Ver. 24071

ODP321

Soil DNA Kit

For extraction of microbial DNA from soil

ISO 13485:2016 ISO 9001:2015

Soil DNA Kit

Spin column
(ODP321)

Kit Contents

Contents	50 Preps
Buffer PL	40mL
Buffer TL	25mL
Buffer NP	25mL
Buffer GD	13mL
Buffer PW	15mL
Buffer TE	10mL
Proteinase K (20mg/mL)	1.25mL
Glass Beads	15g
Spin Columns CB3	50
Collection Tubes (2mL)	50
Handbook	1

Storage

Soil DNA Kit can be stored at room temperature (15-25°C) for up to 8 months without showing any reduction in performance and quality. For longer storage, the kit can be stored at 2-8°C.
Proteinase K should be stored at -20°C.
(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary.)

Introduction

Soil DNA Kit is suitable for the extraction of microbial DNA from extreme soil environment like cinnamon soil, silt. volcanic ash etc. The buffers induce proper lysis of bacteria and fungi to maximize the retention of microbial DNA polymorphism.

The Humus adsorption material provided with the kit facilitates effective removal of humus components and will not affect the yield of DNA. The extracted DNA will have high purity and can be directly used for variety of routine operations, including enzyme digestion, PCR, library construction, Southern blotting etc.

Important Notes (<u>Please read before use</u>)

1. Fresh soil sample could have a higher yield. Best storage conditions of different soil samples should be checked before sampling.
2. All centrifugation steps should be carried out in a conventional table-top microcentrifuge at room temperature (15-25°C).
3. Avoid pipetting precipitate in any supernatant collection steps, or else the column would be blocked and the product purity would be affected.
4. Appropriate volume of ethanol needs to be added to Buffer PW and Buffer GD as indicated on the bottle tag before use.
   Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.
5. Excessive amount of genomic DNA could inhibit the PCR reaction, so please dilute the genomic DNA to an optimal concentration in such a circumstance.

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly

1. Sample Preparation: Weigh 250mg of soil sample in a 1.5mL micro-centrifuge tube.
2. Add 800µL Buffer PL to the sample and vortex briefly for 15 seconds.
3. Add 250mg glass beads to the sample, mix and vortex for 5 minutes.
4. Add 25µL Proteinase K to the sample and mix manually by inverting the tube and then incubate at 56°C for 10 minutes.
   Note: In case of difficult samples increase the time to 20 minutes.
5. Add 500µL Buffer TL and vortex for 15 seconds.
6. Incubate at 95°C for 5 minutes.
7. Centrifuge at 12000rpm (~13.400 ×g) for 1 minute.
8. Transfer the supernatant to a 1.5 mL tube, without disturbing the pellet.
9. Add 500µL Buffer NP to the supernatant and mix well by vortexing for 15 seconds. Incubate the tubes on ice for 5 minutes.
10. Centrifuge at 12,000 rpm (~13,400 ×g) for 5 minutes.
11. Transfer the supernatant to two new 1.5mL micro-centrifuge tubes (approximately 700µL in each tube) without disturbing the pellet.
12. Add 500µL (96-100%) ethanol to the supernatant in each of the tubes and mix well.
13. Pipette 750µL of the mixture to a spin column CB3 (in a 2mL collection tube) and incubate at room temperature for 5-10 minutes.
14. Centrifuge for 12000rpm (~13.400 ×g) for 40 seconds. Then discard the flow- through and place the spin column CB3 to the collection tube.
15. Transfer the remaining mixture to spin column CB3 and repeat step 14 until all the mixture is transferred to spin column CB3 with incubation time 5-10 minutes.
16. Add 500µL Buffer GD to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds, then discard the flow-through and place the spin column into the collection tube.

Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.

17. Add 700µL Buffer PW to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds. Discard the flow-through and place the spin column into the collection tube.
18. Repeat step 17.
19. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes to dry the membrane completely.
    Note: The resident ethanol of Buffer PW may have some affect in downstream application.
20. Place the spin column CB3 in a new clean 1.5mL. microcentrifuge tube, and incubate for 2 minutes at room temperature.
21. Pipette 100µL Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature (15-25°C) for 2-5 minutes, and then centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g).
    Note: To increase the DNA yield,
    - **Introduce the eluted Buffer TE to the spin column CB3 and centrifuge for 2 minutes at 12,000 rpm.**
    - **Warm Buffer TE at 50-60°C before adding to the spin column CB3.**

    If the volume of eluted buffer is less than 50µL, it may affect recovery efficiency. For long-term storage of DNA, eluting in Buffer TE and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.