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.

MO BIO Laboratories, Inc.

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

Please wear gloves at all times (adjusted for 200ul liquid sample)

1. To the 2ml Bead Solution tubes provided, add 0.25 - 1gm (200ul) 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 (invert 5x).
3. **(Check Solution S1).** If precipitated, heat to 60°C until dissolved.
4. Add 60µl of Solution S1 and invert several times or vortex briefly (invert 5x).
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 (650ul with 200ul sample) of supernatant. Supernatant may still contain some soil particles.
10. Add 250µl (330ul) 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). (there will be a total of 900ul supernatant in the tube. Transfer the two halves into two tubes. You will then have two tubes with 450ul of super into which you add 900ul of Solution S3- step 13).
    (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 (invert 5x).
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 (four) 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 03182003