

# UltraClean-htp™ 96 Well Soil DNA Isolation Kit

Catalog # 12896-4  
(4) 96 Well Preps  
**Instruction Manual**

## Introduction

Use this kit for isolating DNA from 0.25 - 1gm depending on the soil types. For a general guideline please refer to the Hints and Trouble Shooting Guide. Two protocols are available for this kit. One is a vacuum protocol with some centrifugation steps. The other protocol is all centrifugation steps (recommended).

## 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 fire.

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

## Equipment required:

Centrifuge capable of spinning two 96 Well blocks stacked (13 cm x 8 cm x 5.5 cm) at 2500 x g, Multi-channel Pipettor (volumes required 50 µl - 650 µl)

Mechanical Shaker that shakes 96 Well Blocks and Adapter (Mo Bio Catalog number: 11996 and 11999)  
Vortex with 3 inch platform

## Optional Equipment Required:

Reagent reservoirs, Vacuum pump (Mo Bio Catalog Number: 11998), Vacuum manifold (Mo Bio Catalog number: 11997)

## Kit Contents

<u>Description</u>	<u>Amt.</u>
96 Well Bead Plates w/Square Well Mat	4 x 96 Well Plates w/ Beads w/ Mat
Bead Solution	233 ml
Solution S1	26 ml
IRS solution	85 ml
Solution S2	106 ml
Solution S3	550 ml
Solution S4	4 x 30 ml
Solution S5	43 ml
Spin Plate	4
2 ml Collection Plate	8
1 ml Collection Plate	8
0.5 ml Collection Plate	4
Microplate	4
Centrifuge Tape	24
Sealing Tape	8
Elution Sealing Mat	4

## Kit Storage

Room temperature.

**Precautions: Wear gloves. Avoid contact with all reagents**

If eye or skin contact occurs, wash thoroughly with water.

**WARNING: Solution S4 contains ethanol. It is flammable.**

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

## Combination of Vacuum and Centrifuge Protocol

**Before you start:** Please see precautions on previous page.

There are several things that will make this protocol more efficient to use.

- ◆ First, be sure to measure the centrifuge and rotor you plan to use and be sure they will accommodate the plates used in this kit. For this Centrifuge Protocol it is best to stack a Spin Plate on top of a 0.5 ml Collection Plate. Place this in the plate holder rotor. **DO NOT** start the centrifuge or possible injury or centrifuge damage may occur. Turn the centrifuge by hand slowly and be sure the stacked plates will clear the rotor and centrifuge.
  - ◆ Make sure you have a multi-channel pipettor that can accommodate all the required volumes (10µl-1000µl).
  - ◆ This protocol assumes you will be processing 192 samples (2-96 well preps). If you plan to process less than this number, divide your samples between two plates evenly so that you always have a balance. See Hints section.
  - ◆ Please read all precautions on 96 well plate shaker instruction manual before using it. Failure to do so may result in personal injury or damage to the shaker.
1. Remove Square Well Mat from Bead Plate. To the Bead Plates add 0.25 - 1gm of soil sample. (For larger sample sizes up to 10 grams, try using our single prep Mega Soil Prep Kit, catalog number 12900-10).
  2. **Add 550µl of Bead Solution** to the wells of the Bead Plate.
  3. **Check Solution S1.** If Solution S1 is precipitated, heat solution to 60°C until dissolved before use.
  4. **Add 60µl of Solution S1.** Place mat on plate without sealing completely. Gently vortex on a 3 inch platform.
  5. Remove mat and **add 200µl of Solution IRS** (Inhibitor Removal Solution). Secure mat tightly to plate.
  6. Place Bead Plate with mat securely fastened, to the 96 Well Plate Shaker (Catalog # 11996). Note: The final order of all components is: Aluminum plate, Square Well mat, 96 Well Bead Plate and Aluminum plate.
  7. Shake at speed 20 for 20 min.
  8. Centrifuge 10 minutes at 2500 x g.
  9. Remove and discard Square Well Mat. Transfer the supernatant to a clean 1ml Collection Plate.
  10. **Note:** Supernatant may still contain some soil particles.
  11. **Add 250µl of Solution S2** and apply Sealing Tape to plate.
  12. Vortex for 5 seconds and Incubate at 4°C for 10 min.
  13. Centrifuge the plate for 30 minutes at 2500 x g.
  14. Avoiding the pellet, transfer entire volume of supernatant to a new 1ml Collection Plate.
  15. Apply Sealing Tape to plate.
  16. Centrifuge 10 min at 2500 x g.
  17. Remove and discard Sealing Tape.
  18. Transfer entire volume of supernatant to a 2 ml Collection Plate avoiding any residual pellet.
  19. Place a new 2 ml Collection Plate in bottom of vacuum manifold.
  20. Replace top of manifold. Now place Spin Plate onto top of manifold.
  21. Turn the vacuum pump on.
  22. To maximize pipet tip efficiency, please process 8 samples (1 row) at a time.
  23. **Add 650 µl of Solution S3** to a row of the 2 ml Collection plate containing the supernatant.
  24. **Add a second volume of 650 µl of Solution S3** to the same row.
  25. Pipet up and down to mix.
  26. Load approximately 650µl onto the first row of the Spin Plate and apply vacuum.
  27. Keep loading 650µl onto Spin Plate until entire volume is used.
  28. Repeat steps 23-27 for each row until all samples have been processed.
  29. Turn off the vacuum pump.

30. Discard the flow through from the 2 ml Collection Plate in the bottom of the vacuum manifold and put the 2 ml Collection Plate back in the manifold.
  31. Place the Spin Plate back on top of the manifold, and turn vacuum on.
  32. **Add 300µl of Solution S4** to each well of the Spin Plate.
  33. After entire volume of Solution S4 has passed through the Spin Plate, turn off the vacuum.
  34. Apply Centrifuge Tape to the Spin Plate to cover the wells.
  35. Place a 0.5 ml Collection Plate under the Spin Plate.
  36. Centrifuge for 10 minutes at 2500 x *g*.
  37. Carefully place Spin Plate onto a Microplate.
  38. Remove Centrifuge Tape and discard.
  39. **Add 100µl of Solution S5** to the center of the white filter membrane of Spin Plate.
  40. Apply Centrifuge Tape.
  41. Centrifuge for 5 minutes. Remove Centrifuge Tape and discard.
  42. Cover wells of Microplate with Elution Sealing Mat provided.
- DNA in the Microplate 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 03252005

## Centrifuge Protocol

### Please wear gloves at all times

**Before you start:** Please see precautions on previous page.

There are several things that will make this protocol more efficient to use.

- ◆ First, be sure to measure the centrifuge and rotor you plan to use and be sure they will accommodate the plates used in this kit. For this Centrifuge Protocol it is best to stack a Spin Plate on top of a 0.5 ml Collection Plate. Place this in the plate holder rotor. **DO NOT** start the centrifuge or possible injury or centrifuge damage may occur. Turn the centrifuge by hand slowly and be sure the stacked plates will clear the rotor and centrifuge.
  - ◆ Make sure you have a multi-channel pipettor that can accommodate all the required volumes (10µl-1000µl).
  - ◆ This protocol assumes you will be processing 192 samples (2-96 well preps). If you plan to process less than this number, divide your samples between two plates evenly so that you always have a balance. See Hints section.
  - ◆ Please read all precautions on 96 well plate shaker instruction manual before using it. Failure to do so may result in personal injury or damage to the shaker.
1. Remove Square Well Mat from Bead Plate. To the Bead Plates add 0.25 - 1gm of soil sample. (For larger sample sizes up to 10 grams, try using our single prep Mega Soil Prep Kit, catalog number 12900-10).
  2. **Add 550µl of Bead Solution** to the wells of the Bead Plate.
  3. **Check Solution S1.** If Solution S1 is precipitated, heat solution to 60°C until dissolved before use.
  4. **Add 60µl of Solution S1.** Place mat on plate without sealing completely. Gently vortex on a 3 inch platform.
  5. Remove mat and **add 200µl of Solution IRS** (Inhibitor Removal Solution). Secure mat tightly to plate.
  6. Place Bead Plate with mat securely fastened, to the 96 Well Plate Shaker (Catalog # 11996). Note: The final order of all components is: Aluminum plate, Square Well mat, 96 Well Bead Plate and Aluminum plate.
  7. Shake at speed 20 for 20 min.
  8. Centrifuge 10 minutes at 2500 x g.
  9. Remove and discard Square Well Mat.
  10. Transfer the supernatant to a clean 1ml Collection Plate.
  11. **Note:** Supernatant may still contain some soil particles.
  12. **Add 250µl of Solution S2** and apply Sealing Tape to plate.
  13. Vortex for 5 seconds and Incubate at 4°C for 10 min.
  14. Centrifuge the plate for 30 minutes at 2500 x g.
  15. Avoiding the pellet, transfer entire volume of supernatant to a new 1ml Collection Plate.
  16. Apply Sealing Tape to plate.
  17. Centrifuge 10 min at 2500 x g. Remove and discard Sealing Tape.
  18. Transfer entire volume to a 2 ml Collection Plate avoiding any residual pellet.
  19. **Add 650 µl of Solution S3** to the 2 ml Collection Plate containing the supernatant.
  20. **Add a second volume of 650 µl of Solution S3** to the same wells of the Collection Plate.
  21. Pipet up and down to mix.
  22. Place SpinPlate onto a new 0.5ml Collection Plate.
  23. Load approximately 650µl onto the Spin Plate.
  24. Apply Centrifuge Tape.
  25. Centrifuge for 5 minutes at 2500 x g.
  26. Discard flow through from the 0.5 ml Collection Plate and replace same 0.5 ml Collection Plate beneath Spin Plate.
  27. Discard Centrifuge Tape.



28. Repeat steps 23 to 27 until all supernatant has been processed.
  29. **Add 300µl of Solution S4** to Spin Plate.
  30. Apply Centrifuge Tape to Spin Plate.
  31. Centrifuge for 3 minutes at 2500 x g.
  32. Discard flow through in 0.5 ml Collection Plate.
  33. Replace same 0.5 ml Collection Plate beneath Spin Plate.
  34. Centrifuge for 10 minutes at 2500 x g.
  35. Carefully place Spin Plate onto a Microplate.
  36. Remove Centrifuge Tape and discard.
  37. **Add 100µl of Solution S5** to the center of the white filter membrane of Spin Plate.
  38. Apply a new piece of Centrifuge Tape to Spin Plate.
  39. Centrifuge for 5 minutes.
  40. Remove and discard Centrifuge Tape.
  41. Cover wells of Microplate with Elution Sealing Mat provided.
- DNA in the Microplate 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 03252005

## Hints and Troubleshooting Guide

### Vacuum hints

If a vacuum step seems to be taking a long time, turn off the vacuum source. Lift the filter plate off the vacuum to release any back pressure. Replace the filter plate and turn the vacuum source back on. Be sure there are no air leaks around the plate. If slow vacuum continues, you can centrifuge the filter plate as an alternative. Be sure any unused wells are covered with the sealing tape provided.

### 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.

### General guideline on some soil types and the amount processed using this kit

Soil Types	Soil Weight (g)
Sediments	0.250
Clay	0.250
Potting Soil	0.10
Garden Soil	Up to 1.0
Sandy Soil	Up to 1.0

### Elution sample still brown

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

### 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. Caution: This has the disadvantage of co-precipitating the Humic Acids along with DNA.

### 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 not to transfer Solution S4 onto the bottom of the Spin Plate. 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.

**Other UltraClean™ Kits 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



**Technical information:**

Call Mo Bio Laboratories, Inc. Toll free 1-800-606-6246, or 1-760-929-9911 email [technical@mobio.com](mailto: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](mailto: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/](http://www.mobio.com/distributors/)