PowerMag®
Soil DNA Isolation Kit
(Optimized for KingFisher®)

Catalog No.	Quantity	Total Purifications
27000-4-KF	4 x 96 Preps (Flex) or 32 x 12 Preps (Duo)	384

Instruction Manual

Please recycle

Version: 09182014

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Introduction

The PowerMag® Soil DNA Isolation Kit is optimized for use with the Thermo Scientific KingFisher® Flex and KingFisher® Duo platforms.

The PowerMag® Soil DNA Isolation Kit can be used for automated isolation of microbial DNA from all types of soil as well as other environmental samples high in humic acids like compost, sediment, and manure. The kit can be used to process as many as 384 samples of up to 0.25 g each. The method employs patented Inhibitor Removal Technology® (IRT) to remove PCR inhibitors released during the extraction process. Additionally, a novel, proprietary magnetic bead system is used to isolate nucleic acids from the IRT treated lysate, without binding residual contaminants. The result is 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). Homogenization may also be performed in 2 ml bead tubes using a Vortex Genie® 2 or a high powered bead beater such as the PowerLyzer® 24.

> **NOTE**: The order of placement of components and reagents for the platform portion of the protocol will be described in the downloaded software specific to your KingFisher® platform.

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

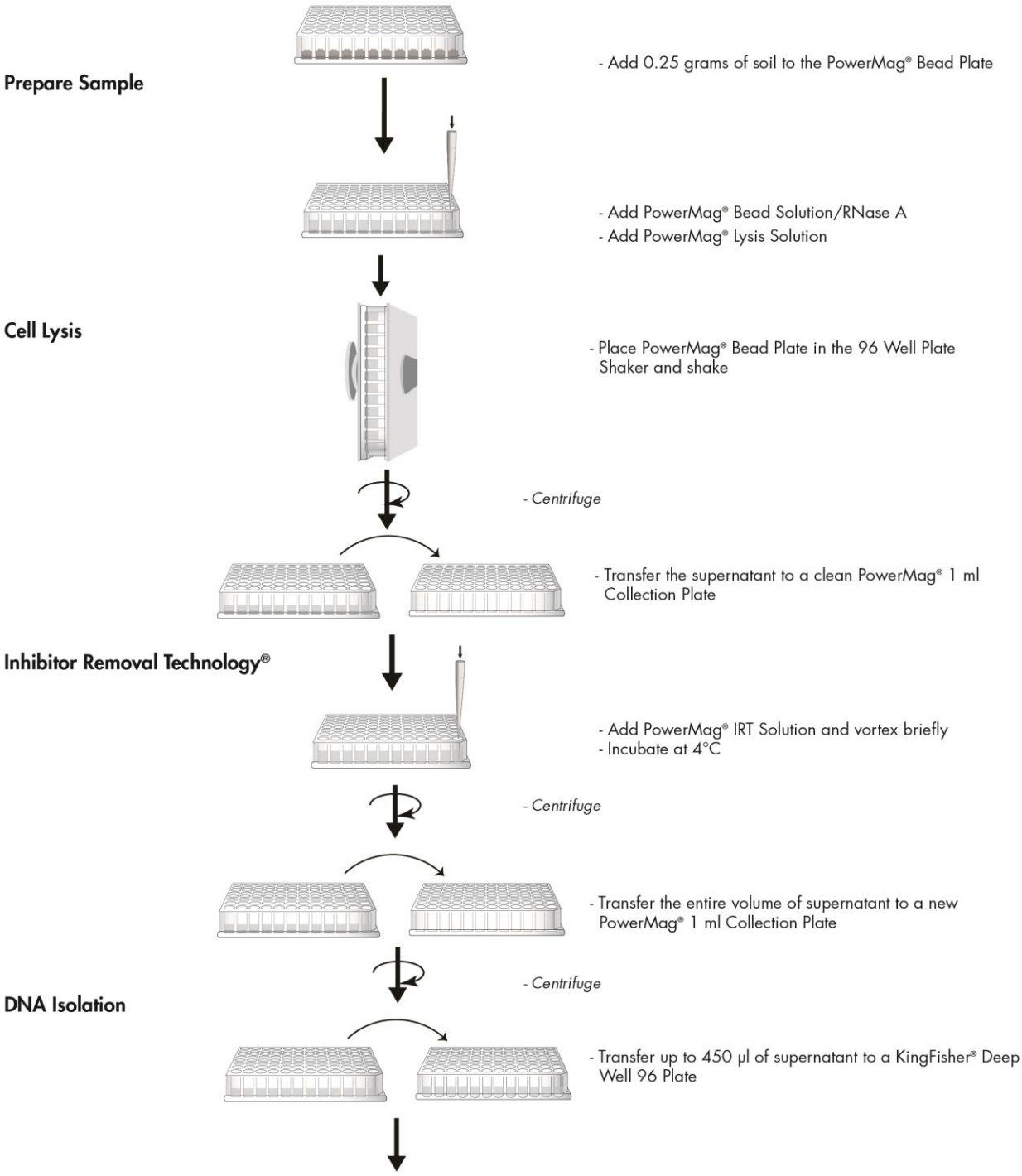
Environmental samples are added to a 96 well bead beating plate for rapid and thorough homogenization. Cell lysis occurs by a combination of mechanical and chemical methods. Humic acids are removed using the patented Inhibitor Removal Technology®. Prepared lysates are transferred to the KingFisher® Flex or KingFisher® Duo platforms where 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 a 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.

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

PowerMag® Soil DNA Isolation Kit



Equipment 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 100 µl - 850 µl)
  Note: The KingFisher® Duo applications require a 12 channel pipettor if multi-channel pipetting is desired on that platform.
- Single Pipettor(s) (volumes of 5 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)
- Optional (for KingFisher® Duo applications): Vortex Adapter (MO BIO Catalog# 13000-V1 or 13000-V1-24)

Consumables not Included

- Contact your Thermo Scientific representative for the KingFisher® Flex and Duo consumables specific to your platform. Go to www.mobio.com/powermag for links to the necessary KingFisher® products on the ThermoFisher website.
- Multi-channel pipettor reagent reservoirs for 10 – 150 ml volumes.
- 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# 27000-4-KF	
Component	**Catalog #**	**Amount**
PowerMag® Bead Plate (w/Square Well Mat)	27000-4-KF-BP	4
PowerMag® Bead Solution	27000-4-KF-BS	320 ml
PowerMag® Lysis Solution	27000-4-KF-1	26 ml
PowerMag® IRT Solution	27000-4-KF-2	200 ml
ClearMag® Binding Solution	27000-4-KF-3	200 ml
ClearMag® Beads	27000-4-KF-4	9 ml
ClearMag® Wash Solution	27000-4-KF-5	640 ml
ClearMag® Elution Buffer	27000-4-KF-6	44 ml
RNase A Solution (25 mg/ml)	27000-4-KF-7	2 x 1 ml
PowerMag® 1 ml Collection Plates	27000-4-KF-1CP	8
Sealing Tape	27000-4-KF-ST	32

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 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

Please wear gloves 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. Taking care to avoid any residual pellet, transfer no more than **450 µl** of supernatant from each well to the wells on a clean KingFisher® Deep Well 96 Plate. If you wish to use the remaining 400 µl of supernatant left in each well you should transfer them to another KingFisher® Deep Well 96 Plate and store at 4°C until they can be processed on the robot.

    Note: Any prepared lysates at this point that cannot be processed immediately on the robot should be transferred to and stored in clean KingFisher® Deep Well 96 Plates at 4°C.

12. Open the appropriate protocol on your instrument specific to your platform and then proceed. For the KingFisher® Flex go to page 8. For the KingFisher® Duo go to page 9.

KingFisher® Flex Protocol (continued from step 12)

13. For each 96 well plate to be processed, resuspend the **ClearMag® Beads** by vortexing the bottle and add 2 ml of the resuspended **ClearMag® Beads** to 45 ml of the **ClearMag® Binding Solution** in an appropriate vessel (user provided) and mix well. Immediately transfer to a multi channel reservoir.

    Note: As time progresses the **ClearMag® Beads/ClearMag® Binding Solution** will slowly settle. Maintain the beads in suspension for uniform distribution to each well in the next step.

14. Add 470 µl of the **ClearMag® Beads/ClearMag® Binding Solution** to each well of lysate in a KingFisher® Microtiter Deep Well 96 Plate.

15. Place the KingFisher® Microtiter Deep Well 96 Plate containing the lysate and ClearMag® Beads/ ClearMag® Binding Solution onto the robotic deck at the specified location indicated in the program.

16. Place 500 µl of **ClearMag® Wash Solution** into each well of three clean KingFisher® Microtiter Deep Well 96 plates and place on the deck at the specified locations indicated in the program.

17. Place 100 µl of **ClearMag® Elution Buffer** into each well of a KingFisher® 96 KF plate and place on the deck at the specified location. Initiate the robotic program.

18. Upon completion of the robotic program, cover the wells of the KingFisher® 96 KF plate with an appropriate storage seal. 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 is 10 mM Tris pH 8.0 and does not contain EDTA.

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

KingFisher® Duo Protocol (continued from step 12)

13. Transfer lysate from up to twelve (12) wells to the first long row (A) on a clean KingFisher® Microtiter Deep Well 96 Plate.

14. Add 450 µl of the **ClearMag® Binding Solution** to each well in row (A) containing lysate.

15. Prepare the **ClearMag® Beads** by vortexing the bottle to resuspend**.** Immediately add 20 µl of the resuspended **ClearMag® Beads** to each well containing the lysate/ClearMag® Binding Solution mixture**.**

    Note: The beads will slowly settle so it is critical to make sure the beads stay in suspension.

16. Place a KingFisher® Duo 12-tip comb into the second row (B) of the KingFisher® Microtiter Deep Well 96 Plate.

17. Place 500 µl of **ClearMag® Wash Solution** into each well of the next three rows (C, D & E) on the plate and place onto the deck.

18. Place 100 µl of **ClearMag® Elution Buffer** into each well of a KingFisher® Duo Elution Strip and place the strip onto the deck.

19. Initiate the KingFisher® MO BIO PowerMag® Soil robotic program.

20. Upon completion of the robotic program, cover the wells of the KingFisher® Duo Elution Strip with an appropriate storage seal. 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 is 10 mM Tris pH 8.0 and 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 the 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, dessicator, 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

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 Microplates 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:
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Email: technical@mobio.com
Fax: 760-929-0109
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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