USER GUIDE

applied biosystems
life technologies

PrepSEQ® Sample Preparation Kits

PrepSEQ® 1-2-3 Nucleic Acid Extraction Kit

PrepSEQ® 1-2-3 *Mycoplasma* Nucleic Acid Extraction Kit

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Contents

About This Guide

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

Revision history

Revision	Date	Description
B	March 2015	Update storage temperature for the Magnetic Particles. Remove Cat. no. 4460627.

Purpose

This guide provides protocols for cell lysis and nucleic acid extraction for *Mycoplasma* cells, Mouse Minute Virus (MMV), or Vesivirus, for use with the following PrepSEQ® Sample Preparation Kits:

- PrepSEQ® 1-2-3 Nucleic Acid Extraction Kit
- PrepSEQ® 1-2-3 *Mycoplasma* Nucleic Acid Extraction Kit

User attention words

Five user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation or accurate chemistry kit use.

CAUTION! Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

PrepSEQ® Sample Preparation Kits

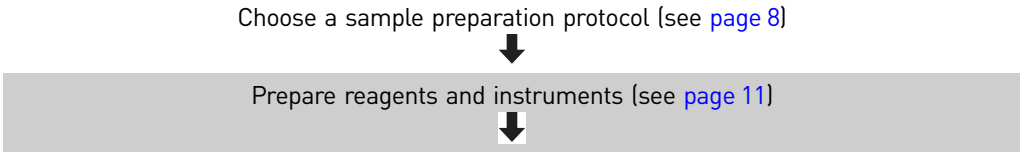
Product description

The PrepSEQ® Sample Preparation Kits use Magnetic Particle-based separation technology to extract DNA and/or RNA from *Mycoplasma* cells, Mouse Minute Virus (MMV), or Vesivirus isolated from a variety of starting material, such as infected cell cultures or *Mycoplasma* liquid cultures.

For information on kit contents and other required materials, see Appendix A, "Required Materials" on page 23.

Workflow

Using one of the PrepSEQ® Sample Preparation Kits, you prepare sample lysate, then extract target nucleic acids. The workflow for the kits is shown below:



After sample preparation, perform the appropriate PCR assay using the *Mycoplasma*, Myco Scan, MMV, or Vesivirus PCR detection kit protocol (see "Documentation and Support" on page 31 for a list of documents).

Choose a sample preparation protocol

There are three sample lysis protocols for use with the PrepSEQ® Sample Preparation Kits. Use Table 1 to select the appropriate protocol and kit based on your sample type and detection task.

Table 1 Select a sample preparation protocol

Protocol	Purpose	For use with kit
PrepSEQ® 1-2-3 protocol for Mycoplasma and/or MMV detection	To process 100 µL (up to 10^6 cells) of sample volume for detection of *Mycoplasma* and/or MMV†	PrepSEQ® 1-2-3 Nucleic Acid Extraction Kit
PrepSEQ® large-scale protocol for Mycoplasma detection	To process up to 50 mL (up to 2×10^8 cells) of sample volume for detection of *Mycoplasma*	PrepSEQ® 1-2-3 *Mycoplasma* Nucleic Acid Extraction Kit
PrepSEQ® 3-in-1 protocol for Mycoplasma, MMV, and Vesivirus detection	To process 100 µL (up to 10^6 cells) of sample volume for detection of *Mycoplasma*, MMV, and Vesivirus†	PrepSEQ® 1-2-3 Nucleic Acid Extraction Kit

† For samples with greater than 10^6 total cells: Centrifuge the sample at 500 × *g* for 2 minutes, then use 100 µL of the supernatant.

Extraction positive control guidelines

The MycoSEQ™ Discriminatory Positive/Extraction Control provided with the MycoSEQ® *Mycoplasma* Detection Kits is a multi-purpose control that can be used as an extraction positive control.

We recommend that you prepare and analyze separate reactions for test samples and extraction positive controls, because the presence of the control DNA in a test sample may affect the detection assay sensitivity for low levels of *Mycoplasma* DNA.

- Extract and analyze one sample replicate with no control added (this is the test sample).
- Extract and analyze one sample replicate spiked with the Discriminatory Positive/Extraction Control. The amount to spike should be appropriate to your application. The recommended range is 100–1000 copies per sample.

  If you use:
  - The 1-2-3 protocol, spike the sample before you perform step 1 of "Prepare sample lysate" on page 12.
  - The large-scale protocol, spike the sample lysate before you perform step 1 of "Bind DNA" on page 18.
  - The 3-in-1 protocol, spike the sample before you perform step 1 of "Prepare sample lysate" on page 20.

Sample preparation guidelines

Before you begin the protocol, review the following sample preparation and handling 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.
- **Cell culture media sampling** – Avoid taking viscous material from the culture into the sample preparation reaction. This material is very likely chromosomal DNA released as a result of cell lysis.
- In the large-scale protocol, while processing the mammalian cell pellet, keep the cell pellet 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 protocol, 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 supernatant, avoid touching the pellet, which contains nuclei and viscous material that may be generated from lysis of nuclei. If necessary, use a P200 pipette to perform the transfer.
  - In the final transfer of supernatant, avoid contact with or transfer of the viscous material. If necessary, recentrifuge the tube at 1000 × *g* for 3 minutes at 4°C, then very carefully transfer 300 µL with a P200 pipette.

Guidelines for working with Magnetic Particles

- Incubate the Magnetic Particle suspension at 37°C for a minimum of 10 minutes with intermittent vortexing at setting #7, or until the particles are completely suspended. White precipitate occasionally forms in the Magnetic Particles tube after storage at 2–8°C, due to precipitation of a salt used in bead formulation. Extraction experiments show that precipitate formation does not affect performance. However, the precipitate may cause the beads to become resistant to resuspension and difficult to pipet.
- When you place tubes into the Magnetic Stand, always orient the Magnetic Particles pellet toward the magnet.
- Except where noted, the Magnetic Particles capture of the DNA is complete after ~1 minute in the Magnetic Stand.

- When separating the liquid phase or eluate from the Magnetic Particles, do not disturb the Magnetic Particles. Magnetic particles can inhibit PCR.
- During wash steps, it is not necessary to detach the Magnetic Particles from the tube wall. Particle adherence to the tube wall does not affect DNA recovery. Although some test samples cause the beads to adhere very firmly to the tube wall and to other samples, the particles form loose aggregates that readily detach during the vortex steps. The particles disperse into a slurry during heating and vortexing in the elution step of the protocol.

Guidelines for working with Wash Buffer

Follow the instructions for air-drying the Magnetic Particles at the end of the wash step to remove any remaining ethanol, which is contained in the Wash Solution. Ethanol decreases DNA recovery and causes PCR inhibition. Do not dry the particles for longer than the recommended time.

Guidelines for 1-2-3 and 3-in-1 protocols

For samples with greater than 10^6 cells, centrifuge at 500 × *g* for 2 minutes, then use 100 µL of the supernatant to avoid a tight aggregate of Magnetic Particles and total nucleic acid from cells, which reduces recovery rate.

Prepare reagents and instruments

Before beginning the sample preparation protocol:

1. Review "Required Materials" on page 23 to confirm that you have all kit components and other required materials.
2. Review "Sample preparation guidelines" on page 9.
3. Prepare the following reagents before their first-time use:
   - **Binding Solution** – Add 30 mL of 100% isopropanol to the empty Binding Solution bottle. Mark the bottle label to indicate that isopropanol has been added.
   - **Wash Buffer** – Add 74 mL of 95% ethanol to the Wash Buffer Concentrate bottle, mix well, then mark the bottle label to indicate that ethanol has been added.
4. Incubate the Magnetic Particle suspension at 37°C for a minimum of 10 minutes with intermittent vortexing at setting #7, or until the particles are completely suspended.

   Note: During extraction, when you place tubes into the Magnetic Stand, always orient the Magnetic Particles pellet toward the magnet.
5. If you are performing the large-scale protocol:
   - Place aliquots of 1X PBS on ice. You need 300 µL of PBS per sample. When not in use, store 1X PBS at 2–8°C.
   - Power on the refrigerated centrifuge to allow it to cool down before use.
6. Power on the heat blocks. We recommend two heat blocks, one set to each temperature:
   - **1-2-3 protocol or large-scale protocol** – Heat block settings for required incubation: 37°C, 56°C, and 70°C.
   - **3-in-1 protocol** – Heat block settings for required incubation: 37°C and 45°C.

PrepSEQ® 1-2-3 protocol for *Mycoplasma* and/or MMV detection

Use this protocol to process 100 µL (up to 10^6 cells) of sample volume for detection of *Mycoplasma* and/or MMV.

Prepare samples

Place the following in a new safe-lock 2-mL microcentrifuge tube:

- **For samples with up to 10^6 total cells** – Use 100 µL of sample
- **For samples with greater than 10^6 total cells** – Centrifuge the sample at 500 × *g* for 2 minutes, then use 100 µL of the supernatant.

Prepare sample lysate

For each sample tube:

1. Add 200 µL of Lysis Buffer, then vortex for ~5 seconds to mix.
2. Add:
   - 2 µL of 0.5 M EDTA
   - 18 µL of RNase Cocktail

   Briefly vortex to mix.
3. Incubate at 56°C for 15 minutes.
4. Add 2 µL of Proteinase K, then briefly vortex to mix.
5. Incubate at 56°C for 10 minutes.
6. Incubate at room temperature for 5 minutes.
7. Add 700 µL of Lysis Solution. Vortex for ~5 seconds to mix.

Bind DNA

For each tube of sample lysate:

1. Add 30 µL of Magnetic Particles, then vortex.
2. Add 525 µL of Binding Solution, then invert the tube to mix.
3. Using a vortex adaptor, vortex the tube vertically at medium speed for 5 minutes to capture the nucleic acid.
4. Centrifuge in a microcentrifuge by pressing the short spin button for 15 seconds, then releasing the button. During this time, the microcentrifuge should reach top speed.
5. Place in the Magnetic Stand for 5 minutes.
6. Aspirate the supernatant without disturbing the Magnetic Particles, then discard the supernatant.

Wash DNA

For each tube of Magnetic Particles pellet (bound DNA):

1. Add 300 µL of Wash Buffer.
2. Vortex for ~5 seconds.

3. Centrifuge in a microcentrifuge by pressing the short spin button for 15 seconds, then releasing the button. During this time, the microcentrifuge should reach top speed.
4. Place in the Magnetic Stand for 1 minute.
5. Aspirate the supernatant without disturbing the Magnetic Particles, then discard the supernatant.
6. Repeat steps 1 through 5.
7. Use a P200 pipette to aspirate the residual supernatant from the bottom of the tube, then discard the supernatant.
8. With the lid open, air-dry the Magnetic Particles pellet at room temperature for 5 minutes to remove any remaining ethanol.

Elute DNA

For each sample:

1. Add 100 µL of Elution Buffer.
2. Vortex for ~10 seconds.
3. Incubate at 70°C for 7 minutes. Vortex 2 to 3 times during incubation to ensure complete resuspension of the Magnetic Particles.
4. Centrifuge at top speed for 5 minutes.
5. Place in the Magnetic Stand for 3 minutes.
6. Transfer the eluate to a non-stick 1.5-mL microcentrifuge tube.

Next steps

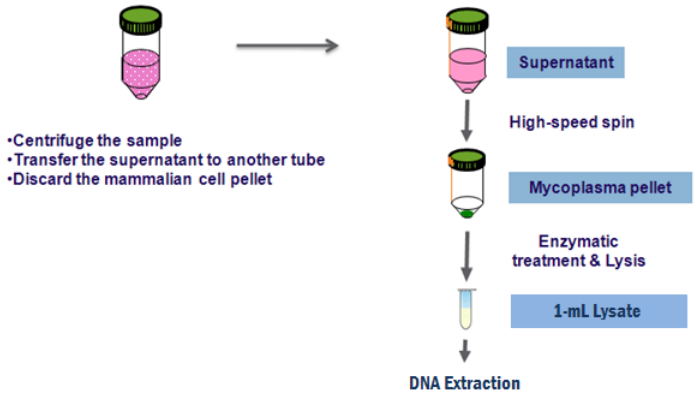
The extracted DNA is now ready for use in the appropriate PCR assay. If not used immediately, it can be stored at –20°C.

PrepSEQ® large-scale protocol for *Mycoplasma* detection

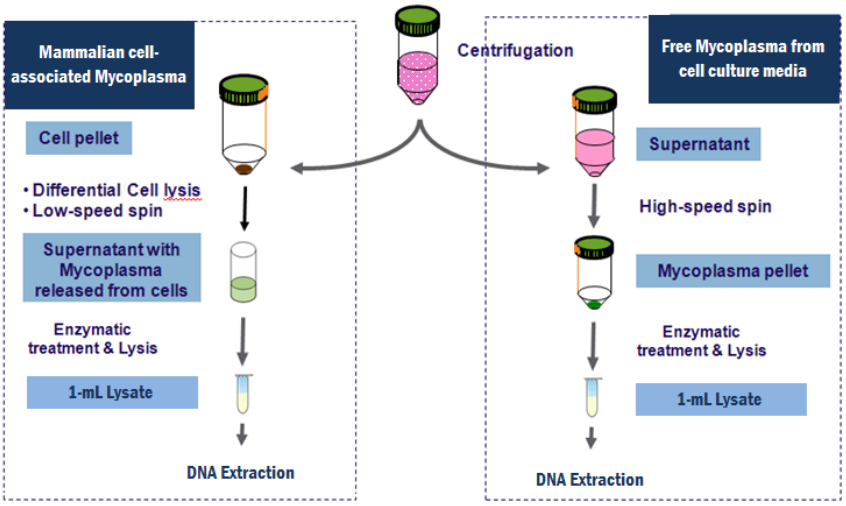
Use this protocol to process up to 10 mL (up to 2×10^8 cells) of sample volume for detection of *Mycoplasma*.

1. Prepare the sample lysate using one of the three options shown below, following the appropriate procedure on pages 15 through 17.
2. Extract the DNA (see procedure on 18).

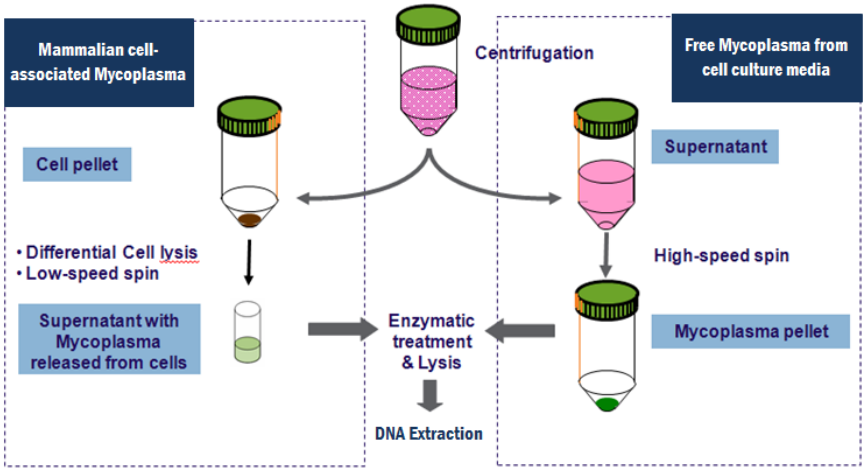
Option 1: Process cell culture media only (see page 15)



Option 2: Process cell culture media and mammalian cells separately (see page 16)



Option 3: Process cell culture media and mammalian cells pooled together (see page 17)



Option 1: Process cell culture media only

Separate mammalian cells from cell culture media

1. Place the cell culture sample (up to ~2 × 10^8 total cells) in a conical tube.
2. Centrifuge the tube at 1000 × *g* for 5 minutes to pellet the mammalian cells.
3. Transfer the supernatant to a new conical tube, and keep on ice. The supernatant contains free *Mycoplasma*.
4. Discard the mammalian cell pellet.

Process the supernatant (cell culture media)

1. Centrifuge the conical tube with the supernatant at 16,000 × *g* for 30 minutes to pellet the *Mycoplasma*.
2. Aspirate and discard the supernatant without disturbing the *Mycoplasma* pellet.
3. Add 300 µL of PBS, then mix thoroughly by vortexing to resuspend the *Mycoplasma* pellet.
4. Transfer the resuspended pellet to a 2-mL microcentrifuge tube.

Treat samples with RNase Cocktail and Proteinase K

1. Add 2 µL of 0.5 M EDTA and 18 µL of RNase Cocktail, then briefly vortex the 2-mL tube to mix.
2. Incubate the tube at 56°C for 30 minutes to digest the cellular RNA. Vortex twice during incubation.
3. Add 5 µL of Proteinase K, then briefly vortex to mix.
4. Incubate at 56°C for 10 minutes.
5. Add 700 µL of Lysis Buffer, then vortex to mix well.

Proceed to "Extract the DNA (for Options 1, 2, and 3)" on page 18.

Option 2: Process cell culture media and mammalian cells separately

Separate mammalian cells from cell culture media

1. Place the cell culture sample (up to ~2 × 10^8 total cells) into a conical tube.
2. Centrifuge the tube at 1000 × *g* for 5 minutes to pellet the mammalian cells.
3. Transfer the supernatant to a new conical tube, and keep on ice. The supernatant contains free *Mycoplasma*.
4. Place the mammalian cell pellet on ice.

Process the supernatant (cell culture media)

1. Centrifuge the conical tube with the supernatant at 16,000 × *g* for 30 minutes to pellet *Mycoplasma*.
2. Aspirate and discard the supernatant without disturbing the *Mycoplasma* pellet.
3. Add 300 µL of PBS, then mix thoroughly by vortexing to resuspend the *Mycoplasma* pellet.
4. Transfer the resuspended pellet to a 2-mL microcentrifuge tube.
5. Keep the resuspended *Mycoplasma* pellet on ice while you process the mammalian cell pellet, then proceed to "Treat samples with RNase Cocktail and Proteinase K" on page 16.

Process the mammalian cell pellet

1. Add 550 µL of ice-cold Cell Fractionation Buffer to the mammalian cell pellet. Very gently pipet up and down several times with a P1000 pipette to completely resuspend the mammalian cells.
2. Transfer the mammalian cell suspension to a 2-mL microcentrifuge tube, then incubate on ice for 5 minutes.
3. Centrifuge the 2-mL tube at 1000 × *g* for 10 minutes at 4°C to pellet the cellular membranes and nuclei.
4. Without disturbing the viscous cellular material, use a P200 pipette to transfer 300 µL (two 150-µL aliquots) of the cell fractionation supernatant (mammalian cell lysate) to a new 2-mL microcentrifuge tube. Keep the tube on ice, then proceed to "Treat samples with RNase Cocktail and Proteinase K" on page 16.

Treat samples with RNase Cocktail and Proteinase K

Separately process the resuspended *Mycoplasma* (from the cell culture media) and the cell fractionation supernatant (from the mammalian cell pellet):

1. Add 2 µL of 0.5 M EDTA and 18 µL of RNase Cocktail, then briefly vortex the 2-mL tube to mix.
2. Incubate the tube at 56°C for 30 minutes to digest the cellular RNA. Vortex twice during incubation.
3. Add 5 µL of Proteinase K, then briefly vortex to mix.
4. Incubate at 56°C for 10 minutes.

5. Add 700 µL of Lysis Buffer, then vortex to mix well.

Proceed to "Extract the DNA (for Options 1, 2, and 3)" on page 18.

Option 3: Process cell culture media and mammalian cells pooled together

Separate mammalian cells from cell culture media

1. Place the cell culture sample (up to ~2 × 10^8 total cells) into a conical tube.
2. Centrifuge the tube at 1000 × *g* for 5 minutes to pellet the mammalian cells.
3. Transfer the supernatant to a new conical tube, and keep on ice. The supernatant contains free *Mycoplasma*.
4. Place the mammalian cell pellet on ice.

Process the supernatant (cell culture media)

1. Centrifuge the conical tube with the supernatant at 16,000 × *g* for 30 minutes to pellet *Mycoplasma*.
2. Aspirate and discard the supernatant without disturbing the *Mycoplasma* pellet.
3. Keep the *Mycoplasma* pellet on ice.

Process the mammalian cell pellet

1. Add 550 µL of ice-cold Cell Fractionation Buffer to the mammalian cell pellet. Very gently pipet up and down several times with a P1000 pipette to completely resuspend the mammalian cells.
2. Transfer the mammalian cell suspension to a 2-mL microcentrifuge tube, then incubate on ice for 5 minutes.
3. Centrifuge the 2-mL tube at 1000 × *g* for 10 minutes at 4°C to pellet the cellular membranes and nuclei.
4. Without disturbing the viscous cellular material, use a P200 pipette to transfer 300 µL (two 150-µL aliquots) of the cell fractionation supernatant (mammalian cell lysate) to the *Mycoplasma* pellet tube from step 3 in "Process the supernatant (cell culture media)" above. Pipet up and down to resuspend the *Mycoplasma* pellet.
5. Transfer to a new 2-mL microcentrifuge tube.

Treat samples with RNase Cocktail and Proteinase K

1. Add 2 µL of 0.5 M EDTA and 18 µL of RNase Cocktail, then briefly vortex the 2-mL tube to mix.
2. Incubate the tube at 56°C for 30 minutes to digest the cellular RNA. Vortex twice during incubation.
3. Add 5 µL of Proteinase K, then briefly vortex to mix.
4. Incubate at 56°C for 10 minutes.
5. Add 700 µL of Lysis Buffer, then vortex to mix well.

Proceed to "Extract the DNA (for Options 1, 2, and 3)" on page 18.

Extract the DNA (for Options 1, 2, and 3)

Bind DNA

For each tube of sample lysate:

1. Add 30 µL of Magnetic Particles, then vortex.
2. Add 525 µL of Binding Solution, then invert the tube to mix.
3. Using a vortex adaptor, vortex at medium speed for 5 minutes to capture the nucleic acid.
4. Centrifuge in a microcentrifuge by pressing the short spin button for 15 seconds, then releasing the button. During this time, the microcentrifuge should reach top speed.
5. Place in the Magnetic Stand for 5 minutes.
6. Aspirate the supernatant without disturbing the Magnetic Particles, then discard the supernatant.

Wash DNA

For each tube of Magnetic Particles pellet (bound DNA):

1. (*Optional*) For samples with PCR inhibitors:
   a. Add 300 µL of a 3:2 mixture of 95% ethanol and Lysis Buffer. Invert the tubes three times to mix. Do not incubate the beads in this wash solution for more than 3 minutes.
   b. Centrifuge in a microcentrifuge by pressing the short spin button for 15 seconds, then releasing the button. During this time, the microcentrifuge should reach top speed.
   c. Place the tubes in the Magnetic Stand for 1 minute, then aspirate and discard the liquid.
2. Add 300 µL of Wash Buffer.
3. Vortex for ~5 seconds.
4. Centrifuge in a microcentrifuge by pressing the short spin button for 15 seconds, then releasing the button. During this time, the microcentrifuge should reach top speed.
5. Place in the Magnetic Stand for 1 minute.
6. Aspirate the supernatant without disturbing the Magnetic Particles, then discard the supernatant.
7. Repeat steps 1 through 5.
8. Use a P200 pipette to aspirate the residual supernatant from the bottom of the tube, then discard the supernatant.
9. With the lid open, air-dry the Magnetic Particles pellet at room temperature for 5 minutes to remove any remaining ethanol.

Elute DNA

For each sample:

1. Add 100 µL of Elution Buffer.
2. Vortex for ~10 seconds.
3. Incubate at 70°C for 7 minutes. Vortex 2 to 3 times during incubation to ensure complete resuspension of the Magnetic Particles.
4. Centrifuge at top speed for 5 minutes.
5. Place in the Magnetic Stand for 3 minutes.
6. Transfer the eluate to a non-stick 1.5-mL microcentrifuge tube.

Next steps

The extracted DNA is now ready for use in the appropriate PCR assay. If not used immediately, it can be stored at -20°C.

PrepSEQ® 3-in-1 protocol for *Mycoplasma*, MMV, and Vesivirus detection

Prepare samples

Place the following in a new safe-lock 2-mL microcentrifuge tube:

- **For samples with up to 10^6 total cells** – Use 100 µL of sample
- **For samples with greater than 10^6 total cells** – Centrifuge the sample at 500 × *g* for 2 minutes, then use 100 µL of the supernatant.

Prepare sample lysate

For each sample tube:

1. Add 500 µL of Lysis Buffer, then vortex for ~15 seconds to mix.
2. Incubate at 45°C for 10 minutes.
3. Vortex ~10 seconds to mix.

Bind nucleic acid

For each sample lysate tube:

1. Add 30 µL of Magnetic Particles, then vortex.
2. Add 330 µL of Binding Solution, then invert the tube to mix.
3. Using a vortex adaptor, vortex at medium speed for 10 minutes to capture the nucleic acid.
4. Centrifuge in a microcentrifuge by pressing the short spin button for 15 seconds, then releasing the button. During this time, the microcentrifuge should reach top speed.
5. Place in the Magnetic Stand for 5 minutes.
6. Aspirate the supernatant without disturbing the Magnetic Particles, then discard the supernatant.

Wash nucleic acid

For each tube of Magnetic Particles pellet (bound DNA):

1. Add 300 µL of Wash Buffer.
2. Vortex for ~5 seconds.
3. Centrifuge in a microcentrifuge by pressing the short spin button for 15 seconds, then releasing the button. During this time, the microcentrifuge should reach top speed.
4. Place in the Magnetic Stand for 1 minute.
5. Aspirate the supernatant without disturbing the Magnetic Particles, then discard the supernatant.
6. Repeat steps 1 through 5.
7. Use a P200 pipette to aspirate the residual supernatant from the bottom of the tube, then discard the supernatant.
8. With the lid open, air-dry the Magnetic Particles pellet at room temperature for 5 minutes to remove any remaining ethanol.

Elute nucleic acid

For each sample:

1. Add 100 µL of Elution Buffer.
2. Vortex for ~10 seconds.
3. Incubate at 45°C for 5 minutes. Vortex 2 to 3 times during incubation to ensure complete resuspension of the Magnetic Particles.
4. Centrifuge at top speed for 5 minutes.
5. Place in the Magnetic Stand for 3 minutes.
6. Transfer the eluate to a non-stick 1.5-mL microcentrifuge tube.

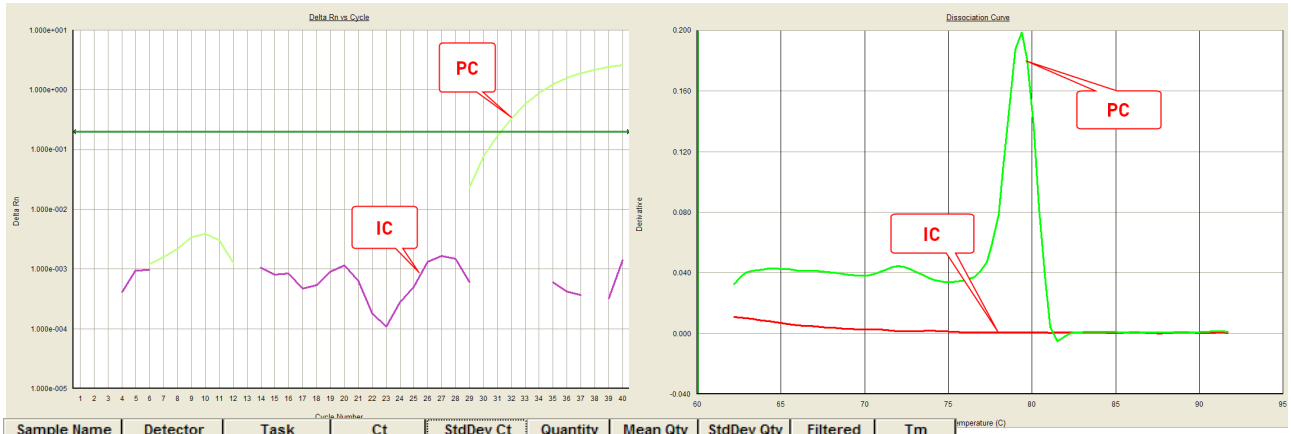
Next Steps

The extracted DNA is now ready for use in the appropriate PCR assay. If not used immediately, it can be stored at –20°C.

Troubleshooting

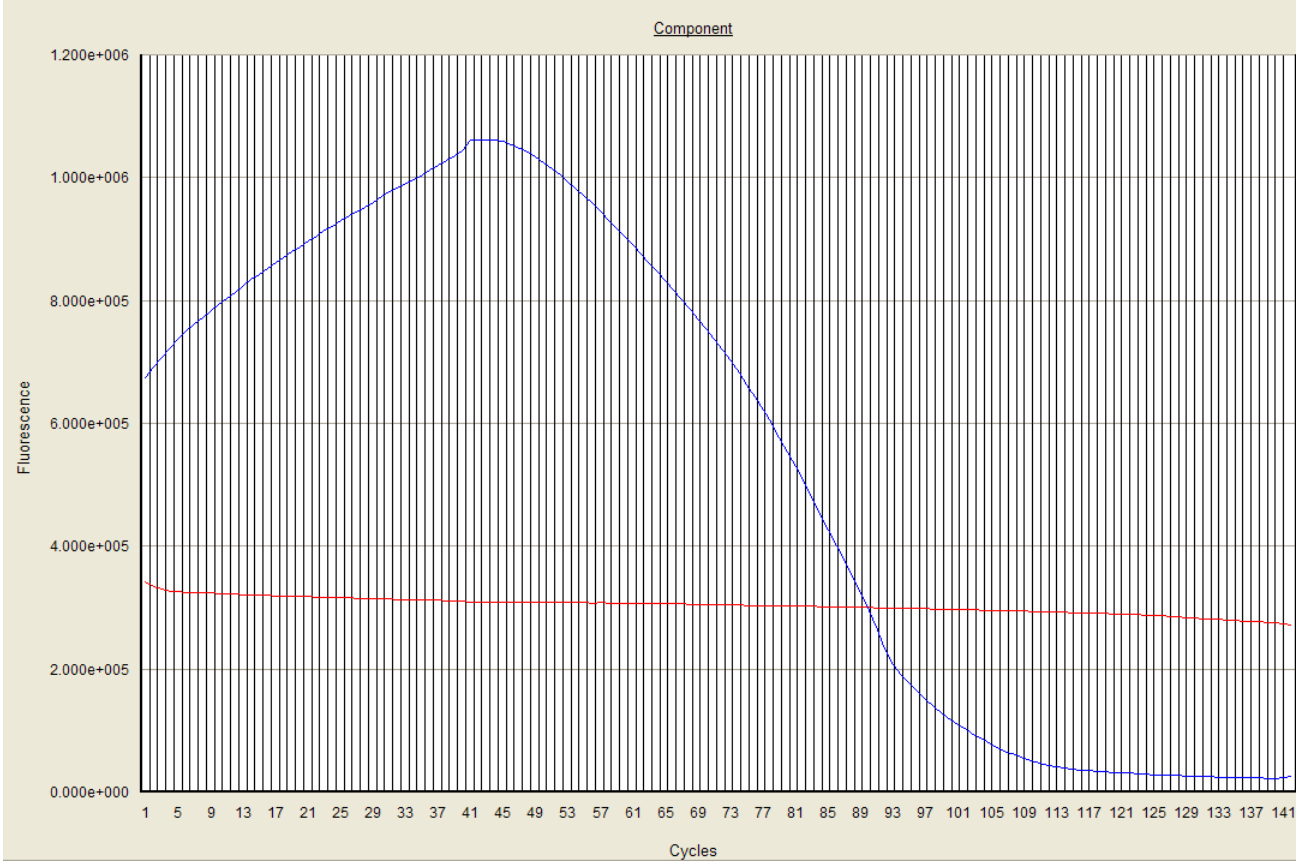
Observation	Possible cause	Recommended action
Poor extraction efficiency (low yields)	Overdrying the magnetic pellet after the final wash step.	Start the timer before removing the Wash Buffer from the first tube. Do not dry longer than 5 minutes. If you are using aspiration or a BSC, no additional drying time is needed.
	Magnetic particles are attached too tightly to the tube wall during the elution (step 1 on page 13 or step 1 on page 19 or step 1 on page 21).	Place the tube in the benchtop microcentrifuge with the Magnetic Particles pellet oriented toward the center. Spin the tube for 30 seconds to detach the Magnetic Particles into the Elution Buffer.
	Magnetic particles are difficult to resuspend during the elution (step 2 on page 13 or step 2 on page 19 or step 2 on page 21).	Incubate the pellets at 70°C for 7 minutes. Vortex the tubes three times during incubation to help resuspension.
PCR inhibition (Figure 1 on page 22) or high background signal (Figure 2 on page 22)	Excess mammalian cell DNA in the sample	Contact your local Field Applications Specialist or Sales Representative.

Figure 1 PCR inhibition; $\Delta C_T > 2$



Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm
3B	SYBR	Unknown	Undet.						65.4 °C
3B_IC	SYBR	Unknown	Undet.						65.4 °C
PC	SYBR	Unknown	31.176						79.4 °C

Figure 2 High background signal



Required Materials

PrepSEQ® 1-2-3 Nucleic Acid Extraction Kit contents and storage

Kit components may be shipped separately depending on the kit configuration and storage conditions. Use the list in Table 2 to confirm that you have received all components.

Table 2 PrepSEQ® 1-2-3 Nucleic Acid Extraction Kit (Cat. no. 4452222) contains reagents for 100 small-scale (100 µL) cell culture extractions

Box label	Component	Description	Catalog number†	Storage
RNase Cocktail	RNase Cocktail	One 1.0-mL tube	AM2286	–20°C
Box 1	Lysis Buffer	Two 50-mL bottles	4400659	Room temperature
	Binding Solution (Isopropanol)	One empty bottle	4400789	
	Wash Buffer Concentrate	Two 26-mL bottles	4400783	
	Elution Buffer	One 25-mL bottle	4400784	
	Proteinase K (PK) Buffer	One 50-mL bottle	4400787	
Box 2	Magnetic Particles	Two 1.5-mL tubes	4401405	Room temperature
Box 3	Proteinase K (20 mg/mL)	One 1.25-mL tube	4403958	–20°C

† These Catalog numbers are provided for identification purposes; the components cannot be ordered separately.

PrepSEQ® 1-2-3 *Mycoplasma* Nucleic Acid Extraction Kit contents and storage

The PrepSEQ® 1-2-3 *Mycoplasma* Nucleic Acid Extraction Kit can be ordered as a standalone kit, or as part of the MycoSEQ™ *Mycoplasma* Detection Kit:

- PrepSEQ® 1-2-3 *Mycoplasma* Nucleic Acid Extraction Kit (Cat. no. 4443789)
- MycoSEQ™ *Mycoplasma* Detection Kit, with Discriminatory Positive Control and sample preparation (Cat. no. 4460626)

Kit components may be shipped separately depending on the kit configuration and storage conditions. Use the list in Table 3 to confirm that you have received all components.

Table 3 The PrepSEQ® 1-2-3 *Mycoplasma* Nucleic Acid Extraction Kit (Cat. no. 4443789) contains reagents for 100 small-scale (100–2000 µL) or 100 large-scale (2–10 mL) cell culture extractions

Box label	Component	Description	Catalog number†	Storage
Cell Fractionation Buffer		Three 25-mL bottles	4405889	2–8°C
RNase Cocktail		Two 1.0-mL tubes	4405890	–20°C
Box 1	Lysis Buffer	Two 50-mL bottles	4400659	Room temperature
	Binding Solution (Isopropanol)	One empty bottle	4400789	
	Wash Buffer Concentrate	Two 26-mL bottles	4400783	
	Elution Buffer	One 25-mL bottle	4400784	
	Proteinase K (PK) Buffer	One 50-mL bottle	4400787	
Box 2	Magnetic Particles	Two 1.5-mL tubes	4401405	Room temperature
Box 3	Proteinase K (20 mg/mL)	One 1.25-mL tube	4403958	–20°C

† These Catalog numbers are provided for identification purposes; the components cannot be ordered separately.

Materials not included in the kit

Table 4 includes materials and equipment that are required to use the PrepSEQ® Sample Preparation Kits, but are not included in the kits. Unless otherwise indicated, items are available from major laboratory suppliers (MLS).

Table 4 Materials not included

Item	Source†
Equipment	
Three block heaters for use with 2-mL tubes: two set at 37°C, and one set at 56°C	MLS
Ice bucket	MLS
16-position Magnetic Stand	Life Technologies Cat. no. 4457858
Refrigerated benchtop microcentrifuge for 1.5- and 2-mL tubes, 2 to 8°C	MLS
Vortex-Genie 2T Mixer	VWR Scientific#14216-188 *or* VWR Scientific #14216-186
Vortex Adapter-60, for use with Vortex-Genie	Life Technologies Cat. no. AM10014
Ultracentrifuge, for use with 50-mL tubes	MLS

Item	Source†
Consumables	
Disposable gloves	MLS
Aerosol-resistant micropipette tips	MLS
Pipettes, P1000 and P200: • Positive-displacement • Air-displacement • Multichannel	MLS
Pipettes	MLS
Conical Tubes, 50 mL (250 tubes)	Life Technologies Cat. no. AM12502
Nonstick, RNase-free Microfuge Tubes, 1.5 mL (250 tubes)	Life Technologies Cat. no. AM12450
Safe-lock PCR clean microcentrifuge tubes, round-bottom, 2 mL (100 tubes)	VWR Scientific #62111-754
Reagents	
SDS, 10%	MLS
1X PBS **IMPORTANT!** Prepare fresh reagent before using the kit.	MLS
EDTA, 0.5 M	MLS
Ethanol, 95% **IMPORTANT!** Do not use denatured ethanol because it contains components that are not compatible with the protocol.	MLS
Isopropanol, 100%	MLS
DNase-free, sterile-filtered water	MLS

† For the SDS of any chemical not distributed by Thermo Fisher Scientific, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

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, and 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! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and Support

Related documentation

Real-time PCR system	Document	Pub. no.	Description
All real-time PCR systems†	*MycoSEQ™ Mycoplasma Detection Kits Quick Reference Card*	4393471	Provides brief, concise instructions on using the MycoSEQ™ *Mycoplasma* Real-Time PCR Detection Kit and MycoSEQ™ Myco Scan *Mycoplasma* Detection Kit.
	MycoSEQ™ Mycoplasma Detection Kits User Guide	4465874	Describes the MycoSEQ™ *Mycoplasma* Detection Kits and provides information on preparing, running, and troubleshooting *Mycoplasma* detection.
	ViralSEQ® Mouse Minute Virus Real-Time PCR Detection Kit Quick Reference Card	4445236	Provides brief, concise instructions on using the ViralSEQ® Mouse Minute Virus Real-Time PCR Detection Kit.
	ViralSEQ® Mouse Minute Virus Real-Time PCR Detection Kit Protocol	4445235	Describes the ViralSEQ® Mouse Minute Virus Real-Time PCR Detection Kit and provides information on preparing, running, and troubleshooting MMV detection.
	PrepSEQ® Sample Preparation Kits Quick Reference Card	4406304	Provides brief, concise instructions on using the PrepSEQ® Sample Preparation Kits.
	PrepSEQ® Sample Preparation Kits User Guide	4465957	Describes the PrepSEQ® Sample Preparation Kits and provides information on preparing, running, and troubleshooting sample preparation.
	PrepSEQ® Nucleic Acid Extraction Kit Quick Reference Card	406303	Provides brief, concise instructions on using the PrepSEQ® Nucleic Acid Extraction Kit.
	PrepSEQ® Nucleic Acid Extraction Kit Protocol	4400739	Describes the PrepSEQ® Nucleic Acid Extraction Kit and provides information on preparing, running, and troubleshooting nucleic acid extractions.

† The preferred platform is a 7500 Fast Real-Time PCR System with the AccuSEQ™ Real-Time PCR Detection Software. However, the assay can be run on any real-time PCR instrument calibrated for the SYBR® Green dye.

Portable document format (PDF) versions of this guide and the documents listed above are available at **www.lifetechnologies.com**.

Note: To open the user documentation available from the Life Technologies web site, use the Adobe® Acrobat® Reader® software available from **www.adobe.com**.

For information on new assays and updated product documentation, contact your local Field Applications Specialist or Sales Representative.

Customer and technical support

Visit **www.lifetechnologies.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - 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

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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