applied biosystems

QuantStudio™ Design and Analysis Software v2

USER GUIDE

for use with:

QuantStudio™ 7 Pro Real-Time PCR System

QuantStudio™ 6 Pro Real-Time PCR System

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Revision H.0

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Revision	Date	Description
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

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 8).

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

Compatible data files

The software is compatible with data files for the following real-time PCR instruments:

- QuantStudio™ 7 Pro Real-Time PCR System (including TaqMan™ Array Card format)
- QuantStudio™ 6 Pro Real-Time PCR System
- QuantStudio™ 5 Real-Time PCR System (if the plate file for the instrument run is created using QuantStudio™ Design and Analysis Software v2)
- QuantStudio™ 3 Real-Time PCR System (if the plate file for the instrument run is created using QuantStudio™ Design and Analysis Software v2)
- QuantStudio™ 1 Real-Time PCR System (if the plate file for the instrument run is created using QuantStudio™ Design and Analysis Software v2)

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

- QuantStudio™ 5 Real-Time PCR System (if the plate file for the instrument run was not created using QuantStudio™ Design and Analysis Software v2)
- QuantStudio™ 3 Real-Time PCR System (if the plate file for the instrument run was not created using QuantStudio™ Design and Analysis Software v2)
- QuantStudio™ 1 Real-Time PCR System (if the plate file for the instrument run was not created using QuantStudio™ Design and Analysis Software v2)
- QuantStudio™ 6 Flex Real-Time PCR System
- QuantStudio™ 7 Flex Real-Time PCR System (including TaqMan™ Array Card format)
- QuantStudio™ 12K Flex Real–Time PCR System (except OpenArray™ format)

- 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**.

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

Chapter 4, "Set up a plate file"

"Select a system template or existing plate file to set up a new plate file" on page 20

▼

"Confirm or edit run method" on page 21

▼

"Confirm or edit plate setup" on page 27

▼

"Review the plate file and send to the instrument run queue" on page 39

▼

Chapter 5, "Review and analyze data"

"Review results in the Amplification Plot" on page 41

▼

"Identify and omit outliers from analysis" on page 45

▼

Chapter 6, "Export results"

3 Use the software with the Security, Auditing, and E-signature (SAE) v2.0 module

Note: SAE functions are only compatible with the QuantStudio™ 7 Pro Real-Time PCR System (see "SAE-enabled system components" on page 11).

Overview of the Security, Auditing, and E-signature (SAE) v2.0 module components

The Security, Auditing, and E-signature (SAE) v2.0 module 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 Security, Auditing, and E-signature (SAE) v2.0 module 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 may not be accessible to you.
- You may see dialog boxes and prompts when you use the software.

SAE-enabled system components

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

- QuantStudio™ Design and Analysis Software v2
- QuantStudio™ 7 Pro Real-Time PCR System

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

We recommend enabling SAE for all system components (see "Enable SAE functions" on page 13). If one or more of the components have conflicting SAE status, some functions may not be available (see "Compatibility between SAE-enabled and SAE-disabled components" on page 12).

Compatibility between SAE-enabled and SAE-disabled components

We recommend enabling SAE for all system components (for more information, see "Enable SAE functions" on page 13). If one or more of the components have conflicting SAE status, some functions may 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 enabled	• The file can be edited depending on SAE configuration. • The audit trail is continued.	• The file is opened in read-only mode. • The file cannot be edited or saved.
QuantStudio™ Design and Analysis Software v2 with SAE 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] • QuantStudio™ 7 Pro Real-Time PCR System forbidden—The file cannot be opened.	The file can be opened, edited, and saved.
QuantStudio™ 7 Pro Real-Time PCR System with SAE enabled	• The file can be opened from the run queue, a USB drive, or other sources.[2] • The file cannot be edited. • The audit record is continued.	The file cannot be opened.
QuantStudio™ 7 Pro Real-Time PCR System with SAE disabled	• The plate file can be opened and edited. • The file can be saved as an invalid SAE file only.[1] • 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 Connect cloud-based platform when the instrument has SAE 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 12).

Enable SAE functions

Workflow: Enable SAE functions

Configure SAE Administrator Console application profiles (page 13)

▼

Enable SAE on the instrument and specify the SAE server (administrator only) (page 14)

▼

Connect to the SAE server (page 15)

▼

Enable SAE functions in QuantStudio™ Design and Analysis Software v2 (page 15)

Configure SAE Administrator Console application profiles

Note: Configuring application profiles in the SAE Administrator Console requires an SAE administrator account.

In the SAE Administrator Console, an application profile contains default settings for an application. Before using the Security, Auditing, and E-signature (SAE) v2.0 module, an administrator must install, then configure profiles for the following applications:

1. QuantStudio™ 7 Pro Real-Time PCR System
2. QuantStudio™ Design and Analysis Software v2

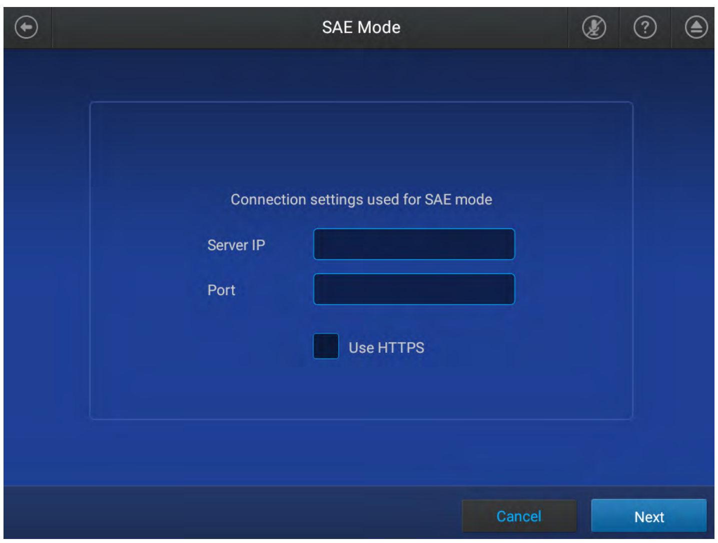
Note: The QuantStudio™ 7 Pro Real-Time PCR System profile must be installed before the QuantStudio™ Design and Analysis Software v2 profile.

For information on configuring application profiles, see *SAE Administrator Console v2.0 User Guide* (Pub. No. MAN0017468).

Enable SAE on the instrument and specify the SAE server (administrator only)

This procedure requires a local administrator profile on the instrument and an SAE administrator 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.



4. Tap the **Port** field, enter the port, then tap **Next**.
5. 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 to the SAE server

- Install the SAE Administrator Console and the SAE server on a computer with a static IP address (*recommended*) or a dynamic IP address.
- Before you connect, close all plate files and data files.

Note: Connect the software and any instruments to the same SAE server to ensure that audit records are maintained across system components.

1. In the menu bar, click **System ▸ SAE Connection Settings**.
2. Enter the IP address and port number of the SAE server.

   Note: If using a dynamic IP address, enter the hostname instead of the IP address to prevent the loss of a connection.

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

Enable SAE functions in QuantStudio™ Design and Analysis Software v2

This procedure requires an SAE administrator account.

Before you enable SAE functions in the QuantStudio™ Design and Analysis Software v2, you must complete the following tasks:

- Connect to the SAE server (see "Connect to the SAE server" on page 15).
- 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 username and password, then click **Sign In**.

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

To sign out of the SAE administrator account, see "Sign out of the software using an SAE account" on page 16.

Note: Signing out of the SAE administrator account does not disable SAE functions in the software. To disable SAE functions in the software, see "Disable SAE functions in QuantStudio™ Design and Analysis Software v2" on page 19.

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 SAE functions in QuantStudio™ Design and Analysis Software v2" on page 15).

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 18.
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 18).

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: • Reviewed and Approved Template • 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 SAE functions in 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

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 "About system templates and plate files" on page 60.

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

2. **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.

   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 67).

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

Tab	Description
System Templates	Contains system templates, non-editable plate files that are included with the software. 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**. Click an existing plate file to automatically generate a new plate file that can be edited, then saved. 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. 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 38).

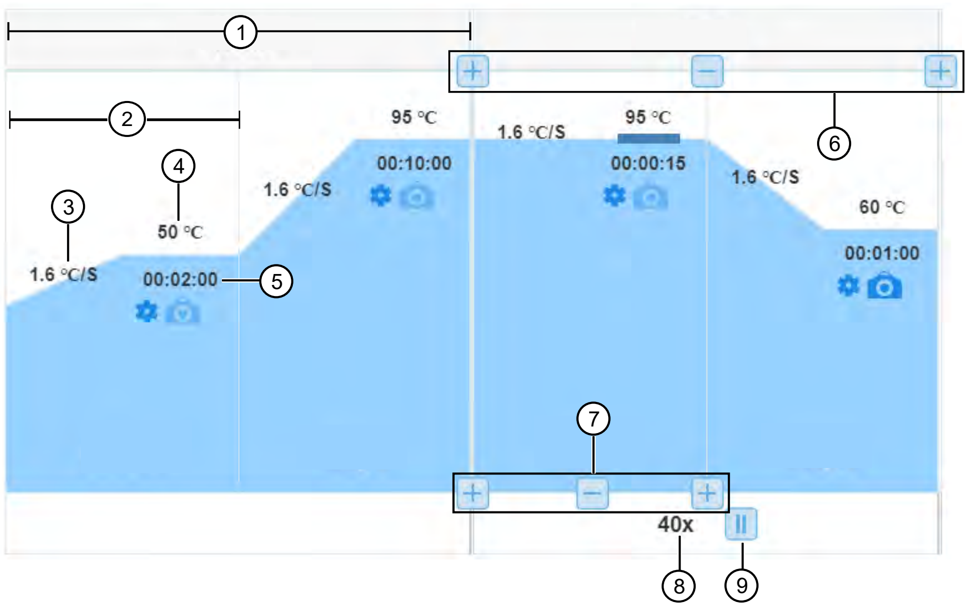
4. Select a system template or plate file to open. To view all options for opening the plate file, hover over the plate file, then click ··· **(Actions)**.

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

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 36).
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 21.
2. Enter the value, then click outside the element to stop editing.

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 21.
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 22).

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 21.

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. • 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. • 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. • 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 22).

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 21.

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

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 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)**.

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 Mac: Cmd-click rows in the table
Deselect a single well	PC: Ctrl-click the selected row Mac: Cmd-click the selected row

Add samples and assign to plate wells

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 39), 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

1. In the **Plate Setup** tab, in the plate layout pane, click ⋯ **(Actions)** ▸ **Import Plate Setup**.
2. Navigate to, then select the plate setup file.

Paste samples, targets, or SNP assays in the plate layout

You can copy samples, targets, or SNP assays information from an Excel™ spreadsheet, then paste it into the plate layout grid.

Note: Copy/paste is not available for TaqMan™ Array Card plate setup.

---

1. Create an Excel™ spreadsheet with the plate setup information. Refer to the plate layout template for the correct format.

   See the software Help to download a spreadsheet that includes a plate layout template.
2. In the spreadsheet, select, then copy the cells of interest.
3. In the **Plate Setup** tab of the software, in the plate layout pane, click **Target** or **SNP**.
4. In the **(Grid View)**, select one or more wells.
5. Use Ctrl+V (PC) or Cmd+V (Mac) to paste the copied information into the plate layout.

The copied information populates the plate layout, **Samples Table**, and **Targets Table** or **SNP Assays Table**.

Note: If the sample, target or SNP assay does not exist in the respective table, the software will automatically create them with the default values for all columns except for the name.

To complete plate setup, edit the **Samples Table**, and **Targets Table** or **SNP Assays Table** if needed.

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.

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	1. Copy the sample information from one of the following sources: • Previously created plate file or data file—Click ⋯ **(Actions)** ▸ **Copy all Samples** in the upper-right corner of **Samples** table. • Excel file—Select, then copy data, including column headers.[1] • Plate setup file—Select, then copy data, including column headers.[1] 2. 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 color and sample type" on page 30).

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 35).

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

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

1. In the **Plate Setup** tab, in the plate layout pane:
   - 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. • 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 color and sample type" on page 30).

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 35).

Edit sample color and sample 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 35).

1. In the **Plate Setup** tab, in the **Samples** table, select a sample color from the **Color** column dropdown list.

2. 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.

Add a custom attribute to samples

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	1. In the custom attribute tab, click + **(Add)**. 2. 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.

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 • Rack/Plate ID

(continued)

Product	Required Information
Fixed TaqMan™ Array Cards	• Part Number • Lot Number
Custom Gene Expression TaqMan™ Array Cards	• Sales Order Number • Lot Number
Custom Advanced miRNA TaqMan™ Array Cards	• Sales Order Number • Lot Number
Fixed TaqMan™ Array Plates	• Part Number • Batch Number
Custom TaqMan™ Array Plates	• Sales Order Number • 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.

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

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

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 31.

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 39), 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

1. In the **Plate Setup** tab, in the plate layout pane, click ⋯ **(Actions)** ▸ **Import Plate Setup**.
2. Navigate to, then select the plate setup file.

Paste samples, targets, or SNP assays in the plate layout

You can copy samples, targets, or SNP assays information from an Excel™ spreadsheet, then paste it into the plate layout grid.

Note: Copy/paste is not available for TaqMan™ Array Card plate setup.

1. Create an Excel™ spreadsheet with the plate setup information. Refer to the plate layout template for the correct format.

   See the software Help to download a spreadsheet that includes a plate layout template.

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

3. In the **Plate Setup** tab of the software, in the plate layout pane, click **Target** or **SNP**.

4. In the **(Grid View)**, select one or more wells.

5. Use Ctrl+V (PC) or Cmd+V (Mac) to paste the copied information into the plate layout.

The copied information populates the plate layout, **Samples Table**, and **Targets Table** or **SNP Assays Table**.

Note: If the sample, target or SNP assay does not exist in the respective table, the software will automatically create them with the default values for all columns except for the name.

To complete plate setup, edit the **Samples Table**, and **Targets Table** or **SNP Assays Table** if needed.

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

Note: 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 32.

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	1. Copy the information from one of the following sources: • **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. • Excel file—Select, then copy data, including column headers.[1] • Plate setup file—Select, then copy data, including column headers.[1] 2. 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

Note: 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 32.

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. • 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 35).

(*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).

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 27).

   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 color and sample type" on page 30).

Task	Description
Unknown (default)	The well contains an unknown sample.
Standard[1]	The well contains a sample with known standard quantities. **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.

Manage target dyes

1. In the **Plate Setup** tab, in the plate layout pane, click ⋯ **(Actions)** ▸ **Manage Dyes**.
2. View system dyes in the **System Dyes** tab.
3. In the **Custom Dyes** tab, click + **(Add)**.
4. Enter or select the **Dye Name**, **Color**, **Type**, **Wavelength**, and **Fluorescence** dye properties.

5. (*Optional*) Click ⊗ **(Remove)** ▶ **OK** to remove a dye from the table.

6. Click **Close**.

Edit reagent information

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 reagent **Name**, **Type**, **Barcode**, **Part Number**, **Lot Number**, and **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 22.

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 28).

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**. **2.** Select a reporter from the dropdown list. **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 ≥1.
   - **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. The biogroup is added to the following locations: • **Samples** table **Biogroup** dropdown list • **Biogroup** table
Add biogroups in the **Biogroup** table	1. Click **Biogroup**. 2. 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

1. Click **Actions ▸ Plate Information**.
2. (*Optional*) Click in the barcode field, then scan the plate barcode.
3. (*Optional*) Add a tag.
4. (*Optional*) Enter a description.
5. Click **Save**.

Save the updated plate file or data file.

Review the plate file and send to the instrument run queue

1. In the **Run Summary** tab, review the run method selections, then edit if needed (see "Confirm or edit run method" on page 21).
2. Review the plate setup, then edit if needed (see "Confirm or edit plate setup" on page 27).
3. (*Optional*) Click the barcode field, then scan the plate barcode.
4. (*Optional*) Select **Add to My Plates**.
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 64).
6. Save the plate file (see "Save a plate file or data file" on page 61).
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" on page 28).

1. In the **Plate Setup** tab, in the plate layout pane, click ⋯ **(Actions) ▸ Export Plate Setup**.
2. Name the file, navigate to the desired folder location, select the file format, then 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 41))

▼

Review data for outliers and (*optional*) omit wells (Identify and omit outliers from analysis (page 45))

▼

(*Optional*) View the Multicomponent Plot to review the dye signal profile (Review results in the Multicomponent Plot (page 46))

▼

(*Optional*) View the Raw Data Plot to review the signal profile (Review results in the Raw Data Plot (page 47))

▼

(*Optional*) Review flags and QC alerts (Review QC alerts in the Well Table (page 48))

▼

(*Optional*) Edit analysis settings (Edit primary analysis settings (page 49))

▼

(*Optional*) Perform additional analysis (Perform additional analysis (page 53))

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.

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 77.

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. Review the overall shape of the curves in the Amplification Plot.
   For more information about the **Amplification Plot**, see “Amplification Plot overview” on page 77.

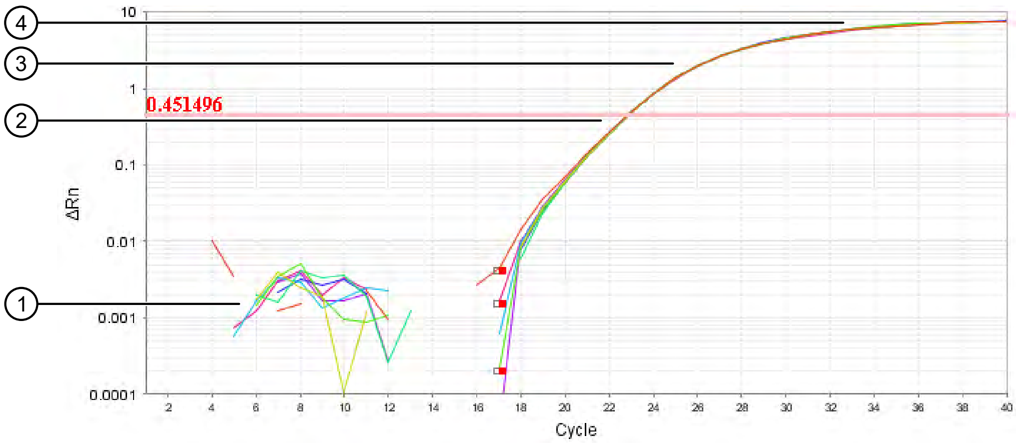


Figure 1 **Typical amplification plot**

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. • The well was omitted from analysis. • Insufficient cycle number to determine if amplification occurred.

Review or edit threshold settings in the Amplification Plot

The default analysis setting is for **Auto Threshold**. To set the threshold manually, see "View or edit Cq settings" on page 49.

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

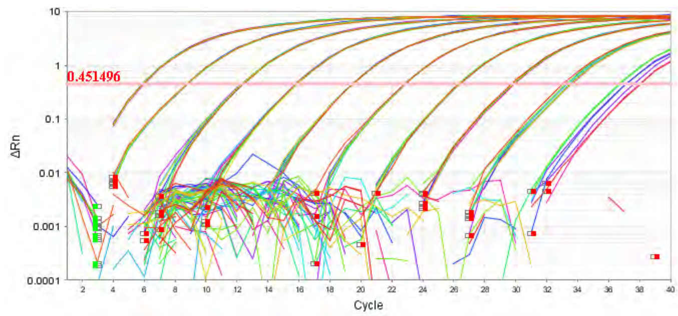
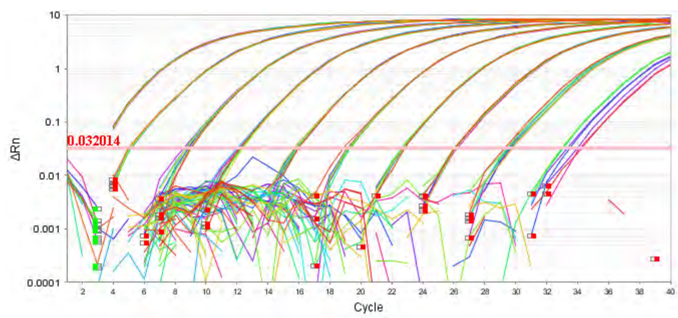
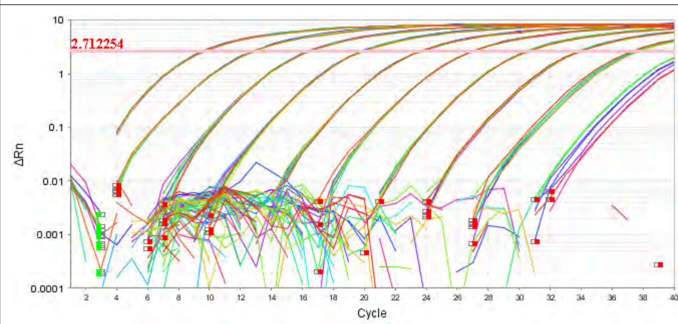
2. Click ⚙ **(Settings)**, then make the following selections:

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

The **Amplification Plot** is displayed for all wells.

3. 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.	

4. (*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 49).

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**
   - Select **Show: Baseline Start / Baseline End**

   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**.
   The start and end cycles display for each well.
3. (*Optional*) Adjust the start and end cycle values for the baseline (see "View or edit Cq settings" on page 49).

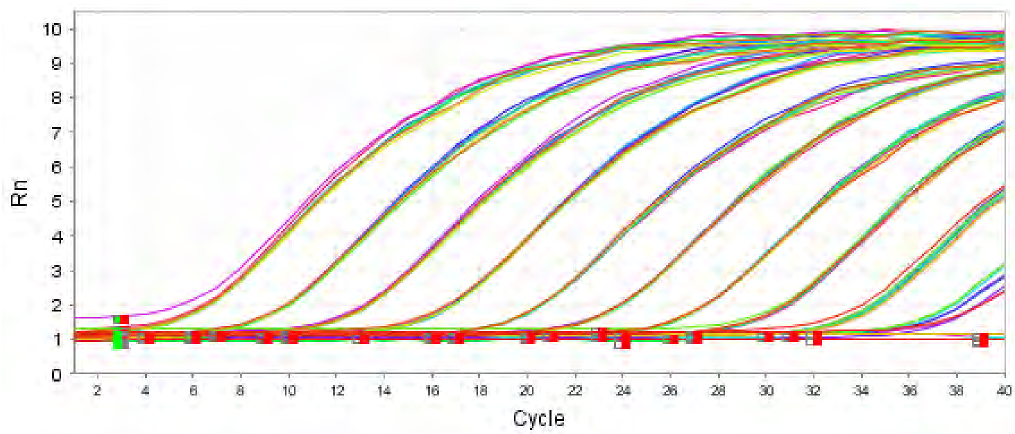


Figure 2 Example of correct baseline

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. In either the **Plate Layout** or **Well Table**, select the negative control wells (wells that should not have amplification for a particular target).

4. 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.

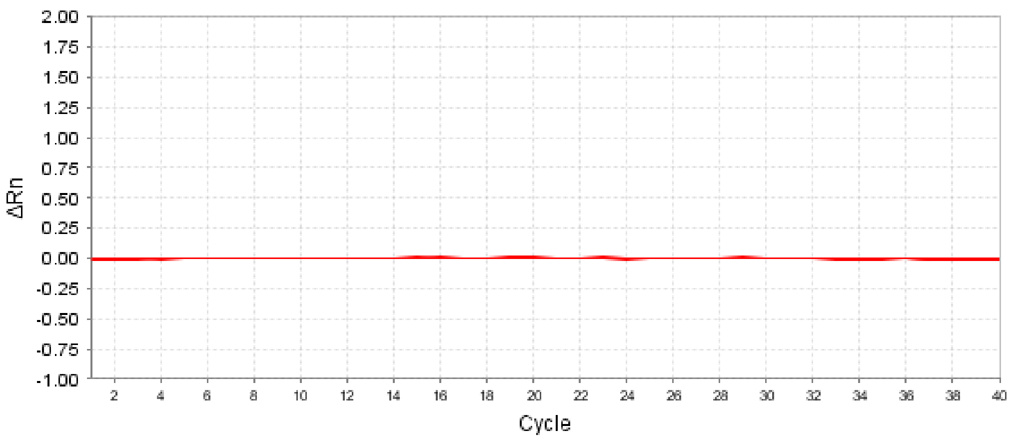


Figure 3 Example amplification plot of negative controls

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. 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.
4. 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**, right-click a well, then select **Omit**.
5. 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. 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.

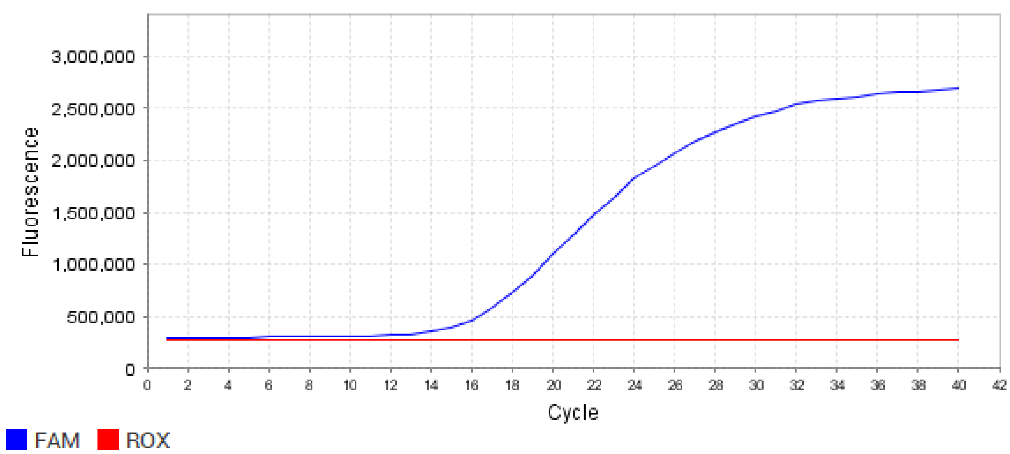


Figure 4 Example multicomponent plot (single well)

Review results in the Raw Data Plot

For more information about the **Raw Data Plot**, see "Raw Data Plot overview" on page 77.

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. 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

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**, see "Melt Curve Plot overview" on page 78.

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 51).

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. Review the plot for evidence of unexpected multiple peaks, which can indicate non-specific amplification or formation of primer-dimers.

4. Review the **Well Table** for the calculated T_m in each well.

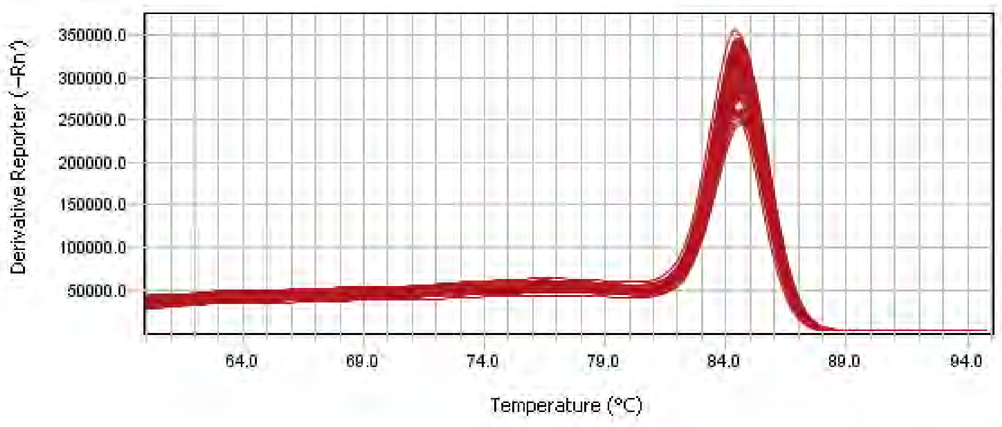


Figure 6 Example Melt Curve Plot

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 following QC alerts in the **Well Table**:
   - Curve Quality
   - Result Quality Issues

   For more information about QC alerts, see "QC alerts overview" on page 75.

2. Adjust QC alert settings as needed (see "View or edit QC alerts settings" on page 50), then reanalyze.

Review results in the Plate Layout

1. In the **Quality Check** tab, in the **Plate Layout**, 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 "QC alerts acceptance criteria" on page 76).

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 73.
- For information about C_q settings, see “Cq settings overview” on page 74.

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.
4. To select the default threshold and baselines settings for a target, select the checkbox in the **Use Default** column.
5. (*Optional*) To edit the **Default Setting** or apply custom C_q settings to a target (Baseline Threshold algorithm only), 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. • 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. • 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** and **Baseline End** column.

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

7. Click **Save**.

8. (*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.

   d. In the right panel, select the acceptance criteria for each sample type and target combination (see "QC alerts acceptance criteria" on page 76).

   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**

3. Click **Save**.

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

View or edit melt analysis settings

For more information about melt analysis settings, see "Melt analysis settings overview" on page 75.

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. Edit the settings as needed.
4. Click **Save**.
5. (*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 8), 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

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.

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.

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 67). For more information about analysis modules, see "About analysis modules" on page 66.
Application on the Connect platform	To perform additional analysis using an application on the 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 54.

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 54.

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 in the QuantStudio™ file format

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 61).

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 70).

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 61).

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. Select the paper size:
   - A4
   - Letter

5. 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: • Analysis Module • Bar Code • File Name • Run Started • Run Finished • Run Duration • Date Modified • Date Created • Operator • Number of Wells Used • Instrument Name • Instrument Type • Instrument Serial Number • Block Type • Stage/Step Analysis • Melt Stage Analysis • Algorithm Settings • Plate Setup Origin • Comments
Well Table	Displays the following information from **Well Table** in the **Quality Check** tab: • Well • Sample • Target • Task • Dyes • C_q • C_q Confidence • Amplification Score • Amplification Status
Replicate Group Results	Displays the following information from **Replicate Table** in the **Quality Check** tab: • Sample • Target • C_q Mean • C_q Standard Deviation
Plate Layout	Displays the sample name, sample color, target(s), and target C_q value(s) for each well.

(continued)

Report Content	Description
Amplification Plot (Rn)	Displays the Amplification Plot (Rn vs Cycle)[1]
Amplification Plot (dRn)	Displays the Amplification Plot (dRn vs Cycle)[1]
Melt Curve Plot	Displays the Melt Curve Plot (Derivative Reporter vs Temperature)[2] This option only displays if the Run Method includes a Melt Curve stage.
TC Protocol	Displays the thermal cycling protocol used in the Run Method.
Analysis Module	Displays the plots and analysis results for the selected analysis modules. This option only displays if an analysis module is selected.

[1] For more information about the Amplification Plot, see "Amplification Plot overview" on page 77.
[2] For more information about the Melt Curve Plot, see "Melt Curve Plot overview" on page 78.

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

Option	Action
Select one well	Click the well
Select multiple contiguous wells	Click-drag over the wells
Select non-contiguous wells	PC: Ctrl-click each well Mac: Cmd-click each well

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**
   - **Samples**
   - **Targets**

- **Biogroups**

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

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

About 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 20).

A plate file can contain the following information:

Information type	Properties
Instrument setup	• Instrument type • Block • Run mode
Run Method	• Thermal protocol • Filter settings
Plate Setup	• Sample definitions and well-assignments • Target or SNP assay definitions and well-assignments • Reagent information **Note:** Plate setup information is not included in the system template and must be defined by the user.
Primary Analysis Settings	• C_q settings • Melt settings • QC settings
Analysis Module	Analysis modules are plugins that enable additional data analysis in the software (see “About analysis modules” on page 66). **Note:** The user can select an analysis module pre- or post-instrument run (“Select an analysis module” on page 67).
Additional Information	Plate information — plate barcode and user-defined description

About 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** • **Operator** and **Block Type** • **Heated Cover S/N** and **Block Serial S/N** • **Instrument Software** and **Instrument Name** • **Run Events** and **Calibration**
Analysis results	• Data plots • C_q and C_q confidence • Amplification score and status • Melting temperature • 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 66.

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 61).

In an open plate file, click **Actions ▸ Add to My Plate Files**.
The plate file appears in the in the **Plate File 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 38).

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.

Batch generate plate files

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

1. In an open plate file in the **Plate Gallery**, click **Actions ▸ Generate Plate Files**.

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

- 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.

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 64.

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

For more information about adding an instrument, see “Add an instrument” on page 64.

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 67.

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.
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
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.
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. **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 “Documentation and support” on page 80.
- 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.

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**). • 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/formas/life-science/quantstudio-6-7-pro-software** to download the file and purchase a license registration code.

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

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 67.

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.

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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/formas/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 70).

Export settings can be applied to data exported by the software or by QuantStudio™ 6 Pro and 7 Pro Real-Time PCR Systems.

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 70

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 70).

1. Open a data file.

2. Click **Actions ▸ Export**.

3. In the **Export Plate** window, 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. Click the data type (Results, Amplification Data, Multicomponent, Raw Data, or Replicate Group Result), then edit the export settings.

Option	Description
Turn data export on or off	Toggle the **Export Results** button on or off. A green checkmark (✓) indicates that the data will be exported.
Select table columns to export	Select columns to include in the exported table in the **Select Columns** pane.
Select well data to export	Select or deselect **Include Empty Wells** or **Include Omitted Wells**.

6. To edit the export settings for analysis module data, select the analysis module from the **Analysis Module** dropdown list.

7. Edit the analysis module data export settings (see step 5).

8. Click **Save Current Setting As**, enter a new export settings file name, then click **Save**.

9. 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 71.
- 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 71).

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

2. Click **Actions** ▸ **Import Setting**.

3. Navigate to the export settings file, then click **Open**.

13

Manage preferences

View or edit preferences for **AIF Import**, **Plate File Format**, **Result Export**, and **Report Generation**.

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 **Plate File Format** pane, select **Create plate file (.edt) in legacy format for QuantStudio™ 1/3/5/6 Flex/7 Flex**, then click **Apply**.

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

4. 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**.

5. 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 67).

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 is calculated using the PCR cycle number at which the fluorescence signal meets the threshold in the amplification plot.
Relative Threshold	C_q 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 74.

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 product's T_m. Multiple peaks in a melt curve experiment indicate additional amplification products, usually from non-specific amplification or formation of primer-dimers.

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).

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 2 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. 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. For recommendations on adjusting baseline and threshold settings, see "Guidelines for manual threshold and baseline settings for Cq analysis" on page 75.
C_q Settings for Target	• **Default Settings** selected—The default C_q settings are used to calculate the C_q values for the target. • **Default Settings** deselected—The software allows manual setting of the baseline or the threshold. For recommendations for adjusting baseline and threshold settings, see "Guidelines for manual threshold and baseline settings for Cq analysis" on page 75.
Relative threshold analysis	
Algorithm Settings – **Relative Threshold**	The **Relative Threshold** Algorithm is used to calculate the C_q values. 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: • Above the background. • Below the plateau and linear phases of the amplification curve. • 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

- Enable or disable **Multi-Peak Calling** the **Melt** tab.

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

- (*For multi-peak calling only*) Adjust the **Peak Level (%)**.
  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 (%)** is set to 40, then peaks above 40% of the tallest peak are reported, and peaks below 40% are regarded as noise.

To edit the melt analysis settings, see "View or edit melt analysis settings" on page 51.

QC alerts overview

Note: A warning message is displayed at the top of the **Quality Check** tab if QC alerts are present.

In the **Curve Quality** column of the **Well Table**, a warning icon is displayed if Curve Quality alerts are present.

In the **Result Quality Issues** column of the **Well Table**, the following can be displayed if Results Quality alerts are present.

- 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

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

QC alerts acceptance criteria

Acceptance criteria	Description
C_q should be	• **Expressed** • **Undetermined** 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. 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. 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. Any well outside of the user-defined minimum and maximum range will be flagged.
Amp Status	• **Amp** • **No Amp** • **Inconclusive** 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. Any well outside of the user-defined minimum and maximum range will be flagged.
Delta Rn Range	The Delta Rn is the calculated normalized delta reporter signal. Any well outside of the user-defined minimum and maximum range will be 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.
Tm Range	Tm is the melting temperature calculated in °C. 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.

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 3 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. • 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. • 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 (not normalized) for each optical filter during each cycle of the real-time PCR.

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 PCR run.

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.

Table 4 Melt Curve plots

Plot	Description
Derivative Reporter vs. Temperature	Displays the derivative reporter signal in the y-axis as a function of temperature. The peaks in the plot indicate significant decrease in SYBR™ Green 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. You can use this plot to check the quality of the fluorescence data.

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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.

Documentation and support

Related documentation for the QuantStudio™ Design and Analysis Software v2

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
QuantStudio™ 6 Pro Real-Time PCR System and QuantStudio™ 7 Pro Real-Time PCR System Site Preparation Guide	MAN0017992
SAE Administrator Console v2.0 User Guide	MAN0017468

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

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Index

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W