PrepSEQ™ Express Nucleic Acid Extraction Kit

Automated sample preparation protocols for *Mycoplasma*, MMV, and Vesivirus detection

Catalog Number 4466351

Pub. No. MAN0017291 **Rev.** A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *PrepSEQ™ Express Nucleic Acid Extraction Kit for Mycoplasma, MMV, and Vesivirus Detection User Guide* (Pub. No. MAN0016799). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The PrepSEQ™ Express Nucleic Acid Extraction Kit (Cat. No. 4466351) is for use with the AutoMate *Express*™ Instrument. The kit includes pre-filled reagent cartridges for automated extraction of DNA and/or RNA from *Mycoplasma* cells or viral particles. A variety of starting material can be used, such as infected cell cultures or *Mycoplasma* liquid cultures.

Kit applications

Organisms	Sample volume	For use with kit	Protocol
Mycoplasma, MMV, and Vesivirus	300 µL (up to 10^6 total cells)	PrepSEQ™ Express Nucleic Acid Extraction Kit and the AutoMate *Express*™ Instrument	"Automated protocol for Mycoplasma, MMV, and/or Vesivirus detection" on page 2
Mycoplasma	Up to 15 mL (up to 10^6 cells/mL)		"Large-scale automated protocol for Mycoplasma detection—Option 1: Direct sample testing" on page 2 *OR* "Large-scale automated protocol for Mycoplasma detection—Option 2: Process pooled cell culture media and mammalian cells" on page 4

Before you begin

Before you begin, review the *PrepSEQ™ Express Nucleic Acid Extraction Kit for Mycoplasma, MMV, and Vesivirus Detection User Guide* (Pub. No. MAN0016799) for required materials and procedural guidelines.

Note: This Quick Reference provides brief procedures for sample preparation and automated DNA extraction. For detailed procedures, see the User Guide.

(Large-scale protocols only) Prepare materials

1. Power on the refrigerated centrifuge to allow it to cool to 4°C before use.
2. Keep the samples on ice as much as possible during the sample lysate steps.

Automated protocol for *Mycoplasma*, MMV, and/or Vesivirus detection

Use this protocol to process 300 µL of sample containing up to 10^6 total cells for the detection of *Mycoplasma*, MMV, and/or Vesivirus.

1 Prepare test samples

a. Add sample to each PrepSEQ™ Express Sample Tube (up to 13 tubes total):
   - For samples with ≤10^6 cells/mL—Add 300 µL of sample to each tube.
   - For samples with >10^6 cells/mL—Spin the sample in a microcentrifuge at 500 × *g* for 2 minutes, then add 300 µL of the supernatant to the tube.

   If needed, add cell culture medium or 1X PBS to the samples to bring the total volume up to 300 µL.

b. Cap the tubes, then spin the tubes in a microcentrifuge at 1,000 × *g* for 3 minutes at room temperature.

c. Carefully remove the tubes from the microcentrifuge. Do not disturb the cell pellet.

d. Remove the screw caps from the tubes.

e. Load the tubes in Row S (fourth row) of the tip and tube rack. (See "Load and insert the tip and tube rack" on page 6.)

f. ***(Optional)*** For an extraction positive control: Spike the appropriate amount of DPC to 300 µL of the prepared test sample to achieve 200 copies per PCR. Extraction controls can be dual- or triple-spiked with DPCs for each assay to conserve sample. You can also use one of the following in place of test samples: 1X PBS solution, Lysis Buffer, Cell Fractionation Buffer, or plain media.

Proceed directly to "Set up and run automated DNA extraction" on page 5.

Large-scale automated protocol for *Mycoplasma* detection—Option 1: Direct sample testing

Use this protocol to process up to 15 mL of sample (up to 10^6 cells/mL) for the detection of *Mycoplasma*.

1 Prepare test samples

Prepare each sample in a new 50-mL conical tube:
- ≤10^6 cells/mL—Add 15 mL of sample to the tube.
- >10^6 cells/mL—Add 15 mL of sample to the tube, centrifuge at 1,000 × *g* for 5 minutes to pellet the cells, then transfer 15 mL of supernatant to a new 50-mL conical tube.

2 Separate mammalian cells from cell culture media

a. Centrifuge each tube at 1,000 × *g* for 5 minutes at 4°C to pellet the mammalian cells.

b. Transfer 15 mL of the supernatant to a new 50-mL conical tube, then place on ice.

   The supernatant contains free *Mycoplasma*.

c. Discard the mammalian cell pellet.

3 Treat with RNase and DNase

If the samples have high SYBR™ Green I dye background because of excess cellular nucleic acid in the supernatant, perform RNase treatment.

IMPORTANT! For some high-density samples, both RNase and DNase treatments are needed. In this case, perform the DNase treatment before the RNase treatment.

a. To treat with DNase:
   1. Add 450 µL of 10× Reaction Buffer and 90 µL of TURBO™ DNase, then gently vortex to mix.
   2. Incubate at 37°C for 30 minutes.

3 Treat with RNase and DNase *(continued)*

b. To treat with RNase:
   1. Add 180 µL of 0.5 M EDTA, 225 µL of RNase Cocktail™ Enzyme Mix, and 150 µL of Proteinase K, gently vortex to mix, then briefly spin.
   2. Incubate at 56°C for a minimum of 30 minutes to digest the cellular RNA and proteins.

4 Process the supernatant to obtain resuspended *Mycoplasma*

a. Centrifuge the tube at 16,000 × *g* for 30 minutes at 4°C to pellet the *Mycoplasma*.

b. Aspirate and discard the supernatant without disturbing the *Mycoplasma* pellet. Do not decant the liquid and do NOT touch the pellet.

c. Add 275 µL of Lysis Buffer, then mix thoroughly by vortexing to resuspend the *Mycoplasma* pellet.

   If the pellet is difficult to dislodge, vigorously agitate the tube.

d. Transfer the resuspended pellet to a PrepSEQ™ Express Sample Tube.

5 Treat the resuspended *Mycoplasma*

Separately process the resuspended *Mycoplasma* pellet in the PrepSEQ™ Express Sample Tube.

a. Add 2 µL of 0.5 M EDTA, 18 µL of RNase Cocktail™ Enzyme Mix, and 5 µL of Proteinase K, then briefly vortex to mix.

b. *(Optional)* Spike with Discriminatory Positive Control (DPC) for optimization or with *Mycoplasma* DNA for lot release validation.

c. ***(Optional)*** For an extraction positive control: Spike the appropriate amount of DPC to 300 µL of the prepared test sample to achieve 200 copies per PCR. Extraction controls can be dual- or triple-spiked with DPCs for each assay to conserve sample. You can also use one of the following in place of test samples: 1X PBS solution, Lysis Buffer, Cell Fractionation Buffer, or plain media.

d. Incubate at 56°C for 15 minutes to digest the cellular RNA. Vortex twice during incubation.

Proceed directly to "Set up and run automated DNA extraction" on page 5.

Large-scale automated protocol for *Mycoplasma* detection—Option 2: Process pooled cell culture media and mammalian cells

Use this protocol to process up to 15 mL of sample (up to 10^6 cells/mL) for the detection of *Mycoplasma*.

1 Prepare test samples

Prepare each sample in a new 50-mL conical tube:

- ≤10^6 cells/mL—Add 15 mL of sample to the tube.

2 Separate mammalian cells from cell culture media

a. Centrifuge each tube at 1,000 × *g* for 5 minutes at 4°C to pellet the mammalian cells.

b. Transfer 15 mL of the supernatant to a new 50-mL conical tube, then place on ice.

The supernatant contains free *Mycoplasma*.

c. Remove residual supernatant from the mammalian cell pellet, then place the cell pellet on ice.

3 Treat with RNase and DNase

If the samples have high SYBR™ Green I dye background because of excess cellular nucleic acid in the supernatant, perform RNase treatment.

IMPORTANT! For some high-density samples, both RNase and DNase treatments are needed. In this case, perform the DNase treatment before the RNase treatment.

a. To treat with DNase:

1. Add 450 µL of 10× Reaction Buffer and 90 µL of TURBO™ DNase, then gently vortex to mix.
2. Incubate at 37°C for 30 minutes.

b. To treat with RNase:

1. Add 180 µL of 0.5 M EDTA, 225 µL of RNase Cocktail™ Enzyme Mix, and 150 µL of Proteinase K, gently vortex to mix, then briefly spin.
2. Incubate at 56°C for a minimum of 30 minutes to digest the cellular RNA and proteins.

4 Process the supernatant to obtain the *Mycoplasma* pellet

a. Centrifuge the supernatant at 16,000 × *g* for 30 minutes at 4°C to pellet the *Mycoplasma*.

b. Carefully remove and discard the supernatant; retain the *Mycoplasma* pellet for use in the next section.

IMPORTANT! Do not decant the liquid and do NOT touch the pellet. Use a P200 pipette to remove the last of the supernatant.

c. Place the 50-mL tube containing the *Mycoplasma* pellet on ice.

5 Process the mammalian cell pellet to obtain free *Mycoplasma* and combine with the *Mycoplasma* pellet

Perform this procedure during the 30-minute centrifugation step in the previous section.

a. Add 550 µL of ice-cold Cell Fractionation Buffer to the mammalian cell pellet. Gently vortex or pipet up and down several times with a P1000 pipette to completely resuspend the mammalian cells.

If the pellet is difficult to dislodge, vigorously agitate the tube.

b. Transfer the mammalian cell suspension to a 2-mL microcentrifuge tube, then place on ice for 5 minutes.

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

5 Process the mammalian cell pellet to obtain free *Mycoplasma* and combine with the *Mycoplasma* pellet *(continued)*

d. Carefully transfer 275 µL (two 137.5-µL aliquots) of the Cell Fractionation Buffer supernatant (mammalian cell lysate) to the *Mycoplasma* pellet obtained in the previous section. Avoid the pellet and viscous material.

e. Resuspend the *Mycoplasma* pellet in the supernatant by pipetting up and down or by vortexing on medium speed.

f. Transfer the resuspended *Mycoplasma* pellet to a new PrepSEQ™ Express Sample Tube.

6 Treat the resuspended *Mycoplasma*

Separately process the resuspended *Mycoplasma* pellet in the PrepSEQ™ Express Sample Tube.

a. Add 2 µL of 0.5 M EDTA, 18 µL of RNase Cocktail™ Enzyme Mix, and 5 µL of Proteinase K, then briefly vortex to mix.

b. *(Optional)* Spike with Discriminatory Positive Control (DPC) for optimization or with *Mycoplasma* DNA for lot release validation.

c. ***(Optional)*** For an extraction positive control: Spike the appropriate amount of DPC to 300 µL of the prepared test sample to achieve 200 copies per PCR. Extraction controls can be dual- or triple-spiked with DPCs for each assay to conserve sample. You can also use one of the following in place of test samples: 1X PBS solution, Lysis Buffer, Cell Fractionation Buffer, or plain media.

d. Incubate at 56°C for 15 minutes to digest the cellular RNA. Vortex twice during incubation.

Proceed directly to "Set up and run automated DNA extraction" on page 5.

Set up and run automated DNA extraction

1 Inspect cartridges

a. Inspect the reagent cartridges to ensure that the contents are in the bottom of the wells and that no precipitate has formed in any of the wells.

b. 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.

2 Insert a protocol card

a. Confirm that the power switch is in the off position.

Note: If you insert the card while the instrument is on, the instrument will not recognize the card.

b. Open the card slot.

c. Insert the protocol card in the slot, with the arrow pointing toward the instrument and the label facing left.

d. Push the card completely into the card slot, then close the card slot.

e. Power on the instrument.

3 Load and insert the cartridge rack

a. Press **Start** to display step-by-step instructions for loading on the touchscreen.

b. Open the instrument door (push up the door), then remove the tip and tube rack and the cartridge rack.

c. Remove up to 13 cartridges from the kit box.

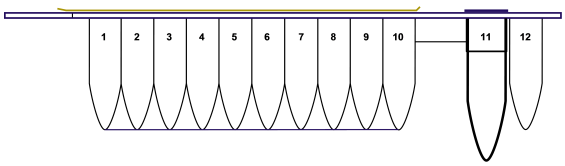
Note: One cartridge is required per sample. Use only PrepSEQ™ Express Cartridges.

d. Prepare the reagent cartridges.

3 Load and insert the cartridge rack *(continued)*

e. Load the reagent cartridges into the cartridge rack by sliding each reagent cartridge along the groove in the direction of the arrow until the reagent cartridge clicks into place. Ensure that the notches in the cartridge align with the notches in the cartridge rack.

f. **(IMPORTANT!)** In each cartridge, insert a Lysis Tube in position 11.



g. Insert the loaded cartridge rack into the instrument.

WARNING! Do not touch the surface of the heat block. Touching the block can cause burns.

4 Load and insert the tip and tube rack

Note: Press ↵ after following each on-screen prompt.

a. Load the tip and tube rack in the following order:

1. **Row E**—Load PrepSEQ™ Express Elution Tubes, with the caps open and secured.
2. **Row T1**—Leave empty.
3. **Row T2**—Load AutoMate *Express*™ tips inserted into tip holders.

   Note: One tip and tip holder set is required per sample.
4. **Row S**—Load PrepSEQ™ Express Sample Tubes containing the lysate.

b. Insert the loaded tip and tube rack into the instrument with row E in the front.

5 Start an automated extraction run

a. Be sure that you have loaded and inserted the cartridge rack and tip and tube rack correctly, then close the instrument door.

b. Press ↵, then press **1** to select the **PS Express 123** option.

c. Select:

- **30 min** for **Lysis Time**
- **100 µL** for **Elution volume**

d. Press **Start**.

The screen shows the steps and the approximate run time remaining.

IMPORTANT! Do not open the door during a protocol run. To pause or cancel the run, see the *AutoMate Express™ Instrument User Guide* (Pub. No. 4441982).

6 Complete the run and store the extracted DNA

At the end of the run, the instrument beeps briefly and the digital display shows "Finished Protocol".

a. Open the instrument door, then remove and cap the Elution Tubes containing the purified DNA.

b. Store the purified DNA at 4°C for same-day use, or at –20°C for longer storage.

c. Run or skip the **WastePooler** protocol.

To...	Do this...
Run the **WastePooler** protocol	1. Close the instrument door, then press **Start**. Wait for the series of beeps indicating the end of the procedure. 2. Press ↵ to return to the **Main** menu.
Skip the **WastePooler** protocol	Press **Esc** to return to the **Main** menu.

6 Complete the run and store the extracted DNA *(continued)*

d. Open the instrument door, then remove the cartridge rack and tip and tube rack.

e. Properly dispose of the used reagent cartridges, tips, and tubes.

> **WARNING!** The used reagent cartridges may contain the following: guanidine thiocyanate, isopropanol, and ethanol. See the Safety Data Sheets and local, state, and national regulations for proper labeling, handling, and disposal.

> **WARNING!** Do not add acids or bases (such as bleach) to any wastes containing Lysis Buffer (present in reagent cartridges or tubes). Acids and bases can react with guanidine thiocyanate in the Lysis Buffer and generate toxic gas.

f. Close the instrument door.

g. After each run, clean the tip and tube rack as needed. Follow the cleaning procedures in the *AutoMate Express™ Instrument User Guide* (Pub. No. 4441982).

Manufacturer: Thermo Fisher Scientific | 7 Kingsland Grange | Warrington, Cheshire WA1 4SR | United Kingdom

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

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Revision history: Pub. No. MAN0017291

Revision	Date	Description
A.0	30 January 2018	New document.

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