MO BIO Laboratories, Inc.

Saving You Time For Life

PowerMag® Soil DNA Isolation Kit

Catalog No. 27100-4-EP

Quantity: 4 x 96 Preps (Flex) or 32 x 12 Preps (Duo)
Total Purifications: 384

INSTRUCTION MANUAL

Version 09182014


Please recycle

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KIT CONTENTS

	Kit Catalog #27100-4-EP	
Component	**Catalog#**	**Amount**
PowerMag® Bead Plates (w/Square Well Mat)	27100-4-EP-BP	4
PowerMag® Bead Solution	27100-4-EP-BS	320 ml
PowerMag® Lysis Solution	27100-4-EP-1	26 ml
PowerMag® IRT Solution	27100-4-EP-2	200 ml
ClearMag® Binding Solution	27100-4-EP-3	2 x 188 ml
ClearMag® Beads	27100-4-EP-4	9 ml
ClearMag® Wash Solution	27100-4-EP-5	765 ml
ClearMag® Elution Buffer	27100-4-EP-6	49 ml
RNase A Solution (25 mg/ml)	27100-4-EP-7	2 x1 ml
MO BIO 2 ml Deep Well Plates (DWP)	27100-4-EP-DWP	4
PowerMag® 1 ml Collection Plates	27100-4-EP-1CP	8
PowerMag® Microplates (MO BIO MTP)	27100-4-EP-MTP	4
Sealing Tape	27100-4-EP-ST	32
Elution Sealing Mats	27100-4-EP-ESM	4

KIT STORAGE

RNase A Solution should be stored at 4°C.
The other 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 SDS 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.

REQUIRED BUT NOT INCLUDED

Equipment

- ☐ 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)

Equipment Required on the Robot Platform

- ☐ Shaker/Heater (The epMotion® 5075 TMX has a thermo-mixer on the deck. For other platforms the heater component is optional.)
- ☐ 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).

PROTOCOL OVERVIEW

PowerMag® Soil DNA Isolation Kit

Catalog No. 27100-4-EP

Introduction

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

The PowerMag® Soil DNA Isolation Kit can be used for automated isolation of microbial DNA from all soil types and other difficult environmental samples containing a high humic acid content, such as compost, sediment, and manure. 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 soil 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. For information outside the USA, contact technical@mobio.com) and Adapters (MO BIO Catalog# 11990).

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. A heating block is optional, but recommended. 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

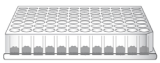
Environmental samples are added to a 96 well bead beating plate for rapid and thorough homogenization. Cell lysis occurs by a combination of mechanical and chemical methods. Humic acids are removed using Inhibitor Removal Technology®. Total genomic DNA is captured on specialized magnetic beads in the presence of buffers that avoid the use of chaotropic salts and ethanol. DNA is washed on the beads and then eluted using 10 mM Tris, pH 8 buffer. 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.

PowerMag® Soil DNA Isolation Kit

Prepare Sample



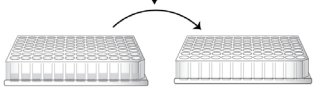
- Add 0.25 grams of soil to the PowerMag® Bead Plate

- Add PowerMag® Bead Solution/RNase A
- Add PowerMag® Lysis Solution

Cell Lysis

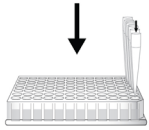
- Place PowerMag® Bead Plate in the 96 Well Plate Shaker and shake

- Centrifuge



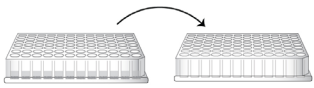
- Transfer the supernatant to a clean PowerMag® 1 ml Collection Plate

Inhibitor Removal Technology®



- Add PowerMag® IRT Solution and vortex briefly
- Incubate at 4°C

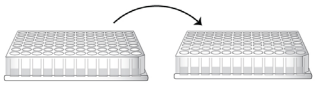
- Centrifuge



- Transfer the entire volume of supernatant to a new PowerMag® 1 ml Collection Plate

- Centrifuge

DNA Isolation



- Transfer up to 850 µl of supernatant to a MO BIO 2 ml Deep Well Plate (DWP)

Place MO BIO DWP on the epMotion® platform and initiate the PowerMag® Soil protocol

PROTOCOL

Please wear gloves and follow Universal Precautions at all times.

Before starting, add 4 µl RNase A Solution to each 750 µl of PowerMag® Bead Solution. Each 96 well plate will require exactly 72 ml of this mixture. To allow for pipetting variations and overage for the reagent reservoir, it is suggested to add 400 µl of the RNase A Solution to 75 ml of the PowerMag® Bead Solution for every 96 well plate you plan to process.

1. Carefully peel off the Square Well Mat that covers the **PowerMag® Bead Plate** and set aside. Add 0.25 grams of soil sample to each well of the **PowerMag® Bead Plate.**

Note

This is an appropriate stopping point. You can store the PowerMag® Bead Plate at 4°C covered with the Square Well Mat.

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 the soil to each well without spilling soil into adjacent wells.

2. Add 750 µl of **PowerMag® Bead Solution / RNase A Solution** to each well of the **PowerMag® Bead Plate.**

3. Check the **PowerMag® Lysis Solution** before using. If the **PowerMag® Lysis Solution** has precipitated, heat the solution at 60°C until the precipitate has dissolved. Mix gently.

Note

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

4. Add 60 µl of **PowerMag® Lysis Solution** to each well. Secure the Square Well Mat (from step 1) tightly to the **PowerMag® Bead Plate**.

Note

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

5. Place each of the **PowerMag® Bead Plates** (with **Square Well 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® Bead Plate** at room temperature for 6 minutes at 4500 x g.

8. Carefully and without splashing remove and discard the Square Well Mat and transfer the supernatant to a clean **PowerMag® 1 ml Collection Plate.**

Note

The supernatant may still contain some soil particles.

9. Add 450 µl of **PowerMag® IRT Solution** to each well and apply Sealing Tape to the **PowerMag 1 ml Collection Plate**. Vortex horizontally for 5 seconds on the vortex ensuring that the solution is well mixed. Incubate at 4°C for 10 minutes. Centrifuge the **PowerMag® 1 ml Collection Plate** at room temperature for 6 minutes at 4500 x g. Remove and discard Sealing Tape.

10. Avoiding the pellet, transfer the entire volume (approximately 850 µl) of supernatant to a new **PowerMag® 1 ml Collection Plate**. 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 **PowerMag® 1 ml Collection Plate**. Centrifuge again at 4500 x g for 6 minutes to clear any residual IRT pellet that may have carried over.

11. Transfer no more than 850 µl of supernatant to the **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.

12. 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.

Protocol (continued from page 12)

13. 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.

14. For each 96 well plate to be processed, place 11 ml of **ClearMag® Elution Buffer** 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.

15. 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.

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

17. 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.

18. Upon completion, cover the wells of the **PowerMag® Microplate (MO BIO MTP)** with the **Elution Sealing 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). ClearMag® Elution Buffer does not contain EDTA.

Thank you for choosing the PowerMag® Soil DNA Isolation Kit.

HINTS AND TROUBLESHOOTING GUIDE

Amount of Soil to Process
This kit is designed to process 0.25 g of soil. For efficient 96 well homogenization, we do not recommend increasing the amount of soil.

Wet Soil Sample
If soil sample is high in water content, weigh the slurry and dispense into the wells. It is suggested to restrict the starting amount to 0.25 g. Increasing the amount used will increase the amount of volume in the subsequent steps.

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® Lysis Solution, can be performed. Continue by proceeding with the mechanical lysis step using the 96 Well Plate Shaker.

Alternative Method for Enhancing Lysis using Freeze/Thaw Cycles
Add the samples to the PowerMag® Bead Plate and maintain at either -70°C or at -20°C until the samples are completely frozen. Immediately float the PowerMag® Bead Plate in a 65°C water bath. Repeat the freeze-thaw a second time and proceed with the addition of PowerMag® Lysis Solution.
Optional: After the second freeze-thaw, PowerMag® Lysis Solution can be added along with Proteinase K Solution (MO BIO Catalog# 1222-2) to improve the lysis efficiency for some organisms.

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 DNA yield by gel electrophoresis and spectrophotometer reading. 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 DNA will be 100 µl. The DNA 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.

Note

This procedure must be done individually after transferring the eluted sample to a microcentrifuge tube.

Storing DNA

DNA is eluted in ClearMag® Elution Buffer (10 mM Tris). Store the DNA at -20°C to prevent degradation. DNA can be eluted in TE without DNA loss, but the EDTA may inhibit downstream reactions such as PCR and automated sequencing. DNA may also be eluted with sterile DNA-Free PCR Grade Water (MO BIO Catalog# 17000-10). DNA that has been eluted into sterile water should be stored at -70°C. 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).

PRODUCTS RECOMMENDED FOR YOU

Product	Catalog#	Amount	This is for you if...
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 250 preps	
PowerLyzer® PowerSoil® DNA Isolation Kit	12855-50 12855-100	50 preps 250 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	

TECHNICAL SUPPORT

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