MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit

Catalog Numbers 4460623, 4460626

Pub. No. MAN0030008 Rev. A.0

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 analyze samples with AccuSEQ™ Software v3.1 or later 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**.

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

   Note: To create a copy of an existing MycoSEQ™ experiment, see the *AccuSEQ™ Real-Time PCR Software v3.2 User Guide* (Pub. No. MAN0029199).

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.

   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 (cannot be changed).

4. Click **Next**.

5. In the **Samples** table in the **Setup** screen, confirm or edit the predefined sample names and control names.
   a. (*Optional*) Click the field in the **Name** column to edit the name of each sample or control.
   b. (*Optional*) Click **Add** to add more samples or controls.

Note: MycoSEQ™ experiments detect presence or absence only, so the values in the remaining columns of the **Samples** table (**Dilution Factor**, etc.) typically remain unchanged.

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

Table 1 Example sample names (rename as appropriate)

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. Define the sample and control wells in the plate layout.

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

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

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

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

   c. Under the **Task** column in the **Targets** table, select the task for each target based on the well type.

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

   e. Repeat for all wells in the experiment.

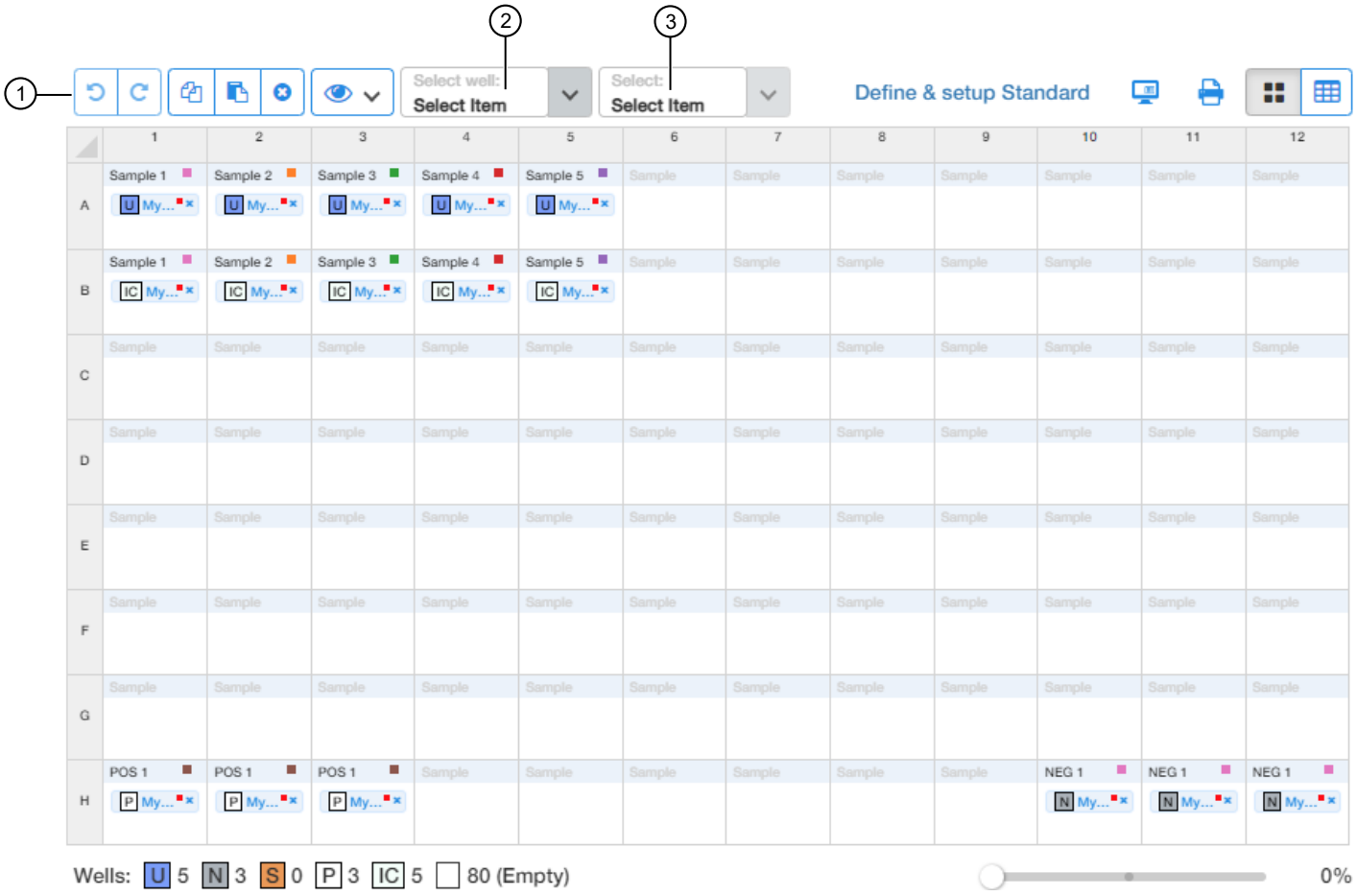


Figure 1 MycoSEQ™ template default sample plate layout

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

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

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

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

8. Click **Next** to open the **Run** screen.
9. To save the experiment, exit the experiment, then click **Yes** when prompted to save changes.

   Note: To create a copy of the experiment, click **Save As**.
10. (*Optional*) Click **Print** to print the plate layout for use in preparing the reactions.
11. Prepare the PCR plate using the plate layout as defined in the experiment, then immediately load the plate in the instrument.

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**.

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

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).

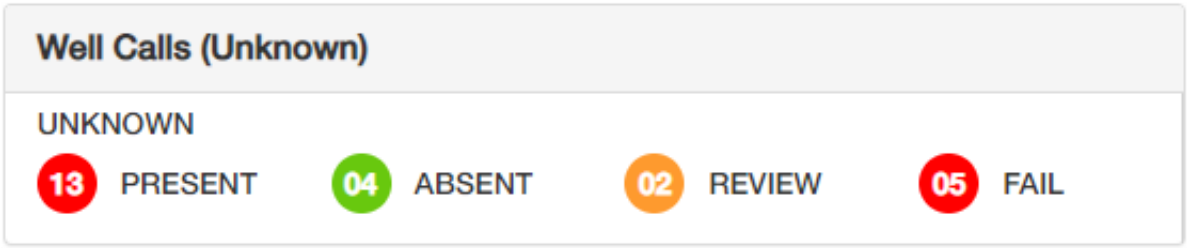
   The number inside the circle indicates the number of controls that passed or failed. The overall plate status (**VALID** or **INVALID**) shown in the **Plate Status** field is determined by the POS and NTC calls.

   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 2 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**).

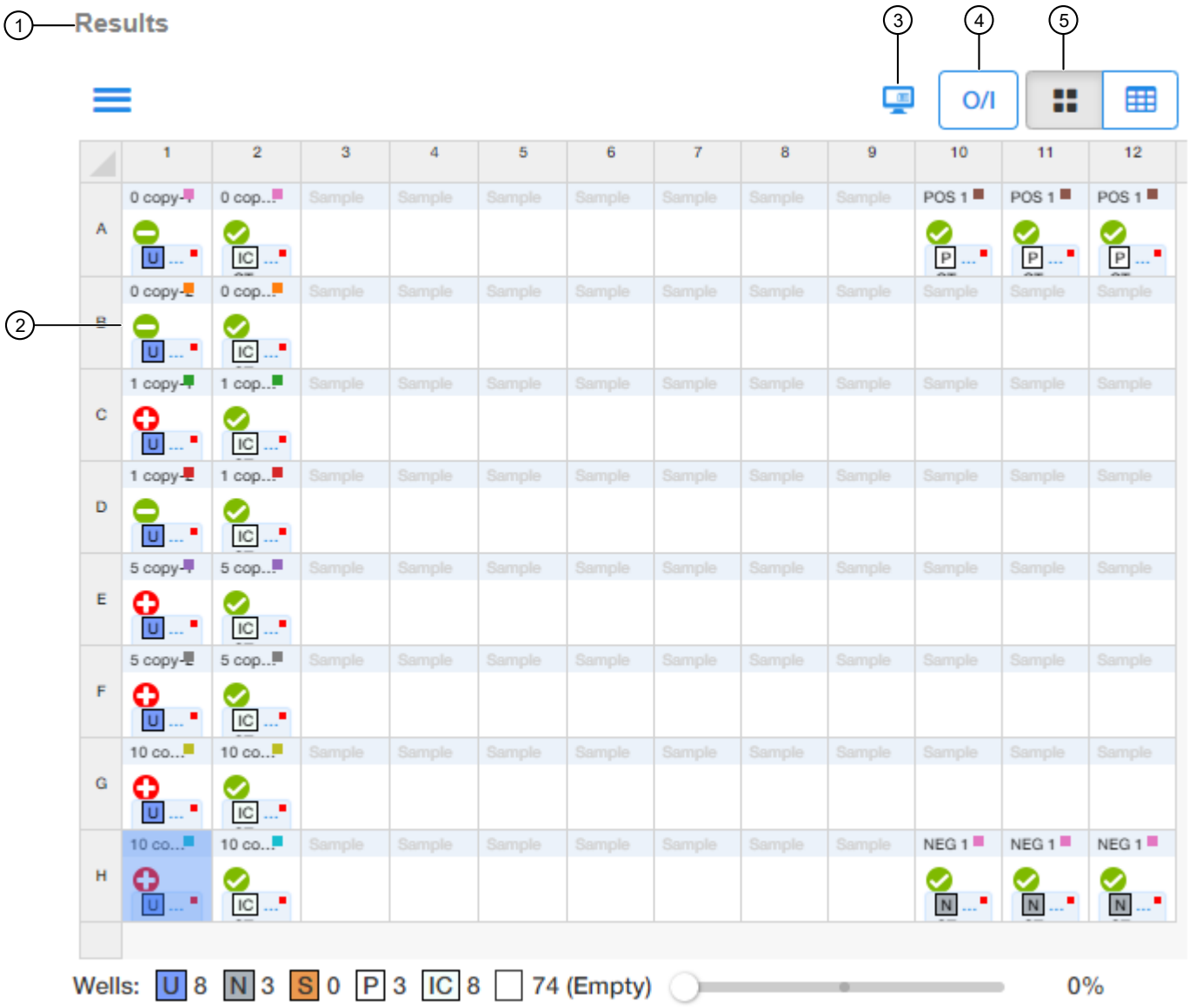


Figure 3 Results pane (example)

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**

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 2 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

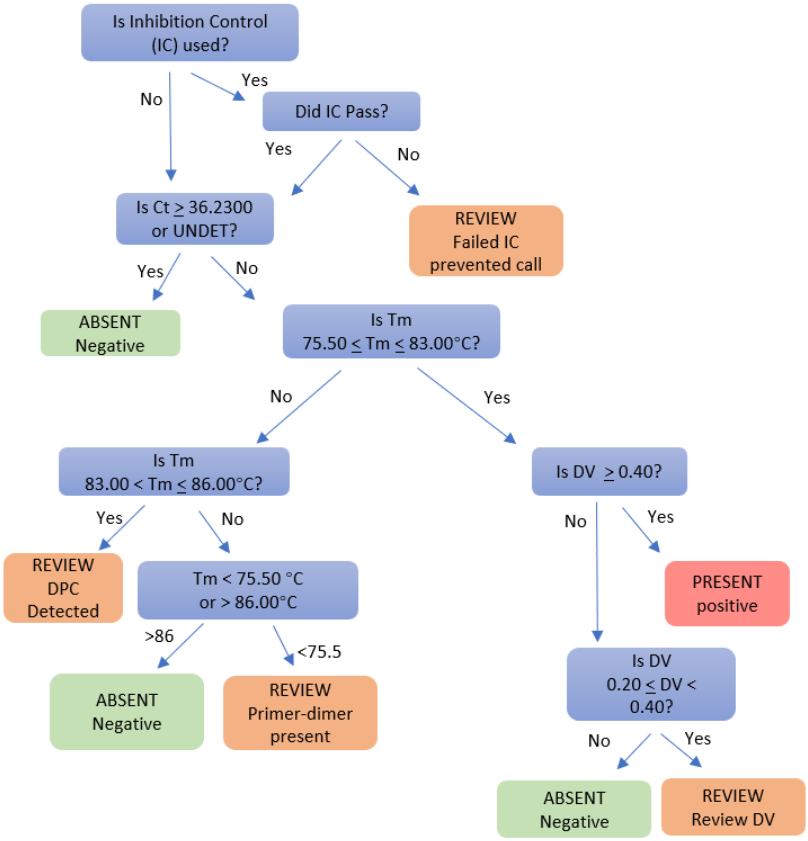


Figure 4 Decision tree for unknown sample calls (with or without an inhibition control [IC])

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. MAN0030008 A.0

Revision	Date	Description
A.0	2 January 2024	MAN0030008 Rev A.0 is updated from 4465876 Rev D. Update to include run and analysis information for AccuSEQ™ Real-Time PCR Software v3.2.
D	28 September 2020	Revision of 4465876, the previous part number for *MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit Quick Reference*. Update to include run and analysis information for AccuSEQ™ Real-Time PCR Software v3.1.
C	24 May 2018	Revision of 4465876, the previous part number for *MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit Quick Reference*. Updated template, legal, and content information. Reorganized content. Added information about using the AccuSEQ™ Software v2.0 Mycoplasma SEQ module.

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

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