |

3. Save the plate file or data file retain the analysis module selection.

# Install a new analysis module plugin

Go to **thermofisher.com/us/en/home/global/forms/life-science/quantstudio-6-7-pro-software** to download the file and purchase a license registration code.

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**Note:** Currently, the High Resolution Melt Analysis Module is the only analysis module available for user-installation.

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1. In any screen, click **System ▸ Plugins**.
2. Click **Actions ▸ Install**.
3. Navigate to, then select the plugin ZIP file.
4. Click **Open**.
5. In the **Install** window, select **Accept Terms of Use** in the lower-left corner, then click **Next**.
6. Enter the license registration information, then click **Install ▸ OK**.

To select and open an analysis module, see "Select an analysis module" on page 84.

# Uninstall an analysis module plugin

---

**Note:** Only user-installed analysis module plugins can be uninstalled. Built-in analysis modules cannot be uninstalled by the user.

---

1. In any screen, click **System ▸ Plugins**.
2. Hover over the analysis module, then click ··· **(Actions) ▸ Uninstall**.
3. Click **OK**.

# 11 Manage language packs

## About language packs

Language packs are plugins that enable different languages in the QuantStudio™ Design and Analysis Software v2.

Language packs are installed by the user and can be uninstalled. To see all of the user-installed language packs, click **System ▸ Plugins**.

To select a different language after installing the language pack, click the language at the bottom of the home screen.

The language packs can be downloaded from **thermofisher.com/us/en/home/global/forms/life-science/quantstudio-6-7-pro-software**.

## Install a language pack

1. In any screen, click **System ▸ Plugins**.
2. Click **Actions ▸ Install**.
3. Navigate to, then select the plugin ZIP file.
4. Click **Open**.
5. In the **Install** window, select **Accept Terms of Use** in the lower-left corner, then click **Install**.

## Uninstall a language pack

1. In any screen, click **System ▸ Plugins**.
2. Hover over the language pack, then click ⋯ **(Actions) ▸ Uninstall**.
3. Click **OK**.

# 12 Manage export settings

## About export settings

Export settings designate the data to include in the exported results. The software includes several non-editable export settings files in the Export Settings library (click **System ▸ Export Settings** to view).

To edit the default settings, you must create a new export settings file (see "View or edit export settings" on page 87).

Export settings can be applied to data exported by the software or by an instrument.

## View files in the Export Settings library

1. In any screen, click **System ▸ Export Settings**.

   **Note:** If you have a data file open, you will be prompted to save, then close the file. To view or edit export settings without closing the current data file, see "View or edit export settings" on page 87

2. Click an export settings file to view.
3. (*Optional*) In the open export settings file, create a new export settings file, or edit an existing custom export settings file.

## View or edit export settings

View or edit export settings while a data file is open. You can also view or edit export settings from the **Export Settings** library (see "View files in the Export Settings library" on page 87).

1. Open a data file.
2. Click **Actions ▸ Export**.
3. In the **Export Plate** dialog box, select an option from the **Export Setting** dropdown list, then click **Customize**.
4. To edit the export settings for Quality Check data, select **Primary** from the **Analysis Module** dropdown list.
5. In the **View** dropdown list, select **Metadata**.

6. In the **Select Fields** pane, select the checkbox associated with each item in order to display it in the exported file.
7. In the **Select Fields** pane, click an item in order to edit the text that is displayed in the exported file.
   The updated name is displayed in the **Metadata Fields** pane.
8. In the **View** dropdown list, select **Result**.
9. In the **Include** dropdown list, select the data to include in the export.
   Each item is exported as a separate file.
10. View the data in each tab.
    - **Results** tab
    - **Amplification Data** tab
    - **Multicomponent** tab
    - **Raw Data** tab
    - **Replicate Group Result** tab
11. In the **Select Columns** pane, select the checkbox associated with the column in the table.
12. In the **Select Columns** pane, click an item in order to edit the text that is displayed in the exported file.
13. Click the **Options** dropdown list to select the options for the values that are displayed in the exported plate.
    a. Select or deselect the **Round values by** checkbox, then enter a number in the **Decimal places** field.
    b. Select or deselect the **Use double quote** checkbox.
    c. Select or deselect the **Use two digits in well position** checkbox.
14. Click the **Options** dropdown list to select the information to include in the exported plate.
    - **Section header** checkbox
    - **Empty wells** checkbox
    - **Omitted wells** checkbox
15. *(Optional)* Click **Save As**, enter a name in the **Export Name** field of the **Save As** dialog box, then click **OK**.
    The saved settings are available as a selection in the **Export Setting** dropdown list.
16. Click **Export** to continue exporting results, or click **Close**.

# Download an export settings file

An export settings file can be imported into the **Export Settings** library or into the QuantStudio™ 6 Pro Real-Time PCR System or the QuantStudio™ 7 Pro Real-Time PCR System.

1. In any screen, click **System ▸ Export Settings**.
2. Hover over the export settings file, then click ⋯ **(Actions) ▸ Download**.
3. Name the export settings file, navigate to the desired folder location, then click **Save**.

- To import an export settings file into the software, see "Import an export settings file" on page 89.
- To import an export settings file into the QuantStudio™ 6 Pro Real-Time PCR System or the QuantStudio™ 7 Pro Real-Time PCR System, see the instrument user guide.

# Import an export settings file

Import a previously downloaded export settings file (see "Download an export settings file" on page 89).

1. In any screen, click **System ▸ Export Settings**.
2. Click **Actions ▸ Import Setting**.
3. Navigate to the export settings file, then click **Open**.

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# Manage preferences

View or edit preferences for the following items:

- Importing an AIF
- Custom dyes
- Plate file format
- Result export
- Report generation

After a custom dye is added to the system, it is available in the **Manage Dyes** dialog box when a plate file is set up.

1. In any screen, click **System ▸ Preferences**.
2. In the **AIF Import** pane, select one of the following, then click **Apply**.
   - **Gene Symbol & Assay ID**
   - **Gene Symbol**
   - **Assay Name**
   - **Assay ID**

   **Note:** To reset to the default settings, click **Reset**.
3. In the **Custom Dyes** pane, click + **(Add)**, edit the following fields, then click **Apply**.
   - Enter a name in the **Dye Name** field.
   - Select a color from the color picker.
   - Select a type from the **Type** dropdown list.
   - Enter a wavelength in the **Wavelength** field.
4. In the **Plate File Format** pane, select **Create plate file (.edt) in legacy format for QuantStudio™ 1/3/5/6 Flex/7 Flex/12K Flex**, then click **Apply**.

   **Note:** To reset to the default settings, click **Reset**.
5. In the **Result Export** pane, select **File name with date and time stamp** or **File name only**, then click **Apply**.

   **Note:** To reset to the default settings, click **Reset**.

6. In the **Report Generation** pane, enter or select the following, then click **Apply**.
   - **Title**
   - **Paper Size**—Select **A4** or **Letter** from the dropdown list.
   - **Logo**—Select **Use the default report logo** or **Use a customized report logo**.

---

**Note:**
- To preview the report generation, click **Preview**.
- To reset to the default settings, click **Reset**.

---

# 14 About data analysis

For information about additional analysis using an analysis module, select the analysis module to view the relevant Help information (see "Select an analysis module" on page 84).

## About the quantification cycle ($C_q$)

The quantification cycle ($C_q$) is used for gene expression metrics quantification analysis. Algorithm-specific calculations of $C_q$ values are used as the primary input values for quantification analysis.

| Algorithm | Description |
|---|---|
| Baseline Threshold | $C_q$ ($C_t$) is calculated using the PCR cycle number at which the fluorescence signal meets the threshold in the amplification plot. |
| Relative Threshold | $C_q$ ($C_{rt}$) is calculated using the PCR cycle number for the threshold calculated from the modeled amplification efficiency profile. |

For more information about $C_q$ analysis settings, see "Cq settings overview" on page 93.

## About melt curve analysis

Use melt curve analysis to determine the melting temperature ($T_m$) of the amplification products of a PCR that used intercalating dyes.

Melting temperature ($T_m$) is the temperature at which 50% of the DNA is double-stranded and 50% is dissociated into single-stranded DNA. The melt curve of a single amplification product displays a single peak at the $T_m$ of the product. Multiple peaks in a melt curve experiment indicate additional amplification products, usually from non-specific amplification or formation of primer-dimers.

Multi-peak calling can be used when more than one product is expected in a reaction.

Melt curve analysis is included in the primary software analysis.

1. The software plots a melt curve based on the fluorescence of the dye with respect to change in temperature.
2. Using the melt curve, the software calculates the melting temperature ($T_m$).

A melt peak parity factor is available. It normalizes the scaling of the melt curve peaks in multiplex assays when the dyes fluoresce at different amplitudes. It helps to make the peak heights more comparable across multiple dyes and targets.

# Primary analysis settings overview

## $C_q$ settings overview

The default $C_q$ settings are appropriate for most applications. Edit the settings for analysis of not typical or unexpected run data.

**Note:** The run data must include a PCR stage to perform $C_q$ analysis.

**Table 4** $C_q$ **settings**

| Setting | Description |
|---|---|
| **PCR Stage/Step** | If there is more than one PCR stage/step with data collection, the user selects PCR stage/step from the dropdown list for $C_q$ or $C_q$ analysis. |
| Baseline threshold analysis | |
| Algorithm Settings – **Baseline Threshold** | The **Baseline Threshold** Algorithm is used to calculate the $C_q$ values.<br>This algorithm is an expression estimation algorithm that subtracts a baseline component and sets a fluorescence threshold in the exponential region. |
| **Default $C_q$ Settings** | Determines how the **Baseline Threshold** Algorithm is set. The default settings are used for targets and wells unless custom values are selected in the **General** or **Well $C_q$** tabs.<br>For recommendations on adjusting baseline and threshold settings, see “Guidelines for manual threshold and baseline settings for Cq analysis” on page 94. |
| **$C_q$ Settings for Target** | • **Default Settings** selected—The default $C_q$ settings are used to calculate the $C_q$ values for the target.<br>• **Default Settings** deselected—The software allows manual setting of the baseline or the threshold.<br>For recommendations for adjusting baseline and threshold settings, see “Guidelines for manual threshold and baseline settings for Cq analysis” on page 94. |
| Relative threshold analysis | |
| Algorithm Settings – **Relative Threshold** | The **Relative Threshold** Algorithm is used to calculate the $C_q$ values.<br>This algorithm is a well-based expression estimation algorithm that sets a threshold for each curve individually. The threshold is based on the shape of the amplification curve, regardless of the height or variability of the curve in its early baseline fluorescence. |
| **Default $C_q$ Settings** | Determines the default start cycle. The default start cycle is used for targets unless a custom start cycle is indicated in the **Well $C_q$** tab. |

## Guidelines for manual threshold and baseline settings for $C_q$ analysis

| Setting | Recommendation |
|---|---|
| Threshold | Enter a value for the threshold so that the threshold is:<br>• Above the background.<br>• Below the plateau and linear phases of the amplification curve.<br>• Within the exponential phase of the amplification curve. |
| Baseline | While in the linear plot view, select the **Start Cycle** and **End Cycle** values so that the baseline ends before significant fluorescence signal is detected. |

## Melt analysis settings overview

- The **Melt Peak Parity Factor** field allows the entry of a factor.

  This feature is for multiplex melt curve experiments. It normalizes the scaling of the melt curve peaks in multiplex assays when the dyes fluoresce at different amplitudes. It helps to make the peak heights more comparable across multiple dyes and targets.

  A factor of ***0*** turns off the feature. Negative values are not permitted.

  If the melt peak parity factor is applied to empty wells of a plate, unexpected $T_m$ values can occur.

  The melt peak parity factor does not affect the High Resolution Melt Analysis Module if this module is enabled.
- Enable or disable **Multi-Peak Calling** the **Melt** tab.

| Multi-Peak Calling | Description |
|---|---|
| Enabled | – More than one PCR product is expected to amplify.<br>– $T_m$ will be determined for more than one peak. |
| Disabled | – A single PCR product is expected to amplify.<br>– $T_m$ will be determined for one peak. |

- (*For multi-peak calling only, using the percentage as the threshold*) Set the value in the **Threshold Type** column to **Percentage**, then adjust the value in the **Peak Level (%)** column.

  Specify a fractional-level value as the additional peak detection threshold. The detected peaks are measured relative to the height of the tallest peak, which has a perfect fractional level of 100%. The default value is 10%.

  For example, if the peak level value is set to 40%, then peaks above 40% of the tallest peak are reported, and peaks below 40% are regarded as noise.
- (*For multi-peak calling only, using the height as the threshold*) Set the value in the **Threshold Type** column to **Height**, then adjust the value in the **Peak Height** column.

  Specify a value as the peak detection threshold.

  The absolute value of the peak is required to be above the value that is set in the **Peak Height** column

To edit the melt analysis settings, see “View or edit melt analysis settings” on page 64.

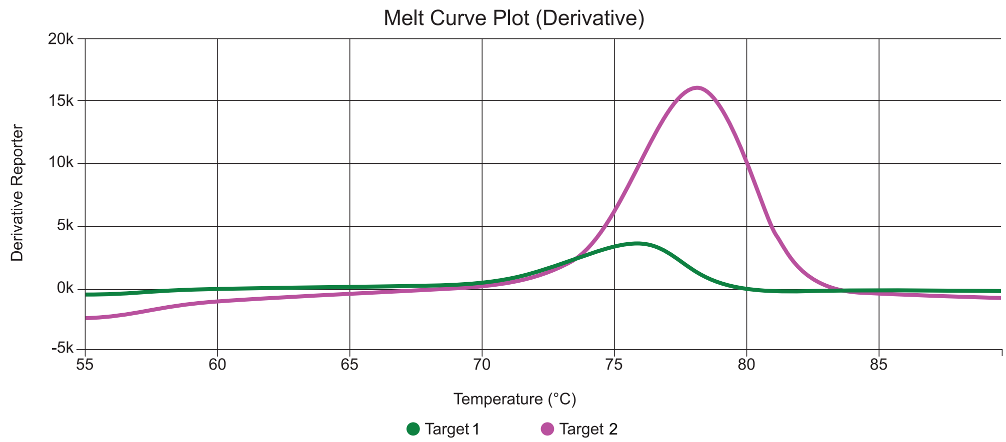


**Figure 7 Melt curve before a melt peak parity factor is applied**

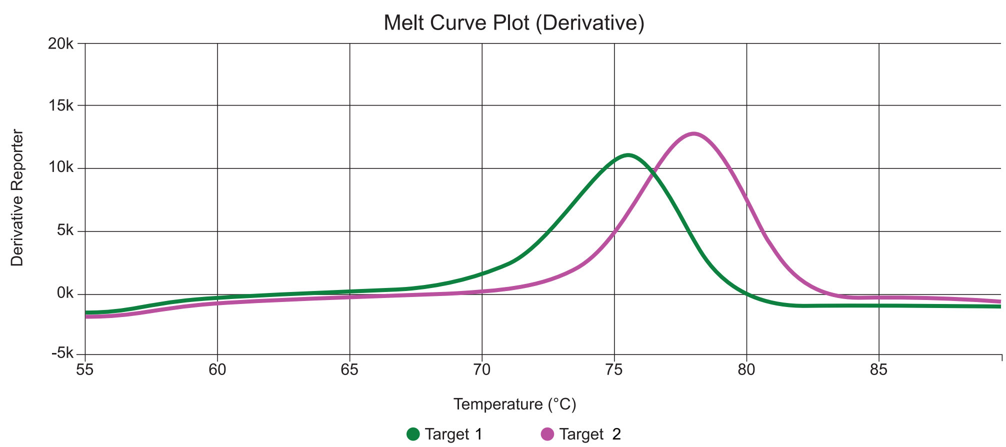


**Figure 8 Melt curve after a melt peak parity factor is applied**

# Quality checks

There are two types of quality checks:

- Result quality check
- Curve quality check

## Overview of the result quality checks

The result quality checks are used to describe the quality of the primary analysis results.

The following items can be displayed in the **Result Quality Issues** column of the **Well Table**:

- Amp Score out of range
- Unexpected Amp Status
- Cq Value out of range
- Cq Confidence out of range

- Cq Standard Deviation out of range
- Unexpected Cq Status
- Delta Rn out of range
- Multiple melt peaks
- Replicate group outlier
- Tm1 out of range
- Pass Ref Change out of range

**Note:** There can be multiple **Result Quality Issues** for a single well.

## Acceptance criteria for result quality checks

| Acceptance criteria | Description |
|---|---|
| **$C_q$ should be** | • **Expressed**<br>• **Undetermined**<br>Any well outside of the user-selection will be flagged. |
| **$C_q$ Value Range** | The $C_q$ Value is the primary input value for quantification analysis.<br>Any well outside of the user-defined minimum and maximum range will be flagged. |
| **$C_q$ Confidence Range** | The $C_q$ Confidence is a value that reflects the reliability of the derived $C_q$.<br>Any well outside of the user-defined minimum and maximum range will be flagged. |
| **$C_q$ Standard Deviation Range** | The $C_q$ Standard Deviation is the standard deviation of the $C_q$ of the replicates.<br>Any well outside of the user-defined minimum and maximum range will be flagged. |
| **Amp Status** | • **Amp**<br>• **No Amp**<br>• **Inconclusive**<br>Any well outside of the user-selection will be flagged. |
| **Amp Score Range** | The Amp Score is a value that indicates the quality of the amplification curve.<br>Any well outside of the user-defined minimum and maximum range will be flagged. |
| **Delta Rn Range** | The Delta Rn is the calculated deviation from the baseline.<br>Any well outside of the user-defined minimum and maximum range will be flagged. |
| **Passive Ref Change (%)** | Any wells with a change in passive reference greater than the threshold are flagged. |
| **Outlier in Replicate Group** | Any wells that have $C_q$ values that differ significantly from the average for the associated replicate wells will be flagged. |

*(continued)*

| Acceptance criteria | Description |
|---|---|
| **Tm Range** | Tm is the melting temperature calculated in °C.<br>Any well outside of the user-defined minimum and maximum range will be flagged. |
| **Multiple Melt Peak Detection** | Any wells that have multiple peaks will be flagged. |

## Overview of the curve quality checks

The curve quality checks are used to describe the quality of the curve, including passive reference signals and smoothness of the curve.

The following items can be displayed in the **Curve Quality** column of the **Well Table**:

- PRFDROP
- PRFLOW
- NOISE
- NOSIGNAL
- OFFSCALE
- SPIKE

## Description of the curve quality flags

| Flag | Description |
|---|---|
| PRFDROP | • Reported for only the PCR data.<br>• The passive reference signal changes near the $C_t$. |
| PRFLOW | • Reported for only the PCR data.<br>• The passive reference signal is low. |
| NOISE | • Reported for only the PCR data.<br>• The noise for a curve is higher than other curves on the plate. |
| NOSIGNAL | • Reported for the whole run.<br>• There is no signal in the well. |
| OFFSCALE | • Reported for the whole run.<br>• The fluorescent signal is off the scale. |
| SPIKE | • Reported for only the PCR data.<br>• There are noise spikes on the curve. |

# Plots overview

## Amplification Plot overview

The **Amplification Plot** displays amplitude of fluorescence by well across a user-defined number of cycles (default 40 cycles). You can use the amplification plot to perform the following tasks:

- Confirm or correct baseline and threshold values.
- Identify outliers.
- Identify and examine abnormal amplification. Abnormal amplification can exhibit one of the following characteristics:
  - Increased fluorescence in negative control wells
  - Absence of detectable fluorescence at an expected cycle

    **Note:** If you notice abnormal amplification or a complete absence of fluorescence, see the instrument user guide for troubleshooting information.

  - Irregularities due to inefficient reactions or sample contaminants

Three plots are available. Some plots can be viewed as a linear or $\log_{10}$ graph.

**Table 5 Amplification Plot types**

| Plot type | Description | Use to |
| --- | --- | --- |
| ΔRn vs Cycle | ΔRn is the magnitude of normalized fluorescence signal, relative to the baseline fluorescence, generated by the reporter at each cycle during the PCR amplification. | • Identify and examine irregular amplification.<br>• View threshold values for the run. |
| Rn vs Cycle | Rn is the fluorescence signal from the reporter dye normalized to the fluorescence signal from the passive reference. | • Identify and examine irregular amplification.<br>• View baseline values for the run. |
| $C_t$ vs Well | $C_t$ is the PCR cycle number at which the fluorescence meets the threshold in the amplification plot. | • Locate outlying amplification (outliers). |

## Raw Data Plot overview

The **Raw Data Plot** displays the raw fluorescence signal for each optical filter during each cycle of the real-time PCR read from the real-time PCR instrument.

Raw data are also collected for plate endpoint reads (genotyping and presence absence runs) and for melt curves. Raw data can be collected at any point of the thermal cycling protocol. Raw data are not specific to PCR reads.

Raw data collection is indicated by the camera icon in the run method (see "Run method elements" on page 26).

Raw data have had background and uniformity calibrations applied. They are the data that are used to produce the multicomponent data. Multicomponent data have dye calibrations and the algorithm to reduce dye signal crosstalk processing implemented.

View the **Raw Data Plot** to perform the following actions:

- Confirm a stable increase in signal (no abrupt changes or dips) from the appropriate filter.
- Confirm that the correct reporter dyes were selected during plate file setup.

## Multicomponent Plot overview

The **Multicomponent Plot** displays the complete spectral contribution of each dye over the duration of the thermal cycling protocol.

Multicomponent data are produced whenever raw data are read from the instrument.

Use the **Multicomponent Plot** to obtain the following information.

- Confirm that the signal from the passive reference dye remains unchanged throughout the run.
- Review reporter dye signal for spikes, dips, and/or sudden changes.
- Confirm that no amplification occurs in the negative control wells.

## Melt Curve Plot overview

The **Melt Curve Plot** displays the melt curve of the amplification products in the selected wells.

Review the **Melt Curve Plot** to confirm that the amplification products in a well display a single melting temperature ($T_m$). Multiple peaks in a melt curve indicate non-specific amplification or primer-dimer formation.

Multi-peak calling is available when more than one peak is expected in a reaction. This is when more than one PCR product is expected to amplify.

**Table 6 Melt Curve plots**

| Plot | Description |
|---|---|
| Derivative Reporter vs. Temperature | Displays the derivative reporter signal in the y-axis as a function of temperature.<br>The peaks in the plot indicate significant decrease in the intercalating dye signal, and therefore the $T_m$ of the amplified products. Use this plot to confirm a single $T_m$ of the amplification products. |
| Normalized Reporter vs. Temperature | Displays the fluorescence signal from the reporter dye normalized to the fluorescence signal of the passive reference, as a function of temperature.<br>You can use this plot to check the quality of the fluorescence data. |

# A Troubleshooting

| Observation | Possible cause | Recommended action |
|---|---|---|
| High fluorescence signal | The reaction volume is not correct. | Ensure that reaction volumes in the plate are correct and match the volume that is entered in the **Run Method** tab. |
| | Signals that exceed the limit of normal fluorescence can indicate fluorescent contaminants on the plate or on the sample block. | Examine the bottom of the reaction plate. If there is contamination, prepare and run new plate. |
| | | Identify the location of contamination on the plate or sample block. For detailed instructions, see the instrument documentation. |
| Inconsistent communication between instrument and computer or instrument and Connect | The instrument is configured for *both* wired and wireless network connection. | Ensure only one connectivity option is plugged into the instrument (either an Ethernet cable *or* a wireless adapter, but not both). |
| | | Configure for wired *or* wireless network connection. |
| | Weak or unstable internet connection, especially if configured for wireless. | Change the configuration to a wired connection. |
| | | Use a wireless network with a stronger or more consistent signal. |
| The connection between the instrument and the computer is not recognized | The connection is not fully established. | Power the instrument off, then power it on again. |
| | If using a networked configuration, the instrument and computer are not on the same subnet mask. | Contact your information technologies department to have them ensure that the instrument and computer are on the same subnet mask. |
| | If using a networked configuration, the instrument or computer has an invalid IP address. | Contact your information technologies department to have them ensure that the IP addresses are valid. |

# B Documentation and support

## Related documentation

| Document | Publication number |
|---|---|
| *QuantStudio™ Design and Analysis Software v2 Standard Curve Analysis Module User Guide* | MAN0018746 |
| *QuantStudio™ Design and Analysis Software v2 Relative Quantification Analysis Module User Guide* | MAN0018747 |
| *QuantStudio™ Design and Analysis Software v2 Presence Absence Analysis Module User Guide* | MAN0018748 |
| *QuantStudio™ Design and Analysis Software v2 Genotyping Analysis Module User Guide* | MAN0018749 |
| *QuantStudio™ Design and Analysis Software v2 High Resolution Melt Analysis Module User Guide* | MAN0018981 |
| *QuantStudio™ 6 Pro Real-Time PCR System and QuantStudio™ 7 Pro Real-Time PCR System User Guide* | MAN0018045 |
| *SAE Administrator Console v2.0 or later User Guide for PCR systems* | MAN0017468 |

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  - Product FAQs
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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

# Limited product warranty

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## D



## E



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