Biospin FFPE Tissue Genomic DNA Extraction Kit

Kit Components (50T)

Component	Amount
Cat#	BSC24S1
Deparaffinization Solution	50mL
Lysis Buffer I	10mL
Binding Buffer	12mL（add 15mL ethanol before use）
PW Buffer	14mL（add 21mL ethanol before use）
Wash Buffer	26mL（add 39mL ethanol before use）
Elution Buffer	10mL
PK Solution	1mL
Spin Column	50
Handbook	1

Storage and transportation

- The Kit has demonstrated stability of 18 months when the PK Solution should be stored at 2-8℃, others at room temperature.
- The kit can be transported at room temperature.

Introduction

This kit used to extract high- purity DNA from FFPE tissue sections, with non-toxic deparaffinization solution, high-performance lysis buffer to release DNA from FFPE efficiently, the high efficient binding of DNA to our spin matrix while proteins and other impurities are removed by wash buffer. Nucleic acids are easily eluted with sterile water or Elution Buffer.

After sample lysis, and removes most of the formaldehyde modifications, the purified DNA is ready for downstream applications such as PCR/Real-Time PCR, SNP, STR etc.

Apparatus and materials to be prepared by the user

* 1.5mL microcentrifuge tubes
* 10μL/100μL/1000μL tips
* centrifuge capable of ≥14,000g
* ethanol（≥95%）
* heating block or water bath

Important note

1）Binding Buffer, PW Buffer ,Wash Buffer: Add the ethanol as the volume marked on bottle label and mix well.

2） Lysis Buffer I precipitates below room temperature. It is critical to warm up the buffer at 50℃ to dissolve the precipitates before use.

3）PK Solution should be stored at 2℃-8℃.

4）Carry out all centrifugations at room temperature.

Procedure

1. Sample processing

a）FFPE sections with a thickness of up to 10μm. Up to 8 sections, each with a thickness of up to 10μm and a surface area of up to 5×5mm, placed in a 1.5mL sterile microcentrifuge tube.

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

c）Formalin fixed tissue samples: Cut the sample to small pieces, placed in a 1.5mL sterile microcentrifuge tube. Add 1mL 10mM pH7.0~7.4 PBS or physiological saline, mix with vortex, centrifuge at full speed for 1min, remove the supernatant by pipetting. Repeat this step again, and then start step 5.

2. Add 1mL Deparaffinization Solution to the 1.5mL tube, close the tube and vortex 10s, place the tube in heating block or water bath at 56℃ for 3 min.

3. Centrifuge at 14,000g for 2min, and remove the supernatant by pipetting.

4. Add 1mL ethanol to the tube, and mix 10s by vortexing. Centrifuge at 14,000g for 2min. Remove the supernatant by pipetting, Open the tube and incubate at room temperature or up to 37 ℃. Incubate for 10min or until all residual ethanol has evaporated.

5. Add 200μL Lysis Buffer I, 20μL PK Solution to the tube，mix 10s by vortexing, briefly centrifuge the 1.5mL tube. Then incubate at 56 ℃ for 1h until the sample has been completely lysed (may overnight).

6. Incubate at 90 ℃ for 1 h.

7. Optional：If RNA free is required, add 2μL RNase A (100mg/ml) mix completely and incubate for 2min at room temperature, then start next step.

8. Add 450μL Binding Buffer (ethanol added), mix by vortexing. Briefly centrifuge the 1.5mL tube.

9. Transfer the mixture to Spin column, centrifuge at 6,000g for 1min. Discard the flow-through.

10. Add 600μL PW Buffer (ethanol added) to the spin column, centrifuge at 6,000g for 1min. Discard the flow-through.

11. Add 600μL Wash Buffer (ethanol added) to the spin column, centrifuge at 6,000g for 1min. Discard the flow-through.

12. Repeat step 11 again.

13. Centrifuge at 14,000g for 2min.

14. Place the column in a clean 1.5mL microcentrifuge tube, and discard the collection tube containing the flow-through. Add 30–100μL Buffer Elution Buffer to the center of the membrane, incubate for 1min at room temperature, and then centrifuge at ≥12,000g for 1min. Transfer the flow-through to the membrane again, and incubate for 5min at room temperature, then centrifuge at ≥12,000g generally increases DNA yield. If not use immediately, please store at -20℃.

Troubleshooting

Q1: Low yield or no DNA

A1：Non or incorrect ethanol add to Binding Buffer, PW Buffer, Wash Buffer. And the PK Solution should be stored at 2 ℃-8℃.

Q2: Precipitation exist in lysate

A2: Depends on the type of the sample and the conditions used for fixation, samples digestion is usually different from fresh or frozen samples. Precipitation exist in lysate is possible, and never affect the extraction efficiency and purity.

Q3: DNA does not perform well in PCR

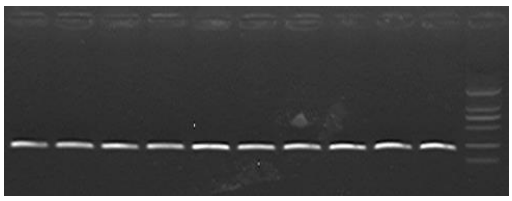
A3: Ethanol remains in DNA solution, make sure step 13 is execute.

Q4: Worry about dumping of liquid in the collection tube can cause cross contamination.

A4: Can buy separate the collection tube, each time change.

Analysis DNA

PCR result of humo β-acting； the marker is DL2,000



Real-Time PCR compare