MagicPure® FFPE Tissue DNA Kit

Cat. No. EC701

Storage: Kits at room temperature (15℃-25℃) for one year; Magnetic FFPE Beads at 2-8℃ for one year; Proteinase K (20 mg/ml) at -20℃ for two years.

Description

MagicPure® FFPE Tissue Genomic DNA Kit is a universal kit for DNA purification of paraffin-embedded tissues. The kit uses enzymatic hydrolysis to lyse cells and magnetic beads to specifically adsorb DNA. It is suitable for extracting genomic DNA from formalin-fixed and paraffin-embedded tissues and paraffin sections. The extracted genomic DNA is suitable for PCR, qPCR, NGS, and other experiments. This kit is suitable for the magnetic rod-type high-throughput nucleic acid extractor.

Features

• Fast, high yield and purity.

• High purity enabled by buffer optimized for paraffin-embedded tissue samples and magnetic beads allowing for high-efficiency and specific DNA adsorption, and effective removal of protein, salt, and other inhibitors.

Kit Contents

Component	EC701-01 (50 rxns)
Lysis Buffer 32 (LB32)	10 ml
Binding Buffer 32 (BB32)	20 ml
Clean Buffer 32 (CB32)	10 ml
Wash Buffer 32 (WB32)	12 ml
Elution Buffer (EB)	5 ml
Magnetic FFPE Beads	1 ml
Proteinase K (20 mg/ml)	1 ml

Starting material

Fresh formalin-fixed and paraffin-embedded tissues.

Procedures

Before starting, add 100% isopropanol into BB32; add 100% ethanol into CB32 and WB32 (see the table below).

Before use, check whether there is crystal precipitation in BB32. If there is precipitation, put it in water bath at 37℃ until the crystals dissolve and the solution becomes transparent, and mix thoroughly.

All magnetic separations are carried out at room temperature. Prepare 56℃ and 90℃ water baths or other heating equipment before use.

Component	EC701
Binding Buffer 32 (BB32)	10 ml 100% isopropanol
Clean Buffer 32 (CB32)	10 ml 100% ethanol
Wash Buffer 32 (WB32)	48 ml 100% ethanol

1. Preparing materials

FFPE sample block: Trim excess paraffin off the tissue and scrap 10-30 mg tissue by a scalpel.

FFPE section: Place 3-10 sections (5-10 μm thick) into a sterile 1.5 ml microcentrifuge tube. (If extraction is to be done later, it is recommended to cut 3-10 pieces of embedded tissue directly ing sections and store it at 4°C).

2. Add 1 ml xylene to the sample, close the lid and vortex vigorously for 10 seconds. Centrifuge at 12,000×g for 2 min, and then remove the supernatant by pipetting. (It is recommended to carefully perform this step in a fume hood because xylene is a highly toxic chemical. Avoid contact with skin, eyes, and respiratory tract. In addition, keep away from flames during operation).

3. Add 1 ml ethanol (96–100%) to the pellet, and mix by vortexing. Centrifuge at 12,000×g for 2 minutes, and then remove the supernatant by pipetting.

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

5. Add 200 μl of LB32 and 20 μl of Proteinase K and mix thoroughly by vortexing. Incubate at 56°C for 1 hour until the sample has been completely lysed.

6. Incubate at 90°C for 1 hour. Briefly centrifuge the tube to collect the droplets condensed from water vapour on the lid. The duration of 90°C water bath should be controlled strictly. Otherwise, more DNA fragments may be generated. So if there is only one water bath or metal bath, we suggest leaving the sample at room temperature first and starting incubation until the water bath or heat block has reached 90°C. If RNA-free genomic DNA is needed, add 10 μl of RNase A to the lysate, and incubate at room temperature for 2 minutes.

7. Add 600 μl of BB32 (make sure isopropanol has been added) to the sample, and mix thoroughly by vortexing.

8. Add 20 μl of Magnetic FFPE Beads (vortex the microcentrifuge tube for 1 minute before use to mix well). Vortex the microcentrifuge tube for 1 minute, and then incubate at room temperature for 2 minutes. Repeat 3 times, and then place the microcentrifuge tube onto the magnetic stand until the solution is clear.

9. Remove the supernatant by pipetting carefully (be careful not to remove any beads). Add 400 μl of CB32 (make sure that ethanol has been added). Vortex the microcentrifuge tube for 2 minutes, and then place it onto the magnetic stand until the solution is clear.

10. Remove the supernatant by pipetting carefully (be careful not to remove any beads). Add 600 μl of WB32 (make sure that ethanol has been added). Vortex the microcentrifuge tube for 2 minutes, and then place it onto the magnetic stand until the solution is clear.

11. Remove the supernatant by pipetting carefully (be careful not to remove any beads). Add 600 μl of WB32 (make sure that ethanol has been added). Vortex the microcentrifuge tube for 2 minutes and mix well, and then place it onto the magnetic stand until the solution is clear.

12. Try to pipet the supernatant thoroughly. Air-dry the uncapped beads at room temperature for 10-15 minutes.

13. Elution: Add 30-100 μl of EB, and vortex the microcentrifuge tube for 30 seconds. Warm it at 65°C for 10 minutes (vortex the microcentrifuge tube for 2-3 times in the process). Then place it onto the magnetic stand until the solution is clear. Transfer the supernatant by pipetting carefully to a new sterile microcentrifuge tube, and store at -20°C.

Notes

• It is not appropriate to use too much starting material, as this affects the extraction performance.

•To ensure the quality of the extracted DNA, please make sure the tissue before fixing is fresh and reliable. Use a fixation time of 14-24 hours (longer fixation times lead to more fragmented DNA). It is not recommended to extract samples that have been stored for too long.

•Use sterile tubes and pipette tips to avoid DNase contamination.

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