【Product Name】HiPure FFPE DNA/RNA Kit

【Cat. No. & Specifications】IVD5116, 50 Preps/Kit

【Intended Use】

The Kit is specially designed for simultaneous purification of genomic DNA and total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections. Purified analytes are suitable for use in applications such as real-time PCR and Pyrosequencing.

【Principle】

FFPE samples are incubated in an optimized lysis buffer, which results in the release of RNA and precipitation of DNA. After centrifugation, the RNA-containing supernatant and DNA-containing pellet are then processed separately to purify RNA and DNA. For RNA Purfication, transfer RNA Lysate to an adsorption column and RNA is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing proteins and other impurities, RNA was finally eluted with low-salt buffer. For DNA Purification, transfer DNA Lysate to an adsorption column and DNA is adsorbed on the membrane, while protein is not adsorbed and is removed with filtration. After washing proteins and other impurities, DNA was finally eluted with low-salt buffer.

【Main Composition】

Product Nanme	50 preps	Contents
HiPure DNA Micro Column	50	Adsorption column
HiPure RNA Mini Column I	50	Adsorption column
2ml Collection Tubes	150	PP Column
Proteinase K	50 mg	Proteinase K
Protease Dissolve Buffer	5 ml	Tris/CaCl2/Glycorel
Buffer DPS	60 ml	Hydrocarbon mixture
Buffer FRL	15 ml	Tris/EDTA/SDS
Buffer ATL	15 ml	Tris/EDTA/SDS
Buffer RLC	15 ml	Guandine
Buffer AL	15 ml	Guandine/NaAc/Tween-20
Buffer VHB*	44 ml	Guandine
Buffer RW2*	25 ml	Tris/Nacl
RNase Free Water	10 ml	DEPCWater
Buffer AE	10 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】

- According to the label, add 2.5ml Protease Dissolve Buffer Blue into the bottle of Proteinase K, then stored at - 20°C after dissolve.
- Dilute Buffer VHB with 56ml absolute ethanol as shown on the label and store at room temperature.
- Dilute Buffer RW2 with 100ml absolute ethanol as shown on the label and store at room temperature.

【Protocol】

1. Using a scalpel, trim excess paraffin off the sample block. Cut sections 10–20 µm thick.
2. Transfer 1- 6 sections to 1.5ml microcentrifuge tube. Add 700µl Buffer DPS (Deparaffinization Solution) to the sample. Vortex for 5s and centrifuge briefly to bring the sample to the bottom of the tube.
   Do not use more than six 10 µm sections of 150 mm2 surface area or three 20 µm sections of 150 mm2 surface area. If the sample surface has been exposed to air, discard the first 2–3 sections.
3. Incubate at 56°C for 5 min and vortex vigorously for 15 s to dissolve the paraffin completely.
   Centrifuge at full speed for 2 min. Remove the supernatant by pipetting without disturbing the pellet.
4. Centrifuge at 14,000 x g for 2 minutes. Aspirate and discard the supernatant carefully, do not disturb the pellet.
5. Resuspend the pellet by adding 180µl Buffer FRL and flicking the tube to loosen the pellet.
6. Add 20µl proteinase K and mix by vortexing. Incubate at 56°C for 15 min.
   Depending on the sample material, the sample may not be completely lysed. This does not affect the procedure.
7. Incubate on ice for 3 min and centrifuge for 15 min at 20,000 x *g*.
8. Carefully transfer the supernatant, without disturbing the pellet, to a new1.5 ml microcentrifuge tube for RNA purification in steps 9–18. Keep the pellet for DNA purification in steps 19–28.
   Depending on the amount and nature of the FFPE sample, the pellet may be very small or difficult to see. If the pellet is aspirated with the supernatant, allow the pellet to drop slowly to the bottom of the tube

and use the pipet tip to reattach the pellet to the tube. Alternatively, centrifuge the supernatant again. The DNA-containing pellet can be stored for 2 h at room temperature, for up to 1 day at 2–8°C or for longer periods at –30 to –15°C.

RNA Purification

9. **Incubate the supernatant from step 7 at 80°C for 15 min.**
This incubation step partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented RNA.

10. **Add 200 µl Buffer RLC, and mix thoroughly by vortexing.**

11. **Add 600 µl ethanol (96–100%), and mix thoroughly again by vortexing.**

12. Insert a HiPure RNA Mini Column I in a 2ml Collection Tube.

13. **Add up to 600µl of the sample from Step 10 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

14. Repeat Step 12 until all of the sample has been transferred to the column.

15. **Add 500µl Buffer VHB to the column, centrifuge at 12,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

16. **Add 650µl Buffer RW2 to the column, Centrifuge at 12,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

17. Centrifuge the empty Column at 12,000 × g for 2 minute at room temperature to dry the column matrix.

18. **Transfer the Column to a clean 1.5ml microcentrifuge tube. Add 30~50µl RNase Free Water directly to the center of the column membrane.** Let sit at room temperature for 2 minutes. Centrifuge at 12,000 × g for 1 minute at room temperature. Store RNA at -20℃.

DNA Purification

19. **Resuspend the pellet from step 7 in 180µl Buffer ATL and add 20 µl proteinase K. Mix by vortexing.**

20. **Incubate at 56°C for 1 h. Incubate at 90°C for 2 h.**
This incubation step partially reverses formaldehyde modification of nucleic acids. Longer incubation times or higher incubation temperatures may result in more fragmented DNA.

21. **Add 200 µl Buffer AL, and mix thoroughly by vortexing.**

22. **Add 200 µl ethanol (96–100%), and mix thoroughly again by vortexing.**

23. Insert a HiPure DNA Micro Column in a 2ml Collection Tube.

24. **Add the sample from Step 21 to the Column.** Centrifuge at 12,000 × g for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

25. **Add 500µl Buffer VHB to the column, centrifuge at 12,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

26. **Add 650µl Buffer RW2 to the column, Centrifuge at 12,000 × g for 1 minute at room temperature.** Discard the filtrate and reuse collection tube.

27. Centrifuge the empty Column at 12,000 × g for 2 minute at room temperature to dry the column matrix.

28. **Transfer the Column to a clean 1.5ml microcentrifuge tube. Add 20~50µl Buffer AE directly to the center of the column membrane.** Let sit at room temperature for 3 minutes. Centrifuge at 12,000 × g for 1 minute at room temperature. Store RNA at -20℃.

【Product performance】

1. Appearance inspection: The kit should be completely composed, the appearance of the package should be clean, no leakage, and no damage; the signs and labels should be clear.

2. Nucleic acid purity: Extract 1mg liver homogenate (PBS, 200µl) according to the instructions. The OD260/280 value is 1.7-2.0, A260/230 value is 1.2-1.8.

3. Nucleic acid yield: Extract 1mg liver homogenate (PBS, 200µl) according to the instructions, the yield is 2~ 5ug.

4. Nucleic acid integrity: 1mg liver homogenate (200µl) was extracted according to the instructions. There was no obvious degradation of DNA/RNA during electrophoresis of the product.