【Product Name】HiPure Stool DNA Kit

【Product Specifications】50 Preps/Kit

【Intended Use】

This product allows rapid and reliable isolation of high-quality genomic DNA from various stool samples. Up to 100 mg stool 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 from stool samples. Purified DNA is suitable for PCR, restriction digestion, and next-generation sequencing.

【Principle】

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 phenol extraction. 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.	IVD3141	Main Composition
Purification Times	50	-
HiPure DNA Mini Columns II	50	Silica Column
2ml Collection Tubes	50	PP Column
2ml Bead Tubes	50	Glass beads
Proteinase K	24 mg	Proteinase K
Protease Dissolve Buffer	1.8 ml	Glycerol/Tris/CaCl2
Buffer SPL	40 ml	SDS/EDTA
Buffer PCI	40 ml	Phenol/Chloroform
Buffer AL	20 ml	Guanidine Salt
Buffer GW1*	22 ml	Guanidine Salt
Buffer GW2*	20 ml	Tris/NaCL
Buffer AE	15 ml	Tris/EDTA

【Storage conditions and Validity】

Proteinase K and Buffer PCI should be stored at 2–8°C upon arrival. However, short-term storage (up to 12 weeks) at room temperature (15–25°C) does not affect their performance. The remaining kit components can be stored at room temperature (15–25°C) and are stable for at least 18 months under these conditions.The entire kit can be stored at 2–8°C, but in this case buffers should be redissolved before use. Make sure that all buffers are at room temperature when used.

【Preparation before Use】

- Add 80ml absolute ethanol to the bottle of Buffer GW2 and store at room temperature.
- Add 28ml absolute ethanol to the bottle of Buffer GW1 and store at room temperature.
- Add 1.2ml Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.

【Protocol】

1. **Transfer 50-100mg Stool sample to 2ml Bead Tubes.**
2. **Add 0.6ml Buffer SPL and 0.6ml Buffer PCI into the sample.** Place on a bead beater machine or vortex at maximum speed for 10 min.
3. Incubate sample at 65℃ for 15 minutes. This step makes bacteria lyse more completely.
4. Centrifuge at 13,000 x g for 10 minutes.
5. **Transfer the cleared supernatant (~250μL) to a new 2.0 mL microcentrifuge tube.**

   If RNA-free genomic DNA is required, add 10 μl RNase A (25mg/ml) and incubate for 10min at room temperature.
6. **Add 250μL Buffer AL and 20μL Proteinase K to the sample.** Mix well and Incubate at 65oC for 10minutes.
7. **Add 250 μl of ethanol (96 – 100%) to the lysate, and mix by vortexing.**
8. Insert a HiPure DNA Mini Column II into a 2.0mL Collection Tube (provided).
9. **Pipet 750μl of the mixture from step 7 into 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 10.

10. **Add 650µl Buffer GW1 and centrifuge for 1 min at 10,000 x *g* .** Discard the flow-through and reuse the collection tube in step11.

11. **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 12.

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

13. 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.

14. **Transfer the column to a 1.5 ml microcentrifuge tube (not supplied), and pipet 50~100µl Buffer AE directly onto the membrane.** Incubate for 5 min at room temperature, and then centrifuge for 1 min at 10,000 x *g* to elute.

15. **Repeat step 14 once.**

    A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the microcentrifuge tube can be reused for the second elution step to combine the eluates.

Troubleshooting Guide

1. Clogged Column

- **Lysate too viscous:** Reduce the amount of starting material.
- **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 SPL and Buffer PCI.
- **Incorrect binding conditions:** Make sure that the amount of lysate is accurately determined so that the correct amount of Buffer AL is added to adjust binding conditions correctly

3. Darkly colored membrane or green/yellow eluate after washing with Buffer GW1

- Too much starting material Reduce the amount of starting material in future preps.

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.