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Paraffin-Embedded Tissue DNA Extraction Kit

Cat No.: D1710

Package: 50T/100T

Storage: Store in dry and temperate condition (15℃-25℃), re-test period for 12 months. Storage at 2℃-8℃for longer. RNase A and Proteinase K will be shipped as accessories, please store at -20℃ after receiving them.

Kit Contents:

Component	D1710-50T	D1710-100T
RNase A	1ml	1ml×2
Proteinase K	1ml	1ml×2
Dewaxing solution	100 mL×2	100 mL×4
Solution A	10ml	20ml
Solution B	10ml	20ml
Washing Buffer	15ml	15ml×2
Elution Buffer	10ml	20ml
Adsorption column	50 units	100 units
Collection tube	50 units	100 units

Product Description

The Paraffin-embedded tissue DNA extraction kit is optimized for purification of DNA from FFPE tissue sections. It uses special method to remove paraffin, and provides unique lysis conditions for DNA release from tissue slice, well removes formalin crosslinking of the released DNA, reducing the damage of formalin to DNA maximally. Combined selective-binding silica-based membrane and flexible elution system, this kit could elute high-quality DNA.

Protocol

Note: Please add Absolute Ethanol to the Washing Buffer before use. Please refer to the label on the bottle for the added volume. Unless otherwise specified, all centrifugation steps are centrifuged at room temperature using a benchtop centrifuge.

1. Sample preparation

a. Paraffin Section: Take Paraffin Section (5-10 μm thick, 1×1 cm^2 in size)5-8 sheets.

b. Paraffin Block: Scrapes about 30 mg of tissue with a scalpel (removing paraffin as much as possible) .

Note: If the surface of the sample is exposed to air, discard the 2-3 pieces that are scraped initially.

c. Samples in formalin and other fixative: Take 30mg samples and cut into several pieces with a scalpel. Place in a 1.5 mL centrifuge tube, add 500μl PBS (0.01 m, PH 7.4) and Vortex Oscillation. Centrifuge at 12,000 rpm for 1 min and discard supernatant. Repeat 3 times, then start with step 7.

2. Dewaxing(alternative)

a. Using Dewaxing solution

Add 1mL dewaxing solution, fully oscillate, water bath at 65 °C for 30 min, then fully oscillate again, centrifuged at 15,000rpm for 15 min at 4 °C, then discarded supernatant. Repeat 3 times, then start with step 7.

b. Using Xylene

Add 1 ml xylene, violent vortex 10-15s. Then start from step 3(xylene needs to be supplied by the customer).

3. Centrifuge at 12000 rpm for 2 min, discard the supernatant.
   Note: Do not pour out the sediment.
4. Adding 1mL Absolute Ethanol into the tube, and mixing it in a vortex for 10s.
5. Centrifuge at 12,000 rpm (~13,400×g) for 2 min, discarding the supernatant.
   Note: Do not pour out the sediment.
6. Leave at room temperature for 5-10 min to volatilize ethanol fully.
7. Add 200 µl buffer A and 20 µl protease K to the precipitate, mix thoroughly, digest in water bath at 56 °C for 1 h-3 h until the sample is completely digested. During digestion, the centrifuge tube can be inverted several times until the sample is completely digested. Indicator of complete digestion: clear and viscous liquid.
8. Add 200 µl solution B, invert and mix thoroughly. White precipitates may form, incubate at 75℃for15-30 min， white precipitates will disappear. It shows that the sample does not digested thoroughly if the solution not clear, which may make the amount and purity of DNA extraction lower and block the column.
9. Add 200 µl Absolute Ethanol, mix thoroughly. Flocculent precipitate may also form on addition of ethanol, which will not affect the DNA extraction. Add the mixture into a adsorption column.
10. Centrifuge at 12000rpm for 1 min, discard the flow-through and re-use the collection tube in the next step.
11. Add 600 µl washing solution to the adsorption column (check if absolute ethanol has been added before use) , centrifuge at 12000 rpm for 1 min, discard the flow-through and re-use the collection tube in the next step.
12. Repeat step 11 with another 600µl Washing buffer.
13. Centrifugate at 12000 rpm for 2 min, incubate at room temperature or 50℃ to dry the column. It is critical for removing ethanol from the column.
14. Put the adsorption column into a clean centrifuge tube, add 50-200µl Elution buffer(pre-heated to 65℃) onto the column matrix, incubate at room temperature for 5min. Centrifuge at 12,000rpm for 1 min..
15. To increase DNA concentration, add the solution obtained from step 14 to the center of membrane again. Incubate at room temperature (15-25℃) for 2 min, and then centrifuge for 2 min at 12,000 rpm.

Note:

1. The sample should avoid repeated freeze-thaw, otherwise the extracted DNA fragments will be smaller and the amount of extracted DNA will decrease.
2. If the solution in the kit appears to precipitate, it can be re-dissolved in a water bath at 65 °C, which will not affect on using.
3. The volume of eluting buffer should be no less than 50μL, too small volume will affect the recovery efficiency: The PH value of eluting solution also affects the elution efficiency, If using distilled water, pH should be controlled at 8.0, NaOH can be used to adjust the PH value of the water to this range, PH below 7.0 will reduce the elution efficiency.
4. The integrity of the DNA in this product depends on sample type, storage time, and fixation conditions. If the fixed time of formaldehyde is too long (more than 24h) or the storage time of sample is too long (> 1 year) , the DNA integrity will be damaged and the long fragment can not be expanded.