applied biosystems

MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit

USER GUIDE

for use with:
PrepSEQ™ 1-2-3 Mycoplasma Nucleic Acid Extraction Kit
PrepSEQ™ 1-2-3 Nucleic Acid Extraction Kit
PrepSEQ™ Express Nucleic Acid Extraction Kit

Catalog Numbers 4460623, 4460626
Publication Number 4465874
Revision G

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: 4465874 G (English)

Revision	Date	Description
G	11 August 2023	Updated to include run and analysis information for AccuSEQ™ Real-Time PCR Software v3.2.
F	22 April 2022	Update to the instructions in "Prepare the PCR reactions (complex or high cell density samples)" on page 39.
E	7 December 2022	Updated Appendix A, "Troubleshooting". Included tips for complex or high cell density samples.
D	28 September 2020	Updated to include run and analysis information for AccuSEQ™ Real-Time PCR Software v3.1.

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 MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit detects *Mycoplasma* species simply, reliably, and rapidly. To detect the presence of these microorganisms, the assay uses the polymerase chain reaction (PCR) to amplify a target unique to a wide variety of *Mycoplasma* species.

Contents and storage

Table 1 MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit (Cat. No. 4460623)

Contents [1]	Cap color	Amount [2]	Storage
Box 1: MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit			
10× *Mycoplasma* Real-Time PCR Primer Mix	blue	325 µL	–25°C to –15°C on receipt 2–8°C after first use
Negative Control	white	1,000 µL	
2× *Power* SYBR™ Green PCR Master Mix	white	2 × 1,000 µL	–25°C to –15°C on receipt, protected from light. 2–8°C after first use, protected from light

Table 1 MycoSEQ Mycoplasma Real-Time PCR Detection Kit (Cat. No. 4460623) *(continued)*

Contents [1]	Cap color	Amount [2]	Storage
Box 2: MycoSEQ™ Discriminatory Positive/Extraction Control			
MycoSEQ™ Discriminatory Positive/ Extraction Control, 1,000 copies/µL	yellow	700 µL	–25°C to –15°C

[1] To purchase the MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit that includes the PrepSEQ™ Mycoplasma Sample Preparation Kit, use Catalog Number 4460626.

[2] The kit contains reagents for 100 reactions.

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
Instrument	
QuantStudio™ 5 Real-Time PCR System with AccuSEQ™ Software v3.1 or later	Contact your local sales representative
Generic consumables	
Disposable gloves	MLS
Aerosol-resistant pipette tips	MLS
Pipettors: • Positive-displacement • Air-displacement • Multichannel • Repeat (1µL–1mL)	MLS
Consumables for the QuantStudio™ 5 Real-Time PCR System	
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4346906
MicroAmp™ 96-Well Base	N8010531
MicroAmp™ Optical Adhesive Film	4360954
MicroAmp™ Adhesive Film Applicator	4333183

Workflow

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2 Methods

IMPORTANT! This chapter describes how to prepare and run PCR samples using AccuSEQ™ Software v3.1 or later on the QuantStudio™ 5 Instrument (0.1 mL block). If you are using AccuSEQ™ software v2.x on the 7500 Fast, see Appendix C, "Use the kit with 7500 Fast System AccuSEQ™ Real-Time PCR Detection Software v2.x". If you are using SDS software v1.4 or later, see Appendix D, "Use the kit with 7500 System SDS Software v1.4 or later".

IMPORTANT! For information on how to avoid PCR contamination, see Appendix G, "Good PCR practices".

Prepare the sample

Prepare the DNA template for the PCR reactions using the PrepSEQ™ 1-2-3 Mycoplasma Nucleic Acid Extraction Kit.

For more information, see:

- The *PrepSEQ™ Sample Preparation Kits for Mycoplasma, MMV, and Vesivirus User Guide* (Pub. No. 4465957)
- The *PrepSEQ™ Express Nucleic Acid Extraction Kit for Mycoplasma, MMV, and Vesivirus Detection User Guide* (Pub. No. MAN0016799)

Prepare the kit reagents and premix solution

1. Thaw all kit reagents completely.
2. Vortex briefly, then spin down the reagents.
3. Prepare the Premix Solution according to the following table.

Component for premix solution	Volume for one 30-µL reaction	Volume for four 30-µL reactions[1]
Power SYBR™ Green PCR Master Mix, 2×	15.0 µL	66.0 µL
Mycoplasma Real-Time PCR Primer Mix, 10×	3.0 µL	13.2 µL
Total premix solution volume	**18.0 µL**	**79.2 µL**

[1] Includes 10% excess to compensate for pipetting errors.

4. Mix the Premix Solution by gently pipetting up and down, then cap the tube.

Prepare the PCR reactions

1. Dispense the following into each well to be used, gently pipetting at the bottom of the well.

To prepare…	In each tube or well…
Negative control reaction	• Add 18 µL of Premix Solution • Add 12 µL of Negative Control (water)
Unknown or spiked sample reaction	• Add 18 µL of Premix Solution • Add 10 µL of unknown sample • Add 2 µL of Negative Control (water)
Inhibition-control reaction	• Add 18 µL of Premix Solution • Add 10 µL of unknown sample • Add 2 µL of the Discriminatory Positive Control (DPC)
Positive control reaction	• Add 18 µL of Premix Solution • Add 2 µL of the DPC • Add 10 µL of Negative Control (water)

Note: The MycoSEQ™ *Mycoplasma* Discriminatory Positive/Extraction Control can be used as a spike control that is added to the unknown sample or lysate before sample preparation.

2. Mix each sample by gently pipetting up and down.
3. Seal the plate with MicroAmp™ Optical Adhesive Film. See “Seal the plates” on page 74.
4. Briefly centrifuge the reaction plate.

Setup, run, and analyze samples with AccuSEQ™ Software v3.1 or later on the QuantStudio™ 5 Instrument

Sign in to the AccuSEQ™ Real-Time PCR Software

Thermo Fisher Scientific recommends configuring the Windows™ screen save feature to require sign in when the screensaver is activated. This prevents other users from accessing the AccuSEQ™ Real-Time PCR Software and making changes.

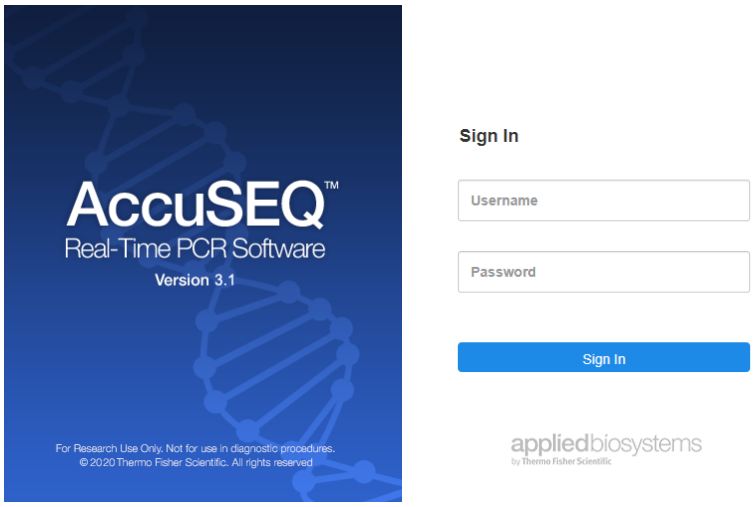
1. Start the AccuSEQ™ Real-Time PCR Software by double-clicking the AccuSEQ icon .

   Note: Ensure that Google Chrome™ is the default browser.

2. Enter the **Username**, then **Password**.

   (First login only) The default username is **Administrator** and the default password is **Administrator**.

3. Click **Sign in**.



The following restrictions may be seen in the software:

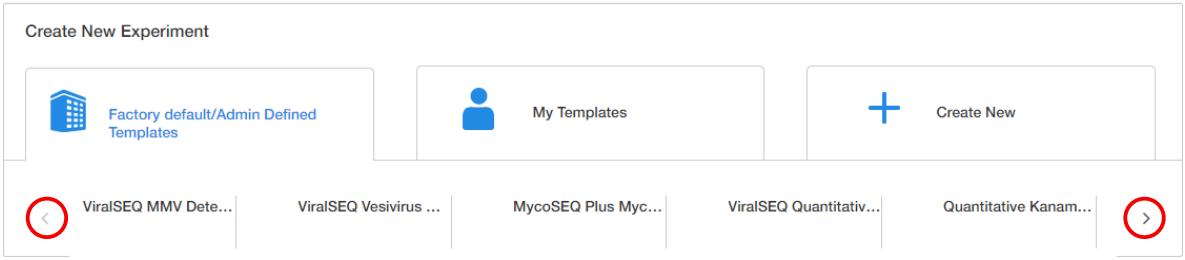
- Access to functions in the software is based on the permissions associated with the individual user account.
- If a user account does not have permission to perform a function, the function is grayed out in the software.
- If the system is configured for password expiration, you will be periodically prompted to change your password. If the system is configured to monitor failed log in attempts, you will be locked out of the software if you incorrectly enter your user name or password more than a specified number of times.

Create a MycoSEQ™ experiment

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

   Note: To create a copy of an existing MycoSEQ™ experiment, see chapter 6 of the *AccuSEQ™ Real-Time PCR Software v3.2 User Guide* (Pub. No. MAN0029199).
   Custom MycoSEQ™ experiments must be set up as **Quantitation-Standard Curve** type experiments with SYBR™ chemistry.

   To navigate to through the available templates click the arrows to either side of the available templates.



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

      Note: Names must be unique. Deleted experiment names can not be reused.

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

      Note: Names and comments are not editable post analysis.

      Note: Comments are not editable post analysis.

      Default MycoSEQ™ settings (These settings cannot be changed in the default template. See the *AccuSEQ™ Real-Time PCR Software v3.2 User Guide* (Pub. No. MAN0029199) for creating custom templates).
      - **Experiment Type—Quantitation-Standard Curve**
      - **Chemistry—SYBR™ Green Reagents**
      - **Ramp Speed—Standard - 2hrs**

   c. Click **Next**.

      Note: Experiment names cannot be changed after this step.

3. In the **qPCR Method** pane of the **Setup** tab, view the default volume and cycling conditions (These settings cannot be changed in the default template. See the *AccuSEQ™ Real-Time PCR Software v3.2 User Guide* (Pub. No. MAN0029199) for creating custom templates).

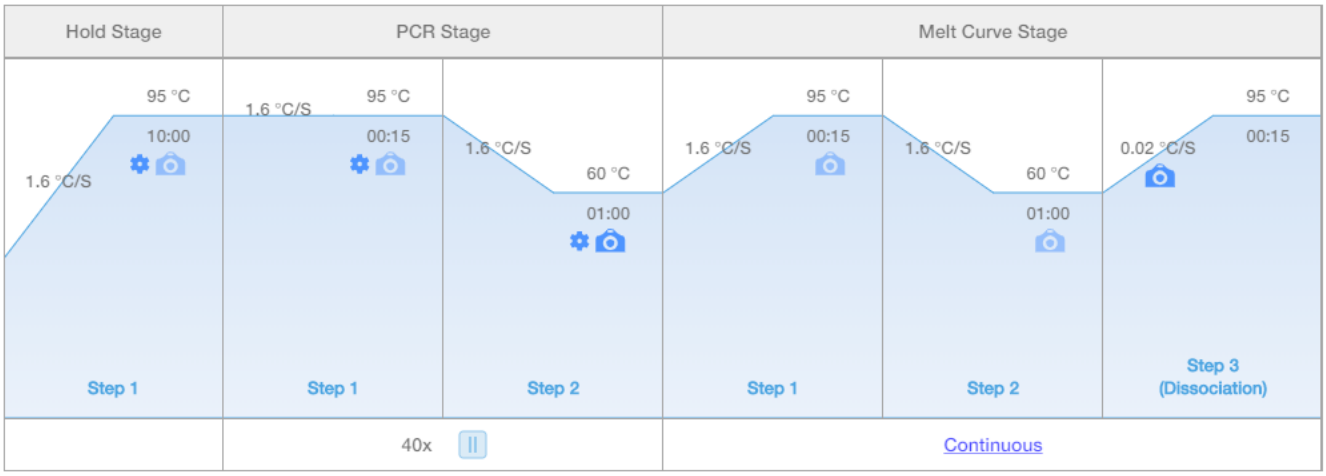


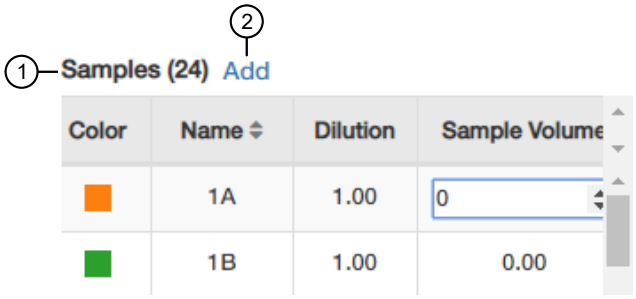
Figure 1 MycoSEQ™ template default cycling conditions

4. Click **Next**.

5. In the **Samples** table in the **Plate Setup** pane, enter the sample name. Click **Add** to add more samples if needed.

Note: Only the sample name is necessary for experiments run from the factory default **MycoSEQ** template.

IMPORTANT! Do not change the **Targets**.



① **Samples** pane
② **Add**—adds more samples

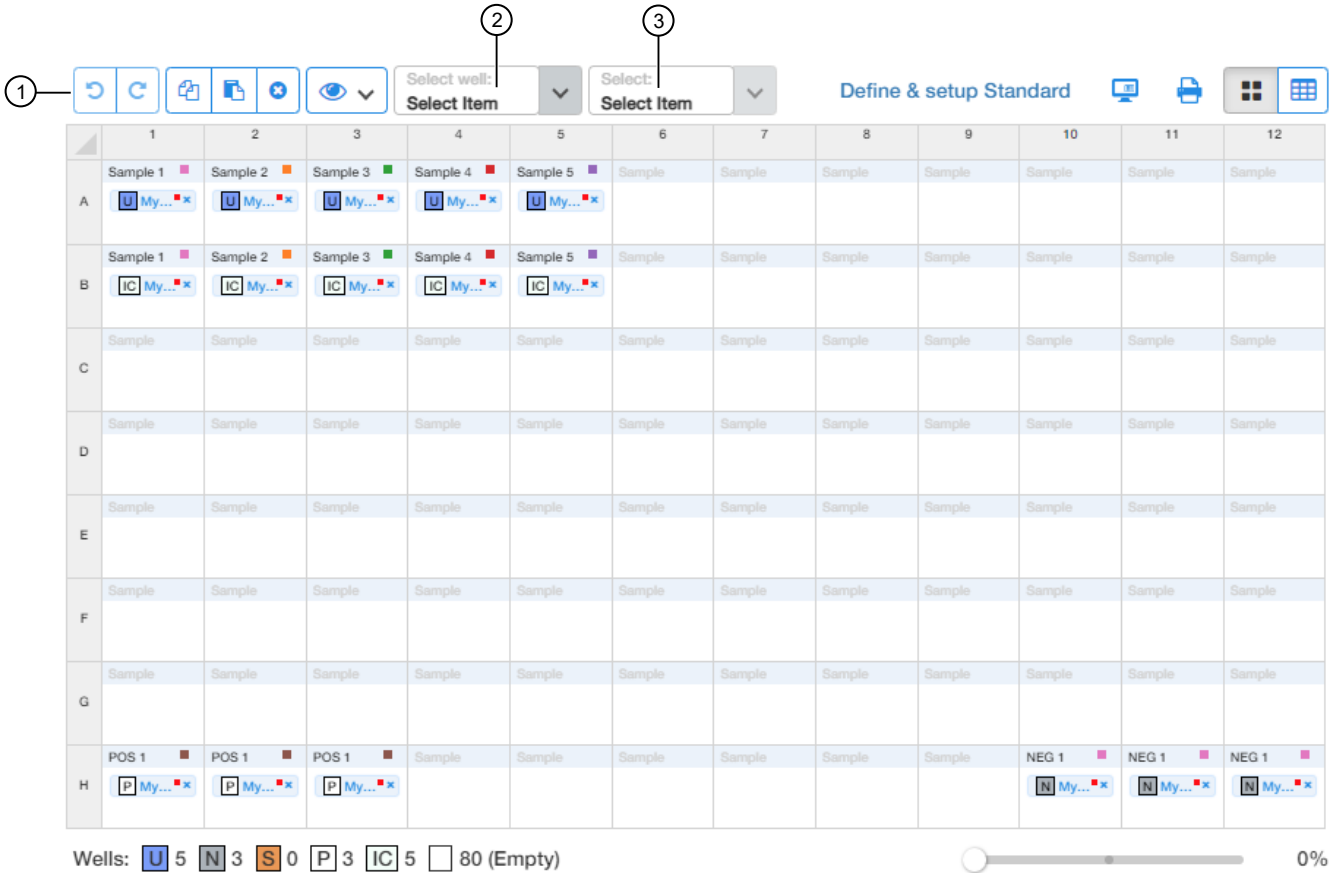


Figure 2 MycoSEQ™ template default sample plate layout

① Toolbar (in order: Undo, Redo, Copy, Paste, Delete, View)

② Select Item to highlight (Sample, Target, or Task).

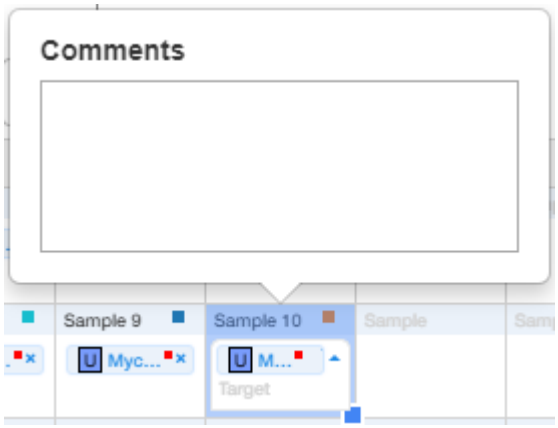
Note: If more samples are added, the **Target** displays as **S** in the wells (experiment type–**Quantitation**).

③ Select Item. For example, Sample 1. Sample 1 replicates are highlighted.

Table 2 Plate well descriptions

Name	Description
Sample 1 to Sample 5	5 default samples with and without inhibition control (IC)
POS 1	Positive control in triplicate
NEG 1	No template control in triplicate

6. (*Optional*) Double-click wells to add comments. Comments can also be added post-analysis.



7. Click **Next**.
   The **Run** tab is displayed.

8. Experiments are auto-saved in the software. To save changes made to the experiment, exit the experiment. The software prompts you to save changes. Click **Yes**.

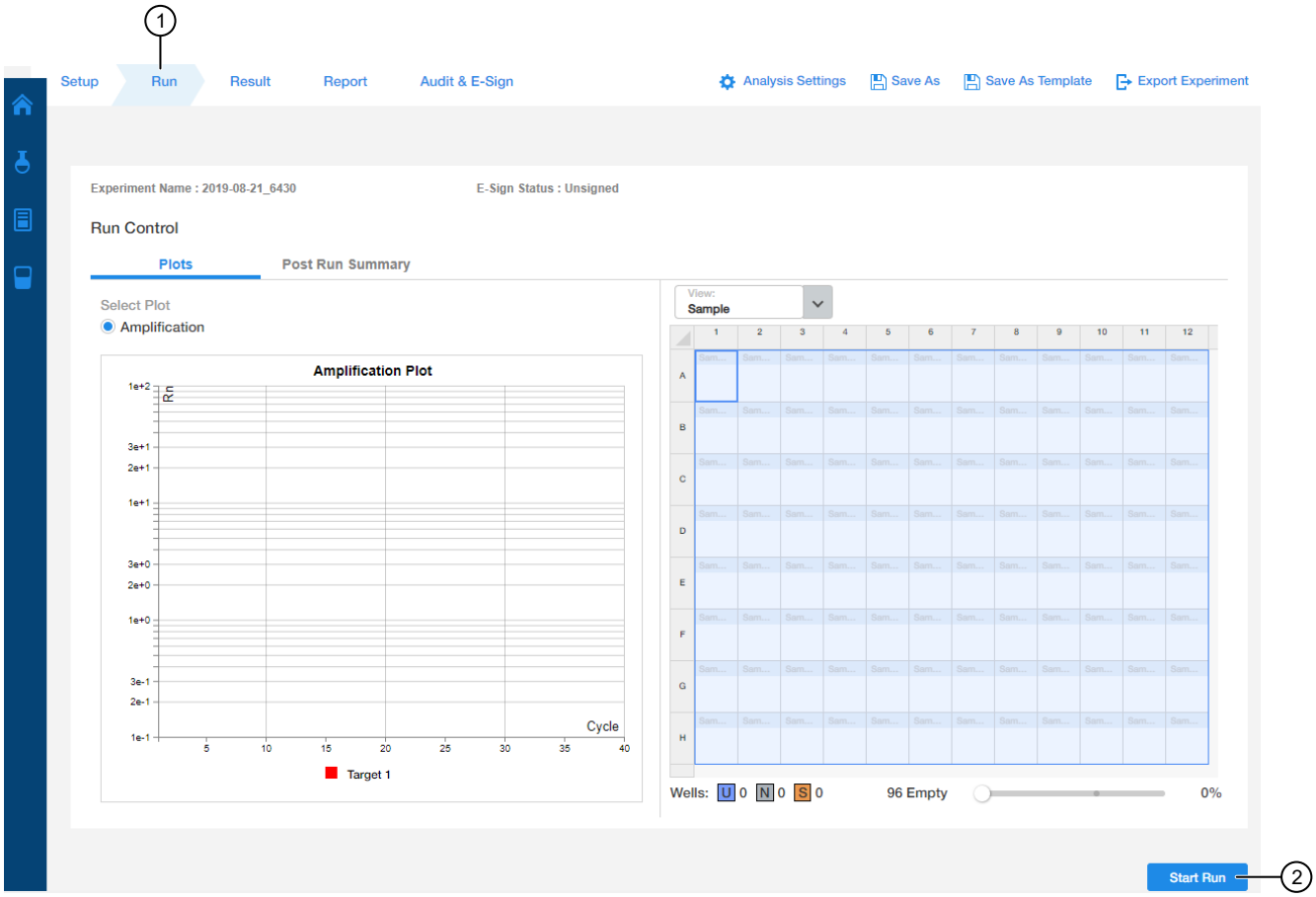
   Note: Clicking **Save As** creates a copy of the experiment.

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

Start the run (QuantStudio™ 5 Real-Time PCR Instrument)

Start the run in the AccuSEQ™ Software v3.1 or later.

Option	Description
If the experiment is open	Click **Start Run**.
If the experiment is closed	a. Open the experiment. b. Click the **Run** tab. c. Click **Start Run**.



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

A message stating **Run has been started successfully** is displayed when the run has started.

Monitor the run

During the instrument run, you can monitor the run from the following places:

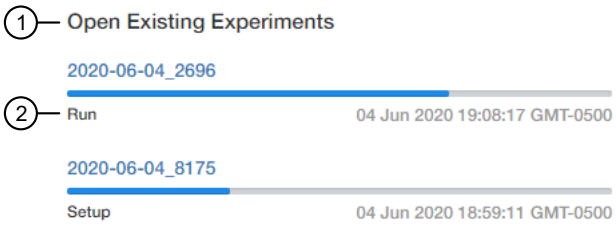
- On the instrument touchscreen.
- In the **Monitor the Run** pane of the AccuSEQ™ Software **(Home)** screen.

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**.

Note: You cannot start another run while the instrument status is **Running**.

When the run is complete, the status changes to **Analysis** and the status bar displays as complete.



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

- In the **Run** tab of the AccuSEQ™ Real-Time PCR 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, by selecting **Sample Name**, **Sample Color**, or **Target** in the **View** dropdown list.

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.

Analyze the results

IMPORTANT! The acceptance criteria that are provided in this section are based on our current knowledge of assay performance in detection of *Mycoplasma* recovered from a wide variety of unknown sample matrices. We recommend that you qualify and validate the assay internally using samples that are specific to your process and manufacturing environment (raw materials, bioreactor, or cell line samples) to ensure that these criteria are appropriate.

For specific sample types, it may be necessary to make slight changes to the acceptance criteria based on specific results. We can provide you with one-on-one support during this process.

Workflow: Review MycoSEQ™ experiments

See the *AccuSEQ™ Real-Time PCR Software v3.2 User Guide* (Pub. No. MAN0029199) for more information about analyzing results.

View the result summary page 19

Evaluate the amplification plot page 22

Evaluate the melt curve plot page 23

View the Result Summary

The **Result** screen is populated when the run is complete.

Calls are made based on the customizable rules settings in the **Analysis Settings** dialog box for MycoSEQ™ experiments. See the *AccuSEQ™ Real-Time PCR Software v3.2 User Guide* (Pub. No. MAN0029199) for more information.

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

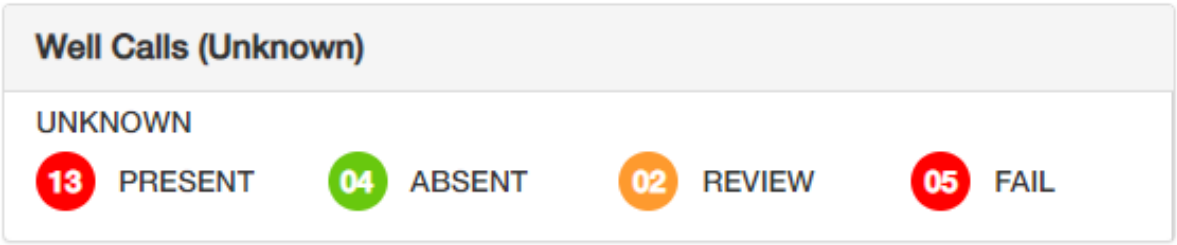
   The number inside the circle indicates the number of controls that passed or failed. The overall plate status (**VALID** or **INVALID**) shown in the **Plate Status** field is determined by the POS and NTC calls.

   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 3 Plate Calls section (example)

① Plate Call
② Positive controls (POS)
③ Plate Status
④ No Template Controls (NTC)

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



3. 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**).

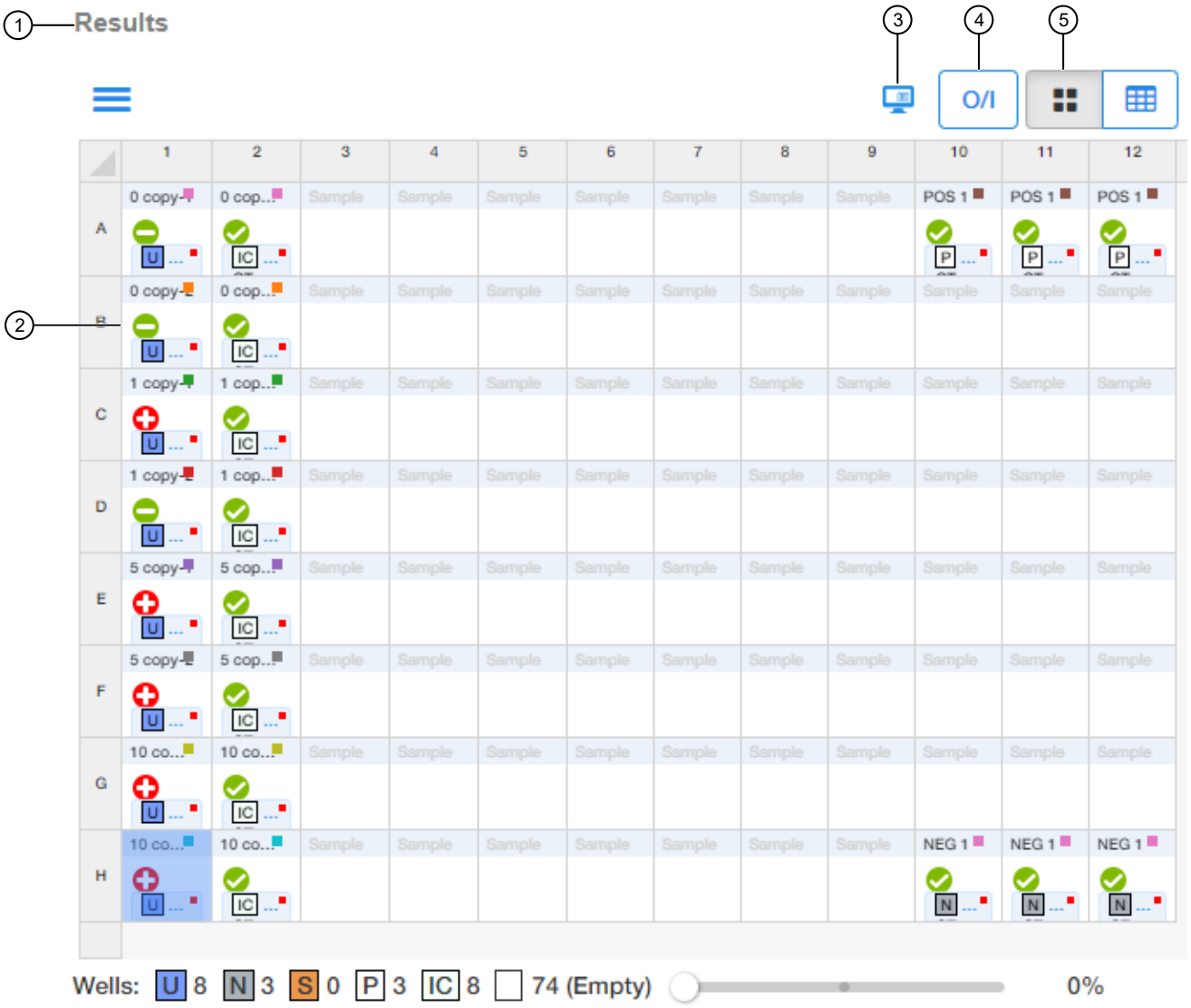


Figure 4 Results pane (example)

① **Results** pane
② Well call
③ **View Legends**—explains the symbols and letters in the wells. The number inside the triangle indicates the number of QC flag calls in the well; review in QC Summary.
④ **Omit/ Include**—omits selected wells from the analysis or includes selected wells in the analysis
⑤ **Grid View**

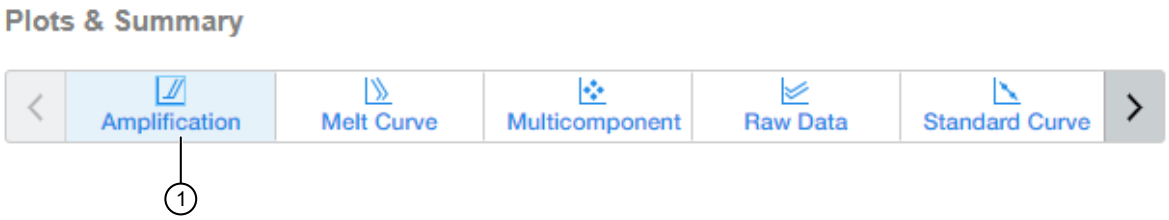
Evaluate the overall shape of the Amplification Plot curves

You can evaluate the overall shape of the amplification plot curves in the **Result** screen. 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 the AccuSEQ™ Software.

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

   Note: If no data are displayed in the **Result Analysis** pane, then click **Analyze**.



   ① Amplification Plot

   The amplification plot is displayed for the selected wells in the grid view.

2. Ensure that the **Target** selected is **Mycoplasma**.

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

   Table 3 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	Select **Auto** or specify a threshold, then decide whether to show threshold.
Baseline	Decide whether to show baseline

4. (*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

      Note: The minimum value must be greater than 0.

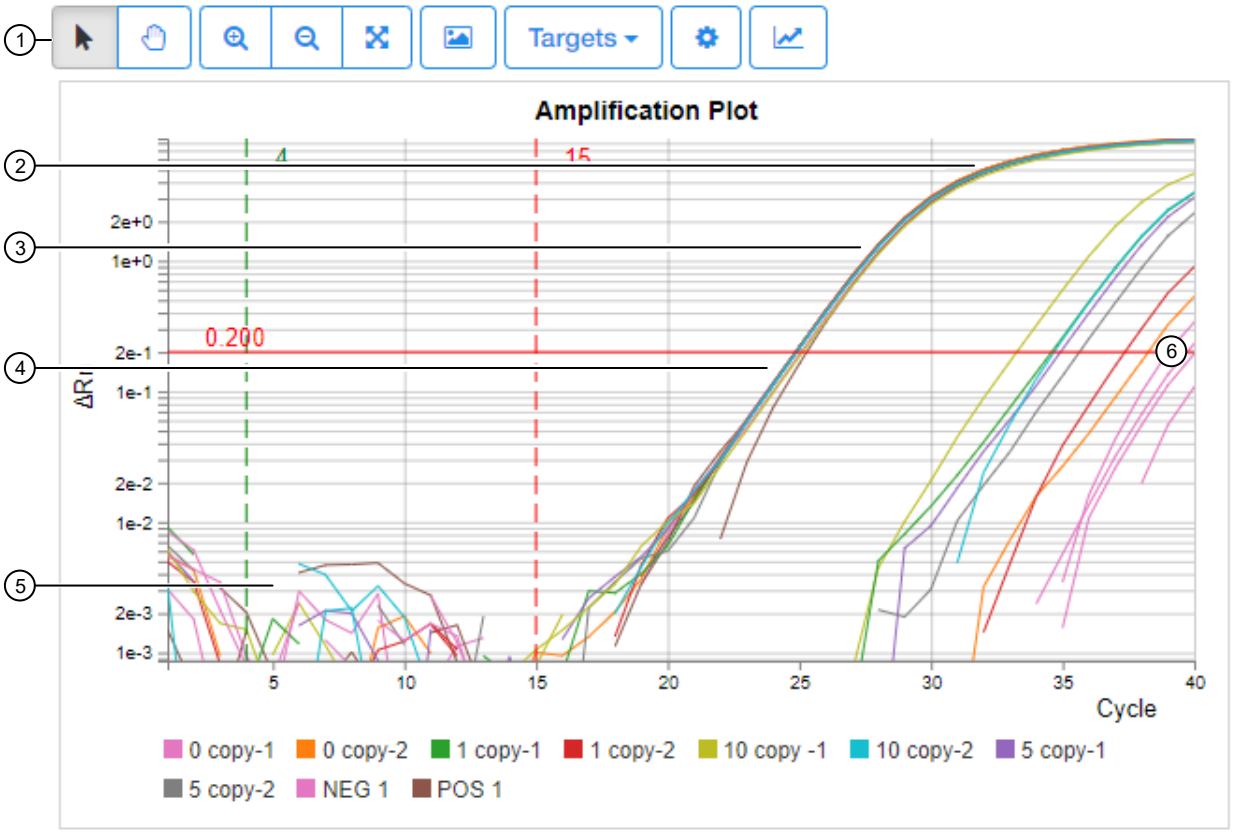
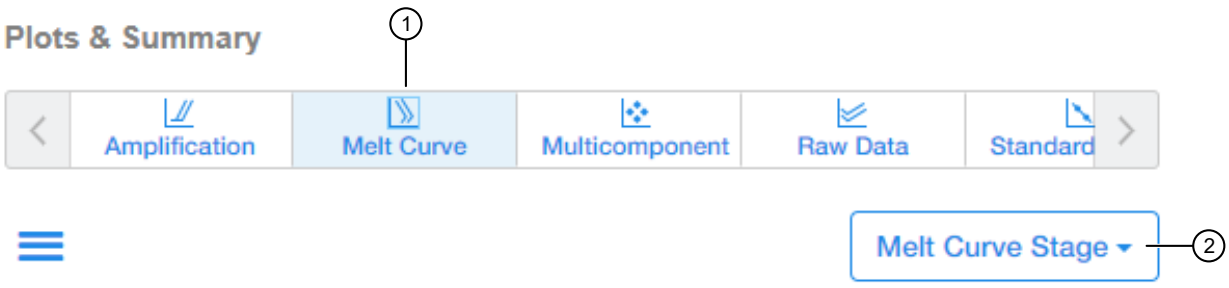


Figure 5 Typical Amplification Plot for a MycoSEQ™ experiment (4 phases)

① **Amplification Plot** tools
② Plateau phase
③ Linear phase
④ Exponential phase
⑤ Baseline phase
⑥ Threshold

View and evaluate the Melt Curve Plot

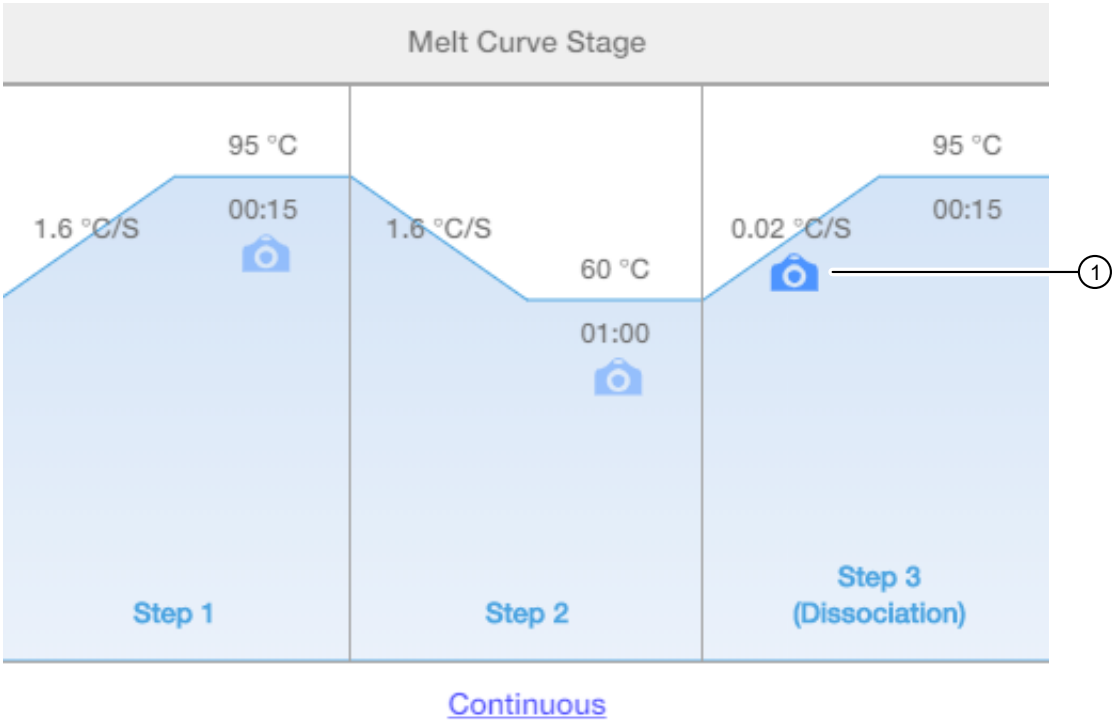
You can view and evaluate the **Melt Curve Plot** in the **Result Analysis** window in the **Result** screen.



① **Melt Curve** plot
② **Melt Curve Stage** dropdown

1. In the **Result Analysis** pane, select **Melt Curve Plot** from the horizontal scroll bar.
2. Keep the default **Melt Curve Stage** selection.

   Custom experiments with multiple data collections in the **Melt Curve Stage**, can select the stage that they want displayed.



① Stage 3 data collection point

3. Click to configure the plot, then make the following selections:
   - **Targets**: Select Mycoplasma.
   - **Plot Settings**: Select the **Plot Type** and **Plot Color**.
   - **Plot Properties**: Edit **Plot Title**, change fonts, colors, and labels.
   - **Save Image**: Save the image (JPG, PNG, or SVG).
   - Use the select, pan, and zoom options to interact with the plot.

   The **Melt Curve Plot** is displayed for data points that are selected in the plot settings. The data points for selected wells in the **Grid View** or **Table View** are highlighted in the plot.
4. (*Optional*) View the default **Melt Curve Stage**.
5. Ensure that amplification in the no template control and IC control wells is as expected. Use one of the following options:
   - Select control wells in the **Grid View** or **Table View**, then confirm the location of the data points in the **Melt Curve Plot**.
   - View the amplification plots for the no template controls.

6. In the **Melt Curve Plot**, view the signal intensity and calls for the unknown samples.

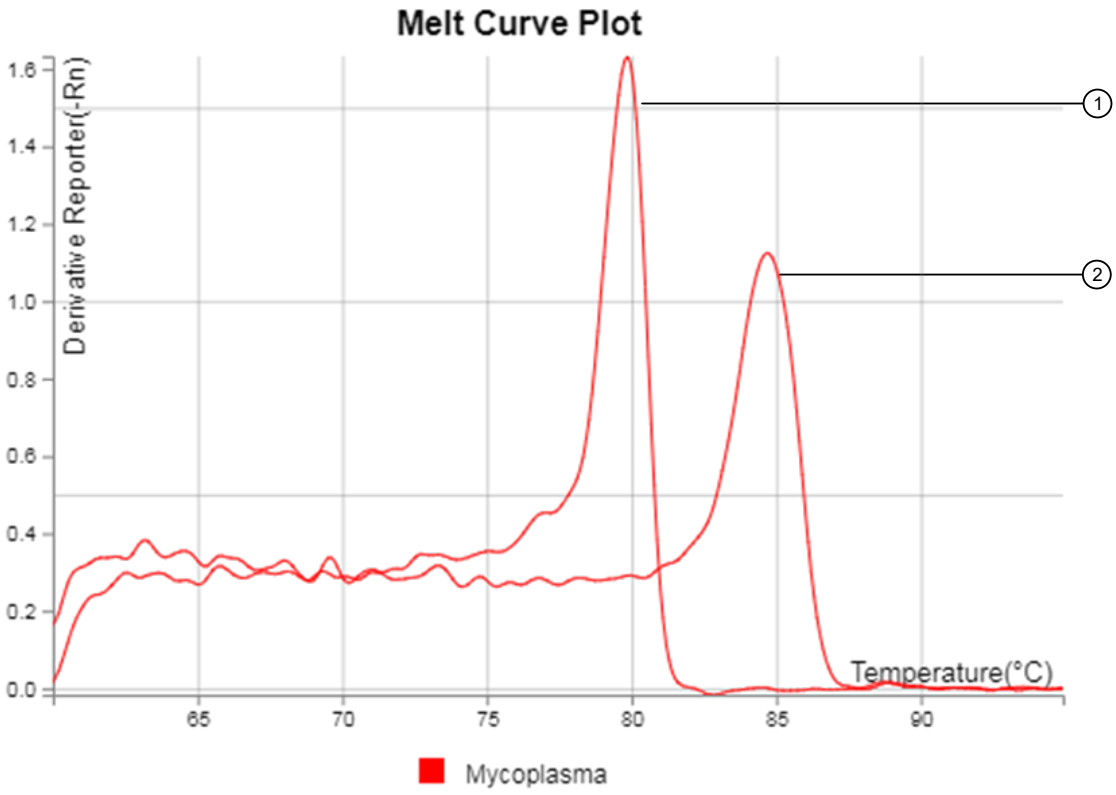


Figure 6 Example Melt Curve Plot

① Melting temperature (Mycoplasma)
② Melting temperature (positive control)

Manually edit unknown well calls

Well calls labeled Unknown 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 well calls labeled Unknown can be manually edited. In the **Result** screen, click the **Results Summary** tab to determine if **Unknown** well calls exist.

1. In the **Result** pane, click to open the table view.
2. Click the **Task** column to group the **Unknown** calls together.
   The **Task** column identifies wells that triggered an **Unknown** call.
3. Click the **Results Analysis** tab to view the well data.
4. Select a well with an **Unknown** call 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 dropdown:
   - **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.

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

Guidance for unknown samples

The table shows criteria for positive and negative calls. A positive call indicates that at least one genome copy of *Mycoplasma* DNA was present in the unknown reaction and the sample is positive for the presence of *Mycoplasma*.

Note: T_m and DV assay acceptance criteria are only relevant if C_t value for present acceptance criteria are met. The AccuSEQ™ Software v3.1 or later flags these as "Review".

Table 4 Example acceptance criteria for unknown samples: AccuSEQ™ Software v3.1 or later

Result	C_t	T_m (°C)	DV
Present	< 36.2300 C_t	75.50 < T_m < 83.00	≥0.40
Absent	≥ 36.2300 C_t	< 75.50	<0.20

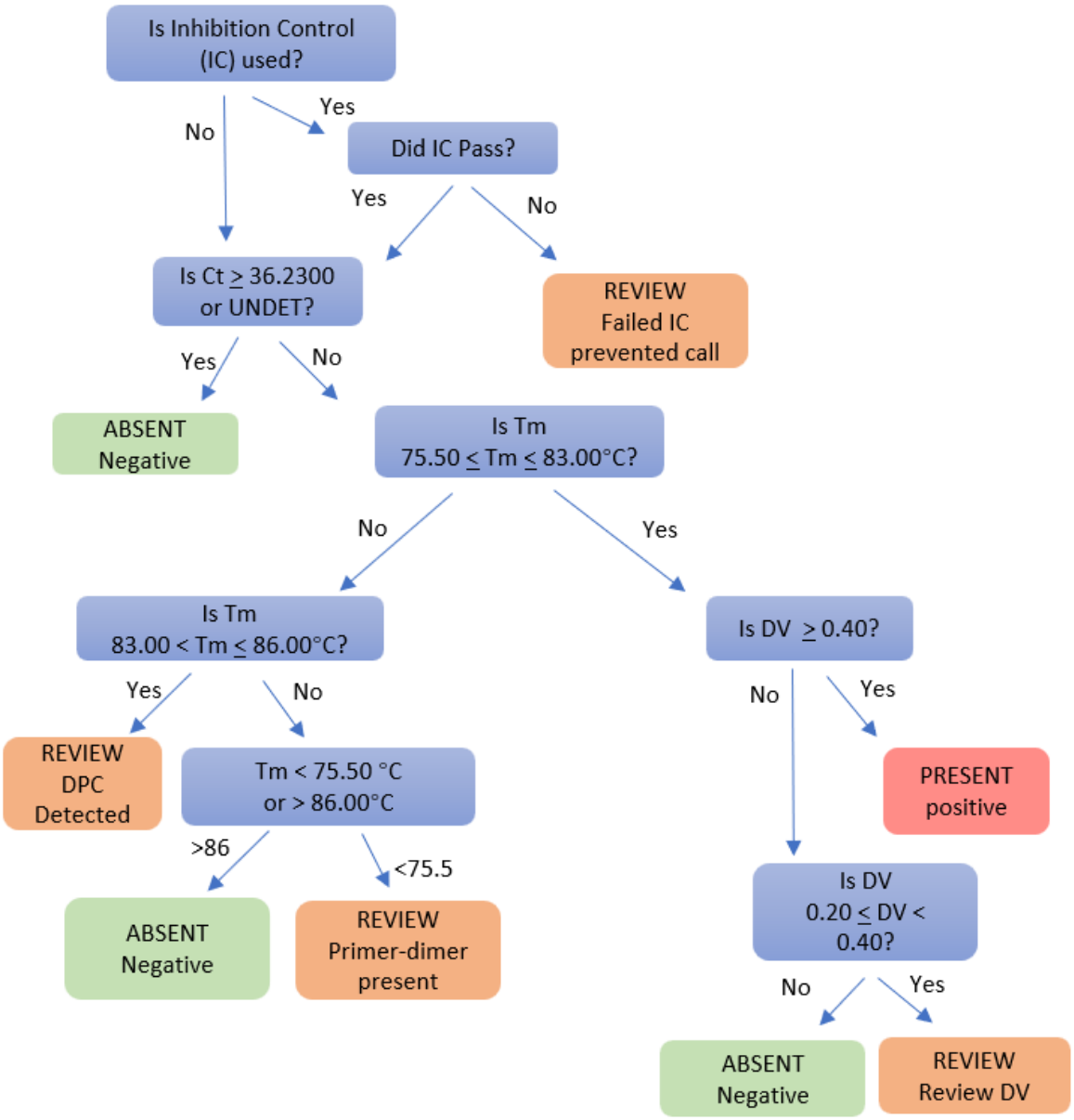


Figure 7 Decision tree for unknown sample calls (with or without an inhibition control [IC])

Note: The presence of a melt peak with a T_m range of 83.00°C ≤ T_m ≤ 86.00°C in wells of unspiked unknown samples indicates presence of DPC contamination. Software flags as **REVIEW**.

Guidance for controls

Note: Assay acceptance criteria are subject to your own validation. T_m and DV assay acceptance criteria are only relevant if C_t acceptance criteria are met.

Table 5 Example acceptance criteria for controls: AccuSEQ™ Real-Time PCR Software v3.1 or later.

Control	C_t	T_m (°C)	DV
PCR positive control	23.5000 ≤ C_t ≤27.5000 [1]	83.00 < T_m ≤86.00	≥0.40
Extraction spike control (if DPC is the spike)	<36.2300	83.00 < T_m ≤86.00	≥0.40
Extraction spike control (if mycoplsma genomic DNA or bacteria is the spike)	<36.2300	75.50 ≤ T_m ≤83.00	≥0.40
No template control[2]	≥36.2300	75.50 ≤ T_m ≤83.00	<0.40
Blank extraction control[2]	≥36.2300	75.50 ≤ T_m ≤83.00	<0.40
Inhibition control	ΔC_t <2.0000 (ΔC_t = C_t sample inhibition control - C_t PCR positive control	83.00 < T_m ≤86.00	N/A

[1] For 2,000 copies; adjust if using lower spike.
[2] When T_m <75.5°C, there is no target with the correct T_m, and therefore C_t and DV values are irrelevant.

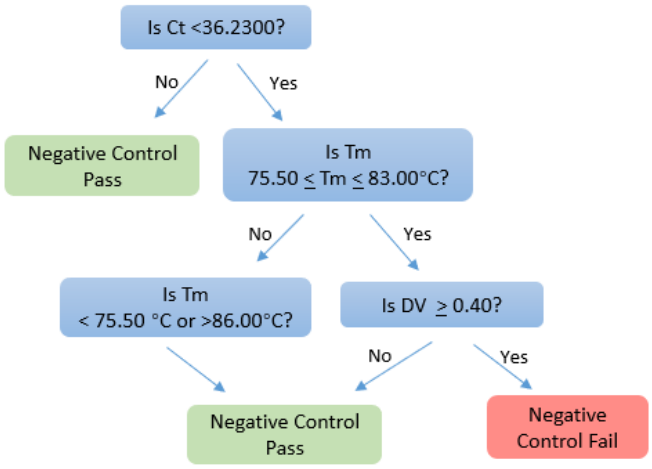


Figure 8 Decision tree for NTC calls

Note: NTC wells can display a melt peak with a T_m <75.5°C or a T_m >86°C. The presence of a melt peak with a T_m range of 83.00°C ≤ T_m ≤86.00°C in NTC wells indicates DPC contamination in the PCR reaction. The presence of a melt peak with a T_m <75.5°C indicates a primer dimer event.

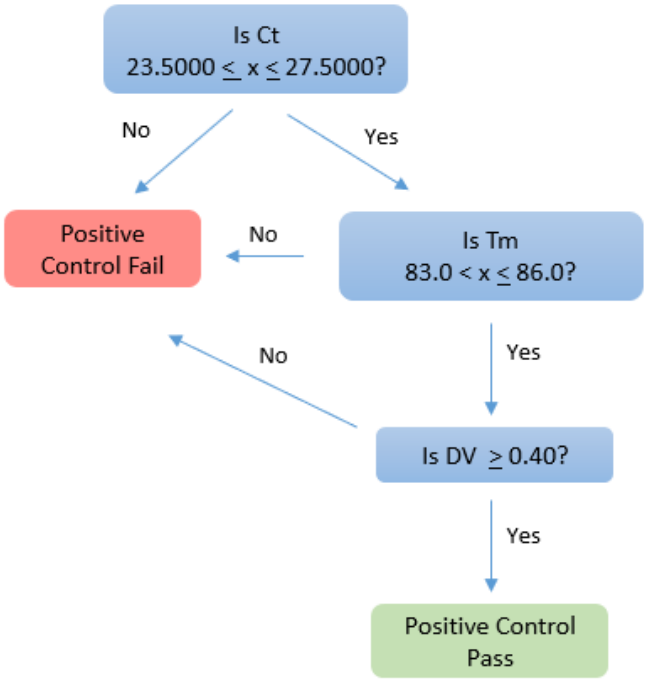


Figure 9 Decision tree for positive control calls with 2,000 copies

Example results with AccuSEQ™ Software v3.1 or later

Positive control (2,000 copies)

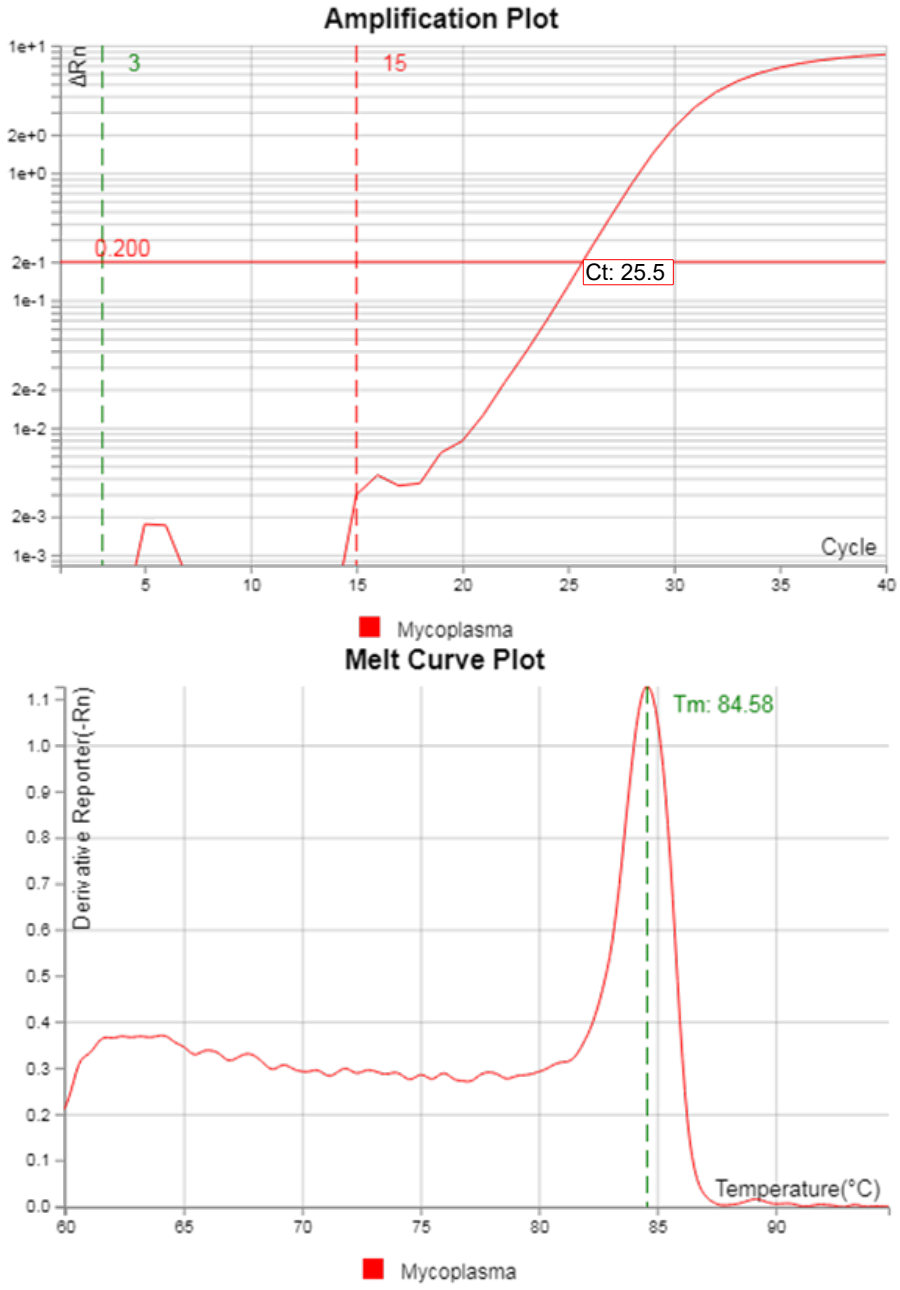


Figure 10 Amplification and melt curve plots of positive control reactions spiked with 2,000 copies of Discriminatory Positive Control.

No template control

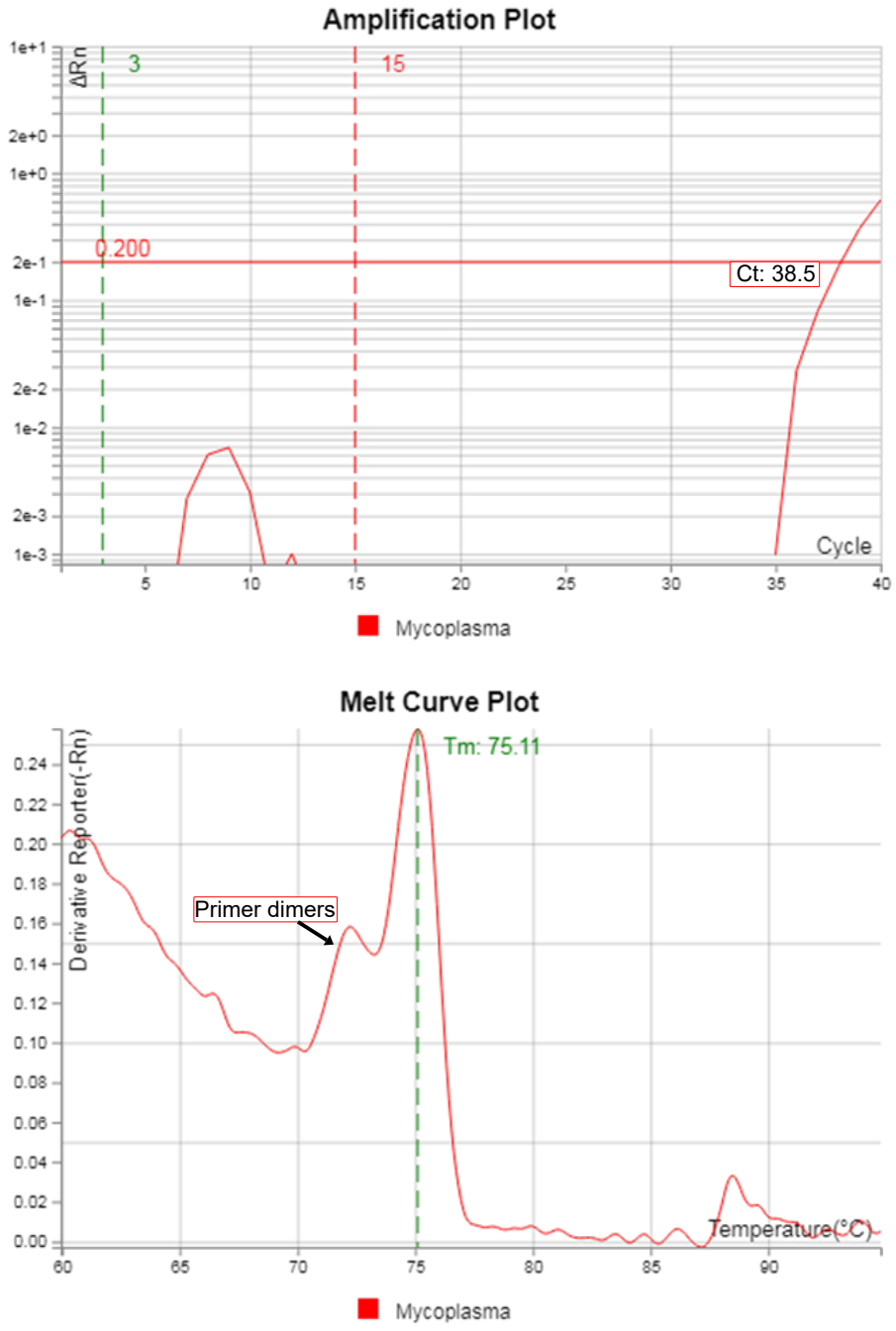


Figure 11 Amplification Plot and Melt Curve Plot of No Template (negative) control reactions.

Blank extraction control

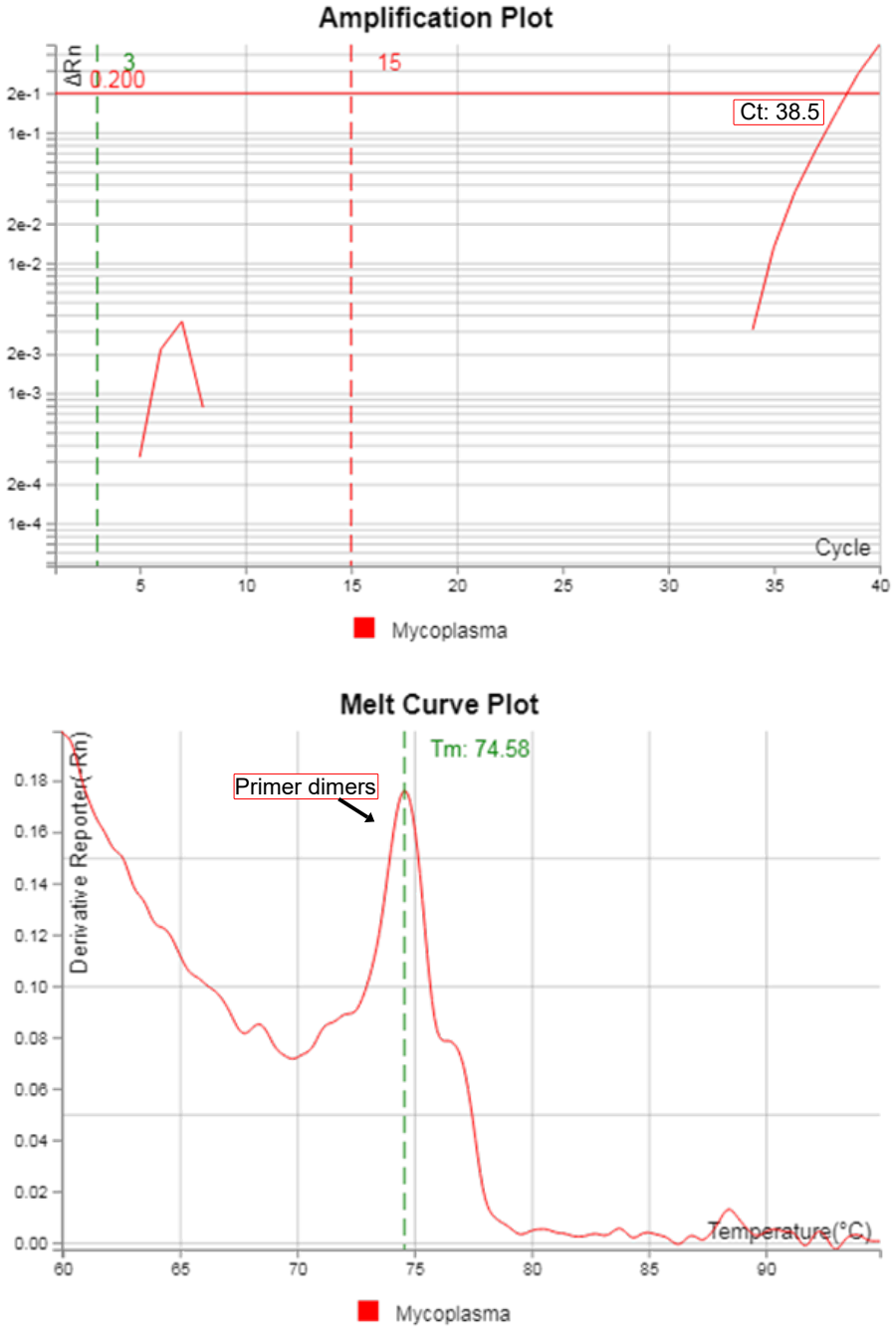


Figure 12 Amplification and Melt Curve plots of blank extraction control with PBS.

Positive unknown sample (about 200 copies mycoplasma)

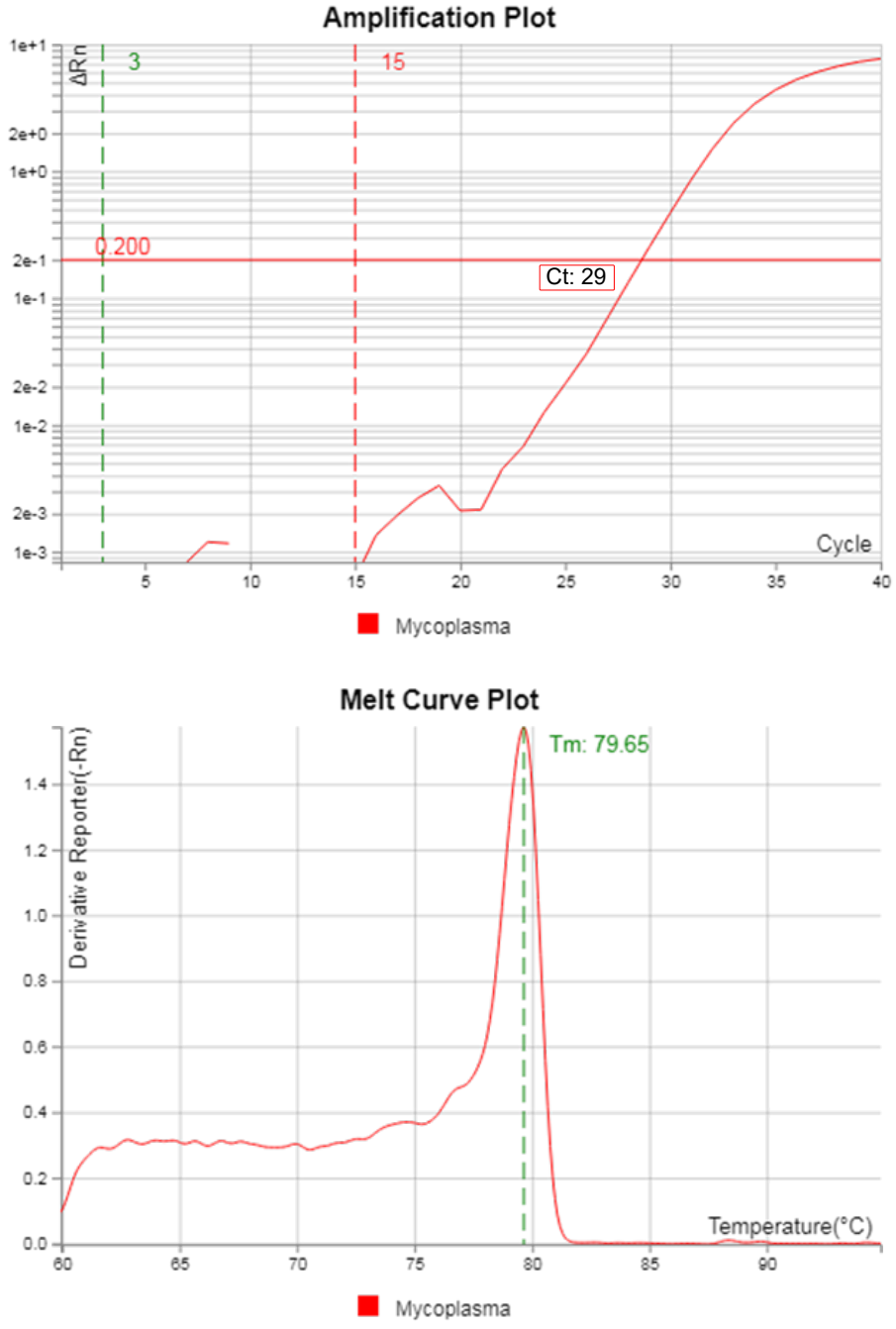


Figure 13 Amplification and Melt Curve plots of positive unknown samples with approximately 200 copies of genomic DNA template

PCR inhibition

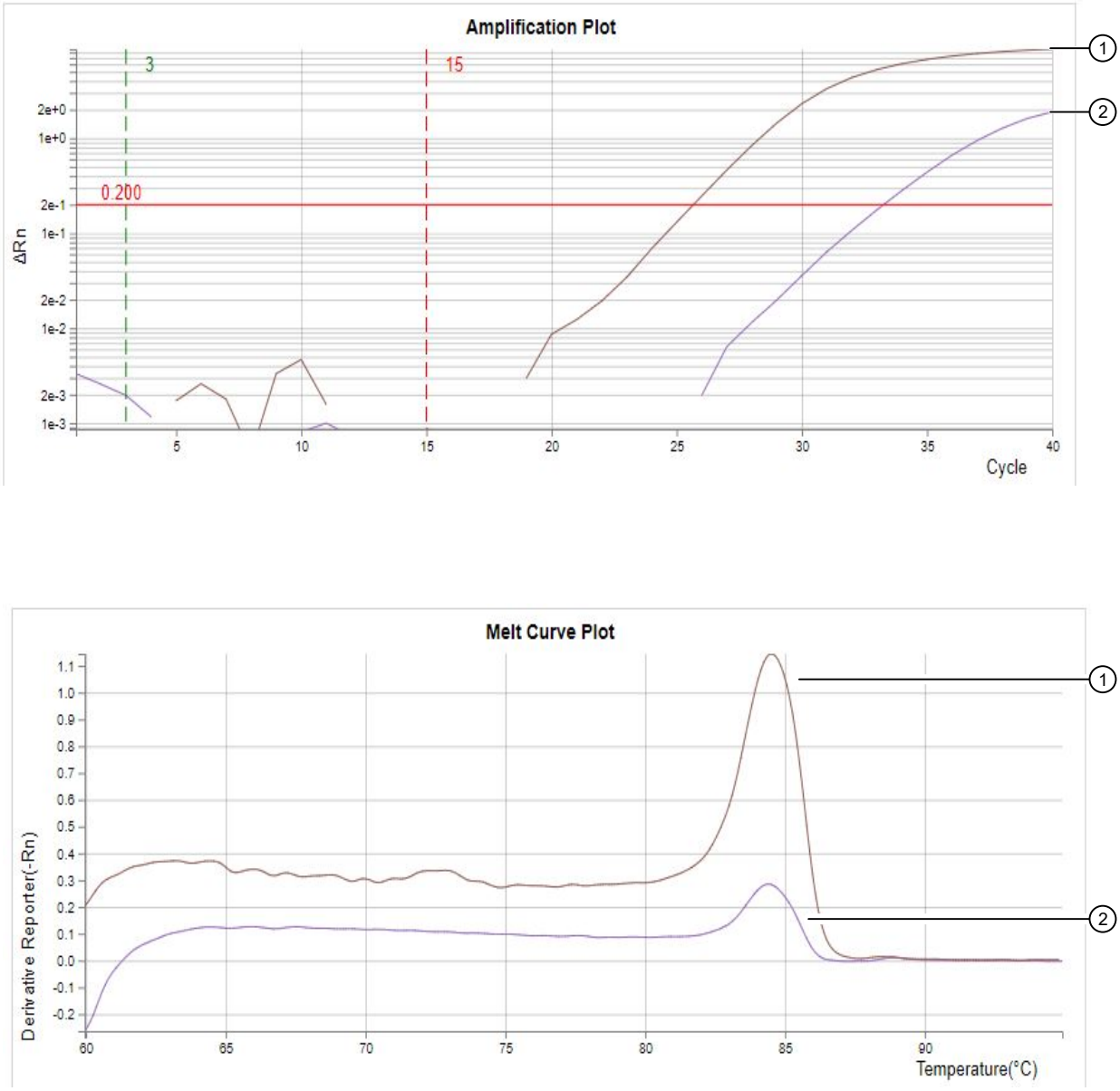


Figure 14 PCR inhibition, $\Delta C_t > 2$ (ΔC_t = C_t sample inhibition control - C_t PCR positive control).

① PCR positive control
② Sample inhibition control

Multicomponent plots

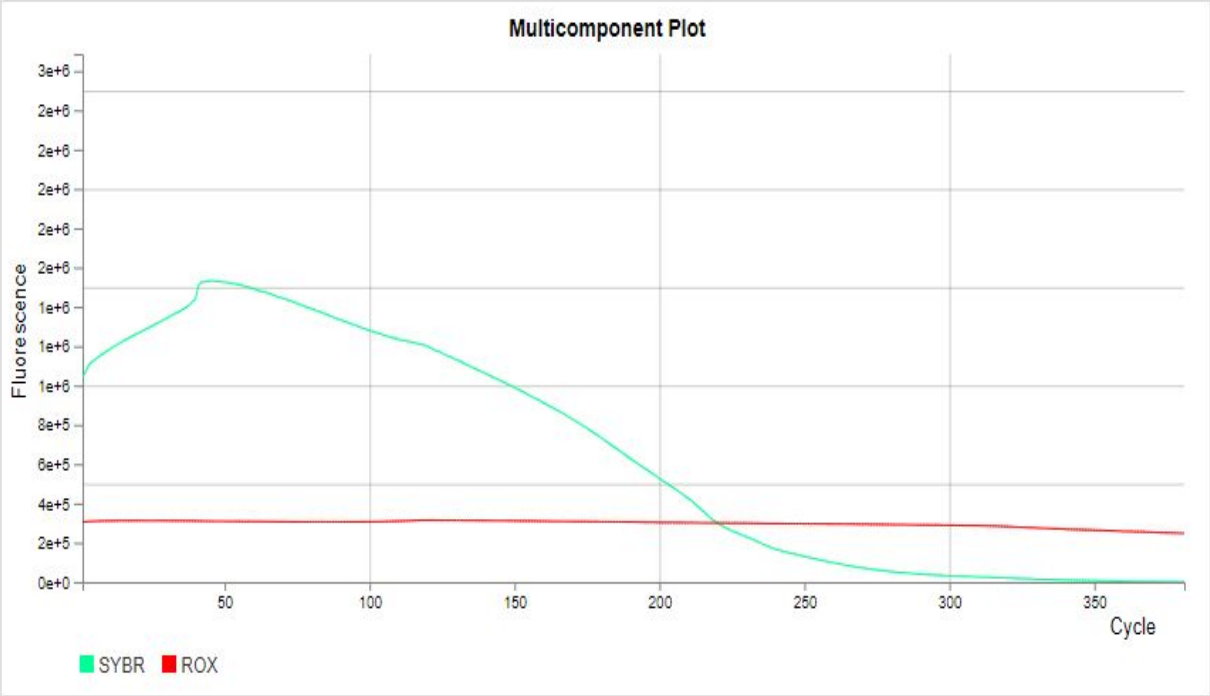


Figure 15 An example of a multicomponent plot.

A

Troubleshooting

Troubleshooting the AccuSEQ™ software

Observation	Possible cause	Recommended action
AccuSEQ™ software **Review** call	Low sample concentration of mycoplasma.	• Call manually according to laboratory guidelines. *or* • Allow the culture to grow for an additional 24 hours, then repurify the sample and repeat the experiment using assay components that were stored correctly.
C_t values that are at or close to the acceptance criteria limits	Incorrect baseline setting applied.	Re-analyze samples using auto baseline, or change to manual start and end baseline settings.
	High SYBR™ signal from contaminating host cell DNA.	Apply RNase treatment during sample preparation. Contact your local FAS for more information.
PCR inhibition • Negative unknown sample *and* • Inhibition control ΔC_t>2 compared to the positive control	Inappropriate sample preparation that results in carryover of chemicals from the media/sample matrix.	Repurify the sample preparation and make sure of appropriate performance of wash and elution that does not carry over chemicals from reagents.
	Excess DNA or RNA. SYBR™ signals will have increased signal in Component View.	Repurify the sample using protocol with RNase and/or DNase treatment. Contact your Field Applications Specialist (FAS) for more information.
	Components in cell culture media or additive (for example, dextran sulfate) may inhibit PCR and change T_m.	• Most cell culture media inhibit PCR or change T_m without sample preparation. • Check if additives to cell culture media inhibit PCR. • To address inhibition from Dextran sulfate or Heparin, contact your FAS for more information.
High background fluorescence signal **Details:** >500,000 fluorescent standard units (FSU). This is visible in the **Multicomponent Plot** (**Results** tab)	The sample block is contaminated.	Run a background calibration to identify the contaminated wells, then decontaminate the sample block.

Observation	Possible cause	Recommended action
High background fluorescence signal **Details:** >500,000 fluorescent standard units (FSU). This is visible in the **Multicomponent Plot** (**Results** tab) *(continued)*	Sample may have high concentration of nucleic acid carried from the cell culture during sample preparation.	Repeat the experiment using assay components that were stored correctly.
		Repeat the sample extraction, using enzymatic treatment of the supernatant (RNase and/or DNase or Proteinase K) before high-speed centrifugation.

Troubleshooting the MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit

Observation	Possible cause	Recommended action
No positive control or target-specific SYBR™ Green dye signal is detected in inhibition control and/or positive control wells	Improper storage of Power SYBR™ Green PCR Master Mix or of target-specific *Mycoplasma* Real-Time PCR Primer Mix (10×).	Repeat the assay using properly stored assay components.
		Avoid freezing and thawing assay components. Protect Power SYBR™ Green PCR Master Mix from light.
	Pipetting error (no premix solution added).	Repeat the assay. Make sure to pipet premix solution into all wells.
	Pipetting error (no positive control added).	Repeat the assay. Make sure to pipet positive control into all positive-control wells.
Target-specific signal is detected in negative control wells	Carryover contamination.	Repeat the assay using fresh aliquots of all reagents and clean pipetting equipment. If the negative control continues to show contamination, repeat the assay using a new kit. If the negative control continues to show contamination, contact your Application Specialist.
	High level of nonspecific product formation.	Check the dissociation curve to confirm. Repeat the assay using properly stored assay components.
		Avoid freezing and thawing assay components. Protect Power SYBR™ Green PCR Master Mix from light.

Troubleshooting complex or high cell density samples

Observation	Possible cause	Recommended action
Non-specific peaks occurring close to the T_m cutoff in complex or high cell density samples	High background signal due to excessive amounts of nucleic acid in complex or high cell density samples.	Prepare sample lysates using the optional enzymatic treatments (DNase and RNase) suggested for the PrepSEQ™ 1-2-3 Mycoplasma Nucleic Acid Extraction Kit (Cat. No. 4443789).
		Optimize the qPCR reaction. • Add 2µg BSA to each qPCR reaction • Increase the annealing temperature to 63°C when analyzing the data with AccuSEQ™ Real-Time PCR Software v3.x. • Increase C_t rule setting for DPC by 1. For detailed reaction setup, see "Prepare the PCR reactions (complex or high cell density samples)" on page 39.

Prepare the PCR reactions (complex or high cell density samples)

Component	Volume	Note
Power SYBR™ Green PCR Master Mix, 2×	15 µL	Unchanged
Mycoplasma Real-Time PCR Primer Mix, 10×	3 µL	Unchanged
Water or DPC control	1 µL	Volume decreased. For DPC, this results in 1,000 copies/ reaction. Inhibition control criterion remains $\Delta C_t < 2.000$. Increase C_t rule setting for DPC by 1 (AccuSEQ™ Real-Time PCR Software v2.x or v3.x).
2 µg BSA[1]	1 µL	1. Prepare the 2 µg (2 mg/mL) BSA. a. Dilute the UltraPure™ BSA (50 mg/mL) (Cat. No. AM2616) 1:5 with nuclease-free water. The resulting solution (D1) has a final concentration of 10 mg/mL. b. Dilute D1 1:5 with nuclease-free water. The resulting solution (D2) has a final concentration of 2 mg/mL. 2. Add 1 µL of the D2 dilution (2 mg/mL) to each reaction.
Sample DNA	10 µL	Unchanged
Total	**30 µL**	Do not increase the reaction volume. Decrease the amount of water or DPC control to 1µL and increase the rule settings in the AccuSEQ™ software by 1 C_t.

[1] Addition of BSA also helps with removing media additives such as dextran sulfate and heparin which co-purify with samples and cause PCR inhibition.

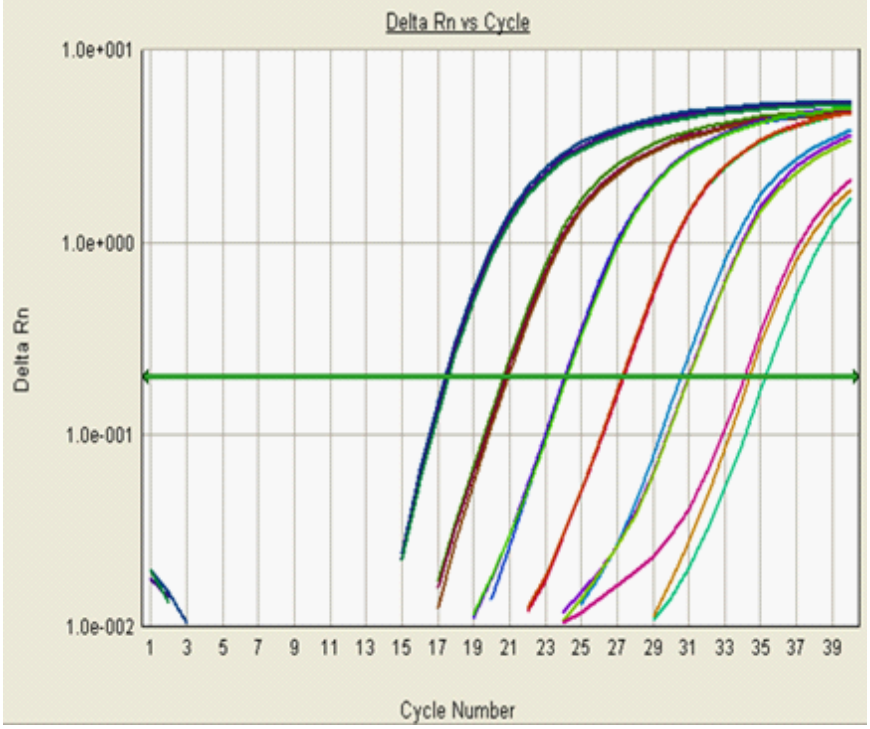
B

Acceptance criteria

Three acceptance criteria are used to determine the presence or absence of mycoplasma with the MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit. This section defines the acceptance criteria in more detail and explains how they are implemented in the assay.

Cycle threshold value (C_t)

The C_t Value (cycle threshold) 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.



Amplification plots generated from analysis of a dilution series of purified Mycoplasma DNA, from 100,000 genome copies per reaction to 1 genome copy per reaction. Reactions with a high concentration of DNA have a lower C_t value.

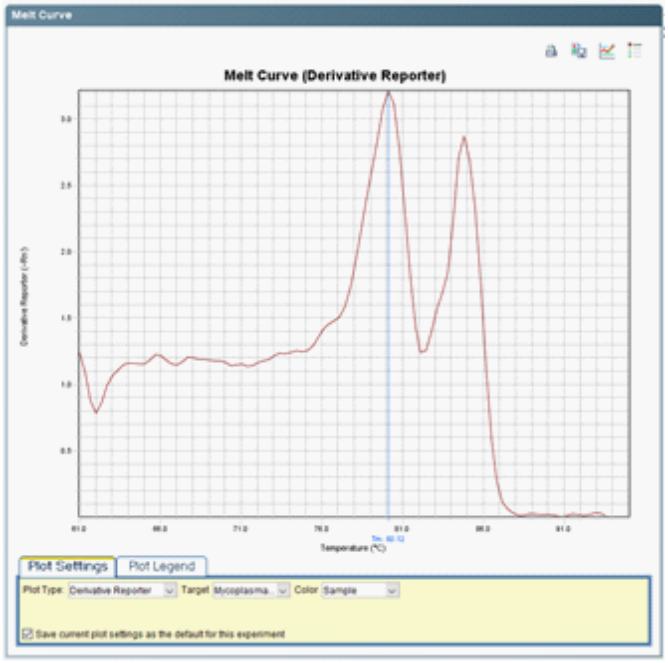
The C_t value is the most important criteria in determination of the presence/absence of mycoplasma. In routine testing with the MycoSEQ assay, unknown samples or negative control reactions with a C_t value greater than the C_t value acceptance criteria (36.23), indicates no amplification of the target occurred and require no additional analysis. The unknown or control sample is negative for Mycoplasma DNA. If the C_t value acceptance criteria are met, amplification has occurred, requiring evaluation of T_m values to determine specificity of mycoplasma DNA amplification.

Melting temperature (T_m)

The T_m (melting temperature) is the temperature at which one-half of the DNA dissociates to become single stranded. As dissociation and melting temperature are functions of DNA composition and length, the melt curve analysis and T_m are critical in demonstrating amplification of the specific target of interest when using non-specific binding dyes, such as SYBR™ Green.

Mycoplasma amplicons generated with the MycoSEQ™ Mycoplasma Detection Kit have a T_m of 75.5–83°C (T_m range may vary, depending on software used for analysis). Reactions with T_m values outside of this range, or not meeting the established T_m acceptance criteria, should be determined to be negative for mycoplasma and require no further analysis.

If peaks are observed in the melt analysis of samples with C_t values below the positive/negative cutoff value, (especially in a negative control or known negative sample), confirm the result by performing an analysis of the melt curve of the inhibition control reaction associated with the unknown sample. Non-mycoplasma related primer-dimer peaks are typically resolved in the presence of assay target. If an unknown sample generating borderline results contains Mycoplasma DNA, two distinct melt peaks will be observed in the inhibition control, one in the melting range of Mycoplasma target and one at the temperature expected for the Discriminatory Positive Control (DPC).

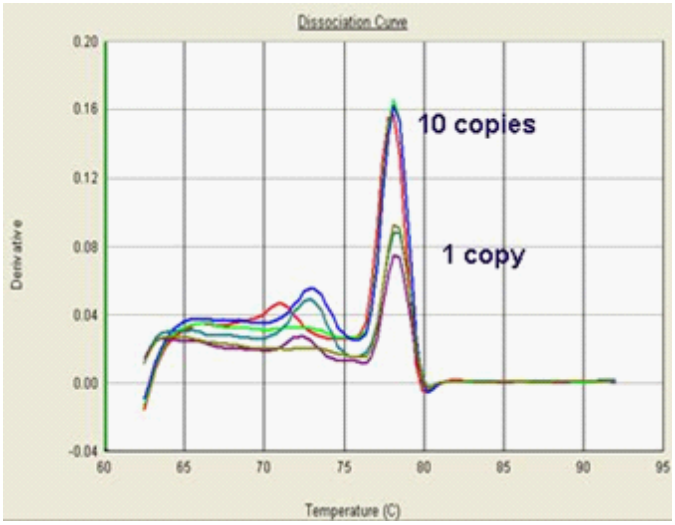


Melt curve with two distinct peaks. One in mycoplasma range and one in DPC range.

Derivative value (DV)

Derivative value (DV) is a measure of how much SYBR™ Green is released from the amplicon during melt analysis and consequently the amount of specific target amplified. The greater the DV, the more amplicon that was generated during PCR.

Note: At very low levels of Mycoplasma sample DNA, usually between 1 and 10 genome copies, there are observable differences in the DV, with lower DNA levels generating lower DVs.



Observable differences in DV between 1 and 10 genome copies of DNA.

While the DV is used as an assay acceptance criterion, it is most critical for unknown samples containing a very low amount of DNA, generally near the limit of detection of the assay. An amplicon generated from the presence of Mycoplasma DNA in an unknown sample will generate a melt curve with a DV that is above the baseline fluorescence observed between the temperatures 65-70°C. Any melt curve peaks observed with a fluorescence level below the baseline fluorescence level (65-70°C) are not related to the presence of Mycoplasma DNA in the unknown sample.

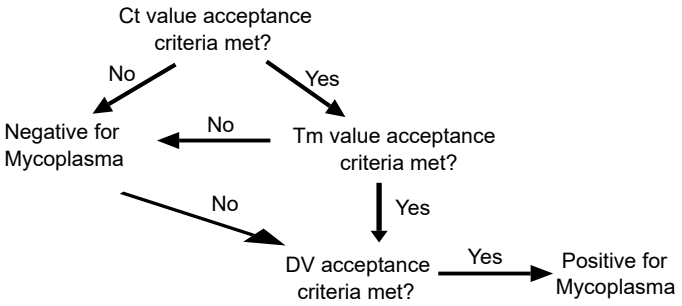
With higher levels of Mycoplasma DNA in an unknown sample, there is generally a maximum DV that is achieved. This value is usually equal to, or greater than, the DV observed in the analysis of the melt curve of the positive control reaction. The instrument software calculates this value. Different software versions might assign different values for the DV, so customers should use the values from the software version that they are using when creating acceptance criteria for their unknown samples. The examples provided in this document are from data that was collected and analyzed using the SDS v1.4 software.

The DV cutoff for positive/negative detection of Mycoplasma was assigned based on analysis of testing results from testing of multiple Mycoplasma species tested at multiple concentrations per qPCR reaction, from high concentrations to concentrations at or below the lowest limit of detection of the MycoSEQ assay analyzed using AccuSEQ analysis software.

Results interpretation using the acceptance criteria

Specific acceptance criteria values in this guide 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 prep optimization and establishment of sample specific acceptance criteria and positive/negative cut-off values.

For a reaction to be determined positive for mycoplasma, all three criteria must be met. If any single criterion is not met, the reaction is negative for the presence of mycoplasma. Figure 2 represents a simplified decision tree that can be utilized for the determination of positive/negative calls.



MycoSEQ acceptance criteria decision tree. All three criteria (C_t, T_m, and DV) must be met for a positive mycoplasma call.

Other acceptance criteria considerations

- The MycoSEQ™ Mycoplasma Detection Kit is optimized to minimize primer-dimers. However, some sample types can give rise to an amplification plot and C_t value due to the formation of primer-dimer during the PCR reaction. In general, if primer dimer formation causes an amplification plot, the C_t value is >36.23 and the T_m is <75.5°C, which is outside the range for Mycoplasma amplicons generated with this assay. This phenomenon is generally only present in samples that do not contain any Mycoplasma target. T_m values can be used to discriminate a result generated by primer-dimer formation from detection of Mycoplasma DNA.
- No two cell culture sample types are the same. The positive and negative cutoff values provided in this guide were established from the analysis of several thousand unknown samples. However, there are cases where unknown, sample-specific, positive and negative cutoff values should be established and applied. For some sample types, it is possible that the values (C_t, T_m, and DV) 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 cutoff values. These values can be established for one or more results parameters to provide the user the highest possible level of confidence in both the negative and positive results obtained during routine testing.

C

Use the kit with 7500 Fast System AccuSEQ™ Real-Time PCR Detection Software v2.x

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
Instruments	
7500 Fast Real-Time PCR System with AccuSEQ™ software v2.x	Contact your local sales representative
Consumables	
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, 20 plates, 0.2-mL well; for use with 7300, 7500, and 7900HT Fast Real-Time PCR Systems	4306737[1]
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL, 20 plates; for use with 7500 Fast Real-Time PCR System	4346906

(continued)

Item	Source
MicroAmp™ Optical 96-Well Reaction Plate with Barcode & Optical Adhesive Films, 100 plates with covers; for use with 7300 and 7500 Fast Real-Time PCR Systems	4314320
MicroAmp™ Optical 8-Cap Strips, 300 strips	4323032
MicroAmp™ Optical Adhesive Film Kit, 20 covers, 1 applicator, 1 optical cover compression pad	4313663
MicroAmp™ Optical Adhesive Film, 25 or 100 covers	4360954, 25 covers 4311971, 100 covers

[1] Not recommended for use with the 7500 Fast system. For 7500 Fast system reactions, use Cat. No. 4346906.

Prepare the sample

Prepare the DNA template for the PCR reactions using the PrepSEQ™ 1-2-3 Mycoplasma Nucleic Acid Extraction Kit.

For more information, see:

- The *PrepSEQ™ Sample Preparation Kits for Mycoplasma, MMV, and Vesivirus User Guide* (Pub. No. 4465957)
- The *PrepSEQ™ Express Nucleic Acid Extraction Kit for Mycoplasma, MMV, and Vesivirus Detection User Guide* (Pub. No. MAN0016799)

Prepare the kit reagents and premix solution

1. Thaw all kit reagents completely.
2. Vortex briefly, then spin down the reagents.
3. Prepare the Premix Solution according to the following table.

Component for premix solution	Volume for one 30-µL reaction	Volume for four 30-µL reactions[1]
Power SYBR™ Green PCR Master Mix, 2×	15.0 µL	66.0 µL
Mycoplasma Real-Time PCR Primer Mix, 10×	3.0 µL	13.2 µL
Total premix solution volume	**18.0 µL**	**79.2 µL**

[1] Includes 10% excess to compensate for pipetting errors.

4. Mix the Premix Solution by gently pipetting up and down, then cap the tube.

Prepare the PCR reactions

1. Dispense the following into each well to be used, gently pipetting at the bottom of the well.

To prepare…	In each tube or well…
Negative control reaction	• Add 18 µL of Premix Solution • Add 12 µL of Negative Control (water)
Unknown or spiked sample reaction	• Add 18 µL of Premix Solution • Add 10 µL of unknown sample • Add 2 µL of Negative Control (water)
Inhibition-control reaction	• Add 18 µL of Premix Solution • Add 10 µL of unknown sample • Add 2 µL of the Discriminatory Positive Control (DPC)
Positive control reaction	• Add 18 µL of Premix Solution • Add 2 µL of the DPC • Add 10 µL of Negative Control (water)

Note: The MycoSEQ™ *Mycoplasma* Discriminatory Positive/Extraction Control can be used as a spike control that is added to the unknown sample or lysate before sample preparation.

2. Mix each sample by gently pipetting up and down.
3. Seal the plate with MicroAmp™ Optical Adhesive Film. See “Seal the plates” on page 74.
4. Briefly centrifuge the reaction plate.

Create a new experiment in AccuSEQ™ software v2.0 or v2.1

1. In the desktop, double–click the AccuSEQ™ software icon to start the software.
2. Sign in to the software.
3. In the **Home** screen, click **Create MycoSEQ Experiment** to open the **Mycoplasma Assay v2.0** workflow.

Create a new experiment in AccuSEQ™ software v2.2 or later

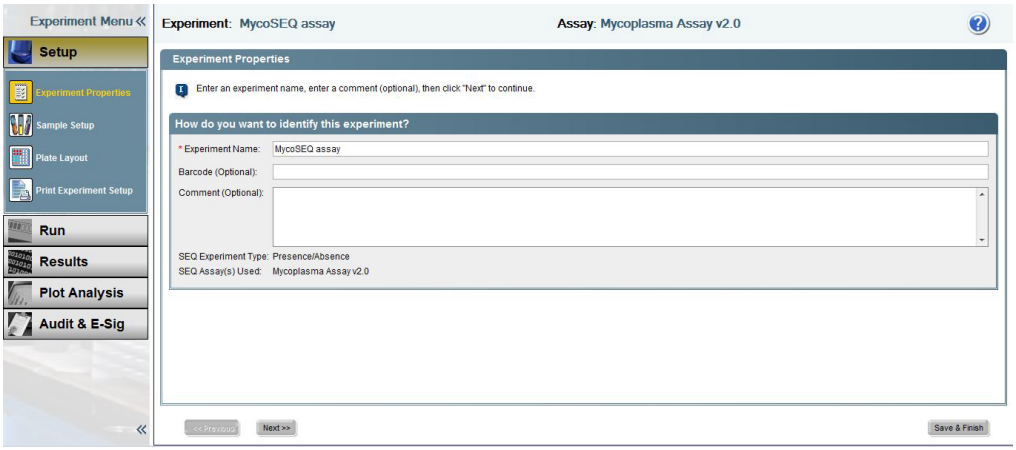
1. In the desktop, double–click the AccuSEQ™ software icon to start the software.
2. Sign in to the software.
3. In the **Home** screen, click **Create SEQ Experiment**.
4. Select the **MycoSEQ Mycoplasma SYBR** assay from the assay list, then click **Next** .
   The **Experiment Properties** tab opens.

Define the experiment properties

In the **Experiment properties** screen:

1. Enter the experiment name.

   Note: The experiment name can be up to 100 letters and numbers. Spaces are not allowed.

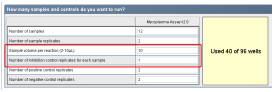


2. *(Optional)* Enter a plate barcode and comments.
3. Verify the SEQ experiment type and assay to use, then click **Next**.

Setup the samples and controls

In the **Sample Setup** screen:

1. Specify the number of samples and replicates:



Field	Minimum entry[1]
Number of samples	1
Number of sample replicates	1
Number of Inhibition control replicates for each sample	0
Number of positive control replicates	1
Number of negative control replicates	1

[1] We recommend that you use at least one negative and one positive control per run, and at least one inhibition control per sample.

Note: All unknown samples have the same number of replicates, as specified in the **Sample replicates** field.

2. Enter 10 µL per reaction in the **Sample volume per reaction** field.

3. In the **Define Samples** table, enter sample names in the **Sample Name** field.

   To use name fill click the **Sample Name** field in the top row to be filled, then click **Name Fill**.

   The software fills rows below with the entered sample name, then adds to each name an incrementing suffix starting with _1.

Note: Samples names must be assigned for **Call** and **Call Assessment** to be assessed in the results.

4. (*Optional*) Click the arrow in the **Plot Color** field to change the color for a sample.

5. Click **Next**.
   The **Plate Layout** tab opens.

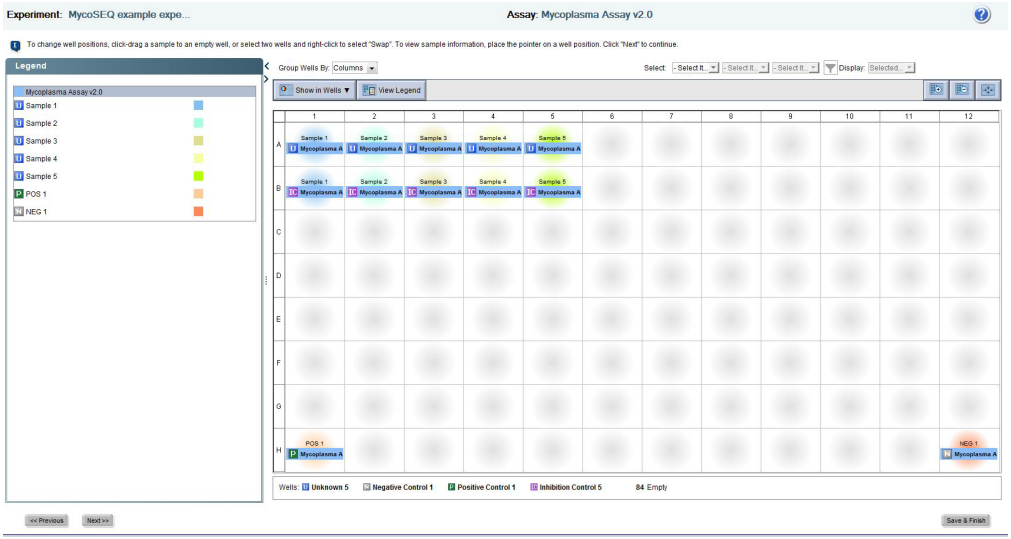
View the plate layout

The AccuSEQ™ software v2.0 or later uses the sample information that you enter in the **Sample Setup** screen to fill the wells in the plate layout and to calculate the needed reaction component volumes for each sample type, based on the Mycoplasma Real-Time PCR Detection Kit guidelines.

To view and edit the plate layout before starting an instrument run:

1. Click **Setup** ▸ **Plate Layout** in the navigation pane.

2. Review the initial well selections in the **Plate Layout** screen. Drag-and-drop samples to create the layout of your choice.



3. Review the **Sample Setup** window to ensure that the number of Unknowns, Inhibition Controls, Positive Controls, and Negative Controls match your experiment sample setup. In the example, this is 5 Unknowns, 5 Inhibition Controls, 1 Positive Control, and 1 Negative Control.

4. *(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**.

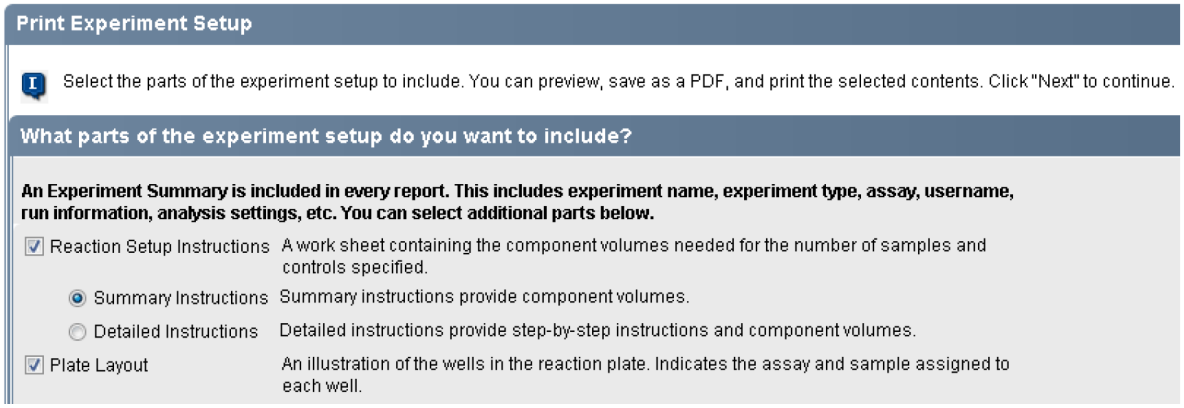
   Note: Electronic signature information is not included in an image file. Use Print Report to include Electronic signature information with the plate layout.

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

6. Click **Next**.

Print and save the experiment

1. Select the experiment setup elements to include in the report.



2. *(Optional)* Click **Preview** to display the report on-screen.

   From the **Preview** screen, you can page through the report, print, or save an electronic copy of the report in PDF or HTML format.

3. *(Optional)* Click **Save as PDF** to save an electronic copy of the report in PDF or HTML format.

4. *(Optional)* Click **Print** to print the report.

5. Click **Save & Finish**, specify a name and location for the EDS file, then click **OK**.

Start the run (7500 Fast Real-Time PCR Instrument)

1. Double-click Mycoplasma SEQ Example Setup.eds to open the example experiment file you created in “Print and save the experiment” on page 51.

2. Load the reaction plate into the instrument.

3. To start your instrument:

   1. Click **Run** in the navigation pane.
   2. Click **START RUN** at the top of any run screen.

Analyze the results

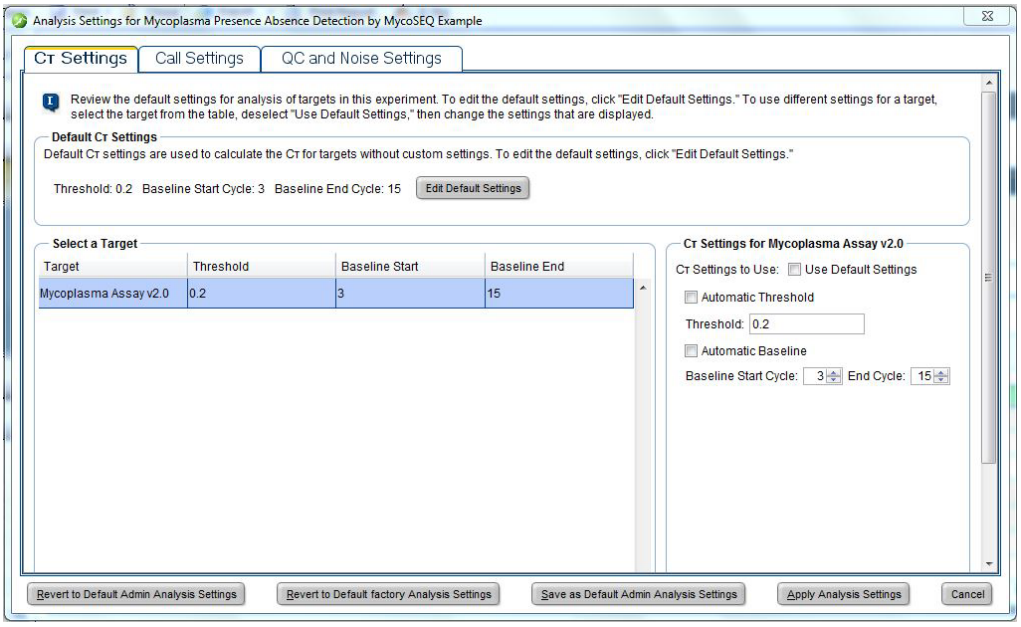
IMPORTANT! The acceptance criteria that are provided in this section are based on our current knowledge of assay performance in detection of *Mycoplasma* recovered from a wide variety of unknown sample matrices. We recommend that you qualify and validate the assay internally using samples that are specific to your process and manufacturing environment (raw materials, bioreactor, or cell line samples) to ensure that these criteria are appropriate.

For specific sample types, it may be necessary to make slight changes to the acceptance criteria based on specific results. We can provide you with one-on-one support during this process.

Set the baseline and threshold values

For all reactions, use the default Analysis Settings:

1. Select **Manual C_t**, then set Threshold to **0.2**.
2. Select **Manual Baseline**, then enter the following settings:
   - Start (cycle): **3**
   - End (cycle): **15**



Note: Autobaseline can also be used. To edit the baseline go to **Analysis ▸ Analysis Settings**.

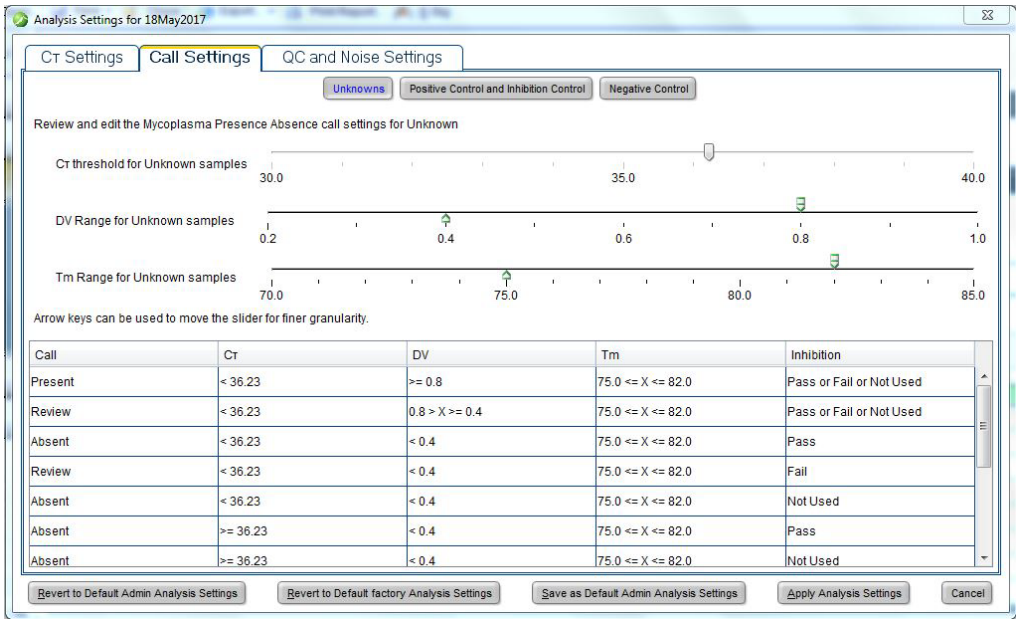
Review the Results Summary

AccuSEQ™ v2.0 or later software uses the acceptance criteria in "Guidance for unknown samples" on page 54 and "Guidance for controls" on page 55 to provide an automated call summary for each reaction. Use the Call Summary, Plate Layout, and Table views in the **Results Summary** screen to review the experiment results.

1. From the navigation pane, select **Results** ▸ **Results Summary**.

2. Review the **Call Summary** for results.

3. *(Optional)* Adjust the *Mycoplasma* presence/absence analysis to meet your method qualifications. In the **Call Settings** tab, modify the values of the threshold cycle (C_t), derivative value (DV), and melting temperature (T_m) values. Use the slider interface at the top-half of the screen, to automatically update the comprehensive table at the bottom half of the screen.



Guidance for unknown samples

The table shows criteria for positive and negative calls. A positive call indicates that at least one genome copy of *Mycoplasma* DNA was present in the unknown reaction and the sample is positive for the presence of *Mycoplasma*. The automated threshold setting for derivative value (DV) of 0.8 for AccuSEQ™ software v2.0 (or later) is equivalent to the 0.05 setting for SDS v1.4 (or later) software.

Note: Assay acceptance criteria are subject to your own validation. T_m and DV assay acceptance criteria are only relevant if C_t acceptance criteria are met.

Table 6 Example acceptance criteria for unknown samples: AccuSEQ™ software v2.0 or later

Result	C_t	T_m (°C)	DV
Positive	<36.23 C_t	75 – 82	≥0.8
Negative	≥36.23 C_t	<75	N/A

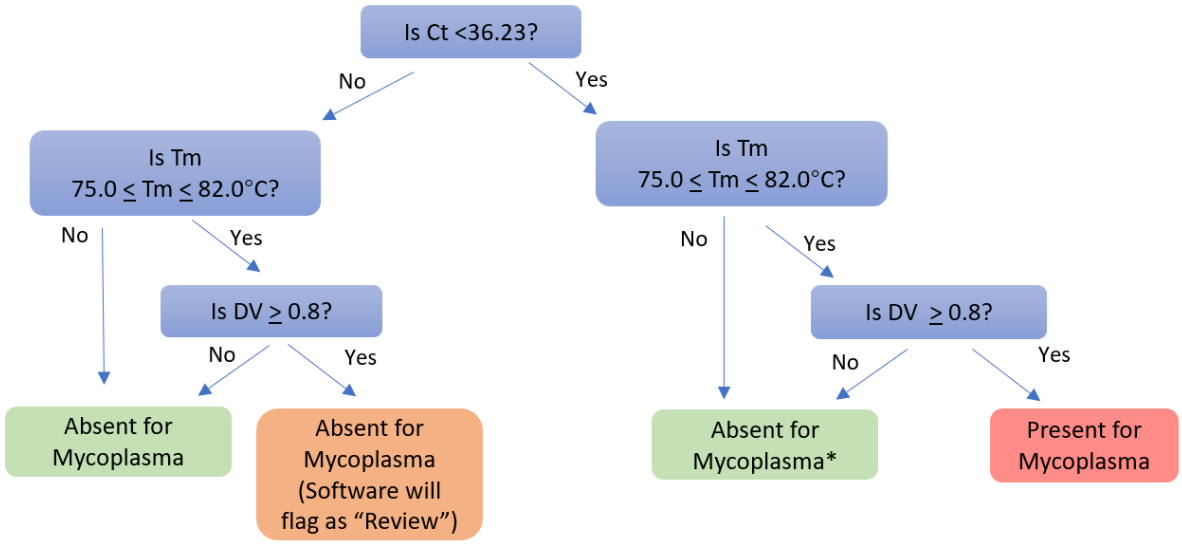


Figure 16 Decision tree for unknown sample calls (without inhibition control)

*When unknown sample C_t is <36.23 and T_m is <75°C, the result is typically caused by primer dimers.

Note: When unknown sample DV is ≥0.8 and T_m >82°C the typical cause is the presence of DPC, this is reported by the software as a **Fail**

Guidance for controls

Note: Assay acceptance criteria are subject to your own validation. T_m and DV assay acceptance criteria are only relevant if C_t acceptance criteria are met.

Table 7 Example acceptance criteria for controls: AccuSEQ™ software v2.0 or later.

Control	C_t	T_m (°C)	DV
PCR positive control	< 36.23 C_t	82–86	> 0.8
Extraction spike control	< 36.23 C_t	82–86	> 0.8
No template control	≥ 36.23 C_t	< 75	N/A
Blank extraction control	≥ 36.23 C_t	< 75	N/A
Inhibition control	ΔC_t < 2	82–86	N/A

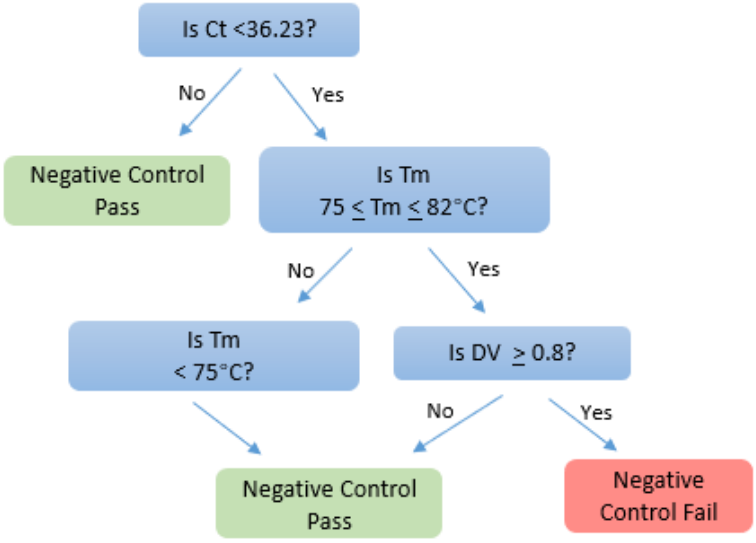


Figure 17 Decision tree for NTC calls

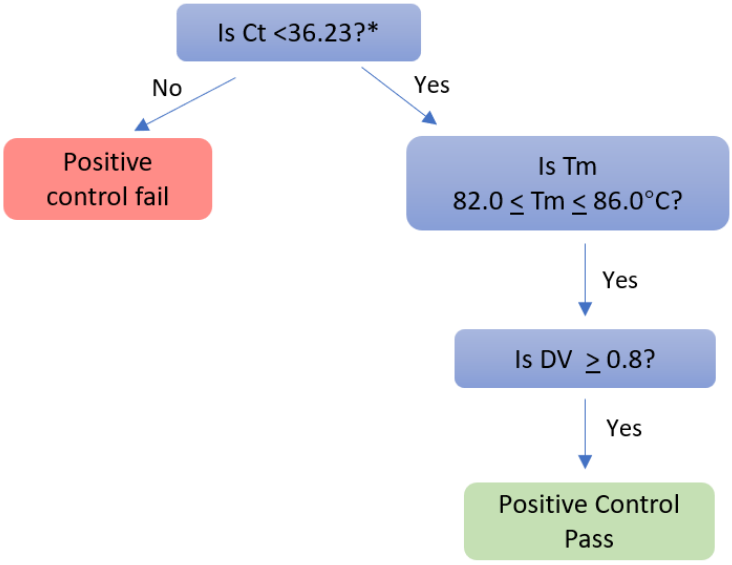


Figure 18 Decision tree for positive control calls

Note: In AccuSEQ™ software v2.2 the C_t condition for a Positive control is set between 23.5≤X≤27.5, this range can be edited to reflect customer preference.

Review call assessments

Call Assessment	Description	Possible Cause	Recommended Action
Positive control failed	All positive controls yield fail result.	No positive control DNA was added to the positive control well. Possible error in pipetting.	Repeat the experiment.
Negative control failed	All negative controls yield fail result.	Reagent contamination. Work area and/or pipette contamination.	

(continued)

Call Assessment	Description	Possible Cause	Recommended Action
Bad passive reference signal	Passive reference signal is abnormal.	Droplets on the sides of the wells. Improper sealing or seal leaks. Condensation on the reaction plate. Inconsistent volumes across the plate.	If a well is flagged, ensure the results: 1. Select the flagged well or wells in the plate layout or well table. 2. View the multicomponent plot, and review the passive reference signal for abnormalities. 3. View the amplification plot (Rn vs. Cycle), and review the data in the C_T region for abnormalities. 4. Examine the reaction plate, and check for: 5. **Droplets:** Repeat the experiment, and ensure that you briefly centrifuge the plate before loading it into the instrument. 6. **Improper sealing, condensation, or inconsistent volumes:** Repeat the reactions, and ensure that you properly seal the plate.
		Pipetting errors.	Calibrate the pipettors, then repeat the experiment.
Baseline algorithm failed	The software cannot calculate the best fit baseline for the data.	The automatic baseline algorithm failed, and the software cannot calculate the best fit baseline for the data.	If a well is flagged, ensure the results: 1. Select the flagged well or wells in the plate layout or well table. 2. View the amplification plot (Rn vs. Cycle and ΔRn vs. Cycle), and check for late amplification or no amplification. 3. Manually set the baseline and threshold values, then click **Analyze**. **Note:** If you manually set the baseline and threshold values, the settings are applied to the C_T settings for the target. Advanced settings are maintained.
C_T algorithm failed	The software cannot calculate C_T.	The automatic C_T algorithm failed, and the software cannot calculate the threshold.	If a well is flagged, ensure the results: 1. Select the flagged well or wells in the plate layout or well table. 2. View the amplification plot (Rn vs. Cycle and ΔRn vs. Cycle), and check for:

(continued)

Call Assessment	Description	Possible Cause	Recommended Action
Exponential algorithm failed	The software cannot identify the exponential region of the amplification plot.	The automatic C_T algorithm failed, and the software cannot identify the exponential region of the amplification plot.	**3.** Amplification too early **4.** Amplification too late **5.** Low amplification **6.** No amplification **7.** If the amplification looks acceptable, manually set the baseline and threshold values, then click **Analyze**. **Note:** If you manually set the baseline and threshold values, the settings are applied to the C_T settings for the target. Advanced settings are maintained.
Failed IC prevents call	Values for DV, T_m, and C_T met requirements for negative call but detection of inhibition in IC prevents definitive absent call.	Excess host cell genomic DNA remains in the sample matrix.	Verify the elevated SYBR™ signal in the component view. If elevated, re-extract using the enzymatic treatment. Contact technical support for the suggested re-extraction protocol.
High background signal	The background fluorescence signal is greater than 1.5 M fluorescent standard units (FSU).	Excess host cell genomic DNA remains in sample matrix.	Verify elevated SYBR™ signal in the component view. If elevated, re-extract using the enzymatic treatment. Contact technical support for the suggested re-extraction protocol.
Inhibition detected	Delta C_T and T_m did not meet specified values needed for "Pass".	Excess host cell genomic DNA remains in the sample matrix or T_m was in the range specified for discriminatory positive control (DPC).	Verify T_m of DPC in the Melt Curve view. Verify elevated SYBR™ signal in the component view. If elevated, re-extract using the DNase treatment. Contact technical support for the suggested re-extraction protocol.
NC criteria failed	C_T, DV, or T_m did not meet specified values needed for "Pass".	Sample was contaminated, or master mix was not added to the sample.	If contaminated, clean all work areas and pipettes. Reprocess the sample with new reagents and repeat the experiment. If master mix was not added, add master mix and repeat the experiment.

(continued)

Call Assessment	Description	Possible Cause	Recommended Action
No signal in well	The well produced low or no fluorescence.	Absence of fluorescent reporter dye (FAM™, VIC™, SYBR™ Green, etc.) due to pipetting error. Reagents and sample template were pipetted in a different well that was not specified in the plate layout	Repeat the experiment; ensure that you add all necessary reagents. Check all plate wells to see if there is signal in a well that was not specified in the plate layout. If there is, specify the well as a sample and reanalyze.
Not supported by IC	C_T, DV, and T_m results support absent call, but inhibition control (IC) was not run. Absent call should be supported by more data demonstrating a absence of matrix inhibition.	Experiment run without inhibition control. We recommend that an IC be run for every unknown.	Verify that an inhibition control for matrix was previously run with no inhibition before accepting the absence call. Repeat the experiment with IC control.
PC criteria failed	C_T, DV, or T_m did not meet specified values needed for "Pass."	Master mix was not added to the sample (failed reaction) or discriminatory positive control (DPC) was not added to the sample at the recommended levels.	Prepare new samples and repeat the experiment.
Positive control signal detected	Peak for discriminatory positive was found in a sample labeled as an unknown, indicating potential error in sample preparation or plate setup.	Sample is cross-contaminated with discriminatory positive control.	

(continued)

Call Assessment	Description	Possible Cause	Recommended Action
Review C_T and DV values	Significant peak found inside specified temperature range and inside specified C_T threshold, but DV magnitude is insufficient for positive call. And/Or C_T and T_m results support absent call, but DV magnitude is too high for absent call.	An not correct volume of discriminatory positive control (DPC) was added to the positive control well.	Verify pipette calibration, prepare new samples, and repeat the experiment.
Thresholding algorithm failed	The software cannot calculate a threshold.	The automatic C_T algorithm failed, and the software cannot calculate the threshold.	If a well is flagged, ensure the results: **1.** Select the flagged well or wells in the plate layout or well table. **2.** View the amplification plot (Rn vs. Cycle and ΔRn vs. Cycle), and check for: • Amplification too early • Amplification too late • Low amplification • No amplification **3.** If the amplification looks acceptable, manually set the baseline and threshold values, then click **Analyze**. **Note:** If you manually set the baseline and threshold values, the settings are applied to the C_T settings for the target. Advanced settings are maintained.

Guidance for inconclusive results with AccuSEQ™ software v2.0

If a MycoSEQ™ assay does not meet all of the criteria for a positive or negative automatic call, the well displays (inconclusive). For information about these results:

- Click Quality Summary (Quality Summary) in the Results navigation pane of the AccuSEQ™ software v2.0 screen.
- Click (Help) in the toolbar at the top of the AccuSEQ™ software v2.0 screen.
- See Appendix A, “Troubleshooting”.
- Refer to the *AccuSEQ™ Real-Time PCR Detection Software Mycoplasma SEQ Experiments Getting Started Guide*.

Example results with AccuSEQ™ Software v. 2.1.1

Positive control

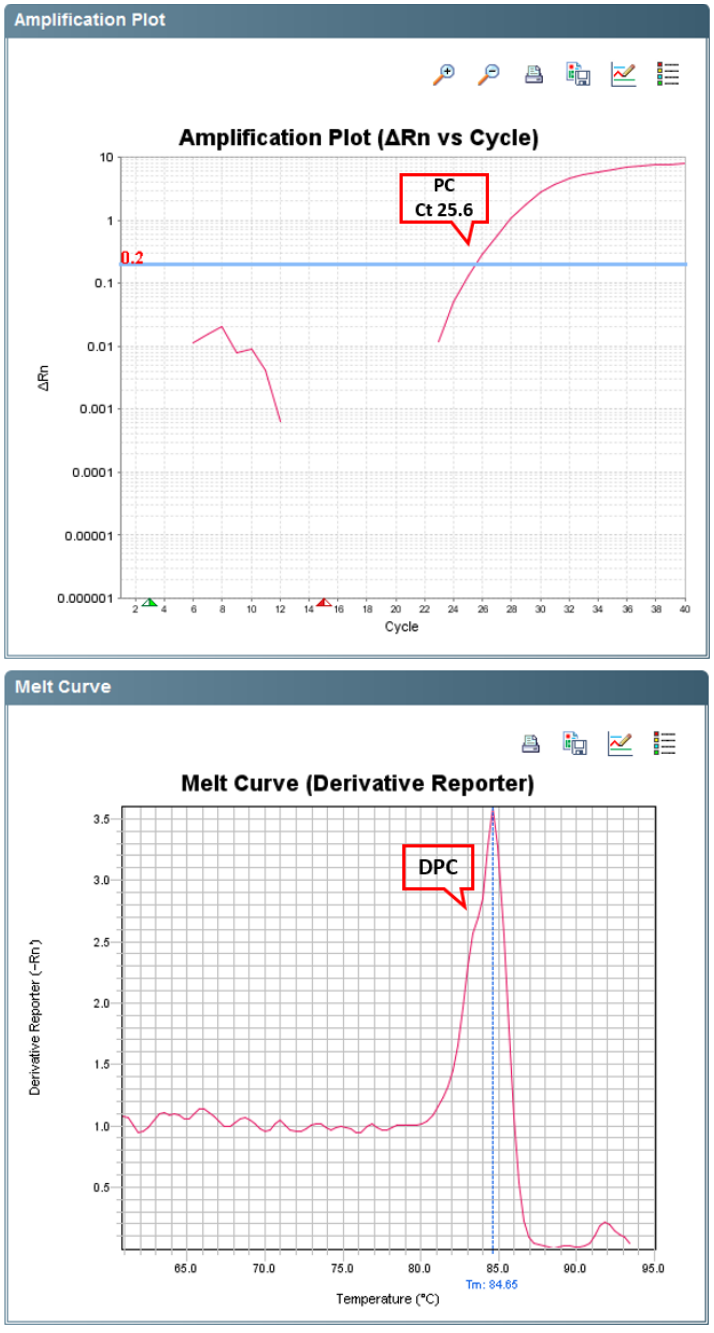


Figure 19 PCR positive control spiked with 2,000 copies of DPC.

Negative control

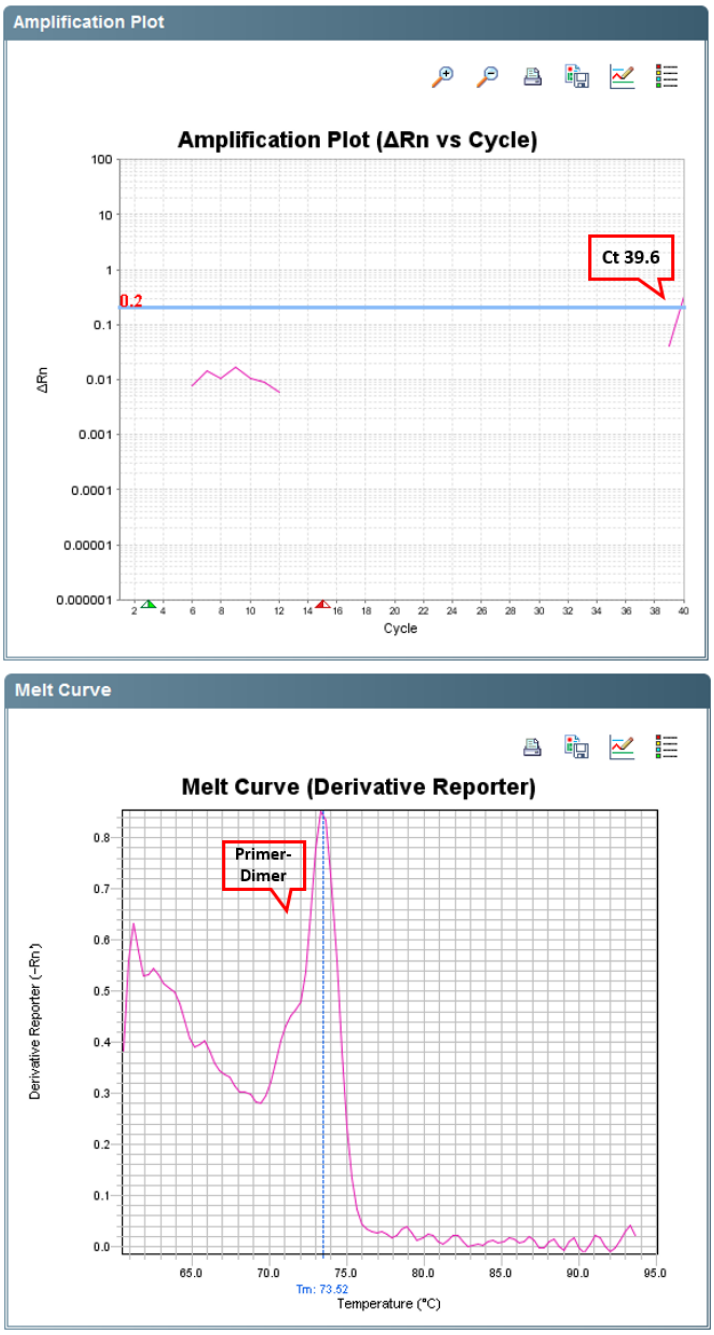


Figure 20 No template PCR control.

Blank extraction control

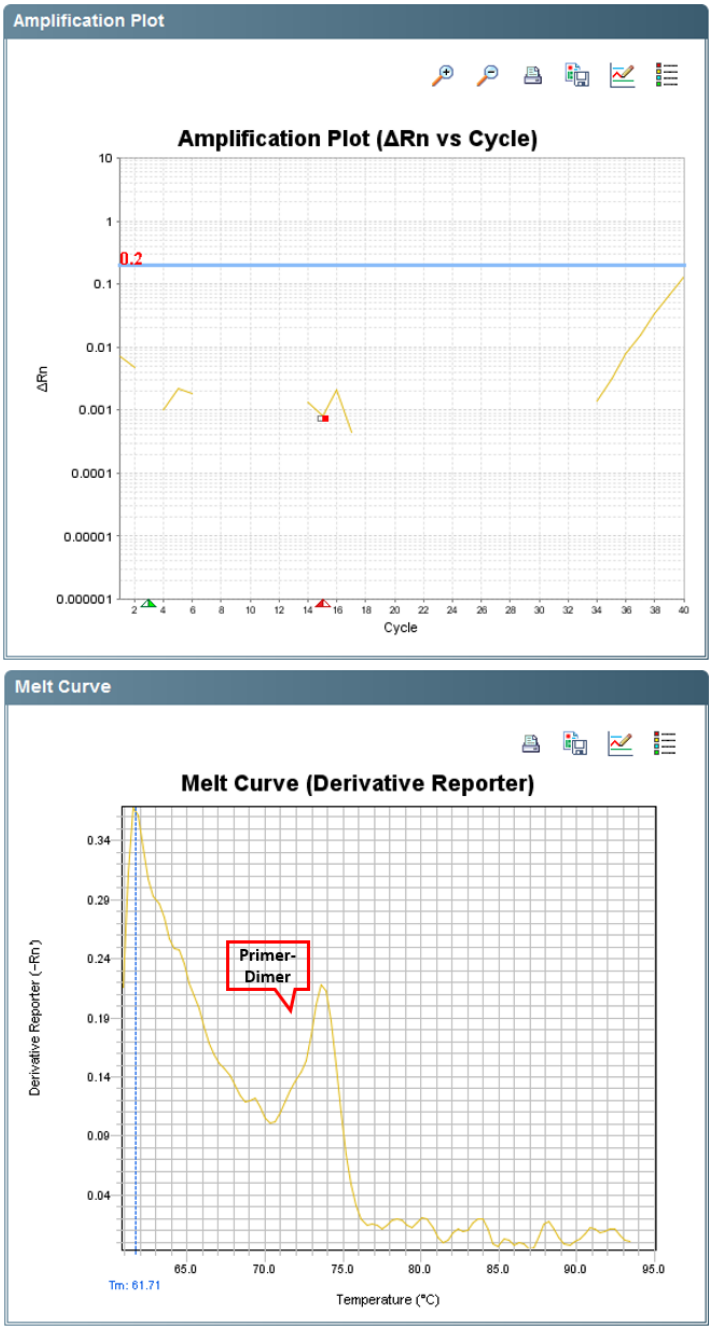


Figure 21 Blank extraction control with PBS.

Positive extraction control

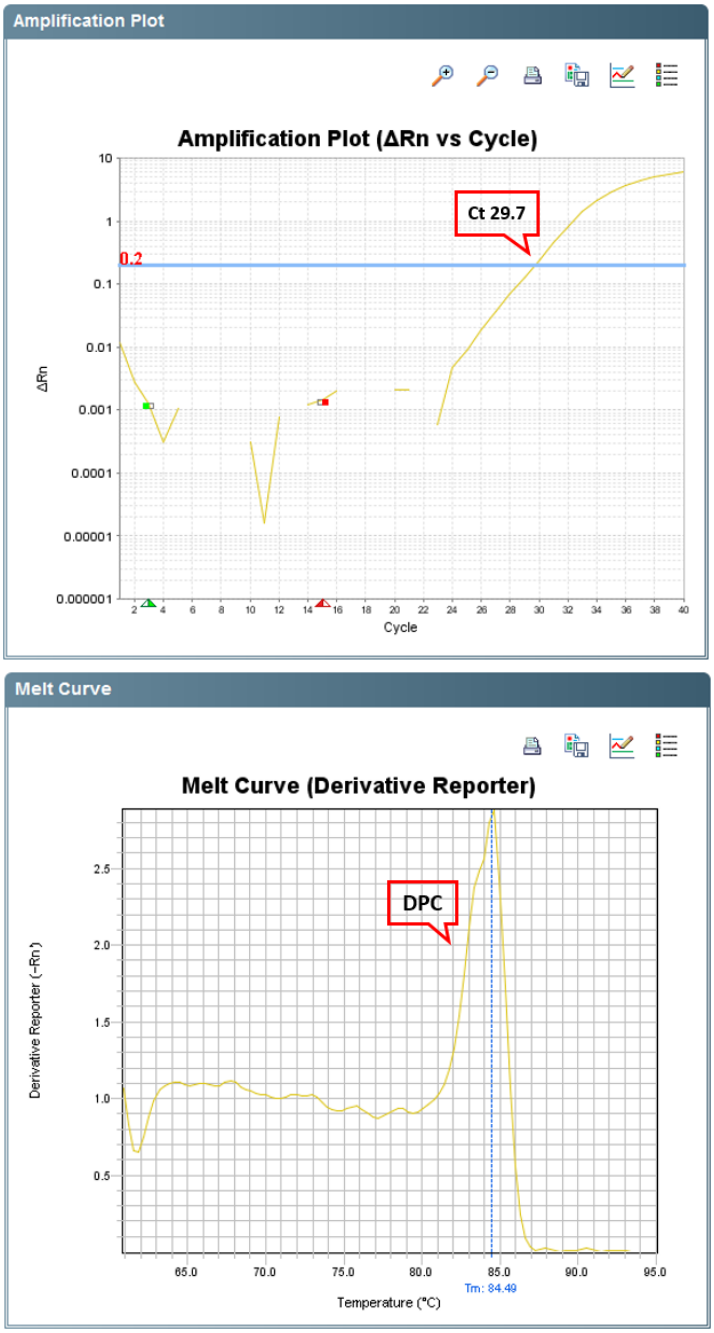


Figure 22 Sample spiked with 2,000 copies of DPC before DNA extraction.

Inhibition control and positive control

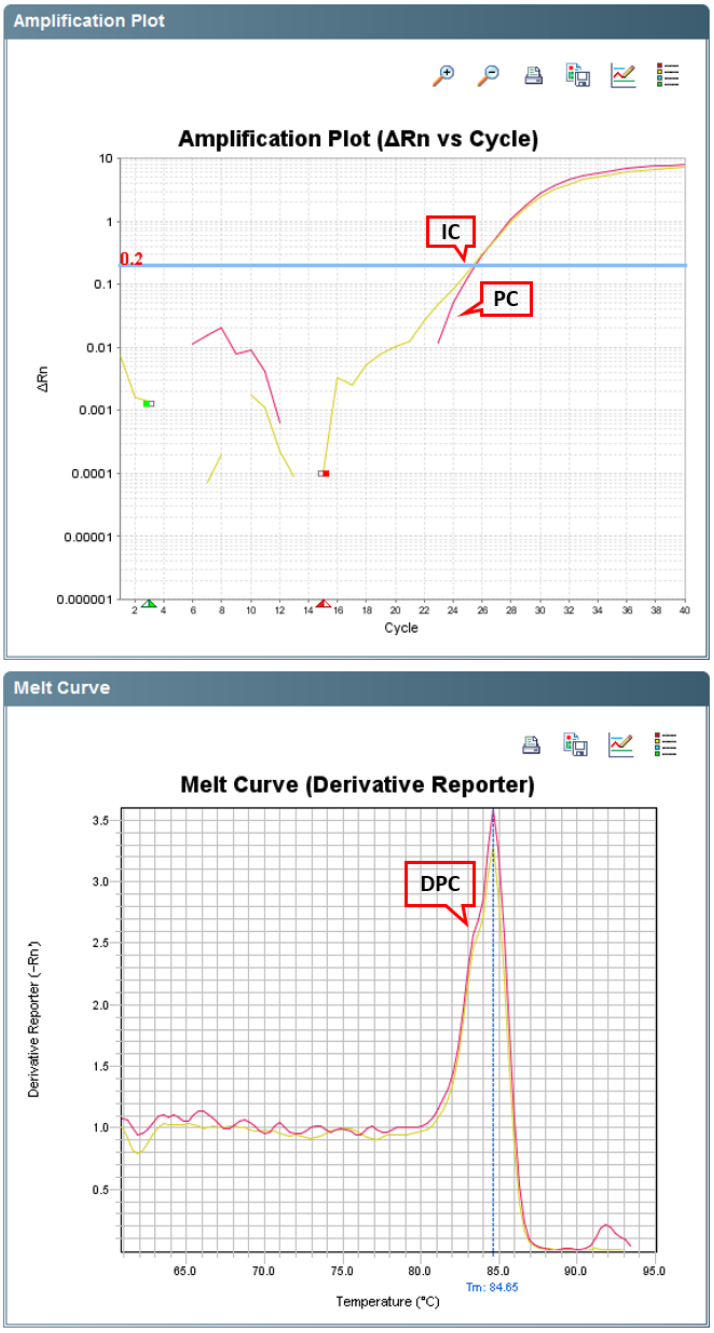


Figure 23 No PCR inhibition present; inhibition control and PCR positive control overlaid, with a $\Delta C_t < 2$.

Unknown sample: Negative result

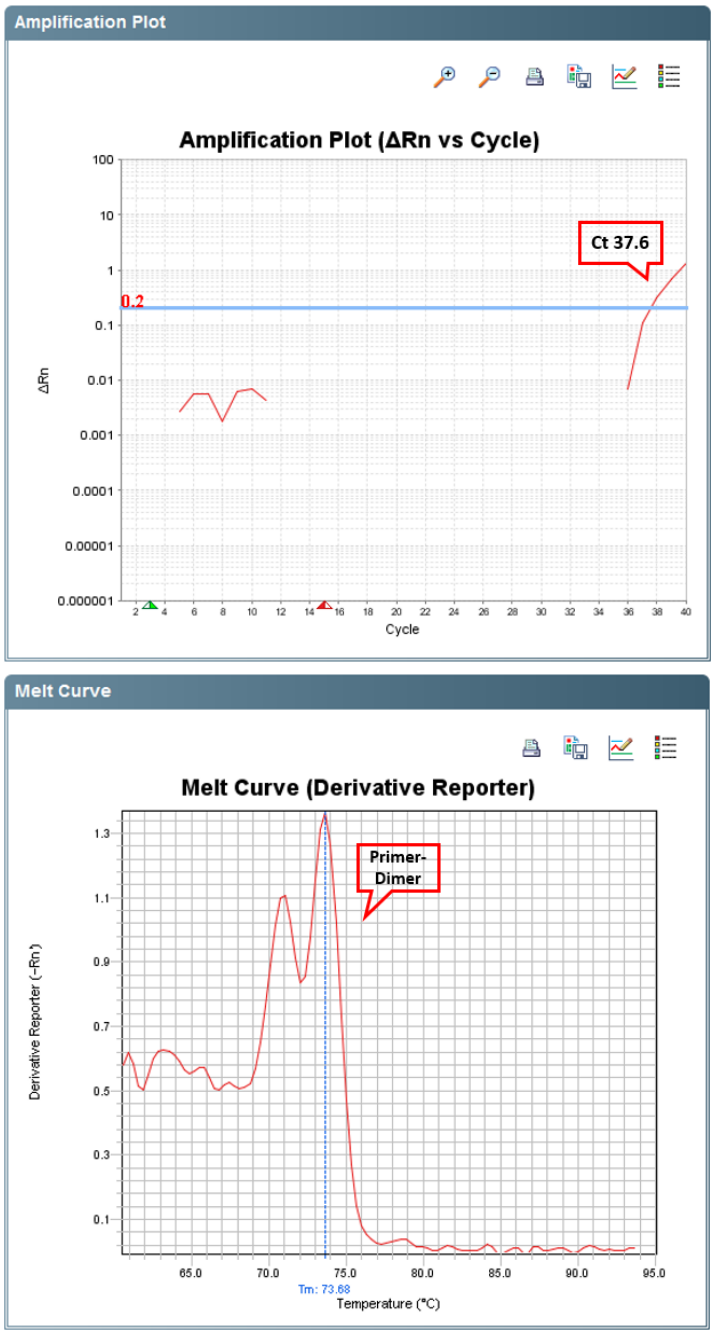


Figure 24 Negative result; C_t > 36.23 and T_m < 75°C.

Unknown sample: Positive result

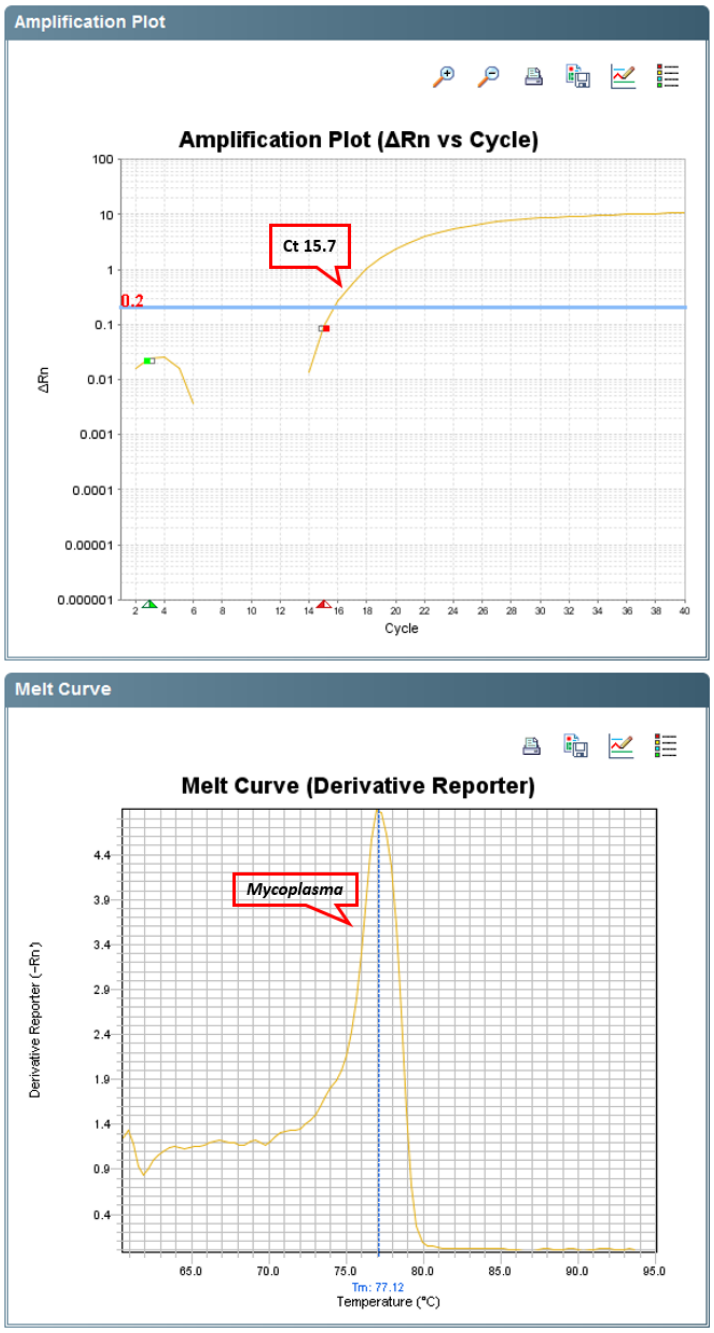


Figure 25 Positive result; C_t = 15.69, T_m > 75°C, and Derivative Reporter >0.8.

Unknown sample: Positive result with decreased detection of DPC

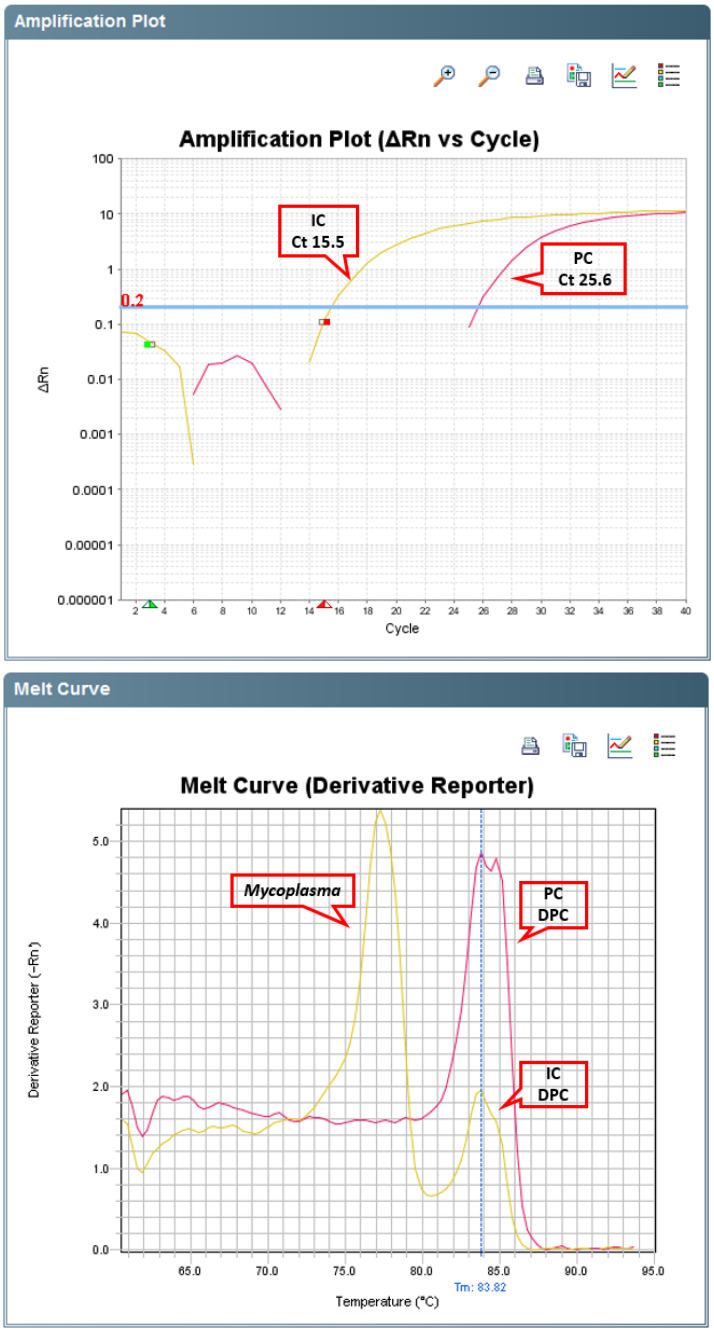


Figure 26 Decreased DPC signal can be observed in the presence of very high *mycoplasma* contamination.

PCR inhibition

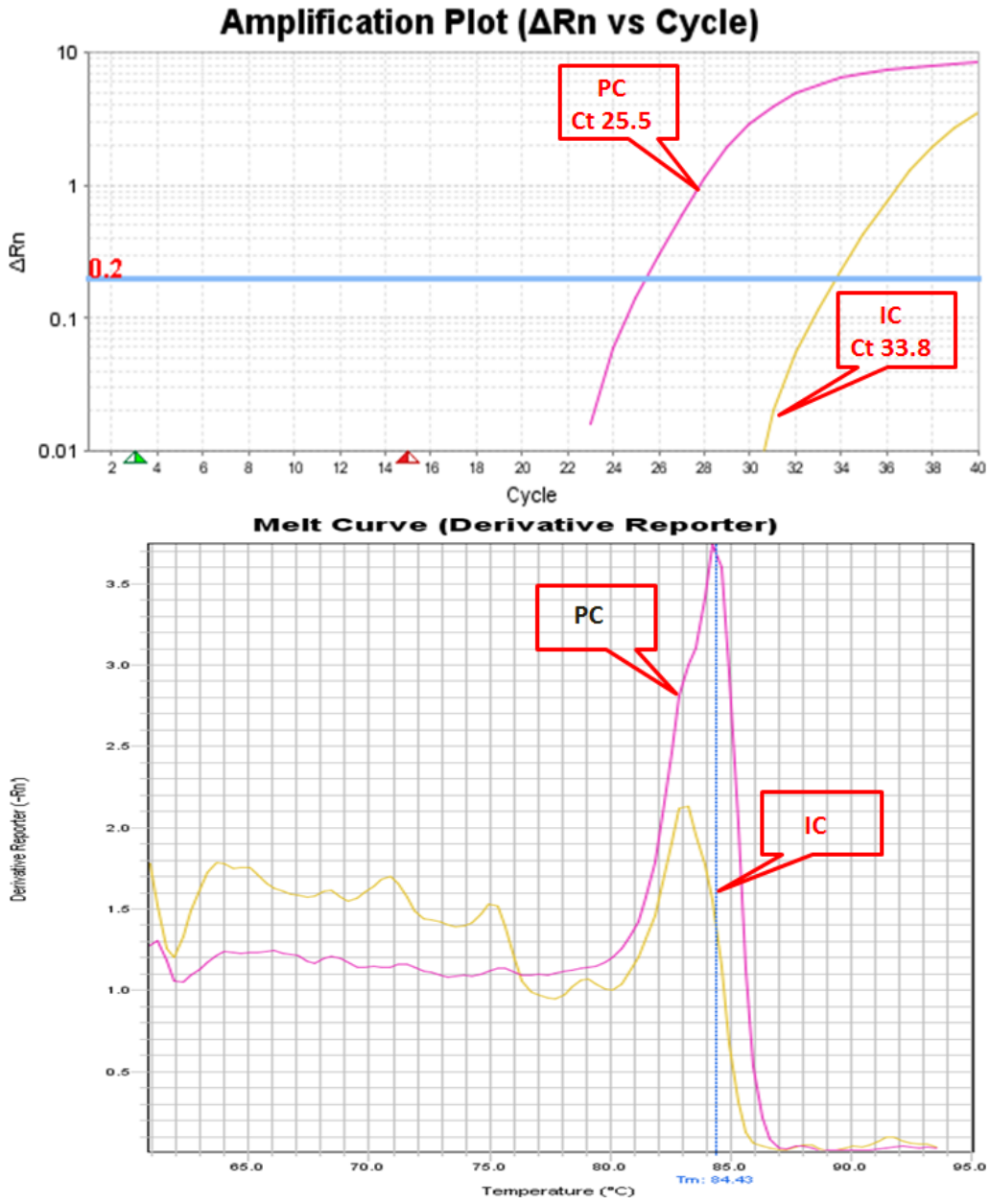


Figure 27 PCR inhibition, $\Delta C_t > 2$.

Multicomponent plots

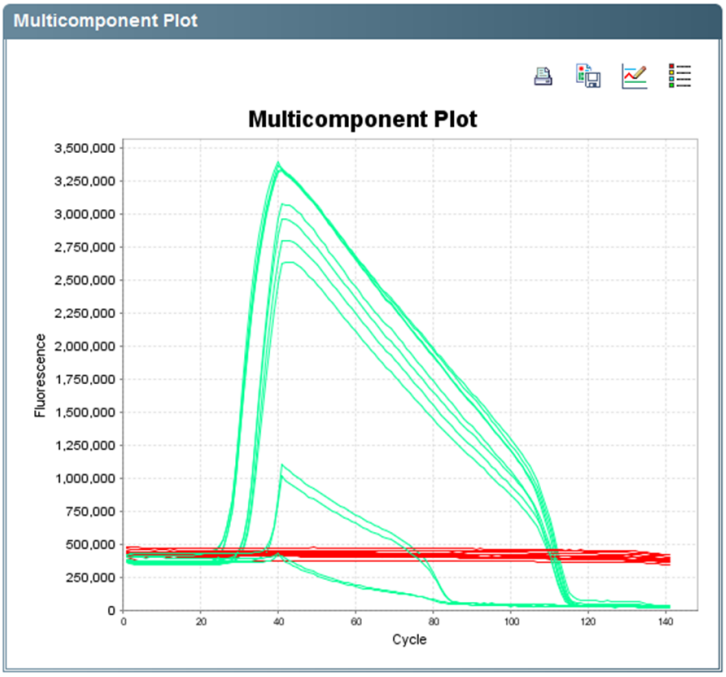


Figure 28 An example of a multicomponent plot.

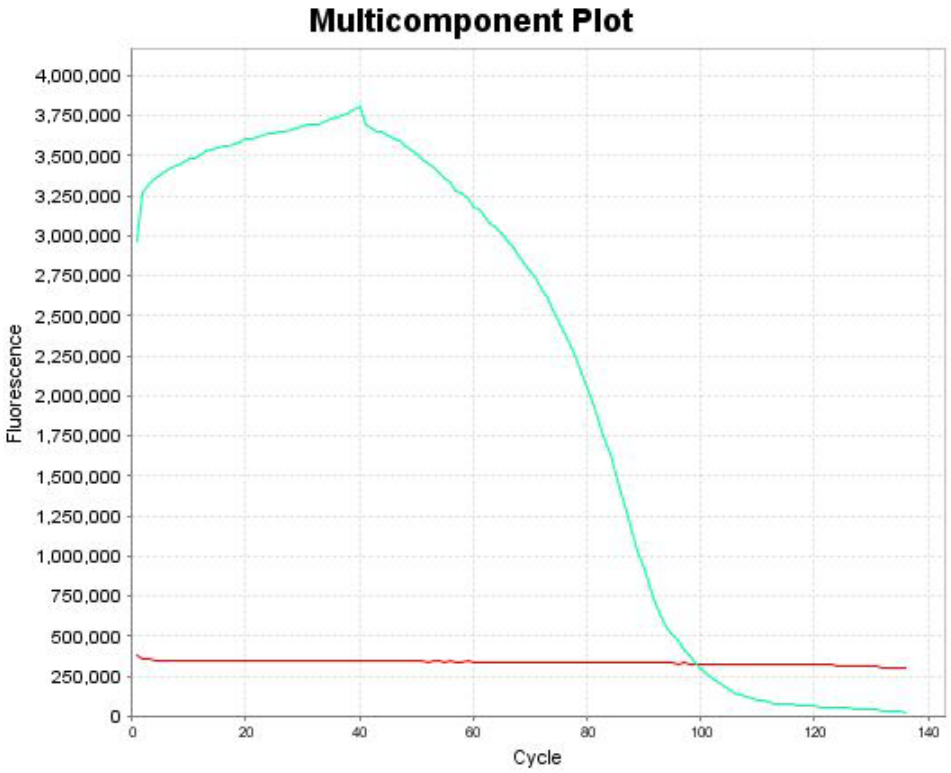


Figure 29 A multicomponent plot with high background signal which will result in PCR inhibition.

D Use the kit with 7500 System SDS Software v1.4 or later

Prepare the kit reagents and premix solution

1. Thaw all kit reagents completely.
2. Vortex briefly, then spin down the reagents.
3. Prepare the Premix Solution according to the following table.

Component for premix solution	Volume for one 30-µL reaction	Volume for four 30-µL reactions[1]
Power SYBR™ Green PCR Master Mix, 2×	15.0 µL	66.0 µL
Mycoplasma Real-Time PCR Primer Mix, 10×	3.0 µL	13.2 µL
Total premix solution volume	**18.0 µL**	**79.2 µL**

[1] Includes 10% excess to compensate for pipetting errors.

4. Mix the Premix Solution by gently pipetting up and down, then cap the tube.

Prepare the PCR reactions

1. Dispense the following into each well to be used, gently pipetting at the bottom of the well.

To prepare…	In each tube or well…
Negative control reaction	• Add 18 µL of Premix Solution • Add 12 µL of Negative Control (water)
Your unknown sample reaction	• Add 18 µL of Premix Solution • Add 10 µL of unknown sample • Add 2 µL of Negative Control (water)
Inhibition-control reaction	• Add 18 µL of Premix Solution • Add 10 µL of unknown sample • Add 2 µL of the Discriminatory Positive Control (DPC)
Positive control reaction	• Add 18 µL of Premix Solution • Add 2 µL of the DPC • Add 10 µL of Negative Control (water)

Note: The MycoSEQ™ *Mycoplasma* Discriminatory Positive/Extraction Control can be used as a spike control that is added to the unknown sample or lysate before sample preparation

For units:

- With standard 0.2-mL block – Dispense into a standard optical 96-well plate (Cat. No. 4306737).
- With Fast 0.1-mL block – Dispense into a Fast optical 96-well plate (Cat. No. 4346906).

2. For each row of wells that you use, place in sequence from left to right the negative control, unknown sample, inhibition control, then positive control. See “Plate layout suggestions” on page 86 for more information.

   Pipetting guidelines:

   - Use at least one negative and one positive control per run.
   - Mix each sample gently by pipetting up and down.
   - Use a new tip for each well, even when aliquoting the same solution.

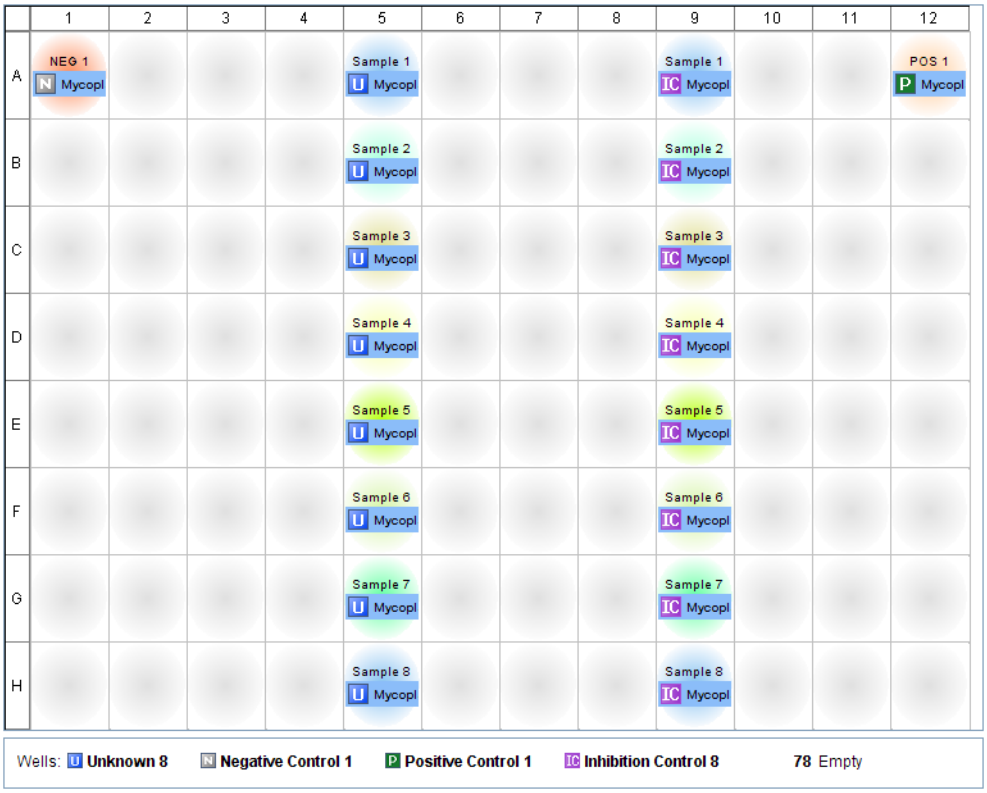
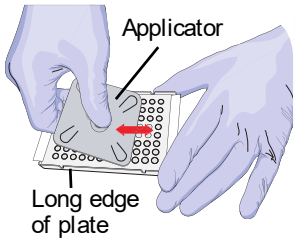


Figure 30 Example plate layout.

Seal the plates

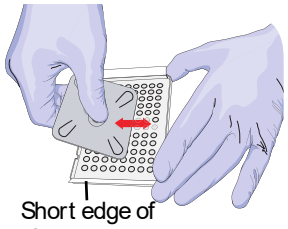
1. Place an optical adhesive cover on the plate, then rub the flat edge of the applicator back and forth along the **long** edge of the plate.



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

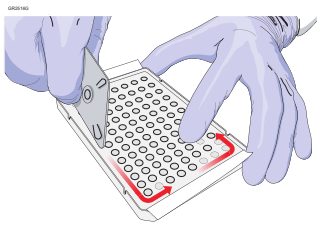
2. Rub the flat edge of the applicator back and forth along the **short** edge (width) of the plate.

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.

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.

Prepare the plate document

Set up the plate document in the SDS software. For more details, see the *Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve* (Pub. No. 4347825).

1. In the **Assay** drop-down list, select **Absolute Quantification**.

2. Select **SYBR™ detector** with:
   - Quencher Dye set to **none** or **Non Fluorescent**
   - Passive Reference set to **ROX™**

3. Set thermal-cycling conditions as indicated in the table below.

Note: For instruments using the AccuSEQ™ 2.x Real-Time PCR Software *Mycoplasma* Module, the cycling conditions are pre-programmed in the software.

Step	AmpliTaq Gold™ enzyme activation	PCR		Dissociation[1,2,3]			
	HOLD	Cycle (40 cycles)		Melt			
		Denature	Anneal/ extend				
Temp	95°C	95°C	60°C	95°C	60°C	95°C	60°C
Time	10 min	15 sec	1 min	15 sec	1 min	15 sec	15 sec

[1] 7500 and 7500 Fast Systems: from the Instrument tab, click **Add Dissociation Stage** (see Figure 31).

[2] Applied Biosystems™ Real-Time PCR Instruments: from the Instrument tab, click **Add Dissociation Stage** (see Figure 31). Use default settings..

[3] For other instruments, refer to their corresponding user guides for dissociation-curve setup information.

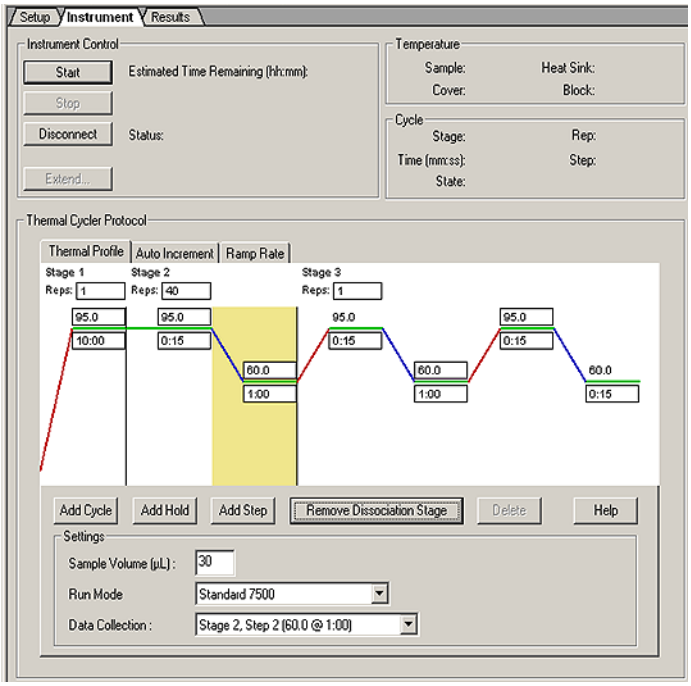


Figure 31 The instrument tab for 7500 Fast Real-Time PCR platform with SDS v1.4 software. The run mode is set to Standard 7500.

4. Set Sample Volume to **30** µL.

5. Select the Standard Run Mode for use with SYBR™ Green I dye.

Perform PCR

On an Applied Biosystems™ Real-Time PCR System:

1. Open the plate document that corresponds to the reaction plate (“Prepare the plate document” on page 74).
2. Load the reaction plate into the real-time PCR system.
3. Start the run.

Analyze the results

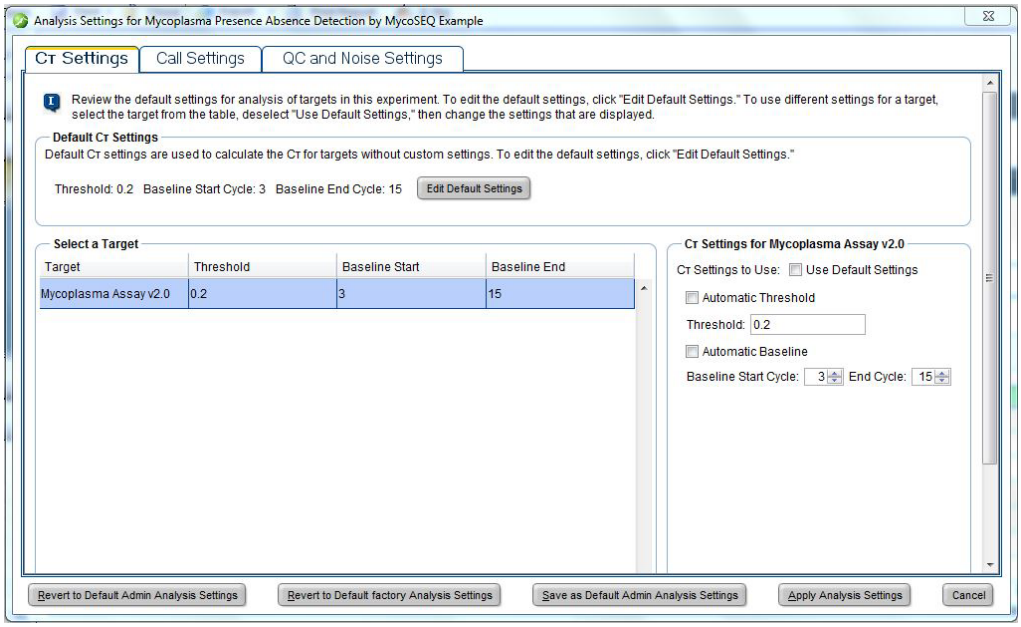
IMPORTANT! The acceptance criteria that are provided in this section are based on our current knowledge of assay performance in detection of *Mycoplasma* recovered from a wide variety of unknown sample matrices. We recommend that you qualify and validate the assay internally using samples that are specific to your process and manufacturing environment (raw materials, bioreactor, or cell line samples) to ensure that these criteria are appropriate.

For specific sample types, it may be necessary to make slight changes to the acceptance criteria based on specific results. We can provide you with one-on-one support during this process.

Set the baseline and threshold values

For all reactions, use the default Analysis Settings:

1. Select **Manual C_t**, then set Threshold to **0.2**.
2. Select **Manual Baseline**, then enter the following settings:
   - Start (cycle): **3**
   - End (cycle): **15**



Note: Autobaseline can also be used. To edit the baseline go to **Analysis ▸ Analysis Settings**.

Guidance for unknown samples

The table shows criteria for positive and negative calls. A positive call indicates that at least one genome copy of *Mycoplasma* DNA was present in the unknown reaction and the sample is positive for the presence of *Mycoplasma*. The automated threshold setting for derivative value (DV) of 0.8 for AccuSEQ™ 2.0 (or later) software is equivalent to the 0.05 setting for SDS v1.4 (or later) software.

Note: The values in the tables are subject to your own validation.

Table 8 Recommended acceptance criteria for unknown samples: SDS software v1.4 or later.

Result	C_t	T_m	DV
Positive	< 36 C_t	75°C – 81°C	≥0.05
Negative	≥ 36 C_t	< 75°C	N/A

Guidance for controls

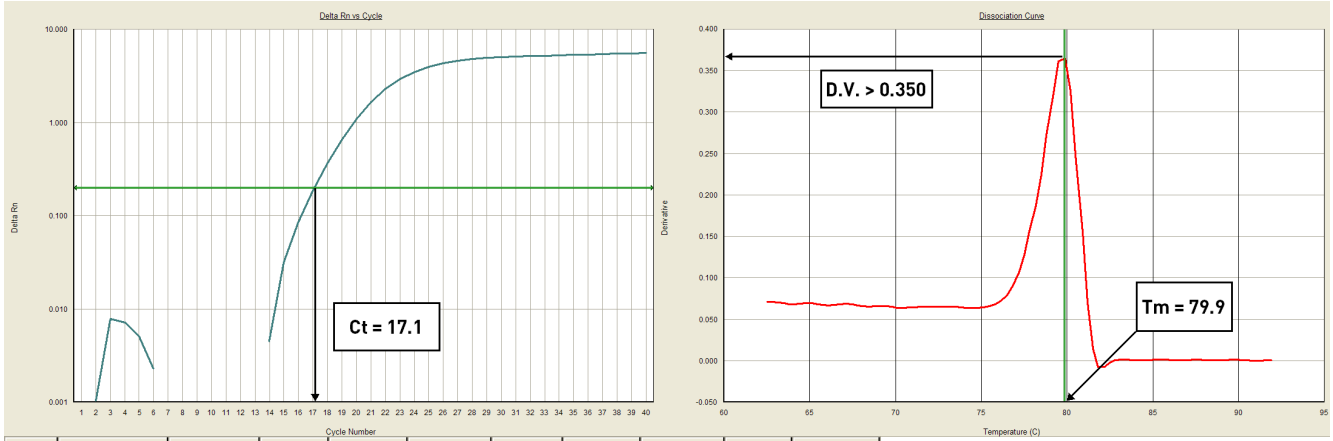
The values in the tables are subject to your own validation.

Table 9 Recommended acceptance criteria for controls: SDS software v1.4 or later.

Control	C_t	T_m	DV
PCR positive control	< 36 C_t	≈84°C	> 0.05
Extraction spike control	< 36 C_t	≈84°C	> 0.05
No template control	≥ 36 C_t	< 75°C	N/A
Blank extraction control	≥ 36 C_t	< 75°C	N/A
Inhibition control	ΔC_t < 2	≈84°C	N/A

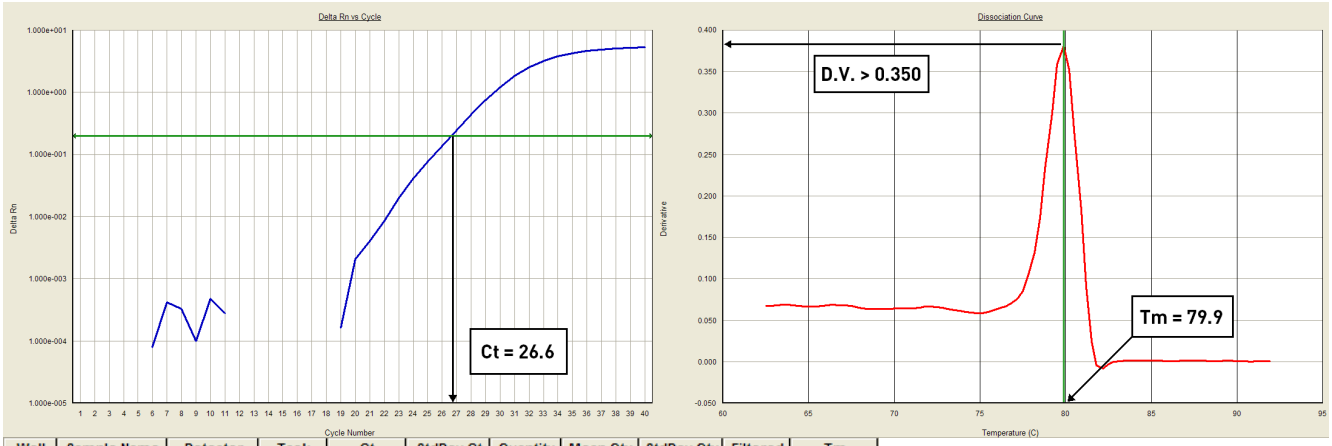
- Both the PCR positive control and the extraction spike control may present extra peaks with T_m < 75°C. These peaks represent primer dimer formation, and they do not interfere with the final results.
- The difference in C_t between the DPC and the inhibition control reaction should be less than 2 cycles. If the unknown sample is negative and the inhibition control shows a ΔC_t > 2 when compared to the positive control, then the PCR is likely inhibited. The sample should be re-purified and the assay repeated. The ΔC_t is calculated by C_t (of inhibition control reaction) – C_t (of positive control reaction).

Example positive results with SDS v1.4 software



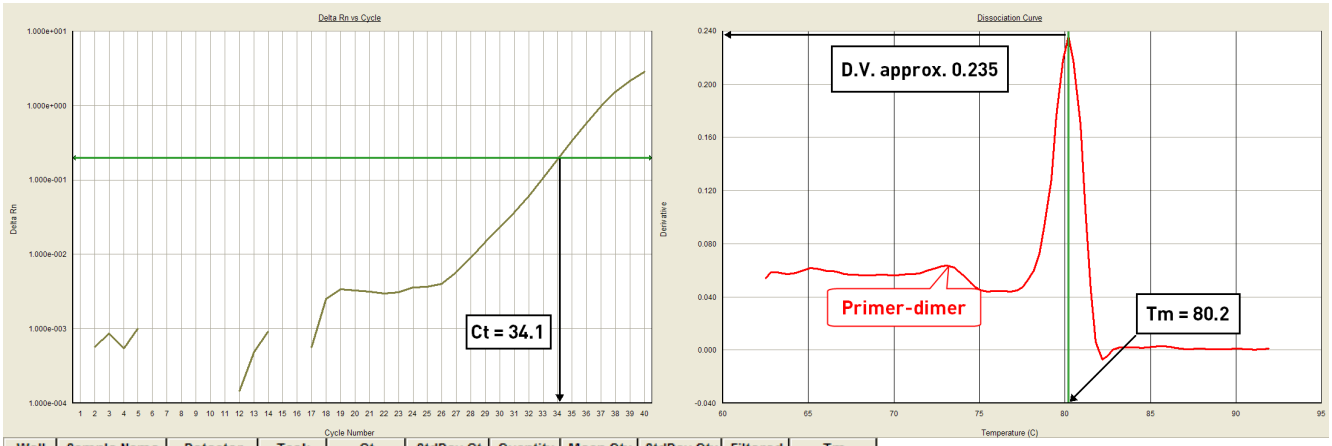
Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm
E1	3	Mycoplasma	Unknown	17.0813						79.9 °C

Figure 32 *Mycoplasma* contamination (approximately 3 × 10^6 copies per PCR reaction).



Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm
A3	5	Mycoplasma	Unknown	26.5762						79.9 °C

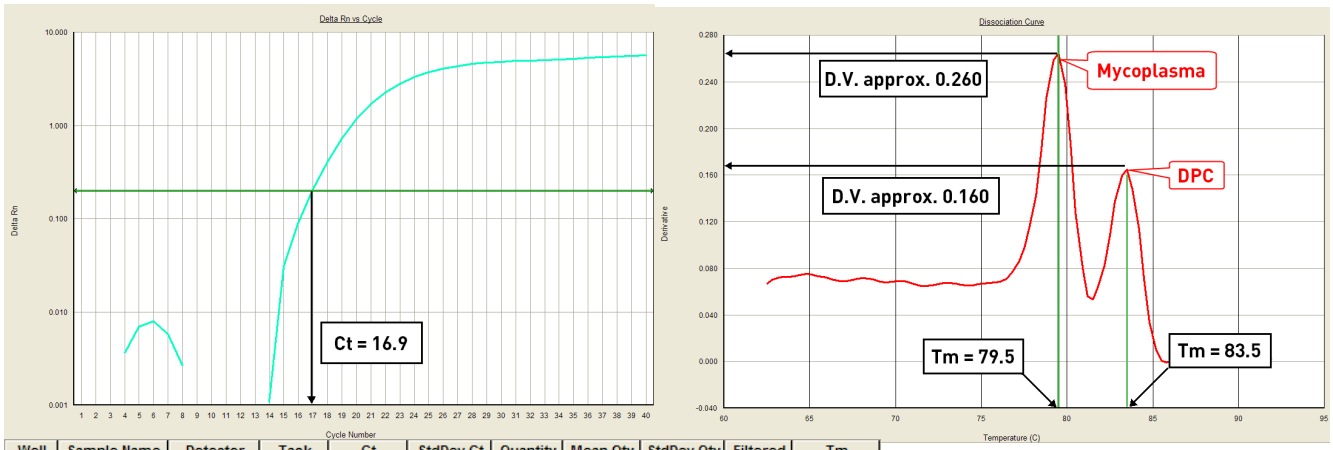
Figure 33 ***Mycoplasma*** **contamination (approximately 2,000 copies per PCR reaction).**



Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm
E3	7	Mycoplasma	Unknown	34.0907						80.2 °C

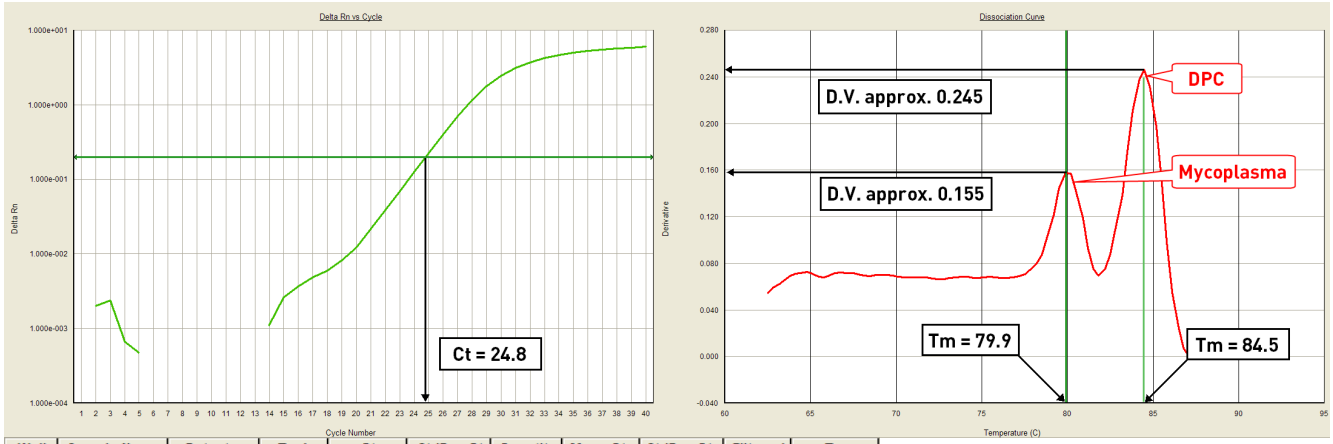
Figure 34 ***Mycoplasma*** **contamination (less than 10 copies per PCR reaction).**

Example positive control extraction results with SDS v1.4 software



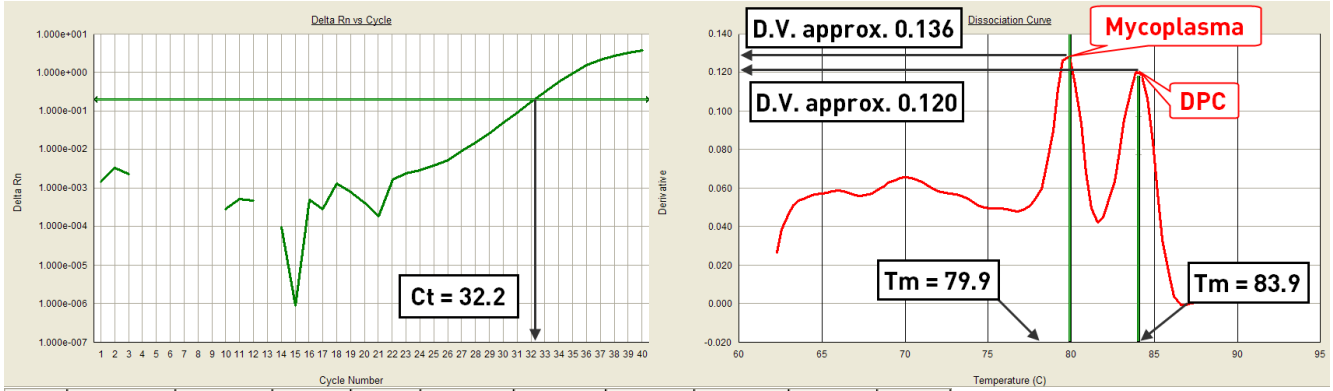
Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm
G1	4	Mycoplasma	Unknown	16.9588						79.5 °C

Figure 35 Sample spiked with 2,000 copies of DPC and contaminated with *Mycoplasma* (3 × 10^6 copies).



Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm
C3	6	Mycoplasma	Unknown	24.7689						84.5 °C

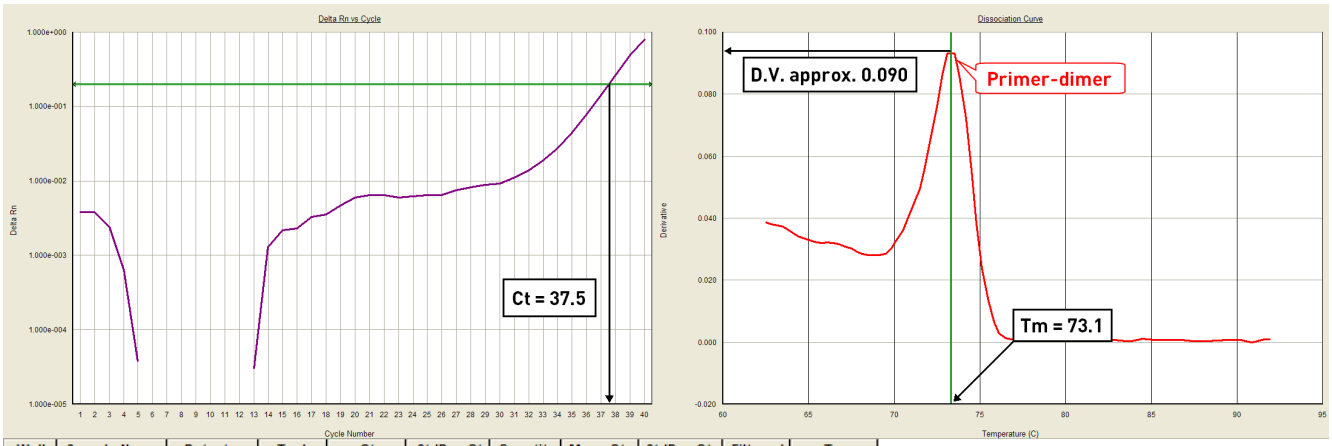
Figure 36 Sample spiked with 2,000 copies of DPC and contaminated with *Mycoplasma* (approximately 2,000 copies).



Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm
A5	Sample D	Mycoplasma	Unknown	32.2065						79.9 °C

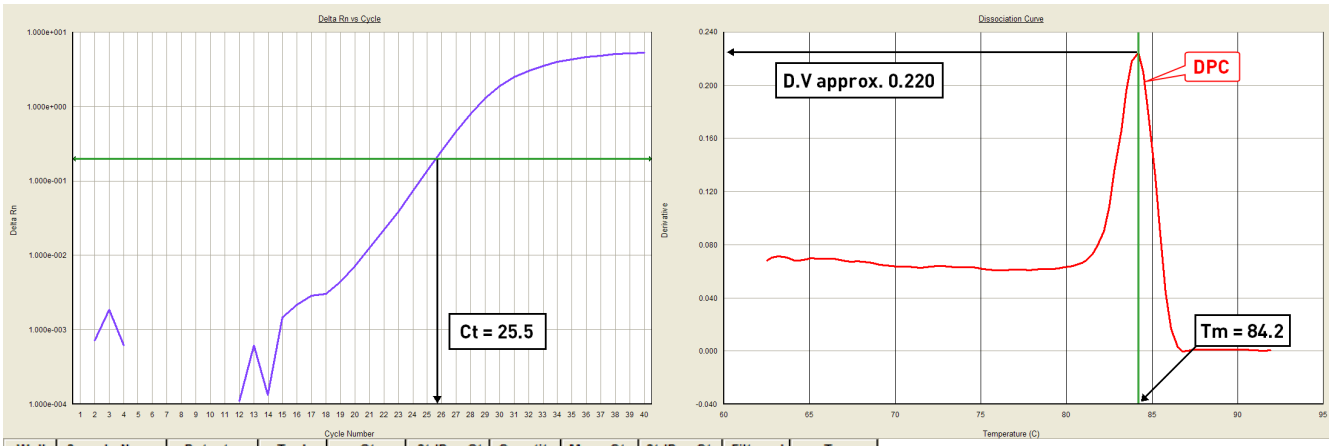
Figure 37 Sample containing 25 copies of *Mycoplasma* and 25 copies of DPC.

Example negative results with SDS v1.4 software



Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm
E5	11	Mycoplasma	Unknown	37.4719						73.1 °C

Figure 38 Negative result.



Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm
G5	12	Mycoplasma	Unknown	25.5402						84.2 °C

Figure 39 Negative sample spiked with 2,000 copies of DPC.

Background information

Mycoplasmas are the smallest and simplest self-replicating organisms. Their genome sizes range from about 540 kb to 1300 kb, with a G+C content of 23 mol to 41 mol%. Although mycoplasmas are derived from the gram-positive branch of walled eubacteria, their evolution from these walled bacteria resulted in a substantial reduction in genome size and loss of the functions required for synthesis and maintenance of a bacterial cell wall.

Mycoplasmas are a common bacterial contaminant of cell culture samples. Infection is persistent, difficult to detect and diagnose, and very difficult to cure. Mycoplasmas vary in size from 0.2 µm to 0.8 µm, so they can pass through some filters that are used to remove bacteria. Mycoplasma in infected cell cultures can change many cell processes, including altering cell growth rate, inducing morphological changes or cell transformation, and mimicking virus infection. Cell culture in pharmaceutical production must be *Mycoplasma*-free as required by the U.S. Pharmacopoeia and FDA regulatory requirements. Therefore, there is an absolute requirement for routine, periodic testing of possible contamination of all cell cultures used in pharmaceutical manufacturing. Because mycoplasmas grow slowly (the colonies can take up to 3 weeks to develop), traditional culture methods are unacceptable for rapid high-throughput testing. The recently introduced and validated rapid bacterial testing methods that are used in this kit provide for fast *Mycoplasma* screening.

F

Kit specificity

Sensitivity

The sensitivity of the PCR using this kit is 1 to 10 copies of the target DNA per reaction. Sensitivity of the assay in real culture samples depends on the quality of the sample preparation method that is used. The sample preparation procedure in the *PrepSEQ™ Sample Preparation Kits for Mycoplasma, MMV, and Vesivirus User Guide* (Pub. No. 4465957) allows you to detect:

- 4 to 10 CFU/mL of *Mycoplasma* from 10 mL of cell culture

  or
- 4 CFU/mL of *Mycoplasma* from 1 mL of media

Note: Detection sensitivity can be affected by factors present in the extracted DNA.

- PCR inhibitors that affects PCR efficiency
- Recovery from the sample preparation method
- Additives in cell culture media that may interfere with recovery of mycoplasma DNA extraction or PCR efficiency
- Cell culture conditions that affect the ratio of genome copy number to CFU

Kit specificity

The MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit can detect more than 90 different *Mycoplasma* species, including *Acholeplasma laidlawii* and *Spiroplasma citri*. The kit does not detect other genera or cell-line DNA.

Inclusivity – detectable species

The kit procedure in this protocol is designed to detect over 90 species, including the 14 shown below in the first table. For a complete list of species, contact Technical Support.

Species	Strain/source
Acholeplasma laidlawii	ATCC 23206D
Mycoplasma arginini	ATCC 23838D
Mycoplasma fermentans	ATCC 19989D
Mycoplasma gallisepticum	ATCC 15302
Mycoplasma genitalium	ATCC 33530D

(continued)

Species	Strain/source
Mycoplasma hominis	ATCC 23114D
Mycoplasma hyorhinis	ATCC 17981D
Mycoplasma hyponeumoniae	ATCC 25095
Mycoplasma orale	ATCC 23714D
Mycoplasma pirum	ATCC 25960D
Mycoplasma pneumoniae	ATCC 15531D
Mycoplasma salivarium	ATCC 23064D
Mycoplasma sinoviae	ATCC 25204
Spiroplasma citri	ATCC 27556D

Exclusivity – undetectable organisms

Organism	Strain/source
Bacillus cereus	ATCC 10876
Bacillus subtilis	ATCC 6051
Campylobacter jejuni	ATCC 29428
Citrobacter freundii	6879
Clostridium perfringens	ATCC 12915
Enterobacter aerogenes	Q87
Enterobacter sakazaki	ATCC 51329
Enterococcus faecalis	ATCC 29212
Escherichia coli O157:H7	43888
Klebsiella oxytoca	ATCC 43165
Lactobacillus bulgaris	ATCC 11842
Listeria ivanovii	ATCC 19119
Listeria monocytogenes	ATCC 7644
Pseudomonas aeruginosa	ATCC 27853
Pseudomonas aeruginosa	ATCC 17423
Shigella	Sfla 395

(continued)

Organism	Strain/source
Shigella	SFL 153
Shigella dysenteriae	ATCC 13313
Shigella dysenteriae	ESCL7-JHH
Staphylococcus aureus	ATCC 43300
Staphylococcus aureus aureus	PE491
Streptococcus faecalis	ATCC 9790
Vibrio cholerae	O36
Yersinia enterocolitica	ATCC 9610
Cat	Novagen™, Cat. No. 69235-3
Cow	Novagen™, Cat. No. 69238-3
Chicken	Novagen™, Cat. No. 69233-3
Chimpanzee	Bios, Inc.[1]
CHO	ATCC CCL-61
HeLa	ATCC CCL-2
Horse	Pel-Freez Biologicals, Cat. No. 39339-5
Mouse	Novagen™, Cat. No. 69239
Orangutang	Bios, Inc.[1]
Pig	Novagen™, Cat. No. 69230-3
Rabbit	Pel-Freez Biologicals, Cat. No. 31130-1
Rat	Novagen™, Cat. No. 69238-3
Sheep	Novagen™, Cat. No. 69231-3

[1] No longer available

Good PCR practices

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule. Follow the guidelines below to prevent contamination and nonspecific amplification.

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

Plate layout suggestions

- For each plate row, dispense in sequence from left to right: negative controls, unknown samples, inhibition controls, and positive controls (at the end of the row or column).
- Place positive controls in one of the outer columns.
- If possible, separate all samples from each other by at least one well. If space is limited, place at least one well between unknown samples and controls.

Documentation and support

Related documentation

Document	Pub. No.	Description
MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit Quick Reference	4465876	Provides brief, concise instructions about using the MycoSEQ™ Mycoplasma Detection Kit.
ViralSEQ™ Mouse Minute Virus (MMV) Real-Time PCR Detection Kit Quick Reference	4445236	Provides brief, concise instructions about using the ViralSEQ™ Mouse Minute Virus Real-Time PCR Detection Kit.
ViralSEQ™ Mouse Minute Virus (MMV) Real-Time PCR Detection Kit User Guide	4445235	Describes the ViralSEQ™ Mouse Minute Virus Real-Time PCR Detection Kit and provides information about preparing, running, and troubleshooting MMV detection.
PrepSEQ™ Sample Preparation Kits for Mycoplasma, MMV, and Vesivirus Quick Reference	4465875	Provides brief, concise instructions about using the PrepSEQ™ Sample Preparation Kits.
PrepSEQ™ Sample Preparation Kits for Mycoplasma, MMV, and Vesivirus User Guide	4465957	Describes the PrepSEQ™ Sample Preparation Kits and provides information about preparing, running, and troubleshooting sample preparation.
PrepSEQ™ Nucleic Acid Extraction Kit Quick Reference	4406303	Provides brief, concise instructions about using the PrepSEQ™ Nucleic Acid Extraction Kit.
PrepSEQ™ Nucleic Acid Extraction Kit User Guide	4400739	Describes the PrepSEQ™ Nucleic Acid Extraction Kit and provides information about preparing, running, and troubleshooting nucleic acid extractions.
Introduction to TaqMan™ and SYBR™ Green Chemistries for Real-Time PCR Protocol	4407003	Describes the TaqMan™ and SYBR™ Green Chemistries for Real-Time PCR and provides information about preparing, running, and troubleshooting PCR.
AccuSEQ™ Real-Time PCR Software v3.1 User Guide	100094287	Provides step-by-step procedures for *Mycoplasma* detection with the AccuSEQ™ Real-Time PCR Software v3.1.
AccuSEQ™ Real-Time PCR Software v3.2 Quick Reference	MAN0029200	Provides brief, step-by-step procedures for *Mycoplasma* detection. It is designed to help you quickly learn to use the AccuSEQ™ Real-Time PCR Detection Software for Mycoplasma SEQ Experiments.
AccuSEQ™ Real-Time PCR Software v3.2 User Guide	MAN0029199	Provides step-by-step procedures for *Mycoplasma* detection with the AccuSEQ™ Real-Time PCR Software v3.2.

(continued)

Document	Pub. No.	Description
AccuSEQ™ Real-Time PCR Software v2.2 User Guide	MAN0029201	Provides step-by-step procedures for *Mycoplasma* detection with the AccuSEQ™ Real-Time PCR Software v2.2.
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407	Provides information about the QuantStudio™ 5 Real-Time PCR Instrument.
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Getting Started Guide: Absolute Quantitation using Standard Curve	4347825	Provides brief, step-by-step procedures for absolute quantitation using a standard curve. It is designed to help you quickly learn to use the Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System.

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

PDF versions of this guide and the documents listed above are available at **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**.