PowerMag®
Microbiome RNA/DNA Isolation Kit
(Optimized for epMotion®)

Catalog No.	Quantity	Total Purifications
27500-4-EP	4 x 96 Preps	384

Instruction Manual

Please recycle

Version: 09182014

Table of Contents

Introduction

The PowerMag® Microbiome RNA/DNA Isolation Kit is a magnetic bead based nucleic acid isolation kit optimized for use with the Eppendorf epMotion® 5075 TMX platform.

The PowerMag® Microbiome Kit can be used for automated isolation of microbial RNA and DNA from all stool, gut, and similar sample types and other difficult environmental samples containing high inhibitor content, such as bile, bilirubin, digested food, and humic acids. The kit, which can process up to 0.25 g of sample, employs 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 that is ready to use in the most demanding downstream applications including PCR, qPCR and next generation sequencing.

This kit requires the use of a specialized plate shaker in order to facilitate the bead beating process in the PowerMag® Bead Plates. We recommend the Retsch 96 Well Plate Shaker (MO BIO Catalog# 11996 in the USA only) and Adapters (MO BIO Catalog# 11990). For information outside the USA, contact technical@mobio.com.

This kit was optimized on the Eppendorf epMotion® 5075 TMX robot for isolation of DNA 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 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 for the platform portion of the protocol will be described in the downloaded software.

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

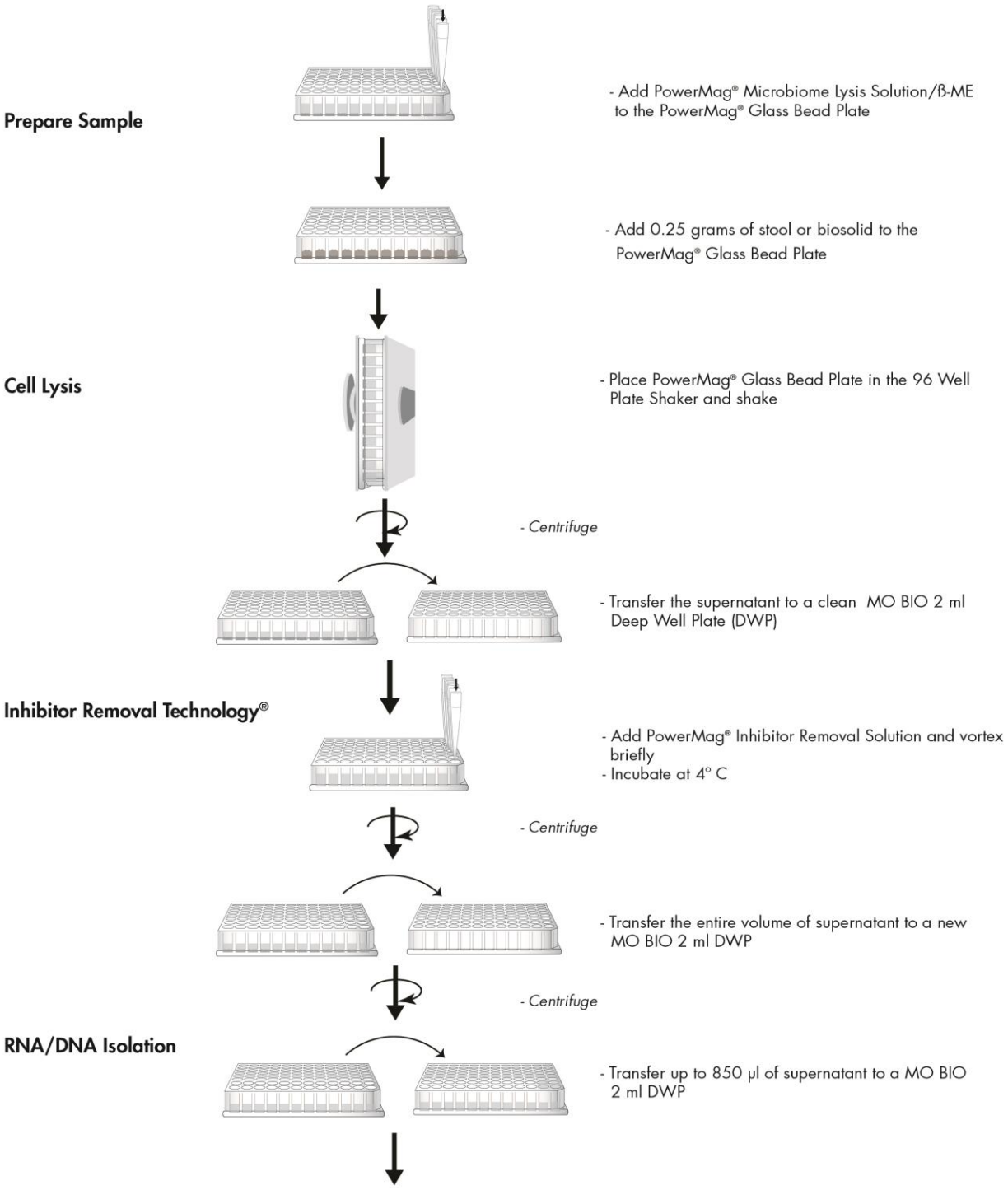
Microbiome samples are added to a 96 well bead beating plate for rapid and thorough homogenization. Cell lysis occurs by a combination of mechanical and chemical methods. Inhibitory compounds are removed using Inhibitor Removal Technology®. Total nucleic acids are captured on specialized magnetic beads in the presence of buffers that avoid the use of chaotropic salts and ethanol. RNA and DNA are washed on the beads and then eluted using RNase-Free Water. The eluted DNA is ready for qPCR, next generation sequencing and other downstream applications.

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 or interfere with next generation sequencing.

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

Other Related Products	Catalog No.	Quantity
96 Well Plate Shaker	11996	1 unit (120 V)
PowerMag® Magnetic Separator	27400	1 unit
Plate Adapter Set	11990	1 set
Anti-Static Polypropylene Weighing Funnels, Small	23302-50	1 bag of 50

PowerMag® Microbiome RNA/DNA Isolation Kit



Equipment and Reagents Required

- Centrifuge capable of handling 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)
- Mechanical Shaker for 96 Well Blocks and Plate Adapters (MO BIO Catalog# 11996 and 11990)
- Vortex-Genie® 2 Vortex with 3 inch platform (MO BIO Catalog# 13111-V or 13111-V-220)
- B-mercaptoethanol
- Optional - Phenol:Chloroform:Isoamyl Alcohol (PCI) (25:24:1, pH 6.5 – 8)

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).

Kit Contents

	Kit Catalog# 27500-4-EP	
Component	**Catalog #**	**Amount**
PowerMag® Glass Bead Plates (w/Sealing Mat)	27500-4-EP-BP	4
Bead Plate Sealing Mats	27500-4-EP-SM	4
PowerMag® Microbiome Lysis Solution	27500-4-EP-1	2 x 141 ml
PowerMag® Inhibitor Removal Solution	27500-4-EP-2	3 x 22 ml
ClearMag® Binding Solution	27500-4-EP-3	2 x 188 ml
ClearMag® Beads	27500-4-EP-4	9 ml
ClearMag® Wash Solution	27500-4-EP-5	765 ml
ClearMag® RNase-Free Water	27500-4-EP-6	49 ml
MO BIO 2 ml Deep Well Plates (DWP)	27500-4-EP-DWP	12
PowerMag® Microplates (MO BIO MTP)	27500-4-EP-MTP	4
Sealing Tape	27500-4-EP-ST	32
Round Well Mats	27500-4-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.

Protocol

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

Add β-mercaptoethanol (β-ME) at a ratio of 25 µl per ml of the PowerMag® Microbiome Lysis Solution. You will need 64 ml of PowerMag® Microbiome Lysis Solution/ β-ME per 96 well plate.

1. Briefly centrifuge (1 minute at 4500 x *g*) the **PowerMag® Glass Bead Plate** to bring all of the glass beads down into the well. Carefully peel off the Sealing Mat that covers the **PowerMag® Glass Bead Plate** and discard.

2. Add 650 µl of warmed **PowerMag® Microbiome Lysis Solution/ β-ME** to each well of the **PowerMag® Glass Bead Plate.**

   Note: PowerMag® Microbiome Lysis Solution contains SDS. If it gets cold, it will precipitate. Heating at 60°C will dissolve the SDS. PowerMag® Microbiome Lysis Solution can be used while it is still warm.

   Optional: To enhance the recovery and integrity of RNA, addition of 100 µl of phenol:chloroform:isoamyl alcohol (PCI) (25:24:1, pH 6.5-8) to the wells of the bead plate pre-loaded with 650 µl of PowerMag® Microbiome Lysis Solution/ β-ME before filling with stool samples will allow for fast nuclease inactivation during the filling process.

3. Add 0.25 grams of stool or environmental sample to each well of the **PowerMag® Glass Bead Plate.**

   Note: This is the most time consuming step of the protocol. Care must be taken to avoid cross contamination between sample wells. Use of an Anti-Static Polypropylene Weighing Funnel (MO BIO Catalog# 23302-50) can make it easier to weigh and add some sample types to each well without spilling into adjacent wells.

4. Secure a new **Bead Plate Sealing Mat** tightly to the **PowerMag® Glass Bead Plate**. Vortex horizontally for 5 seconds on the vortex ensuring that the solution / sample is well mixed.

   Note: A proper seal of the mat is critical to prevent loss of sample and leakage that might cause damage to your shaker.

   This is an appropriate stopping point. You can store the PowerMag® Glass Bead Plate at 4°C or -20°C covered with a new Bead Plate Sealing Mat.

5. Place each of the **PowerMag® Glass Bead Plates** (with **Sealing Mats** securely affixed) between 2 adapter plates (MO BIO Catalog# 11990) and place on the 96 Well Plate Shaker (MO BIO Catalog# 11996). Reference the protocol provided with the adapter plates for proper placement. Shake at speed 20 for 10 minutes.

6. After the first 10 minute cycle, remove the block and rotate it so that the side closest to the machine body is now furthest from the machine. Shake again at speed 20 for 10 more minutes.

   Note: The block needs to be rotated to ensure that bead beating is uniform for all of the wells.

7. Centrifuge the **PowerMag® Glass Bead Plate** at room temperature for 6 minutes at 4500 x *g*.

8. Carefully and without splashing remove and discard the Sealing Mat and transfer the supernatant to a clean **MO BIO 2 ml Deep Well Plate (DWP)**.

   Note: The supernatant may still contain some bio-solid particles.

9. Add 150 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 4°C 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.

    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.

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.

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 is now ready for any downstream application. No further steps are required.

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

    Thank you for choosing the PowerMag® Microbiome RNA/DNA Isolation Kit.

Hints and Troubleshooting Guide

Amount of Sample to Process
This kit is designed to process 0.25 g of bio-solid or soil. For efficient 96 well homogenization, we do not recommend increasing the amount of sample.

Stabilizing Samples for Storage and During Processing
Addition of 100 µl of phenol:chloroform:isoamyl alcohol (PCI) (25:24:1, pH 6.5-8) to the wells of the bead plate pre-loaded with 650 µl of PowerMag® Microbiome Lysis Solution/ β-ME before filling with stool samples will allow for fast nuclease inactivation and sample stabilization during the filling process and storage at -20°C overnight if desired. If the use of PCI is not desired, pre-loading the wells with PowerMag® Microbiome Lysis Solution/ β-ME before adding the samples and then storage overnight in lysis buffer will offer additional protection during the time while samples are sitting in the block at room temperature during filling.

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 70°C, after adding PowerMag® Microbiome Lysis Solution, can be performed. 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 yield by gel electrophoresis and spectrophotometer reading. DNA template is typically added to 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 the DNA
The final volume of eluted RNA and DNA 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* for 15 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.

Hints and Troubleshooting Guide cont.

Storing DNA

The RNA and DNA are eluted in ClearMag® RNase-Free Water. Store the RNA/DNA 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).

Contact Information

Technical Support:
Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911
Email: technical@mobio.com
Fax: 760-929-0109
Mail: MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010

Ordering Information:
Direct: Phone MO BIO Laboratories, Inc. Toll Free 800-606-6246, or 760-929-9911
Email: orders@mobio.com
Fax: 760-929-0109
Mail: MO BIO Laboratories, Inc, 2746 Loker Ave West, Carlsbad, CA 92010

For the distributor nearest you, visit our website at www.mobio.com/distributors

Products recommended for you

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

Description	Catalog No.	Quantity
96 Well Plate Shaker	11996	1 unit (120 V)
Plate Adapter Set	11990	1 set
PowerSoil® DNA Isolation Kit	12888-50 12888-100	50 preps 100 preps
PowerLyzer® PowerSoil® DNA Isolation Kit	12855-50 12855-100	50 preps 100 preps
PowerMax® Soil DNA Isolation Kit	12988-10	10 preps
PowerSoil®-htp 96 Well Soil DNA Isolation Kit	12955-4 12955-12	4 x 96 preps 12 x 96 preps
PowerLyzer® 24 Bench Top Bead-Based Homogenizer	13155	1 unit