PowerMax® Soil DNA Isolation Kit

Catalog No.	Quantity
12988-10	10 Preps

Instruction Manual

Inhibitor Removal Technology® (IRT) is a registered trademark of MO BIO Laboratories, Inc. and is covered by the following patents USA US 7,459,548 B2, Australia 2005323451, Japan 5112064 and India 246946.

Please recycle

Version: 07272016

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Introduction

The PowerMax® Soil DNA Isolation Kit provides researchers with a novel and proprietary method for isolating genomic DNA from environmental samples utilizing our patented Inhibitor Removal Technology® (IRT). With this kit, it is possible to process samples that have in the past proven difficult due to high levels of humic like substances. The isolated DNA has a high level of purity allowing for successful PCR amplification from the sample. Total DNA isolated from various soil types has been successfully amplified in PCR with primers specific for bacteria (*Bacillus subtilis, Bacillus anthracis*), fungi (yeast, mold), and Actinomycetes (*Streptomyces*).

The PowerMax® Soil DNA Isolation Kit distinguishes itself from MO BIO Laboratories UltraClean® Mega Soil DNA Isolation Kit with a humic substance/brown color removal procedure. This procedure is effective at removing PCR inhibitors from even the most difficult soil types, including compost, sediment and manure.

Protocol Overview

Using this kit, environmental samples are added to a bead beating tube with a kit supplied proprietary buffer for rapid and thorough homogenization. Cell lysis and DNA exposure occurs by mechanical and chemical methods. Extracted genomic DNA is captured on a silica membrane in a spin column format. DNA is washed and eluted from the membrane and DNA is ready for PCR and other downstream applications.

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

Other Related Products	Catalog No.	Quantity
PowerSoil® DNA Isolation Kit	12888-50 12888-100	50 Preps 100 Preps
UltraClean® PCR Clean-Up Kit	12500-50 12500-100 12500-250	50 preps 100 preps 250 preps
Vortex Adapters, holds 24 (1.5-2.0 ml) tubes	13000-V1-24	1 unit

Equipment Required

Centrifuge capable of spinning 50 ml tubes (2500 x *g*)
Pipettes (1 ml and 10 ml)
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)
Vortex Adapter (Catalog# 13000-V1-50 for a Vortex Genie 2 or 13000-LV2-50 for Labnet Vortex)

Kit Contents

	Kit Catalog# 12988-10	
Component	**Catalog #**	**Amount**
PowerMax® Soil PowerBead Tubes	12988-10-PBT	10
PowerMax® Soil PowerBead Solution	12988-10-BS	165 ml
PowerMax® Soil Solution C1	12988-10-1	14 ml
PowerMax® Soil Solution C2	12988-10-2	55 ml
PowerMax® Soil Solution C3	12988-10-3	44 ml
PowerMax® Soil Solution C4	12988-10-4	330 ml
PowerMax® Soil Solution C5	12988-10-5	120 ml
PowerMax® Soil Solution C6	12988-10-6	55 ml
PowerMax® Soil Spin Filters (units in 50 ml tubes)	12988-10-SF	10
PowerMax® Soil Collection Tubes (50 ml)	12988-10-T	40

Kit Storage

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.

WARNING: Solution C5 contains ethanol. It is flammable.

IMPORTANT NOTE FOR USE: Shake to mix Solution C4 before use.

Experienced User Protocol
Please wear gloves at all times

1. Add 15 ml of **PowerBead Solution** to a **PowerBead Tube**. **These tubes will now be referred to as PowerMax® Bead Solution Tubes.**
2. Add up to 10 g of soil sample to **PowerMax® Bead Solution Tube**. Vortex vigorously for 1 minute.
   Note: Please refer to the Hints and Troubleshooting Guide before deciding on the amount of soil to process.
3. Check **Solution C1.** If **Solution C1** is precipitated, heat the solution at 60°C until the precipitate has dissolved. Add 1.2 ml of **Solution C1** to the **PowerMax® Bead Solution Tube** and vortex vigorously for 30 seconds.
4. Place **PowerMax® Bead Solution Tubes** on the MO BIO Laboratories, Inc. Vortex Adapter (MO BIO Catalog# 13000-V1) and vortex for 10 minutes at the highest speed. *Alternatively, you can place the tubes in a shaking water bath set at 65°C and shake at maximum speed for 30 minutes.*
5. Centrifuge tubes at 2500 x *g* for 3 minutes at room temperature.
6. Transfer supernatant to a clean **Collection Tube** (provided). The supernatant may still contain some soil particles and color.
7. Add 5 ml of **Solution C2** and invert twice to mix. Incubate at 4°C for 10 minutes.
8. Centrifuge tubes at 2500 x *g* for 4 minutes at room temperature.
9. Avoiding pellet, transfer supernatant to a clean **Collection Tube** (provided).
10. Add 4 ml of **Solution C3** and invert twice to mix. Incubate at 4°C for 10 minutes.
11. Centrifuge tubes at 2500 x *g* for 4 minutes at room temperature.
12. Avoiding pellet, transfer supernatant to a clean **Collection Tube** (provided).
13. Shake to mix Solution C4. Add 30 ml of **Solution C4** to supernatant and invert twice.
14. This step requires <u>three</u> centrifugations. First, fill **Spin Filter** with solution from Step 13. Centrifuge at 2500 x *g* for 2 minutes at room temperature. Discard flow through and add second volume of supernatant to same **Spin Filter** and centrifuge at 2500 x *g* for 2 minutes at room temperature. Discard flow through. Repeat until entire volume has been processed.
15. Add 10 ml of **Solution C5** to **Spin Filter** and centrifuge at 2500 x *g* for 3 minutes at room temperature. Discard flow through.
16. Centrifuge **Spin Filter** at 2500 x *g* for 5 minutes at room temperature.
17. Carefully place **Spin Filter** in a new **Collection Tube** (provided). Avoid splashing **Solution C5** onto **Spin Filter**.
18. Add 5 ml of sterile **Solution C6** to the center of **Spin Filter** membrane and centrifuge at 2500 x *g* for 3 minutes at room temperature.
19. Discard **Spin Filter**. The DNA in the tube is now ready for any downstream application. No further steps are required.

We recommend storing DNA frozen (-20°C). **Solution C6** does not contain EDTA. To concentrate DNA see the Hints and Troubleshooting Guide.

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

Detailed Protocol (Describes what is happening at each step)
Please wear gloves at all times

1. Add 15 ml of **PowerBead Solution** to a **PowerMax® Bead Tube**. **These tubes will now be referred to as PowerMax® Bead Solution Tubes.**

2. Add up to 10 g of soil sample to the **PowerMax® Bead Solution Tube**. Vortex vigorously for 1 minute to mix.
   Note: Please refer to Hints and Troubleshooting Guide before deciding on the amount of soil to process.

   What's happening: There are many types of soil. Be sure to get a clear idea of how much soil to process before beginning the DNA isolation procedure. After your sample has been loaded into the PowerMax™ Bead Solution Tube, the next step is homogenization and lysis. The PowerMax™ Bead Solution Tube contains a buffer that will (a) help disperse the soil particles, (b) begin to dissolve humic acids and (c) protect nucleic acids from degradation. Vortexing mixes the components in the PowerMax™ Bead Solution Tube and begins to disperse the sample in the Solution.

3. Check **Solution C1.** If **Solution C1** is precipitated, heat the solution to 60°C until the precipitate has dissolved before use. Add 1.2 ml of **Solution C1** to a **PowerMax® Bead Solution Tube** and vortex vigorously for 30 seconds.

   What's happening: Solution C1 contains SDS and other disruption agents required for complete cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. If it gets cold, it will form a white precipitate in the bottle. Heating to 60°C will dissolve the SDS and will not harm the SDS or the other disruption agents. Solution C1 can be used while it is still warm.

4. Place the **PowerMax® Bead Solution Tubes** on the MO BIO Laboratories, Inc. Vortex Adapter (MO BIO Catalog# 13000-V1) and vortex for 10 minutes at highest speed.

   Note: *The vortexing step is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from steps 1-3 and mechanical shaking introduced at this step. By randomly shaking the beads in the presence of disruption agents, collision of the beads with microbial cells will cause the cells to break open.*

   What's happening: The MO BIO Laboratories, Inc. Vortex Adapter is designed to be a simple platform to facilitate keeping the tubes tightly attached to the vortex. It should be noted that although you can attach tubes to a vortex with tape, often the tape becomes loose and not all tubes will shake evenly or efficiently. This may lead to inconsistent results or lower DNA yield. Therefore, the use of the MO BIO Laboratories, Inc. Vortex Adapter is a highly recommended and cost effective way to obtain maximum DNA yields.

   Alternatively, you can place the tubes in a shaking water bath set at 65°C and shake at maximum speed for 30 minutes.

5. Centrifuge the tubes at 2500 x *g* for 3 minutes at room temperature.

6. Transfer the supernatant to a clean **Collection Tube** (provided). The supernatant may still contain some soil particles and color.

Note: *The supernatant volume may vary with soil type and may also be dark in appearance due to soil particles and humic substance carry-over. The presence of carry-over soil or a dark color in the mixture is expected in many soil types at this step. Subsequent steps in the protocol will remove both carry-over soil and coloration.*

7. Add 5 ml of **Solution C2** to the supernatant and invert twice to mix. Incubate at 4°C for 10 minutes.

   What's happening: Solution C2 is patented Inhibitor Removal Technology® (IRT). It contains a reagent to precipitate non-DNA organic and inorganic material, including humic substances, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

8. Centrifuge the tubes at 2500 x *g* for 4 minutes at room temperature.

9. Avoiding the pellet, transfer the supernatant to a clean **Collection Tube** (provided).

   What's happening: The pellet at this point contains non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yield and quality, avoid transferring any of the pellet.

10. Add 4 ml of **Solution C3** to the supernatant and invert twice to mix. Incubate at 4°C for 10 minutes.

    What's happening: Solution C3 is patented Inhibitor Removal Technology® (IRT) and is a second reagent to precipitate additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

11. Centrifuge the tubes at 2500 x *g* for 4 minutes at room temperature.

12. Avoiding the pellet, transfer supernatant to a clean **Collection Tube** (provided).

    What's happening: The pellet contains additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yield and quality, avoid transferring any of the pellet.

13. Shake to mix Solution C4. Add 30 ml of **Solution C4** to the supernatant and invert twice.

    What's happening: Solution C4 is a high concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, this will adjust the DNA solution salt concentration to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the Spin Filters.

14. This step requires <u>three</u> centrifugations. First, fill the **Spin Filter** with solution from Step 13. Centrifuge at 2500 x *g* for 2 minutes at room temperature. Discard the flow through and add a second volume of supernatant to the same **Spin Filter** and centrifuge at 2500 x *g* for 2 minutes at room temperature. Discard the flow through. Repeat until the entire volume has been processed.

    What's happening: DNA is selectively bound to the silica membrane in the spin filter device in the high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.

15. Add 10 ml of **Solution C5** to the **Spin Filter** and centrifuge at 2500 x *g* for 3 minutes. Discard the flow through.

    What's happening: Solution C5 is an ethanol based wash solution used to further clean the DNA that is bound to the silica filter membrane in the Spin Filter. This wash solution removes residual salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane.

16. Centrifuge the **Spin Filter** at 2500 x *g* for 5 minutes at room temperature.

    Note: *The second centrifugation removes residual Solution C5 (ethanol wash solution). It is critical to remove all traces of wash solution because the ethanol in Solution C5 can interfere with many downstream DNA applications such as PCR, restriction digests and gel electrophoresis.*

17. Carefully place the **Spin Filter** in a new **Collection Tube** (provided). Avoid splashing **Solution C5** onto the **Spin Filter**.

18. Add 5 ml of **Solution C6** to the center of the **Spin Filter** membrane. Centrifuge at 2500 x g for 3 minutes at room temperature.

    Note: *Placing Solution C6 (sterile elution buffer) in the center of the white membrane will ensure the entire membrane is wetted. This will result in a more efficient and complete release of the DNA from the silica Spin Filter membrane. As Solution C6 (elution buffer) passes through the silica membrane, DNA that was bound in the presence of high salt (Steps 13 and 14) is selectively released by Solution C6 (10mM Tris) which lacks salt.*

    Alternatively, sterile DNA-Free PCR Grade Water may be used for DNA elution from the silica Spin Filter membrane at this step (MO BIO Laboratories, Inc., Catalog No. 17000-10). Solution C6 contains no EDTA. If DNA degradation is a concern, sterile TE may also be used instead of Solution C6 for elution of DNA from the Spin Filter.

19. Discard the **Spin Filter**. The DNA in the tube is now ready for any downstream application. No further steps are required.

    We recommend storing DNA frozen (-20°C). **Solution C6** does not contain EDTA. To concentrate DNA see the Hints and Troubleshooting Guide.

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

Hints and Troubleshooting Guide

Concentrating the DNA

The final volume of eluted DNA will be 5 ml. The DNA may be concentrated by adding 0.2 ml of 5M NaCl and inverting 3-5 times to mix. Next, add 10.4 ml of 100% cold ethanol and invert 3-5 times to mix. Centrifuge at 2500 x *g* for 30 minutes at room temperature. Decant all liquid. (If sterile DNA is desired, wash the DNA pellet with 70% cold ethanol. Be sure not to disturb the pellet.) Remove residual ethanol in a speed vac, desiccator, or ambient air. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.

Amount of Soil to Process

The amount of soil to process will depend on the soil type. 5 g is typically recommended, although dry soils may require less starting material (*1 g*) and wet soils may require more (*up to 10 g*). For mulch and potting mixtures, we recommend up to 2.5 g and for composts up to 5.0 g. Up to 10 g of sandy soil may be processed.

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) may be needed.

DNA Floats Out of Well When Loaded on a Gel

This usually occurs because residual Solution C5 remains in the final sample. Prevent this by being careful in step 17 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation (described in “Concentrating the DNA”) is the best way to remove residual Solution C5.

Storing DNA

DNA is eluted in Solution C6 (10mM 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 that has been eluted into sterile water should be stored at -70°C.

Contact Information

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For the distributor nearest you, visit our web site 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
PowerSoil® DNA Isolation Kit	12888-50 12888-100	50 preps 100 preps
RNA PowerSoil® Total RNA Isolation Kit	12866-25	25 preps
PowerClean® DNA Clean-Up Kit	12877-50	50 preps
Vortex-Genie® 2 Vortex	13111-V 13111-V-220	1 unit (120V) 1 unit (220V)
Vortex Adapter for Vortex Genie® 2	13000-V1-50	Holds 2 (50ml) Tubes
UltraClean® PCR Clean-Up Kit	12500-50 12500-100 12500-250	50 preps 100 preps 250 preps