ARPENspin FFPE Tissue DNA Extraction Kit

Cat# ARP-FTDES50

Kit Components

COMPONENTS	
Deparaffinization Solution	50 mL
Lysis Buffer I	10 mL
Binding Buffer	6.25 mL (Add 18.75 mL of ethanol before use)
PW Buffer	14 mL (Add 21 mL of ethanol before use)
Wash Buffer	26 mL (Add 39 mL of ethanol before use)
Elution Buffer	10 mL
PK Solution	1 mL
Spin Columns	50

Storage and transportation

• The Kit stability of 18 months when the PK Solution is stored at 2-8°C, while others should be kept at room temperature.

• The kit can be transported at room temperature.

Introduction

This kit is designed for extracting high-purity DNA from FFPE (formalin-fixed, paraffin-embedded) tissue sections. It includes a nontoxic deparaffinization solution and a high-performance lysis buffer that efficiently releases DNA from the FFPE samples. The DNA binds effectively to our spin column while proteins and other impurities are removed using a wash buffer. Nucleic acids can be easily eluted with either sterile water or an Elution Buffer.

After sample lysis and removal of most formaldehyde modifications, the purified DNA is prepared for downstream applications such as PCR, Real-Time PCR, SNP, and STR analysis.

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
- heating block or water bath

Important Notes

1. For Binding Buffer, PW Buffer, and Wash Buffer: Add the ethanol to the volume indicated on the bottle label and mix well.l.
2. Lysis Buffer I forms precipitates when stored below room temperature. It is essential to heat the buffer to 50°C to dissolve any precipitates prior to use.
3. PK Solution should be stored at a temperature between 2°C and 8°C.
4. Carry out all centrifugations at room temperature.

Protocol

1. Sample Processing

a) FFPE sections should have a thickness of up to 10µm. Up to 8 sections, each with a thickness of 10µm and a surface area of up to 5×5 mm, should be placed in a 1.5 mL sterile 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 the sample, avoiding paraffin as much as possible. Place it in a 1.5 mL sterile microcentrifuge tube.

c) Paraffin-embedded blocks: Use a sterile scalpel to cut away the paraffin surface that is in contact with the air. Scrape approximately 30 mg of the sample, avoiding paraffin as much as possible, and place it in a 1.5 mL sterile microcentrifuge tube.

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 14,000g for 2 minutes. 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 14,000g for 2 minutes. 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. Add 200 µl of Lysis Buffer I and 20 µl of PK Solution to the tube. Mix for 10 seconds by vortexing, then briefly centrifuge the 1.5 mL tube. Incubate at 56°C for 1 hour or until the sample has been completely lysed; overnight incubation is also acceptable.
6. Incubate the sample at 90°C for 1 hour.
7. Optional: If RNA free is required, add 2µl RNase A (100mg/ml), mix completely, and incubate for 2min at room temperature before proceeding to the next step.
8. Add 450 µl of Binding Buffer (with added ethanol) and mix thoroughly using a vortex mixer. Then, briefly centrifuge the 1.5 mL tube.
9. Transfer the mixture to the Spin column and centrifuge at 6,000 g for 1 minute. Discard the flow-through.
10. Add 600 µl of PW Buffer (with ethanol added) to the spin column, then centrifuge at 6,000 g for 1 minute. Discard the flow-through.
11. Add 600 µl of Wash Buffer (with added ethanol) to the spin column, then centrifuge at 6,000 g for 1 minute. Discard the flow-through..
12. Please repeat step 11 once more.
13. Centrifuge the sample at 14,000 g for 2 minutes.

14. Place the column in a clean 1.5 mL microcentrifuge tube and discard the collection tube that contains the flow-through. Add 30-100 µL of Elution Buffer to the center of the membrane. Incubate for 1 minute at room temperature, then centrifuge at 12,000 g for 1 minute. Next, transfer the flow-through back onto the membrane and incubate for 5 minutes at room temperature before centrifuging again at 12,000 g. This process generally increases DNA yield. If not using immediately, please store at -20°C.

Troubleshooting

Q1: Low yield of DNA or absence of DNA.

A: Incorrect or no ethanol was added to the Binding Buffer, PW Buffer, and Wash Buffer. Additionally, the PK Solution should be stored at 2-8°C.

Q2: Precipitation exists in lysate

A: Sample digestion processes vary based on fixation type and conditions.

Q3: DNA is not very effective in the PCR.

A: Ethanol is present in the DNA solution, so please ensure that step 13 is performed.

Q4: Concerns about liquid being dumped in the collection tube can lead to cross-contamination.

A: You can purchase the collection tube separately and change it each time.