SOIL DNA PURIFICATION KIT

Product Manual

26-016.....5 preps
26-013G.....50 preps
26-013B.....50 preps
P/N 03-293-7 REV. B

OMNI INTERNATIONAL

TABLE OF CONTENTS

INTRODUCTION

The Omni Soil DNA Kit allows rapid and reliable isolation of high-quality genomic DNA from various soil samples. Up to 1 gram of soil samples can be processed in less than 60 minutes. The system combines the reversible nucleic acid-binding properties of Omni matrix with the speed and versatility of spin column technology to eliminate PCR inhibiting compounds such as humic acid from soil samples. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

OVERVIEW

If using the Omni Soil DNA Kit for the first time, please read this booklet to become familiar with the procedure. Soil samples are homogenized and then treated in a specially formulated buffer containing detergent. Humic acid, proteins, polysaccharides, and other contaminants are subsequently precipitated after a heat-freeze step. Contaminants are further removed by extraction steps. Binding conditions are then adjusted and the sample is applied to an Omni DNA Mini Column. Two rapid wash steps remove trace contaminants and pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

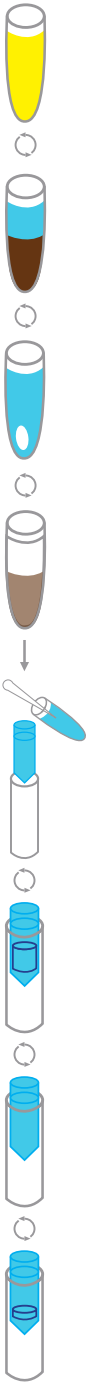
KIT CONTENTS

Product Number	26-016	26-013G	26-013B
Purifications	5	50	50
DNA Mini Columns	5	50	50
2 mL Collection Tubes	10	100	100
2 mL bead kit 0.7 mm garnet	5	50	
RTH Reagent	1.2 mL	12 mL	12 mL
XLSM Buffer	6 mL	60 mL	60 mL
CBH Buffer	4 mL	25 mL	25 mL
PX1 Buffer	4 mL	40 mL	40 mL
EB Buffer	3 mL	30 mL	30 mL
SD Buffer	0.6 mL	6 mL	6 mL
PS2 Buffer	3 mL	25 mL	25 mL
DW Buffer	2 mL	20 mL	20 mL
0.5 mm Glass Beads	3 g		30 g
User Manual	✓	✓	✓

STORAGE AND STABILITY

Most components of the Soil DNA Kit should be stored at 22-25°C. Store the RTH Reagent at 2-8° C. During shipment, or storage in cool ambient conditions, precipitates may form in some buffers. It is possible to dissolve such deposits by warming the buffer to 55° C.

ILLUSTRATED PROTOCOLS



Homogenize Soil

Extract DNA

Precipitate DNA

Remove Inhibitors

Transfer Supernatant to a DNA Mini Column to bind DNA

Wash Column x 2

Dry the Column

Elute DNA

PREPARING REAGENTS

- Dilute DW Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% EtOH to be added
26-016	8 mL
26-013G and 26-013B	80 mL

- Dilute CBH Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be added
26-016	1.6 mL
26-013 and 26-013B	10 mL

Materials and Equipment to be Supplied by User:

- Micro-centrifuge capable of at least 13,000 x g and 4°C
- Centrifuge with rotor for 2mL or 15 mL centrifuge tubes
- Vortexer
- 1.5 mL micro-centrifuge tubes
- 2 mL micro-centrifuge tubes
- 15 mL centrifuge tubes
- Incubator capable of 70°C
- 100% ethanol
- 100% Isopropanol
- Ice Bucket

BEFORE STARTING:

- Prepare the DW Buffer and CBH Buffer as instructed in the "Preparing Reagents" section
- Set a incubator to 70°C
- Heat EB Buffer to 70°C
- Pre-chill PS2 Buffer in ice bucket.

OMNI SOIL DNA PURIFICATION KIT DIRECTIONS

26-013G - Soil Mini Kit with 0.7mm garnet 2 mL bead tubes

1. Obtain 0.7 mm garnet 2 mL bead tube
2. Add 0.1- 0.25 g soil sample to 2 mL bead tube containing 0.7mm garnet.
3. Add 725 µL XLSM Buffer. Lyse samples at 5 m/s for 1 minute on the Bead Ruptor Bead Mill Homogenizer or Vortex at maximum speed for 3-5 minutes.

Note: Bead Mill speed/power and time settings should be adjusted based on the equipment manufacturer's recommendations for the specific sample type.

4. Add 72 µL SD Buffer. Vortex to mix thoroughly.
5. Incubate at 70°C for 10 min. Briefly vortex the tube once during the incubation.
6. Centrifuge at 10,000 x g for 5 min.
7. Transfer 400 µL to new 1.5 mL tube.

26-013B - Soil Mini Kit with bulk 0.5 mm glass beads

1. Transfer 500 mg glass beads to a 15 mL centrifuge tube.
2. Add 0.2 - 1.0 g soil sample to the glass beads.
3. Add 1 mL XLSM Buffer. Lyse samples at 5 m/s for 1 minute on the Bead Ruptor Bead Mill Homogenizer or Vortex at maximum speed for 3-5 minutes.

Note: Bead Mill speed/power and time settings should be adjusted based on the equipment manufacturer's recommendations for the specific sample type.

4. Add 100 µL SD Buffer. Vortex to mix thoroughly.
5. Incubate at 70°C for 10 min. Briefly vortex the tube once during the incubation.
6. Centrifuge at 3,000 x g for 5 min.
7. Transfer 800 µL to a new 1.5 mL tube.

26-013 - Soil Mini Kit with 0.7mm garnet 2 mL bead tubes

8. Add 135 µL pre-chilled PS2 Buffer. Vortex to mix thoroughly.

26-013B - Soil Midi Kit with bulk 0.5 mm glass beads

8. Add 270 µL pre-chilled PS2 Buffer. Vortex to mix thoroughly.

9. Incubate on ice for 3 minutes.
10. Centrifuge at ≥13,000 x g for: **1 minute for the mini kit**
    5 minutes for the midi kit
11. Carefully transfer the supernatant to a new 1.5 mL micro-centrifuge tube.

12. Add 200 µL RTH Reagent. Vortex to mix thoroughly.

Note: Completely re-suspend RTH Reagent by shaking the bottle before use.

13. Let sit at room temperature for 2 minutes.

14. Centrifuge at ≥13,000 x g for 1 minute.

15. Transfer cleared supernatant to a new 1.5 mL micro-centrifuge tube.

Note: If supernatant still has a dark color from the soil, repeat Steps 12-15 for a second RTH Reagent step.

16. Add an equal volume PX1 Buffer. Vortex to mix thoroughly.

17. Insert a Omni DNA Mini Column into a 2 mL Collection Tube provided in this kit.

18. Transfer the sample from Step 16 to the Omni DNA Mini Column.

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

20. Discard the filtrate and reuse the Collection Tube.

21. 500 µL CBH Buffer to the DNA mini column.

22. Centrifuge at 10,000 x g for 1 minute.

23. Discard the filtrate and the Collection Tube.

24. Transfer the Omni DNA Mini Column into a new 2 mL Collection Tube.

25. Add 700 µL DW Buffer to the DNA mini column.

Note: DW Buffer must be diluted with ethanol before use. Please see the "Preparing Reagents" section on Page 4 for instructions.

26. Centrifuge at 10,000 x g for 1 minute.

27. Discard the filtrate and reuse the Collection Tube.

28. Repeat steps 25-27 for a 2nd wash step

29. Centrifuge the empty Omni DNA Mini Column at ≥13,000 x g for 2 minutes at room temperature.

Note: This step is critical in removing residual ethanol that may interfere with downstream applications.

30. Transfer the Omni DNA Mini Column into a clean 1.5 mL micro-centrifuge tube.

31. Add 50 -100 µL EB Buffer heated to 70° C directly onto the center of Omni column membrane.

32. Incubate at room temperature for 1-2 minutes.

33. Centrifuge at ≥13,000 x g for 1 minute.

34. Take flow through and place on same mini column.

35. Incubate at room temperature for 1 minute.

36. Centrifuge at ≥ 13,000 x g for 1 minute.

37. Discard DNA mini column and store eluted DNA at -20°C.

TROUBLE SHOOTING GUIDE

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at 1-800-776-4431.

Problem	Cause	Suggestion
A_{260}/A_{230} ratio is low	Inefficient elimination of inhibitory compounds	Repeat with a new sample, be sure to mix the sample with RTH Reagent thoroughly.
	Salt contamination	Make sure the column is dried before the elution. Wash the column with extra WPS Buffer
Low DNA Yield or no DNA Yield	Poor homogenization of sample	Repeat the DNA isolation with a new sample, be sure to vortex the sample with XLSM Buffer in the bead tube .
	DNA washed off	WPS Buffer must be diluted with ethanol before use
	Column matrix lost binding capacity during storage	Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x g for 30 seconds. Discard the filtrate.
Little or no supernatant after initial centrifuge step	Insufficient centrifugal force	Check the centrifugal force and increase the centrifugal time if necessary
Sample cannot pass through the column	Clogged column	Check the centrifugal force and increase the time of centrifugation
Problems in downstream applications	BSA not added to PCR mixture	Add BSA to a final concentration of 0.1 µg/mL to the PCR mixture.
	Too much DNA inhibits PCR reactions	Dilute the DNA used in the downstream application if possible
	Non-specific bands in downstream PCR	Use hot-start Taq polymerase mixture
	Inhibitory substance in the eluted DNA.	Check the A260/A230 ratio. Dilute the elute to 1:50 if necessary
	Residual ethanol in the elute	Completely dry the column before elution

NOTES

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