【Product Name】SolPure Blood DNA Kit

【Product specifications】50ml, 300ml, 1000ml

【Intended Use】

This product are designed for purification of high-molecular-weight genomic or mitochondrial DNA from a variety of blood samples. High-quality DNA can be purified from sample types including whole blood, buffy coat, bone marrow, body fluids in as little as 25 minutes. 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. 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.	D331101	D311102	D311103
Purification Volumes	50 ml	300 ml	1000 ml
10 x Buffer RBC	20 ml	100 ml	3 x 100 ml
Buffer WTL	60 ml	350 ml	1000 ml
Buffer PPS	20 ml	120 ml	350 ml
RNase Solution	300 µl	1.8 ml	6 ml
Buffer TE	10 ml	30 ml	120 ml

【Storage conditions and Validity】

RNase Solution 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 their 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
- Add ddH2O to 10 x Buffer RBC to final 1 x Buffer RBC

Procedure

1. Dispense ■ 900 µl, ▲ 9 ml, or ● 30 ml 1 x Buffer RBC into a ■ 1.5 ml microcentrifuge tube, ▲ 15 ml centrifuge tube, or ● 50 ml centrifuge tube.
2. Add ■ 300 µl, ▲ 3 ml, or ● 10 ml whole blood or bone marrow, and mix by inverting 10 times.
3. Incubate ■ 1 min, ▲ 5 min, or ● 5 min at room temperature (15–25°C). Invert at least once during the incubation.
   For fresh blood (collected within 1 h before starting the protocol), increase incubation time to 10 min to ensure complete red blood cell lysis.
4. Centrifuge for ■ 20 s at 13,000–16,000 x g, ▲ 2 min at 2000 x g, or ● 2 min at 2000 x g to pellet the white blood cells.
5. Carefully discard the supernatant by pipetting or pouring, leaving approximately ■ 10 µl, ▲ 200 µl, or ● 200 µl of the residual liquid and the white blood cell pellet.
6. Vortex the tube vigorously to resuspend the pellet in the residual liquid. Vortexing greatly facilitates cell lysis in the next step. The pellet should be completely dispersed after vortexing.
7. Add ■ 300 µl, ▲ 3 ml, or ● 10 ml Buffer WTL, and pipet up and down to lyse the cells or vortex vigorously for 10 s.

Usually no incubation is required; however, if cell clumps are visible, incubate at 37°C until the solution is homogeneous. Samples are stable in Buffer WTL for at least 2 years at room temperature.

8. add ■ 1.5 µl, ▲ 15 µl, or ● 50 µl RNase Solution, and mix by inverting 25 times. Incubate for 15 min at 37°C. Then incubate for ■ 1 min, ▲ 3 min, or ● 3 min on ice to quickly cool the sample.

9. Add ■ 100 µl, ▲ 1 ml, or ● 3.33 ml Buffer PPS, and vortex vigorously for 20 s at high speed.

10. Centrifuge for ■ 1 min at 13,000–16,000 x g, ▲ 5 min at 2000 x g, or ● 5 min at 2000 x g. The precipitated proteins should form a tight, dark brown pellet. If the protein pellet is not tight, incubate on ice for 5 min and repeat the centrifugation.

11. Pipet ■ 300 µl isopropanol into a clean 1.5 ml tube, ▲ 3 ml isopropanol into a clean 15 ml tube, or ● 10 ml isopropanol into a clean 50 ml tube and add the supernatant from the previous step by pouring carefully. Be sure the protein pellet is not dislodged during pouring.

12. Mix by inverting gently 50 times until the DNA is visible as threads or a clump.

13. Centrifuge for ■ 1 min at 13,000–16,000 x g, ▲ 3 min at 2000 x g, or ● 3 min at 2000 x g. The DNA may be visible as a small white pellet.

14. 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.

15. Add ■ 300 µl, ▲ 3 ml, or ● 10 ml of 70% ethanol and invert several times to wash the DNA pellet.

16. Centrifuge for ■ 1 min at 13,000–16,000 x g, ▲ 1 min at 2000 x g, or ● 1 min at 2000 x g. Whole Blood

17. Carefully discard the supernatant. Drain the tube on a clean piece of absorbent paper, taking care that the pellet remains in the tube. Air dry the pellet for ■ 5 min, ▲ 5–10 min, or ● 5–10 min. The pellet might be loose and easily dislodged. Avoid over-drying the DNA pellet, as the DNA will be difficult to dissolve.

18. Add ■ 100 µl, ▲ 300 µl, or ● 1 ml DNA Hydration Solution and vortex for 5 s at medium speed to mix.

19. Incubate at 65°C for ■ 5 min, ▲ 1 h, or ● 1 h to dissolve the DNA.

20. Incubate at room temperature overnight with gentle shaking. Ensure tube cap is tightly closed to avoid leakage. Samples can then be centrifuged briefly and transferred to a storage tube.

Troubleshooting Guide

1. Red blood cells in the sample were not completely lysed

- Higher than average number of red blood cells in the sample: Repeat the incubation with Buffer RBC to lyse the remaining red blood cells. Add 3 volumes RBC Buffer for each volume of sample, incubate for 10 min at room temperature, and then centrifuge according to the original protocol followed.

2. White blood cell pellet is loose after centrifugation

- Centrifuge settings need to be optimized: Centrifuge settings need to If following a large volume protocol (15 ml or 50 ml tube) increase centrifugation time from 2 to 5 min. Set centrifuge speed to the g-force specified in the protocol. For microcentrifuge tube preps, set the centrifuge speed to maximum. For tabletop and other centrifuges, the speed should usually be set to 2000 x g. If a g-force of 2000 x g cannot be attained by your centrifuge, increase centrifug

3. Cells are incompletely lyse

- Too many cells were used: The amount of Buffer WTL used was insufficient for the number of cells. If too many cells 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.

4. 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.

5. 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.