【Product Name】HiPure Tissue&Blood DNA Midi Kit

【Product specifications】20 Preps/Kit, 100 Preps/Kit

【Intended Use】

This product provide fast and easy methods for purification of total DNA for reliable PCR and Southern blotting.Total DNA(e.g., genomic, viral, mitochondrial) can be purified from tissue and culture cells.

【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.	D311302	D311303	Main Composition
Purification Times	20	100	-
HiPure gDNA Midi Columns	20	100	Silicon Column
15ml Collection Tubes	40	200	PP Column
Buffer ATL	50 ml	250 ml	Tris/EDTA/SDS
Buffer AL	50 ml	250 ml	Tween-20/Guanidine Salt
Buffer GW1*	22 ml	110 ml	Guanidine Salt
Buffer GW2*	12 ml	50 ml	Tris/NaCl
RNase A	20 mg	90 mg	Ribonuclease
Proteinase K	100 mg	440 mg	Proteinase K
Protease Dissolve Buffer	10 ml	30 ml	Glycerol/Tris/CaCl2
Buffer AE	20 ml	120 ml	Tris/EDTA

【Storage conditions and Validity】

Proteinase K,RNase A 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.

【Preparation before Use】

- Add 48ml (20Preps) or 200ml (100Preps) absolute ethanol to the bottle of Buffer GW2.
- Add 28ml (20Preps) or 140ml (100Preps) absolute ethanol to the bottle of Buffer GW1.
- Add 5ml (20Preps) or 22ml (100Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- Add 1.3ml (20Preps) or 6ml (100Preps) Protease Dissolve Buffer to the RNase A and store at -20~8℃ after dissolve.
- Ethanol (96 – 100%)

【Protocol 】

1. **Determine the amount of Blood，tissue and cells. Do not use more than 200 mg (50 mg spleen), more than 5 x 10^7 culture cells, more than 2ml whole blood, plasma or other body fluids.**
The yield of DNA will depend on both the amount and the type of tissue and cell processed. 1 mg of tissue will yield approximately 0.2–1.2µg of DNA.

2. **Cut up (step 2a) or grind (step 2b) the tissue sample, culture cell (step 2c) or whole blood (step 2d)**
- 2a: Cut up to 200 mg of tissue (up to 50 mg spleen) into small pieces. Place in a 15 crocentrifuge tube and add 2ml of Buffer ATL. Proceed with step 3. It is important to cut the tissue into small pieces to decrease lysis time.
- 2b: Place up to 200 mg of tissue (50 mg spleen) in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into 15 ml centrifuge tube. Allow the liquid nitrogen to evaporate, and add 2ml of Buffer ATL. Proceed with step 3.
- 2c: Determine the number of cells. Centrifuge the appropriate number of cells for 5 min at 300 x g in a 15 ml centrifuge tube. Remove the supernatant completely and discard. Add 1ml Buffer PBS to resuspend cell pellet completely and then add 1ml Buffer ATL and proceed with step 3.
- 2d: Add 200µl Proteinase K to 15 ml centrifuge tube and add up to 2ml whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x 10^7 lymphocytes in 2ml PBS, follow step 4.

3. **Add 200µl proteinase K, mix by vortexing, and incubate at 55°C until the tissue is completely lysed.**
Vortex occasionally during incubation to disperse the sample, or place in a shaking water bath or on a rocking platform. Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. Lysis overnight is possible and does not influence the preparation. To ensure efficient lysis, a shaking water bath or a rocking platform should be used. If not available, vortexing 2–3 times per hour during incubation is recommended.

4. If RNA-free genomic DNA is required, add 50µl RNase A to the sample, mix by pulse-vortexing for 15 s, and incubate for 10~20 min at room temperature.

5. Add 2.2ml Buffer AL to the sample and mix thoroughly by inverting the tube 15 times, followed by additional vigorous shaking for at least 1 min. Incubate at 70°C for 10 min.
It is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of Buffer AL. it will dissolve during incubation at 70°C.

6. Add 2.2ml ethanol (96–100%) to the sample, and mix by inverting the tube 10 times, followed by additional vigorous shaking.
To ensure efficient binding, it is essential that the sample is mixed thoroughly after addition of ethanol to yield a homogeneous solution.

7. Insert a HiPure gDNA Midi Column into a 15mL Collection Tube (provided).

8. Apply one half of the mixture from step 6 to the column. Close the cap and centrifuge at 4,000 x *g* for 3 min.
If the solution has not completely passed through the membrane, centrifuge again at a slightly higher speed. Do not overtighten caps. Always hold the closed Midi columns in an upright position as liquid may pass through the ventilation slots on the rims of the columns even if caps are closed.

9. Remove the Midi column, discard the filtrate, and place the Midi column back into the 15 ml centrifuge tube. Load the remainder of the solution from step 6 onto the column. Close the cap and centrifuge again at 4,000 x g for 3 min.

10. Remove the column, discard the filtrate, and place the column back into the 15 ml centrifuge tube. Add 2ml Buffer GW1to the column. Close the cap and centrifuge at 4,000 x g for 3 min.

11. Carefully, without moistening the rim, add 2 ml Buffer GW2 to the Midi column. Close the cap and centrifuge at 4500 x g (5000 rpm) for 15 min.
The increased centrifugation time should remove all traces of Buffer GW2 from the Midi column before elution. If the centrifugal force is below 4000 x g, incubating the Midi column for 10 min at 55°C in an incubator to evaporate residual ethanol is recommended. Residual ethanol in the eluate may cause inhibition of PCR leading to false-negative results.

12. Place the Midi column in a clean 15 ml centrifuge tube (provided), and discard the collection tube containing the filtrate.
Use a wet tissue paper to wipe any spillage off the Midi column before insertion into the 15 ml centrifuge tube.

13. Pipet 300~500µl Buffer AE directly onto the membrane of the Midi column and close the cap.
Incubate at room temperature for 5 min, and centrifuge at 4500 x g for 3 min.

14. For maximum concentration/Yield: Reload the eluate containing the DNA or pipet a new 300~500µl Buffer AE onto the membrane of th Midi column. Close the cap and incubate at room temperature for 5 min. Centrifuge at 4500 x g (5000 rpm) for 3 min.

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 AL: Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and Buffer AL 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 AL: 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 AL.
- 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.