MO BIO Laboratories, Inc.

Saving You Time For Life

PowerMag® Air & Water DNA/RNA Isolation Kit

(Optimized for epMotion®)

Catalog No. 27800-4-EP

Quantity: 4 x 96 Preps

Total Purifications: 384

INSTRUCTION MANUAL

Version 09102014

Please recycle

www.mobio.com | T: 800.606-6246 | T: 760.929-9911 | technical@mobio.com

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KIT CONTENTS

	Kit Catalog #27800-4-EP	
Component	**Catalog#**	**Amount**
5 ml Bead Tubes	27800-4-EP-BT	384
PowerMag® Lysis Solution	27800-4-EP-1	424 ml
PowerMag® Inhibitor Removal Solution	27800-4-EP-2	85 ml
ClearMag® Binding Solution	27800-4-EP-3	2 x188 ml
ClearMag® Beads	27800-4-EP-4	9 ml
ClearMag® Wash Solution	27800-4-EP-5	765 ml
ClearMag® RNase-Free Water	27800-4-EP-6	49 ml
MO BIO 2 ml Deep Well Plates (DWP)	27800-4-EP-DWP	12
PowerMag® Microplates (MO BIO MTP)	27800-4-EP-MTP	4
Sealing Tape	27800-4-EP-ST	32
Round Well Mats	27800-4-EP-RWM	4

KIT STORAGE

The kit reagents and components should be stored at room temperature (15-30°C).

PRECAUTIONS

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

This kit is for research purposes only. Not for diagnostic use.

EQUIPMENT AND REAGENTS REQUIRED

- ☐ Disposable/reusable filter funnels or air sampling unit
- ☐ Filter membranes (if using a reusable filter funnel)
- ☐ Pipettors
- ☐ Centrifuge capable of handling the 5 ml Tube Centrifuge Blocks (MO BIO Catalog# 11981) and two 96 Well Blocks (13 cm x 8.5 cm x 6.0 cm) at 4500 x g

Note

If you have a centrifuge with a maximum speed less than 4500 x g see the Hints and Troubleshooting Guide.

- ☐ Multi-channel Pipettor(s) (volumes of 50 µl - 1000 µl)
- ☐ Appropriate pipet tips for the Multi-channel pipettors to be used in the lysate preparation steps
- ☐ 96 Well Plate Shaker for 96 Well Blocks and 5 ml Tube Adapter Set (MO BIO Catalog# 11996, #11980)
- ☐ Vortex-Genie® 2 Vortex with 3 inch platform (MO BIO Catalog# 13111-V or 13111-V-220)
- ☐ ß-mercaptoethanol (ß-ME)

EQUIPMENT REQUIRED ON THE ROBOT PLATFORM

- ☐ Shaker (The epMotion® 5075 TMX has a thermo-mixer on the deck)
- ☐ Magnetic Separator, the MO BIO PowerMag® Magnetic Separator is recommended (MO BIO Catalog# 27400).

PLASTIC DISPOSABLES NOT INCLUDED

- Contact your Eppendorf representative for the epMotion® plastic disposables specific to your platform. Go to www.mobio.com/powermag for links to the necessary epMotion® products on the Eppendorf website.
- Appropriate pipet tips for the Multi-channel pipettors to be used in the lysate preparation steps.

Note

The tips must fit in the round wells of the 1 ml blocks (examples of these are Molecular Bioproducts ART Catalog# 2179-HR, Eppendorf Catalog# 0030 077.750 and Rainin Catalog# RT-1000F).

INTRODUCTION

The PowerMag® Air & Water DNA/RNA Isolation Kit is a magnetic bead based nucleic acid isolation kit optimized for use with the Eppendorf epMotion® 5075 TMX platform. It is compatible with other automated platforms with similar features.

The PowerMag® Air & Water Kit can be used for automated isolation of microbial DNA and RNA from any liquid or air sample that is concentrated on a filter membrane. Both 25 mm and 47 mm filter membranes of a variety of materials can be processed with this kit. Filtered samples, containing a variety of inhibitors, such as salts, metals, humic substances and other organic materials are easily processed using patented Inhibitor Removal Technology® (IRT) to remove PCR inhibitors released during the extraction process. A novel, proprietary magnetic bead system is used for the isolation of nucleic acids without the binding of residual contaminants, for inhibitor-free DNA and RNA that is ready to use in the most demanding downstream applications including PCR, qPCR, RT-qPCR and next- generation sequencing.

DNA and RNA have been isolated from a variety of different membrane types used for air and water sampling.

Membrane Type	Air	Water
Mixed Cellulose Ester (MCE)	✔	✔
Cellulose Acetate (CA)	✔	✔
Cellulose Nitrate (CN)	✔	✔
Polyethersulfone (PES)	✔	✔
Glass Fiber (GF/F)*	✔	✔
Polycarbonate (PC)	✔	✔
Nylon	✔	
Gelatin*	✔	

*The use of glass fiber filters (GF/F) and gelatin membranes require modifications to the protocol to ensure the optimal recovery of DNA and RNA. Please read the notes at every step and refer to the Using GF/F Membranes section or the Using Gelatin Membranes in the Hints and Troubleshooting Guide.

For maximum sample processing, this kit requires the use of a specialized plate shaker in order to facilitate the bead beating process in 5 ml Bead Tubes, containing a mixture of 0.7 mm and 0.15 mm RNase and DNase-free garnet beads. We recommend the 96 Well Plate Shaker (MO BIO Catalog# 11996 in North America only. In other countries, contact your local MO BIO distributor for an equivalent product recommendation) and the 5 ml Tube Adapter Set (MO BIO Catalog# 11980). The 5 ml Tube Adapter Set can accommodate up to 16 bead tubes each thereby allowing for the processing of 32 samples at once. To centrifuge all 32 samples at the same time, we recommend the 5 ml Tube Centrifuge Blocks (MO BIO Catalog# 11981) that fit into a 96 well plate bucket.

This kit was optimized on the Eppendorf epMotion® 5075 TMX robot for isolation of DNA and RNA from up to 850 µl of lysate per well in the provided MO BIO 2 ml Deep Well Plate (DWP). This kit requires the use of a plate shaker on the robotic deck. We highly recommend the use of the PowerMag® Magnetic Separator (MO BIO Catalog# 27400) with large open-platform robots for best results. However, other magnetic separators that efficiently pull the magnetic beads away from the center of the well may also be used.

The plastic blocks recommended for use with this chemistry are provided. These are thin-walled plastics that permit the best conductivity of the magnetic field through the plastic block and allow for faster and more complete separation of the magnetic beads from solution.

Note

The order and placement of components and reagents on the robotic deck are described in the software protocol that can be downloaded from our website.
Other open platform robots may be used with this kit. However, you may need to contact your local field application scientist for the manufacturer of your robot for help in adapting this protocol to your system.

PROTOCOL OVERVIEW

PowerMag® Air & Water DNA/RNA Isolation Kit

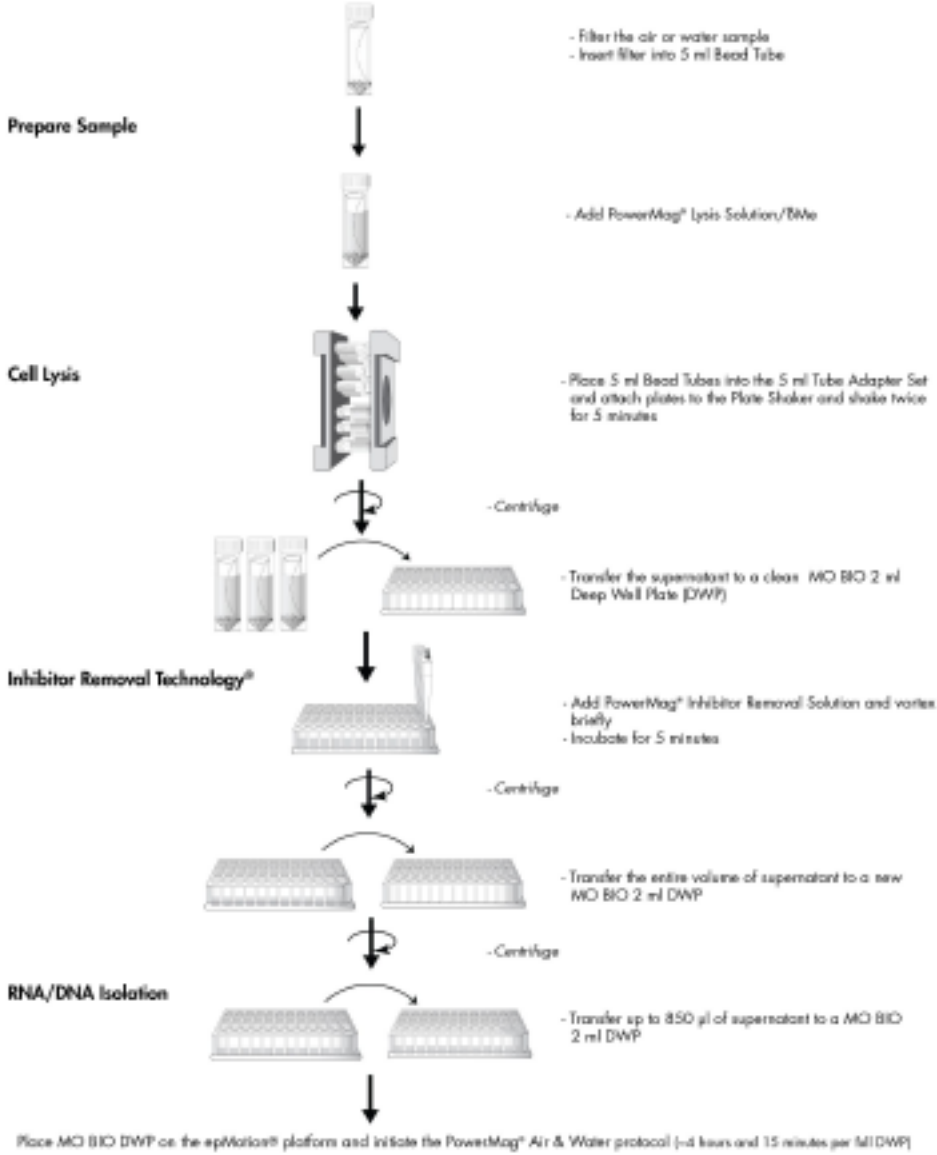
(Optimized for epMotion®)

Catalog No. 27800-4-EP

Individual 25 mm or 47 mm filter membranes are placed into 5 ml Bead Tubes that fit into 5 ml Tube Adapter Set (MO BIO Catalog# 11980) for rapid and thorough homogenization on the 96 Well Plate Shaker. Cell lysis occurs by a combination of mechanical and chemical methods. Inhibitory compounds are removed using Inhibitor Removal Technology®. Total nucleic acids are captured on specialized magnetic beads in the presence of buffers that avoid the use of chaotropic salts and ethanol. DNA and RNA are washed on the beads and then eluted using RNase-Free Water.

It is important to note that quantification of the DNA using PicoGreen® will be approximately 15% lower than the actual yield due to the presence of residual wash solution in the DNA. The wash solution does not inhibit PCR, cDNA synthesis, RT-qPCR or interfere with next generation sequencing and other downstream applications.

PowerMag® Air & Water DNA/RNA Isolation Kit



PROTOCOL

Before starting, warm the PowerMag® Lysis Solution at 60°C for 15-20 minutes before starting to dissolve any precipitates.

Both DNA and RNA can be co-purified with this protocol.
A- For the extraction of both DNA and RNA OR if only RNA is desired, add ß-mercaptoethanol (ß-ME) at a ratio of 25 µl per ml of the PowerMag® Lysis Solution. You will need 98 ml of PowerMag® Lysis Solution / ß-ME per 96 samples (1 ml / sample + 2 ml to account for loss during pipetting).

B- If only DNA is desired, add RNase A at a ratio of 9 µl per ml of PowerMag® Lysis Solution. You will need 98 ml of PowerMag® Lysis Solution / RNase A per 96 samples. ß-mercaptoethanol (ß-ME) is not required.

Note

If you are using glass fiber filter (GF/F) membranes or gelatin filters refer to the Using GF/F Membranes or Using Gelatin Filters in the Hints and Troubleshooting Guide before continuing with the protocol.

1. Filter air or water sample through a 25 mm or 47 mm membrane.

2. Using two sets of sterile forceps, pick up the filter membrane at opposite edges and roll the filter into a cylinder with the top side facing inward.

3. Insert the rolled filter membrane into the 5 ml Bead Tube.

4. Add 1 ml of warmed PowerMag® Lysis Solution / β-ME to each 5 ml Bead Tube (For DNA only, add 1 ml of warmed PowerMag® Lysis Solution / RNase A).

Note

PowerMag® Lysis Solution contains SDS which will precipitate at room temperature. Heating at 60°C will dissolve the SDS. PowerMag® Lysis Solution can be used while it is still warm.

5. Place 16 of the 5 ml Bead Tubes into each 5 ml Tube Adapter (MO BIO Catalog# 11980) and place on the 96 Well Plate Shaker (MO BIO Catalog# 11996). Reference the protocol provided with the 5 ml Tube Adapter Set for proper placement. Shake at speed 20 for 5 minutes.

Note

If using GF/F membranes shake at speed 10 for 5 minutes.

6. After the first 5 minute cycle, remove the Tube Adapter assemblies and rotate them so that the side closest to the machine body is now furthest from the machine body. Shake again at speed 20 for 5 more minutes.

Note

The Tube Adapter assemblies need to be rotated to ensure that bead beating is uniform for all of the 5 ml Bead Tubes. For assistance with loading and unloading the Tube Adapter assemblies please see the online video at the MO BIO website (http://www.mobio.com/5mladapterplates) or contact MO BIO technical support.

Note II

If using GF/F membranes shake at speed 10 for 5 more minutes.

7. Centrifuge the 5 ml Bead Tubes at room temperature for 1 minute at 4500 x g. (We recommend the use of the 5 ml Tube Centrifuge Blocks, MO BIO Catalog# 11981, that fit into a 96 well plate bucket to allow simultaneous centrifugation of each group of 32, 5 ml Bead Tubes).

Note

If using GF/F membranes transfer the entire contents of the bead tube to a 10 ml syringe barrel. Place the syringe barrel in a 15 ml tube and centrifuge for 3 minutes at 4000 x g to recover the supernatant.

8. Transfer the supernatant to a clean MO BIO 2 ml Deep Well Plate (DWP). It will be necessary to push the pipette tip through the beads into the bottom of the bead tube in order to recover as much supernatant as possible.

Note

The supernatant may still contain some bio-solid particles.

9. Add 200 µl of PowerMag® Inhibitor Removal Solution to each well and apply Sealing Tape to the MO BIO 2 ml Deep Well Plate (DWP). Vortex horizontally for 5 seconds on the vortex ensuring that the solution is well mixed. Incubate at room temperature for 5 minutes.

10. Centrifuge the MO BIO 2 ml Deep Well Plate (DWP) at room temperature for 6 minutes at 4500 x g. Remove and discard Sealing Tape.

11. Avoiding the pellet, transfer the entire volume of supernatant to a new MO BIO 2 ml Deep Well Plate (DWP). For the wells at the center of the plate, it may help to mark a line on the pipet tip to show how far to insert the tip without touching the pellet. Apply Sealing Tape to the MO BIO 2 ml Deep Well Plate (DWP). Centrifuge again at 4500 x g for 6 minutes to clear any residual particulates that may have carried over.

12. Transfer no more than 850 µl of supernatant to a new MO BIO 2 ml Deep Well Plate (DWP) again avoiding any residual pellet.

13. Place the MO BIO 2 ml Deep Well Plate (DWP) containing the supernatant on the epMotion® robotic deck as indicated on the worktable in the epMotion® program.

Note

You may place the supernatant in the MO BIO 2 ml Deep Well Plate (DWP) at 4°C for several hours if you need to stop during the protocol or if you can only process one 96 well plate at a time.
If you are using gelatin filters, add 30 µl of Proteinase K (20 mg/ml) and incubate at 65°C for 10 minutes.

14. For each 96 well plate to be processed, place 174 ml of ClearMag® Wash Solution into an Eppendorf 400 ml reservoir placed at the appropriate location on the deck as indicated on the worktable in the epMotion® program.

15. For each 96 well plate to be processed, place 11 ml of ClearMag® RNase-Free Water into an Eppendorf 30 ml reservoir placed in an Eppendorf tub holder located at the appropriate location on the deck as indicated on the worktable.

16. For each 96 well plate to be processed, prepare the ClearMag® Binding Solution / ClearMag® Beads by first vortexing the bottle containing the ClearMag® Beads until all beads are resuspended, followed by adding 2 ml of the now resuspended ClearMag® Beads to 85 ml of the ClearMag® Binding Solution in an appropriate mixing vessel (user provided). Vortex well to mix.

17. Transfer the entire volume of ClearMag® Binding Solution / ClearMag® Beads into an Eppendorf 100 ml reservoir placed in an Eppendorf tub holder located at the appropriate location on the deck as indicated on the worktable.

18. Initiate the protocol.

Note

It is imperative to start the protocol immediately otherwise the beads will begin to settle. If there is a significant delay (in excess of 3 minutes) then re-agitate the beads.

19. Upon completion, cover the wells of the PowerMag® Microplate (MO BIO MTP) with the Round Well Mat provided. DNA/RNA is now ready for any downstream application. No further steps are required.

We recommend storing nucleic acids frozen (-20°C or -80°C).

Thank you for choosing the PowerMag® Air & Water DNA/RNA Isolation Kit.

HINTS AND TROUBLESHOOTING GUIDE

Water & Air Sampling

The amount of water or air that can be filtered through a particular type of filter membrane varies depending on the sample source and the filter membrane type, diameter, and pore size.

- Clear Water Samples: Water samples may vary from clear to highly turbid. Larger volumes of clear water can be processed because there is less chance of filter clogging. Potable drinking water will generally allow for very high volumes depending on the quality and particulate count. In most cases, 100 ml to 10 liters can be processed. Some users report processing even higher volumes.

- Turbid Water Samples: Turbid samples with high levels of suspended solids or sediments will tend to quickly clog filters with a smaller pore size (0.22 micron). Use of 0.45 micron filters is recommended for theses types of samples. Prior to filtering, samples can be stored in a container to allow suspended solids to settle out. For samples where settling does not occur or is not desired, a method involving stacking filters with larger pore sizes on top of a filter membrane of the desired pore size is recommended. A common set-up is to stack a sterile 1 micron filter on top of a membrane with a smaller pore size (0.22 or 0.4 microns). This layering will filter out large debris and allow the smaller micron filter to trap microorganisms. The layered filter system can be washed with sterile saline or sterile phosphate buffer to knock down some of the trapped microorganisms on the larger pore size filters. Although this is not 100% efficient, it will increase the overall yield of microbial DNA.

- Air Samples: Air is typically considered a low biomass sample and utilizes 25 – 35 mm filter membranes. Extended sampling times and sample heating during the protocol (see the section below on Difficult to Lyse Cells) may be required to improve nucleic acid yields. Membrane pore sizes typically range from 0.45 micron to 5 micron and are dependent on the filter membrane type and the air collection device. Depending on the air collection device and pump, smaller pore sizes may result in increased back pressure and reduced air flow. To reduce this problem select a pore size that is recommended for the air sampling device or results in the optimal flow rate.

Stabilizing Samples for Storage and During Processing

Filter membranes will remain stable if stored dry. When possible, store the Bead Tubes containing filter membranes without lysis buffer at -20°C.

Using GF/F Membrane

Glass fiber filter (GF/F) membranes are highly absorbent and will retain most if not all of the PowerMag® Lysis Solution/ß-ME, regardless of membrane size (25 mm, 47 mm). To recover the supernatant after the mechanical lysis step, the membrane must be placed into a column that will retain the membrane but allow the supernatant to be centrifuged out for use in subsequent steps. To do this, remove the plunger from a 10 ml syringe barrel and discard. Carefully transfer the contents of the 5 ml Bead Tube into the syringe barrel and place the barrel into a 15 ml tube. Centrifuge for 3 minutes at 4,000 x g. The supernatant should be at the bottom of the 15 ml tube while the membrane is still retained in the syringe barrel.

When GF/F membranes are mechanically homogenized with the same settings used for other filter membrane types, the DNA becomes sheared in comparison. To reduce DNA shearing, it is recommended to reduce the 96 Well Plate Shaker setting to speed 10.

Using Gelatin Membranes

Gelatin membranes are typically used for sampling of microorganisms including viral particles that are viable and can be cultured. These membranes dissolve in aqueous buffers and on agar plates for culturing. For extraction of DNA and RNA from these membranes, a Proteinase K digestion prior to bead beating is required. After the addition of the PowerMag® Lysis solution / ß-ME, add 30 µl of Proteinase K (20 mg/ml) and incubate for 10 minutes at 65°C. Continue with Step 5 in the protocol.

Difficult to Lyse Cells

When working with organisms that have proven to be difficult to lyse using mechanical or chemical methods, a 10 minute incubation at 65°C, after adding PowerMag® Lysis Solution, can be performed. However, this could result in some degradation of the RNA depending on the microorganisms present. After heating, continue by proceeding with the mechanical lysis step using the 96 Well Plate Shaker.

Centrifuge with a Maximum Speed Less Than 4500 x g

Multiply the protocol time and speed to determine the total force (or speed) required (x g). Divide the total by the maximum speed of your centrifuge (round up if necessary). This will be the number of minutes your centrifuge will need to run to achieve the appropriate overall force.
Example: 10 minutes at 4500 x g = 45000.

If your centrifuge has a maximum speed of 2500 x g, divide 45000 ÷ 2500 = 18 minutes of centrifugation.

If DNA Does Not PCR Amplify

- Check RNA and DNA yields by gel electrophoresis and spectrophotometer readings. DNA template is typically added at 10 ng per reaction, although more or less may be needed depending on the reaction conditions, enzyme activity, and copy number of the target sequence.
- If DNA does not amplify after altering the amount of template in the reaction, PCR optimization (i.e. changing reaction conditions, validating primers, or testing a different polymerase) should be attempted.

Concentrating DNA/RNA

The final volume of eluted DNA and RNA will be 100 µl. Nucleic acids may be concentrated by adding 5 µl of 5M NaCl and inverting 3-5 times to mix. Next, add 200 µl of 100% cold ethanol and invert 3-5 times to mix. Incubate at -20°C for at least 10 minutes to overnight. Centrifuge at 13,000 x g for15 minutes. Decant all liquid. Wash the DNA pellet with 70% cold ethanol. Centrifuge at 13,000 x g for 10 minutes to re-pellet the sample. Decant ethanol and dry in a speed vacuum, desiccator, or ambient air. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.
Note: This procedure must be done individually after transferring the eluted sample to a microcentrifuge tube.

Storing DNA

The RNA and DNA are eluted in ClearMag® RNase-Free Water. Store the DNA/RNA at -20°C to prevent degradation and at -80°C for long term storage. RNA and DNA can be eluted in 10 mM Tris buffer pH 7, or TE without loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. Prolonged storage in the PowerMag® Microplates (MO BIO MTP) at 4°C will result in the loss of liquid due to evaporation.
MO BIO offers TE-4 (10 mM Tris, 0.1 mM EDTA, pH 8.0) which will allow for maximal protection of DNA during storage with no PCR inhibition (Catalog# 17320-1000).

OTHER RELATED PRODUCTS

Product	Catalog#	Amount
96 Well Plate Shaker	11996	1 unit (120 V)
PowerMag® Magnetic Separator	27400	1 unit
5 ml Tube Adapter Set	11980	1 set
5 ml Tube Centrifuge Blocks	11981	1 set
Water Filter Adapter	14800-10-WFA	1 adapter
Water Filter (0.45 µm)	14800-50-WF	50 units
Water Filter (0.22 µm)	14880-50-WF	50 units
RTS DNase™ Kit	15200-50	50 preps
RNase A Solution (25 mg/ml)	1202-1	1 ml

For a complete list of products available from MO BIO Laboratories, Inc., visit www.mobio.com

PRODUCTS RECOMMENDED FOR YOU

Product	Catalog#	Amount
PowerWater® DNA Isolation Kit	14900-50-NF	50 preps
	14900-100-NF	100 preps
PowerWater® RNA Isolation Kit	14700-50-NF	50 preps
PowerWater® Sterivex™ DNA Isolation Kit	14600-50-NF	50 preps
PowerMag® Soil DNA Isolation Kit (Optimized for epMotion®)	27100-4-EP	4 x 96 preps
PowerMag® Microbial DNA Isolation Kit	27200-4	4 x 96 preps
PowerMag® epMotion® Accessory Pack	27300-4-EP	1 pack
PowerMag® Microbiome RNA/DNA Isolation Kit (Optimized for epMotion®)	27500-4-EP	4 x 96 preps

TECHNICAL SUPPORT

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Committed to resolving your technical questions promptly, our technical support team is trained to work with you to rapidly and effectively trouble shoots any issues. We commit to providing you with relevant online support resources that help you complete your research projects.

Frequently Asked Questions:
www.mobio.com/faq

SDS:
www.mobio.com/sds

Protocols:
www.mobio.com/protocols

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