Code No. 212-006

DNA extraction kit for environmental samples

Extrap Soil DNA Kit Plus Ver.2

Manual

NIPPON STEEL & SUMIKIN Eco-Tech Corporation

【Feature】

Extrap Soil DNA Kit Plus Ver.2 is a kit for the extraction of DNA of microorganisms in environmental samples, such as soils and activated sludge. DNA of high purity is recoverable by high yield from the extensive environmental samples. The DNA obtained by this kit is suitable for the template such as a structural analysis of microorganisms and real-time PCR.

- Bead beating is adopted as a method of crushing microbes. Cell of extensive microorganisms can be crushed and DNA can be obtained.
- The original agent which controls adsorption of DNA to soil particles is included. So, DNA is recoverable by high yield.
- By adopting magnetic beads for the purification of DNA, DNA in soil can be obtained by a simple operation.
- Since this kit does not employ organic solvent such as phenol and chloroform, you can use it safely.

【The contents of the kit】

If opened, please check whether there are any abnormalities in content of the package, appearances, quantity, solution, etc.

A part for 50 times

・Beads tubes	50
・Extraction Buffer	71 ml
・Lysis Solution	4 ml
・PP Solution	18 ml
・MBs Solution	3 ml
・Binding Solution	54 ml
・Washing Solution	48 ml

※Although, as for Lysis Solution, Binding Solution, and Washing Solution, crystal may deposit, this is not a problem of quality of this kit. When the crystal deposits, please warm the whole container by a 45-60 ℃ and let the crystal dissolve completely.

【Preservation】

- Please preserve at room temperature.(15～30℃)
- Please avoid the humid location and the location where direct sunlight hit.

【The reagents, an instrument,and apparatus required in addition to this product】

1.Reagents

・70% ethanol
・Elution buffer(TE buffer, sterile water, etc.)

2. Instruments

・Micropipette
・Micropipette tip
・1.5 mL tube
・2 mL tube
・Magnetic stand for 1.5 mL tube
Recommended products: MaguneSphere® Technology Magnetic Separation Stands (Promega, Cat No.Z5332, or Z5342)

3. Apparatus

・Beads beater (for 2 mL tube)
Recommended products: FastPrep series (MP Biomedicals), beads crusher μT-01(TAITEC)
・Vortex mixer
・Centrifuge (RCF(xg) is more than 14,000xg)
・Heating block or water bath

【Attention】

- In use of Product, please observe laboratory general precautions strictly and care about safety.
- This Product is for research use only. Be careful not to use it for the purpose of clinical examinations and others.
- Responsibility cannot be taken about the trouble by different handling from statement in the manual.
- The substances stimulate skin are contained in this kit.
- In case you work, please equip with a glove.
- Please do not use what breakage of the container and the foreign substance were acknowledged in.
- The original agent inhibits the adsorption of DNA to soil is already included in this kit. So, please do not use the skimmed milk generally used as an adsorption inhibitors.

【Protocol】

1. Environmental samples 0.5 g (in the case of liquid samples, 500 µL), Extraction Buffer 950 µL, and Lysis Solution 50 µL are added to Beads tube.
2. Agitates for 5 seconds with a vortex mixer.
3. Bead beating (4-6 m / second or 4,200-6,800 rpm for 30 to 45 seconds)
4. Centrifuge (14,000xg, 5 minutes, 4 ℃)
5. Supernatant (600 µL) is transferred to a 1.5 mL tube, and PP Solution 300 µL is added to the tube.
6. Invert the tube for 10 times.
7. Centrifuge (14,000xg, 5 minutes, 4 ℃)
8. Supernatant (800 µL) is transferred to a 2.0 mL tube.
9. MBs Solution (50 µL) and Binding Solution (890 µL) are added to the tube.
10. Invert the tube for 2 minutes gently.
11. After collecting magnetic beads by setting a tube to a magnetic stand, supernatant is removed by a micropipette.
12. Washing Solution (800 µL) is added to the tube, and fully agitates the tube with a vortex mixer (low speed).
13. After collecting magnetic beads by setting a tube to a magnetic stand, supernatant is removed by a micropipette.
14. One ml of 70% ethanol is added in the tube, and fully agitates the tube with a vortex mixer (low speed).
15. After collecting magnetic beads by setting a tube to a magnetic stand, ethanol is removed by a micropipette.
16. The process of 14-15 is repeated again.
17. Magnetic beads dry at room temperature for 10 minutes, with tube cover opened.
18. Agitate the tube with a vortex mixer (low speed) after adding 100 µL of elution buffer (TE buffer, sterile water, etc.).
19. Heat the tube by heat block (or water bath) for 5 to 10 minutes, inverting several times on the way.
20. After collecting magnetic beads by setting a tube to a magnetic stand, the eluate is transfer to new tube.

※ Operational considerations

(Operation 1)

When the subjected sample is a liquid sample expected that the concentration of microbes is quite low, the filtration of the sample by a membrane filter is recommended. After the filtration, the membrane filter is inserted to Beads tube using forceps etc. After that, DNA of the microbes filtrated on the membrane is extracted according to the usual protocol.

(Operation 11)

Since PCR inhibitors are contained in supernatant, please remove supernatant as much as possible. However, since the black magnetic beads adsorb DNA, be careful not to remove