【Product Name】HiPure Tissue 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(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 plate and filter 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.

【Kit Contents】

Cat.No.	D312102	D312103	Main Composition
Purification Times	50	250	-
HiPure gDNA Mini Columns	50	2 x 125	Silicon Column
2ml Collection Tubes	100	5 x 100	PP Column
Buffer ATL	15 ml	65 ml	Tris/EDTA/SDS
Buffer DL	15 ml	65 ml	Tween-20/Guanidine Salt
Buffer GW1*	22 ml	110 ml	Guanidine Salt
Buffer GW2*	12 ml	50 ml	Tris/NaCl
RNase A	10 mg	50 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】

RNase A and Proteinase K should be stored at 2–8°C upon arrival. However, short-term storage (up to 24 weeks) at room temperature (15–25°C) does not affect its 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 (250Preps) absolute ethanol to the bottle of Buffer GW2 and store at room temperature.
- Add 28ml (50Preps) or 140ml (250Preps) absolute ethanol to the bottle of Buffer GW1 and store at room temperature.
- Add 1.2ml (50Preps) or 6ml (250Preps) Protease Dissolve Buffer to the Proteinase K and store at -20~8℃ after dissolve.
- Add 0.6ml (50Preps) or 3ml (250Preps) Protease Dissolve Buffer to the RNase A and store at -20~8℃ after dissolve.
- Ethanol (96 – 100%)
- Phosphate-buffered saline (PBS) may be required for some samples

【Protocol for tissue and cells】

1. **Determine the amount of tissue and cells. Do not use more than 25 mg (10 mg spleen) or more than 5 x 10^6 culture cells.**
   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), grind (step 2b), or mechanically disrupt (step 2c) the tissue sample or culture Cell (step 2d).** The procedure requires no mechanical disruption of the tissue sample, but lysis time will be reduced if the sample is ground in liquid nitrogen (step 2b) or mechanically homogenized (step 2c) in advance.

- 2a: Cut up to 25 mg of tissue (up to 10 mg spleen) into small pieces. Place in a 1.5 ml microcentrifuge tube and add 220μl of Buffer ATL. Proceed with step 3. It is important to cut the tissue into small pieces to decrease lysis time. 2 ml microcentrifuge tubes may be better suited for lysis.
- 2b: Place up to 25 mg of tissue (10 mg spleen) in liquid nitrogen, and grind thoroughly with a mortar and pestle. Decant tissue powder and liquid nitrogen into 1.5 ml microcentrifuge tube. Allow the liquid nitrogen to evaporate, and add 220μl of Buffer ATL. Proceed with step 3.
- 2c: Add up to 25 mg of tissue (10 mg spleen) to a 1.5 ml microcentrifuge tube containing no more than 100μl PBS. Homogenize the sample using the TissueRuptor or equivalent rotor–stator homogenizer. Add 120μl Buffer ATL, and proceed with step 3.
- 2d: Determine the number of cells. Centrifuge the appropriate number of cells for 5 min at 300 x g in a1.5 ml microcentrifuge tube. Remove the supernatant completely and discard. Add 150 μl Buffer PBS to resuspend cell pellet completely. Add 50μl Buffer ATL, and proceed with step 3.

3. **Add 20µl 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 10µl RNase A to the sample, mix by pulse-vortexing for 15 s, and incubate for 10 min at room temperature.**

5. **Add 220µl Buffer DL to the sample, mix by pulse-vortexing for 15 s, and incubate at 70°C for 10 min.**
It is essential that the sample and Buffer DL are mixed thoroughly to yield a homogeneous solution. A white precipitate may form on addition of Buffer DL. it will dissolve during incubation at 70°C.

6. **Add 220 µL ethanol (96–100%) to the sample, and mix by pulse-vortexing for 15 s.**
It is essential that the sample, Buffer DL, 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 HiPure DNA Mini column.

7. Insert a HiPure gDNA Mini Column into a 2.0mL 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.**
Place the column in a new 2 ml collection tube (provided), and discard the tube containing the filtrate. Close each spin column to avoid aerosol formation during centrifugation. Centrifugation is performed at 10000 x *g* to reduce noise. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the mini column is empty.

9. **Open the column and add 650µl Buffer GW1. Close the cap and centrifuge at 10000 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 10000 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 (not provided), and discard the collection tube containing the filtrate. Add 100~200µl Buffer AE or distilled water. Incubate at room temperature for 3 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%. Elution with volumes of less than 100µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield). For samples containing less than 1µg of DNA, elution in 50µl Buffer AE or water is recommended. For long-term storage of DNA, eluting in Buffer AE and storing at –30 to –15°C is recommended, since DNA stored in water is subject to acid hydrolysis.

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 DL 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. Be sure to store the stock solution at -20–8°C immediately after use. Ensure that Proteinase K is not added directly to Buffer ATL.
- **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.