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:

- 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..
4. Briefly centrifuge the reaction plate.

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

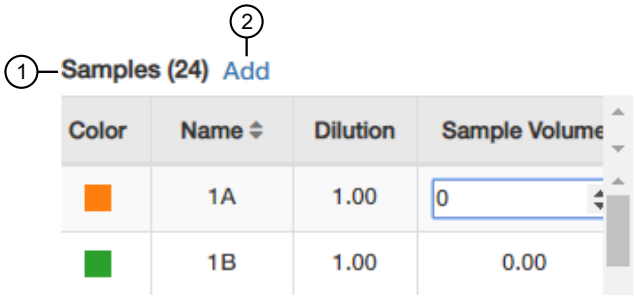
Create a MycoSEQ™ experiment

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).



① **Samples** pane

② **Add**—adds additional samples

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.

Option	Description
If the experiment is open	Click **Start Run**.
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.

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.

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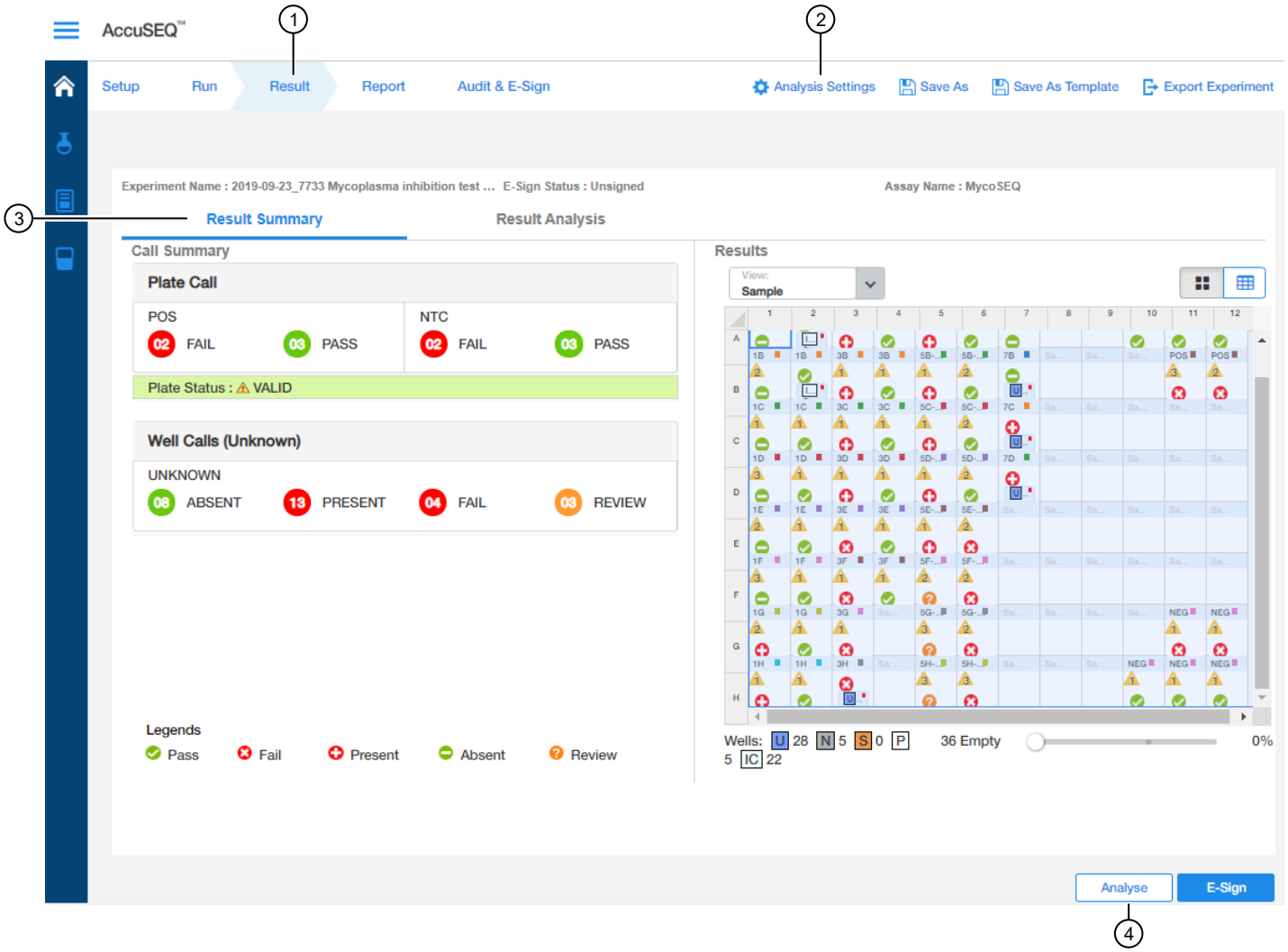
1. In the AccuSEQ™ Real-Time PCR Software, open your experiment, then navigate to the **Result** tab.



① **Result** tab
② **Analysis Settings**
③ **Result Summary** tab
④ **Analyze** button

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: 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 1 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

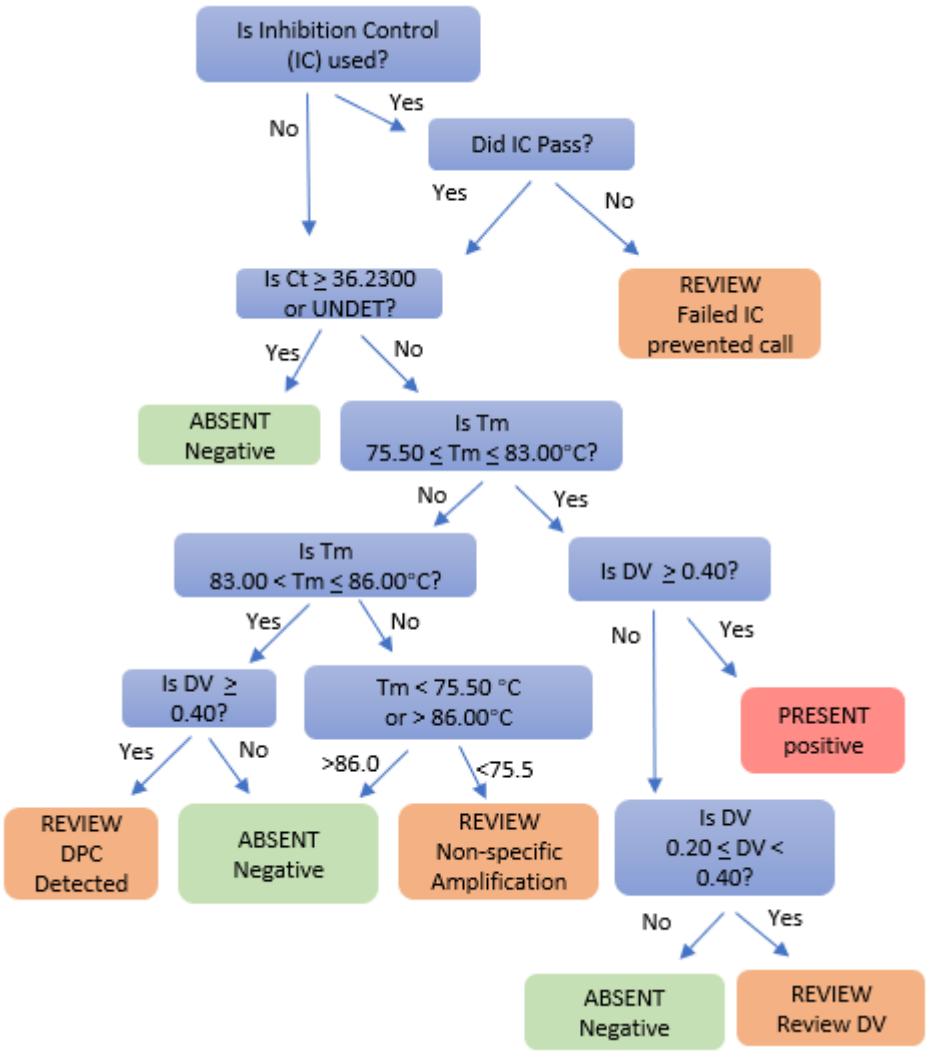


Figure 1 Decision tree for unknown sample calls (with or without an inhibition control [IC])

Note: The presence of a melt peak with a T_m range of 83.00°C ≤ T_m ≤ 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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Revision history: Pub. No. 4465876

Revision	Date	Description
D	28 September 2020	Update to include run and analysis information for AccuSEQ™ Real-Time PCR Software v3.1.
C	24 May 2018	Updated template, legal, and content information. Reorganized content. Added information about using the AccuSEQ™ Software v2.0 Mycoplasma SEQ module.