【Product Name】HiPure Microbiome DNA Kit

【Product specifications】50 Preps/Kit, 250 Preps/Kit

【Intended Use】

This product provide an easy-to-use workflow for selective isolation of bacterial DNA from samples that are intrinsically rich in host DNA, such as bodily fluids or swabs. The method is specific for the identification of intact bacteria so it prevents false results due to nucleic acids from dead bacteria. The Kit allows isolation of enriched bacterial DNA suitable for a variety of applications, including qPCR and whole metagenome or 16S rRNA gene sequencing.

【Principle】

This product is based on silica Column purification. This product efficiently depletes human and animal host DNA and yields enriched bacterial DNA. An optimized combination of mechanical and chemical lysis allows efficient disruption of bacterial cells. Target DNA is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing proteins and other impurities, Nucleic acid was finally eluted with low-salt buffer (10mm Tris,pH9.0, 0.5mm EDTA).

【Kit Contents】

Cat.No.	D314802	D314803	Main Composition
Purification Times	50	250	-
HiPure DNA Mini Columns I	50	2 x 125	Silicon Column
2ml Collection Tubes	50	2 x 125	PP Column
2ml beads Tubes	50	250	Glass beads
Buffer DRB	15 ml	60 ml	Tris/EDTA/SDS
Buffer ES	6 ml	30 ml	Tween-20/Guanidine Salt
Reagent DX	0.5 ml	1 ml	Anti-former
Buffer DL	30 ml	120 ml	Guanidine Salt
Buffer GW1*	22 ml	110 ml	Guanidine Salt
Buffer GW2*	12 ml	50 ml	Tris/NaCl
DNase I (Powder)	6 mg	30 mg	nuclease
Proteinase K	24 mg	120 mg	Proteinase K
Protease Dissolve Buffer	5 ml	15 ml	Glycerol/Tris/CaCl2
Buffer AE	15 ml	60 ml	Tris/EDTA

【Storage conditions and Validity】

DNase I and Proteinase K 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 dry 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 48ml (50Preps) or 200ml (250 Preps) absolute ethanol to the bottle of Buffer GW2 and store at room temperature.
- Add 28ml (50Preps) or 140ml (250 Preps) absolute ethanol to the bottle of Buffer GW1 and store at room temperature.
- Add 1.2ml (50Preps) or 6ml (250 Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- Add 0.6ml (50Preps) or 3ml (250 Preps) Protease Dissolve Buffer to the DNase I and store at -20℃ after dissolve.

【Protocol】

- Large Volume

1. **Add 500μl Water , 150μl Buffer DRB to 1 ml of sample in a new 2 ml microcentrifuge tube (not provided) , mix well and incubate for 10 min at room temperature.** Mix 3-5 times during incubation by inverting tube.
   If the sample volume is greater than1ml, increase the amount of Water and Buffer DRB proportionally.
2. Centrifuge the tube at 10,000 x g for 10 min. Carefully discard the supernatant by pipetting and **leave 0.4ml of the supernatant and the pellet in the tube. Vortex the tube vigorously to resuspend the pellet in the residual liquid.**
3. Add 10μl DNase I to the sample, mix well and incubate at 37°C for 30 min. Mix 3-5 times during incubation by inverting tube and Proceed step 4.

- Small volume

1. **Add 400 μl of the sample to a new microcentrifuge tube.** If the sample volume is less than 400μl, add the appropriate volume of Water.

2. Add 40µl Buffer DRB to the sample and mix well.

3. Add 10 µl DNase I to the sampe and mix well. Incubate at room temperature for 20 min. Mix 2–3 times during incubation by inverting tube and Proceed step 4.

4. Transfer the sample from step 3 to 2 ml beads tube.

5. Add 50µl Buffer ES/Buffer DX to 2ml beads Tubes and lyse bacterial cells by votex for 10 min at maximum speed or by FastPrep-24 (6.5 m/s twice for 45s).

   Before use, add 40µl Reagent DX to 6ml Buffer ES. Mix well after adding Reagent DX. After preparation, the mixture is stable for 6 months at room temperature.

6. Centrifuge at 10,000 x g for 1 min. Transfer 400µl of the supernatant to a fresh microcentrifuge tube.

7. Add 400µl Buffer DL and 20µL Proteinase K to the sample. Mix by vortexing and incubate at 70°C for 10mintues.

8. Add 400µL ethanol (96–100%) to the sample, and mix by vortexing for 15s.

9. Insert a HiPure DNA Mini Column I into a 2mL Collection Tube (provided).

10. Pipet 650 µl of the mixture from step 8, including any precipitate that may have formed, into the column. Centrifuge for 1 min at ⩾10000 x *g* and discard the flow-through. Reuse the collection tube in step 11.

11. Repeat step 10 with remaining sample. Discard flow-through and Reuse the collection tube.

12. Discard the flow through and reuse the collection Tubes. Add 650µl Buffer GW1. Close the cap and centrifuge at 10,000 x g for 1 min.

13. Discard the flow through and reuse the collection Tubes. Add 650µl Buffer GW2. Close the cap and centrifuge at 10,000 x g for 1 min.

14. 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.

15. Place the column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Add 50~100µl Buffer AE or distilled water. Incubate at room temperature for 2 min, and then centrifuge at 10000 x *g* for 1 min.
    A second elution step with a further 50~100µl Buffer AE will increase yields by up to 15%. For samples containing less than 1µg of DNA, elution in 30µl Buffer AE or water is recommended.

Troubleshooting Guide

1. Low or no recovery

- Buffer GW1/GW2 did not contain ethanol: Ethanol must be added to Buffer GW1/GW2 before used. Repeat procedure with correctly prepare Buffer.
- Low concentration of target DNA in the Sample: Samples were standing at room temperature for too long. Repeated freezing and thawing should be avoided.
- Inefficient cell lysis due to insufficient mixing with Buffer DL: Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer DL immediately and thoroughly by pulse-vortexing.
- Low-percentage ethanol used instead of 100%: Repeat the purification procedure with a new sample. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

2. A260/A280 ratio for purified nucleic acids is low

- Inefficient cell lysis due to insufficient mixing with Buffer DL: Repeat the procedure with a new sample. Be sure to mix the sample and Buffer AL immediately and thoroughly by pulse vortexing.
- No ethanol added to the lysate before loading onto the column: Repeat the purification procedure with a new sample.

3. 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 3 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 >10,000 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.