MO BIO Laboratories, Inc.

UltraClean™ Soil DNA Isolation Kit

Catalog # 12800-50
50 preps
New improved PCR inhibitor removal solution (IRS) included

Instruction Manual

(New Alternative Protocol maximizes yields)

Introduction

Use this kit for isolating DNA from 0.25 - 1gm soil samples.

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. 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:

Micro centrifuge (10,000 x g)
Pipettor (volumes required 50 µl - 500 µl), vortex

Kit Contents

Description	Amt.
2 ml Bead Solution tubes (contains 550µl solution)	50
Solution S1	3.3 ml
IRS solution	11 ml
Solution S2	14 ml
Solution S3	72 ml
Solution S4	16.5 ml
Solution S5	3 ml
Spin filters units in 2 ml tubes	50
Collection tubes (2 ml)	150

Kit Storage

Room temperature.

Make sure the 2 ml Bead Solution screw cap tubes rotate freely in your centrifuge without rubbing.

WARNING: Solution S4 contains ethanol. It is flammable.

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Protocol (To maximize yields, follow Alternative Protocol on next page.)

Please wear gloves at all times

1. To the 2ml Bead Solution tubes provided, add 0.25 - 1gm of soil sample. (For larger sample sizes up to 10 grams, try using our Mega Prep Kit, catalog number 12900-10).
2. Gently vortex to mix.
3. **Check Solution S1.** If Solution S1 is precipitated, heat solution to 60°C until dissolved before use.
4. Add 60µl of Solution S1 and invert several times or vortex briefly.
5. Add 200µl of Solution IRS (Inhibitor Removal Solution). Only required if DNA is to be used for PCR.
6. Secure bead tubes horizontally using the Mo Bio Vortex Adapter tube holder for the vortex (cat.13000-V1. Call 1-800-606-6246 for information) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. (See alternative lysis method for less DNA shearing).
7. Make sure the 2ml tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds. **CAUTION:** Be sure not to exceed 10,000 x g or tubes may break.
8. Transfer the supernatant to a clean microcentrifuge tube (provided).
9. **Note**: With 0.25gm of soil and depending upon soil type, expect between 400 to 450µl of supernatant. Supernatant may still contain some soil particles.
10. Add 250µl of Solution S2 and vortex for 5 sec. Incubate 4°C for 5 min.
11. Centrifuge the tubes for 1 minute at 10,000 x *g.*
12. Avoiding the pellet, transfer 450µl of supernatant to a clean microcentrifuge tube (provided). ***(To transfer entire volume, follow alternative protocol steps 12 through 21.)***
13. Add 900µl of Solution S3 to the supernatant and vortex for 5 seconds.
14. Load approximately 700µl onto a spin filter and centrifuge at 10,000 x *g* for 1 minute. Discard the flow through and add the remaining supernatant to the spin filter and centrifuge at 10,000 x *g* for 1 minute. **Note**: A total of two loads for each sample processed are required.
15. Add 300µl of Solution S4 and centrifuge for 30 seconds at 10,000 x *g*.
16. Discard the flow through.
17. Centrifuge again for 1 minute.
18. Carefully place spin filter in a new clean tube (provided). Avoid splashing any Solution S4 onto the spin filter.
19. Add 50µl of Solution S5 to the center of the white filter membrane.
20. Centrifuge for 30 seconds.
21. Discard the 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 Soil DNA Isolation Kit.

Version 03222005

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Alternative Protocol (For maximum yields)

Please wear gloves at all times

1. To the 2ml Bead Solution tubes provided, add 0.25 - 1gm of soil sample. (For larger sample sizes up to 10 grams, try using our Mega Prep Kit, catalog number 12900-10. For amounts of sample to process see Hints and Troubleshooting Guide).
2. Gently vortex to mix.
3. **Check Solution S1.** If Solution S1 is precipitated, heat solution to 60°C until dissolved before use.
4. Add 60µl of Solution S1 and invert several times or vortex briefly.
5. Add 200µl of Solution IRS (Inhibitor Removal Solution). Only required if DNA is to be used for PCR.
6. Secure bead tubes horizontally using the Mo Bio Vortex Adapter tube holder for the vortex (cat.13000-V1. Call 1-800-606-6246 for information) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. (See alternative lysis method for less DNA shearing).
7. Make sure the 2ml tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds. **CAUTION:** Be sure not to exceed 10,000 x g or tubes may break.
8. Transfer the supernatant to a clean microcentrifuge tube (provided).
9. **Note**: With 0.25gm of soil and depending upon soil type, expect between 400 to 450µl of supernatant. Supernatant may still contain some soil particles.
10. Add 250µl of Solution S2 and vortex for 5 sec. Incubate 4°C for 5 min.
11. Centrifuge the tubes for 1 minute at 10,000 x *g.*
12. Avoiding the pellet, transfer entire volume of supernatant to a clean microcentrifuge tube (provided).
13. Add 1.3ml of Solution S3 to the supernatant (careful, volume touches rim of tube) and vortex for 5 seconds.
14. Load approximately 700µl onto a spin filter and centrifuge at 10,000 x *g* for 1 minute. Discard the flow through, add the remaining supernatant to the spin filter, and centrifuge at 10,000 x *g* for 1 minute. Repeat until all supernatant has passed through the spin filter. **Note**: A total of three loads for each sample processed is required.
15. Add 300µl of Solution S4 and centrifuge for 30 seconds at 10,000 x *g*.
16. Discard the flow through.
17. Centrifuge again for 1 minute.
18. Carefully place spin filter in a new clean tube (provided). Avoid splashing any Solution S4 onto the spin filter.
19. Add 50µl of Solution S5 to the center of the white filter membrane.
20. Centrifuge for 30 seconds.
21. Discard the 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 Soil DNA Isolation Kit.

Version 03222005

MO BIO Laboratories, Inc.

Detailed Protocol (Describes each step)

Please wear gloves at all times

1. To the 2ml Bead Solution tubes provided, add 0.25 - 1gm of soil sample. (For larger sample sizes up to 10 grams, try using our Mega Prep Kit, catalog number 12900-10. For amounts of sample to process see Hints and Troubleshooting Guide).

What's happening: Your 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.

2. Gently vortex to mix.

What's happening: This step mixes the sample and Bead Solution.

3. **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 hot.

4. Add 60µl of Solution S1 and invert several times or vortex briefly.

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.

5. Add 200µl 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.

6. Secure bead tubes horizontally using the Mo Bio Vortex Adapter tube holder for the vortex (cat.13000-V1. Call 1-800-606-6246 for information) or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 minutes. (See alternative lysis method for less DNA shearing).

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.

7. Make sure the 2ml tubes rotate freely in your centrifuge without rubbing. Centrifuge tubes at 10,000 x *g* for 30 seconds. **CAUTION:** Be sure not to exceed 10,000 x g or tubes may break.

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.

8. Transfer the supernatant to a clean microcentrifuge tube (provided).
9. **Note**: With 0.25gm of soil and depending upon soil type, expect between 400 to 450µl of supernatant. Supernatant may still contain some soil particles.
10. Add 250µl of Solution S2 and vortex for 5 sec. Incubate 4°C for 5 min.

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.

11. Centrifuge the tubes for 1 minute at 10,000 x *g*.
12. Avoiding the pellet, transfer entire volume of supernatant to a clean microcentrifuge 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.

13. Add 1.3ml of Solution S3 to the supernatant (careful, volume touches rim of tube) and vortex for 5 seconds.

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

14. Load approximately 700µl onto a spin filter and centrifuge at 10,000 x *g* for 1 minute. Discard the flow through, add the remaining supernatant to the spin filter, and centrifuge at 10,000 x *g* for 1 minute. Repeat until all supernatant has passed through the spin filter. **Note**: A total of three loads for each sample processed is required.

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.

15. Add 300µl of Solution S4 and centrifuge for 30 seconds at 10,000 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.

16. Discard the flow through from the collection tube.

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.

17. Centrifuge again for 1 minute.

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 down stream applications for the DNA.

18. 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.

19. Add 50µl of Solution S5 to the center of the white 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.

20. Centrifuge for 30 seconds.

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.

21. Discard the 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 Soil DNA Isolation Kit.

Version 03222005

Hints and Troubleshooting Guide

Amount of soil to process

Depending on soil type, usually 0.25gm -1gm works well. Typically, only 0.25 g of the more absorbent soil types, such as potting soils, can be processed. For wet soils, see "Wet soil sample" below.

Wet soil sample

If soil sample is high in water content remove contents from bead tube (beads and solution) and set aside. Add soil sample to bead tube and centrifuge 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 bead tube and follow protocol starting at step 2.

If DNA does not amplify

This is due to high humic acid content in soil sample. If the humic acid content is 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 steps 15 through 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.

Elution sample still brown
This is due to high humic acid content in soil sample. If the humic acid content is sample is high, you can do two to three washes of Solution S4 in steps 15 through 18. If elution solution is still brown, dilute the elution three fold and add two volumes of Solution S3. Run through spin filter, wash and elute.

Alternative lysis method
After adding Solution S1, vortex 3-4 seconds. Add the IRS Solution, vortex 3-4 seconds then heat to 70°C for 5 min. Vortex 3-4 seconds. Heat another 5 minutes. Vortex 3-4 seconds. This alternative procedure will reduce shearing but may reduce yield.

Concentrating the DNA
Your final volume will be 50µl. If this is too dilute for your purposes, add 2µl of 5M NaCl and mix. Add 100µl of 100% cold ethanol and mix. Centrifuge at 10,000 x g for 5 min. Decant all liquid. Dry residual ethanol in a speed vac, dessicator, or air dry. Resuspend precipitated DNA in desired volume.

DNA floats out of well when loaded on a gel
You may have inadvertently transferred some residual Solution S4 into 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 is the best way to remove Solution S4 residue. (See concentrating DNA above)

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

Cells are difficult to lyse
If cells are difficult to lyse, a 10-min incubation at 70°C, after adding Solution S1, can be performed. Follow by continuing with protocol step 5.

MO BIO Laboratories, Inc.

Other UltraClean™ Kits available from Mo Bio Laboratories, Inc.

Kit description	Cat. number
Plasmid Prep Kits	
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
Endotoxin-Free Plasmid Prep Kits	
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
DNA Purification Kits	
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
DNA Isolation Kits	
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
RNA Isolation Kits	
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
Growth Media	
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

Technical information:
Call Mo Bio Laboratories, Inc. Toll free 1-800-606-6246, or 1-760-929-9911**email** technical@mobio.com
Fax: 760-929-0109 Mail: Mo Bio Laboratories, Inc., 2746 Loker Avenue West, Carlsbad, CA 92008

Ordering Information
Direct: Call Mo Bio Laboratories, Inc. Toll free 1-800-606-6246, or 1-760-929-9911
email: orders@mobio.com
Fax: 760-929-0109 Mail: Mo Bio Laboratories, Inc. 2746 Loker Avenue West, Carlsbad CA 92008
For the distributor nearest you, go to our web site at www.mobio.com/distributors/