【Product Name】HiPure Soil DNA Kit

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

【Intended Use】

This product allows rapid and reliable isolation of high-quality genomic DNA from various soil samples. Up to 500 mg soil samples can be processed in 60 minute. The system combines the reversible nucleic acid binding properties of HiPure 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 next-generation sequencing. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

【Principle】

Soil sample is homogenized and then treated in a specially formulated buffer containing detergent to lyse bacteria, yeast, and fungal samples. Humic acid, proteins, polysaccharides, and other contaminants are removed using our propietary Absorber Solution. Binding conditions are then adjusted and the sample is applied to an DNA Mini Column. Two rapid wash steps remove trace contaminants and pure DNA is eluted in low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

【Kit Contents】

Cat.No.	D314202	D314203	Main Composition
Purification Times	50	250	-
HiPure DNA Mini Columns II	50	250	Silicon Column
2ml Collection Tubes	50	250	PP Column
2ml Bead Tubes	50	250	Glass beads
Buffer SOL	60 ml	250 ml	PBS/EDTA
Buffer SDS	5 ml	20 ml	SDS
Buffer PS	10 ml	50 ml	KAC
Absorber Solution	10 ml	50 ml	Tris/NaC
Buffer GWP	40 ml	220 ml	Guanidine Salt
Buffer DW1	30 ml	150 ml	
Buffer GW2*	20 ml	2 x 50 ml	Tris/NaCL
Buffer AE	15 ml	30 ml	Tris/EDTA

【Storage conditions and Validity】

Absorber Solution should be stored at 2 – 8° C upon arrival. However, short-term storage (up to 24 weeks) at room temperature (15 – 25° C) does not affect their performance. The remaining kit components can be stored dry at room temperature (15 – 25° C) and are stable for at least 18 months under these conditions.

【Preparation before Use】

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

【Protocol】

1. **Transfer 250-500mg soil, 50-100mg Stool or 200-500mg other environmental samples to 2ml Bead Tubes.**
2. **Add 800µL Buffer SOL to the sample. Lyse sample by vortex at maximum speed for 10 minutes or by Fastpreps 24 (6.5 m/s twice for 45s).**
   For best results, a mixer mill, such as GenoGrinder 2010, Fastprep-24®, or Omni Bead Ruptor should be used.
3. Centrifuge for 5 seconds to remove drops of liquid from the lid. **Add 60µL Buffer SDS and vortex to mix thoroughly. Incubate at 65°C for 10 minutes.**
4. Centrifuge at 13,000 x g for 1 min at room temperature.
5. **Transfer 600µL supernatant into a new 1.5 mL microcentrifuge tube (not provided).**
6. **Add 150µL Buffer PS and vortex to mix thoroughly. Add 150µL Absorber Solution and vortex to mix thoroughly.** Incubate for 3 minutes.
   If the nucleic acid content of the sample is very low or the color of the supernatant is light, it is not necessary to add Absorber Solution. Absorber solution can effectively adsorb pigments or other inhibitors, but it will also adsorb a small amount of nucleic acid.
7. Centrifuge at maximum speed (≥13,000 x g) for 5 min.
8. **Transfer the cleared supernatant (~750µL) to a new 2.0 mL microcentrifuge tube.**
9. **Add an equal volume Buffer GWP and mix by inverting the tube 4-6 times.**

10. Insert a HiPure DNA Mini Column II into a 2.0mL Collection Tube (provided).

11. **Pipet 750µl of the mixture from step 9into the column placed in a 2 ml collection tube (supplied).** Centrifuge for 1 min at 10,000 x *g* and discard the flow-through. Reuse the collection tube in step 12.

12. Repeat step 11 with remaining sample. Discard flow-through and reuse the collection tube.

13. **Add 500µl Buffer DW1 and incbuate for 1 min. Centrifuge for 1 min at 10,000 x *g* .** Discard the flow-through and reuse the collection tube in step 14.

14. **Add 650µl Buffer GW2 to the column and centrifuge for 1 min at 10,000 x *g* .** Discard the flow-through and reuse the collection tube in step 15.

15. **Add 650µl Buffer GW2 to the column, and centrifuge for 1 min at 10,000 x *g*.**

16. Discard the flow through and reuse the collection Tubes. Centrifuge at 10,000 x g for 1 min. This step helps to eliminate the chance of possible Buffer GW2 carryover.

17. **Transfer the column to a 1.5 ml microcentrifuge tube (not supplied), and pipet 50µl Buffer AE (Preheated at 65oC) directly onto the membrane.** Incubate for 3 min at room temperature, and then centrifuge for 1 min at 10,000 x *g* to elute.

18. **Pipet another 50µl Buffer AE(Preheated at 65oC) or eluate directly onto the membrane.** Incubate for 3 min at room temperature, and then centrifuge for 1 min at 10,000 x *g* to elute.

19. Discard the column and store the pure DNA at -20oC.

【Fast Protocol】

1. **Transfer 250-500mg Soil sample, 50-100mg Stool or 200-500mg other environmental samples to 2ml Bead Tubes.**

2. **Add 800µL Buffer SOL and 60µL Buffer SDS to the sample.**

3. Lyse sample by vortex at maximum speed for 10 minutes or by Fastpreps 24 (6.5 m/s twice for 45s).

4. Centrifuge at 13,000 x g for 1 min at room temperature.

5. **Transfer 600µL supernatant into a new 1.5 mL microcentrifuge tube (not provided).**

6. Add 150µL Buffer PS and 150µL Absorber Solution to the sample. vortex to mix thoroughly.

7. Centrifuge at 13,000 x g for 5 min.

8. **Transfer up to 750 µl of supernatant to a clean 2 ml Microcentrifuge Tube (provided). Add an equal volume Buffer GWP and mix by inverting the tube 4-6 times. Follow step 10-19.**

Troubleshooting Guide

1. Clogged DNeasy membrane

- **Lysate too viscous:** Reduce the amount of starting material and/or increase the amounts of Buffer SOL and Buffer PS.
- **Insufficient centrifugation:** Increase the g-force and centrifugation time.

2. Low or no recovery

- **Buffer GW2 did not contain ethanol:** Ethanol must be added to Buffer GW2 before used. Repeat procedure with correctly prepare Buffer.
- **Insufficient disruption:** Ensure that the starting material is completely disrupted.
- **Insufficient lysis:** Reduce the amount of starting material and/or increase the amounts of Buffer SOL and Buffer PS.
- **Incorrect binding conditions:** Make sure that the amount of lysate is accurately determined so that the correct amount of Buffer GWP is added to adjust binding conditions correctly

3. Darkly colored membrane or green/yellow eluate after washing with Buffer GWP

- Too much starting material Reduce the amount of starting material in future preps.
- Insufficient washing of the membrane: After washing with Buffer GWP (step 15), perform an additional wash with 500 µl Buffer GWP and Proceed step 16.

4. DNA does not perform well (e.g. in ligation reaction)

- **Salt concentration in eluate too high**: Modify the wash step by incubating the column for 5 min at room temperature after adding Buffer GW2, then centriufge or Vacuum.
- **Eluate contains residual ethanol:** Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at >12,000 x g for 1min, then dry.
- **Inappropriate elution volume used:** Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly.