【Product Name】SolPure Tissue DNA Kit

【Product specifications】1g, 5g, 50g

【Intended Use】

This product are designed for purification of high-molecular-weight genomic or mitochondrial DNA from a variety of Tissue and culture cells. The convenient, scalable purification procedure removes contaminants and enzyme inhibitors such as proteins and divalent cations, and purified DNA is ready for immediate use in sensitive downstream applications or for archiving.

【Principle】

Cells are lysed with an anionic detergent in the presence of a DNA stabilizer and Proteinase K. The DNA stabilizer limits the activity of intracellular DNases and also DNases found elsewhere in the environment. RNA is then removed by treatment with an RNA digesting enzyme. Other contaminants, such as proteins, are removed by salt precipitation. Finally, the genomic DNA is recovered by precipitation with alcohol and dissolved in Buffer TE. Purified DNA typically has an A260/A280 ratio between 1.7 and 1.9, and is up to 200 kb in size. The DNA can be safely stored at 2–8°C, –20°C, or –80°C.

【Kit Contents】

Cat.No.	D331201	D311202	D311203
Purification Times	1g	5 g	50 g
Proteinase K	330 µl	1.8 ml	18 ml
Buffer WTL	33 ml	160 ml	2 x 800 ml
Buffer PPS	12 ml	55 ml	500 ml
RNase Solution	330 µl	1.8 ml	18 ml
Buffer TE	12 ml	60 ml	200 ml

【Storage conditions and Validity】

RNase Solution and Proteinase K should be stored at 2–8°C upon arrival. However, short-term storage (up to 4 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】

- Isopropanol
- 70% Ethanol

【Procedure】

- Animal Tissue

1. Dissect tissue sample quickly and freeze in liquid nitrogen.
2. Grind ■ 5–10 mg or ▲ 50–100 mg frozen or fresh tissue in liquid nitrogen with a mortar and pestle. Work quickly and keep tissue on ice at all times, including when tissue is being weighed.
3. Dispense ■ 300µl or ▲ 3ml Buffer WTL into a ■ 1.5 ml or ▲ 15 ml grinder tube on ice, and add the ground tissue from the previous step.
4. Add ■ 3µl or ▲ 30 µl Proteinase K, mix by inverting 25 times, and incubate at 55°C for 3 h or until tissue has completely lysed. Invert tube periodically during the incubation and process step 5.

- Culture Cell

1. Add ■ 1–2 x 10^6 or ▲ 1–2 x 10^7 cells in balanced salt solution or culture medium to a ■ 1.5 ml microcentrifuge tube or ▲ 15 ml centrifuge tube. Centrifuge for ■ 5 s at 13,000–16,000 x g or ▲ 3 min at 500 x g to pellet cells.
2. Carefully discard the supernatant by pipetting or pouring, leaving approximately ■ 20 µl or ▲ 200 µl residual liquid. Vortex vigorously to resuspend the cells in the residual supernatant.
3. Add ■ 300 µl or ▲ 3 ml Buffer WTL to the resuspended cells and pipet up and down or vortex on high speed for 10 s to lyse the cells.
4. Add ■ 3µl or ▲ 30 µl Proteinase K, mix by inverting 25 times, and incubate at 55°C for 30 min. Invert tube periodically during the incubation and process step 5.
5. Incubate for ■ 1 min or ▲ 3 min on ice to quickly cool the sample.

6. Add ■ 3μl or ▲ 30μl RNase Solution , and mix the sample by inverting 25 times. Incubate at 37°C for 30~60 min.

7. **Add ■ 100μl or ▲ 1ml Buffer PPS, and vortex vigorously for 20 s at high speed.** Centrifuge for ■ 3 min at 13,000–16,000 x g or ▲ 10 min at 2000 x g.

   The precipitated proteins should form a tight pellet. If the protein pellet is not tight, incubate on ice for 5 min and vortex to mix 15s. Repeat the centrifugation.

8. Pipet ■ 300μl or ▲ 3ml isopropanol into a clean ■ 1.5 ml microcentrifuge tube or ▲ 15 ml centrifuge tube.

9. Add the supernatant from step 7 by pouring or pipetting carefully. Be sure the protein pellet is not dislodged during pouring. Mix by inverting gently 50 times.

10. Centrifuge for ■ 1 min at 13,000–16,000 x g or ▲ 3 min at 2000 x g.

11. Carefully discard the supernatant, and drain the tube by inverting on a clean piece of absorbent paper, taking care that the pellet remains in the tube.

12. Add ■ 300 μl or ▲ 3 ml of 70% ethanol and invert several times to wash the DNA pellet. Centrifuge for ■ 1 min at 13,000–16,000 x g or ▲ 1 min at 2000 x g.

13. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube.

14. Allow to air dry for ■ 5 min or ▲ 5–10 min. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.

15. Add ■ 100 μl or ▲ 400 μl Buffer TE and vortex for 5 s at medium speed to mix. Incubate at 65°C for 1 h to dissolve the DNA.

16. Incubate at room temperature (15–25°C) overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storag

Troubleshooting Guide

1. **Tissue or Cells are incompletely lyse**

- Too many cells/Tissue were used： The amount of Buffer WTL used was insufficient for the number of cells/Tissue. If too many cells/Tissue are used, cell lysis will be incomplete; the lysate will become very viscous and cells will clump. Add more Buffer WTL to completely lyse the cells. To prevent incomplete cell lysis, either count cells with a hemacytometer or other cell counter or weigh tissue samples prior to adding Buffer WTL
- Cell clumps were present after add Buffer WTL： Cells may clump if cells are not completely resuspended prior to addition of Buffer WTL. To lyse the cells in the clumps, incubate sample at either 37°C or room temperature (15–25°C) with periodic mixing until the solution is homogeneous.

2. **Protein pellet soft, loose, or absent**

- Sample was not cooled sufficiently before adding Protein Precipitation Solution： To obtain a tight protein pellet be sure that the sample is cooled to room temperature or below (≤20–22°C) prior to adding Protein Precipitation Solution. To obtain a tight protein pellet.
- Protein Precipitation Solution was not mixed uniformly with the cell lysate: Be sure to vortex vigorously for the full 20 s as specified in the protocol.

3. **Samples are slow to rehydrate**

- Samples were not mixed during the hydration step: Incubate with gentle shaking to facilitate hydration of the DNA.
- The DNA pellet was dried too long prior to adding Buffer TE： DNA pellets that are too dry will require a longer time to rehydrate completely. Incubate at 65°C for 1 h and at room temperature overnight. DNA in Buffer TE can be stored at room temperature for up to 1 year.
- Protein contamination in the rehydrated DNA: Protein contamination usually results from sample exceeding the recommended amount of sample material.

4. **A260/A280 too high**

- RNA contamination: Ratios above 2.0 may indicate the presence of RNA. RNA can be moved with one of the following: Increase RNase incubation time in cell lysate from 15 min to 30~60 min.