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**Revision history:** Pub. No. 4465874

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>22 April 2022</td>
<td>Update to the instructions in “Prepare the PCR reactions (complex or high cell density samples)” on page 36.</td>
</tr>
<tr>
<td>E</td>
<td>7 December 2021</td>
<td>Updated Appendix A, “Troubleshooting”. Included tips for complex or high cell density samples.</td>
</tr>
<tr>
<td>D</td>
<td>28 September 2020</td>
<td>Updated to include run and analysis information for AccuSEQ™ Real-Time PCR Software v3.1.</td>
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</tbody>
</table>

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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)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10X <em>Mycoplasma</em> Real-Time PCR Primer Mix</td>
<td>blue</td>
<td>325 µL</td>
<td>–25°C to –15°C on receipt 2–8°C after first use</td>
</tr>
<tr>
<td>Negative Control</td>
<td>white</td>
<td>1,000 µL</td>
<td></td>
</tr>
<tr>
<td>2X <em>Power SYBR</em>™ Green PCR Master Mix</td>
<td>white</td>
<td>2 × 1,000 µL</td>
<td>–25°C to –15°C on receipt, protected from light. 2–8°C after first use, protected from light</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Box 2: MycoSEQ™ Discriminatory Positive/Extraction Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>MycoSEQ™ Discriminatory Positive/Extraction Control, 1,000 copies/µL</td>
</tr>
</tbody>
</table>

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

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.

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

Prepare the sample (page 9)

▼

Prepare the kit reagents and premix solution (page 9)

▼

Prepare the PCR reactions (page 64)

▼

Create a MycoSEQ™ experiment (page 12)

▼

Start the run (QuantStudio™ 5 Real-Time PCR Instrument) (page 15)

▼

Monitor the run (page 16)

▼

Analyze the results (page 17)
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™ Mycoplasma Nucleic Acid Extraction Kit.

For more information, see:


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.

<table>
<thead>
<tr>
<th>Component for premix solution</th>
<th>Volume for one 30-µL reaction</th>
<th>Volume for four 30-µL reactions([1])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power SYBR™ Green PCR Master Mix, 2×</td>
<td>15.0 µL</td>
<td>66.0 µL</td>
</tr>
<tr>
<td>Mycoplasma Real-Time PCR Primer Mix, 10×</td>
<td>3.0 µL</td>
<td>13.2 µL</td>
</tr>
<tr>
<td>Total premix solution volume</td>
<td>18.0 µL</td>
<td>79.2 µL</td>
</tr>
</tbody>
</table>

\([1]\) 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.

<table>
<thead>
<tr>
<th>To prepare...</th>
<th>In each tube or well...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control reaction</td>
<td>• Add 18 µL of Premix Solution</td>
</tr>
<tr>
<td></td>
<td>• Add 12 µL of Negative Control (water)</td>
</tr>
<tr>
<td>Unknown or spiked sample reaction</td>
<td>• Add 18 µL of Premix Solution</td>
</tr>
<tr>
<td></td>
<td>• Add 10 µL of unknown sample</td>
</tr>
<tr>
<td></td>
<td>• Add 2 µL of Negative Control (water)</td>
</tr>
<tr>
<td>Inhibition-control reaction</td>
<td>• Add 18 µL of Premix Solution</td>
</tr>
<tr>
<td></td>
<td>• Add 10 µL of unknown sample</td>
</tr>
<tr>
<td></td>
<td>• Add 2 µL of the Discriminatory Positive Control (DPC)</td>
</tr>
<tr>
<td>Positive control reaction</td>
<td>• Add 18 µL of Premix Solution</td>
</tr>
<tr>
<td></td>
<td>• Add 2 µL of the DPC</td>
</tr>
<tr>
<td></td>
<td>• Add 10 µL of Negative Control (water)</td>
</tr>
</tbody>
</table>

**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 65.

4. Briefly centrifuge the reaction plate.
Setup, run, and analyze samples with AccuSEQ™ Software v3.1 on the QuantStudio™ 5 Instrument

Sign in to the AccuSEQ™ Real-Time PCR Software

Thermo Fisher Scientific recommends configuring the Windows™ 10 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. Launch the AccuSEQ™ Real-Time PCR Software by double-clicking the AccuSEQ icon.

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 v3.1 User Guide (Pub. No. 100094287).

Custom MycoSEQ™ experiments must be set up as Quantitation-Standard Curve type experiments with SYBR™ chemistry.

2. In the Experiment Properties pane of the Setup tab:
   a. *(Optional)* Change the system-generated name of the experiment.
   
   b. *(Optional)* Enter the plate Barcode, then add Comments.

**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 v3.1 User Guide (Pub. No. 100094287) for creating custom templates).

- Experiment Type—Quantitation-Standard Curve
- Chemistry—SYBR™ Green Reagents
- Ramp Speed—Standard - 2hrs

C. Click Next.
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 v3.1 User Guide (Pub. No. 100094287) for creating custom templates).

4. Click Next.

5. In the Samples pane of the Setup tab, enter the sample Name. Add additional 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.

Figure 1  MycoSEQ™ template default cycling conditions

1. Samples pane
2. Add—adds additional samples
Chapter 2  Methods
Setup, run, and analyze samples with AccuSEQ™ Software v3.1 on the QuantStudio™ 5 Instrument

Figure 2  MycoSEQ™ template default sample plate layout

1. Toolbar (in order:  Undo,  Redo,  Copy,  Paste,  Delete,  View)
2. 5 default Samples with and without an inhibition control (IC)
3. Positive control (P)
4. Selected samples. Click-drag to add additional samples.
5. No template control (N)

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 additional changes made to the experiment, exit the experiment. The software prompts you to save changes. Click **Yes**.

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

---

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

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

<table>
<thead>
<tr>
<th>Option</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>If the experiment is open</td>
<td>Click <strong>Start Run</strong>.</td>
</tr>
</tbody>
</table>
| If the experiment is closed | 1. Open the experiment.  
                            | 2. Click the Run tab.  
                            | 3. Click **Start Run**. |

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.

![Monitor the Run](image)

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**
   - 2020-06-04_2696
   - 04 Jun 2020 19:08:17 GMT-0500

2. **Run**
   - 2020-06-04_8175
   - 04 Jun 2020 18:59:11 GMT-0500

- **Open Experiment** pane
- **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**

- “View the Result Summary” on page 18
- “Evaluate the overall shape of the Amplification Plot curves” on page 19
- “View and evaluate the Melt Curve Plot” on page 21

See the **AccuSEQ™ Real-Time PCR Software v3.0 User Guide** (Pub. No. 100084348) for more information on analyzing results.
View the Result Summary

The Result tab is populated when the run is complete.

Calls are made based on the customizable Rule Settings within the Analysis Settings for MycoSEQ™ experiments. See the AccuSEQ™ Real-Time PCR Software v3.1 User Guide (Pub. No. 100094287) for more information.

1. View the Plate Call.
   a. View the calls for the positive controls (POS).
   b. View the calls for the no template controls (NTC).

The number inside the circle indicates the number of samples that passed or failed. The overall Plate Status (VALID or INVALID) is determined by the POS and NTC calls.

Note: One passing POS and NTC call are required for a Plate Status to be VALID. This requirement is not editable.

2. View the Well Calls (Unknown).
   a. View the total number of wells for each call—Present, Absent, Review, or Fail.

   b. Use the Legends to view the calls in the plate.
Results pane

Well call

Flags—the number within the triangle indicates the number of QC flag calls in the well; review in QC Summary.

Grid View

Evaluate the overall shape of the Amplification Plot curves

You can evaluate the overall shape of the Amplification curves in the Result tab. Evaluating the amplification curve in wells with a Review call can help determine if the sample should be rerun, or whether to 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 tab, click Amplification in the horizontal scroll bar.

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

The Amplification Plot is displayed for the selected wells in the (Grid View).

2. Ensure that the Target selected is Mycoplasma.

3. (Optional) Click (Settings), then make the following selections:
   - **Plot Type**: ΔRn, Rn, or C<sub>T</sub>
   - **Graph Type**: Log or Linear
   - **Show**: 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) 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 whether you want Tick Marks
      - Select Auto-adjust range or enter minimum and maximum values

Note: The minimum value must be greater than 0.
Figure 4  Typical Amplification Plot (4 phases)

1. Amplification Plot tools
2. Plateau phase
3. Linear phase
4. Exponential (geometric) phase
5. Baseline
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 tab.

Plots & Summary

1. Melt Curve plot
2. Melt Curve Stage dropdown
1. In the **Result Analysis** window, select [Melt Curve](#) Plot from the horizontal scroll bar.

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

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 (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. Confirm 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.

![Melt Curve Plot]

**Figure 5  Example Melt Curve Plot**

1. Melting temperature (Mycoplasma)
2. Melting temperature (positive control)
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 flags these as "Review".

**Table 2** Example acceptance criteria for unknown samples: AccuSEQ™ Software v3.1 or later

<table>
<thead>
<tr>
<th>Result</th>
<th>$C_t$</th>
<th>$T_m$ (°C)</th>
<th>DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>$&lt; 36.2300$</td>
<td>$75.50 &lt; T_m &lt; 83.00$</td>
<td>$\geq 0.40$</td>
</tr>
<tr>
<td>Absent</td>
<td>$\geq 36.2300$</td>
<td>$&lt; 75.50$</td>
<td>$&lt; 0.20$</td>
</tr>
</tbody>
</table>

**Figure 6** 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 $83.00^\circ C \leq T_m \leq 86.00^\circ C$ 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 3  Example acceptance criteria for controls: AccuSEQ™ Real-Time PCR Software v3.1 or later.

<table>
<thead>
<tr>
<th>Control</th>
<th>$C_t$</th>
<th>$T_m$ (°C)</th>
<th>DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR positive control</td>
<td>$23.5000 \leq C_t \leq 27.5000$</td>
<td>$83.00 &lt; T_m \leq 86.00$</td>
<td>$\geq 0.40$</td>
</tr>
<tr>
<td>Extraction spike control (if DPC is the spike)</td>
<td>&lt; 36.2300</td>
<td>$83.00 &lt; T_m \leq 86.00$</td>
<td>$\geq 0.40$</td>
</tr>
<tr>
<td>Extraction spike control (if mycoplasma genomic DNA or bacteria is the spike)</td>
<td>&lt; 36.2300</td>
<td>$75.50 \leq T_m \leq 83.00$</td>
<td>$\geq 0.40$</td>
</tr>
<tr>
<td>No template control[1]</td>
<td>$\geq 36.2300$</td>
<td>$75.50 \leq T_m \leq 83.00$</td>
<td>$&lt; 0.40$</td>
</tr>
<tr>
<td>Blank extraction control[1]</td>
<td>$\geq 36.2300$</td>
<td>$75.50 \leq T_m \leq 83.00$</td>
<td>$&lt; 0.40$</td>
</tr>
<tr>
<td>Inhibition control</td>
<td>$\Delta C_t &lt; 2.0000$</td>
<td>$83.00 &lt; T_m \leq 86.00$</td>
<td>N/A</td>
</tr>
</tbody>
</table>

[1] When $T_m < 75.5^\circ C$, there is no target with the correct $T_m$, and therefore $C_t$ and DV values are irrelevant.

![Decision tree for NTC calls](image)

Note: NTC wells can display a melt peak with a $T_m < 75.5^\circ C$ or a $T_m > 86^\circ C$. The presence of a melt peak with a $T_m$ range of $83.00^\circ C \leq T_m \leq 86.00^\circ C$ in NTC wells indicates DPC contamination in the PCR reaction.
Chapter 2 Methods
Setup, run, and analyze samples with AccuSEQ™ Software v3.1 on the QuantStudio™ 5 Instrument

Figure 8 Decision tree for positive control calls
Example results with AccuSEQ™ Software v3.1 or later

Positive control

Amplification and melt curve plots of positive control reactions spiked with 2,000 copies of Discriminatory Positive
Chapter 2 Methods

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

Control.
No template control

**Amplification Plot**

Ct: 38.5

**Melt Curve Plot**

Tm: 75.11

Primer dimers
Chapter 2 Methods
Setup, run, and analyze samples with AccuSEQ™ Software v3.1 on the QuantStudio™ 5 Instrument

Blank extraction control

![Amplification Plot](image)

**Amplification Plot**

![Melt Curve Plot](image)

**Melt Curve Plot**

Figure 10: Amplification and Melt Curve plots of blank extraction control with PBS.
Positive unknown sample

**Amplification Plot**

- ΔRn: 3
- ΔRn: 0.200
- Ct: 29

**Melt Curve Plot**

- Tm: 79.65

Chapter 2 Methods

Setup, run, and analyze samples with AccuSEQ™ Software v3.1 on the QuantStudio™ 5 Instrument
Chapter 2 Methods

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

PCR inhibition

Figure 12  PCR inhibition, ΔC>T > 2.

1. No inhibition
2. Inhibition
Multicomponent plots

Figure 13   An example of a multicomponent plot.
## Troubleshooting the AccuSEQ™ software

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| **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<sub>t</sub> 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**                                                         | 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.    |
| • Negative unknown sample and                                               | 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.         |
| • Inhibition control ΔC<sub>t</sub> > 2 compared to the positive control   | Components in cell culture media or additive (for example, dextran sulfate) may inhibit PCR and change T<sub>m</sub>. | • Most cell culture media inhibit PCR or change T<sub>m</sub> 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**                                     | The sample block is contaminated.                                             | Run a background calibration to identify the contaminated wells, then decontaminate the sample block.                                           |

**Details:** >500,000 fluorescent standard units (FSU). This is visible in the Multicomponent Plot (Results tab)
## Troubleshooting the MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
<tbody>
<tr>
<td>High background fluorescence signal</td>
<td>Sample may have high concentration of nucleic acid carried from the cell culture during sample preparation.</td>
<td>Repeat the experiment using assay components that were stored correctly. Repeat sample prep with RNase and DNase treatment (if RNase alone does not resolve issue) to solubilize excess nucleic acid from the host cell.</td>
</tr>
<tr>
<td>No positive control or target-specific SYBR™ Green dye signal is detected in inhibition control and/or positive control wells</td>
<td>Improper storage of Power SYBR™ Green PCR Master Mix or of target-specific Mycoplasma Real-Time PCR Primer Mix (10×).</td>
<td>Repeat the assay using properly stored assay components. Avoid freezing and thawing assay components. Protect Power SYBR™ Green PCR Master Mix from light.</td>
</tr>
<tr>
<td>Pipetting error (no premix solution added).</td>
<td>Repeat the assay. Make sure to pipet premix solution into all wells.</td>
<td></td>
</tr>
<tr>
<td>Pipetting error (no positive control added).</td>
<td>Repeat the assay. Make sure to pipet positive control into all positive-control wells.</td>
<td></td>
</tr>
<tr>
<td>Target-specific signal is detected in negative control wells</td>
<td>Carryover contamination.</td>
<td>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.</td>
</tr>
<tr>
<td>High level of nonspecific product formation.</td>
<td>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.</td>
<td></td>
</tr>
</tbody>
</table>
Troubleshooting complex or high cell density samples

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommended action</th>
</tr>
</thead>
</table>
| 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™ 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 36. |

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power SYBR™ Green PCR Master Mix, 2×</td>
<td>15 µL</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Mycoplasma Real-Time PCR Primer Mix, 10×</td>
<td>3 µL</td>
<td>Unchanged</td>
</tr>
<tr>
<td>Water or DPC control</td>
<td>1 µL</td>
<td>Volume decreased. For DPC, this results in 1,000 copies/ reaction. Inhibition control criterion remains ΔC_t &lt; 2.000. Increase C_t rule setting for DPC by 1 (AccuSEQ™ Real-Time PCR Software v2.x or v3.x).</td>
</tr>
</tbody>
</table>
| 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.
Acceptance criteria

- Cycle threshold value (Ct) ............................................................. 38
- Melting temperature (Tm) .............................................................. 38
- Derivative value (DV) ................................................................. 40
- Results interpretation using the acceptance criteria ......................... 41
- Other acceptance criteria considerations ........................................ 41

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\textsubscript{t})**

The C\textsubscript{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\textsubscript{t} values are inversely proportional to the amount of target nucleic acid in the reaction. The lower the C\textsubscript{t} value, the greater the amount of target in the reaction.

![Amplification plots](image)

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\textsubscript{t} value.

The C\textsubscript{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\textsubscript{t} value greater than the C\textsubscript{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\textsubscript{t} value acceptance criteria are met, amplification has occurred, requiring evaluation of T\textsubscript{m} values to determine specificity of mycoplasma DNA amplification.

**Melting temperature (T\textsubscript{m})**

The T\textsubscript{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\textsubscript{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\textsubscript{m} of 75.5–83°C (T\textsubscript{m} range may vary, depending on software used for analysis). Reactions with T\textsubscript{m} values outside of this range, or not meeting the established T\textsubscript{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.](image_url)
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.

![Graph showing observable differences in DV between 1 and 10 genome copies of DNA.](image)

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 (Ct, Tm, 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 Ct value due to the formation of primer-dimer during the PCR reaction. In general, if primer dimer formation gives rise to an amplification plot, the Ct value is >36.23 and the Tm 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. Tm 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 (Ct, Tm, 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 in order to provide the user the highest possible level of confidence in both the negative and positive results obtained during routine testing.
Use the kit with 7500 Fast System AccuSEQ™ Real-Time PCR Detection Software v2.x

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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.

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Instruments</strong></td>
<td></td>
</tr>
<tr>
<td>7500 Fast Real-Time PCR System with AccuSEQ™ software v2.x</td>
<td>Contact your local sales representative</td>
</tr>
<tr>
<td><strong>Consumables</strong></td>
<td></td>
</tr>
<tr>
<td>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</td>
<td>4306737[^1]</td>
</tr>
<tr>
<td>MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL, 20 plates; for use with 7500 Fast Real-Time PCR System</td>
<td>4346906</td>
</tr>
<tr>
<td>MicroAmp™ Optical 96-Well Reaction Plate with Barcode &amp; Optical Adhesive Films, 100 plates with covers; for use with 7300 and 7500 Fast Real-Time PCR Systems</td>
<td>4314320</td>
</tr>
<tr>
<td>MicroAmp™ Optical 8-Cap Strips, 300 strips</td>
<td>4323032</td>
</tr>
</tbody>
</table>

[^1]: MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit User Guide
Prepare the sample

Prepare the DNA template for the PCR reactions using the PrepSEQ™ 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.

<table>
<thead>
<tr>
<th>Component for premix solution</th>
<th>Volume for one 30-µL reaction</th>
<th>Volume for four 30-µL reactions[^1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power SYBR™ Green PCR Master Mix, 2×</td>
<td>15.0 µL</td>
<td>66.0 µL</td>
</tr>
<tr>
<td><em>Mycoplasma</em> Real-Time PCR Primer Mix, 10×</td>
<td>3.0 µL</td>
<td>13.2 µL</td>
</tr>
<tr>
<td>Total premix solution volume</td>
<td>18.0 µL</td>
<td>79.2 µL</td>
</tr>
</tbody>
</table>

[^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.

<table>
<thead>
<tr>
<th>To prepare...</th>
<th>In each tube or well...</th>
</tr>
</thead>
</table>
| 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 65.

4. Briefly centrifuge the reaction plate.

Create a new experiment

1. In the desktop, double-click the AccuSEQ™ software icon to start the software.

2. Log into the software. In the Home screen, click Create MycoSEQ Experiment to open the Mycoplasma Assay v2.0 workflow.
Define the experiment properties

In the Experiment properties screen:

1. Enter an experiment name.

2. *(Optional)* Enter a barcode to identify the reaction plate.

3. *(Optional)* Enter comments to describe the experiment.

4. 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:
Create a new experiment

<table>
<thead>
<tr>
<th>Field</th>
<th>Minimum entry[^1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>1</td>
</tr>
<tr>
<td>Sample replicates</td>
<td>1</td>
</tr>
<tr>
<td>Inhibition control replicates for each sample</td>
<td>0</td>
</tr>
<tr>
<td>Positive control replicates</td>
<td>1</td>
</tr>
<tr>
<td>Negative control replicates</td>
<td>1</td>
</tr>
</tbody>
</table>

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

2. Set the Sample volume to 10 µL per reaction.

3. Enter sample names, and *(optional)* set plot colors.

4. Click **Next**.

**View the plate layout**

The AccuSEQ™ software v2.0 uses the sample information that you enter in the **Sample Setup** screen to fill the wells in the plate layout and to calculate the required 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.

Here is an example:

---

[^1]: We recommend that you use at least one negative and one positive control per run, and at least one inhibition control per sample.
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. Review the run method and click **Next**.

### Save the experiment

1. At the bottom of the AccuSEQ™ software screen, click **Save & Finish**.
2. In the **Save Experiment** dialog box, verify the Mycoplasma Presence Absence Detection by MycoSEQ Example.eds file name, then click **Save**.

### 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 “Save the experiment” on page 47.
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<sub>t</sub>**, then set Threshold to **0.2**.
2. Select **Manual Baseline**, then enter the following settings:
   - Start (cycle): **3**
   - End (cycle): **15**
Review the Results Summary

AccuSEQ™ v2.0 software uses the acceptance criteria in Table 5 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 (Cₜ), derivative value (DV), and melting temperature (Tₘ) 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 4** Example acceptance criteria for unknown samples: AccuSEQ™ software v2.0 or later

<table>
<thead>
<tr>
<th>Result</th>
<th>C_t</th>
<th>T_m (°C)</th>
<th>DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>&lt; 36.23 C_t</td>
<td>75 – 82</td>
<td>≥ 0.8</td>
</tr>
<tr>
<td>Negative</td>
<td>≥ 36 C_t</td>
<td>&lt; 75</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Figure 14** Decision tree for unknown sample calls
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™ software v2.0 or later.

<table>
<thead>
<tr>
<th>Control</th>
<th>C_t</th>
<th>T_m (°C)</th>
<th>DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR positive control</td>
<td>&lt; 36.23 C_t</td>
<td>82–86</td>
<td>&gt; 0.8</td>
</tr>
<tr>
<td>Extraction spike control</td>
<td>&lt; 36.23 C_t</td>
<td>82–86</td>
<td>&gt; 0.8</td>
</tr>
<tr>
<td>No template control</td>
<td>≥ 36.23 C_t</td>
<td>&lt; 75</td>
<td>N/A</td>
</tr>
<tr>
<td>Blank extraction control</td>
<td>≥ 36.23 C_t</td>
<td>&lt; 75</td>
<td>N/A</td>
</tr>
<tr>
<td>Inhibition control</td>
<td>ΔC_t &lt; 2</td>
<td>82–86</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Figure 15  Decision tree for NTC calls
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) 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 17  PCR positive control spiked with 2,000 copies of DPC.
Negative control

Figure 18  No template PCR control.
Blank extraction control

Figure 19  Blank extraction control with PBS.
Positive extraction control

Figure 20  Sample spiked with 2,000 copies of DPC before DNA extraction.
Inhibition control and positive control

Figure 21  No PCR inhibition present; inhibition control and PCR positive control overlaid, with a $\Delta C_t < 2$. 
Unknown sample: Negative result

Figure 22  Negative result; $C_t > 36.23$ and $T_m < 75^\circ C$. 
Unknown sample: Positive result

**Figure 23** Positive result; $C_t = 15.69$, $T_m > 75^\circ C$, and Derivative Reporter $>0.8$. 
Unknown sample: Positive result with decreased detection of DPC

Figure 24 Decreased DPC signal can be observed in the presence of very high *mycoplasma* contamination.
PCR inhibition

Figure 25  PCR inhibition, $\Delta C_t > 2$. 
Multicomponent plots

Figure 26 An example of a multicomponent plot.

Figure 27 A multicomponent plot with high background signal which will result in PCR inhibition.
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.

<table>
<thead>
<tr>
<th>Component for premix solution</th>
<th>Volume for one 30-µL reaction</th>
<th>Volume for four 30-µL reactions[^1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power SYBR™ Green PCR Master Mix, 2X</td>
<td>15.0 µL</td>
<td>66.0 µL</td>
</tr>
<tr>
<td>Mycoplasma Real-Time PCR Primer Mix, 10X</td>
<td>3.0 µL</td>
<td>13.2 µL</td>
</tr>
<tr>
<td>Total premix solution volume</td>
<td>18.0 µL</td>
<td>79.2 µL</td>
</tr>
</tbody>
</table>

[^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.

<table>
<thead>
<tr>
<th>To prepare…</th>
<th>In each tube or well…</th>
</tr>
</thead>
</table>
| 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 78 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.

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Wells: Unknown, Negative Control, Positive Control, Inhibition Control, 79 Empty

Figure 28  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


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.

<table>
<thead>
<tr>
<th>Step</th>
<th>AmpliTaq Gold™ enzyme activation</th>
<th>PCR</th>
<th>Dissociation(^{[1,2,3]})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HOLD</td>
<td>Cycle (40 cycles)</td>
<td>Melt</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Denature</td>
<td>95°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anneal/extend</td>
<td>60°C</td>
</tr>
<tr>
<td>Temp</td>
<td>95°C</td>
<td>95°C</td>
<td>95°C</td>
</tr>
<tr>
<td>Time</td>
<td>10 min</td>
<td>15 sec</td>
<td>1 min</td>
</tr>
</tbody>
</table>
<pre><code>                             |                                 | 1 min         | 15 sec                     |
                             |                                 |               | 15 sec                     |
                             |                                 |               | 15 sec                     |
</code></pre>

\(^{[1]}\) 7500 and 7500 Fast Systems: from the Instrument tab, click Add Dissociation Stage (see Figure 29).
\(^{[2]}\) Applied Biosystems™ Real-Time PCR Instruments: from the Instrument tab, click Add Dissociation Stage (see Figure 29). Use default settings.
\(^{[3]}\) For other instruments, refer to their corresponding user guides for dissociation-curve setup information.
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 66).

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

![Analysis Settings](image)

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>
<thead>
<tr>
<th>Result</th>
<th>C&lt;sub&gt;t&lt;/sub&gt;</th>
<th>T&lt;sub&gt;m&lt;/sub&gt;</th>
<th>DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>&lt; 36 C&lt;sub&gt;t&lt;/sub&gt;</td>
<td>75°C – 81°C</td>
<td>≥0.05</td>
</tr>
<tr>
<td>Negative</td>
<td>≥ 36 C&lt;sub&gt;t&lt;/sub&gt;</td>
<td>&lt; 75°C</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Guidance for controls

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

<table>
<thead>
<tr>
<th>Control</th>
<th>C&lt;sub&gt;t&lt;/sub&gt;</th>
<th>T&lt;sub&gt;m&lt;/sub&gt;</th>
<th>DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR positive control</td>
<td>&lt; 36 C&lt;sub&gt;t&lt;/sub&gt;</td>
<td>≈84°C</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Extraction spike control</td>
<td>&lt; 36 C&lt;sub&gt;t&lt;/sub&gt;</td>
<td>≈84°C</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>No template control</td>
<td>≥ 36 C&lt;sub&gt;t&lt;/sub&gt;</td>
<td>&lt; 75°C</td>
<td>N/A</td>
</tr>
<tr>
<td>Blank extraction control</td>
<td>≥ 36 C&lt;sub&gt;t&lt;/sub&gt;</td>
<td>&lt; 75°C</td>
<td>N/A</td>
</tr>
<tr>
<td>Inhibition control</td>
<td>ΔC&lt;sub&gt;t&lt;/sub&gt; &lt; 2</td>
<td>≈84°C</td>
<td>N/A</td>
</tr>
</tbody>
</table>

- Both the PCR positive control and the extraction spike control may present extra peaks with T<sub>m</sub> < 75°C. These peaks represent primer dimer formation, and they do not interfere with the final results.
- The difference in C<sub>t</sub> 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<sub>t</sub> > 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<sub>t</sub> is calculated by C<sub>t</sub> (of inhibition control reaction) – C<sub>t</sub> (of positive control reaction).
Example positive results with SDS v1.4 software

Figure 30  *Mycoplasma* contamination (approximately $3 \times 10^6$ copies per PCR reaction).

Figure 31  *Mycoplasma* contamination (approximately 2,000 copies per PCR reaction).
Figure 32  *Mycoplasma* contamination (less than 10 copies per PCR reaction).

**Example positive control extraction results with SDS v1.4 software**

![Graph showing PCR results](image)

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample Name</th>
<th>Detection</th>
<th>Task</th>
<th>C1</th>
<th>StDev Ct</th>
<th>Quantity</th>
<th>Mean Ct</th>
<th>StDev Qty</th>
<th>Filtered</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Mycoplasma</td>
<td>Unknown</td>
<td>1.854</td>
<td>8</td>
<td>1.758</td>
<td>1.010</td>
<td>1.758</td>
<td>0.050</td>
<td>76.6°C</td>
<td>79.5</td>
</tr>
</tbody>
</table>

Figure 33  Sample spiked with 2,000 copies of DPC and contaminated with *Mycoplasma* (3 x 10⁶ copies).
Appendix D Use the kit with 7500 System SDS Software v1.4 or later

Analyze the results

Figure 34  Sample spiked with 2,000 copies of DPC and contaminated with Mycoplasma (approximately 2,000 copies).

Figure 35  Sample containing 25 copies of Mycoplasma and 25 copies of DPC.

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample Name</th>
<th>Detector</th>
<th>Task</th>
<th>Ct</th>
<th>StdDev Ct</th>
<th>Quantity</th>
<th>Mean Qty</th>
<th>StdDev Qty</th>
<th>Filtered</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>Mycoplasma</td>
<td>unknown</td>
<td>24.2829</td>
<td>30.21</td>
<td>2.18</td>
<td>20.12</td>
<td>1.35</td>
<td>2.18</td>
<td>1.15</td>
<td>74.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample Name</th>
<th>Detector</th>
<th>Task</th>
<th>Ct</th>
<th>StdDev Ct</th>
<th>Quantity</th>
<th>Mean Qty</th>
<th>StdDev Qty</th>
<th>Filtered</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>A5</td>
<td>Sample D</td>
<td>Mycoplasma unknown</td>
<td>32.2065</td>
<td>32.21</td>
<td>2.18</td>
<td>20.12</td>
<td>1.35</td>
<td>2.18</td>
<td>1.15</td>
<td>75.5</td>
</tr>
</tbody>
</table>

MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit User Guide
Example negative results with SDS v1.4 software

Figure 36  Negative result.

Figure 37  Negative sample spiked with 2,000 copies of DPC.
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.
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
- 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 affect 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain/source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acholeplasma laidlawii</td>
<td>ATCC 23206D</td>
</tr>
<tr>
<td>Mycoplasma arginini</td>
<td>ATCC 23838D</td>
</tr>
<tr>
<td>Mycoplasma fermentans</td>
<td>ATCC 19989D</td>
</tr>
<tr>
<td>Mycoplasma gallisepticum</td>
<td>ATCC 15302</td>
</tr>
<tr>
<td>Mycoplasma genitalium</td>
<td>ATCC 33530D</td>
</tr>
</tbody>
</table>
(continued)

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain/source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma hominis</td>
<td>ATCC 23114D</td>
</tr>
<tr>
<td>Mycoplasma hyorhinis</td>
<td>ATCC 17981D</td>
</tr>
<tr>
<td>Mycoplasma hyponeumoniae</td>
<td>ATCC 25095</td>
</tr>
<tr>
<td>Mycoplasma orale</td>
<td>ATCC 23714D</td>
</tr>
<tr>
<td>Mycoplasma pirum</td>
<td>ATCC 25960D</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>ATCC 15531D</td>
</tr>
<tr>
<td>Mycoplasma salivarium</td>
<td>ATCC 23064D</td>
</tr>
<tr>
<td>Mycoplasma sinoviae</td>
<td>ATCC 25204</td>
</tr>
<tr>
<td>Spiroplasma citri</td>
<td>ATCC 27556D</td>
</tr>
</tbody>
</table>

Exclusivity – undetectable organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain/source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>ATCC 10876</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>ATCC 6051</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>ATCC 29428</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>6879</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>ATCC 12915</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>Q87</td>
</tr>
<tr>
<td>Enterobacter sakazaki</td>
<td>ATCC 51329</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>ATCC 29212</td>
</tr>
<tr>
<td>Escherichia coli O157:H7</td>
<td>43888</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>ATCC 43165</td>
</tr>
<tr>
<td>Lactobacillus bulgaris</td>
<td>ATCC 11842</td>
</tr>
<tr>
<td>Listeria ivanovii</td>
<td>ATCC 19119</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>ATCC 7644</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC 27853</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>ATCC 17423</td>
</tr>
<tr>
<td>Shigella</td>
<td>Sfla 395</td>
</tr>
<tr>
<td>Organism</td>
<td>Strain/source</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Shigella</td>
<td>SFL 153</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>ATCC 13313</td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>ESCL7-JHH</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC 43300</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>PE491</td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em></td>
<td>ATCC 9790</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>O36</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>ATCC 9610</td>
</tr>
<tr>
<td>Cat</td>
<td>Novagen™, Cat. No. 69235-3</td>
</tr>
<tr>
<td>Cow</td>
<td>Novagen™, Cat. No. 69238-3</td>
</tr>
<tr>
<td>Chicken</td>
<td>Novagen™, Cat. No. 69233-3</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>Bios, Inc.[1]</td>
</tr>
<tr>
<td>CHO</td>
<td>ATCC CCL-61</td>
</tr>
<tr>
<td>HeLa</td>
<td>ATCC CCL-2</td>
</tr>
<tr>
<td>Horse</td>
<td>Pel-Freez Biologicals, Cat. No. 39339-5</td>
</tr>
<tr>
<td>Mouse</td>
<td>Novagen™, Cat. No. 69239</td>
</tr>
<tr>
<td>Orangutan</td>
<td>Bios, Inc.[1]</td>
</tr>
<tr>
<td>Pig</td>
<td>Novagen™, Cat. No. 69230-3</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Pel-Freez Biologicals, Cat. No. 31130-1</td>
</tr>
<tr>
<td>Rat</td>
<td>Novagen™, Cat. No. 69238-3</td>
</tr>
<tr>
<td>Sheep</td>
<td>Novagen™, Cat. No. 69231-3</td>
</tr>
</tbody>
</table>

[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

<table>
<thead>
<tr>
<th>Document</th>
<th>Pub. No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit Quick Reference</strong></td>
<td>4465876</td>
<td>Provides brief, concise instructions on using the MycoSEQ™ Mycoplasma Detection Kit.</td>
</tr>
<tr>
<td><strong>ViralSEQ™ Mouse Minute Virus (MMV) Real-Time PCR Detection Kit Quick Reference</strong></td>
<td>4445236</td>
<td>Provides brief, concise instructions on using the ViralSEQ™ Mouse Minute Virus Real-Time PCR Detection Kit.</td>
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<tr>
<td><strong>ViralSEQ™ Mouse Minute Virus (MMV) Real-Time PCR Detection Kit User Guide</strong></td>
<td>4445235</td>
<td>Describes the ViralSEQ™ Mouse Minute Virus Real-Time PCR Detection Kit and provides information on preparing, running, and troubleshooting MMV detection.</td>
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<tr>
<td><strong>PrepSEQ™ Sample Preparation Kits for Mycoplasma, MMV, and Vesivirus Quick Reference</strong></td>
<td>4465875</td>
<td>Provides brief, concise instructions on using the PrepSEQ™ Sample Preparation Kits.</td>
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<td><strong>PrepSEQ™ Sample Preparation Kits for Mycoplasma, MMV, and Vesivirus User Guide</strong></td>
<td>4465957</td>
<td>Describes the PrepSEQ™ Sample Preparation Kits and provides information on preparing, running, and troubleshooting sample preparation.</td>
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<td><strong>PrepSEQ™ Nucleic Acid Extraction Kit Quick Reference</strong></td>
<td>4406303</td>
<td>Provides brief, concise instructions on using the PrepSEQ™ Nucleic Acid Extraction Kit.</td>
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<td><strong>PrepSEQ™ Nucleic Acid Extraction Kit User Guide</strong></td>
<td>4400739</td>
<td>Describes the PrepSEQ™ Nucleic Acid Extraction Kit and provides information on preparing, running, and troubleshooting nucleic acid extractions.</td>
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<tr>
<td><strong>Introduction to TaqMan™ and SYBR™ Green Chemistries for Real-Time PCR Protocol</strong></td>
<td>4407003</td>
<td>Describes the TaqMan™ and SYBR™ Green Chemistries for Real-Time PCR and provides information on preparing, running, and troubleshooting PCR.</td>
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<tr>
<td><strong>AccuSEQ™ Real-Time PCR Software v3.1 Quick Reference</strong></td>
<td>100094288</td>
<td>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.</td>
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</tbody>
</table>
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