【Product Name】HiPure Tissue&Blood DNA 96 Kit

【Product specifications】4 x 96 Preps, 20 x 96 Preps

【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,whole blood, plasma, serum, buffy coat, bone marrow, other body fluids, lymphocytes, cultured 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.	D311701	D311702	Main Composition
Purification Times	1 x 96	4 x 96	-
HiPure gDNA Plate	1	4	Silicon Column
96 well Plate (2.2ml)	1	4	PP Column
1.6ml Collection Plate	1	4	PP Plate
0.5ml Collection Plate	1	4	PP Plate
Silicon Seal Tape	1	4	PP Plate
Seal Film	5	25	PP Plate
Buffer ATL	30 ml	100 ml	Tris/SDS
Buffer AL	30 ml	100 ml	Tween-20/Guanidine Salt
Buffer DW1	60 ml	250 ml	Guanidine Salt
Buffer GW2*	50 ml	2 x 100 ml	Tris/NaCl
Proteinase K	50 mg	200 mg	Proteinase K
Protease Dissolve Buffer	5 ml	15 ml	Glycerol/Tris/CaCl2
Buffer AE	30 ml	120 ml	Tris/EDTA

【Storage conditions and Validity】

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 at room temperature (15–25°C) and are stable for at least 18 months under these conditions.

【Preparation before Use】

- Add 200ml (96Preps) or 2 x 400ml (4 x 96Preps) absolute ethanol to the bottle of Buffer GW2 and store at room temperature.
- Add 2.5ml (96Preps) or 10ml (4 x 96 Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- Ethanol (96 – 100%)*
- Phosphate-buffered saline (PBS) may be required for some samples

【Protocol】

- Whole blood, plasma, serum and body fluids.

1. Pipet 20µl Proteinase K into the bottom of a 96 well Plate (2.2ml).
2. Add 200 µl sample to the 96 well plate. Use up to 200 µl whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x 10^6 lymphocytes or Culture Cells in 200 µl PBS. Proceed step 5.
   If the sample volume is less than 200µl, add the appropriate volume of PBS. HiPure DNA Plate copurify RNA and DNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 10µl of an RNase A (25 mg/ml) should be added to the sample before addition of Buffer AL.

- Tissue

1. Pipet 20 µl Proteinase K into the bottom of a 96 well Plate (2.2ml).
2. Cut up to 15 mg of tissue (up to 5 mg spleen) into small pieces and place it in bottom of 96 well Plate.
3. Add 200µl of Buffer ATL and seal the wells thoroughly using the Silica Seal tape. Incubate at 55°C until the tissue is completely lysed in an incubator oven.
   Vortex occasionally during incubation to disperse the sample. Lysis time varies depending on the type of tissue processed. Lysis is usually complete in 1–3 h. If RNA-free genomic DNA is required, add 10µl RNase A to the sample, mix by pulse-vortexing for 15 s, and incubate for 10 min at room temperature.
4. Centrifuge briefly at 3000 rpm to collect any solution from the tape. Proceed step 5.

- Dried blood spots

1. Place 3 punched-out circles(3mm) from a dried blood spot to the bottom of one well of a 96 well plate (2.2ml) and add 220µl of Buffer ATL and Incubate at 90°C for 10min in an incubator oven.
2. Centrifuge briefly at 3000 rpm to collect any solution from the caps.

3. **Add 20µl Proteinase K to the 96 well Plate and seal the wells with Silicon Seal Tape.** Mix by shaking the plate for 60s at 1200rpm. Incubate at 56°C for 1 h in an incubator oven.

4. Centrifuge briefly at 3000 rpm to collect any solution from the tape. Proceed step 5.

5. **Add 200 µl Buffer AL to the sample and seal 96-well plate with seal film. Mix by shaking at 900~1200rpm for 2 min.**
To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

6. Incubate at 65°C for 20 min in an incubator oven.

7. **Remove the seal film and add 200 µl ethanol (96–100%) to the sample. Seal 96-well plate with an new seal film and mix again by shaking at 700~900rpm for 3 min.**
it is essential that the sample, Buffer AL and 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 HiPure gDNA Plate.

8. Insert a HiPure gDNA Plate onto a 1.6mL Collection Plate (provided).

9. **Pipet 600µl of the mixture from step 7 into HiPure gDNA plate placed in a 1.6ml collection plate (supplied). Seal the DNA plate with an Seal film.** Centrifuge for 5 min at >4,000 x *g* and discard the flow-through. Reuse collection plate.

10. **Remove the seal film and add 500µl Buffer DW1 to each well of plate. Seal gDNA Plate with seal film and centrifuge for 3 min at >4,000 x *g* .** Discard the flow-through and reuse the collection plate.

11. **Remove the seal film and add 650µl Buffer GW2 to each well of the plate . Seal HiPure gDNA Plae with seal film and centrifuge for 3 min at >4,000 x *g*.**

12. **Remove the seal film and add 650µl Buffer GW2 to each well of the plate . Seal HiPure gDNA Plae with seal film and centrifuge for 3 min at >4,000 x *g*.**

13. Discard the flow through and reuse the collection plate. Centrifuge at >4,000 x g for 10 min.

14. Allow to air dry for 10 min at room temperature.

15. **Transfer the plate onto a 0.5 ml Collection Plate, and pipet 100~150µl Buffer AE directly onto the membrane.** Incubate for 5 min at room temperature, and then centrifuge for 3 min at >4,000 x *g* to elute.
A second elution step with a further 100~150µl Buffer AE increases yields by up to 20%. For samples containing less than 1 µg DNA, elution in 80 µl Buffer AE or water is recommended.

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. Low concentration of cells or viruses in the sample
- **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. A260/A280 ratio for purified nucleic acids is high

- **High level of residual RNA:** In future DNA preparations, use the optional RNase step included in the protocols.

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 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 Plate 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.