PowerSoil™ DNA Isolation Kit

Catalog No.	Quantity
12888-50	50 Preps
12888-100	100 Preps

Instruction Manual

Introduction

The PowerSoil™ DNA Isolation Kit* is comprised of a novel and proprietary method for isolating genomic DNA from environmental samples. The kit is intended for use with environmental samples containing a high humic acid content including difficult soil types such as compost, sediment, and manure. Other more common soil types have also been used successfully with this kit. The isolated DNA has a high level of purity allowing for more successful PCR amplification of organisms from the sample. PCR analysis has been performed to detect a variety of organisms including bacteria (e.g. *Bacillus subtilis, Bacillus anthracis*), fungi (e.g. yeasts, molds), algae and Actinomycetes (e.g. *Streptomyces*).

The PowerSoil™ DNA Isolation Kit distinguishes itself from MO BIO's UltraClean™ Soil DNA Isolation Kit with a **NEW** humic substance/brown color removal procedure. This new procedure is effective at removing PCR inhibitors from even the most difficult soil types.

Environmental samples are added to a bead beating tube for rapid and thorough homogenization. Cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the membrane. DNA is then ready for PCR analysis and other downstream applications.

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

***PATENT PENDING**

Version: 05182007

Technical Information: Toll free 1-800-606-6246, or 1-760-929-9911 Email: technical@mobio.com

Required Equipment:

Microcentrifuge (10,000 x *g*)
Pipettors (50 µl - 500 µl)
Vortex
Vortex Adapter (MO BIO Catalog # 13000-V1)

Kit Contents

	Kit Catalog # 12888-50		Kit Catalog # 12888-100	
Component	**Catalog #**	**Amount**	**Catalog #**	**Amount**
PowerBead Tubes (contain 750µl solution)	12888-50-PBT	50	12888-100-PBT	100
PowerSoil Solution C1	12888-50-1	3.3 ml	12888-100-1	6.6 ml
PowerSoil Solution C2	12888-50-2	14 ml	12888-100-2	28 ml
PowerSoil Solution C3	12888-50-3	11 ml	12888-100-3	22 ml
PowerSoil Solution C4	12888-50-4	72 ml	12888-100-4	144 ml
PowerSoil Solution C5	12888-50-5	30 ml	12888-100-5	2 x 30 ml
PowerSoil Solution C6	12888-50-6	6 ml	12888-100-6	12 ml
PowerSoil Spin Filters (units in 2 ml tubes)	12888-50-SF	50	12888-100-SF	100
PowerSoil 2 ml Collection Tubes	12888-50-T	200	12888-100-T	400

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: Make sure the 2 ml PowerBead Tubes rotate freely in your centrifuge without rubbing.

Experienced User Protocol

Please wear gloves at all times

1. To the PowerBead Tubes provided, add 0.25 gm of soil sample.
2. Gently vortex to mix.
3. **Check Solution C1.** If Solution C1 is precipitated, heat solution to 60°C until dissolved before use.
4. Add 60µl of Solution C1 and invert several times or vortex briefly.
5. Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog No. 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.
6. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds at room temperature. **CAUTION:** Be sure not to exceed 10,000 x *g* or tubes may break.
7. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect between 400 to 500µl of supernatant. Supernatant may still contain some soil particles.
8. Add 250µl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.
9. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g.*
10. Avoiding the pellet, transfer up to, but no more than, 600µl of supernatant to a clean 2 ml Collection Tube (provided).
11. Add 200µl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes.
12. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g.*
13. Avoiding the pellet, transfer up to, but no more than, 750µl of supernatant into a clean 2 ml Collection Tube (provided).
14. Add 1200µl of Solution C4 to the supernatant and vortex for 5 seconds.
15. Load approximately 675µl onto a Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow through and add an additional 675µl of supernatant to the Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. **Note**: A total of three loads for each sample processed are required.
16. Add 500µl of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x *g*.
17. Discard the flow through.
18. Centrifuge again at room temperature for 1 minute at 10,000 x *g*.
19. Carefully place Spin Filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the Spin Filter.
20. Add 100µl of Solution C6 to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog No. 17000-10).
21. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.
22. 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° to -80°C). Solution C6 contains no EDTA. To concentrate the DNA see the Additional Information Section.

Thank you for choosing the PowerSoil™ DNA Isolation Kit.

Detailed Protocol

Please wear gloves at all times

1. To the PowerBead Tubes provided, add 0.25 gm of soil sample.

   After your sample has been loaded into the PowerBead Tube, the next step is a homogenization and lysis procedure. The PowerBead 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.

2. Gently vortex to mix.

   Gentle vortexing mixes the components in the PowerBead Tube and begins to disperse the sample in the PowerBead Solution.

3. **Check Solution C1**. If Solution C1 is precipitated, heat solution to 60°C until the precipitate has dissolved before use.

   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. Add 60µl of Solution C1 and invert several times or vortex briefly.

5. Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO Catalog No. 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes.

   Note: *The vortexing step is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from steps 1-4 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.*

   The MO BIO 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 with tape, often the tape becomes loose and not all tubes will shake evenly or efficiently. This may lead to inconsistent results or lower yields. Therefore, the use of the MO BIO Vortex Adapter is a highly recommended and cost effective way to obtain maximum DNA yields.

6. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds at room temperature. **CAUTION:** Be sure not to exceed 10,000 x *g* or tubes may break.

7. Transfer the supernatant to a clean 2 ml Collection Tube (provided).

   Note: *Expect between 400 to 500µl of supernatant at this step. The exact recovered volume depends on the absorbancy of your starting material and is not critical for the procedure to be effective. The supernatant may be dark in appearance and still contain some soil particles. 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 of the mixture.*

8. Add 250µl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes.

   Solution C2 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.

9. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g.*

10. Avoiding the pellet, transfer up to 600µl of supernatant to a clean 2 ml Collection Tube (provided).

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

11. Add 200µl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes.

    Solution C3 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.

12. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g.*

13. Transfer up to 750µl of supernatant to a clean 2 ml Collection Tube (provided).

    The pellet at this point contains additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellet.

14. Add 1.2ml of Solution C4 to the supernatant (be careful solution doesn't exceed rim of tube) and vortex for 5 seconds.

    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 concentrations 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.

15. Load approximately 675µl onto a Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow through and add an additional 675µl of supernatant to the Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 x *g* for 1 minute at room temperature. **Note**: A total of three loads for each sample processed are required.

    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.

16. Add 500µl of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x *g*.

    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.

17. Discard the flow through from the 2 ml Collection tube.

    This flow through fraction is just non-DNA organic and inorganic waste removed from the silica Spin Filter membrane by the ethanol wash solution.

18. Centrifuge at room temperature for 1 minute at 10,000 x *g*.

    This second spin 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.

19. Carefully place Spin Filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the Spin Filter.

    Note: *It is important to avoid any traces of the ethanol based wash solution.*

20. Add 100µl of Solution C6 to the center of the white filter membrane.

    Note: *Placing the Solution C6 (sterile elution buffer) in the center of the small white membrane will make sure 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 is selectively released by Solution C6 (10 mM 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 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.

21. Centrifuge at room temperature for 30 seconds at 10,000 x *g*.

22. 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° to -80°C). Solution C6 does not contain any EDTA. To concentrate DNA see the Additional Information Section.

Thank you for choosing the PowerSoil™ DNA Isolation Kit.

Additional Information

Amount of Soil to Process
This kit is designed to process 0.25 g of soil. For inquiries regarding the use of larger sample amounts, please contact technical support for suggestions. For wet soils, see information under "Wet Soil Sample" below.

Wet Soil Sample
If soil sample is high in water content, remove contents from PowerBead Tube (beads and solution) and transfer into another sterile microcentrifuge tube (not provided). Add soil sample to PowerBead Tube and centrifuge at room temperature for 30 seconds at 10,000 x g. Remove as much liquid as possible with a pipet tip. Add beads and bead solution back to PowerBead Tube and follow protocol starting at step 2.

If DNA Does Not Amplify
- Make sure to check DNA yields by gel electrophoresis or spectrophotometer reading. An excess amount of DNA will inhibit a PCR reaction.
- Diluting the template DNA should not be necessary with DNA isolated with the PowerSoil DNA Isolation Kit; however, it should still be attempted.
- If DNA will still not amplify after trying the steps above, then PCR optimization (changing reaction conditions and primer choice) may be needed.

Eluted DNA Sample Is Brown
We have not observed any coloration in DNAs isolated using the PowerSoil DNA Isolation kit. If you observe coloration in your samples, please contact technical support for suggestions.

Alternative Lysis Methods
- After adding Solution C1, vortex 3-4 seconds, then heat to 70°C for 5 minutes. Vortex 3-4 seconds. Heat another 5 minutes. Vortex 3-4 seconds. This alternative procedure will reduce shearing but may also reduce yield.
- If cells are difficult to lyse, a 10 minute incubation at 70°C, after adding Solution C1, can be performed. Follow by continuing with protocol step 5.

Concentrating the DNA
The final volume of eluted DNA will be 100µl. The DNA may be concentrated by adding 4µl of 5M NaCl and inverting 3-5 times to mix. Next, add 200µl of 100% cold ethanol and invert 3-5 times to mix. Centrifuge at 10,000 x g for 5 minutes at room temperature. Decant all liquid. Remove residual ethanol in a speed vac, dessicator, or air dry. Resuspend precipitated DNA in sterile water or sterile 10 mM Tris.

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 19 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) and must be stored at -20° to -80°C to prevent degradation. DNA can be eluted in TE without 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 No. 17000-10).

Other Quality Products Available from MO BIO Laboratories, Inc.

Product Description	Catalog No.
DNA Isolation Kits	
UltraClean™ Soil DNA Isolation Kit (50 preps)	12800-50
UltraClean™ Mega Soil DNA Isolation Kit (10 preps)	12900-10
UltraClean-htp™ 96 Well Soil DNA Isolation Kit (4 x 96 preps)	12896-4
UltraClean™ Fecal DNA Isolation Kit (50 preps)	12811-50
UltraClean™ Microbial DNA Isolation Kit (50 preps)	12224-50
RNA Isolation Kits	
UltraClean™ Microbial RNA Isolation Kit (50 preps)	15800-50
DNA Purification Kits	
UltraClean™ 15 DNA Purification Kit (300 preps)	12100-300
UltraClean™ GelSpin™ DNA Extraction Kit (100 preps)	12400-100
UltraClean™ PCR Clean-Up Kit (100 preps)	12500-100

Contact Information

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