【Product Name】HiPure Soil DNA 96 Kit

【Product Specifications】1 x 96 Preps/Kit, 4 x 96 Preps/Kit, 20 x 96 Preps/Kits

【Intended Use】

This product allows rapid and reliable isolation of high-quality genomic DNA from various soil and stool samples. Up to 100mg stool sample and 500 mg soil samples can be processed in 60 minute. The system combines the reversible nucleic acidbinding 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/Stool 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.	D314401	D314402	D314403
Purification Times	1 x 96	4 x 96	20 x 96
HiPure DNA Plate	1	4	20
96 Well Plate (2.2ml)	1	4	20
1.6ml Collection Plate	1	4	20
0.8ml Collection Plate	1	4	20
2ml Bead Tubes	100	400	2000
Buffer SOL	100 ml	360 ml	2 x 900 ml
Buffer SDS	10 ml	36 ml	180 ml
Reagent DX	1 ml	1.8 ml	9 ml
Buffer PS	20 ml	80 ml	400 ml
Absorber Solution	20 ml	80 ml	400 ml
Buffer GDP	150 ml	500 ml	3 x 900 ml
Buffer GW2*	50 ml	100 ml	4 x 200 ml
Buffer AE	30 ml	120 ml	500 ml

【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 200ml (D314401) or 400ml (D314402) or 4 x 800ml (D314403) absolute ethanol to the bottle of Buffer GW2 and store at room temperature.

【Protocol】

1. **Transfer 250-500mg Soil sample，50~100mg stool sample or 100-500mg environmental sample to 2ml Bead Tubes. Add 800μL Buffer SOL, 80μL Buffer SDS and 4μL Reagent DX to the sample.**

   Before use, mix Buffer SOL,Buffer SDS and Reagent DX and mix well. After preparation, the mixture is stable for 1 months at room temperature.

2. Lyse sample by vortex at maximum speed for 10 minutes or by Fastpreps 24 (6.5 m/s twice for 45s). **Incubate at 65°C for 10 minutes.**
3. Centrifuge at 13,000 x g for 2 min at room temperature.
4. **Transfer 600μL supernatant into a new 1.5 mL microcentrifuge tube (not provided).**
5. **Add 150μL Buffer PS and 150μL Absorber Solution and vortex to mix thoroughly.** Let sit on ice for 5 minutes.
6. Centrifuge at maximum speed (≥13,000 x g) for 5 min.
7. **Transfer the cleared supernatant (~700μL) to 96 well Plate (2.2ml).**
8. **Add an equal volume Buffer GDP and mix well by pipet up and down.**

Spin Procedure

9. Insert a HiPure DNA Plate onto a 1.6mL Collection Tube (provided).
10. **Pipet 700μl of the mixture from step 8 into the plate placed in a 1.6ml collection plate (supplied).** Centrifuge for 3 min at >4,000 x *g* and discard the flow-through. Reuse collection plate in step 13.
11. Repeat step 10 with remaining sample. Discard flow-through and reuse the collection plate.

12. **Add 500µl Buffer GDP and incbuate for 1 min. Centrifuge for 3 min at 4,000 x *g* .** Discard the flow-through and reuse the collection plate in step 13.
13. **Add 650µl Buffer GW2 to each well of plate and centrifuge for 3 min at >4,000 x *g* .** Discard the flow-through and reuse the collection tube in step 14.
14. **Add 650µl Buffer GW2 to each well of the plate, and centrifuge for 3 min at >4,000 x *g*.**
15. Discard the flow through and reuse the collection plate. Centrifuge at >4,000 x g for 10 min. Allow to air dry for 10 min at room temperature.
16. **Transfer the plate onto a 0.8ml Collection tube, and pipet 75~100µl Buffer AE directly onto the membrane.** Incubate for 5 min at room temperature, and then centrifuge for 3 min at >4,000 x *g* to elute.

    Important: Ensure that the buffer AE is dispensed directly onto the center of HiPure membrane for complete elution of bound DNA. Please note that the average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume. A second elution step with a further 75-100µl Buffer AE increases yields by up to 20~30%.

Vacuum Procedure:

9. Plate a new HiPure gDNA Plate into Vacuum Manifold such as QiaVac 96.
10. load the samples from step 8 into the plate by pipetting,and then apply vacuum. After the samples have passed through the column, switch off the vacuum source. The maximum loading volume of the column is 800 µl. For sample volumes greater than 1000 µl, simply load again.
11. **To wash, add 0.5ml of Buffer GDP and incubate for 1 min. Apply vacuum.**
12. **To wash, add 0.75 ml of Buffer GW2 to each column and apply vacuum.**
13. **To wash, add 0.75 ml of Buffer GW2 to each column and apply vacuum.**
14. **To wash, add 0.5ml of absoluet ethanol to each column and apply vacuum.** After Absolute ethanol in all wells has passed through the membrane, apply maximum vacuum for an additional 10 min to dry the membrane.

    Important: This step removes residual ethanol from the membrane. The removal is only effective when maximum vacuum is used (i.e., turn off vacuum regulator valves or leakage valves if they are used), allowing maximum airflow to go through the wells.
15. Switch off vacuum source. **Vigorously tap the HiPure DNA plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the plate with clean absorbent paper.**

    This step removes residual Absoluet ethanol from around the outlet nozzles and collars of the k plate. Residual ethanol may inhibit subsequent enzymatic reactions, e.g., sequencing.

16. Transfer the HiPure DNA Plate into a new 0.8ml Collection Plate. Add 75~100µl Buffer AE to the center of the membrane. let the column stand for 2 min. Centrifuge at 4,000 × g for 3 minute at room temperature.

    Important: Ensure that the buffer AE is dispensed directly onto the center of HiPure membrane for complete elution of bound DNA. Please note that the average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume. A second elution step with a further 75-100µl Buffer AE increases yields by up to 20~30%.

Troubleshooting Guide

1. Clogged DNeasy membrane

- **Lysate too viscous:** Reduce the amount of starting material and/or increase the amounts of Buffer SPL 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 GDP is added to adjust binding conditions correctly

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

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