MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit

Catalog Numbers 4460623, 4460626
Pub. No. 4465876 Rev. D

Note: For safety and biohazard guidelines, see the “Safety” appendix in the MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit User Guide (Pub. No. 4465874). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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.

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, 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)                  |
| 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.

4. Briefly centrifuge the reaction plate.

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

Create a MycoSEQ™ experiment

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

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.
      Default MycoSEQ™ settings (cannot be changed).
      • 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 (cannot be changed).

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.

For more information on plate setup, see the AccuSEQ™ Real-Time PCR Software v3.1 User Guide (Pub. No. 100094287).
6. Click Next.

The Run tab is displayed.

7. Experiments are auto-saved in the software. To save, 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

Start the run in the AccuSEQ™ Software.

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

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

### Analyze the results

After the qPCR run is finished, use the following general procedure to analyze the results. For more detailed instructions see the AccuSEQ™ Real-Time PCR Software v3.1 User Guide (Pub. No. 100094287).

**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 test 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.
1. In the AccuSEQ™ Real-Time PCR Software, open your experiment, then navigate to the Result tab.

2. In the Result Summary tab, review the Plate Call and Well Calls.

3. In the Result Analysis tab, review the Amplification Curve plots for amplification profiles in the controls and samples.

4. In the Result Analysis tab, review the QC Summary for any flags in wells.

5. In the Result Analysis tab, review the Melt Curve plot.

6. (Optional) Navigate to the Report tab to generate a report of the experiment, or to export results.

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: Tₘ and DV assay acceptance criteria are only relevant if Cᵥ value for present acceptance criteria are met. The AccuSEQ™ Software v3.1 flags these as "Review".

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

<table>
<thead>
<tr>
<th>Result</th>
<th>Cᵥ</th>
<th>Tₘ (°C)</th>
<th>DV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>&lt; 36.2300</td>
<td>75.50 &lt; Tₘ &lt; 83.00</td>
<td>≥0.40</td>
</tr>
<tr>
<td>Absent</td>
<td>≥ 36.2300</td>
<td>&lt; 75.50</td>
<td>&lt;0.20</td>
</tr>
</tbody>
</table>
Figure 1  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 $83.00°C \leq T_m \leq 86.00°C$ in wells of unspiked unknown samples indicates presence of DPC contamination. Software flags as REVIEW.

Limited product warranty

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