SteriSEQ™ Rapid Sterility Testing Kit

USER GUIDE

for use with:
AccuSEQ™ Real-Time PCR Software 3.2.1 or later
AccuSEQ™ Real-Time PCR Software 2.2.1 or later

Catalog Numbers A57185, A57186
Publication Number MAN0029388
Revision A.0

ThermoFisher SCIENTIFIC

Life Technologies Ltd | 7 Kingsland Grange | Woolston, Warrington WA1 4SR | United Kingdom
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0029388 A.0 (English)

Revision	Date	Description
A.0	7 March 2024	New document for SteriSEQ™ Rapid Sterility Testing Kit.

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

Contents

1 Product information

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

Product description

The SteriSEQ™ Rapid Sterility Testing Kit is a TaqMan™-based quantitative PCR (qPCR) kit for the detection of bacteria and fungi in complex bioproduction samples. Designed and tested using criteria for rapid bacteria and fungi detection in biotherapeutic manufacturing cell culture lots, the kit meets or exceeds sensitivity and specificity guidance provided in European Pharmacopoeia (E.P 2.6.27) and U.S. Pharmacopoeia (USP1071). The kit can detect 5 to 25 genome copies per reaction or the equivalent of 5 to 25 CFU per reaction.

This multiplexed assay enables simultaneous screening of over 16,000 species of bacteria and 2,500 species of fungi (determined by in silico analysis). An internal positive control (IPC) is included to check for PCR inhibition. An assay-specific discriminatory positive control (DPC) is provided as a positive plate control and sample extraction control. This control also generates a discriminatory signal to distinguish a contamination event from a true positive sample call.

The SteriSEQ™ Rapid Sterility Testing Kit is part of an integrated workflow for adventitious agent, impurity, and contaminant testing during biopharmaceutical manufacturing. Kit performance has been tested and confirmed on the QuantStudio™ 5 Real-Time PCR System and 7500 Fast Real-Time PCR Instrument. Data analysis using AccuSEQ™ Real-Time PCR Software provides accurate detection and security, audit, and e-signature capabilities to enable 21 CFR Pt 11 compliance.

The SteriSEQ™ Rapid Sterility Testing Kit uses predefined experiment templates that are included with the following versions of AccuSEQ™ Real-Time PCR Software for PCR set up, operation, and post-run analysis:

- AccuSEQ™ Real-Time PCR Software 3.2.1 or later (used with the QuantStudio™ 5 Real-Time PCR System)
- AccuSEQ™ Real-Time PCR Software 2.2.1 or later (used with the 7500 Fast Real-Time PCR Instrument)

Contents and storage

The SteriSEQ™ Rapid Sterility Testing Kit is sold as a 100-reaction kit (Cat. No. A57185) or a 50-reaction kit (Cat. No. A57186).

Kit components are listed below.

Table 1 SteriSEQ™ Rapid Sterility Testing Kit

Contents	Cap color	Amount (50 reaction kit)	Amount (100 reaction kit)	Storage
SteriSEQ™ Assay Mix	green	110 µL	2 × 110 µL	–25°C to –15°C. Protect from light.
2x qPCR Master Mix Plus	blue	750 µL	2 × 750 µL	–25°C to –15°C until first thawed, then store at 2–8°C. Protect from light.
SteriSEQ™ Discriminatory Positive Control (1 x 10^5 copies/µL)	red	30 µL	2 × 30 µL	–25°C to –15°C
SteriSEQ™ DNA Dilution Buffer	clear	7 mL	7 mL	–25°C to –15°C until first thawed, then store at room temperature.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Equipment	
QuantStudio™ 5 Real-Time PCR System with AccuSEQ™ Software v3.2.1 or later *or* 7500 Fast Real-Time PCR Instrument with AccuSEQ™ Real-Time PCR Software v2.2.1 or later	Contact local sales representative
Vortex-Genie 2 (required by some sample preparation kits)	Scientific Industries™
Vortex Adapter (required by some sample preparation kits)	AM10014 or **MLS**
Microtube centrifuge	**MLS**
Plate centrifuge	**MLS**

(continued)

Item	Source
Generic consumables	
Disposable gloves	**MLS**
Aerosol-resistant, DNA-free filtered pipette tips	**MLS**
Pipettors: • Positive-displacement • Air-displacement • Multichannel • Repeat (1 µL–1 mL)	**MLS**
2.0-mL Screw cap Micro Tubes or equivalent sterile, DNA- and nuclease-free tubes	3469-11 or **MLS**
DNA*Zap*™ PCR DNA Degradation Solutions	AM9890
Consumables for use with real-time PCR instruments	
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4346906
MicroAmp™ Optical Adhesive Film	4360954 (25 covers) 4311971 (100 covers)
MicroAmp™ Adhesive Film Applicator	4333183
MicroAmp™ 96-Well Base	N8010531
Spectral Calibration Plate, Alexa Fluor™ 647, Fast 96-well	A50599
ABY™ Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well (7500 Fast Real-Time PCR Instrument only)	A24734

Dyes in the kit and their use

IMPORTANT! Alexa Fluor™ 647 calibration is not included in the standard system dye calibration service provided with either the QuantStudio™ 5 Real-Time PCR System or the 7500 Fast Real-Time PCR Instrument. ABY™ calibration is not included with the standard system dye calibration provided with the 7500 Fast Real-Time PCR Instrument. These dyes must be calibrated separately. For more information about custom dye calibration, see “Required custom dyes” on page 10.

Dye	Detected in	Use
FAM™	Test samples that contain fungi Discriminatory positive control (DPC)	Detects the presence of fungi in test samples, and verifies amplification of the fungi target sequence in DPC.
VIC™	Test samples that contain bacteria Discriminatory positive control (DPC)	Detects the presence of bacteria in test samples, and verifies amplification of the bacteria target sequence in DPC.
ABY™	All samples and controls (positive and negative)	Negative or severely-delayed ABY™ C_t indicates PCR inhibition or incorrect set up of the reaction mix. If there is PCR inhibition in test sample wells and the target-specific signal (FAM™/VIC™ dye) is present, the IPC signal (ABY™ dye) can be ignored and the sample considered positive for the presence of bacteria and/or fungi.
Alexa Fluor™ 647	Discriminatory positive control (DPC) Test samples and negative controls that have been contaminated by the DPC	Verifies the presence of DPC in positive control wells and positive extraction control wells. Detects contamination of test samples or negative controls by the DPC. The DPC includes a specific sequence whose presence is indicated by Alexa Fluor™ 647 during PCR amplification.
ROX™	Not reported by the software	Used by the software for normalization (passive reference).

Required custom dyes

Custom dyes

Alexa Fluor™ 647 calibration is not included in the standard system dye calibration service provided with either the QuantStudio™ 5 Real-Time PCR System or the 7500 Fast Real-Time PCR Instrument. ABY™ calibration is not included with the standard system dye calibration provided with the 7500 Fast Real-Time PCR Instrument. These dyes have to be calibrated separately by users.

Cat. number	Dye	For use with (equipment)[1]	Block format
A24734	ABY™	7500 Fast Real-Time PCR Instrument[2]	96-well 0.1 mL plate
A50599	Alexa Fluor™ 647	QuantStudio™ 5 Real-Time PCR System, 7500 Fast Real-Time PCR Instrument	96-well 0.1 mL plate

[1] Review the instrument calibration history to ensure that calibrations are current and unexpired. Proper maintenance enables the real-time PCR system to accurately detect the fluorescent spectra. See the instrument user guide for general instrument calibrations, frequency, and procedures for optimal system performance.

[2] Calibration of ABY™ dye is included in the standard calibration list for the QuantStudio™ 5 Real-Time PCR System.

Calibrating Alexa Fluor™ 647 dye for SteriSEQ™ experiments

For instructions about calibrating Alexa Fluor™ 647 dye, see *Spectral calibration plates, Alexa Fluor™ 647 Product Information Sheet* (Pub. No. MAN0028449).

For Alexa Fluor™ 647 calibration on the QuantStudio™ 5 Real-Time PCR System for use with AccuSEQ™ Real-Time PCR Software, use the following parameters for the dye information:

Item	Action
Custom Dye Name	Enter dye name: • AccuSEQ™ Software v3.1: **OTHER** • AccuSEQ™ Software v3.2 or later: **ALEXA647** • Other qPCR software: **ALEXA 647** **IMPORTANT!** The dye name is case-sensitive and must be written in capital letters.
Type	Select **Reporter**—The dye works with a quencher dye to report an increase of PCR product.

For Alexa Fluor™ 647 calibration on the 7500 Fast Real-Time PCR Instrument for use with AccuSEQ™ Real-Time PCR Software, use the following parameters for the dye information:

Item	Action
Custom Dye Name	Enter dye name: • AccuSEQ™ Software v2.1 or later: **ALEXA647** • Other qPCR software: **ALEXA 647** **IMPORTANT!** The dye name is case-sensitive and must be written in capital letters.

(continued)

Item	Action
Wavelength (Optional)	Leave blank
Type	Select **Reporter**—The dye works with a quencher dye to report an increase of PCR product.

Calibrating ABY™ dye for SteriSEQ™ experiments

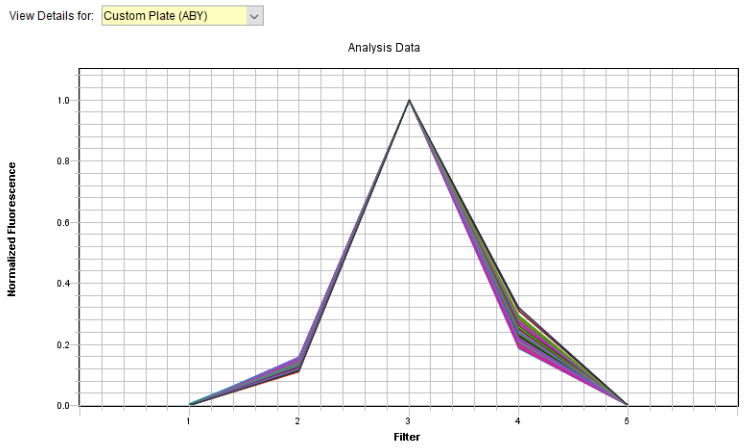
For instructions about calibrating ABY™ dye, see the custom dye calibration section of the *AccuSEQ™ Real-Time PCR Software v2.2 User Guide* (Pub. No. MAN0029201).

For ABY™ calibration on the 7500 Fast Real-Time PCR Instrument for use with AccuSEQ™ Real-Time PCR Software v2.1 or later, use the following parameters for the dye information:

Item	Action
Custom Dye Name	Enter **ABY**. **IMPORTANT!** • Dye name is case sensitive. • Do not add spaces after the dye name.
Wavelength (Optional)	Leave blank
Type	Select **Reporter**—The dye works with a quencher dye to report an increase of PCR product.

Passing ABY™ calibration results show uniform signals with a peak that is aligned with the dye wavelength for the filter x3-m3.

Peak filter	Excitation filter wavelength (nm)	Emission filter wavelength (nm)
x1-m1	470 ± 15 nm	520 ± 15 nm
x2-m2	520 ± 10 nm	558 ± 12 nm
x3-m3	550 ± 10 nm	587 ± 10 nm
x4-m4	580 ± 10 nm	623 ± 14 nm
x5-m5	640 ± 10 nm	682 ± 14 nm
x6-m6	662 ± 10 nm	711 ± 12 nm

Instrument model	Example results indicating successful calibration[1]
7500 Real-Time PCR Instrument and 7500 Fast Real-Time PCR Instrument with 96-well block	

[1] The central wavelengths are the optimized wavelengths.

Testing options and applications

The SteriSEQ™ Rapid Sterility Testing Kit was developed for testing in process and accelerated lot release cell therapy samples. The workflow described in this guide is targeted towards samples with up to 10^6 cells, as well as cell culture media typically used for the production of cell therapy products. However, customers should qualify and validate the workflow described here for their own products, processes, and applications.

SteriSEQ™ Rapid Sterility Testing Kit abbreviations

Abbreviation	Description
CFU	Colony forming unit
IPC	Internal positive control
DPC	Discriminatory positive control
PEC	Positive extraction control
NEC	Negative extraction control
SDDB	SteriSEQ™ DNA Dilution Buffer
S_PEC	Sample-level positive extraction control
S_NEC	Sample-level negative extraction control
PTC	Positive template control
NTC	No template control

Workflow

Sample preparation guidelines (page 14)

Create an experiment, prepare the reactions, and set up the PCR plate (page 18)

Run the PCR and review the results—QuantStudio™ 5 Real-Time PCR System (page 28)

OR

Run the PCR and review the results—7500 Fast Real-Time PCR Instrument (page 48)

2 Guidelines for sample preparation and PCR

Guidelines to prevent contamination

- Store kit reagents in a separate location from DNA samples.
- Handle samples in sterile environments that are free of potential bacteria or fungi DNA contamination.
- Perform sample preparation and qPCR plating using aseptic techniques in a biosafety cabinet (BSC) or PCR cabinet.
- BSC or PCR cabinets should be thoroughly decontaminated before use with an appropriate nucleic acid decontamination agent such as 10% household bleach or DNA*Zap*™ PCR DNA Degradation Solutions (Cat. No. AM9890).
- Use water that has been autoclaved for an extended period (80 minutes at 121°C) to wipe surfaces after bleach-based disinfection.
- Do not bring amplified products into the reaction setup area.
- Wear clean gloves, a clean laboratory coat, and protective eyewear.
- Do not reuse gloves and coats between amplification and preparation reactions.
- Change gloves if you suspect that they are contaminated.
- Wipe gloves regularly with DNA*Zap*™ or a few drops of 10% household bleach.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Immediately discard pipette tips after one dispense cycle.
- Use low-retention tips when preparing unknown samples.
- Use sterile, DNA-free and nuclease-free tubes when preparing unknown samples.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Always include a no template control (NTC) reaction to detect contamination.
- Do not unseal reaction plates after amplification is complete. Unsealing the plates increases the risk of contaminating subsequent reactions with amplified product.
- Clean laboratory benches and equipment periodically with a nucleic acid decontamination solution such as such as 10% household bleach or DNA*Zap*™.

Guidelines for sample preparation

Sample preparation kits that are designed for extraction of microbial DNA and include a DNA purification step should be used with the SteriSEQ™ Rapid Sterility Testing Kit. Most sample preparation kits have residual bacteria or fungi DNA background contamination. Users should include a negative extraction control during sample preparation to establish an appropriate C_t cut-off for sample preparation kit-specific background contamination and true positive detection.

Note:

- The recommended volume for each test sample replicate is 1 mL.
- For cell culture samples, the maximum recommended cell density is 10^6 mammalian cells per mL.
- We recommend performing sample preparation using 3 replicates from the test sample. If samples are limited, 1 or 2 extraction replicates can be used.

IMPORTANT! High amounts of cellular DNA and/or RNA can cause PCR inhibition and reduce detection of low-copy targets. We recommend testing on samples containing no more than 10^6 mammalian cells.

1. For samples with cell suspension (≤10^6 mammalian cells in approximately 1 mL):
    a. Centrifuge cell suspension at 15,000 x *g* for 5 minutes to create a sample pellet.
    b. Discard the supernatant, being careful not to disturb the pellet.
    c. Suspend the pellet in 90 µL of SteriSEQ™ DNA Dilution Buffer (SDDB).
    d. Continue with sample preparation using the sample preparation kit manufacturer's instructions.
    e. Elute using a volume of 80 µL (sufficient for up to 5 qPCR replicates).
    f. After the sample is eluted into the collection tube, remove the spin column and transfer the eluted sample into a sterile, clean microcentrifuge tube without capping the collection tube.
2. For samples without cell suspension (for example cell culture media/supernatant):
    a. Centrifuge 1 mL of sample at 15,000 x *g* for 5 minutes.
    b. Remove 910 µL of supernatant and discard, leaving behind 90 µL of supernatant.
    c. Briefly pipet up and down to resuspend any pellet.
    d. Continue with sample preparation using the sample preparation kit manufacturer's instructions.
    e. Elute using a volume of 80 µL (sufficient for up to 5 qPCR replicates).
    f. After the sample is eluted into the collection tube, remove the spin column and transfer the eluted sample into a sterile, clean microcentrifuge tube without capping the collection tube.

Guidelines for extraction control

The SteriSEQ™ Discriminatory Positive Control (DPC) provided in the kit is a multipurpose control that can be used as both a positive extraction control (PEC) and a positive template control (PTC). The SteriSEQ™ DNA Dilution Buffer (SDDB) provided in the kit can be used for negative extraction control (NEC), as well as diluting the DPC for PEC. If using alternative buffers for NEC and PEC, ensure that the buffers used are free from contaminating bacteria or fungi genomic material. Extended autoclaving (121°C, 80 minutes) can be performed to degrade DNA present in buffers.

We recommend extracting 1 replicate of each extraction control. Each extraction sample should be plated in triplicate on the qPCR plate, providing 3 total data points per control.

Type of control	Description	Number to run	Purpose
Positive extraction control (PEC)[1]	10 µL DPC (diluted in SDDB)	1 per extraction run	• Verify reagent and system performance. • Evaluate the efficiency of DNA extraction.
Negative extraction control (NEC)	100 µL SDDB	1 per extraction run	• Monitor for contamination of the extraction reagents, equipment, and work areas. • Establish background residual contamination of the sample preparation kit.
Sample with positive extraction control (S_PEC)[1]	10 µL DPC (diluted in SDDB and spiked into the test sample matrix.)	1 per extraction run	• Evaluate the efficiency of DNA extraction. • Evaluate presence of PCR inhibitors in matrix.
Sample negative extraction control (S_NEC)[1,2]	Sample matrix of test sample that is known to be sterile (for example, fresh cell culture media)	1 per extraction run	• Establish background residual DNA contamination of the sample matrix

[1] Optional during routine testing.

[2] Some sample matrices such as cell culture media, antibiotics or supplements can carry residual bacteria or fungi DNA. We recommend establishing the baseline of the residual DNA contamination to set an appropriate C_t cut-off for true positive bacteria or fungi presence.

Prepare the Negative Extraction Control (NEC) and Positive Extraction Control (PEC)

1. Dilute discriminatory positive control (DPC) using the SteriSEQ™ DNA Dilution Buffer (SDDB) to an appropriate concentration for spiking positive extraction control (PEC) and sample positive extraction control (S_PEC) samples. Copy numbers for dilutions labelled “Spike_1000” and “Spike_100” correspond to 1000 and 100 copies per qPCR reaction when using the recommended spike volume of 10 µL per extraction sample and elution volume of 80 µL.

   Note: Volumes can be scaled up if needed. Dilutions for spiking should be prepared fresh at the start of each extraction and stored on ice until use.

Name	Dilutions	Copies/ µL
Pre-dilution (D1, see “Prepare the positive template control (PTC)” on page 26)	5 µL DPC stock + 45 µL SDDB	10,000
Spike_1000	4 µL Predilution + 61 µL SDDB	615.4
Spike_100	6 µL Spike_1000 + 54 µL SDDB	61.54

2. For PEC samples, add in 10 µL of either Spike_1000 or Spike_100 dilutions to 90 µL SDDB.
3. For S_PEC or S_NEC samples, centrifuge 1 mL of the sample matrix.
   a. For sample matrices with mammalian cells (≤10^6 cells per mL), remove all supernatant without disturbing the pellet, then suspend the pellet in 90 µL SDDB.
   b. For sample matrices without mammalian cells, remove 910 µL of supernatant, leaving behind 90 µL, then gently pipet up and down to mix.
   c. For information about sample preparation, see “Guidelines for sample preparation” on page 15.
4. For S_PEC samples, add in 10 µL of either Spike_1000 or Spike_100 dilutions to 90 µL matrix.
5. For S_NEC samples, add in 10 µL of SDDB to 90 µL matrix.
6. For NEC samples, use 100 µL of SDDB for extraction.
7. Proceed with sample preparation using third-party kit according to manufacturer’s instructions.
8. Perform elution using a volume of 80 µL.
9. After the sample is eluted into the collection tube, remove the spin column and transfer the eluted sample into a sterile, clean microcentrifuge tube without capping the collection tube.

3 Set up the PCR

This chapter describes how to set up SteriSEQ™ experiments in AccuSEQ™ Real-Time PCR Software v3.2.1 or later (QuantStudio™ 5 Real-Time PCR System) or 2.2.1 or later (7500 Fast Real-Time PCR Instrument), then prepare the PCR plate.

IMPORTANT! Review the guidelines to prevent contamination before proceeding (see "Guidelines to prevent contamination" on page 14).

Set up a SteriSEQ™ Rapid Sterility experiment in AccuSEQ™ Real-Time PCR Software v3.2.1 or later

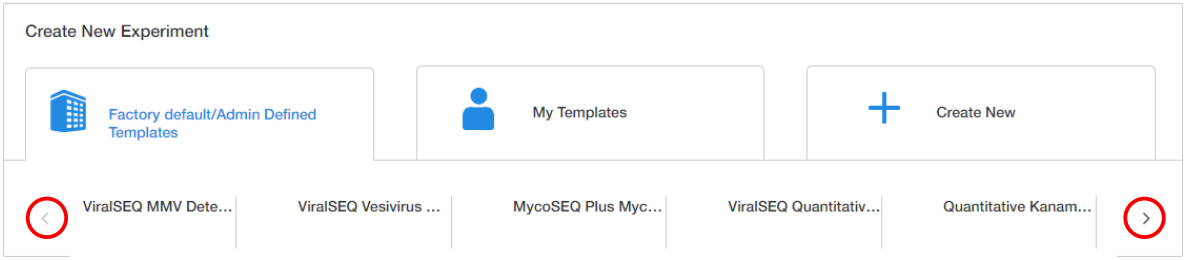
AccuSEQ™ Real-Time PCR Software v3.2.1 or later includes a template for running SteriSEQ™ experiments on the QuantStudio™ 5 Real-Time PCR System. The template includes an example plate layout with predefined control and sample wells (which can be edited), and preset thermal cycling and analysis settings to make automated calls for the presence or absence of bacteria and fungi.

For detailed instructions about using the software, see *AccuSEQ™ Real-Time PCR Software v3.2 Help*.

IMPORTANT! The **SteriSEQ Rapid Sterility** experiment template contains default volumes, cycling conditions, and analysis settings. Do not change these settings.

1. In the **Home** screen, click the **Factory default/Admin Defined Template** tab, then select **SteriSEQ Rapid Sterility**.

   Click the arrows to navigate through the available templates.



2. In the **Experiment Properties** pane of the **Setup** screen:
   a. (*Optional*) Change the system-generated name of the experiment.

      Names must be unique. Deleted experiment names cannot be reused.

   b. (*Optional*) Enter the plate barcode in the **Barcode** field, then add comments in the **Comments** field.

      Names and comments are not editable post-analysis.

c. Click **Next**.

Experiment names cannot be changed after this step.

3. In the **qPCR Method** pane of the **Setup** screen, view the default volume and cycling conditions (cannot be changed).

This assay is run with a standard ramp speed.

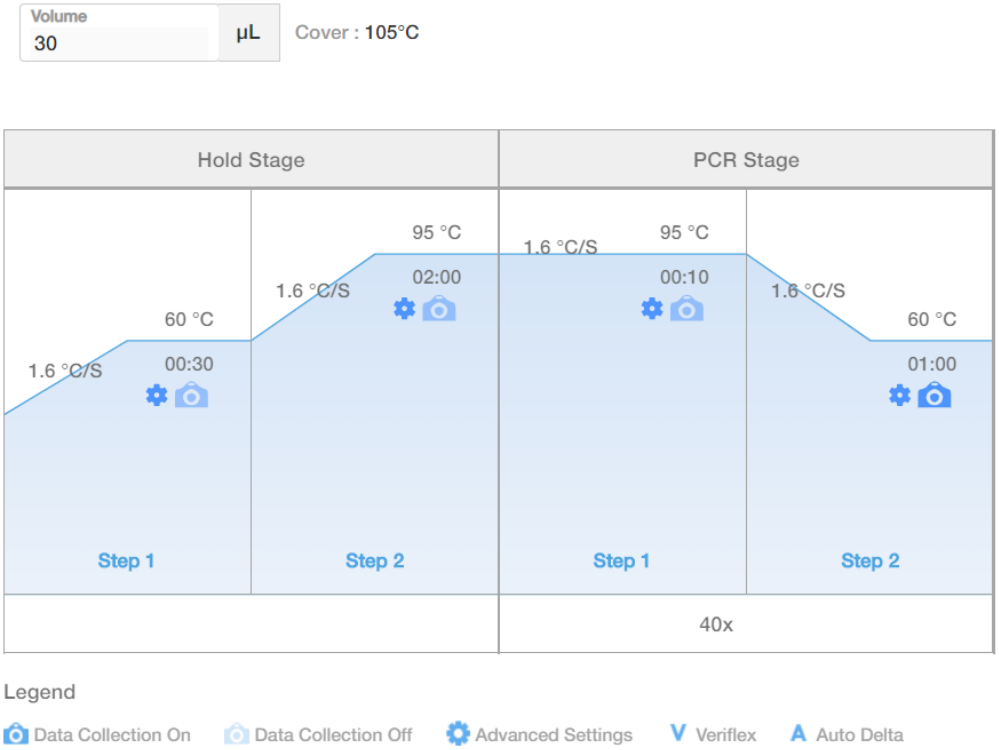


Figure 1 Default cycling conditions

4. Click **Next**.

5. In the **Samples** table in the **Setup** screen, confirm or edit the predefined sample name (S1) and control names:

a. (Optional) Click the field in the **Name** column to edit the name of each sample or control.

b. (Optional) Click **Add** to add more samples or controls.

SteriSEQ™ experiments detect presence or absence only, so the values in the remaining columns of the **Samples** table (**Dilution Factor**, and so on) typically remain unchanged.

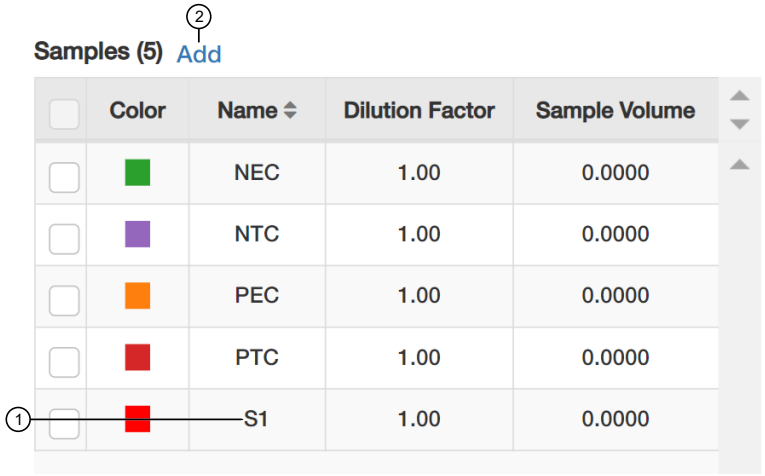


Figure 2 Samples table

① Sample name
② Click **Add** to add more samples or controls

Table 2 Example sample names (rename as appropriate)

Name	Description
PTC	Positive template control
NTC	No template control
NEC	Plate-level negative extraction control (with SDDB)
PEC	Plate-level positive extraction control (with SDDB)
S_NEC	Quality-control sample that does not contain bacteria or fungi
S_PEC	Sample-level positive extraction control
S1	Unknown sample

6. Define the sample and control wells in the plate layout. For more information, see “Define and assign plate and well attributes” on page 72.

   The template includes a predefined example plate layout that can be modified.

   a. With **(Grid View)** selected, click or drag to select the plate wells for a particular sample or control.

   b. In the **Targets** table, select the checkboxes for all four targets to add them to the selected well or wells.

      Each assigned well must include all four targets. Do not change the target names, reporter dyes, or quenchers in the table.

c. Under the **Task** column in the **Targets** table, select the task for each target based on the well type.
   - **U**—Unknown sample
   - **N**—Negative template control
   - **P**—Positive template control
   - **NEC**—Negative extraction control (in SDDB)
   - **PEC**—Positive extraction control (in SDDB)
   - **S_PEC**—Positive extraction control (in sample matrix)
   - **S_NEC**—Negative extraction control (in sample matrix)

d. In the **Samples** table, select the checkbox next to a sample or control to add it to the selected well or wells.

e. Repeat for all wells in the experiment.

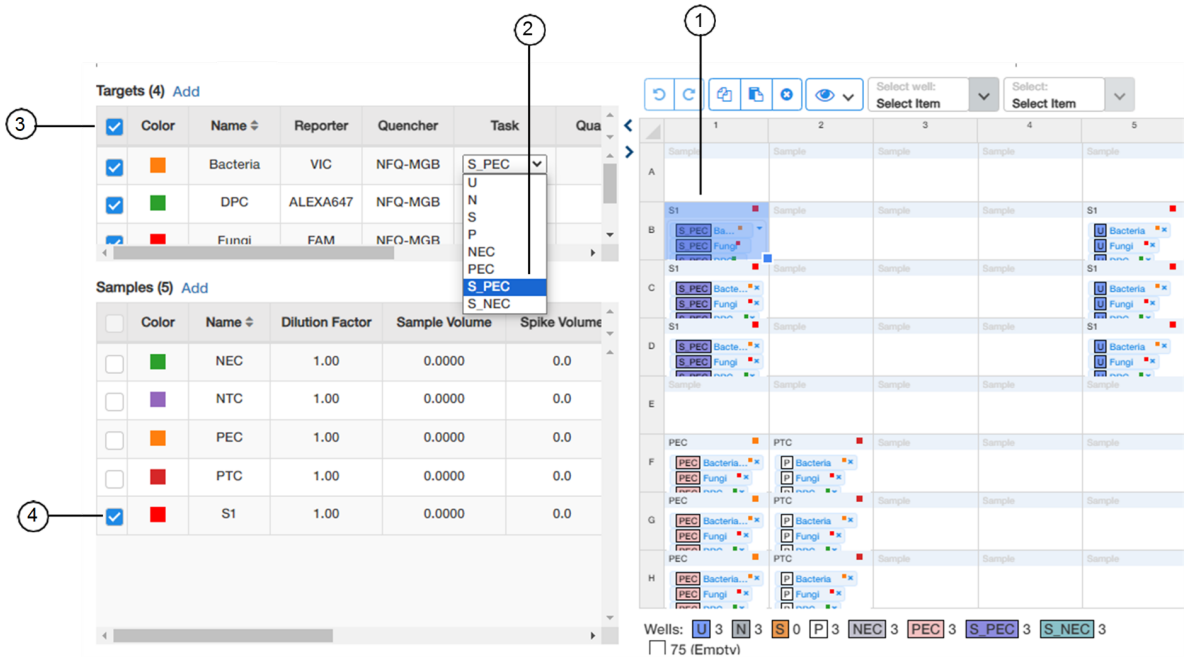


Figure 3 Example plate layout

① Selected well
② Selected task for each target for the well
③ Selected targets for the well (select all 4 targets)
④ Selected sample or control for the well

7. (*Optional*) Double-click each well to add a comment. Comments can also be added post-analysis.

8. Click **Next** to open the **Run** screen.

9. To save the experiment, exit the experiment, then click **Yes** when prompted to save changes.
   To create a copy of the experiment, click **Save As**.

10. (*Optional*) Click **Print** to print the plate layout for use in preparing the reactions.

Proceed to "Prepare the PCR master mix" on page 25 using the plate layout as defined in the experiment.

Create a SteriSEQ™ Rapid Sterility experiment in AccuSEQ™ Real-Time PCR Software v2.2.1 or later

AccuSEQ™ Real-Time PCR Software v2.2.1 or later includes a default SEQ experiment for running SteriSEQ™ experiments on the 7500 Fast Real-Time PCR Instrument. Each default SEQ experiment includes an example plate layout with predefined control and sample wells (which can be edited), and preset thermal cycling and analysis settings to make automated calls for the presence or absence of bacteria and fungi.

For detailed instructions about using the software, see *AccuSEQ™ Real-Time PCR Software v2.2 Help*.

IMPORTANT! The experiment templates contain default volumes, cycling conditions, and analysis settings. Do not change these settings.

1. In the **Home** screen, click **Create SEQ Experiment**.

2. Select **SteriSEQ Rapid Sterility** from the assay list, then click **Next** .

3. In the **Experiment Menu** navigation bar, under **Setup**, click **Experiment Properties**, then enter a name in the **Experiment Name** field.
   The experiment name can be up to 100 letters and numbers. Spaces are not allowed.

4. *(Optional)* Enter a plate barcode and comments.

5. Click **Plate Setup**.

6. In the **Plate Setup** pane, under the **Define Targets and Samples** tab, under **Define Samples**, confirm or edit the predefined sample name (S1) and control names. Sample volume is not needed.
   a. (Optional) In the **Sample Name** column, click the name to edit the sample name or control.
   b. (Optional) Click **Add New Sample** to add more samples or controls.

   For more information, see "Add and remove samples" on page 79 and "Define samples" on page 79

   IMPORTANT! Do not modify the targets.

7. Click the **Assign Targets and Samples** tab.

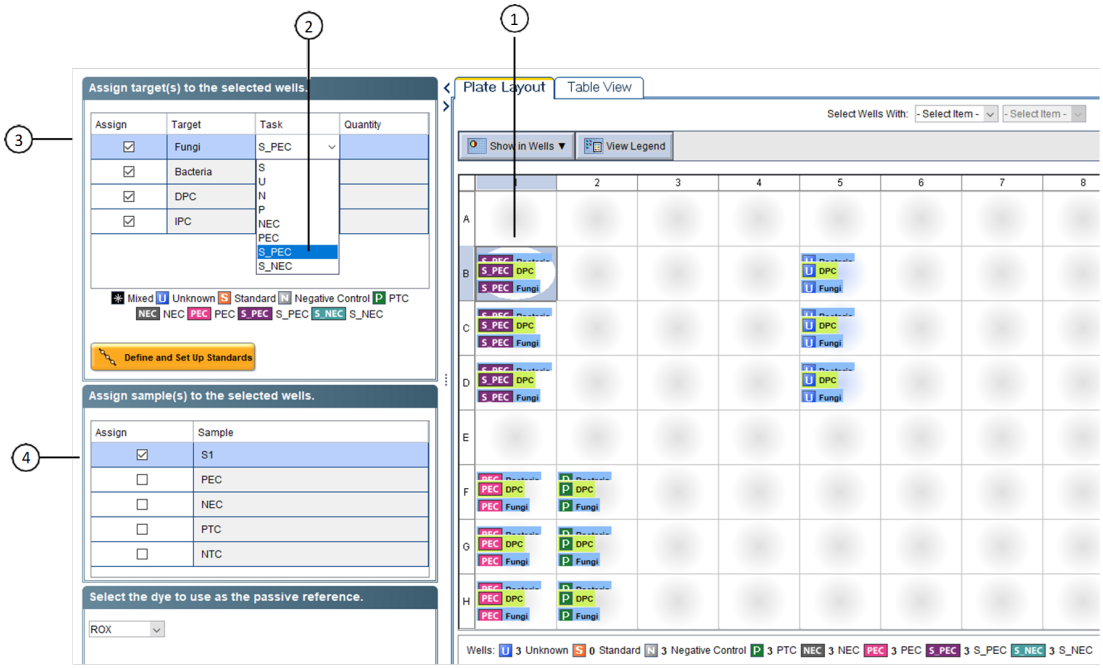


Figure 4 Example plate layout

① Selected well
② Selected task for each target for the well
③ Selected targets for the well (select all 4 targets)
④ Selected sample or control for the well

(Optional) To change the plate layout display, see *AccuSEQ™ Real-Time PCR Software v2.2 Help*.

8. With the **Plate Layout** tab selected, click or drag to select the plate wells for a particular sample or control.

9. Click the checkbox next to each target name to assign all 4 targets to the selected well or wells, then assign tasks to those targets

   See “Assign targets to wells” on page 80.

10. Check the checkbox next to the sample or control name to assign it to the selected well or wells

    See “Assign a sample to a well” on page 80.

11. Repeat step 8 through step 10 for the remaining sample and control wells.

12. Confirm that ROX™ dye is selected as the passive reference.

13. *(Optional)* Save the plate layout as an image.
    a. Right-click the plate layout.
    b. Select **Save As**.

c. Specify a file name and location for the JPEG file, then click **Save**.

   Electronic signature information is not included in an image file. Use **Print Report** to include electronic signature information with the plate layout.

14. *(Optional)* Print the plate layout.
    a. Right click the plate layout.
    b. Click **Print Preview** or **Print**.

15. Click **Reaction Setup** to review the default reaction mix volumes and concentrations.

    A standard dilution series is not included in the **SteriSEQ Rapid Sterility** experiment.

IMPORTANT! Do not change the default cycling conditions or analysis settings. These can be reviewed in the **Experiment Menu** navigation bar under **Run** and **Analysis**.

Proceed to "Prepare the PCR master mix" on page 25.

Set up SteriSEQ™ Rapid Sterility experiments using other qPCR software

Use these guidelines to set up SteriSEQ™ experiments using qPCR software other than AccuSEQ™ Real-Time PCR Software v2.2.1 or v3.2.1 or later.

Use a reaction volume of 30 µL for SteriSEQ™ experiments.

Thermal cycling conditions

Stage	Temperature (°C)	Time	Cycle
Hold	60	30 seconds	1
Hold	95	2 minutes	1
PCR	95	10 seconds	40
	60	60 seconds[1]	

[1] Read after 60 second cycle at 60°C.

- Ensure the heated cover is 105°C.
- Use a ramp rate of 1.6°C/second.

  IMPORTANT! Higher ramp rates can decrease detection of targets in some samples.

- Use a standard run mode.

Set up the PCR plate

Set up the plate using the following targets and reporters:

Target	Reporter	Task
Fungi	FAM™	Test samples and controls: Unknown, NTC: NTC
Bacteria	VIC™	Test samples and controls: Unknown, NTC: NTC
IPC	ABY™	All controls and samples: Unknown
DPC	Alexa Fluor™ 647	Test samples and controls: Unknown, NTC: NTC

Recommended analysis settings

Note: The recommended analysis settings were validated on AccuSEQ™ Real-Time PCR Software v2.2.1 and v3.2.1 or later. If needed, a manual baseline can be used. The use of other qPCR software can require changing these settings.

Target	Reporter	Threshold	Baseline
Fungi	FAM™	0.2	Auto baseline
Bacteria	VIC™	0.05	Auto baseline
IPC	ABY™	0.2	Auto baseline
DPC	Alexa Fluor™ 647	0.1	Auto baseline

Prepare the PCR master mix

Note:

- Protect SteriSEQ™ Assay Mix and 2x qPCR Master Mix Plus from direct exposure to light.
- Minimize freeze-thaw cycles of the reagents.
- Vortex reagents at medium speed (2100 rpm or Setting 7 on analog vortexes).
- Prepare all reagents and reactions using clean techniques to prevent contamination.

1. Thaw 2x qPCR Master Mix Plus and SteriSEQ™ Assay Mix completely on ice.
2. Vortex each tube thoroughly for at least 10 seconds to mix, then briefly centrifuge to collect the contents.

   Note: Place reagents on ice until use. Vortex the reagents again before pipetting.

3. Determine the number of reactions needed for the number of controls and test samples. Prepare a sufficient volume of PCR master mix plus 10% overage to compensate for pipetting errors.

   We recommend each extraction sample be plated in triplicate on the qPCR plate, providing 9 total data points for 3 extraction replicates per test sample.

Table 3 PCR master mix

Component	Volume	
	1 reaction	Example: 10 reactions[1]
2x qPCR Master Mix Plus	15 µL	165 µL
SteriSEQ™ Assay Mix	2 µL	22 µL
Total	**17 µL**	**187 µL**

[1] Includes 10% overage.

4. Vortex the PCR master mix for at least 10 seconds to mix, then briefly centrifuge to collect the contents.

Prepare the positive template control (PTC)

Prepare the following mix fresh for each reaction.

1. Thaw DPC tube for 1 hour at room temperature, protected from light.
2. Vortex for 10 seconds and briefly centrifuge.
3. Perform serial dilutions of the DPC.
   a. When dispensing the DPC, pipet up and down gently five times.
   b. After each transfer, vortex for 10 seconds, then briefly centrifuge.

Dilution (D) tube	Dilution	Dilution factor	Copies/ µL	Copies/ reaction
D1	5 µL DPC stock tube + 45 µL SDDB	10	10,000	N/A
D2	5 µL D1 + 60 µL SDDB	13	769.23	10,000

Note: Only D2 is plated as a positive template control.

Store the dilution tubes on ice for up to 6 hours if not using immediately.

Prepare the PCR plate

IMPORTANT! Protect the prepared plate from extended exposure to light or elevated temperatures before thermal cycling.

Prepare the PCR plate using the plate layout created for the experiment in AccuSEQ™ Real-Time PCR Software v3.2.1 or v2.2.1 or later.

Ensure that filter tips are used for all pipetting.

1. Add 17 µL of PCR master mix to each reaction well.
2. Add 13 µL of SteriSEQ™ DNA Dilution Buffer (SDDB) to each no template control (NTC) well.
3. Add 13 µL of the negative extraction control to each NEC well.
4. Add 13 µL of test sample to each test sample well.
5. Add 13 µL of the positive template control, D2 (prepared as described in "Prepare the positive template control (PTC)" on page 26), to each PTC well.
6. Add 13 µL of the positive extraction control to each PEC well.
7. Seal the plate with MicroAmp™ Optical Adhesive Film (see "Seal the plates" on page 71). Handle the plate by the edges and avoid touching the top of the plate.
8. Centrifuge the plate at 700-1,000 × g for 2 minutes.

   Ensure that the liquid in the plate is at the bottom of each well. If bubbles or air pockets are observed, centrifuge the plate again

Proceed immediately to one of the following:

- Chapter 4, "Run the PCR and review the results—QuantStudio™ 5 Real-Time PCR System"
- Chapter 5, "Run the PCR and review the results—7500 Fast Real-Time PCR Instrument"

4 Run the PCR and review the results —QuantStudio™ 5 Real-Time PCR System

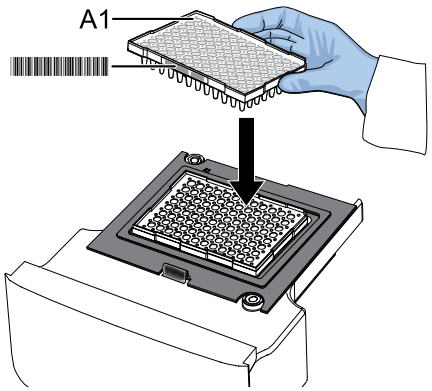
This chapter describes how to load and run the QuantStudio™ 5 Real-Time PCR System and review the results in AccuSEQ™ Real-Time PCR Software v3.2.1 or later.

IMPORTANT! Review the guidelines to prevent contamination before proceeding (see “Guidelines to prevent contamination” on page 14).

Load the plate in the instrument

CAUTION! Use optical flat caps for tubes. Rounded caps can damage the heated cover.

1. Touch ⏏ to eject the instrument drawer.
2. Load the plate onto the plate adapter so that:
   - Well A1 of the plate is in the top-left corner of the plate adapter.
   - The barcode faces the front of the instrument.



IMPORTANT! The instrument should be used by trained operators who have been warned of the moving parts hazard.

Note: Do not remove the black plate adapter before loading a plate or tube strips. If used, tube strips can fit loosely in the adapter, but the heated cover applies the appropriate pressure to seat the tube strips securely in the adapter.

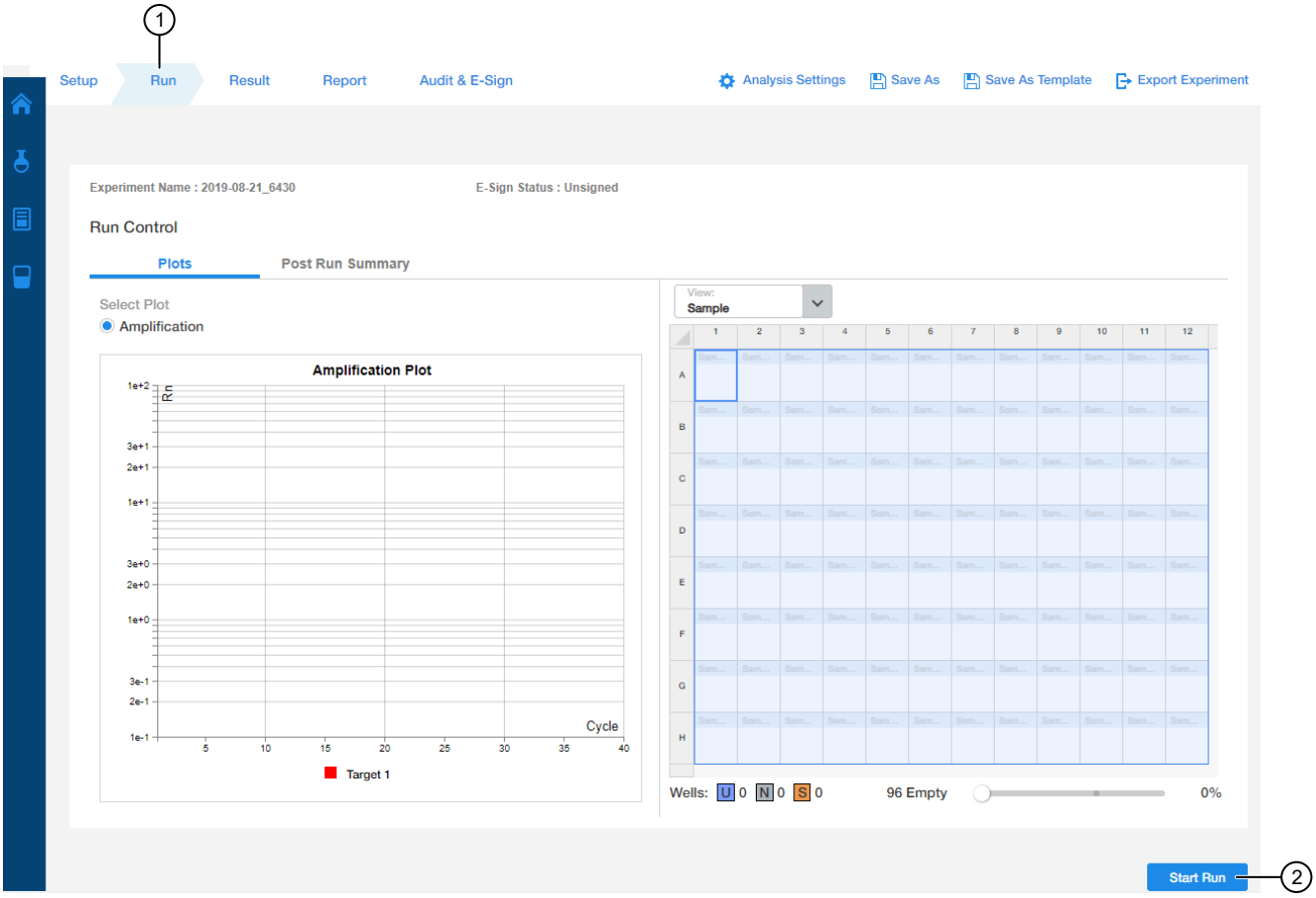
The black plate adapter is used only with 0.2 mL blocks. The black plate adapter is not needed when 0.1 mL blocks are used.

CAUTION! PHYSICAL INJURY HAZARD. During instrument operation, the plate temperature can reach 100°C. Allow it to cool to room temperature before handling.

3. Touch ⏏ to close the instrument drawer.

Start the run

With the experiment open in AccuSEQ™ Software, in the **Run** tab, click **Start Run**.



1 **Run** tab
2 **Start Run** button

A message notifies you when the run has started.

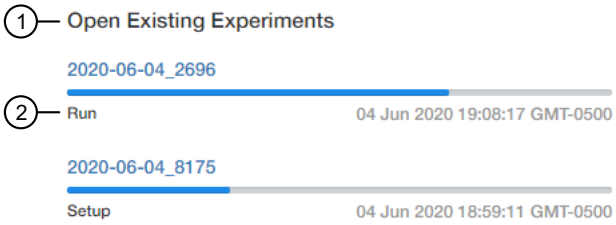
Monitor the run

Monitor the run from one of the following places.

- On the instrument touchscreen. See the *QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide*.
- In the **Monitor the Run** pane of the AccuSEQ™ Software **(Home)** screen.

1. Instrument name
2. Instrument status (Ready, Running, Offline)
3. Calibration status:
   Calibrated—All required calibrations are "Current". Required calibrations include: Background, ROI/Uniformity, and Dyes.
   Not calibrated—None of the required calibrations are complete.
   Requires calibration—One or more dyes are not calibrated.
4. Time lapsed (if a run is in progress)
5. Total run time
6. Experiment name

- In the **Open Existing Experiments** pane of the AccuSEQ™ Software **(Home)** screen. The experiment being run is the first experiment listed. Status is **Run**.
  When the run is complete, the status changes to **Analysis** and the bar changes to completely blue.



1. **Open Existing Experiments** pane
2. Experiment status

- In the **Run** tab of the software, you can perform the following actions.
  - Select wells in the plate layout to highlight respective curves in the plot.
  - Hover over curves in the plot for well information.
  - (*Optional*) Change what is displayed in the table wells of the plate layout, by selecting from the dropdown lists.

  For more information, see *AccuSEQ™ Real-Time PCR Software v3.2 Help*.

On run completion, the **Post Run Summary** displays the run length, user and instrument information, and a list of any errors that occurred.

When the run is complete, unload the plate from the instrument.

SteriSEQ™ results workflow

Review SteriSEQ™ experiment results

View the results for SteriSEQ™ experiments (page 32)

Evaluate the amplification plot (page 34)

(Optional) **View and evaluate the multicomponent plot** (page 35) **to review the dye signal profile**

(Optional) **Review the flags in the QC Summary** (page 36)

(Optional) **Manually edit unknown well calls** (page 37)

IMPORTANT! If you manually edit well calls, do not click Analyze to reanalyze the data. Well calls revert to their default call based on the rule settings.

(Optional) **Modify call thresholds** (page 38)

View the results for SteriSEQ™ experiments

When a run is complete, the **Result** screen displays the calls for all the plate wells in an experiment. If the experiment is not open, see “Open an experiment” on page 72.

The **Result** screen is divided into the **Result Summary** and **Result Analysis** tabs on the left and the **Results** pane on the right.

Calls are made based on the default analysis settings for SteriSEQ™ experiments. See *AccuSEQ™ Real-Time PCR Software v3.2 Help* for more information.

1. In the **Result Summary** tab, in the **Plate Calls** section, review the calls for the controls (PTC, NTC, PEC, and NEC).

   The number inside the circle indicates the number of controls that passed or failed. The overall plate status (**VALID** or **INVALID**) shown in the **Plate Status** field is determined by the PTC, NTC, PEC, and NEC calls.

   Note: If there are multiple replicates for a control, at least one replicate must pass for the plate status to be labeled **VALID**. This requirement is not editable.

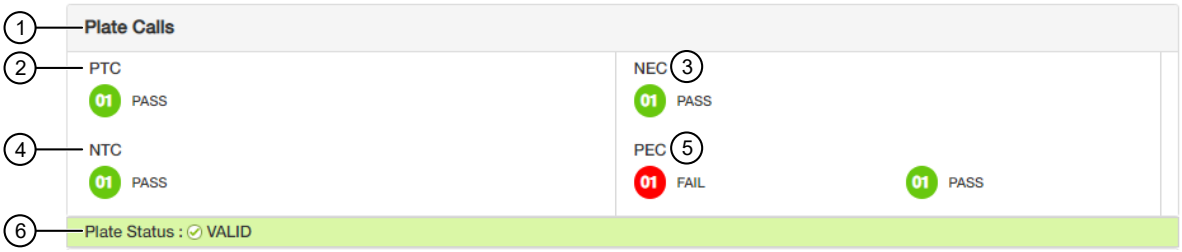


Figure 5 Plate Calls section (example)

1. Plate Calls pane
2. Positive template controls (PTC)
3. Plate-level negative extraction control (NEC)
4. No template control (NTC)
5. Plate-level positive extraction control (PEC)
6. Plate status

2. In the **Result Summary** tab, in the **Sample Level PEC/NEC Calls** section, review the total number of wells for each S_PEC or S_NEC call (**PASS** or **FAIL**).

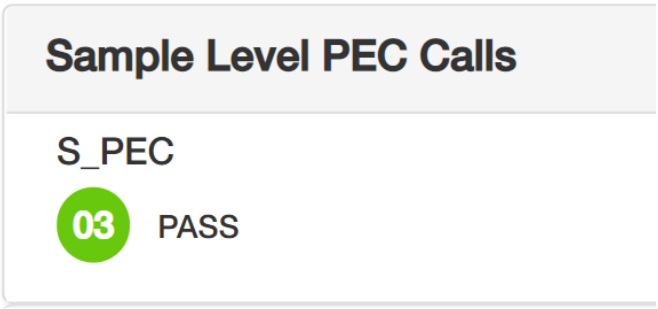


Figure 6 Sample Level PEC Call section (example)

3. In the **Result Summary** tab, in the **Well Calls** section, review the total number of wells for each call—**Present**, **Absent**, **Review**, or **Fail**.

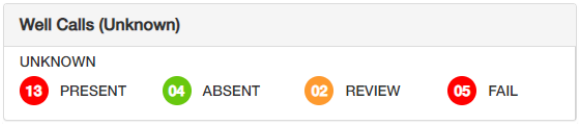


Figure 7 Well Calls section (example)

4. In the **Results** pane, review the calls for all the plate wells (samples and controls) as a plate layout (**Grid View**) or a table (**Table View**).

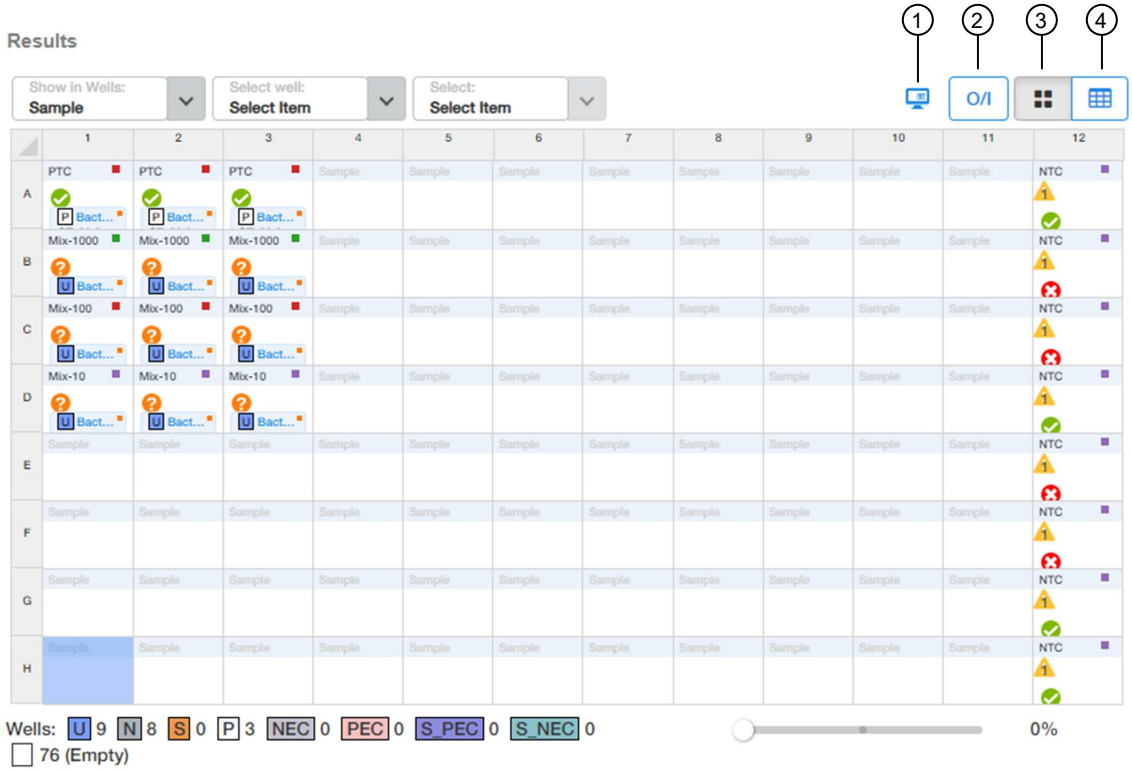


Figure 8 Results pane (example)

① **View Legends**—explains the symbols and letters in the wells. See *AccuSEQ™ Real-Time PCR Software v3.2 Help* for information about the flags.

② **Omit/ Include**—omits selected wells from the analysis or includes selected wells in the analysis

③ **Grid View**

④ **Table View**

Evaluate the amplification plot

You can evaluate the overall shape of the **Amplification Plot** curves in the **Result** tab. Evaluating the shape in wells with a **Review** call can help determine if the sample needs to be run again, or whether you can edit the call to **Present** or **Absent**.

Ensure that the experiment is open in AccuSEQ™ Software. For more information about opening an experiment, see “Open an experiment” on page 72.

1. In the **Results Analysis** pane of the **Result** tab, click **Amplification** in the horizontal scroll bar.

   If no data are displayed in the **Result Analysis** pane, click **Analyze**. See “Results screen” on page 78.

① Amplification Plot

   The **Amplification Plot** is displayed for the selected wells in the grid view.

2. (*Optional*) Click , to adjust the plot settings.

Table 4 Amplification Plot settings

Setting	Options
Plot type	ΔRn, Rn, or C_T
Graph type	Log or Linear
Show on plot	Legend, Cq Mark (the cycle at which the curvature of the amplification curve is maximal), or unselected.
Plot color	Target, Sample, Well, or Flag_Status
Threshold	Decide whether to show thresholds. Thresholds are preset in SteriSEQ™ template files, but can be changed.
Baseline	Decide whether to show baseline

3. (*Optional*) Click to adjust the plot properties.
   a. (*Optional*) In the **General** tab, add a plot title, adjust the font and color, then click **Apply**.
   b. In the **X Axis** or **Y Axis** tabs you can:
      - Add a label
      - Select if you want tick marks
      - Select **Auto-adjust range** or enter minimum and maximum values
        The minimum value must be greater than 0.

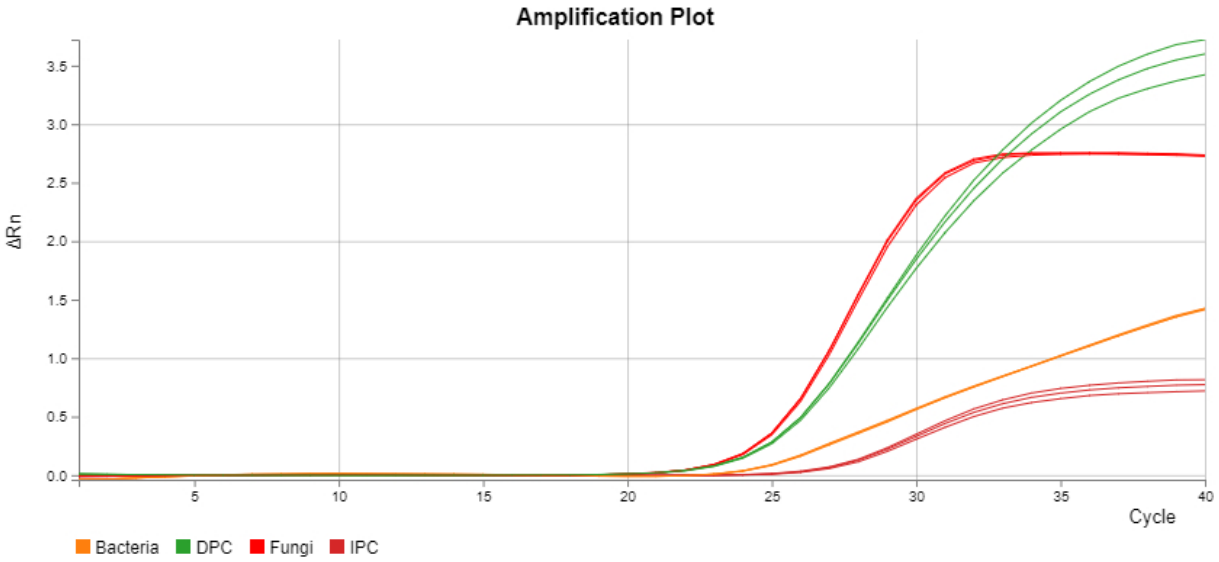


Figure 9 Typical Amplification Plot for a SteriSEQ™ experiment

View and evaluate the multicomponent plot

The **Multicomponent Plot** in the **Result** tab displays the complete spectral contribution of each dye over the length of the PCR run. Use the plot to perform the following tasks:

- Ensure that the signal from the passive reference dye remains unchanged throughout the run.
- Review reporter dye signal for spikes, dips, and/or sudden changes.
- Ensure that Alexa Fluor™ 647 has no amplification in the negative control wells.
- Ensure that internal positive control signal (ABY™ dye) is present in all sample wells.

If no data are displayed in the **Result** tab, click **Result Analysis**, then click **Analyze**.

1. In the **Result** tab, select **Multicomponent** in the horizontal scroll bar.
2. Click to configure the plot, then make the following selections:
   - **Plot Color**: Dye, target, or well.

   The **Multicomponent Plot** is displayed for the selected wells in the grid view.

3. In the grid view, 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 (ROX™ dye) should remain relatively constant throughout the PCR process.
Reporter dye	For positive reactions, the reporter dye fluorescence signal (FAM™, VIC™, ABY™, or Alexa Fluor™ 647 dye) should show 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 effect on the data.
Negative control wells	The negative control wells should show no significant increase in fluorescence signal for Alexa Fluor™ 647 dye.
Internal positive control	All wells should show amplification of ABY™ dye as it is an internal positive control.

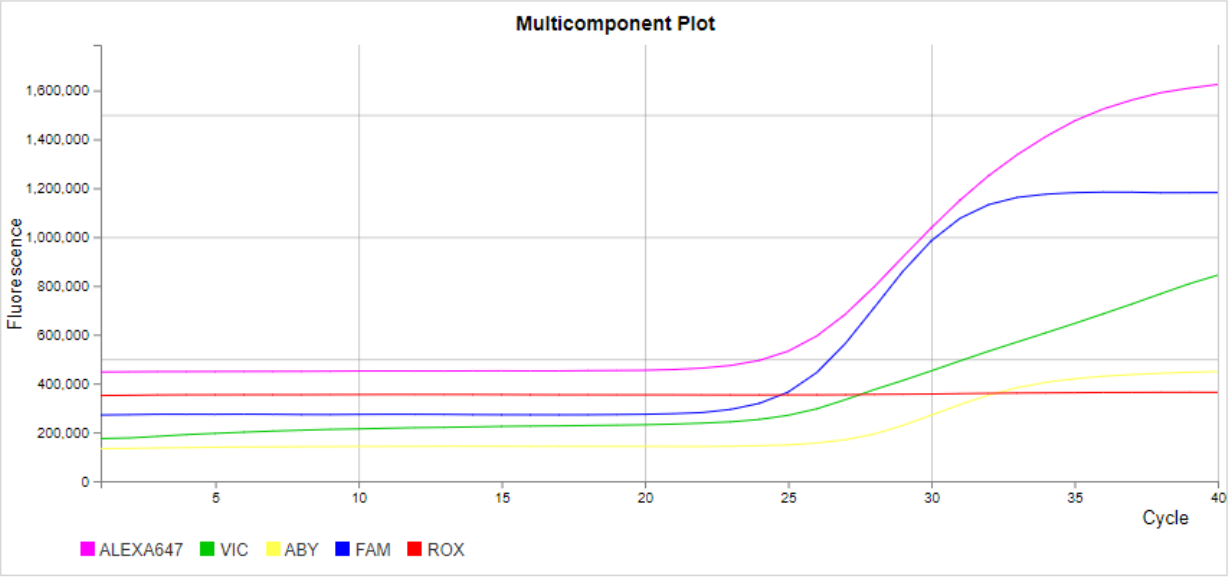


Figure 10 Example Multicomponent Plot (single well)

Review the flags in the QC Summary

The **QC Summary** tab in the **Result** screen displays a list of the QC flags, including the flag frequency and location.

If no data are displayed in the **Result** screen, click **Analyze**.

1. In the **Result** screen, click **Result Analysis**, then select the **QC Summary** tab in the horizontal scroll bar.
2. Review the **Flag** column.
   The **Wells** column identifies wells that triggered a flag.
3. (*Optional*) View the **Description** column for a description of the flag.

4. (*Optional*) View the **Frequency** column for the number of times the flag occurred.

5. (*Optional*) Click the flag of interest for information text about the flag, including the **Flag** name, the **Flag Detail** field, the **Flag Criteria** field, and the **Flagged Wells** field.

Manually edit unknown well calls

Calls for wells assigned as **Unknown** task in factory default presence/absence assays can be manually edited. Any change in the well call is recorded by the silent audit function.

IMPORTANT! AccuSEQ™ Real-Time PCR Software Administrator access is needed to perform this workflow.

Only wells assigned as **Unknown** task can be manually edited. In the **Result** screen, click the **Results Summary** tab to determine if **Unknown** tasks exist.

1. In the **Result** pane, click  to open the table view.

2. Click the **Task** column to group the **Unknown** tasks together.

3. Click the **Results Analysis** tab to view the well data.

4. Select an **Unknown** well in the **Task** column.

5. Determine if a call can be made based on the available data.

6. Click the **Call** column in the highlighted row to modify the well call.

7. Select a well call option from the list.
   - **Review** can become **Review**, **Present with Review**, or **Absent with Review**.
   - **Absent** can become **Absent with Review** or **Present with Review**.
   - **Present** can become **Present with Review** or **Absent with Review**.

   A **Fail** call cannot be edited.

   The call assessment is updated and the icons for the edited call appears in orange.

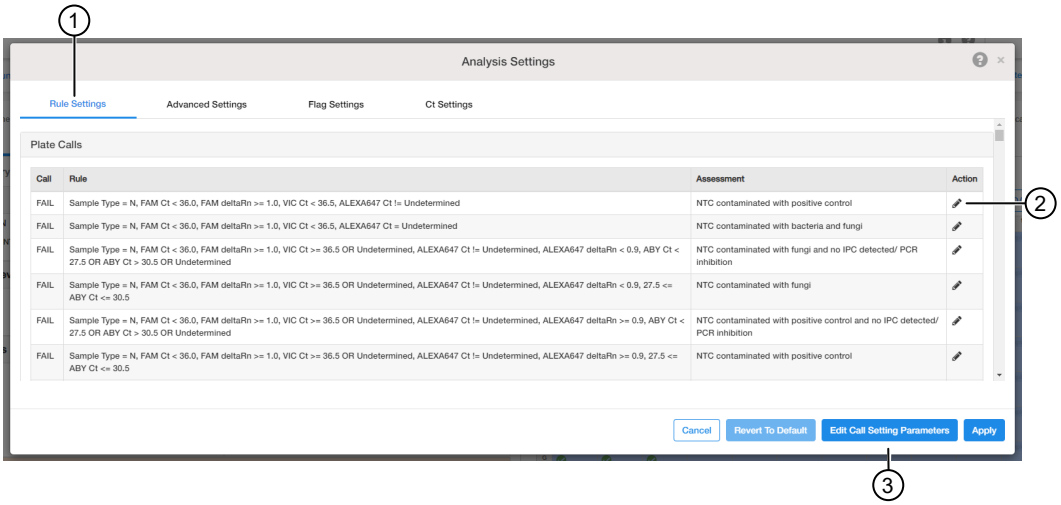
Table view, exported results, and printed reports include the manually edited call and call assessment values. An experiment imported as an EDS file retains manual calls that were edited

If the experiment is reanalyzed, the edited well calls revert to the default calls in accordance with the rule settings.

Modify call thresholds

Calls are made based on the default analysis settings for SteriSEQ™ experiments. The passing criteria for all sample types can be modified. The need to change passing criteria can occur when a different cut-off is established for a sample preparation kit or for a particular type of sample matrix. If a different cut-off is established, FAM™ (fungi) and VIC™ (bacteria) acceptance criteria may need to be updated for NEC, S_NEC, PEC, S_PEC, and unknown test samples.

1. In the **Result** screen, click **Analysis Settings** in the top-right.
   The **Analysis Settings** dialog box opens to the **Rule Settings** tab.



① **Rule Settings** tab
② Edit call button
③ **Edit Call Setting Parameters** button

The thresholds can be updated collectively using **Edit Call Setting Parameters** or individually using the edit call button ✎.

2. Click **Edit Call Setting Parameters** in the bottom-right to view all thresholds.

3. Edit C_t and ΔRn cut-off values.

   a. Enter the new value in the **Value** column for each parameter.

      Detailed parameter descriptions and default call setting values are listed below.

Table 5 Default call setting parameters

Parameter name	Details	Default passing criteria[1]
ct_aby_lower	Lower limit of IPC (ABY™) acceptance criteria for NTC	$C_t \geq 27.5$
ct_aby_upper	Upper limit of IPC (ABY™) acceptance criteria for NTC	$C_t \leq 30.5$
ct_alexa647	DPC (Alexa Fluor™ 647) acceptance criteria for PTC	$C_t \leq 25$
ct_fam_nec	Fungi (FAM™) acceptance criteria for NEC and S_NEC	$C_t \geq 33$
ct_fam_ntc	Fungi (FAM™) acceptance criteria for NTC	$C_t \geq 36$
ct_fam_p	Fungi (FAM™) acceptance criteria for PTC	$C_t \leq 25$
ct_fam_pec	Fungi (FAM™) acceptance criteria for PEC and S_PEC	$C_t < 33$
ct_fam_u	Fungi (FAM™) acceptance criteria for extracted test samples	$C_t < 33$: Fungi present $C_t \geq 33$: No fungi contamination

Table 5 Default call setting parameters *(continued)*

Parameter name	Details	Default passing criteria[1]
ct_vic_nec	Bacteria (VIC™) acceptance criteria for NEC and S_NEC	$C_t \geq$ **34**
ct_vic_ntc	Bacteria (VIC™) acceptance criteria for NTC	$C_t \geq$ **35.5**
ct_vic_p	Bacteria (VIC™) acceptance criteria for PTC	$C_t \leq$ **26**
ct_vic_pec	Bacteria (VIC™) acceptance criteria for PEC and S_PEC	$C_t <$ **34**
ct_vic_u	Bacteria (VIC™) acceptance criteria for extracted test samples	$C_t <$ **34**: Bacteria present $C_t \geq$ **34**: No bacteria contamination
deltaRn_alexa647	DPC (Alexa Fluor™ 647) acceptance criteria for all samples	ΔRn ≥ **0.9**: DPC present
deltaRn_fam	FAM™ acceptance criteria for all samples	ΔRn ≥ **1**: Fungi present
IPCdifference	IPC (ABY™) acceptance criteria for all samples except NTC	$C_t \leq$ NTC ABY™ mean C_t + **2**

[1] Bold values can be edited.

b. Click **Save Changes**.

4. in the **Analysis Settings** dialog box, click **Apply** when all desired rule changes are complete.

Acceptance criteria—AccuSEQ™ Real-Time PCR Software v3.2.1 or later

The following table shows default criteria for presence or absence calls of unknown samples and passing calls of controls. These criteria are included in the analysis settings in the SteriSEQ™ experiment template provided with AccuSEQ™ Real-Time PCR Software v3.2.1 or later.

- A call of "present" indicates that at least one genomic copy of bacteria or fungi DNA was present in the unknown (test sample) well and the well is positive for the presence of bacteria or fungi.
- For the plate to be valid, at least one replicate of each type of plate-level control (PTC, NTC, PEC, and NEC) must pass.
- We recommended establishing acceptance criteria threshold based on sample preparation kit and sample matrix background.
- For information about modifying calling rules, see “Modify call thresholds” on page 38.

Note: Assay acceptance criteria are subject to your own validation.

Table 6 Default acceptance criteria for AccuSEQ™ Real-Time PCR Software v3.2.1 or later

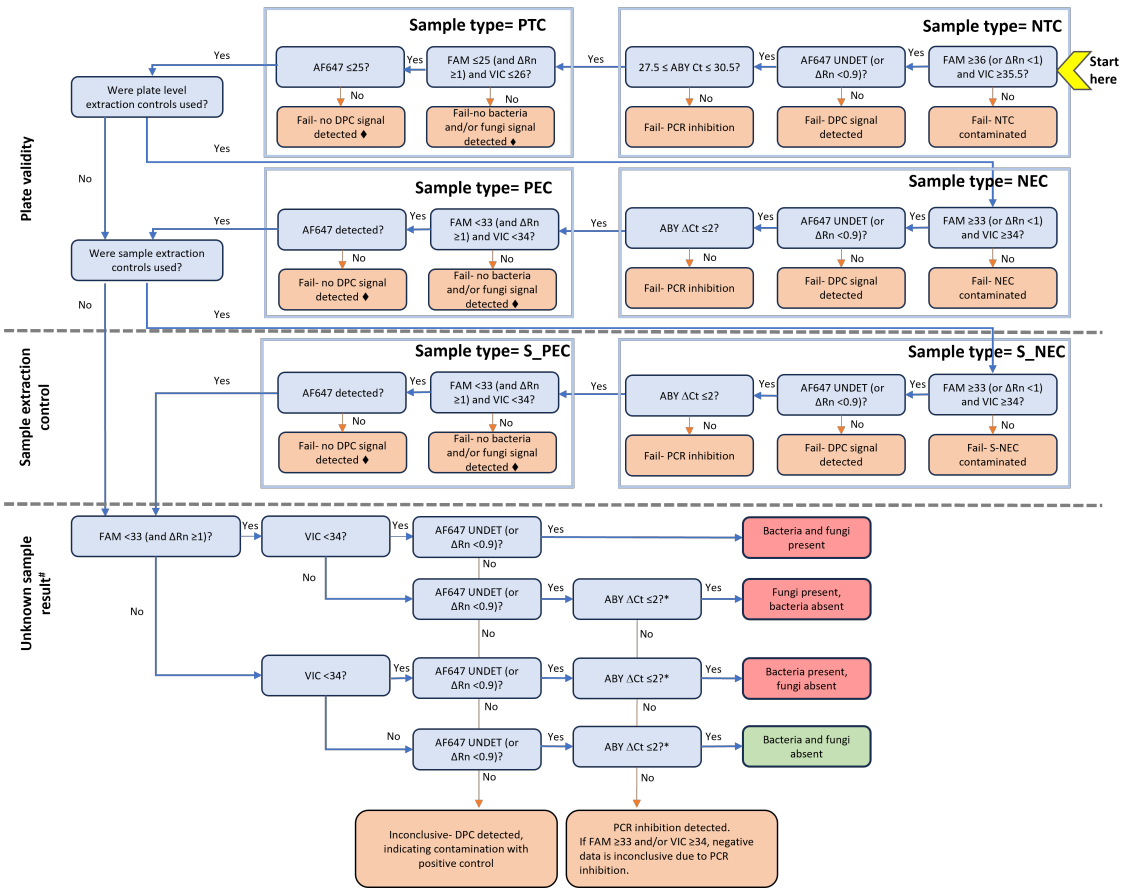
Sample type	Result	FAM™[1]	VIC™	ABY™	Alexa Fluor™ 647[2]
Unknown	Fungi and bacteria present	C_t <33.0[3]	C_t <34.0[3]	ΔC_t ≤2.0[4]	Undetermined
	Fungi present, bacteria absent	C_t <33.0[3]	C_t ≥34.0		
	Fungi absent, bacteria present	C_t ≥33.0	C_t <34.0[3]		
	Fungi and bacteria absent	C_t ≥33.0	C_t ≥34.0		
NTC	Pass	C_t ≥36.0	C_t ≥35.5	27.5 ≤ mean C_t ≤30.5	Undetermined
PTC	Pass	C_t ≤25.0	C_t ≤26.0	ΔC_t ≤2.0[4]	C_t ≤25.0
NEC/ S_NEC	Pass	C_t ≥33.0	C_t ≥34.0	ΔC_t ≤2.0[4]	Undetermined
PEC/ S_PEC	Pass	C_t <33.0	C_t<34.0	ΔC_t ≤2.0[4]	C_t detected

[1] For samples where the FAM™ C_t is detected, the Δ Rn has to be ≥1, otherwise the FAM™ is negative.
[2] For samples where the Alexa Fluor™ 647 C_t is detected, the Δ Rn has to be ≥0.9, otherwise, the Alexa Fluor™ 647 signal is negative.
[3] For unknown samples where the FAM™ C_t is <33.0 and VIC™C_t is <34.0, ABY™ signal can be ignored.
[4] ΔC_t equals sample ABY™ C_t – NTC ABY™ mean C_t.

Note:

1. NTC well failures indicate contamination during qPCR plating. We recommend including 3 NTC wells in each qPCR plate. AccuSEQ™ Real-Time PCR Software requires at least 1 NTC well to pass for a plate to be valid. However, if 3 NTC wells are present, at least 2 wells should pass for greater reliability of data.
2. We recommend 3 extraction replicates for unknown test samples. Each extraction replicate can be plated in triplicate on the qPCR plate, producing 9 data points. If samples are limited, 1 or 2 extraction replicates can be used, producing 3 or 6 data points.

   Low contamination levels in test samples can show positive detection in only some qPCR replicate wells. Test samples that are positive for contamination in more than one-third of wells can be determined to be contaminated. Samples which have one-third or fewer wells showing positive contamination are inconclusive, and can benefit from repeated testing after 24 hours to allow for growth of any existing contamination to levels that are more easily detected.



Note: ABY™ C_t values may increase with high loads of bacteria or fungi target. In such cases, ABY™ can be disregarded and the unknown sample is determined to be contaminated. Ensure that all channels for unknown samples are checked before determining the presence of contamination.

Figure 11 Decision tree

- ◆—For failed PEC and S_PEC check ABY™ C_t. If ABY™ ΔC_t >2.0, the failures may be caused by PCR inhibition in the well.
- #—Ensure that all channels for unknown samples are checked before reaching a decision.
- *—The ABY™ C_t values may increase when there are high levels of bacteria or fungi present. In such cases, ABY™ C_t may not provide accurate results and the unknown sample may be determined to be contaminated.

Example results with AccuSEQ™ Software v3.2.1 or later

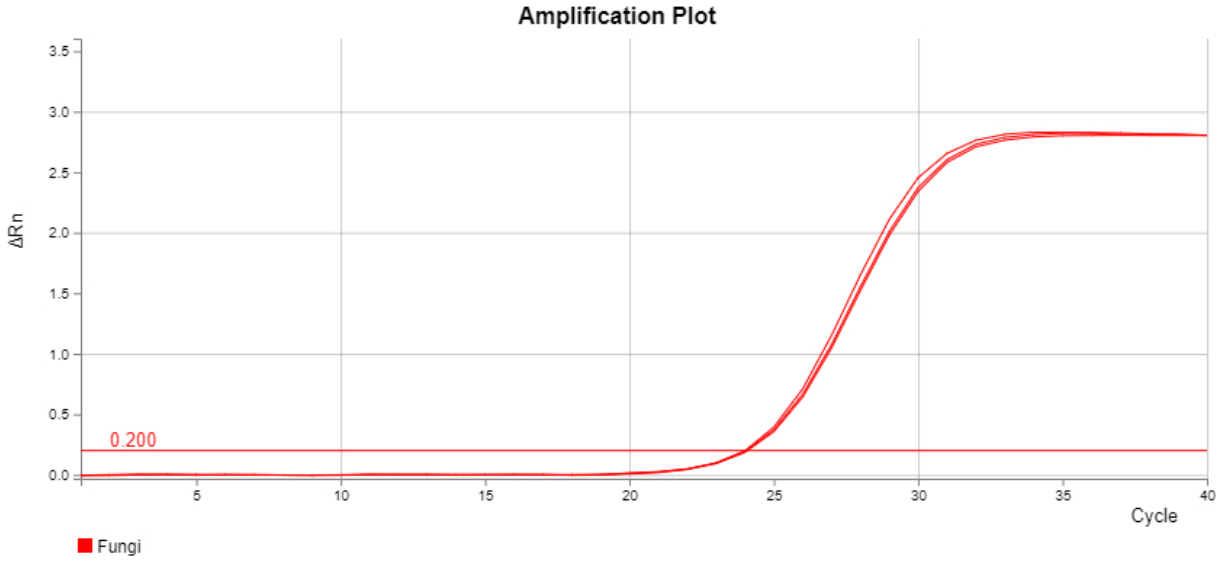


Figure 12 Positive template control (10,000 copies, 3 replicates)—FAM™ target

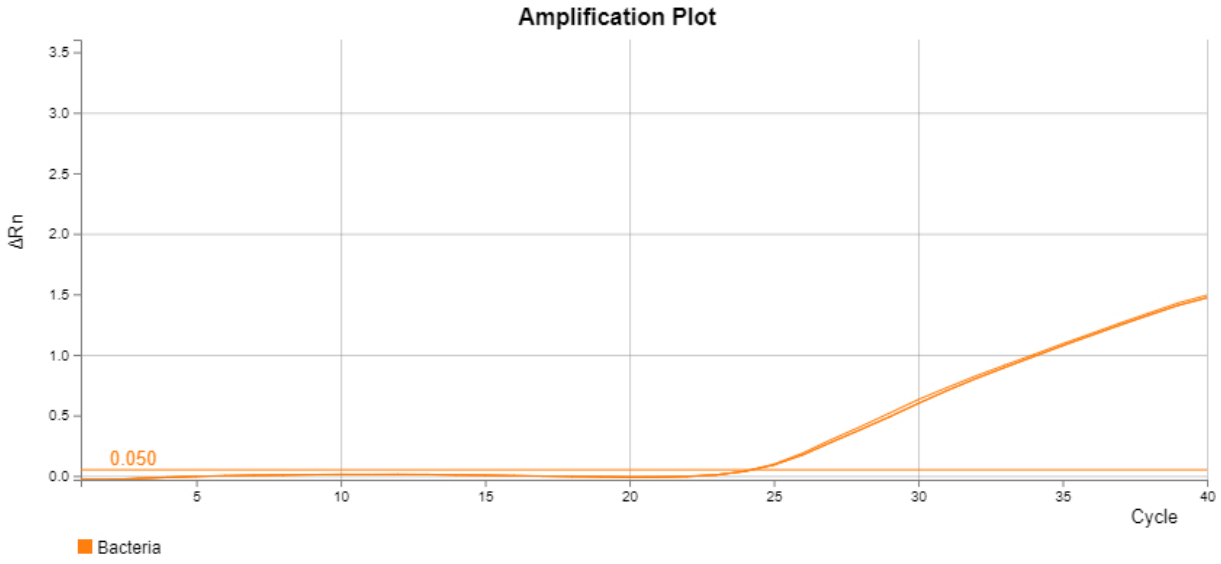


Figure 13 Positive template control (10,000 copies, 3 replicates)—VIC™ target

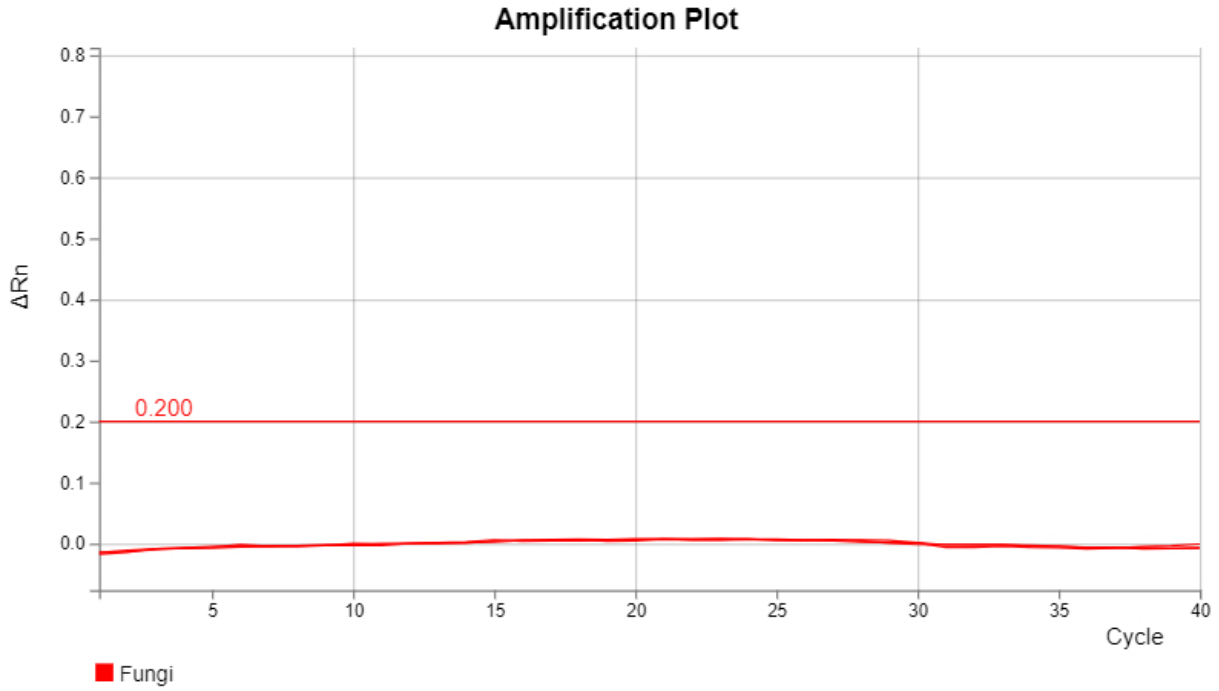


Figure 14 Negative template control (3 replicates)—FAM™ target

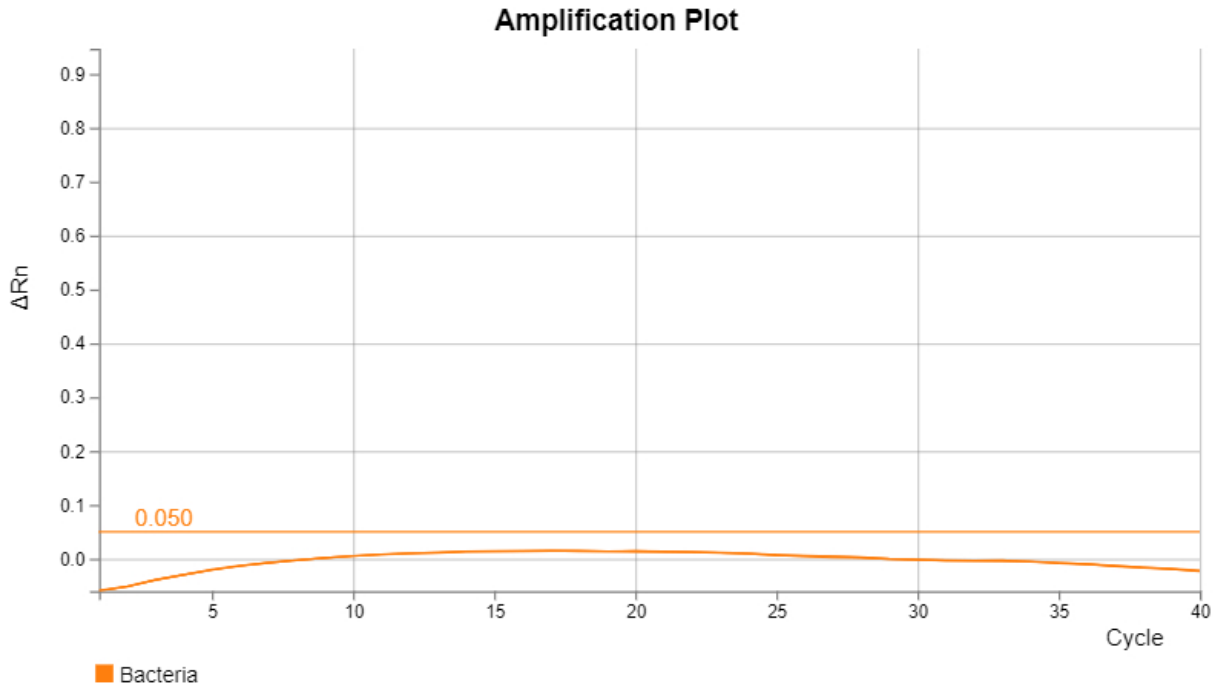


Figure 15 Negative template control (3 replicates)—VIC™ target

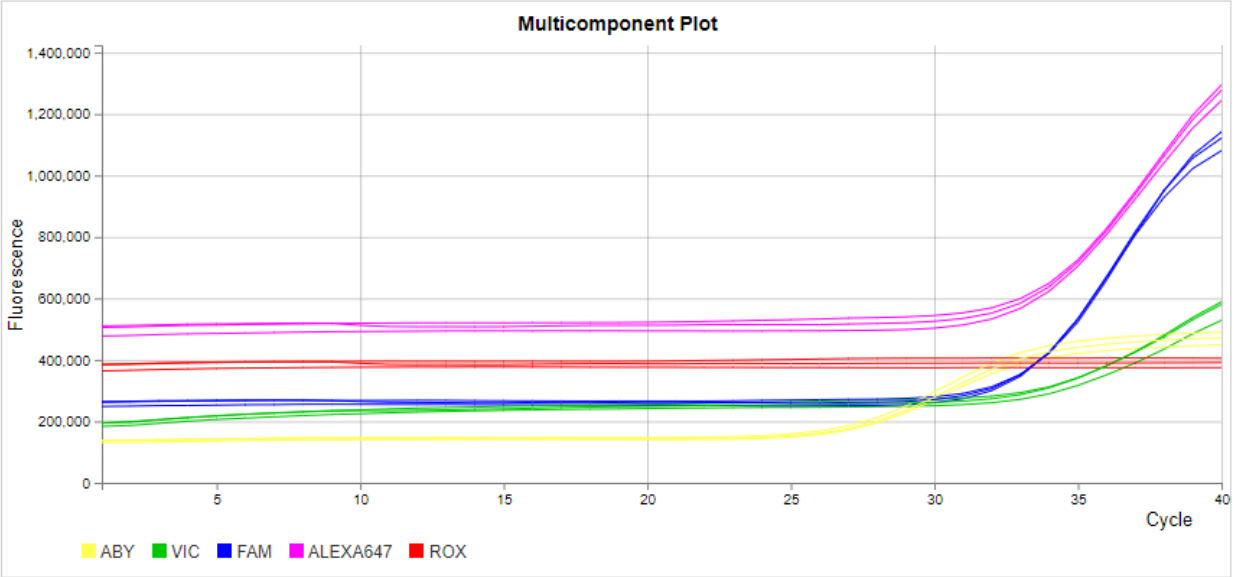


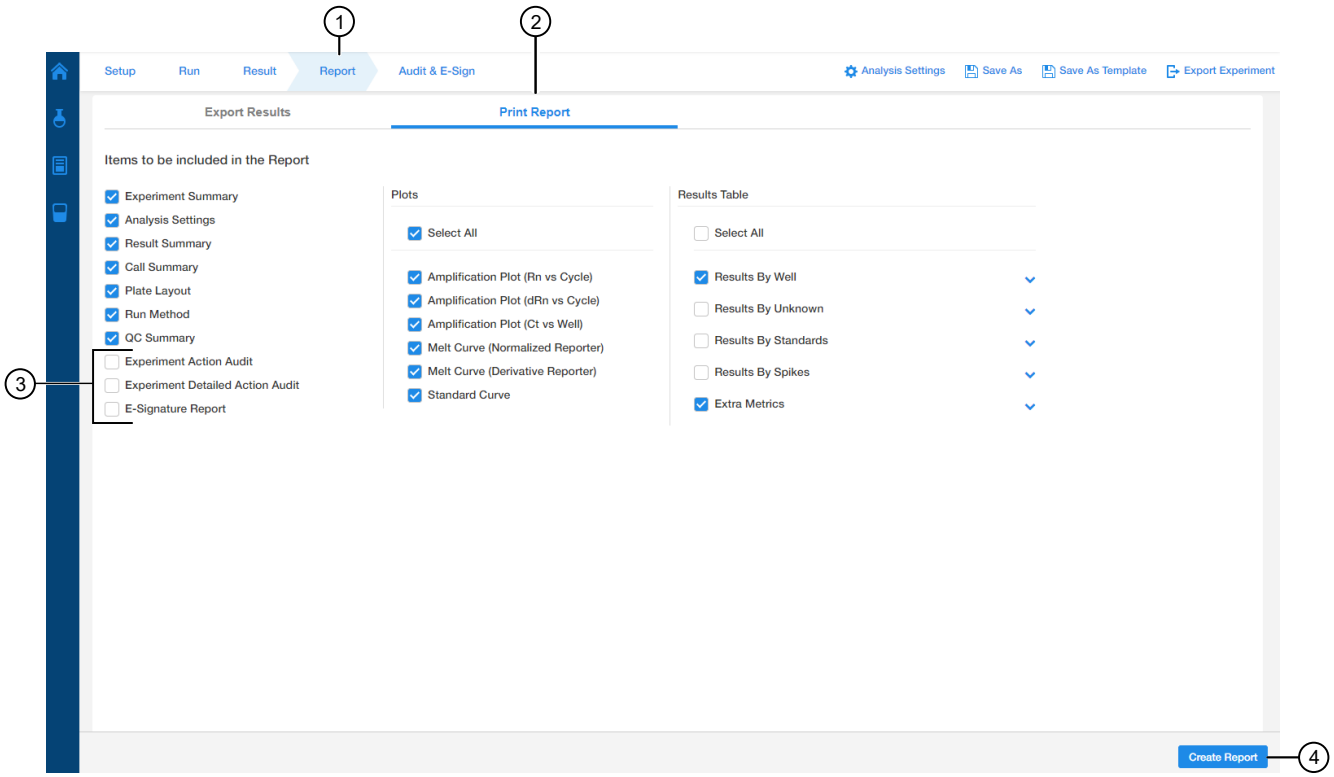
Figure 16 Multicomponent plot—Positive template control (10,000 copies, 3 replicates)

Print reports and export results

Create and print a report

To create a report, an experiment must be analyzed.

1. Open the experiment in the AccuSEQ™ Software. See “Open an experiment” on page 72.
2. Navigate to the **Report** screen, then the **Print Report** tab.



① **Report** screen
② **Print Report** tab
③ SAE attributes
④ **Create Report** button

3. Click the checkbox next to the items to be included in the report.

   Default attributes are preselected when using factory default templates. The order of items in the report can vary from what is shown in the software.

   IMPORTANT! To select and print SAE attributes, the user must have permission to print.

4. Click **Create Report**, then **View PDF**.

   The times shown in the **Run started** and **Run completed** fields vary slightly from times shown in instrument status due to the time delay between the instrument and AccuSEQ™ Software.

The PDF report name differs from the experiment name. Rename the file using the laboratory standards and practices.

5. (*Optional*) Click **Previous** to go back and make different selections for the report.

Export results

To export results, an experiment must have been analyzed.

1. Open the experiment in the AccuSEQ™ Software.
   See "Open an experiment" on page 72.
2. Navigate to the **Report** screen, then the **Export Results** tab.
3. Enter a file name, then select the file type (XLS, XLSX, TXT, or CSV).
   TXT and CSV files can be exported as combined or separate files.
4. Click the checkbox next to the content to export, then click **Export**.
5. (*Optional*) Click **Customize Export** to select more columns to include in the report.
   a. Select the type of content to customize from the **Content** dropdown, then click the checkbox next to the table fields to include.
   b. Click **Apply**.
6. Click **Export**.
   The results are downloaded to the default download folder.

5 Run the PCR and review the results—7500 Fast Real-Time PCR Instrument

This chapter describes how to load and run the 7500 Fast Real-Time PCR Instrument and review the results in AccuSEQ™ Real-Time PCR Software v2.2.1 or later.

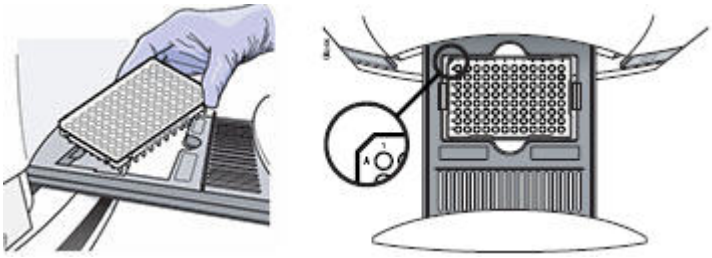
IMPORTANT! Review the guidelines to prevent contamination before proceeding (see "Guidelines to prevent contamination" on page 14).

Load the plate in the instrument

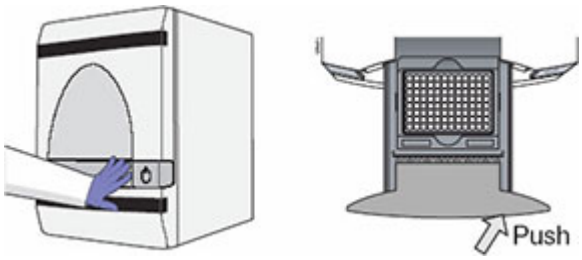
CAUTION! PHYSICAL INJURY HAZARD During instrument operation, the sample block can be heated to 100°C. Before performing this procedure, wait until the sample block reaches room temperature.

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

1. Push the tray door to open it.
2. Load the plate into the plate holder in the instrument with the notched A1 position at the top-left of the tray. Ensure that the plate is properly aligned in the holder.



3. To close the tray door, press the right side of the door at an angle.



Start the instrument run

Load the instrument before starting a run.

IMPORTANT! While the 7500 Fast Real-Time PCR Instrument is performing a run, do not create experiments, perform maintenance, print experiment data, or allow the computer to run antivirus software or to enter hibernation mode. Performing such activities while the instrument is running an experiment causes gaps in data collection.

1. If the experiment is not already open, select **File** ▸ **Open**. In the dialog box that opens, select the experiment, then click **Open**.
2. In the **Experiment** menu, select **Run**.
3. Ensure that the run status is not started.
   You cannot start the run if the run is complete.
4. Ensure that the instrument status is connected.
5. Click **START RUN** .

   Note: The button is disabled if the experiment file contains any run data.

For information about monitoring the run, stopping the run, and unloading the instrument, see the *AccuSEQ™ Real-Time PCR Software v2.2 Help*.

Review SteriSEQ™ results

Review the call summary

When a run is complete, the **Analysis** screen displays the calls and data for the plate wells in an experiment.

Calls are made based on the default analysis settings for SteriSEQ™ experiments. For more information about analysis settings, see “Modify call thresholds” on page 57.

1. Click **Analysis** in the navigation pane, then click **Call Summary**.
2. In the **Call Summary** pane, in the **Plate Calls** section, review the calls for the controls : positive template controls (P), negative template controls (NTC), positive extraction controls (PEC), and negative extraction controls (NEC).

   The number of controls that passed or failed is shown to the left of the PASS or FAIL icons. The overall plate status (**VALID** or **INVALID**) shown in the **Plate Status** field is determined by the P, NTC, PEC, and NEC calls.

   If there are multiple replicates for a control, at least one replicate must pass for the plate status to be labeled **VALID**. This requirement is not editable.
3. In the **Call Summary** pane, in the **Sample Level PEC/NEC Calls** section, review the total number of wells for each S_PEC or S_NEC call (**PASS** or **FAIL**).
4. In the **Call Summary** pane, in the **Well Calls** section, review the total number of wells for each call—**Present**, **Absent**, **Review**, or **Fail**.
5. To review more information about the calls, see “Review the QC Summary” on page 51.

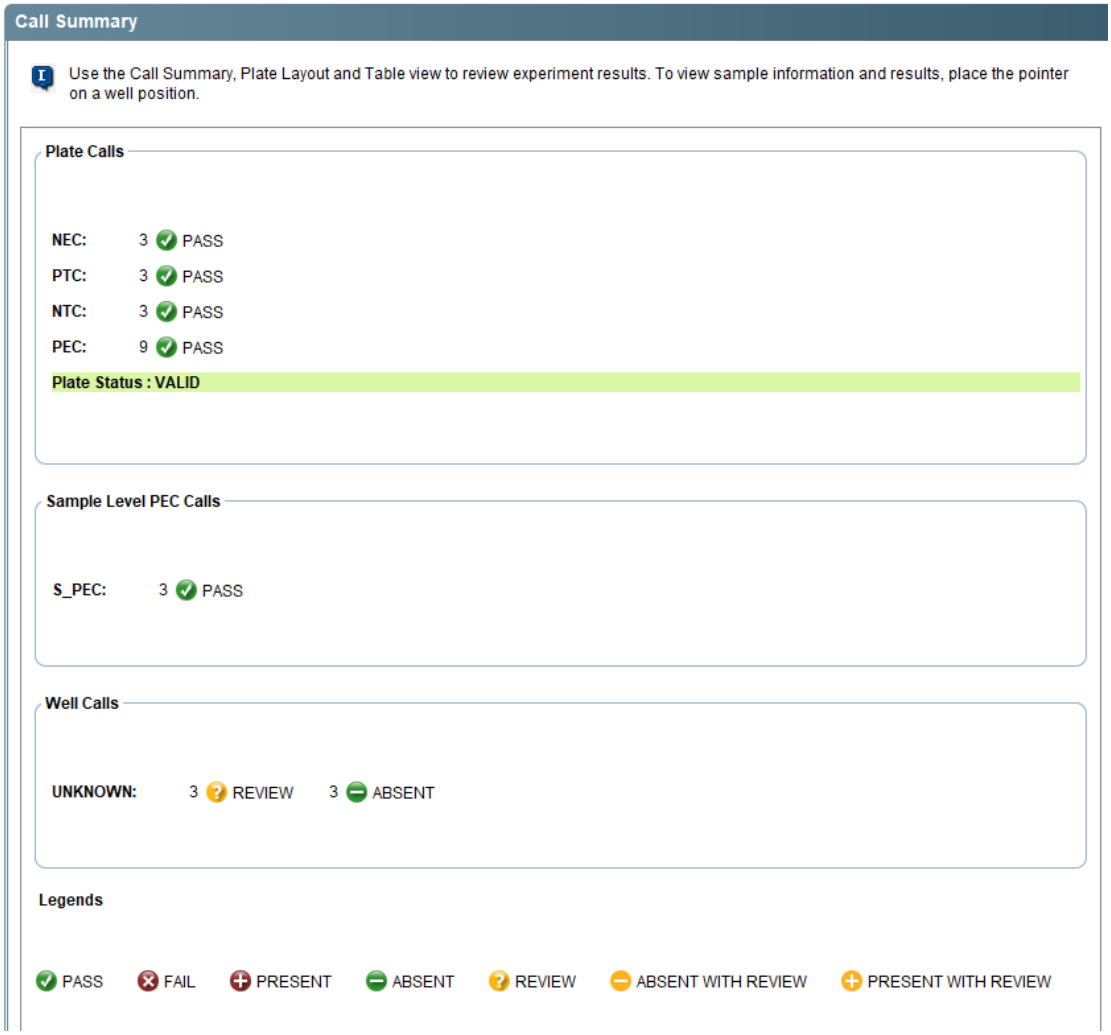


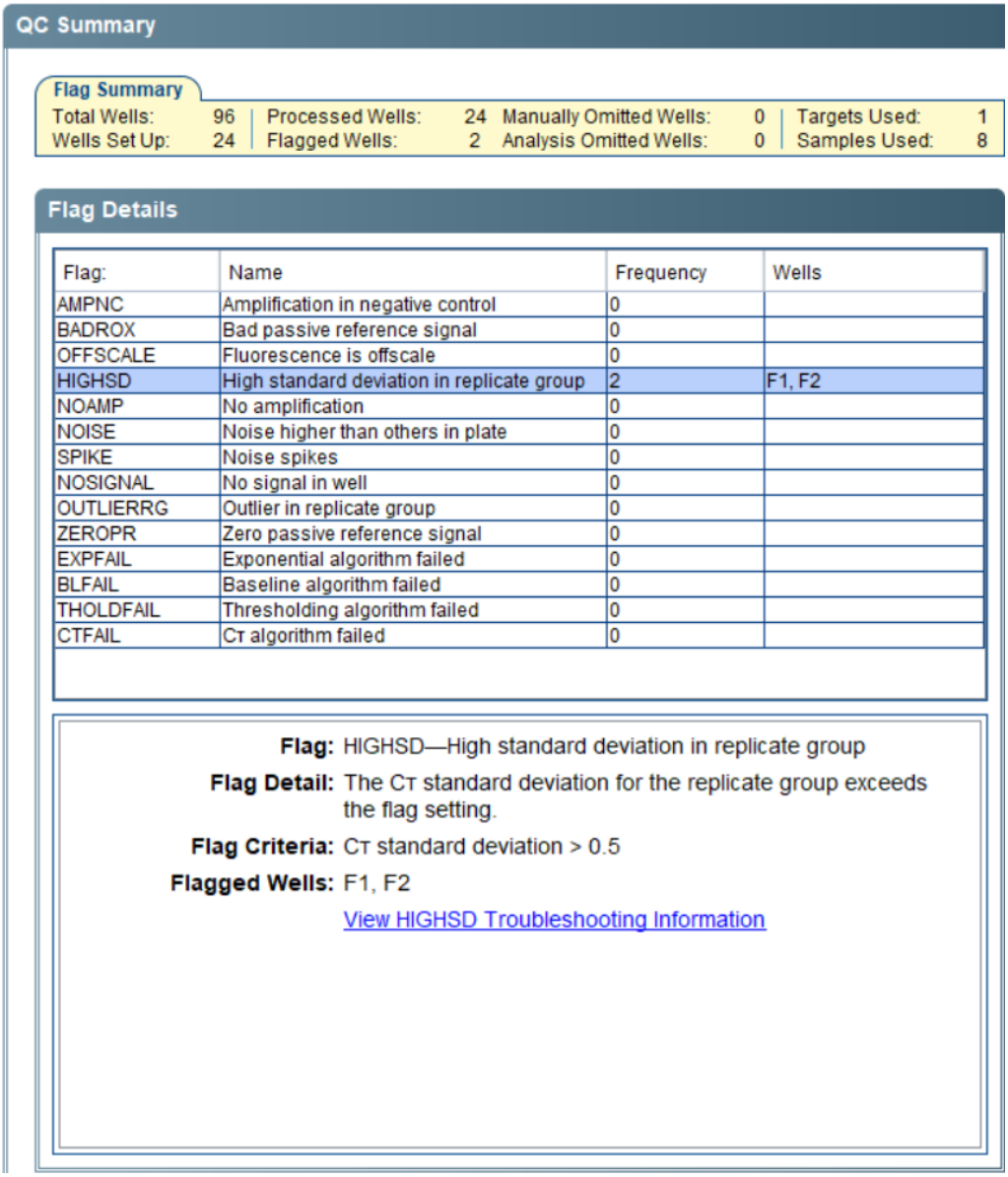
Figure 17 Call Summary pane (example)

Review the QC Summary

The **QC Summary** provides a description and troubleshooting information to help review results.

1. Click **Analysis** in the **Experiment Menu** pane, then click **Quality Summary**.
2. In the **QC Summary**, look in the **Frequency** and **Wells** columns to determine which flags appear in the experiment.

3. Click a call assessment in the table to show more information about the assessment.



QC Summary

Flag Summary

Total Wells:	96	Processed Wells:	24	Manually Omitted Wells:	0	Targets Used:	1
Wells Set Up:	24	Flagged Wells:	2	Analysis Omitted Wells:	0	Samples Used:	8

Flag Details

Flag:	Name	Frequency	Wells
AMPNC	Amplification in negative control	0	
BADROX	Bad passive reference signal	0	
OFFSCALE	Fluorescence is offscale	0	
HIGHSD	High standard deviation in replicate group	2	F1, F2
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	0	
SPIKE	Noise spikes	0	
NOSIGNAL	No signal in well	0	
OUTLIERRG	Outlier in replicate group	0	
ZEROPR	Zero passive reference signal	0	
EXPFAIL	Exponential algorithm failed	0	
BLFAIL	Baseline algorithm failed	0	
THOLDFAIL	Thresholding algorithm failed	0	
CTFAIL	CT algorithm failed	0	

Flag: HIGHSD—High standard deviation in replicate group

Flag Detail: The CT standard deviation for the replicate group exceeds the flag setting.

Flag Criteria: CT standard deviation > 0.5

Flagged Wells: F1, F2

View HIGHSD Troubleshooting Information

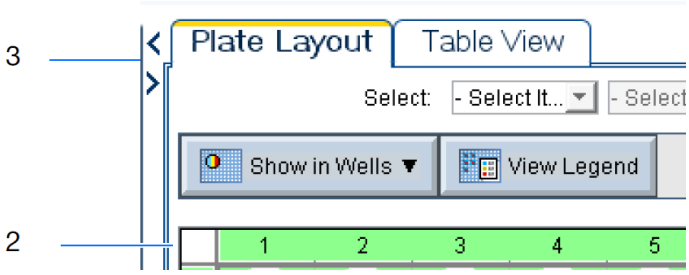
4. Click the troubleshooting link below the table to view information about correcting the flag.

View the Amplification Plot

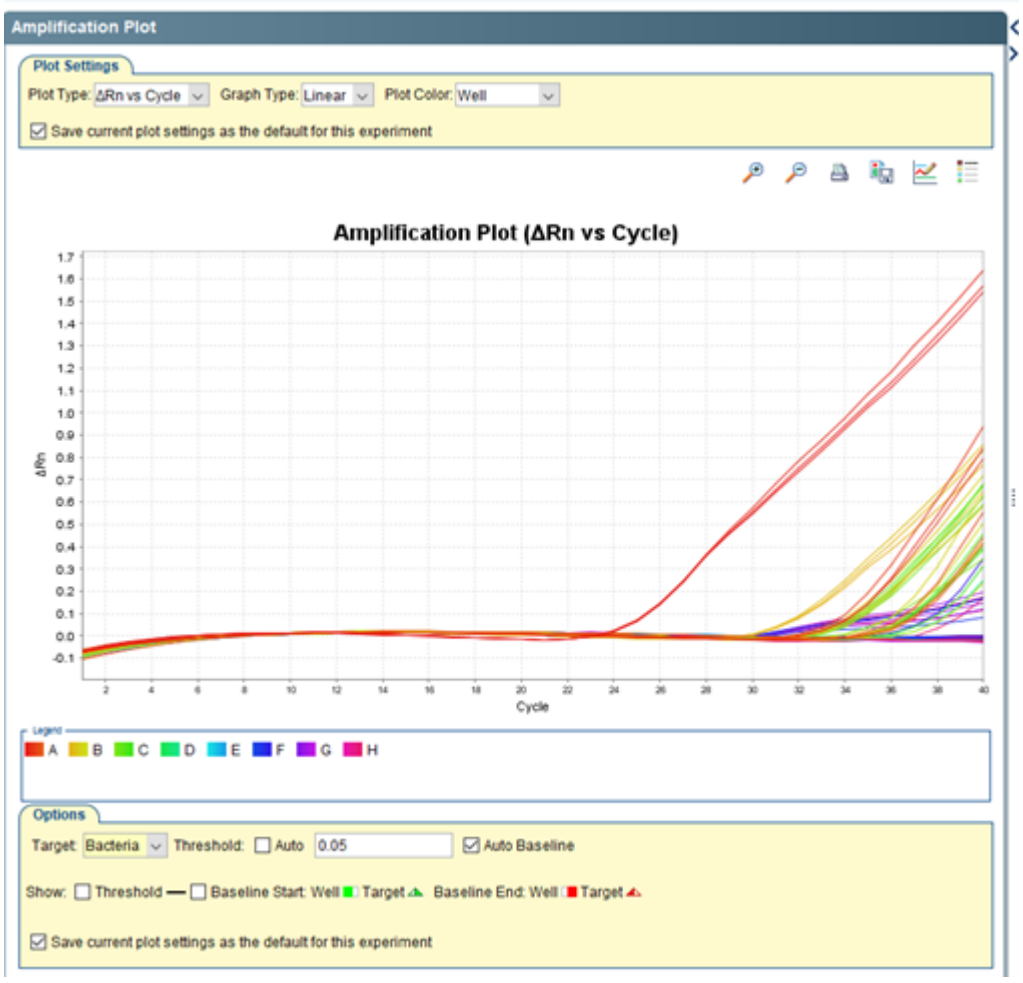
1. From the navigation pane, select **Plot Analysis** ▸ **Amplification Plot**.

2. Show all 96 wells in the amplification plot by clicking the upper left corner of the plate layout in the **Plate Layout** tab.

3. *(Optional)* For an expanded view of the amplification plot, click the top-left corner of the plate layout in the **Plate Layout** tab.



4. Use the **Plot Settings** tab to change the plot type, graph type, and plot color.



5. Use the **Options** tab below the plot to show the **Threshold** and **Baseline Start**.

6. Select wells in the plate layout to view in the amplification plot .

7. *(Optional)* To view an area of the amplification plot in detail, click:
   a. A quadrant in the plot to the left of the area you want to review.

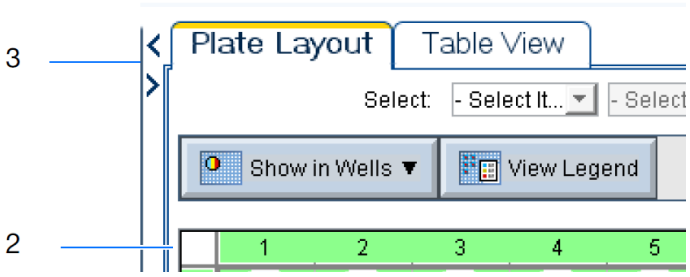
b. (Zoom in).

8. *(Optional)* Click (Hide the plot legend).

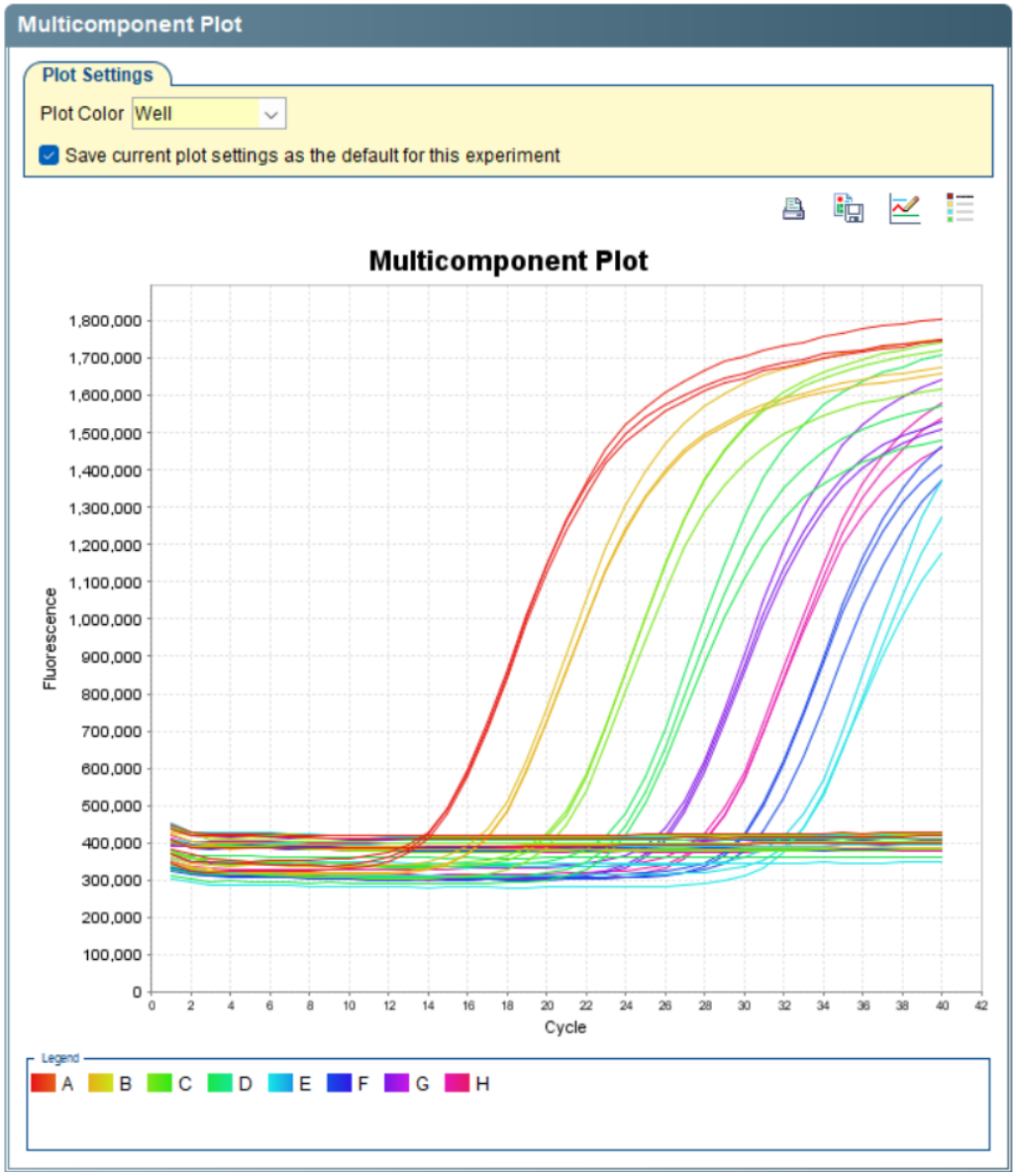
Note: This is a toggle button. When the legend is hidden, the button changes to Show a legend for the plot.

View the Multicomponent Plot

1. From the navigation pane, select **Plot Analysis** ▸ **Multicomponent Plot**.
2. Show all 96 wells in the **Multicomponent Plot** by clicking the upper left corner of the plate layout in the **Plate Layout** tab.
3. *(Optional)* For an expanded view of the plot, click the top-left corner of the plate layout in the **Plate Layout** tab.



4. Use the **Plot Settings** tab to change the plot color.



5. Select wells in the plate layout to view in the multicomponent plot .

6. *(Optional)* Click (Hide the plot legend).

   This is a toggle button. When the legend is hidden, the button changes to Show a legend for the plot.

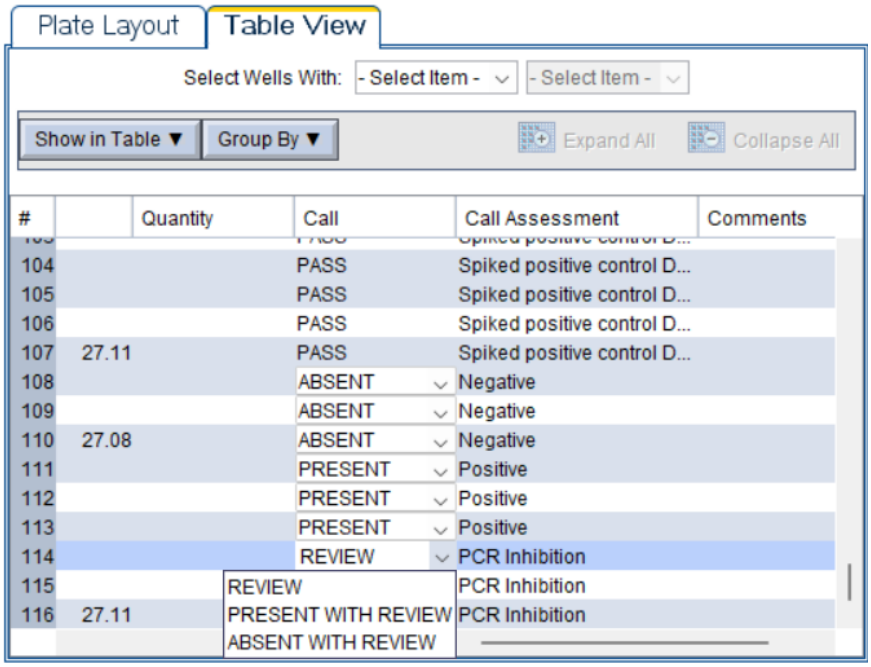
Manually edit unknown well calls

Calls for wells assigned as **Unknown** task in factory default SEQ detection assays can be manually edited. Any change in the well call is recorded by the silent audit function.

Only **Unknown** wells can be manually edited. In the **Experiment Menu** pane, click **Analysis** ▸ **Call Summary** to determine if **Unknown** wells exist.

IMPORTANT! You must have administrator privileges to perform this task.

1. In the **Call Summary** screen, click the **Table View** tab to open the table view.
2. Click the **Task** column to group wells with **Unknown** tasks together.
3. Select an **Unknown** well in the **Task** column.
4. Use the plots available in the **Analysis** section of the **Experiment Menu** to view the well data.
5. Determine if a call can be made based on the available data.
6. Click the **Call** column in the highlighted row to modify the well call.
7. Select an option from the dropdown:



- Review call can become **Review**, **Present with Review**, or **Absent with Review**.
- Absent call can become **Absent** or **Present with Review.**
- Present call can become **Present** or **Absent with Review**.

A **Fail** call cannot be edited.

The call assessment is updated and the icons for the edited call appears in orange.

ABSENT WITH REVIEW PRESENT WITH REVIEW

Table view, exported results, and printed reports include the manually edited call and call assessment values. An experiment imported as an EDS file keeps manual calls that were edited.

If the experiment is reanalyzed, the edited well calls revert to the default calls in accordance with the rule settings.

Modify call thresholds

Calls are made based on the default analysis settings for SteriSEQ™ experiments. The passing criteria for all sample types can be modified. The need to change passing criteria can occur when a different cut-off is established for a sample preparation kit or for a particular type of sample matrix. If a different cut-off is established, FAM™ (fungi) and VIC™ (bacteria) acceptance criteria may need to be updated for NEC, S_NEC, PEC, S_PEC, and unknown test samples.

1. In the **Result** screen, click **Analysis Settings** in the top-right.
   The **Analysis Settings** dialog box opens to the **Rule Settings** tab.

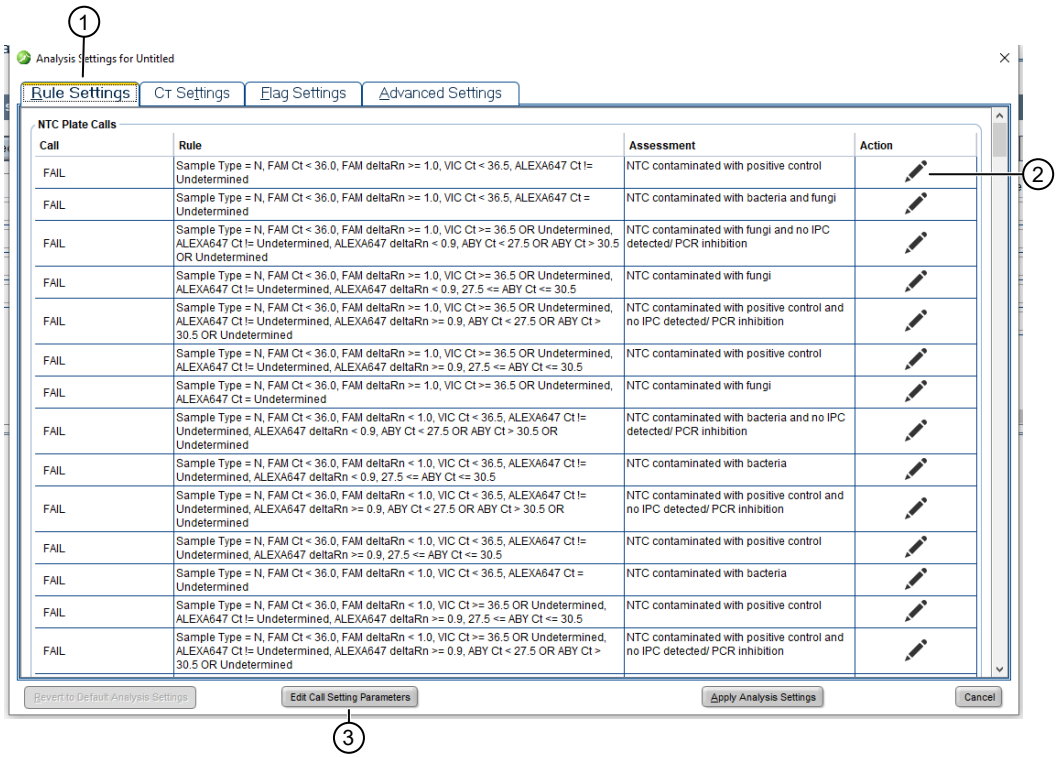


Figure 18 Example analysis settings

① **Rule Settings** tab
② Edit rule
③ **Edit Call Setting Parameters** button

The thresholds can be updated collectively using **Edit Call Setting Parameters** or individually using ✎.

2. Click **Edit Call Setting Parameters**.

3. Edit the rule parameters.

   Rule parameters than can be edited include Ct and ΔRn cut-offs that determine Pass/Fail results.

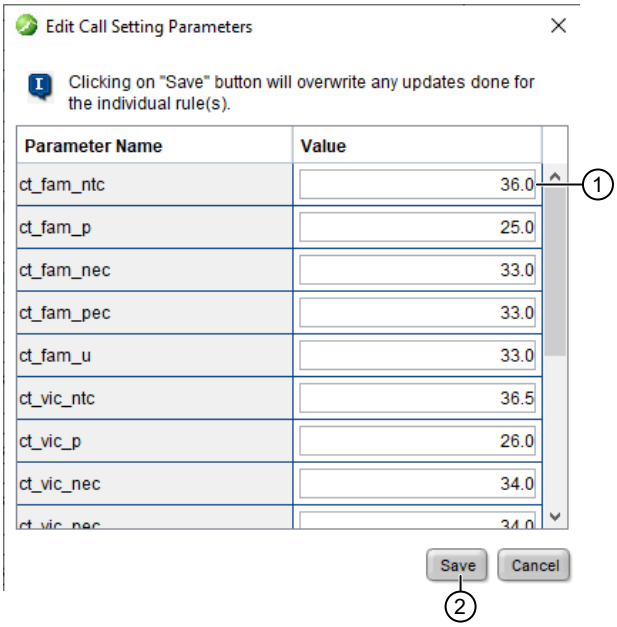


Figure 19 Example call setting parameters

① Edit parameter value
② **Save** button

   a. Enter the new value in the **Value** column for each parameter.

      Detailed parameter descriptions and default call setting values are listed below.

Table 7 Default call setting parameters

Parameter name	Details	Default passing criteria[1]
ct_aby_lower	Lower limit of IPC (ABY™) acceptance criteria for NTC	$C_t \geq$ **27.5**
ct_aby_upper	Upper limit of IPC (ABY™) acceptance criteria for NTC	$C_t \leq$ **30.5**
ct_alexa647	DPC (Alexa Fluor™ 647) acceptance criteria for PTC	$C_t \leq$ **25**
ct_fam_nec	Fungi (FAM™) acceptance criteria for NEC and S_NEC	$C_t \geq$ **33**
ct_fam_ntc	Fungi (FAM™) acceptance criteria for NTC	$C_t \geq$ **36**
ct_fam_p	Fungi (FAM™) acceptance criteria for PTC	$C_t \leq$ **25**
ct_fam_pec	Fungi (FAM™) acceptance criteria for PEC and S_PEC	$C_t <$ **33**
ct_fam_u	Fungi (FAM™) acceptance criteria for extracted test samples	$C_t <$ **33**: Fungi present $C_t \geq$ **33**: No contamination

Table 7 Default call setting parameters *(continued)*

Parameter name	Details	Default passing criteria[1]
ct_vic_nec	Bacteria (VIC™) acceptance criteria for NEC and S_NEC	$C_t \geq$ **34**
ct_vic_ntc	Bacteria (VIC™) acceptance criteria for NTC	$C_t \geq$ **35.5**
ct_vic_p	Bacteria (VIC™) acceptance criteria for PTC	$C_t \leq$ **26**
ct_vic_pec	Bacteria (VIC™) acceptance criteria for PEC and S_PEC	$C_t <$ **34**
ct_vic_u	Bacteria (VIC™) acceptance criteria for extracted test samples	$C_t <$ **34**: Bacteria present $C_t \geq$ **34**: No contamination
deltaRn_alexa647	DPC (Alexa Fluor™ 647) acceptance criteria for all samples	ΔRn ≥ **0.9**: DPC present
deltaRn_fam	FAM™ acceptance criteria for all samples	ΔRn ≥ **1**: Fungi present
IPCdifference	IPC (ABY™) acceptance criteria for all samples except NTC	$C_t \leq$ NTC ABY™ mean C_t + **2**

[1] Bold values can be edited.

b. Click **Save**.

4. In the **Analysis Settings** dialog box, click **Apply Analysis Settings**.

5. In the upper toolbar, click **Save** to save the settings to the experiment file.

Acceptance criteria—AccuSEQ™ Real-Time PCR Software v2.2.1 or later

The following table shows default criteria for presence or absence calls of unknown samples and passing calls of controls. These criteria are included in the analysis settings in the SteriSEQ™ experiment template provided with AccuSEQ™ Real-Time PCR Software v2.2.1 or later.

- A call of "present" indicates that at least one genomic copy of bacteria or fungi DNA was present in the unknown reaction and the sample is positive for the presence of bacteria or fungi.
- For the plate to be valid, at least one replicate of each type of plate-level control (PTC, NTC, PEC, and NEC) must pass.
- We recommended establishing acceptance criteria threshold based on sample preparation kit and sample matrix background.
- For information about modifying calling rules, see “Modify call thresholds” on page 57.

Note: Assay acceptance criteria are subject to your own validation.

Table 8 Default acceptance criteria for AccuSEQ™ Real-Time PCR Software v2.2.1 or later

Sample type	Result	FAM™[1]	VIC™	ABY™	Alexa Fluor™ 647[2]
Unknown	Fungi and bacteria present	C_t <33.0[3]	C_t <34.0[3]	ΔC_t ≤2.0[4]	Undetermined
	Fungi present, bacteria absent	C_t <33.0[3]	C_t ≥34.0		
	Fungi absent, bacteria present	C_t ≥33.0	C_t <34.0[3]		
	Fungi and bacteria absent	C_t ≥33.0	C_t ≥34.0		
NTC	Pass	C_t ≥36.0	C_t ≥35.5	27.5 ≤ mean C_t ≤30.5	Undetermined
PTC	Pass	C_t ≤25.0	C_t ≤26.0	ΔC_t ≤2.0[4]	C_t ≤25.0
NEC/ S_NEC	Pass	C_t ≥33.0	C_t ≥34.0	ΔC_t ≤2.0[4]	Undetermined
PEC/ S_PEC	Pass	C_t <33.0	C_t<34.0	ΔC_t ≤2.0[4]	C_t detected

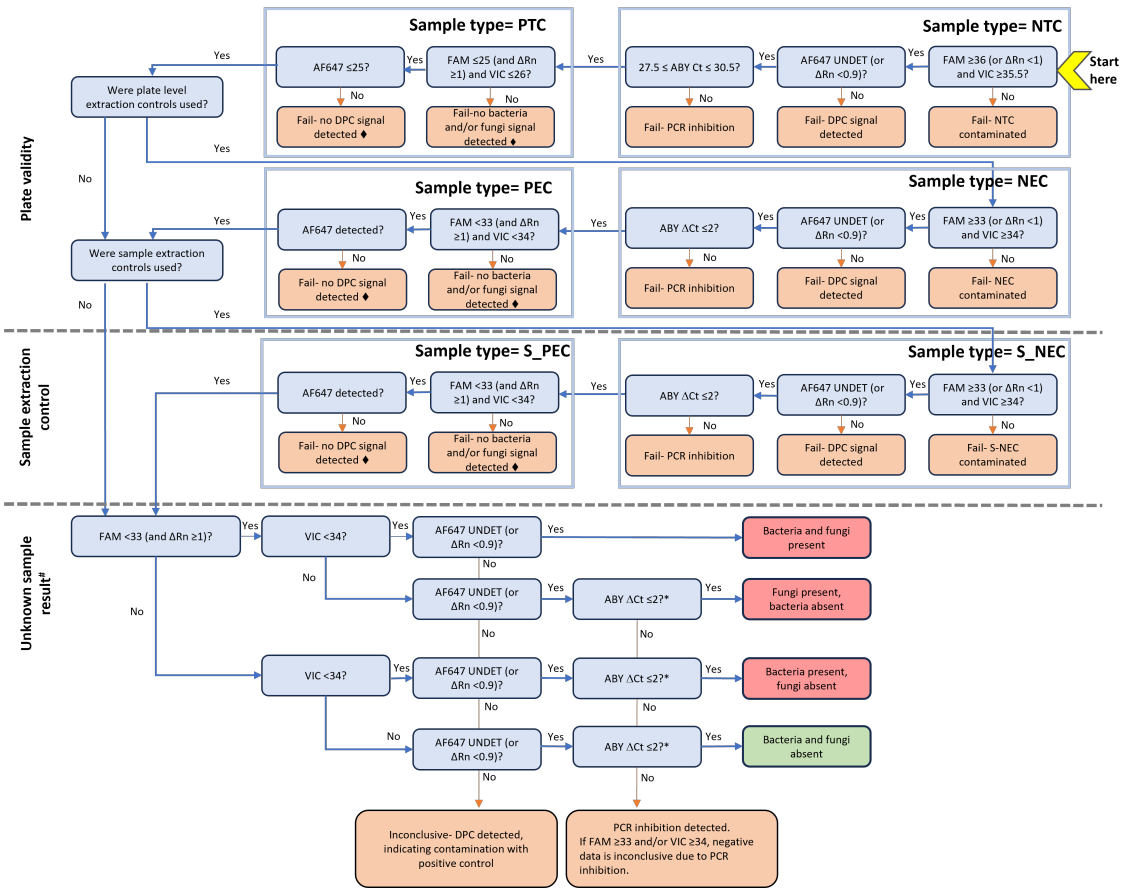
[1] For samples where the FAM™ C_t is detected, the Δ Rn has to be ≥1, otherwise the FAM™ is negative.
[2] For samples where the Alexa Fluor™ 647 C_t is detected, the Δ Rn has to be ≥0.9, otherwise, the Alexa Fluor™ 647 signal is negative.
[3] For unknown samples where the FAM™ C_t is <33.0 or VIC™C_t is <34.0, ABY™ signal can be ignored.
[4] ΔC_t equals sample ABY™ C_t – NTC ABY™ mean C_t

Note:

1. NTC well failures indicate contamination during qPCR plating. We recommend including 3 NTC wells in each qPCR plate. AccuSEQ™ Real-Time PCR Software requires at least 1 NTC well to pass for a plate to be valid. However, if 3 NTC wells are present, at least 2 wells should pass for greater reliability of data.
2. We recommend 3 extraction replicates for unknown test samples. Each extraction replicate can be plated in triplicate on the qPCR plate, producing 9 data points. If samples are limited, 1 or 2 extraction replicates can be used, producing 3 or 6 data points.

   Low contamination levels in test samples can show positive detection in only some qPCR replicate wells. Test samples that are positive for contamination in more than one-third of wells can be determined to be contaminated. Samples which have one-third or fewer wells showing positive contamination are

inconclusive, and can benefit from repeated testing after 24 hours to allow for growth of any existing contamination to levels that are more easily detected.



Note: ABY™ C_t values may increase with high loads of bacteria or fungi target. In such cases, ABY™ can be disregarded and the unknown sample is determined to be contaminated. Ensure that all channels for unknown samples are checked before determining the presence of contamination.

Figure 20 Decision tree

- ◆—For failed PEC and S_PEC check ABY™ C_t. If ABY™ ΔC_t >2.0, the failures may be caused by PCR inhibition in the well.
- #—Ensure that all channels for unknown samples are checked before reaching a decision.
- *—The ABY™ C_t values may increase when there are high levels of bacteria or fungi present. In such cases, ABY™ C_t may not provide accurate results and the unknown sample may be determined to be contaminated.

Example results with AccuSEQ™ Software v2.2.1 or later

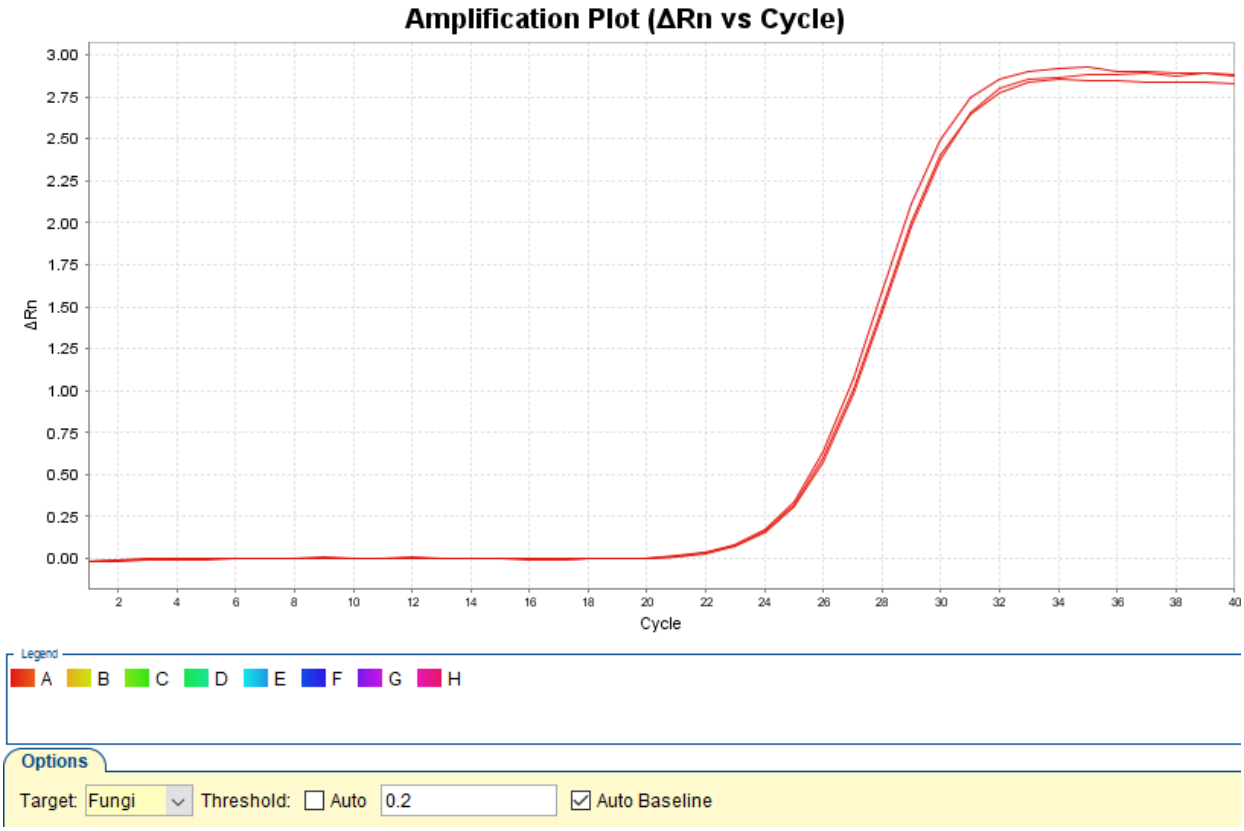


Figure 21 Positive control (10,000 copies, 3 replicates)—FAM™ target

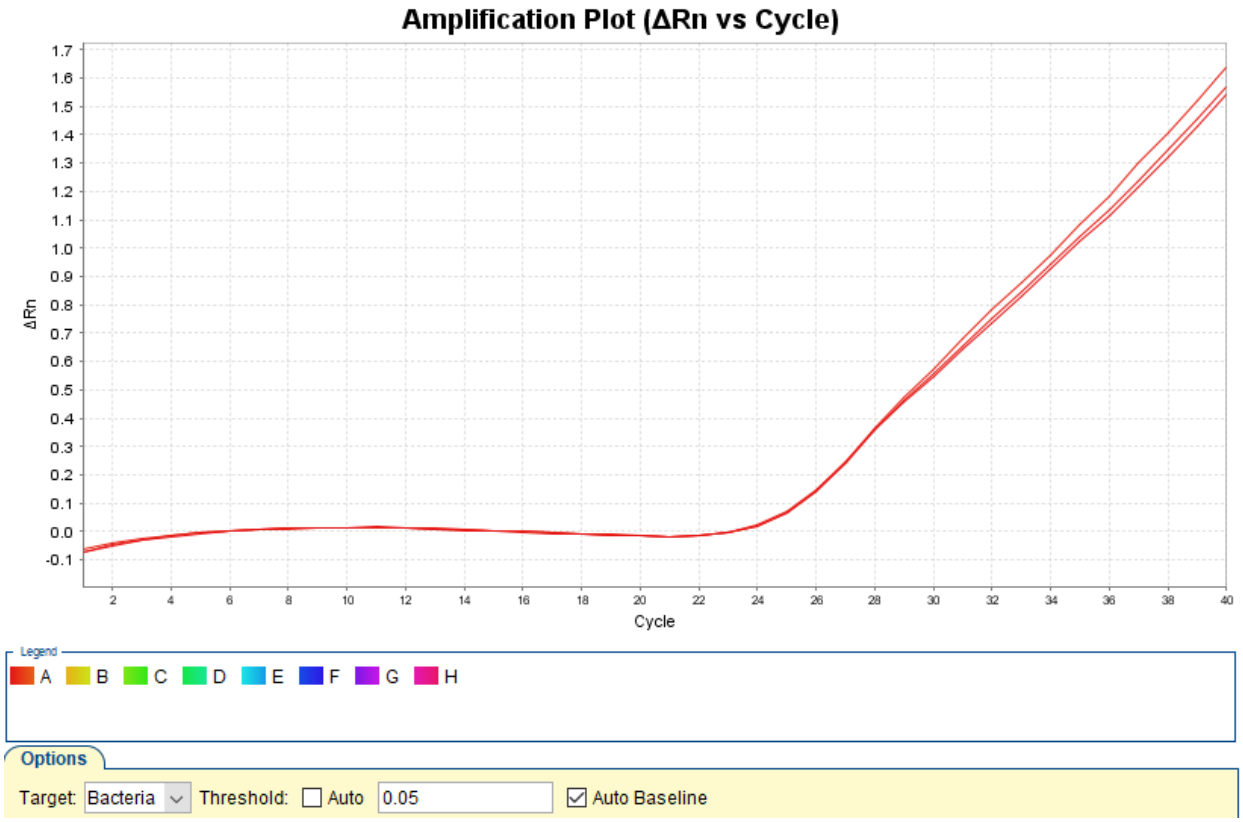


Figure 22 Positive control (10,000 copies, 3 replicates)—VIC™ target

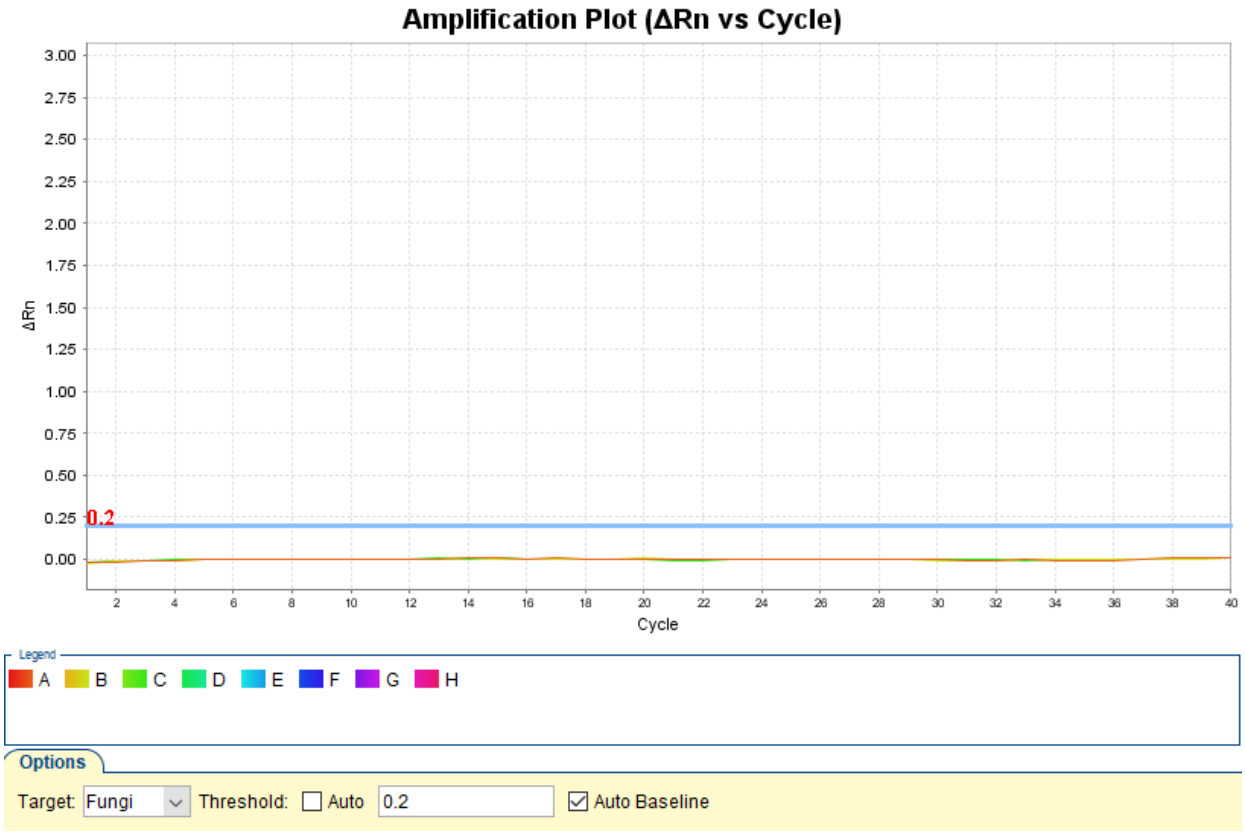


Figure 23 Negative template control (3 replicates)—FAM™ target

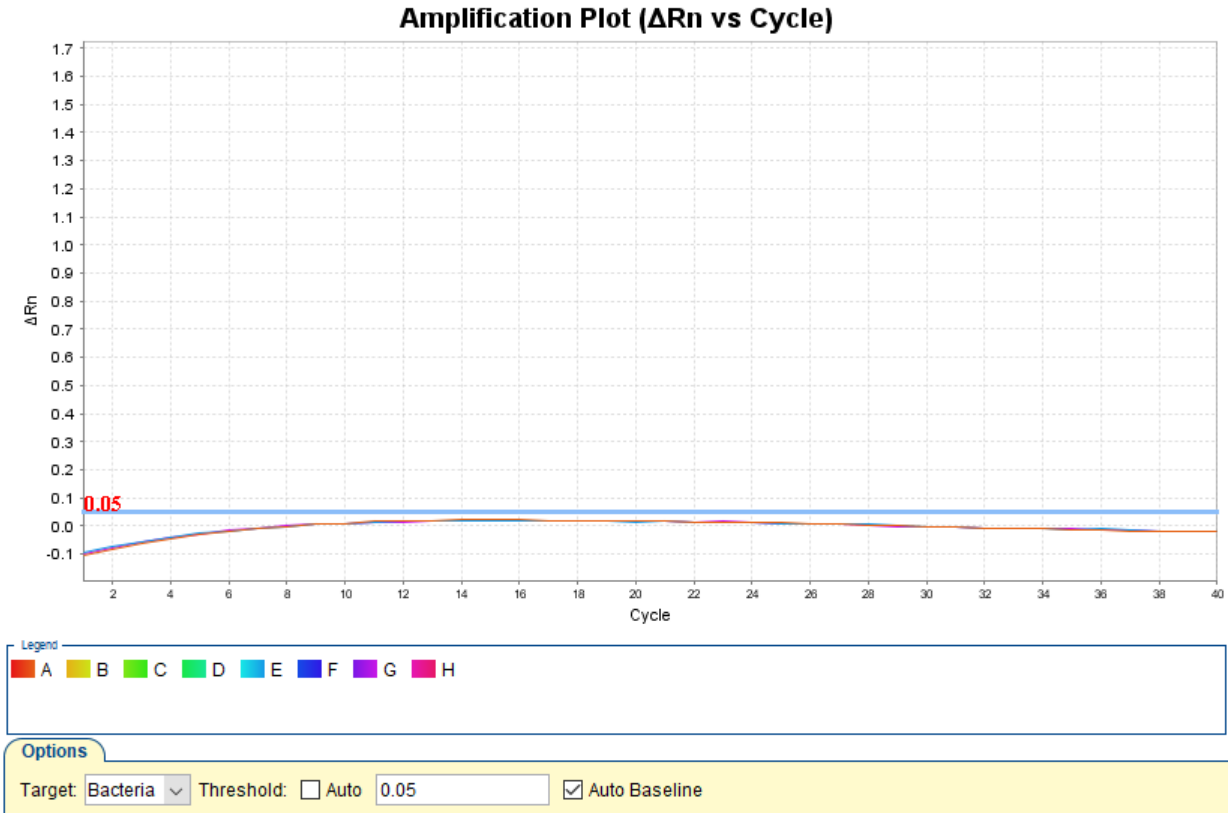


Figure 24 Negative template control (3 replicates)—VIC™ target

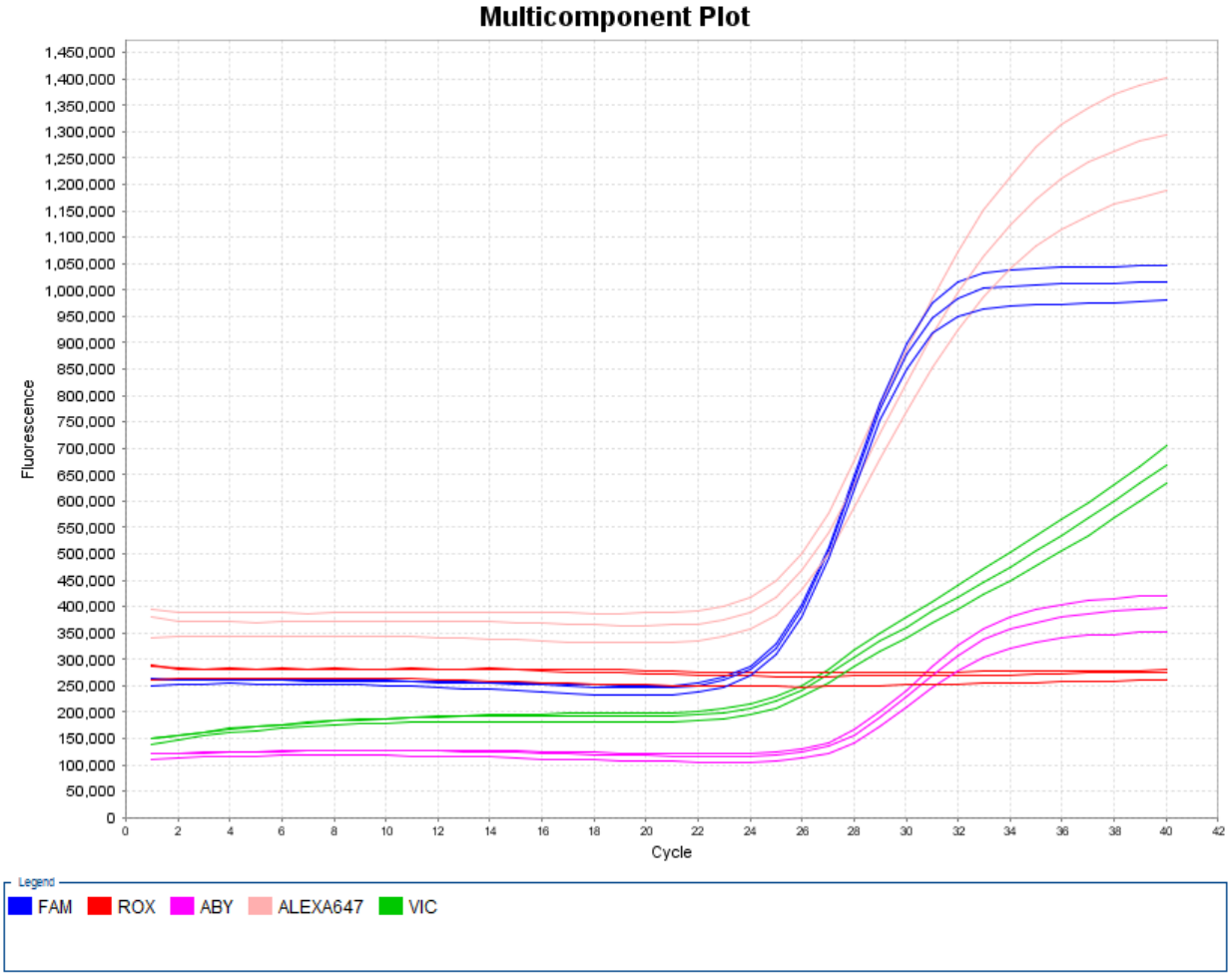


Figure 25 Multicomponent plot—Positive control (10,000 copies, 3 replicates)

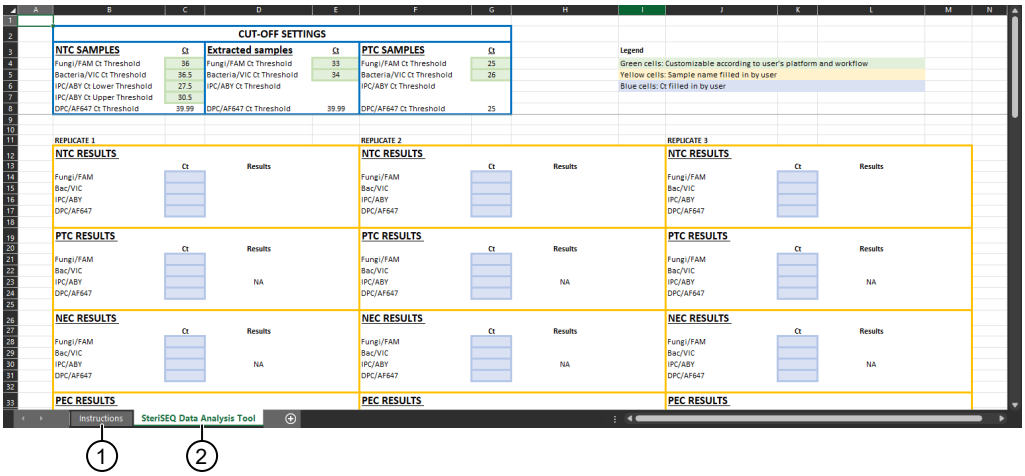
A Manual data analysis tool

Data analysis tool for SteriSEQ™

Use this data analysis tool to run the SteriSEQ™ assay on earlier versions of AccuSEQ™ Real-Time PCR Software (without the SteriSEQ™ experiment template) or other qPCR software.

1. Download the **SteriSEQ™ Data Analysis Tool** from the SteriSEQ™ Rapid Sterility Testing Kit product page to manually analyze data.

2. Open the data analysis tool in Microsoft™ Excel™.



① **Instructions** tab

② **SteriSEQ Data Analysis Tool** tab

3. Follow the steps in the **Instructions** tab.

   The data analysis tool determines the presence or absence of contamination in unknown sample wells.

B

Troubleshooting

Troubleshooting the SteriSEQ™ Rapid Sterility Testing Kit

Observation	Possible cause	Recommended action
Positive control does not meet acceptance criteria or C_t values are at or near to acceptance criteria limits. Abnormal amplification curve that begins too early then dips below the baseline and reappears.	Improper PCR setup or degradation of reagents.	Check the Amplification Plot and Multicomponent Plot. Repeat the assay using properly stored assay components.
		Work in a PCR hood or BSC and clean all work surfaces thoroughly.
	Incorrect baseline setting applied.	Re-analyze samples using auto baseline, or change to manual start and end baseline settings.
PCR inhibition • IPC ΔC_t>2 compared to the NTC *and/or* • S_PEC failure	Inappropriate sample preparation that results in carryover of chemicals from the media/sample matrix.	Repurify the sample preparation and ensure appropriate performance of wash and elution that does not carry over chemicals from reagents.
	High cell number or background nucleic acid.	Dilute sample to less than 1 million cells per extraction
		Reduce cellular background using an appropriate sample preparation method or kit.
		Ensure that the PCR ramp rate is not faster than 1.6°C per second.
NEC or S_NEC Failure	Sample preparation method has background levels of bacteria or fungi genomic DNA that differs from default C_t cut-offs in software.	Use a sample preparation kit with minimal background contamination.
		If needed, establish C_t cut-offs that are better suited to the sample preparation kit. Modify the calling rules in the software to match the new acceptance criteria.
	The matrices or buffers used contain background levels of bacteria/fungi genomic DNA that differs from default C_t cut-offs in the software.	Use samples that have passed internal QC testing to establish cut-offs that are relevant for specific matrices. Modify the calling rules in the software to match the new acceptance criteria. **Note:** Some components added into cell culture preparations (for example plasmids or antibiotics) may carry background bacteria/fungi genomic DNA due to the microbial sources that they are obtained from. These components show lower C_t values for bacteria/fungi targets.

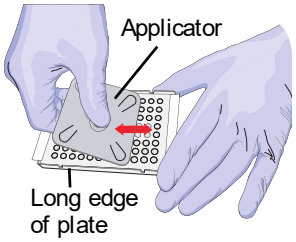
Observation	Possible cause	Recommended action
NEC or S_NEC Failure *(continued)*	External contamination from environment.	If NEC failed while NTC passed, environmental contamination may have occurred during extraction. Clean the contained environment (PCR hood or BSC) and re-extract the samples, then repeat the assay with fresh reagents. For more information, see "Guidelines to prevent contamination" on page 14.
		If both NTC and NEC failed, see "NTC failure" on page 70.
Inconclusive call	Incorrect well assignment, run setting or analysis settings	Check well assignment, run settings and analysis settings (baseline and C_t threshold) are correct and reanalyze.
NTC failure	Positive control unintentionally added.	If NTC wells are positive for Alexa Fluor™ 647, contamination is due to the positive control. Repeat the qPCR. Ensure that the positive control is added into only the PTC wells during plating.
	External contamination from environment.	Clean the contained environment (PCR hood or BSC), and then repeat the assay with fresh reagents. For more information, see "Guidelines to prevent contamination" on page 14. If the negative control continues to show contamination, repeat the assay using a new kit. If the negative control continues to show contamination after changing kits, contact technical support.
No positive control or target-specific dye signal is detected in PTC wells	Improper storage of assay kit components.	Repeat the assay using properly stored assay components.
	Pipetting error	Repeat the assay.
Large variation in C_t values across replicates	Pipetting inaccuracies.	Use calibrated pipettors. Pipet up and down gently five times when dispensing samples to minimize sample retention in pipette tips. Change the pipette tip for each well.

C

Supplemental procedures

Seal the plates

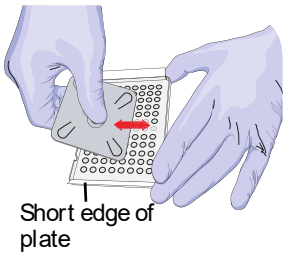
1. Place an optical adhesive cover on the plate, then rub the flat edge of the applicator back and forth along the **long** edge of the plate.



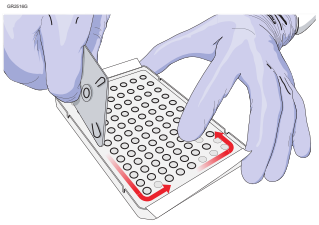
IMPORTANT! Apply significant downward pressure on the applicator to completely seal the wells. Pressure is required to activate the adhesive on the optical cover.

2. Rub the flat edge of the applicator back and forth along the **short** edge (width) of the plate.

3. Rub the edge of the applicator horizontally and vertically between all wells.



4. Rub the edge of the applicator around all outside edges of the plate using small back and forth motions to completely seal around the outside wells. After the seal is applied to the plate, remove the white seal tabs from the short edges of the plate.



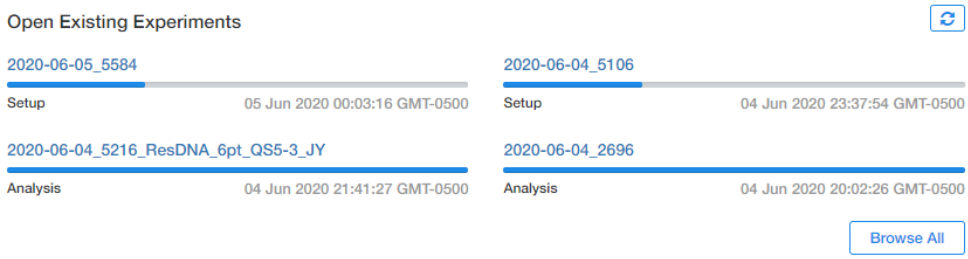
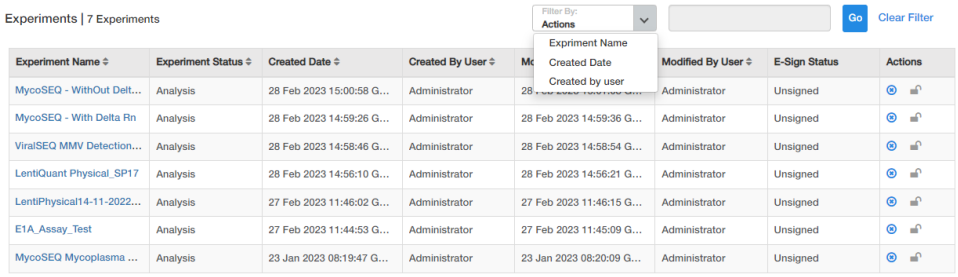
5. Briefly spin down the plate using a centrifuge with a plate adapter.

IMPORTANT! Make sure that the reagents (and no bubbles) are in the bottom of the wells.

Supplemental software procedures (AccuSEQ™ Real-Time PCR Software v3.2 or later)

Open an experiment

You can open an experiment from 2 locations:

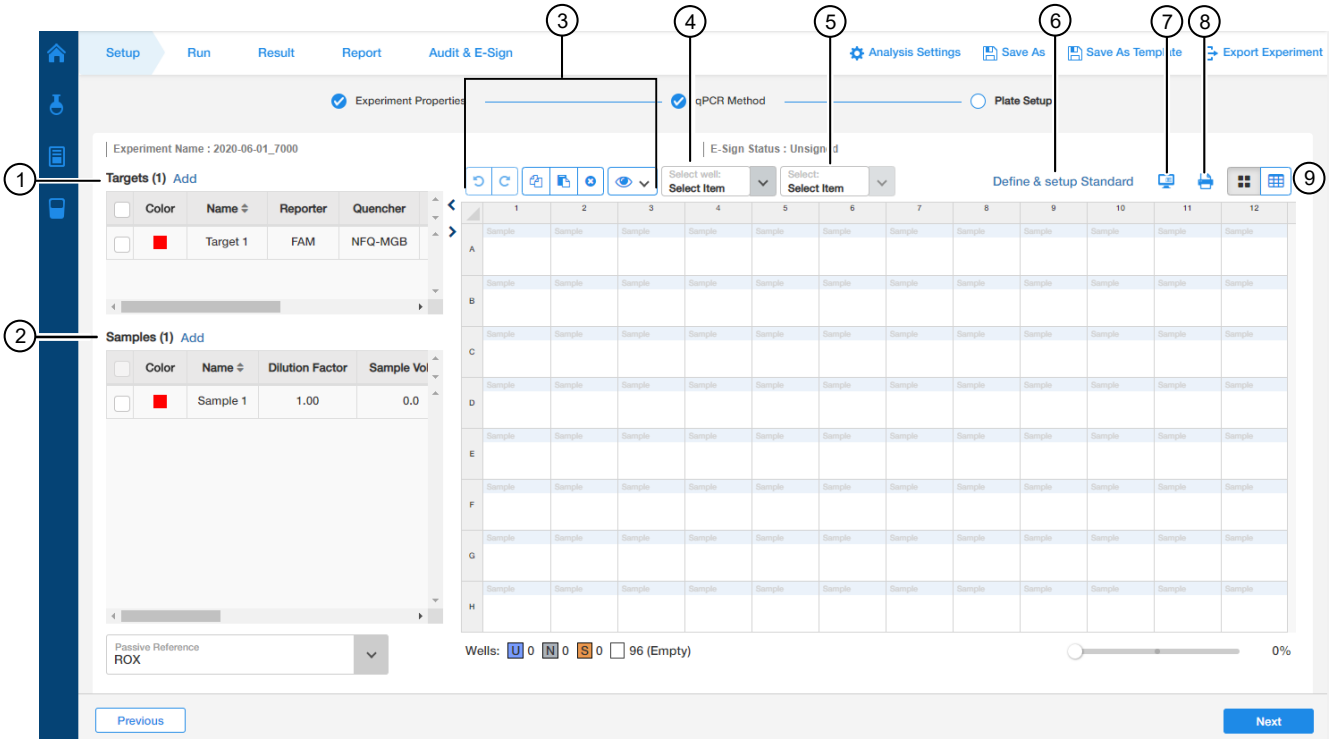
Location	Description
Home screen	Open the most recently run experiments. a. Click (**Home**). b. Click an experiment name, or click **Browse All**. 
Experiments screen	Open previously run experiments. a. Click (**Experiments**). b. If needed, filter, or click the next pages of the list. c. Click an experiment name. 

Define and assign plate and well attributes

This section provides general procedures to set up the plate layout.

IMPORTANT!

- Do not change the targets in the **Targets** pane for predefined assay templates, and do not set up standard curves for presence/absence assays.



1. **Targets** pane
2. **Samples** pane
3. Plate setup toolbar
4. **Select Item** to highlight (Sample, Target, or Task).
5. **Select Item**. For example, Sample 1. Sample 1 replicates are highlighted.
6. **Define & setup Standard**
7. **(View Legend)**
8. **(Print Preview)**
9. **View (Grid View or Table View)**

1. In the **Plate Setup** screen, add targets to the experiment in the **Targets** pane.
   a. Click **Add** above the table of targets.

   b. Enter a target name in the **Name** column.

      When a sample or target name is edited, two entries are added to the audit trail (**Delete** and **Create**).

   c. Click the default dye name (**FAM**) in the **Reporter** column to select a reporter dye from the dropdown.

      The reporter is the dye used to detect the target sequence. See the assay user guide for information about which reporter to use for each target.

   d. Click the default dye name (**NFQ-MGB**) in the **Quencher** column to select a quencher dye from the dropdown.

      The quencher is the dye used to quench the reporter dye signal.

   e. (*Optional*) Click the color in the **Color** column to select a different color to represent the target.

f. Repeat this workflow for all targets in the experiment.

2. In **Plate Setup** screen, add samples to the experiment.
   a. Click **Add** above the sample table.
   b. Enter a sample name in the **Name** column.
      When a sample or target name is edited, two entries are added to the audit trail (**Delete** and **Create**).
   c. Enter the dilution factor, sample volume, spike volume, spike standard concentration, spike input, and comments if needed.
   d. (*Optional*) Click the color in the **Color** column to select a different color to represent the sample.
   e. Repeat the workflow for all samples in the experiment.
3. Add targets to wells.
   a. In **Plate Setup** screen, click or click-drag to select plate wells in the **(Grid View)** of the plate, see “Select plate wells” on page 77.
   b. Check the checkbox next to the desired target in the **Targets** table.
      If the selected wells contain mixed assignments (indicated by a), click the next to the desired target to assign the target to all selected wells (indicated by a).
   c. Click the **U** in the **Task** column to select the detection task for the selected wells.

      Table 9 Detection tasks

Task	Description
U—Unknown (*default*)	Wells contain samples with unknown quantities of target.
S—Standard (*standard curve and relative standard curve experiments only—not used in detection assays*)	Wells contain samples with known standard quantities. If standard is selected as the detection task, enter the standard quantity in the **Quantity** column.
N—Negative control	Wells contain water or buffer instead of sample. Wells do not contain target.

   d. Repeat workflow for all target assignments in the plate layout.
4. Assign samples to wells.
   a. In **Plate Setup** screen, click or click-drag to select plate wells in the **(Grid View)** of the plate, see “Select plate wells” on page 77.
   b. Check the checkbox next to the desired sample in the **Samples** table.
      If the selected wells contain mixed assignments (indicated by a), click the next to the desired sample to assign the sample to all selected wells (indicated by a).
      Each well can only have one sample assigned to it.

c. Repeat for all samples in the experiment.

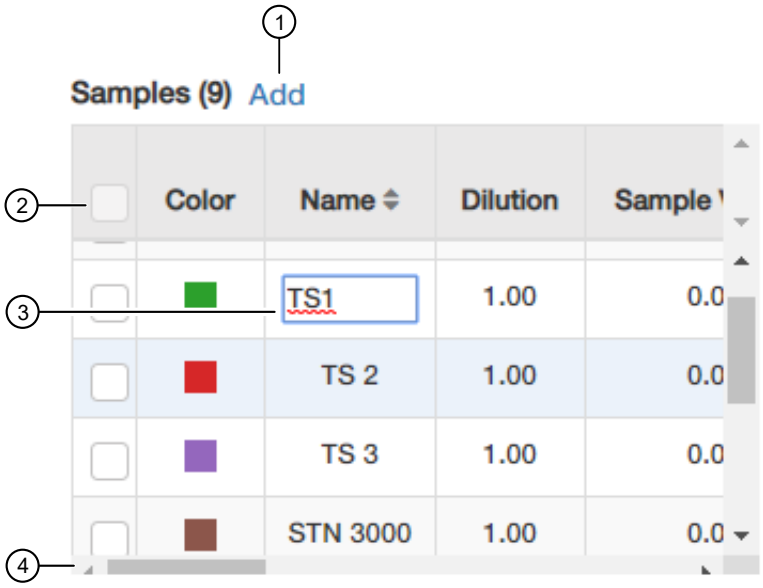


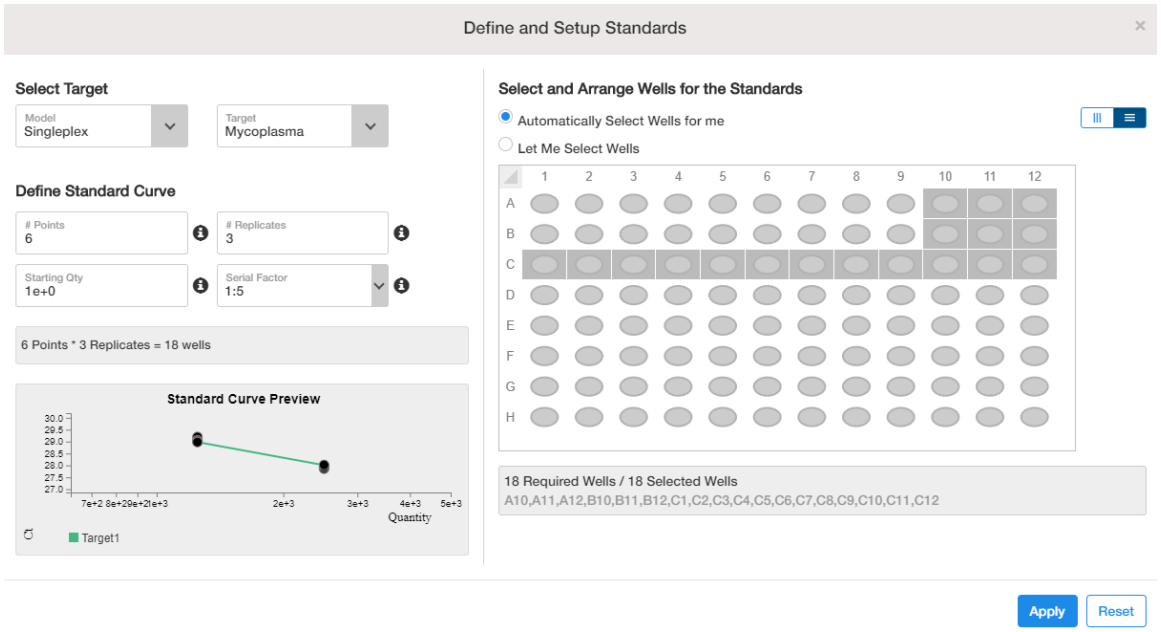
Figure 26 Example Samples table

① **Add** button
② Checkbox—Select **Targets** and **Samples** to go in the selected well.
③ Textbox—Click the name to edit.
④ Scrollbar—Use to scroll to more properties.

5. (*Optional*) Use the plate setup toolbar (above the plate) to make edits to the plate.
   - Click **(View)** to show/hide the sample name, sample color, and target from the view.

6. (*Optional, for assays using standard curves*) Click **Define & setup Standard** to set up a standard curve.

   Do not set up standard curves for presence/absence assays.



a. Select **Singleplex** or **Multiplex**.

b. Select or enter the target.

c. Define the number of points and replicates, the starting quantity, and the serial dilution factor. The **Standard Curve Preview** pane shows a representative example only.

d. (*Optional*) Select **Automatically Select Wells for me** or **Let Me Select Wells** used for the standard curve.

e. Click **Apply** to save before closing the window.

Standards can be renamed using the sample table in the **Plate Setup** pane. For example, SD1, SD2, and SD3.

7. (*Optional*) Double-click a well to enter comments for the selected well.

8. Select a reference dye from the **Passive Reference** dropdown list (lower-left of screen).

Select plate wells

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

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 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 plate wells in the **(Table View)**.

To	Action
Select a single well	Click a row in the table
Select non-contiguous wells	Ctrl+click rows in the table
Deselect a single well	Ctrl+click the selected row

Results screen

Use the **Results** screen to:

- View the plate calls (factory default presence/absence assays).
- View the well calls (factory default presence/absence assays).
- Manually edit **Unknown** well calls (factory default presence/absence assays).
- Omit wells and reanalyze the experiment.

  The **Analyze** button is disabled if the user does not have permission to both omit wells and analyze data.
- View plots.
- E-Sign the experiment.

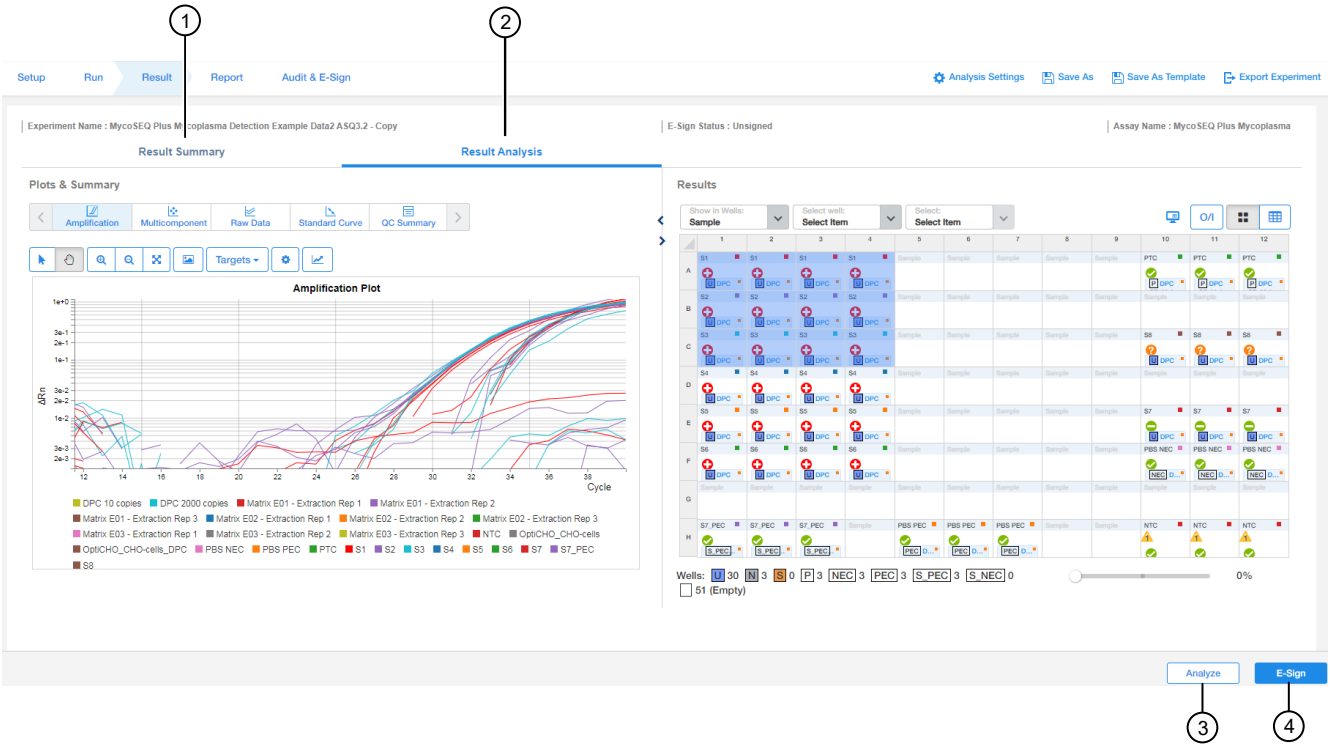


Figure 27 Example of a Results screen for a MycoSEQ™ assay

① **Result Summary** tab—only available for factory default presence/absence assays
② **Result Analysis** tab
③ **Analyze** button—Use after omitting wells.
④ **E-Sign** button

Supplemental software procedures (AccuSEQ™ Real-Time PCR Software v2.2 or later)

Add and remove samples

1. Add a new sample to the experiment.
   a. On the **Plate Setup** screen, click **Add New Sample** in the toolbar above the sample table.
   b. Enter a sample name, then select the sample color.
2. (*Optional*) Add samples from the sample library.
   a. On the **Plate Setup** screen, click **Add Saved Sample** in the toolbar above the sample table.
   b. In the Sample Library, select one or more samples to add to the experiment.
   c. Click **Add Selected Samples**.
3. (*Optional*) Save a sample to the sample library.
   a. On the **Plate Setup** screen, click a row in the sample table to select the sample.
   b. Click **Save Sample** in the toolbar.
      If a sample with the same name is already saved to the Sample Library, the software asks to replace the sample in the library.
4. (*Optional*) Delete a sample from the experiment.
   a. On the **Plate Setup** screen, click a row in the sample table to select the sample.
   b. Click **Delete Sample**.
      If the sample is assigned to wells in the plate, the software asks to remove the sample assignments from the wells.

Define samples

On the **Plate Setup** screen, select the **Define Targets and Samples** tab to define the samples to test in the reaction plate. The samples in the experiment are listed in the sample table.

1. Enter the sample name.
   a. In the sample table, click a cell in the **Sample Name** column for the sample to name
   b. Enter up to 100 characters to identify the sample.
2. Select a sample color.
   a. In the sample table, click ⌄ in the **Color** column for the sample to define.

b. Select a color.

- **None**—Associate no color with the sample
- A color square—Replace the color associated with the sample
- **More Colors**—Select from more colors

Assign targets to wells

1. In the **Plate Setup** screen, select wells using the plate layout or the well table, see “Select wells using the plate layout” on page 81 and “Select wells using the well table” on page 81.
2. Check the **Assign** checkbox next to the desired target in the **Assign targets(s) to the selected wells** pane.

Note: If the selected wells contain mixed assignments (indicated by a), click the next to the desired target to remove the target from the selected wells, then click the checkbox again to assign the target all selected wells (indicated by a).

---

3. Click the dropdown in the **Task** column to select the detection task for the selected wells.

Table 10 Detection Tasks

Task	Description
U– Unknown (*default*)	Wells contain samples with unknown quantities of target. Unknowns can be setup separately, see “Set up unknowns” on page 81.
N– Negative control	Wells contain water or buffer instead of sample. Wells do not contain target. Negative controls can be setup separately, see “Set up negative controls” on page 82.

4. Repeat steps 1–3 for all targets.

Assign a sample to a well

1. On the **Plate Setup** screen, select wells using the plate layout or the well table.

   See “Select wells using the plate layout” on page 81 and “Select wells using the well table” on page 81.

2. Click the checkbox next to the desired sample to assign it to a well.

   When the checkbox is selected, the sample is assigned to the selected wells.

Note: Only 1 sample can be assigned to a well. If the selected wells contain mixed assignments (indicated by a), you must remove the existing sample assignments before making new sample assignments.

Select wells using the plate layout

On the **Plate Setup** screen, select wells using the plate layout to view well information or to make well assignments.

On run screens or analysis screens, select wells using the plate layout to view results in the plots.

1. Click a well in the plate layout to select it.
2. (*Optional*) To select a block of wells, click one well to define a corner, then drag the cursor to the opposite corner until all desired wells are selected.

   A block of wells can also be selected by clicking one well to define a corner, then pressing the Shift key while clicking another well on the opposite corner.
3. (*Optional*) To select all wells in a column, click the column heading .
4. (*Optional*) To select all wells in a row, click the row heading.
5. (*Optional*) To select discontinuous wells, press the Ctrl key while clicking the desired wells.

   If a well is clicked without pressing the Ctrl key, any previously selected wells are deselected.
6. (*Optional*) To select all wells in the plate, click the top-left corner of the plate grid.
7. (*Optional*) Press the Ctrl key while clicking a selected well to deselect it.

Select wells using the well table

On the **Plate Setup** screen, select wells using the well table to view well information or to make well assignments.

On run screens or analysis screens, select wells using the well table to view results in the plots.

1. Click a row in the well table to select a well.
2. (*Optional*) To select continuous rows, click one row in the table and drag the cursor up or down until all desired wells are selected.
3. (*Optional*) To select discontinuous rows, press the Ctrl key while clicking the desired wells.

   If a well is clicked without pressing the Ctrl key, any previously selected wells are deselected.
4. (*Optional*) To deselect a well, press the Ctrl key while clicking a row in the well table.

Set up unknowns

Ensure that targets and samples are already assigned to wells.

1. On the **Plate Setup** screen, select the **Assign Targets and Samples** tab, then select wells using the plate layout or the well table.
2. In the **Assign target(s)** table, click U as the task for the targets.

Set up negative controls

Ensure that targets are already assigned to wells.

1. On the **Plate Setup** screen, select wells using the plate layout or the well table.
   Ensure that no sample is assigned to the selected wells.
2. In the **Assign target(s)** table, click N as the task for the targets.
3. Repeat these steps for all negative controls in the plate.

Edit plot properties

1. On the screen with the plot that you want to edit, click above the plot.
2. Edit the plot properties.
   - Click the **General** tab to edit the plot title text, font, or color and select whether to show the plot title.
   - Click the **X Axis** tab to edit the x axis label text, font, or color; select the tick marks and tick mark labels to show; and select the range to show.
   - Click the **Y Axis** tab to edit the y axis label text, font, or color; select the tick marks and tick mark labels to show; and select the range to show.
3. Click **OK**.

Review the plots

1. Click **Plot Analysis** in the **Experiment Menu** pane, then click **Multiple Plots View**.
   All available plots for the experiment are shown.
2. Select **Amplification Plot**, **Standard CurveMelt Curve**, or **Presence/Absence Plot**.
   The plots available are determined by the type of experiment.
3. Click a sample in the plate layout or the well table to display the corresponding plots.
   To change plot properties, see “Edit plot properties” on page 82.
4. Click **Multicomponent Plot** or **Raw Data** to further investigate results.
5. *(Optional)* Print a plot.
   a. On the analysis screen that displays the desired plot, click above the plot.
   b. In the **Print** dialog box, select the printer and print options, then click **OK**.
6. *(Optional)* Save a plot as an image file.
   a. On the analysis screen that displays the desired plot, click above the plot.
   b. Select a file name and location for the file, then click **Save**.

7. *(Optional)* Create slides from plots.

   You must have administrator or scientist privileges to access this function.

   Analysis plots are not available for slides until the run status is complete and the data are analyzed.

   You must have Microsoft™ PowerPoint™ software installed on your computer to view slides.

   a. If not already open, open the experiment that contains the data that you want to include in a slideshow presentation.

   b. Select **File** ▸ **Create Slide**.

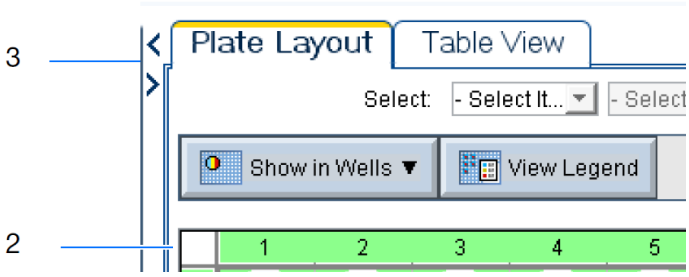
   c. Select the slides for your presentation, then click **Create Slides**. The software opens a Microsoft™ PowerPoint™ presentation (PPT) with the slides you specified.

View the Raw Data Plot

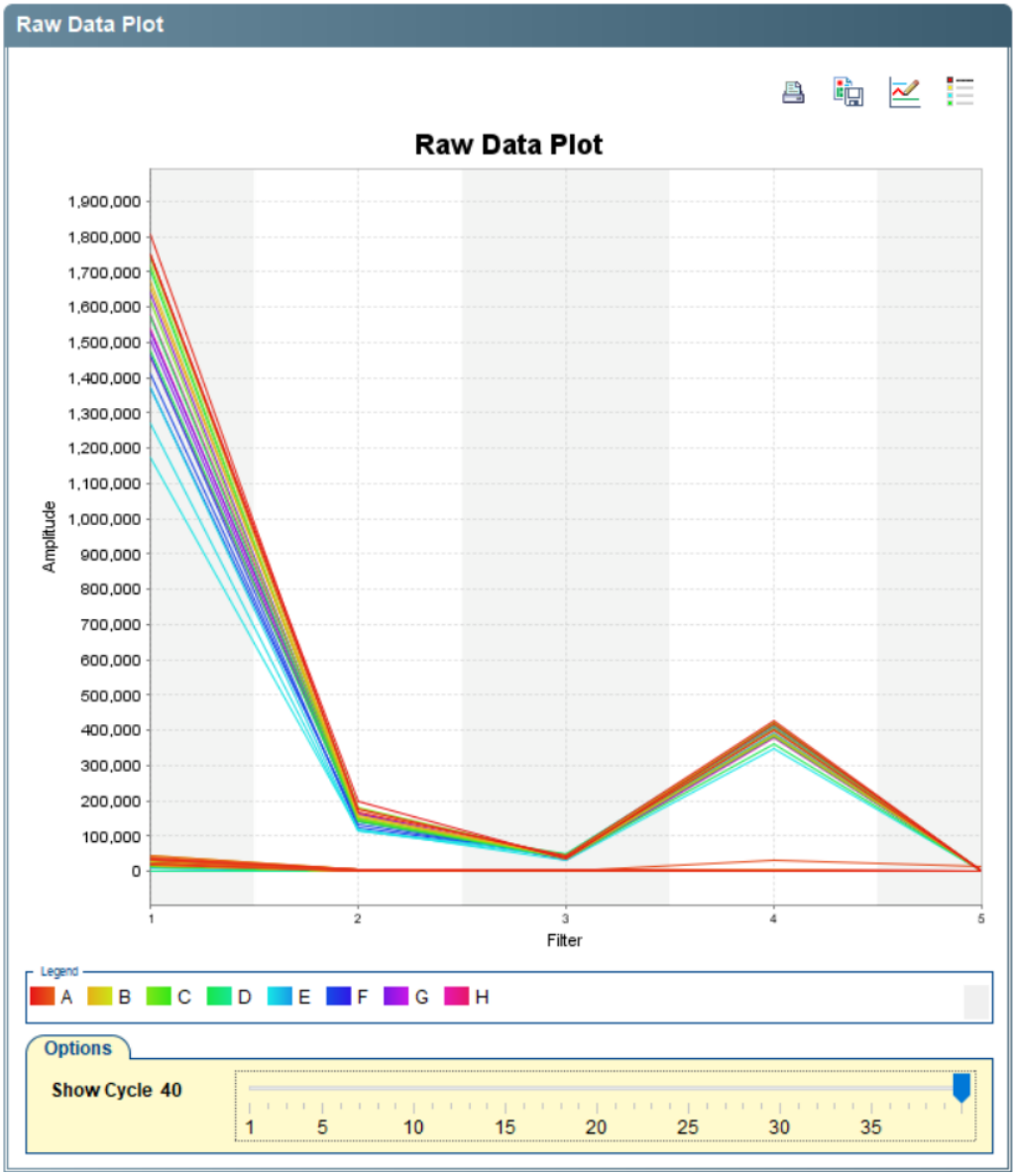
1. From the navigation pane, click **Analysis** or **Plot Analysis**, then click **Raw Data Plot**.

2. Show all 96 wells in the **Amplification Plot** by clicking the upper left corner of the plate layout in the **Plate Layout** tab.

3. *(Optional)* For an expanded view of the plot plot, click the top-left corner of the plate layout in the **Plate Layout** tab.



4. Use the **Options** tab to select the range of raw data shown (Cycle 1 to Cycle 40).



5. Select wells in the plate layout to view in the raw data plot .

6. *(Optional)* Click (Hide the plot legend).

   This is a toggle button. When the legend is hidden, the button changes to Show a legend for the plot.

D Acceptance criteria

PCR cycle threshold (C_t) values are used to determine the presence or absence of the target with the SteriSEQ™ Rapid Sterility Testing Kit. This section defines the acceptance criteria for C_t values in more detail and explains how they are implemented in the assay.

Threshold cycle value (C_t)

The threshold cycle (C_t) value is defined as the number of cycles required for the fluorescent signal to cross the threshold or exceed background fluorescence. C_t values are inversely proportional to the amount of target nucleic acid in the reaction. The lower the C_t value, the greater the amount of target in the reaction.

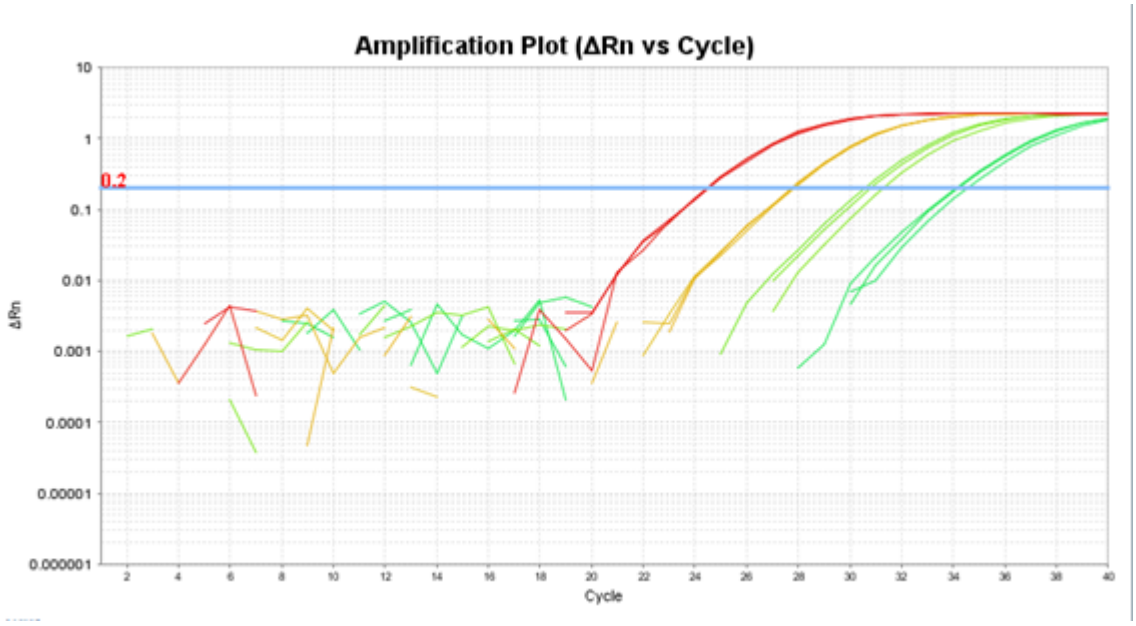


Figure 28 Dilution series of positive template control

Amplification plots generated from a dilution series of positive template control, from 10,000 to 10 genome copies per reaction. Reactions with a high concentration of DNA have a lower C_t value.

In routine testing, if the ABY™ C_t value in unknown samples shows no inhibition when compared to the ABY™ C_t in the NTCs, the wells are determined to have no PCR inhibition and absence of bateria and fungi signal indicates no detected contamination. For samples that do not go through a sample preparation process, a FAM™ C_t value ≥36.00 and VIC™ C_t value ≥35.5 in unknown samples suggests that no amplification of the targets occurred, indicating the sample is negative for fungi and bacteria DNA. No further analysis is needed. For samples that have undergone third-party sample preparation, the appropriate C_t cut-offs can vary depending on the background levels of bacteria and

fungi inherently present in the kits. In this case, NEC and S_NEC samples can be used to establish an appropriate criteria. The sample PEC C_t can also be taken into consideration, if used.

Results interpretation using the acceptance criteria

The specific acceptance criteria values in this guide (see "Acceptance criteria—AccuSEQ™ Real-Time PCR Software v3.2.1 or later" on page 40 and "Acceptance criteria—AccuSEQ™ Real-Time PCR Software v2.2.1 or later" on page 60) are for reference purposes only and should be independently verified or validated specific to the sample matrix being tested. During method development and qualification, detailed analysis of data can be performed as part of the process of sample preparation optimization and establishment of cut-off values for sample-specific acceptance criteria.

Other acceptance criteria considerations

No two cell culture sample types are the same. The positive and negative cut-off values provided in this guide were established from the analysis of hundreds of samples. However, there are cases where unknown, sample-specific cut-off values should be established and applied. For some sample types, it is possible that the C_t values for negative unknown samples will fall outside of the recommended ranges. In these cases, use the guidance above to establish user and sample specific positive and negative cut-off values. These values can be established for one or more results parameters to provide the user with the highest possible level of confidence in both the negative and positive results obtained during routine testing.

E Kit specificity and sensitivity

Sensitivity

Sensitivity of the PCR using this kit is 5 to 25 genome copies of target DNA per reaction. Sensitivity of the assay in real cell culture samples depends on the quality of the sample preparation method.

Primary factors that can affect detection sensitivity:

- Extraction efficiency of DNA from the sample preparation method
- Purity of DNA from sample preparation method
- High non-target genomic background

Secondary factors that may affect detection sensitivity:

- PCR inhibitors that affect PCR efficiency
- High protein or cellular matrix background that can interfere with DNA extraction efficiency or PCR efficiency

Kit specificity

The SteriSEQ™ Rapid Sterility Testing Kit can detect more than 16,000 different bacterial species and more than 2,600 fungal species. The kit does not detect other genera or cell line DNA. Samples derived from bacteria or fungi sources may have residual bacteria or fungi DNA. Some of sources of this type of residual DNA contamination are:

Component	Potential contaminating source
Plasmids	Bacteria
Enzymes (eg. proteinase)	Bacteria
Antibiotics	Fungi
Serum	Bacteria

Inclusivity—Detectable species

The SteriSEQ™ Rapid Sterility Testing Kit is validated for the following genome species. For a complete list of species, contact Technical Support.

Kingdom	Species	Source
Fungi	*Aspergillus brasiliensis*	ATCC 16404D-2
Fungi	*Candida albicans*	ATCC 10231DQ
Bacteria	*Bacillus subtilis*	ATCC 6633DQ
Bacteria	*Clostridium sporogenes*	ATCC 11437DQ
Bacteria	*Staphylococcus aureus*	ATCC 6538DQ
Bacteria	*Pseudomonas aeruginosa*	ATCC 9027DQ
Bacteria	*Escherichia coli*	ATCC 8739DQ
Bacteria	*Salmonella enterica*	ATCC 13311DQ
Bacteria	*Cutibacterium acnes*	ATCC 11827
Bacteria	*Streptococcus pyogenes*	ATCC 19615DQ
Bacteria	*Bacteroides fragilis*	ATCC 25285DQ
Bacteria	*Staphylococcus epidermidis*	ATCC 12228DQ
Bacteria	*Clostridioides difficile*	ATCC 43598DQ
Bacteria	*Enterococcus hirae*	ATCC 10541DQ
Bacteria	*Streptococcus equinus*	ATCC 9812
Bacteria	*Streptococcus pneumoniae*	ATCC 33400
Bacteria	*Kocuria rhizophila*	ATCC 9341D-5

Exclusivity—Undetectable genomes

Species/DNA	Concentration per qPCR reaction	Source
Adenovirus 2	30 ng	ATCC VR-846D
Kanamycin resistance gene	300,000 copies	Synthetic gene block
Ampicillin resistance gene	300,000 copies	Synthetic gene block
HEK 293 genomic DNA	300 ng	ATCC CRL-1573D
CHO genomic DNA	300 ng	A29127

(continued)

Species/DNA	Concentration per qPCR reaction	Source
Baculovirus vector genes	300,000 copies	Synthetic gene block
Lentivirus vector genes	300,000 copies	Synthetic gene block
Bovine genomic DNA	3 ng	Zyagen GB-100M

F

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

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

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

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

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

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

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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

G Documentation and support

Related documentation

Document	Pub. No.
AccuSEQ™ Real-Time PCR Software v3.2 User Guide	MAN0029199
AccuSEQ™ Real-Time PCR Software v2.2 User Guide	MAN0029201
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4347828

For information about new assays and updated product documentation, go to **thermofisher.com**.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

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

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.