MycoSEQ™ Plus Mycoplasma Detection Kit—Automated Sample Preparation

Catalog Numbers A55124, A57925, A57926

Pub. No. MAN0028696 Rev. B

Note: For safety and biohazard guidelines, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference guide provides procedures for experienced users using the MycoSEQ™ Plus Mycoplasma Detection Kit with the following instruments, kits, and software:

- Automated sample preparation: AutoMate *Express*™ Instrument with the PrepSEQ™ Express Nucleic Acid Extraction Kit.
- Real-time PCR: QuantStudio™ 5 Real-Time PCR System with AccuSEQ™ Real-Time PCR Detection Software 3.2 or later.

IMPORTANT! This quick reference is designed for experienced users only, and omits important information for new users. For complete instructions—including kit contents, notes, guidelines, additional procedures, example data, and troubleshooting—see the *MycoSEQ™ Plus Mycoplasma Detection Kit User Guide* (Pub. No. MAN0028695).

Prepare the PrepSEQ™ Express Cartridge

1. Shortly before use (≤1 hour), mix the reagents and resuspend the Magnetic Particles in each PrepSEQ™ Express Cartridge:
   a. Hold the cartridge foil-side up on a vortexer set to maximum speed, then pulse 2–3 times for ~3 seconds each. Repeat with the cartridge foil-side down, then repeat again with the cartridge on its side.
   b. Hold the cartridge foil-side up, then tap the cartridge on the counter several times to deposit any particles or liquid droplets into the bottom of the compartments.
2. Inspect each cartridge to ensure that the contents are in the bottom of the wells and that no precipitate has formed in any of the wells.

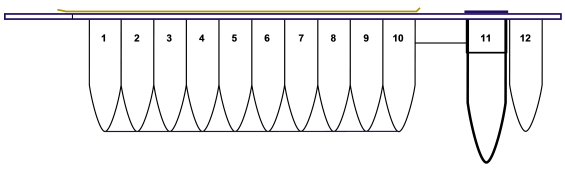

Figure 1 Cartridge compartments

Compartment	Contents
1	Lysis Buffer
2	Magnetic Particles suspension
3	Binding Solution
4 through 6	Wash Buffer
7	Elution Buffer
9	Proteinase K Solution
11	Lysis Tube (added by user)
12	Heated chamber for elution

3. If precipitate forms in compartments 1 or 2 (Lysis Buffer and Magnetic Particles suspension), heat the cartridge in an incubator at 37°C for 30 minutes or until the precipitate is no longer visible. Heat only those cartridges that you plan to use that day.

Option 1: Lot-release method for production harvest—supernatant or spent media only (AutoMate *Express*™ Instrument)

Follow this procedure to test supernatant, spent media, and waste stream samples. This high-sensitivity protocol (LOD ≤ 10 CFU/mL or equivalent in genome copies/mL) is suggested for lot-release testing.

If you are running a positive extraction control for each sample (S-PEC), prepare 2 tubes for each sample: one to be spiked with positive control and one without.

1. (For cell therapy applications only) Add 300,000 cells to 15 mL of sample in a 50-mL conical tube, then proceed to step 4.
2. Add 15 mL of sample to a 50-mL conical tube, then centrifuge at 1,000 × *g* for 5 minutes at 4°C.
3. Transfer the supernatant to a new 50-mL conical tube without disturbing the mammalian cell pellet, then place the supernatant on ice. Discard the pellet.
4. Centrifuge the tube at 16,000 × *g* for 30 minutes at 4°C to collect the mycoplasma.

5. Carefully remove and discard the supernatant without touching or disturbing mycoplasma pellet. Use a pipette to carefully remove any remaining liquid.

6. Add 300 µL of PrepSEQ™ Lysis Buffer to the mycoplasma pellet. Gently vortex for 5 seconds to completely resuspend the pellet. If the pellet is difficult to dislodge, vigorously agitate the tube.

Proceed to "Process samples on the AutoMate *Express*™ Instrument" on page 2.

Option 2: Lot-release method for production harvest—pooled supernatant and mammalian cells (AutoMate *Express*™ Instrument)

Follow this procedure to test supernatant samples pooled with mammalian cell lysate. This high-sensitivity protocol (LOD ≤ 10 CFU/mL or equivalent in genome copies/mL) is suggested for lot-release testing.

If you are running a positive extraction control for each sample (S-PEC), prepare 2 tubes for each sample: one to be spiked with positive control and one without.

1. Add 15 mL of sample to a 50-mL conical tube, then centrifuge at 1,000 × *g* for 5 minutes at 4°C.

2. Transfer the supernatant to a new 50-mL conical tube without disturbing the mammalian cell pellet, then place the supernatant on ice.

3. Remove residual supernatant from the pellet. Reserve the pellet on ice for use in step 6.

4. Centrifuge the supernatant (from step 2) at 16,000 × *g* for 30 minutes at 4°C to collect the mycoplasma.

5. Carefully remove and discard the supernatant without touching or disturbing the mycoplasma pellet. Use a pipette to carefully remove any remaining liquid. Retain the mycoplasma pellet for use in step 9.

6. Add 300 µL of Cell Fractionation Buffer to the mammalian cell pellet from step 3. Gently vortex for 5 seconds to completely resuspend the pellet. If the pellet is difficult to dislodge, vigorously agitate the tube.

   Note: Larger pellets from high-density samples can require up to 550 µL of Cell Fractionation Buffer to completely resuspend.

7. Transfer the cell suspension to a 2-mL tube, then place the tube on ice for 10 minutes.

8. Centrifuge the 2-mL tube at 1,500 × *g* for 10 minutes at 4°C to collect the cellular membranes and nuclei.

9. Carefully transfer the supernatant containing the cell lysate to the mycoplasma pellet from step 5.

10. Resuspend the mycoplasma pellet in the supernatant by pipetting up and down or by vortexing on medium speed.

Proceed to "Process samples on the AutoMate *Express*™ Instrument" on page 2.

Option 3: In-process testing (AutoMate *Express*™ Instrument)

If you are running a positive extraction control for each sample (S-PEC), prepare 2 tubes for each sample: one to be spiked with positive control and one without.

1. If the sample volume is less than 300 µL, dilute with 1X PBS to bring the volume to 300 µL.

2. Add 300 µL of sample to a PrepSEQ™ Express Sample Tube.

3. If the sample contains >1 × 10^6 cells, centrifuge at 1,000 × *g* for 5 minutes at 4°C, then transfer 300 µL of the supernatant to a new PrepSEQ™ Express Sample Tube without disturbing the pellet. Discard the cell pellet.

   If the sample contains ≤1 × 10^6 cells, skip this step.

Proceed to "Process samples on the AutoMate *Express*™ Instrument" on page 2.

Process samples on the AutoMate *Express*™ Instrument

1. For each sample positive extraction control (S-PEC) replicate, add 3 µL (3,000 copies) of MycoSEQ™ Plus Discriminatory Positive/Extraction Control to the sample replicate. Vortex briefly to mix.

   Skip this step for each non-control sample replicate.

2. (Optional) If you are running a plate-level positive extraction control (PEC), add 3 µL (3,000 copies) of MycoSEQ™ Plus Discriminatory Positive/Extraction Control to 300 µL of 1X PBS.

3. If you are running a plate-level negative extraction control (NEC), prepare a tube with 300 µL of 1X PBS instead of sample lysate.

4. Add sample or control to each PrepSEQ™ Express Sample Tube provided in the PrepSEQ™ Express Nucleic Acid Extraction Kit (up to 13 tubes total). Load the tubes in Row S of the AutoMate *Express*™ Instrument Tip and Tube Rack.

5. If necessary, remix the reagents and resuspend the Magnetic Particles in each PrepSEQ™ Express Cartridge (see "Prepare the PrepSEQ™ Express Cartridge" on page 1)

6. After you have prepared the sample tubes and cartridges, set up and run automated extraction on the AutoMate *Express*™ Instrument following the instructions in the *PrepSEQ™ Express Nucleic Acid Extraction Kit for Mycoplasma, MMV, and Vesivirus Detection User Guide* (Pub. No. MAN0016799).

   Note the following:

   - Use the PrepSEQ™ Express Protocol Card during instrument setup.

- Select the **PS Express 123** protocol option, **30 min** for **Lysis Time**, **100 µL** for **Elution volume**.

7. After 80 minutes, when the run has completed, collect 100 µL of eluate.

The extracted DNA is now ready to use in the PCR reaction. To store the extracted DNA, cap the elution tubes and store at 2–8°C for same-day use or –20°C for longer storage.

Note: Storage length is subject to user validation.

Set up a MycoSEQ™ Plus Mycoplasma experiment in AccuSEQ™ Real-Time PCR Detection Software v3.2 or later

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

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

3. In the **qPCR Method** pane of the **Setup** screen, view the default volume and cycling conditions (cannot be changed).

4. In the **Samples** table in the **Setup** screen, confirm or edit the 3 predefined sample names (S1, S2, and S3) and control names.

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

6. To save the experiment, exit the experiment, then click **Yes** when prompted to save changes.

Proceed to “Prepare the PCR master mix” on page 3. When preparing the physical PCR plate, use the plate layout as defined in the experiment.

Prepare the PCR master mix

1. Thaw 2x qPCR Master Mix Plus and MycoSEQ™ Plus qPCR 10X Assay Mix completely on ice.

2. Vortex each tube thoroughly to mix, then briefly centrifuge to collect the contents.

3. Determine the number of reactions needed for the number of controls and test samples. Prepare a sufficient volume of master mix plus 10% overage to compensate for pipetting errors.

Table 1 PCR master mix

Component	Volume	
	1 reaction	Example: 10 reactions[1]
2x qPCR Master Mix Plus	15 µL	165 µL
MycoSEQ™ Plus qPCR 10X Assay Mix	3 µL	33 µL
Water (labeled Negative Control in the kit)	2 µL	22 µL
Total	**20 µL**	**220 µL**

[1] Includes 10% overage.

Prepare the positive template control (PTC)

1. Add 10 µL of MycoSEQ™ Plus Discriminatory Positive/Extraction Control to 40 µL of DNA Dilution Buffer.

2. Vortex and briefly centrifuge.

Prepare the PCR plate

1. Add 20 µL of PCR master mix to each reaction well.

2. Add 10 µL of test sample to each test sample well.

3. Add 10 µL of the positive extraction control to each PEC well.

4. Add 10 µL of the negative extraction control to each NEC well.

5. Add 10 µL of Negative Control (water) to each no-template control (NTC) well.

6. Add 10 µL of the positive template control (prepared as described in “Prepare the positive template control (PTC)” on page 3 to each PTC well.

7. Seal the plate with MicroAmp™ Optical Adhesive Film. Handle the plate by the edges and avoid touching the top of the plate.

8. Centrifuge the plate at 1,000 × g for 2 minutes.

Load the plate in the instrument

CAUTION! Use optical flat caps for tubes. Rounded caps can damage the heated cover.

1. Touch ⏏ to eject the instrument drawer.
2. Load the plate onto the plate adapter so that:
   - Well A1 of the plate is in the top-left corner of the plate adapter.
   - The barcode faces the front of the instrument.
3. Touch ⏏ to close the instrument drawer.

Start the run

With the experiment open in AccuSEQ™ Software, in the **Run** tab, click **Start Run**.

A message notifies you when the run has started.

View the results for MycoSEQ™ Plus Mycoplasma experiments

When a run is complete, the **Result** screen in AccuSEQ™ Software displays the calls for all the plate wells in an experiment.

1. In the **Result Summary** tab, in the **Plate Calls** section, review the calls for the controls (PTC, NTC, PEC, and NEC).
2. In the **Result Summary** tab, in the **Sample Level PEC/NEC Calls** section, review the total number of wells for each PEC or NEC call (**PASS** or **FAIL**).
3. In the **Result Summary** tab, in the **Well Calls** section, review the total number of wells for each call—**Present**, **Absent**, **Review**, or **Fail**.
4. 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**).

Acceptance criteria—AccuSEQ™ Real-Time PCR Detection Software v3.2 or later

- A call of "present" indicates that at least one genomic copy of mycoplasma DNA was present in the unknown reaction and the sample is positive for the presence of mycoplasma.
- For the plate to be valid, at least one replicate of each type of control (PTC, NTC, PEC, and NEC) must pass.

Table 2 Default acceptance criteria for AccuSEQ™ Real-Time PCR Detection Software v3.2 or later

Sample type	Result	FAM™	VIC™	NED™
Unknown	Present	C_t <38.0000	C_t ≥38.0000	Not applicable[1]
	Absent	C_t ≥38.0000	C_t ≥38.0000	ΔC_t <2.0000[2]
NTC or NEC	Pass	C_t ≥38.0000	C_t ≥38.0000	C_t <38.0000
PTC or PEC	Pass	C_t <38.0000	C_t <38.0000	C_t <38.0000

[1] For unknown samples where the FAM™ C_t is <38.0000, NED™ signal can be ignored.

[2] ΔC_t = Sample NED™ C_t – NTC NED™ C_t

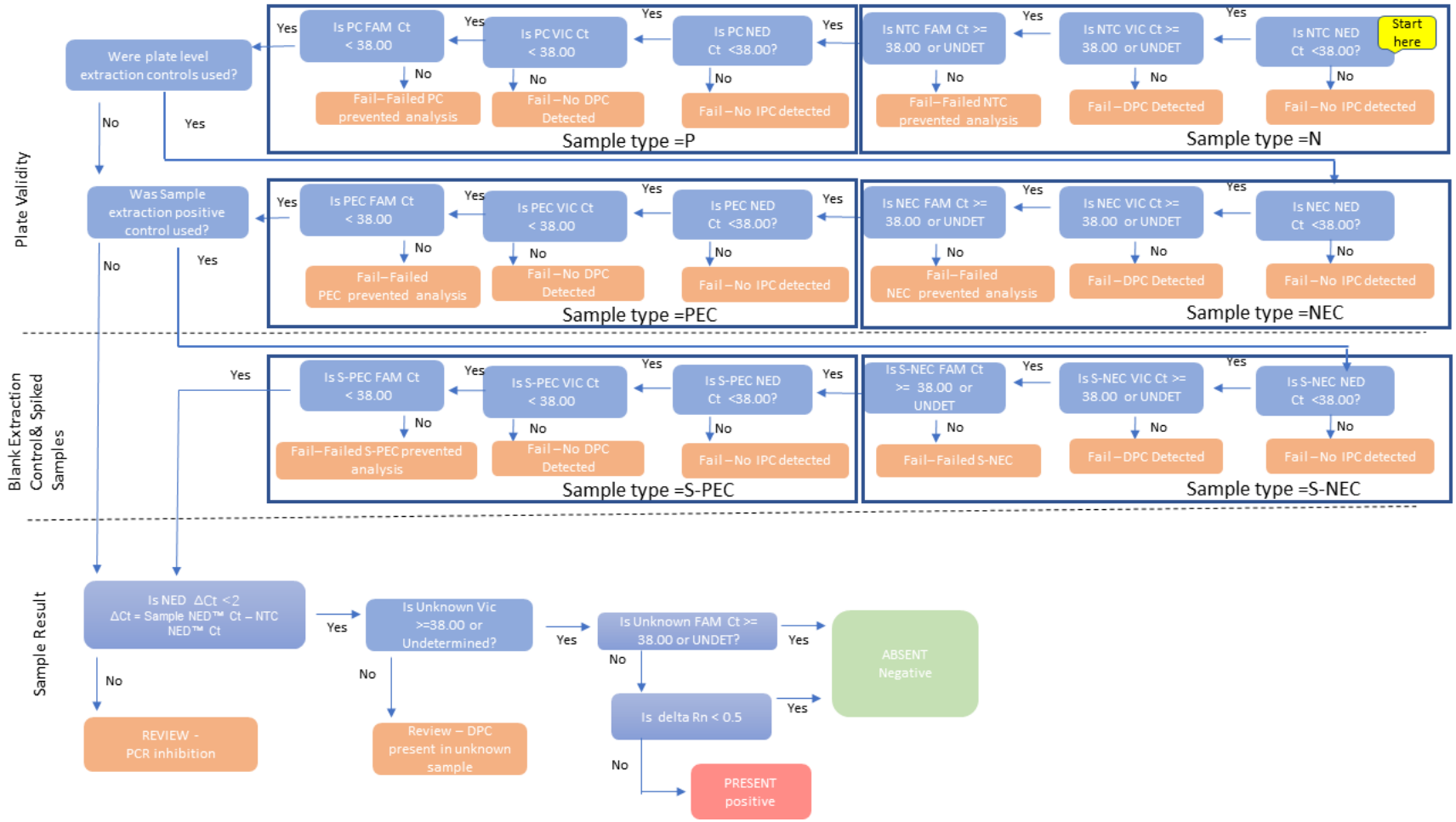

Figure 2 Decision tree

Life Technologies Ltd | 7 Kingsland Grange | Woolston, Warrington WA1 4SR | United Kingdom

Revision history: Pub. No. MAN0028696 B

Revision	Date	Description
B	17 July 2024	• The kit contents for Cat. no. A57925 were updated to include the PrepSEQ™ 1-2-3 Mycoplasma Nucleic Acid Extraction Kit instead of the PrepSEQ™ 1-2-3 Nucleic Acid Extraction Kit, and references to the kit were updated throughout. • Additional information about the default acceptance criteria for presence/absence calls in each version of AccuSEQ™ Software was added, and the corresponding decision trees were updated. • Long-term storage guidance for extracted DNA was clarified.
A.0	12 September 2023	New quick reference for the MycoSEQ™ Plus Mycoplasma Detection Kit.