Product Name: Extrap Soil DNA Kit Plus ver.2

Code No.: 212-006

Size: 50 preps

Storage Conditions: Room temperature
*This product should be stored at room temperature and protected from light (10-30°C). After opening, store at 2-4°C.
* Do not open the cap of bead tube before use.

Shelf life: 1 year

Features:

Extrap Soil DNA Kit Plus ver.2 is a kit for extraction and purification of microbial DNA from environmental samples such as soil and activated sludge. This product is suitable for applications such as microbial community structure analysis in environmental samples and real-time PCR. The kit provides high recovery and high purity of DNA from a wide range of environmental samples.

✓ Purifying DNA from a wide range of microbial species via bead beating disruption.
✓ Our proprietary technology that inhibits the adsorption of DNA on soil particles, enabling high recovery of DNA from environmental samples.
✓ Easy DNA purification with magnetic beads and applicable for automation.
✓ Organic solvents such as phenol or chloroform are not required.

Kit Contents:

Components	Volume
Bead Tubes	50 tubes
Extraction Buffer	71 mL
Lysis Solution	4 mL
PP Solution	18 mL
MBs Solution	3 mL
Binding Solution	54 mL
Washing Solution	48 mL

*Lysis solution, Binding solution, and Washing solution may precipitate at low temperatures.
In that case, warm the bottle at 45-60°C to completely dissolve the precipitate.
*Water droplets may be seen inside the Bead Tubes, but this does not indicate a quality problem.

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Required materials not included with the kit

1. Reagents

✓ 70% ethanol

✓ Elution buffer (TE buffer, sterile water, etc.)

2. Equipment / Instruments

✓ Micropipettes and tips

✓ 1.5 mL tubes

✓ 2 mL tubes

✓ Magnetic rack for 1.5 mL

Recommended product: Magtrap<Magnetic separator> (BDL, Cat No. DD100)

✓ Cell disruptor (for 2 mL tubes)

Recommended products: FastPrep series (MP Biomedicals), Bead Crusher μT-01 (TAITEC)

✓ Vortex Mixer

✓ Centrifuge (>14,000 x g)

✓ Heat block or water bath

Caution:

* When using this product, general laboratory precautions should be taken, and safety precautions should be followed.
* This product is a research use only. Do not use this product for diagnostics or any other purpose.
* BioDynamics Laboratory Inc. is not responsible for any problems caused by using the product in a manner different from that described in the instruction manual.
* Wear appropriate protective equipment.
* Do not use damaged bottles or contaminated solutions.
* Do not add additional inhibitors to the product. This product already contains DNA adsorption inhibitors.
* The contamination of this kit is less than 10 copies of 16S rDNA/μL of DNA extract.
* Do not open the cap of bead tube before use.
* Ensure that the O-ring on the cap is in place before use.
* The DNA binding capacity of magnetic beads is 10 to 20 μg /tube.

Protocol:

1. Add 0.5 g of environmental sample (500 μL for liquid sample), 950 μL of Extraction Buffer, and 50 μL of Lysis Solution to the Bead Tubes.
2. Vortex the tube for 5 seconds.
3. Process the bead beat for 4 to 6 m/sec (or 4,200 to 6,800 rpm for 30 to 45 sec).
4. Centrifuge (14,000 x g, 5 min, 4°C)
5. Transfer 600 μL of supernatant to a 1.5 mL tube and add 300 μL of PP Solution.
6. Mix by inverting for 10 times
7. Centrifuge (14,000 x g, 5 min, 4°C)
8. Transfer 800 μL of supernatant to a 2 mL tube.

9. Add 50 µL of MBs Solution and 890 µL of Binding Solution to the tube.
10. Mix by inverting for 2 minutes.
11. Spin down and place microcentrifuge tube into the magnetic rack.
12. Pull the magnetic beads to the side of the tube at least 1 minute, remove and discard supernatant.
13. Add 800 µL of Washing Solution and vortex the tube in low speed.
14. Spin down and place microcentrifuge tube into the magnetic rack. Pull the magnetic beads to the side of the tube at least 1 minute, remove and discard supernatant.
15. Add 1 mL of 70% ethanol solution and vortex the tube in low speed.
16. Spin down and place microcentrifuge tube into the magnetic rack. Pull the magnetic beads to the side of the tube at least 1 minute, remove and discard supernatant.
17. Repeat steps 15-16.
18. Air-dry the magnetic beads at room temperature for about 10 minutes.
19. Add 100 µL of elution buffer (TE buffer, sterile water, etc.), and vortex the tube in low speed.
20. Heat the tube at 65°C for 5-10 minutes. mix by vortexing the tube several times during the incubation.
21. Place microcentrifuge tube into the magnetic rack. Pull the magnetic beads to the side of the tube at least 1 minute, collect the supernatant to a new tube.

Notes:

(Step 1)

For lightweight samples such as compost, adding sample to the bead tube may interfere the movement of the beads, resulting in inadequate cell disruption and low DNA yield. To avoid this interference, reduce the amount of sample added to the bead tube.

(Step 1)

For liquid samples with low bacterial concentrations, it can be concentrated with membrane filters. Specifically, the liquid sample is filtered through a commercially available membrane filter. After the filtration, add the filtered membrane to a bead tube with tweezers. Then, the extraction can be performed according to the protocol of this kit.

(Step 12)

The supernatant contains PCR inhibitors. Remove it as much as possible.

(Step 16)

Ethanol should be removed as completely as possible. Remaining ethanol may reduce the elution of DNA in subsequent steps resulting lower DNA yield.

(Step 18)

Remove as much ethanol as possible before air-drying.

(Step 19-21)

When eluate is added, DNA is eluted into the eluate.