

【Product Name】 HiPure Stool DNA Mini Kit

【Product Specifications】 20 Preps/Kit, 50 Preps/Kit, 250 Preps/Kits

【Introduction】

This kit combines bead grinding and column purification techniques and is suitable for rapid extraction of high-purity total DNA from ≤ 200 mg stool samples. The purification column selects high-performance ultrafine glass fiber filter membrane as the material, which efficiently and specifically adsorbs DNA. This kit adopts silica gel column purification technology and a unique salt mediated adsorption method, which can efficiently remove RNA and inhibitory factors. The entire process does not require phenol chloroform and can efficiently adsorb humic acid and other inhibitory factors in the solution. Purified DNA can be directly used for experiments such as PCR, Southern hybridization, enzyme digestion, and second-generation sequencing.

【Kit Contents】

Cat.No.	D314101F	D314102F	D314103F
Purification Times	20	50	250
HiPure DNA Mini Columns II	20	50	250
2ml Collection Tubes	20	50	250
2ml Bead Tubes	20	50	250
Proteinase K	12 mg	24 mg	120 mg
Protease Dissolve Buffer	1.8 ml	1.8 ml	10 ml
Buffer STL	20 ml	40 ml	180 ml
Buffer SL	2.5 ml	5 ml	25 ml
Buffer PCI	10 ml	20 ml	90 ml
Buffer GWP	30 ml	70 ml	2 x 170 ml
Buffer GW2*	10 ml	20 ml	2 x 50 ml
Elution Buffer	10 ml	15 ml	60 ml

【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 40ml (20Preps), 80ml (50Preps) or 2 x 200ml (250 Preps) absolute ethanol to the bottle of Buffer GW2 and store at room temperature.
- Add 0.6ml (20Preps), 1.2ml (50Preps) or 6ml (250 Preps) Protease Dissolve Buffer to the Proteinase K and store at -20–8°C after dissolve.

【Protocol】

1. **Transfer 50-200mg stool sample, 100-200 μ l liquid sample, or 200-300 μ l stool suspension containing preservation solution to the 2ml Bead Tubes.**
 - Human stool samples may contain undigested food substances (such as crop or fruit shells, undigested seeds), do not transfer these particles.
 - For animal stool samples, reducing the sample amount may yield better results.
 - Very dry stool samples, such as rabbit or mouse stool, may absorb lysis buffer, resulting in insufficient sample volume after centrifugation. In these cases, it is recommended to reduce the sample amount, such as 50mg.
 - For difficult-to-extract samples, such as samples rich in lipids, polysaccharides, or protein, it is recommended to start extraction with 60-100mg, reducing the starting material may also improve lysis efficiency and DNA purity.
 - For liquid stool samples, it is recommended to transfer 0.2ml for operation. If there is a lot of water in the sample, the transferred sample can be centrifuged to remove excess water. The total volume of residue and residual liquid should not exceed 0.2ml.
 - For samples treated with preservation solution, it is recommended to transfer 0.2-0.4ml for operation..
2. **Add 600 μ l Buffer STL, 60 μ l Buffer SL and 300 μ l Buffer PCI into the sample, screw the cap tightly.**

3. Vortex the tube at high speed for 10 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.
4. Centrifuge at 14,000 x g for 10 minutes at room temperature.
5. Transfer 600µl supernatant to a new 2ml centrifuge tube, add 600µl Buffer GWP and 20µl Proteinase K, invert to mix 6-8 times. Stay at room temperature for 10 minutes.
 - When using Buffer GWP mediation, the maximum loading capacity of HiPure DNA Mini Column II can only reach 20µg. If the DNA yield in the sample exceeds 20µg, another 300µl isopropanol can be added to the mixture, invert to mix for 6-8 times and then follow step 6.
 - As the isopropanol will cause precipitation of pigments or other impurities, do not add isopropanol when the mixture color is dark.
6. Install a HiPure DNA Mini Column II into a 2ml collection tube. Transfer half volume of the mixture into the column. Centrifuge at 13,000x g for 1 minute.
7. Discard the filtrate, install the column back into the collection tube, and transfer the remaining mixture to the column. Centrifuge at 13,000 x g for 1 minute.
8. Discard the filtrate, 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, install the column back into the collection tube. Add 600µl Buffer GW2 to the column. Centrifuge at 13,000 x g for 1 minute.
 - Buffer GW2 should be diluted with absolute ethanol according to the bottle label or instructions.
10. Repeat Step.9 once.
11. Discard the filtrate, install the column back into the collection tube. Centrifuge at 13,000 x g for 2 minutes to dry the column.
12. Install the column in a 1.5ml centrifuge tube. Add 50-100µl Elution Buffer (preheated to 65°C) to the center of the column membrane and place for 3 minutes. Centrifuge at 13,000 x g for 1 minute.
 - Elution Buffer contains 10mM Tris (pH 8.5) , it can be replaced with Buffer TE or sterile water (pH>6.5).
13. Transfer the eluent back to the center of the column membrane and place for 3 minutes. Centrifuge at 13,000 x g for 1 minute.
14. Discard the column. Store DNA at 2-8°C, and for long-term storage, it should be kept at -20°C.

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 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 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 centrifuge 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.