

USER GUIDE

applied
biosystems®
by *life* technologies™

PrepSEQ® Sample Preparation Kits

PrepSEQ® *Mycoplasma* Nucleic Acid Extraction Kit

PrepSEQ® 1-2-3 Nucleic Acid Extraction Kit

Publication Part Number 4465957 Rev. A

Revision Date August 2011

life
technologies™

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

Information in this document is subject to change without notice.

APPLIED BIOSYSTEMS DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. TO THE FULLEST EXTENT ALLOWED BY LAW, IN NO EVENT SHALL APPLIED BIOSYSTEMS BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT APPLIED BIOSYSTEMS IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

LIMITED USE LABEL LICENSE

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product (a) to perform internal research for the sole benefit of the purchaser; and (b) for environmental testing, quality control/quality assurance testing, food and agricultural testing, including reporting results of purchaser's activities in environmental testing, quality control/quality assurance testing, food and agricultural testing for a fee or other commercial consideration. No other right is hereby granted expressly, by implication, or by estoppel. This product is for environmental testing, quality control/ quality assurance testing, food and agricultural testing and research purposes only.

The purchase of this product does not grant the purchaser any additional rights, including (without limitation) the right to transfer or resell the product in any form, the right to use the product as a therapeutic agent or diagnostics test component, or to use the product to perform other tests on a contract or fee per test basis for or on behalf of third parties. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

End Users are specifically not authorized to and are forbidden from reselling, transferring or distributing any products either as a stand alone product or as a component of another product. This product or portions thereof is manufactured and sold under license from GE Healthcare.

End User Terms and Conditions

Acceptance. These terms and conditions shall govern the purchase, use, transfer and acceptance of the products described in the purchase order, quotation or invoice, which products are sold and distributed by Life Technologies Corporation to the buyer/transferee of such products (the "End User"). The transfer/sale of products to the End User is expressly conditional upon End User's acceptance of these terms and conditions.

Restrictions on Use. End Users are specifically not authorized to and are forbidden from reselling, transferring or distributing any products either as a stand alone product or as a component of another product. The right to use the products does not, in and of itself, include or carry any right of the End User to any GE Healthcare Bio-Sciences Corp.'s technology or intellectual property other than expressly provided herein. End Users may not attempt to reverse engineer parameters of any of GE Healthcare Bio-Sciences Corp. proprietary products or services.

DISCLAIMER OF WARRANTIES. GE HEALTHCARE BIO-SCIENCES CORP. PROVIDES NO WARRANTIES TO END USER (STATUTORY OR IMPLIED), INCLUDING WITHOUT LIMITATION, AS TO PRODUCT QUALITY, CONDITION, DESCRIPTION, MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, AND ALL SUCH WARRANTIES ARE HEREBY EXPRESSLY DISCLAIMED. GE HEALTHCARE BIO-SCIENCES CORP. HEREBY EXPRESSLY DISCLAIMS ANY WARRANTY REGARDING RESULTS OBTAINED THROUGH THE USE OF THE PRODUCTS, INCLUDING WITHOUT LIMITATION ANY CLAIM OF INACCURATE, INVALID OR INCOMPLETE RESULTS.

Exclusion of Liability. GE Healthcare Bio-Sciences Corp. and its affiliates shall have no liability to an End User, including, without limitation, for any loss of use or profits, business interruption or any consequential, incidental, special or other indirect damages of any kind, regardless of how caused and regardless of whether an action in contract, tort, strict product liability or otherwise.

TRADEMARKS

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

© 2011 Life Technologies Corporation. All rights reserved.

Publication Part Number 4465957 Rev. A

Revision Date August 2011

Contents

	About This Guide	5
	Purpose	5
	User attention words	5
PROTOCOL	PrepSEQ® Sample Preparation Kits	7
	Product description	7
	Workflow	7
	Choose a sample preparation protocol	8
	Guidelines for extraction positive controls	8
	Prepare reagents and instruments	9
	PrepSEQ® 1-2-3 protocol for <i>Mycoplasma</i> or MMV detection	10
	PrepSEQ® large-scale protocol for <i>Mycoplasma</i> detection	12
	PrepSEQ® 3-in-1 protocol for <i>Mycoplasma</i> , MMV, and Vesivirus detection	18
	Sample preparation guidelines	20
	Troubleshooting	21
APPENDIX A	Background and Ordering Information	23
	Product overview	23
	Required materials	23
APPENDIX B	Safety	27
	Chemical safety	28
	Biological hazard safety	28
	Documentation and Support	31
	Related documentation	31
	Obtaining SDSs	32
	Obtaining support	32
	Index	33

About This Guide

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

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® *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, “Background and Ordering Information”](#) 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 PrepSEQ[®] Sample Preparation Kits is shown below:

Choose a sample preparation protocol (see [page 8](#))



Prepare reagents and instruments (see [page 9](#))



Prepare samples using one of the following methods:

- PrepSEQ[®] 1-2-3 protocol for Mycoplasma or MMV detection (see [page 10](#))
- PrepSEQ[®] large-scale protocol for Mycoplasma detection (see [page 12](#))
- PrepSEQ[®] 3-in-1 protocol for Mycoplasma, MMV, and Vesivirus detection (see [page 18](#))

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 protocols).

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 or MMV detection	To process 100 µL (up to 10 ⁶ cells) sample volume for detection of <i>Mycoplasma</i> and/or MMV [†]	PrepSEQ® 1-2-3 Nucleic Acid Extraction Kit
PrepSEQ® large-scale protocol for Mycoplasma detection	To process sample volume to up to 50 mL (up to 2 × 10 ⁸ cells) for detection of <i>Mycoplasma</i>	PrepSEQ® <i>Mycoplasma</i> Nucleic Acid Extraction Kit
PrepSEQ® 3-in-1 protocol for Mycoplasma, MMV, and Vesivirus detection	To process 100 µL (up to 10 ⁶ cells) sample volume for detection of <i>Mycoplasma</i> , MMV, and Vesivirus [†]	PrepSEQ® 1-2-3 Nucleic Acid Extraction Kit

† For samples with greater than 10⁶ total cells: Centrifuge the sample at 500 × g for 2 minutes, then use 100 µL of the supernatant.

Guidelines for extraction positive controls

The MycoSEQ™ Discriminatory Positive/Extraction Control provided with the MycoSEQ® *Mycoplasma* Detection Kits is a multi-purpose control which 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 10.
- The large-scale protocol, spike the sample lysate before you perform step 1 of “[Bind DNA](#)” on page 16.
- The 3-in-1 protocol, spike the sample before you perform step 1 of “[Prepare sample lysate](#)” on page 18.

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 20.
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 Particles at 37°C for 10 minutes, then vortex the Magnetic Particles at medium speed until the particles are completely resuspended.
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 1× PBS on ice. You need 300 µL PBS per sample. When not in use, store 1× 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* or MMV detection

Use this protocol to process 100 µL (up to 10⁶ cells) sample volume for detection of *Mycoplasma* and/or MMV.

Prepare samples

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

- **For samples with up to 10⁶ total cells** – Use 100 µL of sample
- **For samples with greater than 10⁶ 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 approximately 5 seconds to mix.
2. Add:
 - 2 µL of 0.5M EDTA
 - 18 µL RNase CocktailBriefly 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 approximately 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. Vortex at medium speed for 5 minutes, using a vortex adaptor, 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 approximately 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 one 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 approximately 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 three minutes.
6. Transfer the eluate to a non-stick 1.5-mL microcentrifuge tube.

Next steps

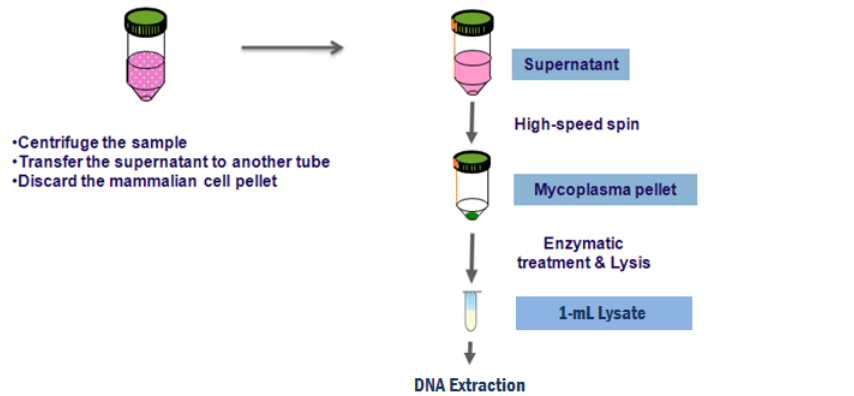
The extracted DNA is now ready for use in the appropriate PCR assay, or it may be stored at -20°C if not used immediately.

PrepSEQ® large-scale protocol for *Mycoplasma* detection

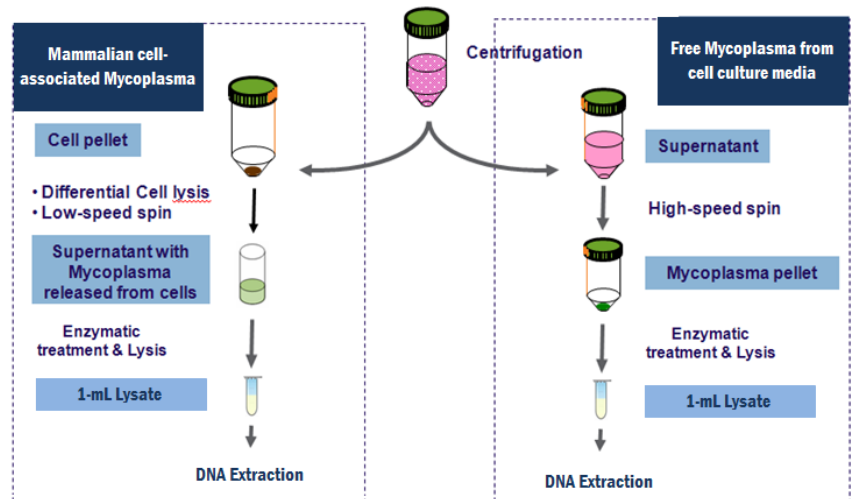
Use this protocol to process sample volumes to up to 10 mL (up to 2×10^8 cells) for detection of *Mycoplasma*:

1. Prepare sample lysate using one of the three options (shown below, with procedures on pages 13 through 15).
2. Extract the DNA (see procedure on 16).

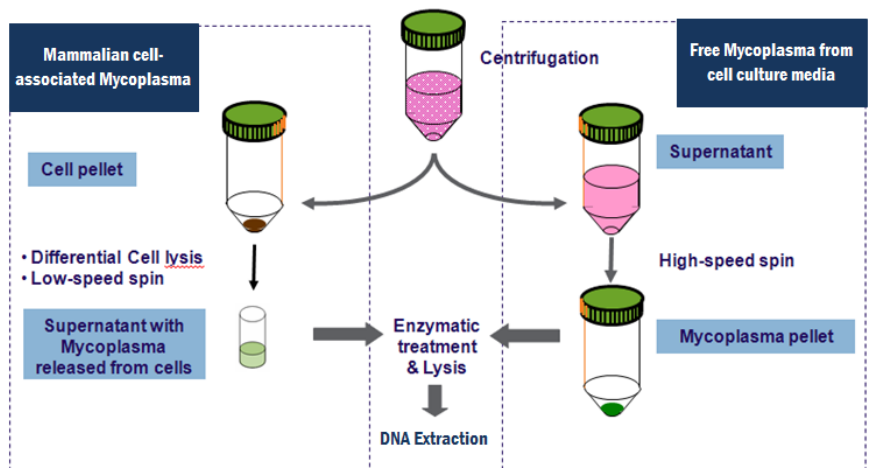
Option 1: Process cell culture media only (see page 13)



Option 2: Process cell culture media and mammalian cells separately (see page 14)



Option 3: Process cell culture media and mammalian cells pooled together (see page 15)



Option 1: Process cell culture media only

Separate mammalian cells from cell culture media

1. Place the cell culture sample (up to $\sim 2 \times 10^8$ total cells) in a conical tube.
2. Centrifuge the tube at $1,000 \times g$ 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 $16,000 \times g$ 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 16.

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 $\sim 2 \times 10^8$ total cells) into a conical tube.
2. Centrifuge the tube at $1,000 \times g$ 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 $16,000 \times g$ 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 14.](#)

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 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 $1000 \times g$ for 10 minutes at 4°C to pellet the cellular membranes and nuclei.
4. 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, without disturbing the viscous cellular material. Keep the tube on ice, then proceed to [“Treat samples with RNase cocktail and Proteinase K” on page 14.](#)

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) as follows:

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 16.

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 $\sim 2 \times 10^8$ total cells) into a conical tube.
2. Centrifuge the tube at $1,000 \times g$ 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 $16,000 \times g$ 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 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 $1000 \times g$ for 10 minutes at 4°C to pellet the cellular membranes and nuclei.
4. 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 without disturbing the viscous cellular material, then pipette 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 16.

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. Vortex at medium speed for 5 minutes, using a vortex adaptor, 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, then aspirate and discard the liquid.
2. Add 300 μL of Wash Buffer.
3. Vortex for approximately 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 one 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 approximately 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 three 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, or it may be stored at -20°C if not used immediately.

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

- Prepare samples** Place the following in a new lock-safe 2-mL microcentrifuge tube:
- **For samples with up to 10⁶ total cells** – Use 100 µL of sample
 - **For samples with greater than 10⁶ 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 approximately 15 seconds to mix.
 2. Incubate at 45°C for 10 minutes.
 3. Vortex approximately 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. Vortex at medium speed for 10 minutes, using a vortex adaptor, 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 approximately 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 one 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 approximately 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 three 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, or it may be stored at –20°C if not used immediately.

Sample preparation guidelines

Before you begin, 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 in turn 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 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.

Guidelines for working with magnetic particles

- Always incubate the tube containing the magnetic particles at 37°C for 10 minutes, vortexing intermittently, before use. White precipitate may form in the magnetic particles tube after prolonged storage at 4°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 approximately 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 washing 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 the heating and vortexing during 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 $>10^6$ cells, centrifuge at $500 \times g$ 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.

Troubleshooting

Observation	Possible cause	Recommended action
Poor extraction efficiency (low yields)	Ethanol in the Wash Solution (step 8 on page 11 or step 9 on page 16 or step 8 on page 18).	Thoroughly air-dry the magnetic particles pellet in the magnetic stand for 5 minutes at room temperature.
	Magnetic particles are attached too tightly to the tube wall during the Elution (step 1 on page 11 or step 1 on page 17 or step 1 on page 19).	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 11 or step 2 on page 17 or step 2 on page 19).	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 21) 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; $\Delta C_T > 2$

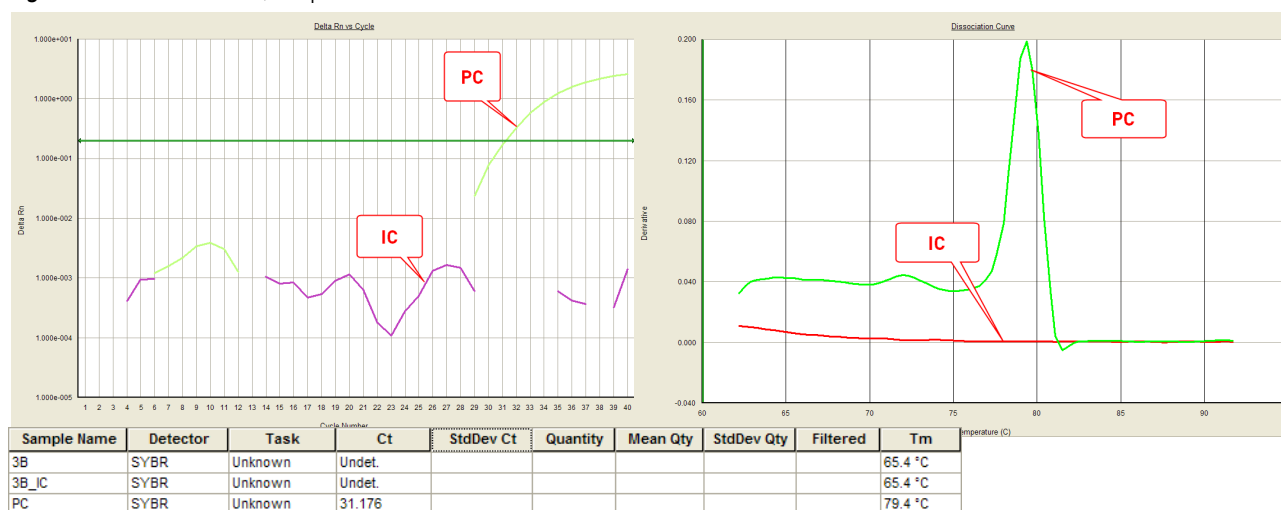
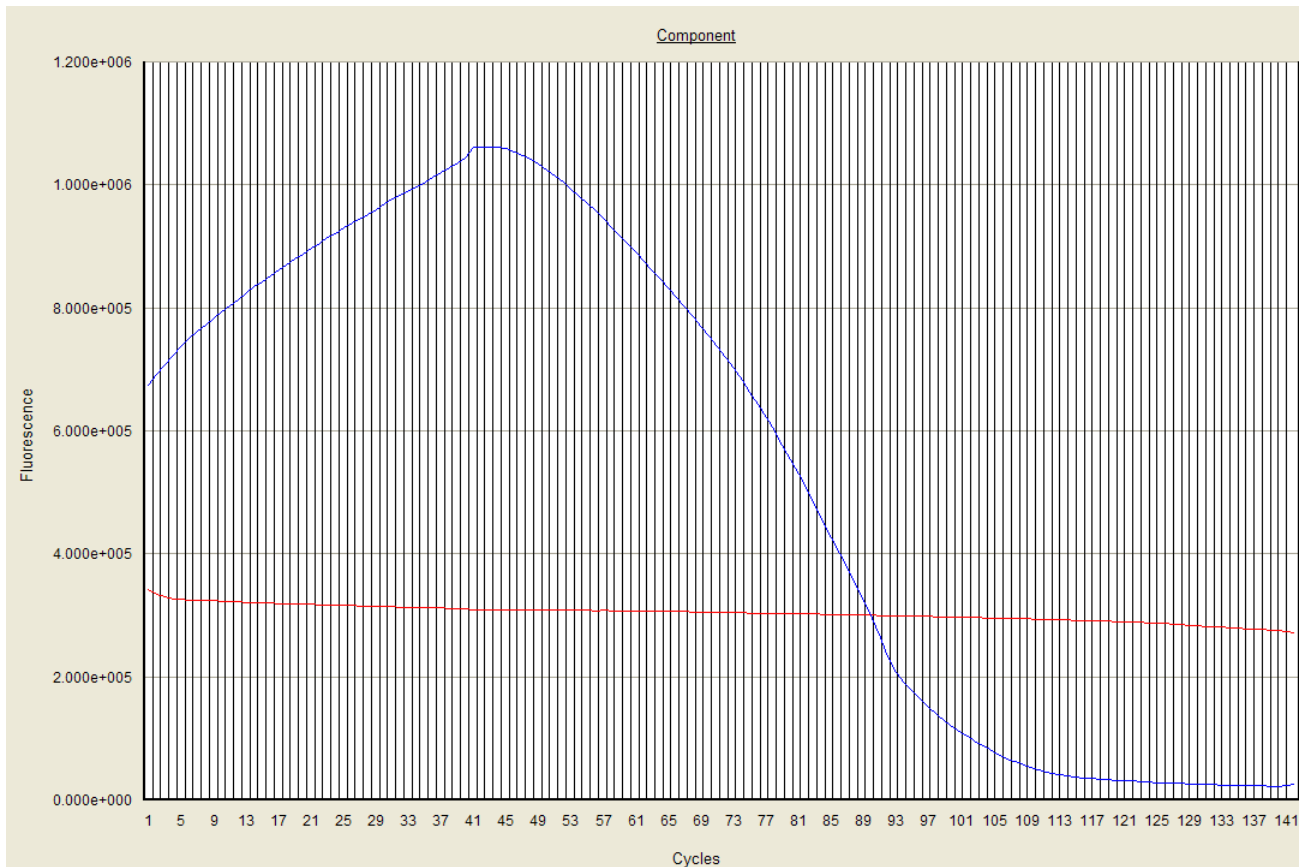


Figure 2 High background signal



Background and Ordering Information

Product overview

The PrepSEQ® Sample Preparation Kits use magnetic particle-based separation technology to extract DNA from *Mycoplasma* cells and virus particles that are isolated from a variety of starting material, such as infected cell cultures or *Mycoplasma* liquid cultures.

Required materials

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

Kit components may be shipped separately depending on 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 (Part No. 4452222) contains reagents for 100 small-scale (100 µL) cell culture extractions

Shipped in box labeled	Component	Description	Part number†	Storage
RNase Cocktail	RNase Cocktail	One 1.0-mL tube	AM2286	- 20°C
Box 1	Lysis Buffer	Two 50-mL bottles	4400659	Room temp.
	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	2-8°C
Box 3	Proteinase K (20 mg/mL)	One 1.25-mL tube	4403958	- 20°C

† These part numbers are provided for identification purposes; they cannot be ordered separately.

PrepSEQ® Mycoplasma Nucleic Acid Extraction Kit contents and storage

The PrepSEQ® *Mycoplasma* Nucleic Acid Extraction Kit must be ordered as part of the one of the following *Mycoplasma* detection kits:

- MycoSEQ™ *Mycoplasma* Detection Assay with Discriminatory Positive Control and PrepSEQ® *Mycoplasma* Sample Preparation Kits User Guide and Quick Reference included. (Part No. 4460627)
- MycoSEQ™ *Mycoplasma* Detection Assay with Discriminatory Positive Control and PrepSEQ® *Mycoplasma* Sample Preparation Kits User Guide and Quick Reference not included (Part No. 4460626)

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

Table 3 PrepSEQ® *Mycoplasma* Nucleic Acid Extraction Kit contains reagents for 100 small-scale (100 to 2000 µL) or 100 large-scale (2 to 10 mL) cell culture extractions

Shipped in box labeled	Description	Description	Part 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 temp.
	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	2–8°C
Box 3	Proteinase K (20 mg/mL)	One 1.25-mL tube	4403958	– 20°C

† These part numbers are provided for identification purposes; they cannot be ordered separately.

Materials not included in the kit

Table 4 includes materials and equipment that are required for using (but not included in) the PrepSEQ Sample Preparation Kits. Unless otherwise indicated, many of the listed 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: 2 set at 37°C, and 1 set at 56°C	MLS
Ice bucket	MLS
Magnetic stand, 16-tube	Applied Biosystems Part 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 <i>or</i> VWR Scientific #14216-186
Vortex Adapter-60, for use with Vortex-Genie	Applied Biosystems Part No. AM10014
Ultracentrifuge, for use with 50-mL tubes	MLS

Item	Source [†]
Consumables	
Disposable gloves	MLS
Aerosol-resistant micropipette tips	MLS
Pipettors, P1000 and P200: <ul style="list-style-type: none"> • Positive-displacement • Air-displacement • Multichannel 	MLS
Pipettes	MLS
Tubes, conical, 50-mL	Applied Biosystems Part No. AM12502
Microcentrifuge tubes, non-stick RNase-free, 1.5-mL, 1 box (250 tubes/box)	Applied Biosystems Part No. AM12450
Safe-Lock PCR clean microcentrifuge tubes, round-bottom, 2-mL, 1 bag (100 tubes/bag)	VWR Scientific #62111-754
Reagents	
SDS, 10%	MLS
1X PBS IMPORTANT! Prepare fresh reagent before using kit.	MLS
EDTA, 0.5 M	MLS
Ethanol, 95% IMPORTANT! Do not use denatured ethanol because it contains components not compatible with the protocol.	MLS
Isopropanol, 100%	MLS
DNase-free, sterile-filtered water	MLS

[†] For the SDS of any chemical not distributed by Applied Biosystems, 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

For information on new assays and updated product documentation, go to www.microseq.com.

The following related documents are shipped with the system:

Real-time PCR system	Document	PN	
All real-time PCR systems	<i>MycoSEQ™ Mycoplasma Detection Kits Quick Reference Card: MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit, MycoSEQ™ Myco Scan Mycoplasma Detection Kit</i>	4393471	Provides brief, concise instructions on using the MycoSEQ™ Mycoplasma Detection Kits.
	<i>MycoSEQ™ Mycoplasma Detection Kits User Guide</i>	4465874	Describes the MycoSEQ™ Mycoplasma Detection Kits and provides information on preparing, running, and troubleshooting Mycoplasma detection.
	<i>ViralSEQ® Mouse Minute Virus Real-Time PCR Detection Kit Quick Reference Card</i>	4445236	Provides brief, concise instructions on using the ViralSEQ® Mouse Minute Virus Real-Time PCR Detection Kit.
	<i>ViralSEQ® Mouse Minute Virus Real-Time PCR Detection Kit Protocol</i>	4445235	Describes the ViralSEQ® Mouse Minute Virus Real-Time PCR Detection Kit and provides information on preparing, running, and troubleshooting MMV detection.
	<i>PrepSEQ® Sample Preparation Kits Quick Reference Card</i>	4406304	Provides brief, concise instructions on using the PrepSEQ® Sample Preparation Kits.
	<i>PrepSEQ® Sample Preparation Kits User Guide</i>	4465957	Describes the PrepSEQ® Sample Preparation Kits and provides information on preparing, running, and troubleshooting sample preparation.
	<i>PrepSEQ® Nucleic Acid Extraction Kit Quick Reference Card</i>	406303	Provides brief, concise instructions on using the PrepSEQ® Nucleic Acid Extraction Kit.
	<i>PrepSEQ® Nucleic Acid Extraction Kit Protocol</i>	4400739	Describes the PrepSEQ® Nucleic Acid Extraction Kit and provides information on preparing, running, and troubleshooting nucleic acid extractions.

Note: To open the user documentation available online, use the Adobe® Reader® software available from www.adobe.com

Note: For additional documentation, see “Obtaining support” on page 32.

Obtaining SDSs

Safety Data Sheets (SDSs) are available from www.appliedbiosystems.com/sds

Note: For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

B

before you begin 9
binding solution, prepare 9
biohazard safety 28
block heater, settings 9

C

chemical safety 28

D

documentation, related 31

E

equipment, prepare 9

G

guidelines
magnetic particles 20
sample preparation 20
wash buffer 21

K

kit
contents and storage 23, 24
description 7
workflow 7

M

magnetic particles
guidelines 20
precipitate in 20
prepare 9
magnetic stand, guidelines for using 9
materials not included 25
MMV sample preparation protocol
1-2-3 10
3-in-1 18

Mycoplasma sample preparation protocol
1-2-3 10
3-in-1 18
large-scale 12

P

PBS, prepare 9
PrepSEQ® 1-2-3 Nucleic Acid Extraction Kit
contents 23
PrepSEQ® 1-2-3 protocol for *Mycoplasma* or MMV
detection 10
PrepSEQ® 3-in-1 protocol for *Mycoplasma*, MMV, and
Vesivirus detection 18
PrepSEQ® large-scale protocol for *Mycoplasma*
detection 12
PrepSEQ® Mycoplasma Nucleic Acid Extraction Kit
contents 24
PrepSEQ® Sample Preparation Kits
description 7
workflow 7
product description 23
protocol, select 8

R

reagents, prepare 9
required materials not included 25

S

safety
biohazard 28
chemical 28
Safety Data Sheets (SDSs), obtaining 32
sample preparation
guidelines 20
select a protocol 8
select a sample preparation protocol 8
storage conditions 23, 24
support, obtaining 32

T

training, information on [32](#)

troubleshooting [21](#)

V

Vesivirus sample preparation protocol [18](#)

W

wash buffer

 guidelines [21](#)

 prepare [9](#)

workflow [7](#)



Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit www.appliedbiosystems.com/support

www.lifetechnologies.com

