applied biosystems

PrepSEQ™ Express Nucleic Acid Extraction Kit

USER GUIDE

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

for use with:

AutoMate *Express*™ Instrument

Catalog Number 4466351

Publication Number MAN0016799

Revision A.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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Revision history: Pub. No. MAN0016799

Revision	Date	Description
A	30 January 2018	New document.

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Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

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	Chapter 3, "Automated protocol for Mycoplasma, MMV, and/or Vesivirus detection"
Mycoplasma	Up to 15 mL (up to 10^6 cells/mL)		Chapter 4, "Large-scale automated protocol for Mycoplasma detection"

Contents and storage

Contents	Amount	Storage
PrepSEQ™ Express Cartridges	1 box of 52	18–25°C (room temperature), foil-side up
Lysis Tubes, capless	1 pack of 52	18–25°C (room temperature)
Sample Tubes	1 pack of 52 1.5-mL tubes and screw caps	
Elution Tubes	1 pack of 52 hinged-cap tubes	
AutoMate *Express*™ Tips and Tip Holders	1 pack of 52 sets	

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Table 1 All protocols

Item	Source
Instrument	
AutoMate *Express*™ Nucleic Acid Extraction System	4467754
Laboratory supplies	
Heat block with block inserts, for use with 2-mL tubes	MLS
Vortex-Genie™ 2T Mixer	VWR™ Scientific Industries (14216-188 or 14216-186)
Eppendorf™ PCR Clean Microcentrifuge Tubes (Safe-Lock, 2 mL, round-bottom)	VWR™ Scientific Industries (62111-754)
Benchtop microcentrifuge (13,000 × *g* or greater), for use with 2-mL tubes	MLS
(Optional) Fisher Scientific™ Mini Plate Spinner Centrifuge	14-100-143
Serological pipettes	MLS
Nonstick, RNase-free Microfuge Tubes (1.5 mL)	AM12450
Reagents	
EDTA, 0.5 M	AM9260G
(Optional) 1X PBS, calcium- and magnesium-free, pH ~7	MLS

Table 2 Large-scale protocols

Item	Source
Laboratory supplies	
Refrigerated centrifuge (2–8°C, 16,000 × *g*), for use with 50-mL tubes	MLS
Conical Tubes (50 mL)	AM12502
Ice bucket	MLS
Reagents	
Proteinase K, 20 mg/mL	AM2548
PrepSEQ™ Lysis Buffer	A29825
Cell Fractionation Buffer	4403461
(Optional) For samples with high SYBR™ dye background: TURBO™ DNase (2 U/µL) (includes TURBO™ DNase and 10✕ Reaction Buffer)	AM2239
(Optional) RNase Cocktail™ Enzyme Mix	AM2286

2 Lysate, Magnetic Particle, and positive control guidelines

Guidelines for preparing sample lysates that contain target DNA

Minimizing cellular DNA and/or RNA in the final extracted DNA is critical to *Mycoplasma* DNA detection. High amounts of cellular DNA and/or RNA cause PCR inhibition and high background of the SYBR™ Green I dye signal, reducing detection of low copy numbers of targets. Factors that affect levels of cellular DNA and/or RNA include:

- **Viability of cell culture sample**—Use fresh culture samples to increase the purity of your extracted target DNA. Avoid conditions such as long-term storage at 4°C (or freezing temperatures). Such temperatures cause increased death or lysis of cells, which contributes to additional background DNA in samples.
- In the large-scale protocols, when processing the mammalian cell pellet, keep it on ice and perform all processing steps at 4°C to avoid host cell nuclei lysis as much as possible. Room temperature increases lysis of nuclei and host DNA in the final extracted DNA, and causes PCR inhibition.
- In the large-scale protocols, if working with the mammalian cell pellet:
  - In some cases, the cell pellet is large and sticky and cannot be resuspended easily. Never vortex to resuspend the cells.
  - When transferring the cell culture supernatant, avoid touching the pellet, which contains nuclei and viscous material that may be generated from lysis of nuclei. If needed, use a P200 pipette to perform the transfer.
  - In the final transfer of the cell pellet supernatant, avoid contact with or transfer of the viscous material. If needed, recentrifuge the tube at 1000 × *g* for 3 minutes at 4°C, then very carefully transfer 300 µL (two 150-µL aliquots) with a P200 pipette.

Guidelines for working with PrepSEQ™ Express Cartridges

- Always use the plastics provided with the kit.
- Do not switch the supplied pre-filled reagents with any other buffers, because the protocols are specifically optimized with the reagents supplied with the kit.
- Mix the reagents and resuspend the Magnetic Particles in each cartridge:
  a. Hold the cartridge foil-side up on a vortexer set to maximum speed, then pulse (~3 seconds) 2–3 times. 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.

  Note: Vortexing may result in foam in compartment 1 (Lysis Buffer). The foam disperses within minutes and does not affect the assay performance.

  After resuspending the Magnetic Particles, use the cartridges within 2 hours, or perform this procedure again before using.

Guidelines for working with the AutoMate *Express*™ Instrument

Before using the instrument, see the *AutoMate Express™ Instrument User Guide* (Pub. No. 4441982) and review the sections on safety and operating the instrument.

(Recommended) Run extraction controls

IMPORTANT! Positive and/or negative extraction controls are primarily used during optimization or pre-validation testing. Extraction controls are not required, but we recommend that you run them.

The MycoSEQ™ Discriminatory Positive Control (DPC) provided with the MycoSEQ™ *Mycoplasma* Detection Kit is a multi-purpose control that can be used as an extraction positive control.

If you are running an extraction positive control, we recommend that you extract and analyze 1 replicate of the sample unspiked and 1 replicate of the sample spiked:

- **Sample 1, tube 1**—Test sample.
- **Sample 1, tube 2**—Test sample + DPC. Spike a volume of DPC to achieve 200 copies per PCR.

3 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.

Prepare test samples

1. 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.

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

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

4. Remove the screw caps from the tubes.

5. 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 23.)

6. ***(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.

Next steps

Proceed directly to Chapter 5, "Set up and run automated DNA extraction".

4 Large-scale automated protocol for *Mycoplasma* detection

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

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.

Prepare test samples

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

- *(Options 1 and 2)* ≤10^6 cells/mL—Add 15 mL of sample to the tube.
- *(Option 1 only)* >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.

Option 1: Direct sample testing

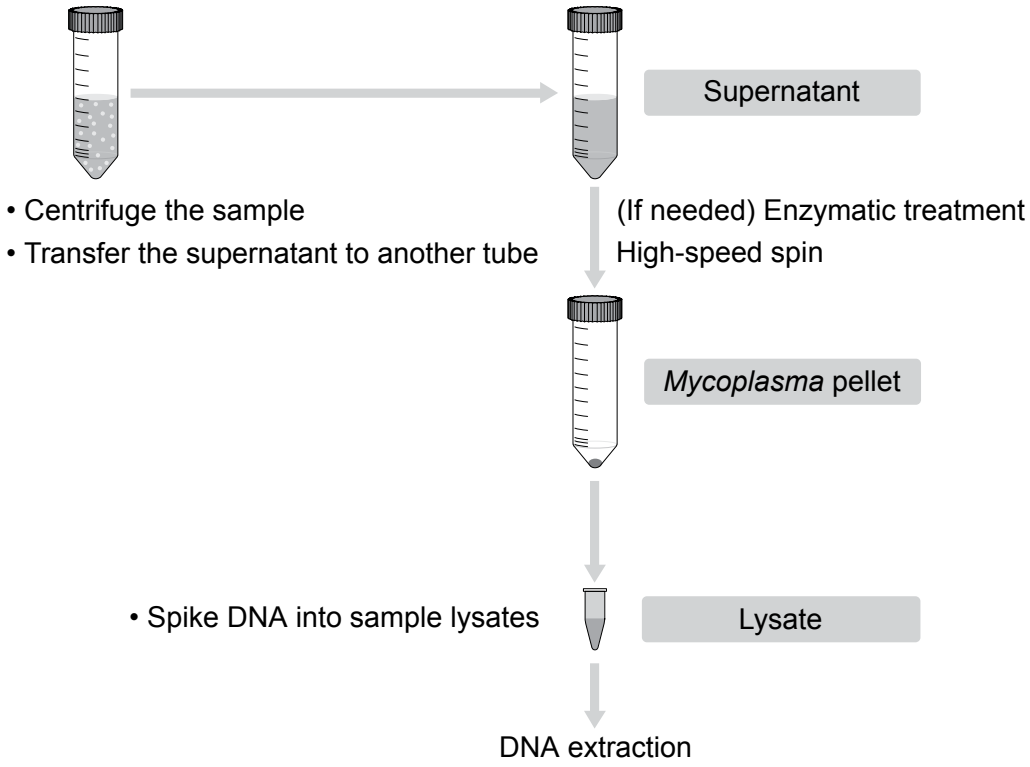


Figure 1 Option 1: Direct sample testing

Separate mammalian cells from cell culture media

1. Obtain the 15-mL samples from "Prepare test samples" on page 11.
2. Centrifuge each tube at 1,000 × *g* for 5 minutes at 4°C to pellet the mammalian cells.
3. Transfer 15 mL of the supernatant to a new 50-mL conical tube, then place on ice. The supernatant contains free *Mycoplasma*.
4. Discard the mammalian cell pellet.

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.

Treat with DNase

1. Add the following TURBO™ DNase (2 U/µL) components, then gently vortex to mix:
   - 450 µL of 10× Reaction Buffer
   - 90 µL of TURBO™ DNase
2. Incubate at 37°C for 30 minutes.

Treat with RNase

1. Add the following components, gently vortex to mix, then briefly spin:
   - 180 µL of 0.5 M EDTA
   - 225 µL of RNase Cocktail™ Enzyme Mix
   - 150 µL of Proteinase K

   Note: Alternatively, you can prepare a stock mix of EDTA, Proteinase K, and RNase Cocktail™ Enzyme Mix, then add 555 µL of stock mix to each sample.
2. Incubate at 56°C for a minimum of 30 minutes to digest the cellular RNA and proteins.

Process the supernatant to obtain resuspended *Mycoplasma*

1. Centrifuge the tube at 16,000 × *g* for 30 minutes at 4°C to pellet the *Mycoplasma*.
2. Aspirate and discard the supernatant without disturbing the *Mycoplasma* pellet. Do not decant the liquid and do NOT touch the pellet.
3. 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.
4. Transfer the resuspended pellet to a PrepSEQ™ Express Sample Tube.

Treat the resuspended *Mycoplasma*

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

1. Add the following volumes, then briefly vortex to mix:
   - 2 µL of 0.5 M EDTA
   - 18 µL of RNase Cocktail™ Enzyme Mix
   - 5 µL of Proteinase K
2. *(Optional)* Spike with Discriminatory Positive Control (DPC) for optimization or with *Mycoplasma* DNA for lot release validation.
3. ***(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.
4. Incubate at 56°C for 15 minutes to digest the cellular RNA. Vortex twice during incubation.

Next steps

Proceed directly to Chapter 5, “Set up and run automated DNA extraction“.

Option 2: Process pooled cell culture media and mammalian cells

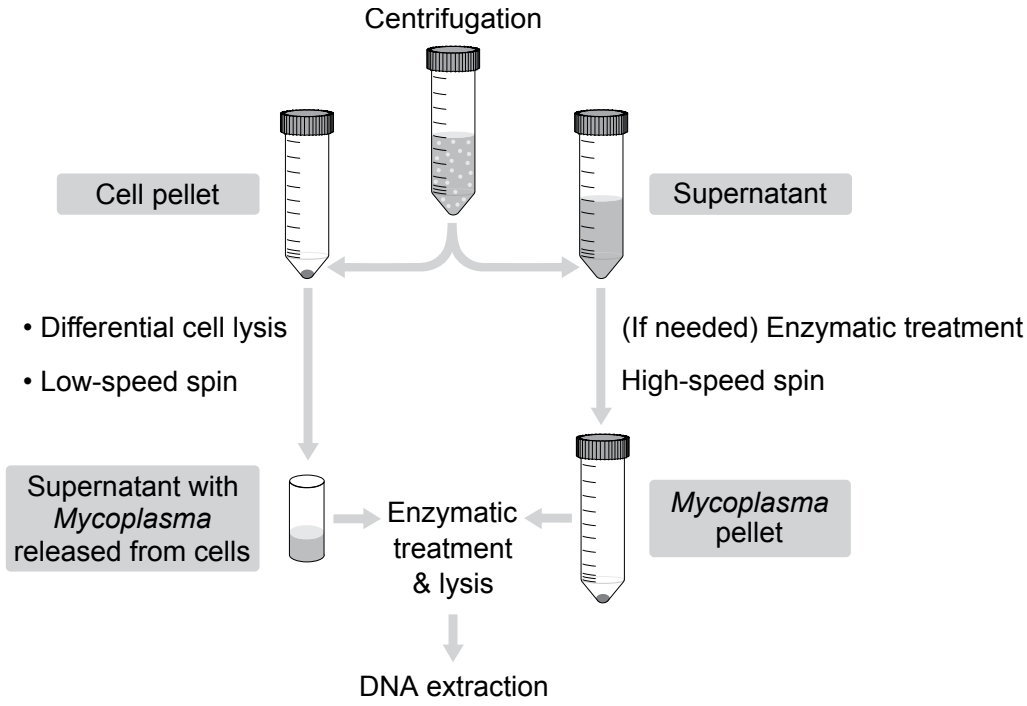


Figure 2 Option 2: Process pooled cell culture media and mammalian cells

Separate mammalian cells from cell culture media

1. Obtain the 15-mL samples from "Prepare test samples" on page 11.
2. Centrifuge each tube at 1,000 × *g* for 5 minutes at 4°C to pellet the mammalian cells.
3. Transfer 15 mL of the supernatant to a new 50-mL conical tube, then place on ice. The supernatant contains free *Mycoplasma*.
4. Remove residual supernatant from the mammalian cell pellet, then place the cell pellet on ice.

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.

Treat with DNase

1. Add the following TURBO™ DNase (2 U/µL) components, then gently vortex to mix:
   - 450 µL of 10× Reaction Buffer
   - 90 µL of TURBO™ DNase
2. Incubate at 37°C for 30 minutes.

Treat with RNase

1. Add the following components, gently vortex to mix, then briefly spin:
   - 180 µL of 0.5 M EDTA
   - 225 µL of RNase Cocktail™ Enzyme Mix
   - 150 µL of Proteinase K

   Note: Alternatively, you can prepare a stock mix of EDTA, Proteinase K, and RNase Cocktail™ Enzyme Mix, then add 555 µL of stock mix to each sample.
2. Incubate at 56°C for a minimum of 30 minutes to digest the cellular RNA and proteins.

Process the supernatant to obtain the *Mycoplasma* pellet

1. Centrifuge the supernatant at 16,000 × *g* for 30 minutes at 4°C to pellet the *Mycoplasma*.
2. 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.
3. Place the 50-mL tube containing the *Mycoplasma* pellet on ice.

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.

1. 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.

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

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

4. 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.

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

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

Treat the resuspended *Mycoplasma*

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

1. Add the following volumes, then briefly vortex to mix:
   - 2 µL of 0.5 M EDTA
   - 18 µL of RNase Cocktail™ Enzyme Mix
   - 5 µL of Proteinase K

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

3. ***(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.

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

Next steps

Proceed directly to Chapter 5, “Set up and run automated DNA extraction“.

5 Set up and run automated DNA extraction

Inspect cartridges

1. 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.

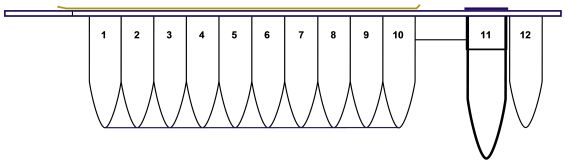


Figure 3 Cartridge compartments

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

2. 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.

Insert a protocol card

For guidelines on handling protocol cards, see the *AutoMate Express™ Instrument User Guide*.

1. 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.

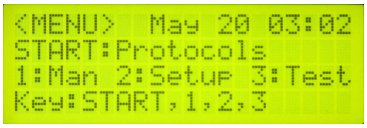
2. Open the card slot.

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

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

5. Power on the instrument.
   When the card is fully inserted in the correct orientation, the display briefly shows information including the instrument version, then shows the **Main** menu.

IMPORTANT! Do not remove or insert the protocol card while the instrument is powered on. Removing the card stops the run, and it may cause instrument data file loss. If the card is removed during a run, immediately power off the instrument to minimize the potential for data loss.



Load and insert the cartridge rack

Wear gloves when you handle samples or load the cartridges, tips, and tubes in the rack.

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

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

① Push up

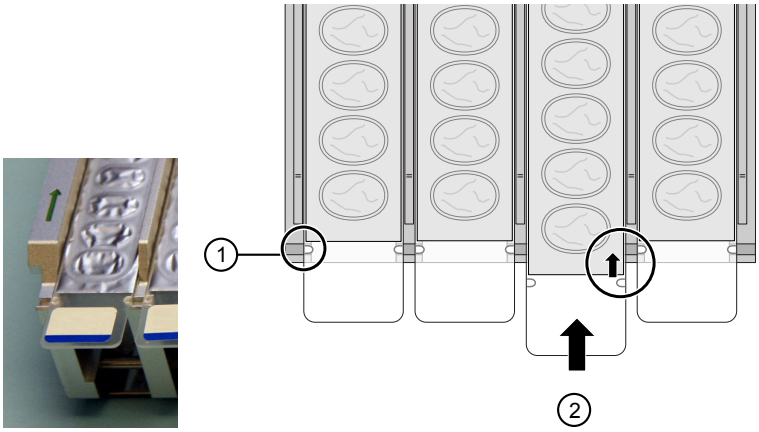
3. Remove up to 13 cartridges from the kit box.

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

4. Prepare the reagent cartridges according to "Guidelines for working with PrepSEQ™ Express Cartridges" on page 9.

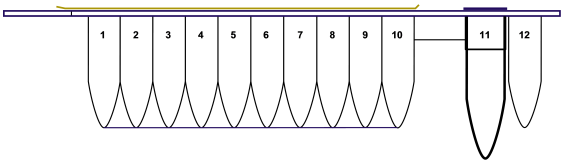
5. Load the reagent cartridges into the cartridge rack by sliding each reagent cartridge along the groove in the direction of the arrow until the reagent cartridge clicks into place. Ensure that the notches in the cartridge align with the notches in the cartridge rack.

   Note: An incorrectly loaded cartridge rack can cause the instrument to stop during a run.



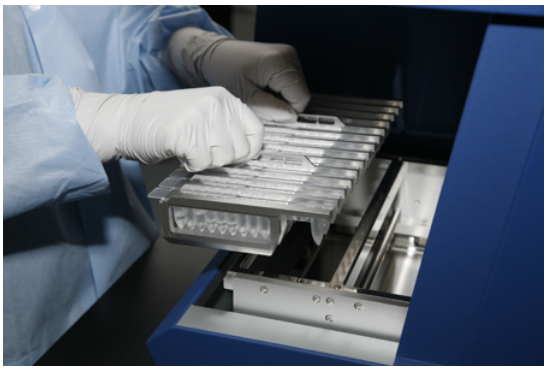
① Correct position
② Slide the cartridge until the notches align and the cartridge clicks into place

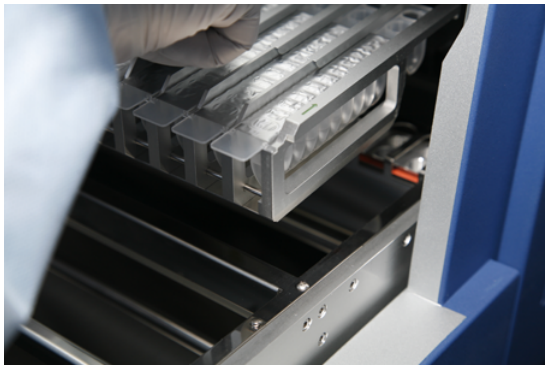
6. **(IMPORTANT!)** In each cartridge, insert a Lysis Tube in position 11.

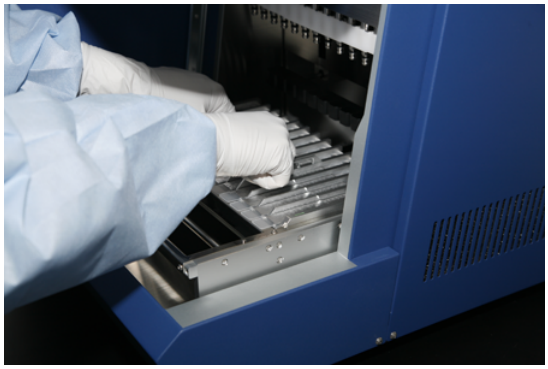


7. Insert the loaded cartridge rack into the instrument.

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







Load and insert the tip and tube rack

IMPORTANT! Follow these guidelines to avoid potential problems during the run:

- Load the cartridge rack into the instrument first, followed by the tip and tube rack. Loading the tip and tube rack first causes the instrument to stop during a run.
- Use only the supplied screw-cap tubes. Using other tubes will result in instrument or experiment failure.
- If you are processing fewer than 13 samples, load the tips and tubes in the same positions as the cartridges in the cartridge rack.

Note: Press  after following each on-screen prompt.

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

   Note: If you are processing fewer than 13 samples, be sure to load the tips and tubes in the same positions as the PrepSEQ™ Express Cartridges that are loaded in the cartridge rack.

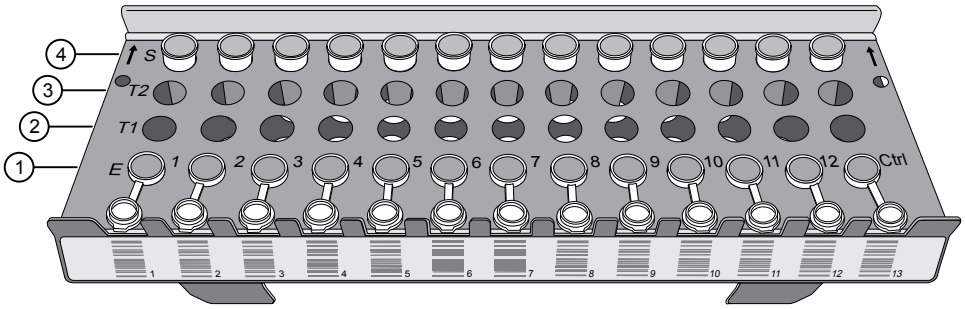
   a. **Row E**—Load PrepSEQ™ Express Elution Tubes, with the caps open and secured as shown in the photo.

   b. **Row T1**—Leave empty.

   c. **Row T2**—Load AutoMate *Express*™ tips inserted into tip holders.

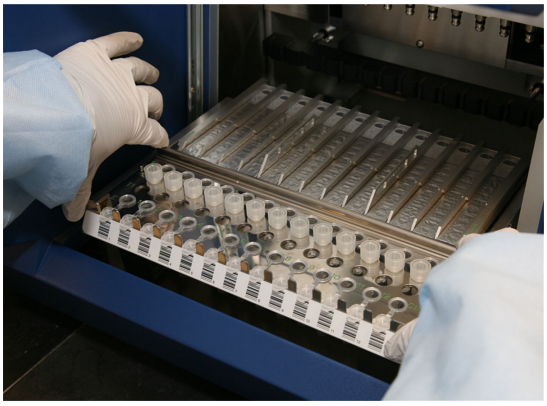
      Note: One tip and tip holder set is required per sample.

d. **Row S**—Load PrepSEQ™ Express Sample Tubes containing the lysate.



① Row E—PrepSEQ™ Express Elution Tubes
② Row T1—Empty row
③ Row T2—Tips and tip holders
④ Row S—PrepSEQ™ Express Sample Tubes

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



Start an automated extraction run

1. Be sure that you have loaded and inserted the cartridge rack and tip and tube rack correctly, then close the instrument door.
2. Press , then press **1** to select the **PS Express 123** option.
3. Select:
   - **30 min** for **Lysis Time**
   - **100 µL** for **Elution volume**
4. 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*.

   Note: If you lose power or the power cord is unplugged, the run stops. When the power resumes, the digital display shows the **Main** menu. You cannot resume the run. If the tips are still on the syringe unit when the power resumes, return the tips to the original positions as described in the *AutoMate Express™ Instrument User Guide*.

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".

1. Open the instrument door, then remove and cap the Elution Tubes containing the purified DNA.
2. Store the purified DNA at 4°C for same-day use, or at –20°C for longer storage.
3. Run or skip the **WastePooler** protocol.

   Note: The **WastePooler** protocol separates the waste reagents containing guanidine thiocyanate from the alcohol-based waste reagents for easier waste disposal.

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.

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

5. 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.

6. Close the instrument door.

7. 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).

   Note: No cooling period is required between runs.

Troubleshooting

Troubleshooting

Review the information below to troubleshoot your experiments using the PrepSEQ™ Express Nucleic Acid Extraction Kit.

To troubleshoot operation of the AutoMate *Express*™ Instrument, see the *AutoMate Express™ Instrument User Guide*.

Observation	Possible cause	Action
Before loading the cartridges in the cartridge rack...		
PrepSEQ™ Express Cartridges contain precipitate in some compartments.	Cartridges were exposed to low temperatures during the shipping or storage.	To dissolve precipitate that may have formed during shipping or storage, incubate the PrepSEQ™ Express Cartridges in a 37°C incubator for 30 minutes or until precipitate is no longer visible. Incubate only the cartridges that you will use that day.
During the automated extraction run...		
The AutoMate *Express*™ Instrument tip filters become wet.	Loose cell pellet or partial dispersion of cell pellet, resulting in partial or full clogging of the pipette tip.	• Centrifuge the samples immediately before you load the Sample Tubes in the instrument. Do not disturb the cell pellet when you move the samples from the centrifuge to the instrument. Start the run immediately after you place the samples in the instrument. • Clean the instrument and/or nozzles according to the *AutoMate Express™ Instrument User Guide*.
	The cartridge was placed into the cartridge rack incorrectly.	Before each run, confirm that cartridges are loaded as shown in step 5.
	Pipette tips are hitting the bottom of the cartridge wells.	• If cartridges were loaded correctly, the instrument may require calibration in the z direction. Contact Technical Support. • Use only the supplied consumables (plastics).
During a run: There is not any liquid in the tip, or the liquid in the tip is not moving. After a run: There is no elution volume.	There is no sample in the tube or tips are cogged, resulting in a wet filter barrier on the tip and blocked nozzles.	Add samples to tubes, load new reagent cartridges, then perform the run again.

Observation	Possible cause	Action
During a run: There is not any liquid in the tip, or the liquid in the tip is not moving. After a run: There is no elution volume.	The sample volume is lower than the recommended volume or tips are clogged, resulting in a wet filter barrier on the tip and blocked nozzles.	In future runs, use the recommended sample volume for the protocol you are using. Long-term operation with lower-than-recommended sample volumes can lead to issues with liquid handling performance.
After quantifying extracted nucleic acid...		
No or low yield of nucleic acid	The biological sample contains no or a low amount of nucleic acid.	Centrifuge samples before performing the automated extraction run.
	Missed the sample centrifugation step before starting the automated extraction run. This can result in carry-over of cells, which may decrease the nucleic acid yield.	
	The nucleic acid eluate contains PCR inhibitors due to excessive amount of inhibitors in the sample.	Run inhibition or internal positive controls according to the detection assay protocol.
	Poor quality of starting material.	Make sure to process the sample immediately after collection or store the sample at the appropriate temperature. The yield and quality of nucleic acid isolated depends on the starting material.
	Insufficient amount of Magnetic Particles added.	During shipping, some Magnetic Particles solution may adhere to the sealing foil of the cartridge. Resuspend the Magnetic Particles as described in "Guidelines for working with PrepSEQ™ Express Cartridges" on page 9.
	Clogged tips resulting in nucleic acid loss.	Ensure that the sample has been centrifuged at 1,000 × *g* for 3 minutes so that the cells are pelleted at the bottom of the Sample Tube. If needed, re-centrifuge the sample before loading the samples in the tip and tube rack.
There is evidence of failure to correctly aspirate and pipette reagents. For example, spent reagents remain in tubes 1–10 after you run the **WastePooler** protocol.	The cartridge was placed into the cartridge rack incorrectly.	Before each run, confirm that the cartridges are loaded as shown in step 5. If the cartridges were loaded correctly, the instrument may require calibration in the z direction. Contact Technical Support.
	Pipette tips are hitting the bottom of the cartridge wells.	

Observation	Possible cause	Action
PCR inhibition (Figure 4) or high background signal (Figure 5).	Excess mammalian cell DNA in the sample.	For some high-density samples, both RNase and DNase treatments are needed. In this case, perform the DNase treatment before the RNase treatment. See "Treat with RNase and DNase" on page 13.
		Contact your local Field Applications Specialist or Sales Representative.

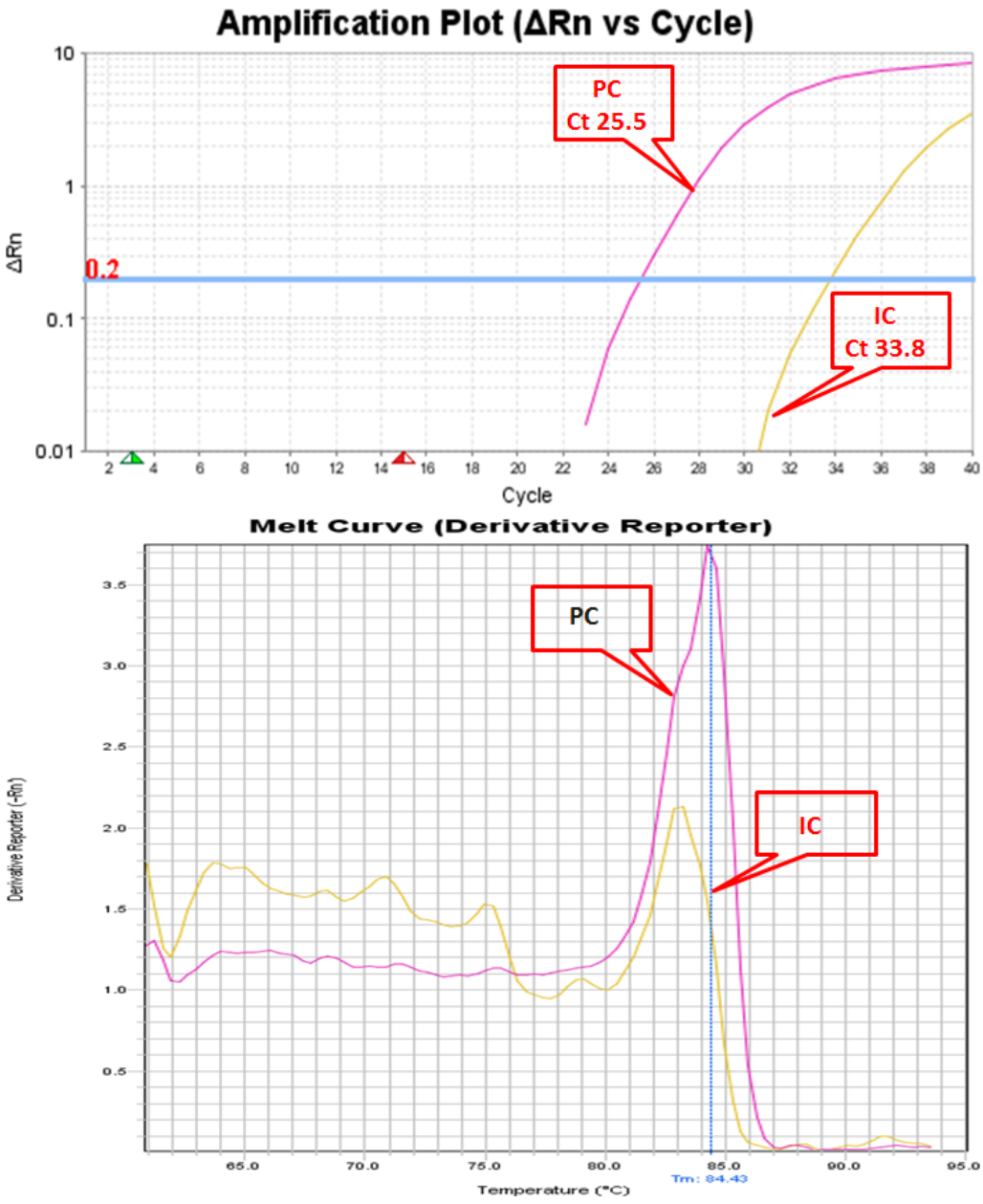


Figure 4 PCR inhibition; $\Delta C_T > 2$

Figure 5 High background signal

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication number
AutoMate Express™ Instrument User Guide	4441982
PrepSEQ™ Express Nucleic Acid Extraction Kit for Mycoplasma, MMV, and Vesivirus Detection Quick Reference	MAN0017291

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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