ARPENspin FFPE Tissue RNA Extraction Kit

Cat# ARP-FTRES50

Kit Components

COMPONENTS	
Deparaffinization Solution	50 mL
Lysis II Buffer	12 mL
Binding Buffer	5 mL
Wash Buffer II	11 mL (Add 44 mL of ethanol before use)
RElution Buffer	10 mL
DNaseI Buffer	2.6 mL
PK Solution	500 µL
Spin Columns	50

Storage and transportation

• The Kit stability of 12 months when the PK Solution is stored at 2-8℃; DNaseI should be stored at -15 ~ -20℃; the other components of the kit were stored at 2 ~30℃ The DNaseI needs to be transported at 2 ~ 8℃, and the other components of the kit can be transported at room temperature.

Introduction

This kit is designed for extracting high-quality RNA from paraffin-embedded tissues or formalin-fixed tissues. It utilizes a safe and non-toxic deparaffinization solution along with a special lysate formula to efficiently release RNA from the sample. With an effective binding and purification system, the kit ensures the extraction of RNA with good integrity and high purity.

Additional apparatus and materials required but not supplied

- Sterile 1.5mL microcentrifuge tubes
- 10μL/200μL/1000μL tips
- Centrifuge capable of 14,000g
- Ethanol (≥95%)
- Vortex mixer

Important Notes

1. Before using the Binding Buffer, please check for the presence of crystals. If the temperature is low enough that the solution may have crystallized, place the Binding Buffer in a 56°C warm bath. While it is warming, continue to shake the solution to ensure that any crystals completely dissolve before use.
2. Absolute ethanol was added to Wash Buffer II before use and mixed as indicated..

Protocol

1. Sample Processing

a) FFPE sections should be cut to a thickness of up to 10 μm. You can use up to 8 sections, each with a thickness of 10 μm and a surface area of up to 5 × 5 mm. These sections should be placed in a 1.5 mL microcentrifuge tube.

b) Paraffin-embedded blocks: Use a sterile scalpel to cut off the paraffin surface that is in contact with air, scrape up to 30 mg of sample, and avoid paraffin as much as possible. Place the sample in a 1.5 mL microcentrifuge tube.

c) Formalin fixed tissu e samples: Cut the sample into small pieces and place them in a 1.5 mL microcentrifuge tube. Add 1 mL of 10 mM PBS (pH 7.0-7.4) or physiological saline. Mix the contents using a vortex mixer, then centrifuge at full speed for 1 minute. Carefully remove the supernatant with a pipette. Repeat this step once more before proceeding to step 5.

2. Add 1 mL of Deparaffinization Solution to a 1.5 mL tube, close the tube, and vortex for 10 seconds. Then, place the tube in a heating block or water bath at 56°C for 3 minutes.
3. Centrifuge the samples at 10,000g for 1 minute. Then, carefully remove the supernatant using a pipette.
4. Add 1 mL of ethanol to the tube and mix for 10 seconds using a vortex mixer. Centrifuge the tube at 10,000g for 1 minute. Carefully remove the supernatant by pipetting. Open the tube and incubate at room temperature or up to 37°C. Allow it to incubate for 10 minutes or until all residual ethanol has evaporated.
5. To the tube, add 240 µL of Lysis II Buffer and 10 µL of PK Solution. Mix thoroughly using a vortex mixer. Incubate the mixture at 56°C in a metal bath at 1400 rpm for 15 minutes. If a metal bath is not available, gently shake the tube every 2 to 3 minutes during the incubation at 56°C).
6. Incubate the sample at 80°C for 15 minutes.
7. Ice bath for 3 min.
8. Centrifuge at 20,000 g for 5 minutes, and transfer the supernatant to a new 2 mL tube.
9. Add 48µL of DNase I Buffer and 2µL of DNase I to the tube, mix by inverting, and incubate at room temperature for 15 minutes.
10. Add 500 µL of Binding Buffer and mix thoroughly by vortexing. Then, add 1200 µL of ethanol, again mixing by vortexing.

11. Transfer 700 µL of the mixture to the spin column, then centrifuge at 10,000 g for 15 seconds. Discard the flow-through.

12. Transfer the remaining mixture to the spin column and centrifuge at 10,000 g for 15 seconds. Discard the flow-through.

13. Add 500 µL of Wash Buffer II (with ethanol) to the spin column, then centrifuge at 10,000 g for 15 seconds. Discard the flow-through.

14. Add 500 µL of Wash Buffer II (with added ethanol) to the spin column, then centrifuge at 10,000g for 2 minutes. Discard the flow-through.

15. Centrifuge at 20,000 g for 3 minutes, then discard the collection tube.

16. Place the column into a clean 1.5 mL microcentrifuge tube and discard the collection tube with the flow-through.

17. Add 30-50 µL of RElution Buffer to the center of the membrane. Incubate for 2 minutes at room temperature, then centrifuge at 20,000 g for 1 minute. If not used immediately, store at -80°C.