



# UltraClean™ Mega Soil DNA Kit

Catalog number: 12900-10  
10 Preps (For up to 10 grams of soil)

## Instruction Manual

### Introduction

Use this kit for isolating DNA from 5-10 g soil samples. The basic procedure is to lyse the microorganisms in the soil by a combination of heat, detergent, and mechanical force against specialized beads. The released DNA is then bound to a spin filter. The filter is washed and then PCR quality DNA is released into a buffer.

### Precautions

Please wear gloves when using this product. Avoid all skin contact with reagents in this kit. 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 on our web site at [www.mobio.com](http://www.mobio.com). Reagents labeled flammable should be kept away from open flames and sparks.

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

### Equipment required:

Shaking water bath set at 65°C. Centrifuge capable of spinning 50 ml tubes (2500 x g)

**Pipettes (volumes required 1 ml and 10 ml), vortex**

### Kit Contents (10 PREPS)

<u>Description</u>	<u>Amt.</u>
Mega Bead Tubes	10
Bead Solution	165 ml
Solution S1	14 ml
Solution IRS	44 ml
Solution S2	22 ml
Solution S3	330 ml
Solution S4	2x30 ml
Solution S5	88 ml
Spin filters units in 50 ml tubes	10
Collection tubes (50 ml)	30

### Kit Storage

Room temperature

Version: 01102007

Technical information: Toll free 1-800-606-6246, or 1-760-929-9911 email: [technical@mobio.com](mailto:technical@mobio.com)



## Experienced User Protocol

### Please wear gloves at all times

1. Adjust a shaking water bath to 65°C before starting (see step 8).
2. **Each prep will require you to add 15 ml of Bead Solution to a 50 Mega Bead Tube. These tubes will now be referred to as Bead Solution Tubes.**
3. Add 5.0 g-10 g of soil sample to a Bead Solution Tube.
4. Vortex vigorously for 1 minute to mix.
5. **Check Solution S1.** If Solution S1 is precipitated, heat solution to 60°C until dissolved before use.
6. Add 1.2 ml of Solution S1 and vortex vigorously 30 seconds.
7. Add 4 ml of Solution IRS (Inhibitor Removal Solution). Only required if DNA is to be used for PCR.
8. Place the 50 ml Bead Solution Tubes on the MO BIO Vortex Adapter, catalog number 13000-V1-50 for a Genie 2 Vortex, or catalog number, 13000-LV2-50, for a Labnet vortex, and vortex for 10 minutes at 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.
9. Centrifuge tubes at 2500 x g for 3 minutes.
10. Transfer the supernatant to a clean centrifuge tube (provided).
11. Supernatant may still contain some soil particles.
12. Add 2 ml of Solution S2, invert twice to mix.
13. Incubate at 4°C for 10 minutes.
14. Centrifuge the tubes for 4 minutes at 2500 x g.
15. Avoiding the pellet, transfer supernatant to a clean centrifuge tube (provided).
16. Add 30 ml of Solution S3 to the supernatant and invert twice.
17. This step requires two spins. First fill Spin Filter with solution from step 16. Centrifuge at 2500 x g for 2 minutes. Discard the flow through and add the remaining supernatant to the same Spin Filter and centrifuge at 2500 x g for 2 minutes. Discard flow through.
18. Add 6 ml of Solution S4 and centrifuge for 3 minutes at 2500 x g.
19. Discard the flow through.
20. Centrifuge again at 2500 x g for 5 minutes.
21. Carefully place Spin Filter in a new clean tube (provided). Avoid splashing any Solution S4 onto the spin filter.
22. Add 8 ml of Solution S5 to the center of Spin Filter membrane.
23. Centrifuge for 3 minutes at 2500 x g.
24. Discard Spin Filter. DNA in the tube is now application ready. No further steps are required.

We recommend storing DNA frozen (-20°C). Solution S5 contains no EDTA. To concentrate the DNA see the Additional Information Section.

**Thank you for choosing the UltraClean™ Mega Soil DNA Kit.**



## Detailed Protocol (Describes each step)

Please wear gloves at all times

1. Adjust a shaking water bath to 65°C before starting (see step 8).
2. **Each prep will require you to add 15 ml of Bead Solution to a 50 Mega Bead Tube. These tubes will now be referred to as Bead Solution Tubes.**
3. Add 5.0 g-10 g of soil sample to a Bead Solution Tube. (For amounts of sample to process see Additional Information Section).  
*What's happening: The soil or fecal sample has now been loaded into the Bead Tube. This is the first part of the lysis procedure. The Bead Solution is a buffer that will disperse the soil particles and begin to dissolve humic acids.*
4. Vortex vigorously for 1 minute to mix.  
*What's happening: This step mixes the sample and Bead Solution.*
5. **Check Solution S1.** If Solution S1 is precipitated, heat solution to 60°C until dissolved before use.  
*What's happening: Solution S1 contains SDS. If it gets cold, it will precipitate. Heating to 60°C will dissolve the SDS. The Solution S1 can be used while it is still warm.*
6. Add 1.2 ml of Solution S1 and vortex vigorously 30 seconds.  
*What's happening: Solution S1 contains SDS. This is a detergent that aids in cell lysis. The detergent breaks down fatty acids and lipids associated with the cell membrane of several organisms.*
7. Add 4 ml of Solution IRS (Inhibitor Removal Solution). Only required if DNA is to be used for PCR.  
*What's happening: IRS is a proprietary reagent designed to precipitate humic acids and other PCR inhibitors. This precipitation step is required if the intended use of the DNA is for PCR. Humic acids are generally brown in color. They belong to a large group of organic compounds associated with most soils that are high in organic content.*
8. Place the 50 ml Bead Solution Tubes on the MO BIO Vortex Adapter, catalog number 13000-V1-50 for a Genie 2 Vortex, or catalog number, 13000-LV2-50, for a Labnet vortex, and vortex for 10 minutes at 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.  
*What's happening: The method you use to secure tubes to the vortex is critical. We have designed the vortex adapter as a simple tool that keeps 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. The use of the vortex adapter is highly recommended for maximum DNA yields.  
Mechanical lysis is introduced at this step. The protocol uses a combination of mechanical and chemical lysis. By randomly shaking the beads, they collide with one another and with microbial cells causing them to break open.*
9. Centrifuge tubes at 2500 x g for 3 minutes.  
*What's happening: Particulates including cell debris, soil, beads, and humic acids, will form a pellet at this point. DNA is in the liquid supernatant at this stage.*
10. Transfer the supernatant to a clean centrifuge tube (provided).
11. Supernatant may still contain some soil particles.
12. Add 2 ml of Solution S2, invert twice to mix.

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13. Incubate at 4°C for 10 minutes.

*What's happening: Solution S2 contains a protein precipitation reagent. It is important to remove contaminating proteins that may reduce DNA purity and inhibit downstream applications for the DNA.*

14. Centrifuge the tubes for 4 minutes at 2500 x g.

15. Avoiding the pellet, transfer supernatant to a clean centrifuge tube (provided).

*What's happening: The pellet at this point contains residues of humic acid, cell debris, and proteins. For the best DNA yields, and quality, avoid transferring any of the pellet.*

16. Add 30 ml of Solution S3 to the supernatant and invert twice.

*What's happening: Solution S3 is a DNA binding salt solution. DNA binds to silica in the presence of high salt concentrations.*

17. This step requires two spins. First fill Spin Filter with solution from step 16. Centrifuge at 2500 x g for 2 minutes. Discard the flow through and add the remaining supernatant to the same Spin Filter and centrifuge at 2500 x g for 2 minutes. Discard flow through.

*What's happening: DNA is selectively bound to the silica membrane in the Spin Filter device. Almost all contaminants pass through the filter membrane, leaving only the desired DNA behind.*

18. Add 6 ml of Solution S4 and centrifuge for 3 minutes at 2500 x g.

*What's happening: Solution S4 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 residues of salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane. Note: You can wash more than one time to further clean DNA if desired. In some cases where soils have very high humic acid content, it will be beneficial to repeat this wash step. There is 10% extra Solution S4 in the bottle for this purpose. Solution S4 is also sold separately.*

19. Remove the Spin Filter and discard the flow through.

*What's happening: This flow through is just waste containing ethanol wash solution and contaminants that did not bind to the silica Spin Filter membrane.*

20. Replace the Spin Filter and centrifuge again for 5 minutes.

*What's happening: This step removes residual Solution S4 (ethanol wash solution). It is critical to remove all traces of wash solution because it can interfere with downstream applications for the DNA.*

21. Carefully place Spin Filter in a new clean tube (provided). Avoid splashing any Solution S4 onto the Spin Filter.

*What's happening: Once again it is important to avoid any traces of the ethanol based wash solution.*

22. Add 8 ml of Solution S5 to the center of Spin Filter membrane.

*What's happening: Placing the Solution S5 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in more efficient release of the desired DNA.*

23. Centrifuge for 3 minutes at 2500 x g.

*What's happening: As the Solution S5 (elution buffer) passes through the silica membrane, DNA is released, and it flows through the membrane, and into the collection tube. The DNA is released because it can only bind to the silica Spin Filter membrane in the presence of salt. Solution S5 is 10mM Tris pH. 8 and does not contain salt.*

24. Discard Spin Filter. DNA in the tube is now application ready. No further steps are required.

We recommend storing DNA frozen (-20°C). Solution S5 contains no EDTA.

**Thank you for choosing the UltraClean™ Mega Soil DNA Kit.**

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## **Additional Information**

### **Concentrating the DNA**

The final volume will be 8 ml. If this is too dilute for your purposes, add 0.32 ml of 5M NaCl and mix. Then add 16.6 ml of 100% cold ethanol. Mix. Centrifuge at 2500 x g for 30 minutes. Decant all liquid. Dry residual ethanol in a speed vac or dessicator or ambient air. Resuspend precipitated DNA in desired volume.

### **Amount of soil to process**

Depends on soil type. Usually 5.0 g - 10 g works well.

### **If DNA does not amplify**

This is due to high humic acid content in soil sample. If the humic acid content in sample is high, you can do the following:

- Diluting template DNA may also work because this will also dilute the inhibitors of the reaction.
- Perform two to three washes of Solution S4 in step 18.
- Dilute the elution three fold and add two volumes of Solution S3. Run through spin filter, wash and elute.
- Make sure to check DNA yields by gel electrophoresis or spectrophotometer reading. An excess amount of DNA will also inhibit a PCR reaction.
- If DNA will still not amplify after trying the steps above, then PCR optimization may be needed.

### **DNA floats out of well when loaded on a gel**

Residual Solution S4 in the final sample. Prevent this by being careful in step 20 not to transfer liquid onto the bottom of the Spin Filter basket. Ethanol precipitation is the best way to remove residues of Solution S4.

### **Storing DNA**

DNA is eluted in Solution S5 (10mM Tris) therefore it must be stored at -20°C or it may degrade. DNA can be eluted in TE but the EDTA may inhibit reactions such as PCR and automated sequencing.



## Other quality products available from MO BIO Laboratories, Inc.

<u>Kit description</u>	<u>Cat. number</u>
<b>Plasmid Prep Kits</b>	
6 minute Mini Plasmid Prep Kit (100 preps)	12300-100
6 minute Mini Plasmid Prep Kit (250 preps)	12300-250
25-50 ml Plasmid Prep Kit (20 preps)	12700-20
25-50 ml Plasmid Prep Kit (50 preps)	12700-50
250-500 ml Plasmid Prep Kit (10 preps)	12600-10
250-500 ml Plasmid Prep Kit (20 preps)	12600-20
<b>Endotoxin-Free Plasmid Prep Kits</b>	
Endotoxin-free Mini Prep Kit (100 preps)	12311-100
Endotoxin-free Mini Prep Kit (250 preps)	12311-250
Endotoxin-free Midi Prep Kit (10 preps)	12711-10
Endotoxin-free Maxi Prep Kit (10 preps)	12611-10
<b>DNA Purification Kits</b>	
Agarose Gel DNA Purification Kit (300 preps)	12100-300
Agarose Gel-Spin DNA Purification (100 preps)	12400-100
Agarose Gel-Spin DNA Purification (250 preps)	12400-250
PCR Clean-Up Kit (100 preps)	12500-100
PCR Clean-Up Kit (250 preps)	12500-250
<b>DNA Isolation Kits</b>	
DNA Blood Isolation Kit (100 preps)	12000-100
DNA BloodSpin Kit (50 preps)	12200-50
DNA BloodSpin Kit (250 preps)	12200-250
Mega BloodSpin Kit (10 preps)	12210-10
Soil DNA Isolation Kit (50 preps)	12800-50
Soil DNA Isolation Kit (100 preps)	12800-100
Soil DNA Mega Prep Kit (10 preps)	12900-10
Fecal DNA Isolation Kit (50 preps)	12811-50
Fecal DNA Isolation Kit (100 preps)	12811-100
Microbial DNA Isolation Kit (50 preps)	12224-50
Microbial DNA Isolation Kit (250 preps)	12224-250
Plant DNA Isolation Kit (50 preps)	13000-50
Plant DNA Isolation Kit (250 preps)	13000-250
Tissue DNA Isolation Kit (50 preps)	12334-50
Tissue DNA Isolation Kit (250 preps)	12334-250
Water DNA Isolation Kit (10 preps)	14800-10
Water DNA Isolation Kit (25 preps)	14800-25
Forensic DNA Kit- Single prep format (10 preps)	14000-10
Forensic DNA Kit- Single prep format (20 preps)	14000-20
<b>RNA Isolation Kits</b>	
Tissue RNA Isolation Kit (50 preps)	15000-50
Tissue RNA Isolation Kit (250 preps)	15000-250
Plant RNA Isolation Kit (20 preps)	13300-20
Plant RNA Isolation Kit (50 preps)	13300-50
Microbial RNA Isolation Kit (50 preps)	15800-50
Microbial RNA Isolation Kit (250 preps)	15800-250
<b>Growth Media</b>	
TB DRY (1 kg) Terrific Broth powder	12105-1
LB (1 kg) LB powder (Miller)	12106-1
LB Agar (1 kg) LB Agar Powder (Miller)	12107-1

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## Contact Information

### Technical information:

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