【Product Name】HiPure Tissue DNA Mini Kit

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

【Intended Use】

This product provides fast and easy methods for the purification of total DNA for reliable PCR, Southern blotting, and virus DNA detection.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.	D3121-01	D3121-02	D3121-03	Main Composition
Purification Times	10	50	250	-
HiPure gDNA Mini Columns	10	50	250	Silicon Column
2 ml Collection Tubes	10	50	250	PP Column
Buffer ATL	5 ml	20 ml	80 ml	Tris/EDTA/SDS
Buffer DL	5 ml	20 ml	80 ml	Tween-20/Guanidine Salt
Buffer GW1*	6.6 ml	13 ml	66 ml	Guanidine Salt
Buffer GW2*	6 ml	20 ml	50 ml	Tris/NaCl
RNase A	5 mg	10 mg	45 mg	Ribonuclease
Proteinase K	6 mg	24 mg	120 mg	Proteinase K
Protease Dissolve Buffer	2 ml	5 ml	15 ml	Glycerol/Tris/$CaCl_2$
Buffer AE	5 ml	15 ml	60 ml	Tris/EDTA, pH 9.0

【Storage conditions and Validity】

This product can be stored at room temperature (15–25℃) for 18 months. Proteinase K and RNase A dry powder can be transported and stored at room temperature for short-term preservation, and at -20–8℃ for long-term preservation (> 6 months). The dissolved Proteinase K and RNase A should be stored at -20–8℃.

【Preparation before Use】

- Set a water bath at 55°C and 70°C.
- Dissolve Proteinase K (20 mg/mL): Add an appropriate amount of Protease Dissolve Buffer to dissolve Proteinase K until the final concentration reaches 20 mg/mL. Invert and mix thoroughly to ensure the Proteinase K is completely dissolved, and then store at -20–8℃.
- Dissolve RNase A (15 mg/mL): Add an appropriate amount of Protease Dissolve Buffer to dissolve RNase A until the final concentration reaches 15 mg/mL. Invert and mix thoroughly to ensure the RNase A is completely dissolved, and then store at -20–8℃.
- Buffer GW1 must be diluted with anhydrous ethanol as indicated on the bottle label before using.
- Buffer GW2 must be diluted with anhydrous ethanol as indicated on the bottle label before using.

【Protocol】

A. Animal tissues

1. Cut 1–20 mg tissue (< 10 mg liver, lung or spleen) into small pieces. Place in a 1.5 ml microcentrifuge tube, add 230 µL Buffer ATL and 20 µL Proteinase K. Mix by vortexing, and incubate at 55°C for 0.5–3 hours or overnight until the tissue is completely lysed. 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.

   Correct starting amount of tissue can achieve the desired result. Excessive starting amount of tissue will lead to a reduction in both the output and purity. For samples with rich DNA (e.g., liver, lung, or spleen), less than 10 mg should be used. For samples with less DNA content, the tissue can be increased to 20–50 mg (e.g., 30 mg can be used for muscle or skin). Correspondingly, the dosages of Buffer ATL, Buffer DL, and anhydrous ethanol should be increased proportionally.

   Lysis time will be reduced if the sample is cut into small pieces, ground in liquid nitrogen, mechanically homogenized, or treated with a glass homogenizer or bead grinding instrument in advance. The lysis time depends on the type of sample and the homogenization effect. Generally, tissues require 0.5–3 hours, while the mouse tail needs 6–8 hours. Overnight lysis has no negative effects.

2. Add 10 µl RNase A to the lysate, mix thoroughly by inversion several times, and incubate at room temperature for 10–20 min.

   The RNA digestion time depends on the type of tissue. For samples with rich RNA (e.g., liver or kidney), the digestion time should be extended to 20–30 min.

3. Add 250 µl Buffer DL, mix thoroughly by vortexing for 10 s, and incubate at 70°C for 10 min. Proceed with step 4.

If there are obvious particles or impurities after incubation, centrifuge at 12,000 x g for 5 min to remove undigested impurities. Transfer the supernatant to a new 1.5 mL microcentrifuge tube, and then proceed with step 4.

B. Culture cells

1. Determine the number of cells, and do not use more than 5 x 10^6 culture cells. Harvest the cells in a 1.5 ml microcentrifuge tube and centrifuge at 500 × g for 10 min, then remove and discard the supernatant. Add 150 µL Buffer PBS, and resuspend the cells by vortexing.

2. Add 100 µl Buffer ATL and 10 µl RNase A, mix thoroughly by inversion several times, and incubate at room temperature for 10–15 min.

3. Add 250 µl Buffer DL and 20 µl Proteinase K, mix thoroughly by vortexing for 10 s, and then incubate in a shaking water bath at 55–65°C for 30 min. Proceed with step 4.

C. Fluid samples (e.g., blood, resuspended fluid)

1. Add 20 µl Proteinase K and 250 µl fluid sample in a 1.5 ml microcentrifuge tube, and mix thoroughly.

2. Add 250 µl Buffer DL, mix thoroughly by vortexing for 10 s.

3. Incubate in a shaking water bath (1200–1400 rpm) at 65–70°C for 10 min. Proceed with step 4.

DNA purification

4. Add 250 µl anhydrous ethanol, and mix by vortexing for 10 s.

It is essential that the sample, Buffer DL, and the ethanol are thoroughly mixed to yield a homogeneous solution. A white precipitate may form upon the addition of ethanol when handling samples rich in DNA (e.g., liver or spleen). Pipette up and down 5–10 times to disperse the precipitate.

5. Insert a HiPure gDNA Mini Column into a 2 ml Collection Tube (provided). Apply the mixture (including all of the precipitate) from step 4 to the column. Close the cap and centrifuge at 12,000 x g for 1 min.

If the mixture has not completely passed through the column after centrifugation, centrifuge again at 14,000 x g for 3–5 min until the mini column is empty. If the mixture exceeds 750 µL, pass it through the column in several portions.

6. Discard the filtrate and reuse the collection tubes. Open the column and add 500 µl Buffer GW1. Close the cap and centrifuge at 12,000 x g for 1 min.

7. Discard the filtrate and reuse the collection tubes. Open the column and add 500 µl Buffer GW2. Close the cap and centrifuge at 12,000 x g for 1 min.

8. Discard the filtrate and reuse the collection tubes. Open the column and add 300 µl Buffer GW2. Close the cap and centrifuge at 12,000 x g for 2 min.

When removing the column, ensure that the bottom of the column does not touch the filtrate. If the column is contaminated by the filtrate, discard the filtrate. Reuse the column and the collection tubes, and centrifuge the column again to eliminate the contamination.

9. Place the column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Add 50–100 µl Buffer AE preheated to 70°C to the center of the column membrane. Incubate at room temperature for 3 min, and then centrifuge at 12,000 x g for 1 min.

10. Apply the eluent from step 9 to the center of the column membrane. Incubate at room temperature for 3 min, and then centrifuge at 12,000 x g for 1 min. Remove and discard the column. Store the purified DNA at 2–8°C for short-term storage or at -20°C for long-term storage.

Troubleshooting Guide

1. Clogged column

- Excessive starting amount of the sample: Reduce the sample amount. For samples with rich DNA (e.g., liver, lung, or spleen), use less than 10 mg.
- Sample incompletely lysis/digestion:

  a. Enhance the lysis/digestion effect by grinding with liquid nitrogen or mechanical homogenization.

  b. Prolong the digestion time with Proteinase K or conduct overnight digestion.

  c. Be sure to immediately mix the sample and Buffer DL well by inverting 3–5 times and thoroughly vortexing by vortexing.

  d. If there are obvious particles or impurities after incubation, centrifuge at 12,000 x g for 5 min to remove undigested impurities.

2. Low or no recovery

- Refer to "Column clogged".
- Low concentration of target DNA in the sample: Extracted from the samples with rich DNA (e.g., liver, lung, or spleen).
- Buffer GW1/GW2 did not add ethanol: Ethanol must be added to Buffer GW1/GW2 before used. Repeat procedure with correctly prepared Buffer.
- A precipitate forms upon the addition of ethanol. Pipetting up and down 5–10 times to disperse the precipitate is conducive to increasing the yield.
- Insufficient elution: Buffer AE should be added to the center of the column membrane. Increase the elution volume or frequency.

3. Low DNA purity

- Inefficient 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 vortexing.

- **Excessive starting amount of the sample:** Repeat the DNA purification procedure with a new sample using the correct starting amount.
- **Complex samples:** For tissues rich in metabolic substances, the samples should be extracted with an equal volume of phenol-chloroform after being digested with Buffer ATL/Proteinase K.

4. RNA contamination

- **Samples with rich DNA:** For samples with rich RNA (e.g., liver, kidney, or culture cells), the RNase A digestion time should be extended to 20–30 min.