EasyPure® FFPE Tissue Genomic DNA Kit

Please read the manual carefully before use

Cat. No. EE191

Storage: At room temperature (15-25°C) for one year.

Description

EasyPure® FFPE Tissue Genomic DNA Kit is designed for extracting genomic DNA from formalin-fixed, paraffin-embedded (FFPE) tissue blocks and sections. It uses xylene deparaffinization method to remove paraffin, followed by enzymatic tissue lysis and a specialized incubation step to effectively reverse formaldehyde-induced cross-linking. DNA is specifically adsorbed using a silica-based column. The purified DNA is suitable for downstream applications including PCR, qPCR.

Features

- Resistant to formaldehyde inhibition, with strong lysis capability, fast extraction speed, and high yield.
- The spin column efficiently and specifically adsorbs DNA, effectively removing impurities such as proteins and salts, resulting in high-purity DNA.

Kit Contents

Components	EE191-01 (50 rxns)
Lysis Buffer 15 (LB15)	11 ml
Binding Buffer 15 (BB15)	11 ml
Clean Buffer 15 (CB15)	6 ml
Wash Buffer 15 (WB15)	12 ml
Elution Buffer (EB)	25 ml
Proteinase K (20 mg/ml)	1 ml
Genomic Spin Column with Collection Tubes	50 each

Before starting

- Add 100% ethanol to CB15 and WB15

	Volume of 100% ethanol to add
Clean Buffer 15 (CB15)	24 ml
Wash Buffer 15 (WB15)	48 ml

Procedures (all centrifugation steps are performed at room temperature)

1. Preparing materials

A. Tissue fixed in formalin, etc.: Place 10-30 mg chopped tissue into a 1.5 ml microcentrifuge tube.

a. Add 500 µl 1×PBS and mix thoroughly by vortexing. Incubate at room temperature for 1 minute. Centrifuge at 12,000×g for 30 seconds and remove the supernatant.

b. Repeat step a twice.

B. Paraffin-embedded tissue block: Trim excess paraffin off the tissue and scrap 10-30 mg tissue by a scalpel.

Paraffin-embedded tissue sections: Scrap tissue form 3-10 sections (5-10 µm thick) and place into a 1.5 ml microcentrifuge tube.

a. Add 1 ml xylene to the sample, close the lid and vortex vigorously for 10 seconds. Centrifuge at 12,000×g for 2 minutes, then remove the supernatant by pipetting.

It is recommended to carefully perform this step in a fume hood because xylene is highly toxic chemical. Avoid contact with skin, eyes, and respiratory tract. Additionally, exercise caution to keep away from open flames during operation.

b. Add 1 ml absolute ethanol to the pellet, and mix by vortexing. Centrifuge at 12,000×g for 2 minutes, then remove the supernatant by pipetting.

c. Open the tube and incubate at room temperature or up to 37°C until all residual ethanol has evaporated.

2. Add 200 µl of LB15 and 20 µl of Proteinase K to resuspend the pellet, mix thoroughly by vortexing. Incubate at 56°C for 1 hour (or until the sample has been completely lysed).
3. Incubate at 90°C for 1 hour. Briefly centrifuge the tube to remove drops from the lid.
   The incubation at 90°C can partially reverse formalin-induced crosslinking of nucleic acids. Longer incubation time and higher temperature may result in more fragmented DNA. Therefore, the heating device must be preheated to 90°C before incubation begins.
   Optional: If RNA-free genomic DNA is needed, add 10 µl of RNase A（20 mg/ml, Cat. No. GE101）to the sample after it has cooled to room temperature, incubate at room temperature for 2 minutes.
4. Add 200 µl of BB15 to the sample, and mix thoroughly by vortexing (white precipitates may form).
5. Add 250 µl of absolute ethanol, and mix thoroughly by vortexing (if there are white precipitates, vortex until they disappear). Briefly centrifuge the 1.5 ml tube and transfer all the lysate to a Genomic Spin Column, then centrifuge the tube at 12,000×g for 1 minute, discard the flow-through.
6. Add 500 µl of CB15 (make sure that ethanol has been absolute ethanol), and centrifuge at 12,000×g for 30 seconds, discard the flow-through.
7. Add 500 µl of WB15 (make sure that ethanol has been absolute ethanol), and centrifuge at 12,000×g for 30 seconds, discard the flow-through.
8. Repeat step 7 once.
9. Centrifuge the Spin Column at 15,000 ×g for 2 minutes. Open the tube and air-dry the Spin Column at room temperature to volatilize the residual ethanol.
10. Place the Spin Column in a clean 1.5 ml microcentrifuge tube. Add 30-100 µl of Elution Buffer (for higher yield, prewarm the buffer to 65°C) or distilled water (pH >7.0) to the center of the membrane. Incubate at room temperature for 1 minute. Centrifuge at 12,000×g for 1 minute to elute the DNA.

Notes

- Too many starting materials will affect DNA yield.
- To ensure the quality of the extracted DNA, it is critical that the tissue is fresh prior to fixation. Use a fixation time of 14-24 hours (longer fixation times lead tomore fragmented DNA). Thoroughly dehydrate samples prior to embedding because residual formalin can inhibit the Proteinase K digestion.
- Use sterile centrifuge tubes and pipette tips to avoid DNase contamination.
- Prolonged sample storage time (>2 years) may lead to extraction failure.
- It is recommended to measure the DNA concentration prior to PCR. Using 20 ng of DNA as template (in a 20 µl reaction system) typically yields optimal amplification.

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