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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Manual

Product: **FFPE Tissue DNA Extraction Kit - Column**
Catalog Number: **K5011350**

Shipping Condition: Shipped with blue ice

Storage Condition: Aliquot proteinase K into appropriate amounts and store aliquots and actin control primer at -20°C upon arrival. Store all the rest of contents at room temperature

Shelf Life: 1 year from the date of receipt under proper storage conditions

Introduction:
Formalin-fixed, paraffin embedded (FFPE) tissue specimens are highly valuable sources for retrospective studies of many pathologies. Nevertheless, the extraction of nucleic acids from FFPE specimens could often be challenging, as nucleic acids become cross-linked and degraded during the archiving process. Nucleic acids obtained are usually highly fragmented and chemically modified from the archiving process.

Features
- No toxic chemicals
- Short and robust protocol
- No inhibition on downstream applications

Description
The FFPE Tissue DNA Extraction Kit - Column allows for facile and efficient deoxyribonucleic acid extraction from FFPE tissues, with potential high throughput capabilities and full compatibility for down-stream applications such as qPCR. Utilizing heat and proteinase K treatment, the FFPE Tissue DNA Extraction Kit - Column is optimized in the removal of paraffin, partial reversal of formalin cross linking, and release of DNA from fixed tissues. After clean up with DNA binding columns, the concentration of the high purity DNA can be determined by spectrophotometer or nanodrop equipment.

Content
All necessary reagents for DNA extractions in FFPE tissue specimens are provided. The kit contains sufficient reagents for 50 FFPE tissue DNA extraction reactions.

Quality Control
All kit components are DNase-, RNase-, and protease-free. Each component has been tested for purity and efficacy.

Important Notes
Starting Material: The starting tissue material shall be freshly cut FFPE tissue sections with thickness of 6 to 10 µm each with surface area of up to 200 mm^2 for each 200 µl reaction. The extraction protocols and reagents are easily scaleable to accommodate larger or smaller amount of input sections.

Recommendations for downstream PCR applications: Due to the highly fragmented nature of the nucleic acids obtained from FFPE tissues, care should be taken in the design of primers. PCR amplification shall be less than 300 bases in length with PCR profiles at 40 amplification cycles to ensure successful amplification. A control actin primer is provided in this kit.

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Protocol for FFPE DNA Extraction

Prior to initial use:

Add 22 ml of 100% Ethanol to the bottle of WB1 and mix well. Mark the bottle.
Add 61.6 ml of 100% Ethanol to the bottle of WB2 and mix well. Mark the bottle

Tissue Lysis

1. Cut sections 5-10 µm thick.
2. Place paraffin sections directly into 1.5 ml microcentrifuge tube.
3. Add 180 µl FFPE Lysis Buffer into the sample tube and vortex to mix. Spin at 10,000 × g for 30 seconds at room temperature. Two phases will be formed, a lower (aqueous) phase and an upper (Dewaxil) phase.
4. Add 20 µl Proteinase K directly to the lower phase and mix the lower phase by pipetting up and down 20-30 times.
5. Incubate sample tube at 56°C for 1.5 hour and then incubate the sample tube at 90°C for 1 hour.
6. Centrifuge the sample tube briefly at room temperature to collect any drops from the inside of the lid and carefully transfer the lower phase to a new tube

RNase A Treatment (Optional)

1. Add 2 µl of RNase A (100 mg/ml) to the sample tube in above step 6 and vortex to mix well.
2. Incubate at room temperature for 2 minutes.

DNA Binding

1. Add 200 µl Binding Buffer to the sample tube and mix well by vortex.
2. Add 400 µl Ethanol (100%) to the sample tube and mix well by vortex.
3. Carefully transfer the entire solution to the column (in a 2 ml collection tube) and close the lid. Centrifuge at 6000 x g (8000 rpm) for 1 min and discard the flow-through from collection tube.

Washing

1. Add 500 µl WB1 to the column. Centrifuge at 13,000 rpm for 1 min and discard the flow-through from collection tube.
2. Add 500 µl WB2 to the column. Centrifuge at 13,000 rpm for 1 min and discard the flow-through from collection tube.
3. Centrifuge at 13,000 rpm for 1 min to dry the membrane completely.

DNA Elution

1. Place the column in a clean 1.5 ml microcentrifuge tube and discard the collection tube containing the flow-through.
2. Add 50 µl Elution Buffer to the center of the membrane of the column. Close the lid and incubate at room temperature (15 - 25°C) for 1 min. Centrifuge at 13,000 rpm for 1 min.
3. Discard the column and close the tube lid.

Storage of Extracted DNA

Store the extracted DNA at -20°C or -80°C

Kit Components

Item	Part #	Amount	Storage
1. Dewaxil	K5011350-1	28 ml	Room Temp
2. Proteinase K	K5011350-2	1.1 ml	-20°C
3. FFPE Lysis Buffer	K5011350-3	10 ml	Room Temp
4. Binding Buffer	K5011350-4	11 ml	Room Temp
5. Wash Buffer 1	K5011350-5	1 bottle	Room Temp
6. Wash Buffer 2	K5011350-6	1 bottle	Room Temp
7. Elution Buffer	K5011350-7	2.8 ml	Room Temp
8. Column	K5011350-8	50	Room Temp
9. Actin control primer	K5011450-9	1 tube	-20°C

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Reference

1. Doleshal M, Magotra AA, Choudhury B Cannon BD, Labourier E, Szafranska AE. “Evaluation and validation of total DNA extraction methods for microRNA expression analyses in formalin-fixed, paraffin-embedded tissues” J Mol Diagn 2008 May; 10(3) : 203-11.

2. Haller AC, Kanakapalli D, Walter R, Alhasan S, Eliason JF, Everson RB. “Transcriptional profiling of degraded RNA in cryopreserved and fixed tissue samples obtained at autopsy” BMC Clin Path 2006 Dec; 6(9).

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