

【Product Name】 HiPure Microbiome DNA Kit

【Product specifications】 10 Preps/Kit, 50 Preps/Kit, 250 Preps/Kit

【Intended Use】

This product provides an easy-to-use workflow for selective isolation of parasitic microbial DNA from samples that are intrinsically rich in host DNA. It is suitable for enrichment and extraction of microbial DNA from anticoagulant blood, tissue and intestinal microbes, and remove cellular DNA. This method is specific for the identification of intact bacteria so it prevents false results due to nucleic acids from dead bacteria. Purified DNA is suitable for a variety of applications, including PCR, enzyme digestion, southern blot, ect.

【Principle】

This product is based on silica Column purification. Eukaryocyte is lysed in lysis buffer LBX1, but bacterial/fungal will not lysed. Microbial cells gets after centrifuge, microbial DNA release in lysis buffer and proteinase K. Target DNA is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. Salts and other impurities are remove by wash buffer 2, nucleic acid is finally eluted with low-salt buffer (Buffer AVE).

【Kit Contents】

Cat.No.	D314801	D314802	D314803
Purification Times	10	50	250
HiPure DNA Mini Columns I	10	50	250
2ml Collection Tubes	10	50	250
2ml beads Tubes	10	50	250
Buffer LBX1	15 ml	60 ml	270 ml
DNase Buffer	5 ml	15 ml	60 ml
Buffer TL	5 ml	15 ml	60 ml
Buffer MLB	10 ml	30 ml	150 ml
Buffer DCW1 *	4.4 ml	22 ml	110 ml
Buffer DCW2 *	5 ml	20 ml	2 x 50 ml
DNase I (Powder)	3 mg	10 mg	4 x 10 mg
Proteinase K	12 mg	24 mg	120 mg
Protease Dissolve Buffer	1.8 ml	3 ml	10 ml
Buffer AVE	3 ml	10 ml	30 ml

Version: 202408

【Storage conditions and Validity】

DNase I and Proteinase K should be stored at 2–8°C upon arrival. However, short-term storage (up to 8 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.

【Preparation before Use】

- Add 20ml (10Preps), 80ml (50Preps) or 200ml (250 Preps) absolute ethanol to the bottle of Buffer DCW2 and store at room temperature.
- Add 5.6ml (10Preps), 28ml (50Preps) or 140ml (250 Preps) absolute ethanol to the bottle of Buffer DCW1 and store at room temperature.
- Dissolve DNase I (10mg/ml): Add 0.3ml (10Preps), 1.0ml (50Preps) or 4x1.0ml (250 Preps) Protease Dissolve Buffer to the DNase I tube and store at -20~8°C after dissolve.
- Dissolve Proteinase K (20mg/ml): Add 0.6ml (10Preps), 1.2ml (50Preps) or 6ml (250 Preps) Protease Dissolve Buffer to the bottle of Proteinase K and store at -20°C after dissolve.

【Protocol 1】 Total DNA extraction from biology sample

1. Add 150µl Buffer TL and 20µl Proteinase K to the 2ml Bead Tubes, then transfer 300µl whole blood, blood water, tissue homogenate, plasma, ascitic fluid, cerebrospinal fluid, swab soaking solution, cell suspension, ect samples to the 2ml bead tubes.
 - Sputum samples: sputum samples should be fully liquefied with 4~5 volumes of normal saline or Buffer PBS with 0.1% DTT before operation. After liquefied, centrifuge at 13,000 x g for 10 minutes to collect microorganism and cells, remove the excess supernatant, remaining 300µl of supernatant and sediment, vortex to resuspend the cells and then transfer to the 2ml Bead Tubes.
 - Solid samples: transfer 30~50mg paste, tissue blocks, intestinal contents or other solid samples to the 2ml Bead tubes directly, add 300µl Buffer AVE to the tube, and then add 150µl Buffer AVE and 20µl Proteinase K to the tube.
2. Vortex the tube at maximum speed for 10min or place on a bead grinding machine for fast grinding with 60~90s.
 - 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 5min, reposition and then repeat once
3. Centrifuge the tube at 13,000 x g for 3 min.
4. Transfer 250 µl supernatant to a new centrifuge tube, add 500µl Buffer MLB, invert 6~8 times to mix.

5. **Insert a HiPure DNA Mini Column I into a 2ml Collection Tube (provided). Transfer all mixture from step 4 to the column.** Centrifuge at 10,000 x g for 1 min and discard the flow-through. Reuse the collection tube into the column.
6. **Add 500 µl Buffer DCW1 to the column,** centrifuge at 10,000 x g for 1 min and discard the flow-through. Reuse the collection tube into the column.
7. **Add 500 µl Buffer DCW2 to the column,** centrifuge at 10,000 x g for 1 min and discard the flow-through. Reuse the collection tube into the column
8. Repeat Step 7 once.
9. Centrifuge at 10,000 x g for 2 minutes..
10. **Place the column into a clean 1.5 ml centrifuge tube (not provided), and discard the collection tube containing the filtrate. Add 50µl Buffer AVE directly to the middle of column membrane, place at room temperature for 3 min.** Centrifuge at 10,000 x g for 1 min.
11. Discard the column and store DNA at 2~8°C, for long time storage, store at -20°C.

【Protocol 2】 Enrich and extraction of microbial DNA from large volume of liquid samples

1. **Transfer 1.0~1.8ml whole blood, blood water, tissue homogenate, ascitic fluid, cerebrospinal fluid, swab soaking solution, cell suspension, ect samples in to a 2ml centrifuge tube. Centrifuge at 13,000 x g for 10 min to collect microbial cells. Pipet to remove the supernatant carefully, remaining 0.7ml of supernatant and sediment, vortex to resuspend the cells.**
 - Sputum samples: sputum samples should be fully liquefied with 4~5 volumes of normal saline or Buffer PBS with 0.1% DTT before operation. After liquefied, centrifuge at 10,000 x g for 10 minutes to collect microorganism and cells. Pipet to remove the supernatant, then add 0.5ml Buffer PBS (not provided) to resuspend the cells, follow step 2 process.
 - Solid samples: transfer 50~200mg paste, tissue blocks, intestinal contents or other solid samples to a 2ml centrifuge tube or homogenize tube (not provided), add 1ml Buffer PBS to the tube for sample homogenization. Stay the homogenate at room temperature for 3 min, transfer 0.5ml supernatant to a 2ml centrifuge tube, follow step 2 process.
 - Whole blood samples: do not use blood exceed 1.0ml, it will cause large amount sediments.
2. **Add 1.0ml lysis Buffer LBX1 and 5µl Proteinase K to the sample, invert to mix 10~15s. Place at room temperature for 10 minutes, during which invert several times to mix. Centrifuge at 13,000 x g for 10 minutes to collect microbial cells. Pipet to remove the supernatant, remaining 50µl liquid and sediment, vortex to resuspend the cells throughly.**

3. **Add 200µl DNase Buffer and 1 µl DNase I to the tube, invert to mix.** Incubate at 37 °C for 15 minutes with oscillating (600~900rpm) to remove eukaryotic cells DNA. (Magen Thermostatic oscillating metal bath machine cat# MagMix B)
4. **Add 200µl Buffer TL and 15µl Proteiase K, vortex to mix throughly. Then transfer all liquid to the 2ml Bead Tubes, screw the lid tightly.** Vortex at maximum speed for 10 minutes or place on a bead grinding machine for fast grinding with 60~90s..
 - 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 5min, reposition and then repeat once
5. Centrifuge the tube at 13,000 x g for 3 min.
6. **Transfer 250 µl supernatant to a new centrifuge tube, add 500µl Buffer MLB,** invert 6~8 times to mix.
7. **Insert a HiPure DNA Mini Column I into a 2ml Collection Tube (provided). Transfer all mixture from step 4 to the column, centrifuge at 10,000 x g for 1 min and discard the flow-through. Reuse the collection tube into the column.**
8. **Add 500 µl Buffer DCW1 to the column,** centrifuge at 10,000 x g for 1 min and discard the flow-through. Reuse the collection tube into the column.
9. **Add 500 µl Buffer DCW2 to the column,** centrifuge at 10,000 x g for 1 min and discard the flow-through. Reuse the collection tube into the column
10. Repeat Step 9 once.
11. Centrifuge at 10,000 x g for 2 minutes..
12. **Place the column into a clean 1.5 ml centrifuge tube (not provided), and discard the collection tube containing the filtrate. Add 30~50µl Buffer AVE directly to the middle of column membrane, place at room temperature for 3 min.** Centrifuge at 10,000 x g for 1 min.
13. Discard the column and store DNA at 2~8°C, for long time storage, store at -20°C.