【Product Name】HiPure Soil DNA Mini Kit C

【Product Specifications】50 Preps/Kit, 250 Preps/Kits

【Introduction】

HiPure Soil DNA kits are specifically designed for soil DNA extraction. The kit adopts silica gel column purification technology combined with innovative humic acid adsorbent technology, which is suitable for extracting high yield and high purity total DNA from various soils such as forest soil, grassland soil, mining soil, sediment and other samples. Humic acid adsorbent can efficiently adsorb humic acid and other inhibitory factors in soil samples. The purified DNA can be directly used for PCR, Southern hybridization, enzyme digestion and other experiments.

【Kit Contents】

Cat.No.	D314202C	D314203C
Purification Times	50	250
HiPure DNA Mini Columns II	50	250
2ml Collection Tubes	50	250
2ml Bead Tubes	50	250
Buffer STL	50 ml	250 ml
Buffer SL	15 ml	60 ml
Buffer GWP	80 ml	2 x 180 ml
Buffer GW2*	20 ml	2 x 50 ml
Buffer AE	15 ml	60 ml

【Storage conditions and Validity】

The kit components can be stored dry at room temperature (15 – 25° C) and are stable for at least 24 months under these conditions.

【Preparation before Use】

Add 80ml (50Preps) or 2 x 200ml (250 Preps) absolute ethanol the bottle of Buffer GW2 and store at room temperature.

【Protocol】

1. Add 0.25-0.5g soil, sediment, fermented sediment, filter residue, 0.1-0.2g stool or environmental samples into a centrifuge tube containing grinding beads, then add 0.8ml Buffer STL.
   - Before using dry environmental samples, a sieve can be used to remove foreign objects such as leaves, stones, or small branches as much as possible. For very dry materials, if the sample material absorbs too much lysis buffer, control the sample amount and increase the amount of Buffer STL appropriately.
   - For very humid materials, centrifuge to remove excess liquid before adding lysis buffer.
   - Control the sample amount to ensure sufficient space in the centrifuge tube for bead grinding. The reduction of starting samples usually helps to improve lysis efficiency and increase DNA purity.
   - Microbial DNA extraction from water: The maximum volume of water sample that can be processed depends on the sample (such as source and mass) and the filtration membrane (such as type, diameter, and pore size). As the turbidity of water sample ranging from clear to highly turbid, generally speaking, it can handle large amounts of transparent drinking water ranging from 100 mL to several liters for the low probability of filter clogging. Turbid water samples containing sediment or suspended particles, such as clay, silt, or other inorganic or organic matter, may cause filter clogging. For these sample types, it is recommended to use a 0.45 μm filter. Use a suitable filter to filter and enrich microorganisms in the water using a 25 mm diameter filter membrane (0.2 um or 0.45 μm). Use sterile forceps to remove the filter membrane from the filtration device, roll the filter membrane into a cylinder (with the bacterial side facing inward) and insert it into a 2ml homogenization tube. Then add 800 μ L Buffer STL1 and follow step
2. Transfer to the vortex mixer, vortex at the highest speed for 10-15 minutes or perform rapid bead grinding on the bead grinder for 30-60 seconds.
   - PowerLyzer grinder: recommend 2000rpm for 30s, pause for 30s and then repeat once.
   - FastPrep 24 grinder: recommend 5m/s for 30s, pause for 30s, and then repeat once.
   - Tissue Lysis II grinder: recommend 25Hz for 5mins, reposition and then repeat once.
3. Briefly Centrifuge for 30 seconds, add 0.2ml Buffer SL to the sample, vortex to mix for 15 seconds.

For environmental samples with low humus content (such as non-soil samples), Buffer SL can be omitted. Buffer SL can efficiently remove inhibitors such as humic acid, but it can also cause loss of trace nucleic acids. **Do not skip this step when processing soil samples.**

4. **Centrifuge at 13,000 x g for 5 minutes and transfer the supernatant to a new centrifuge tube.**

5. **Transfer the supernatant to a 2ml centrifuge tube, add an equal volume of Buffer GWP, invert to mix for 6-8 times.**
   If the volume of the supernatant is 700μl, 700μl Buffer GWP needs to be added. If there are still obvious precipitates in the mixed solution, centrifuge at 13,000xg for 1 minute.

6. **Install the HiPure DNA Mini Columns II column into the collection tube. Transfer half the volume of the mixture into the column.** Centrifuge at 13,000 x g for 1 minute.

7. **Discard the filtrate and install the column back into the collection tube. Transfer the remaining mixture to the column.** Centrifuge at 13,000 x g for 1 minute.

8. **Discard the filtrate and install the column back into the collection tube. Add 600μl Buffer GWP to the column.** Centrifuge at 13,000 x g for 1 minute.

9. **Discard the filtrate and install the column back into the collection tube. Add 600μl Buffer GW2 (diluted with ethanol) to the column.** Centrifuge at 13,000 x g for 1 minute.

10. **Repeat Step 9 once.**

11. **Discard the filtrate and install the column back into the collection tube.** Centrifuge at 13,000 x g for 2 minutes.

12. **Install the column in a 1.5ml centrifuge tube. Add 50-100μl Buffer AE (preheated to 55-70℃) to the center of the membrane.** Place for 3 minutes. Centrifuge at 13,000 × g for 1 minute.

13. **Transfer the eluent to the center of the column membrane and place for 3 minutes. Centrifuge at 13,000 × g for 1 minute.** Discard the column and store the DNA at 2-8℃. Long term storage should be kept at -20℃.

Troubleshooting Guide

1. DNA has color

- **Excessive sample amount:** Forest soil and grassland soil are rich in humic acid, reducing soil sample amount by half.
- **Not thoroughly mixed after adding Buffer SL.**
- **Excessive sample amount:** Reduce the sample amount, when processing complex stool samples, control the sample amount at 50mg.

2. Severe DNA degradation

- **Replace manual vortexing with a bead grinder:** Manual vortexing for a long time can cause DNA breakage.
- **Excessive sample amount:** Forest soil and grassland soil are rich in humic acid, while sediment rich in water is rich in organic matter. When processing these samples, the sample amount should be halved.

3. Low DNA production

- **Low soil DNA content:** Increasing the sample amount, multiple samples can be prepared
- **Insufficient lysis:** Use a bead grinder instead of manual vortexing or adjust the vortex speed to the highest and vortex continuously for 5-10 minutes.
- **Insufficient elution efficiency:** Increase elution volume and elution frequency. Due to the large size of genomic DNA fragments, the water solubility is poor. Recommend a second elution to increase yield or increase the volume of eluent.
- **Inaccurate volume of buffer GWP:** The volume of buffer GWP should be the same as that of the supernatant.