【Product Name】HiPure Bacterial DNA Kit

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

【Intended Use】

This product provide fast and easy methods for purification of total DNA for reliable PCR and Southern blotting.Total DNA can be purified from bacterial culture, body fluids, food and fermentation.

【Principle】

This product is based on silica Column purification. The sample is lysed and digested with lysate and protease, DNA is released into the lysate. Transfer to an adsorption column. Nucleic acid 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.	D314602	D314603	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
Glass Beads (0.1~0.2mm)	20 g	100 g	Glass Beads
Buffer P1	20 ml	100 ml	Tris/EDTA/SDS
Buffer DL	15 ml	80 ml	Guanidine Salt
Buffer GW1*	22 ml	88 ml	Tris/NaCl
Buffer GW2*	12 ml	50 ml	Tris/NaCl
Lysozme	60 mg	300 mg	Ribonuclease
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】

Lysozme 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.

【Preparation before Use】

- Add 48ml (50Preps) or 200ml (250 Preps) absolute ethanol to the bottle of Buffer GW2.
- Add 28ml (50Preps) or 112ml (250 Preps) absolute ethanol to the bottle of Buffer GW1.
- Add 1.2ml (50Preps) or 6ml (250 Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- Add 1.2ml (50Preps) or 6ml (250 Preps) Protease Dissolve Buffer to the Lysozyme and store at -20~8℃ after dissolve.
- Ethanol (96 – 100%)

【Standart Protocol】

1. **Transfer 0.5-2ml bacterial cultures, fermentations, homogenates, body fluids, liquid foods, swab soaking solution and other samples to 2.0ml centrifuge tube. centrifuge at 10,000 x g for 5 minutes to collect bacterials and remove the supernatant.**

- **Plaque:** Scrape off the plaque with the inoculation ring and transfer it to 0.5 ~1ml sterilized water to wash out the colony. Repeat several times until the plaque is fully removed. If the medium substrate is transferred, let it settle to the bottom for a few minutes, and then transfer the supernatant to the microcentrifuge tube.
- **Tissue block or solid sample:** Transfer 50-500mg tissue block or solid into a suitable tool, disperse or homogenize with 1-3ml saline, and let the large particles precipitate after standing for 2 minutes. Transfer ~ 1.5ml supernatant into a new microcentrifuge tube, centrifuge 10,000 x g for 5 minutes to collect bacteria and discard supernatant. Treat sediment volume no more than 100ul; for animal tissue samples, the precipitation volume after centrifugation shall not exceed 50ul.
- **Liquid sample:** Transfer an appropriate amount of liquid sample into the centrifuge tube, and centrifuge at 10,000 x g for 5-15 minutes to collect bacteria. The volume of sediment should not exceed 200ul.
- **Swab sample**: transfer the swab to the centrifuge tube, add 0.5-1ml normal saline or sterilized water, soak for a few minutes, vortex at high speed for 15-30 seconds, and transfer the suspension to the centrifuge tube and centrifuge.
- **Viscous liquid sample (yogurt, honey, etc.):** Transfer 1-2ml viscous liquid sample to a 15-50ml centrifuge tube, add 5-10 times sterilized water for dilution, mix well, transfer to the centrifuge tube, and centrifuge at 10,000 x g for 5-15 minutes to collect bacteria. If there is too much sediment, add sterilized water for dilution and resuspension, centrifuge at 10,000 x g for 5-15 minutes to collect bacteria, and the volume of sediment should not exceed 200ul.

2. **Add 400μl buffer P1 and 20μl lysozyme were added to the precipitate.** The precipitate was fully suspended by vortex and incubate at room temperature for 20 minutes.
Buffer P1/lysozyme can be mixed in proportion in advance. Staphylococcus was treated and 1μl lysostaphin (20mg/ml) was added.

3. (Optional for difficult to lyse sample) Add ~300mg glass beads to the sample and vortex at maximum speed for 10min or by FastPreps 24 (6.5 m/s twice for 45s).
If RNA-free genomic DNA is required, 5µl of an RNase A (25 mg/ml, no provided) should be added to the sample.

4. Add 400µl Buffer DL and 20 µl Proteinase K to the sample. Mix by pulse-vortexing for 15 s. Incubate at 70°C for 10 min.

5. Centrifuge at 10,000 x g for 3 min. Transfer the supernatant into a new microcentrifuge tube.

6. Add 0.5 volume of ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s.
For example, to 800µl supernatant, add 400µl ethanol (96-100%). It is essential that the sample and the ethanol are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the column.

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

8. Apply the mixture from step 6 to the column. Close the cap and centrifuge at 10,000 x *g* for 1 min.
Centrifugation is performed at 10000 x *g* to reduce noise. Centrifugation at full speed will not affect the yield or purity of the DNA. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the mini column is empty.

9. 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.

10. 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.

11. 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.

12. Place the column in a clean 1.5 ml microcentrifuge tube . 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.
Incubating the column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield. A second elution step with a further 100~200µl Buffer AE will increase yields by up to 15%.

【Fast Protocol】

1. Transfer 200µl of cultures, fermentations, bacterial suspensions, plaque suspension, tissue homogenate, body fluid, swab soaking liquid and other samples without obvious solid impurities to 1.5ml centrifuge tube.

2. Add 50µl buffer P1 and 20µl lysozyme and mix well. Leave at room temperature for 10 minutes.

3. (Optional for difficult to lyse sample) Add ~100mg glass beads to the sample and vortex at maximum speed for 10min or by FastPreps 24 (6.5 m/s twice for 45s). let it sit for 3minutes and Transfer the supernatant into a new tube.

4. Add 250µl buffer DL and 20µl proteinase K into the sample and mix by vortexing for 15s. Incubation at 70℃ for 10 minutes.

5. Centrifuge at 10,000 x g for 1 min. Transfer the supernatant into a new microcentrifuge tube.

6. Add 0.5 volume of ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s.
Follow step 7-12.

Troubleshooting Guide

1. 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.
- 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.

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.
- Inefficient cell lysis due to decreased protease activity: Repeat the DNA purification procedure with a new sample and with freshly prepared Proteinase K stock solution. Besure to store the stock solution at -20–8°C immediately after use. Ensure that Proteinase K is not added directly to Buffer DL.
- 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 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 >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.