Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

User's Manual and Instructions

Product: **FFPE Tissue DNA Extraction Kit - Magnetic Beads**

Catalog Number: **K5011450**

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 beads particles at 4°C. 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
- No lost of nucleic acids
- Short and robust protocol
- No inhibition on downstream applications

Description

BioChain's FFPE Tissue DNA Extraction Kit - Magnetic Beads 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, BioChain's FFPE Tissue DNA Extraction Kit- Magnetic beads 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 beads, 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 up 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 scalable 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, carefulness 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 WB1 and mix well. Mark the bottle.
Add 61.6 ml of 100% Ethanol to the WB2 and mix well. Mark the bottle

1. Cut sections 6-10 µm thick
2. Place tissue sections directly into 1.5 ml microcentrifuge tube.
3. Add Dewaxil to the sample
   - For 4-8 sections, add 500 µl of Dewaxil
4. Incubate at 90°C for 1 minute. Vortex to mix.
5. Add 180 µl FFPE Lysis Buffer into each sample tube
6. Spin at 10,000 x g for 30 seconds at room temperature. Two phases will be formed, a lower (aqueous) phase and an upper (Dewaxil) phase.
7. Add 20 µl Proteinase K directly to the lower phase; mix the lower phase by pipetting up and down 20 times.
8. Incubate specimen samples at 56°C for 1 to 1.5 hour with intermittent mixing (shaker/rotator preferred)
9. Incubate specimen samples at 90°C for 1 hour with intermittent mixing
10. Briefly spin down and Immediately place on ice for 2 minutes
11. Centrifuge briefly at room temperature to collect any drops from the inside of the lid.
12. RNase A treatment (optional): add 2 ul of RNase A (100 mg/ml) directly to the lysed sample in the lower phase, mix the lower phase by pipetting up and down 20 times. Incubate at room temperature for 2 minutes.
13. Add 200 µl Binding Buffer and 400ul Ethanol (95-100%), mix well by vortex.
14. Spin at 10,000 x g for 15 seconds at room temperature. Two phases will be formed, a lower (aqueous) phase and an upper (Dewaxil) phase.
15. Carefully transfer the lower phase to a new tube, avoiding white paraffin residues.
16. Add 12 µl beads. Mix well by vortex. Incubate at RT for 30 min.
17. Place the tube on a magnetic separation device. Let it sit at RT until the beads particles are completely clear from solution.
18. Aspirate and discard the clear supernatant e. Do not disturb the beads particles
19. Remove the tube from the magnetic separation device. Add 800 µl WB1 to the tube and resuspend the beads particles by vortex.
20. Place the tube on a magnetic separation device. Let it sit at RT until the beads particles are completely clear from solution. Aspirate and discard the clear supernatant. Do not disturb the beads particles
21. Remove the tube from the magnetic separation device. Add 800 µl WB2 to the tube and resuspend the beads particles by vortex.
22. Place the tube on a magnetic separation device. Let it sit at RT until the beads particles are completely clear from solution. Aspirate and discard the clear supernatant e. Do not disturb the beads particles
23. Remove the tube from the magnetic separation device. Add 800 µl WB2 to the tube and resuspend the beads particles by vortex
24. Place the tube on a magnetic separation device. Let it sit at RT for 10-15 min with lid open until the beads particles are completely clear from solution. Aspirate and discard the clear supernatant. Do not disturb the beads particles.
25. Remove the tube from the magnetic separation device. Add 50 µl Elution buffer to the tube and resuspend the beads particles well by pipetting up and down 30 times. Incubate for 10 min at RT.
26. Place the tube on a magnetic separation device to magnetize the beads particles. Let it sit at RT until the beads particles are completely clear from solution.
27. Transfer the clear supernatant containing purified DNA to a clear tube

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28. Check the concentration either by UV or pico green, and store the DNA at -20°C (BioChain recommends pico green measurement for more accurate FFPE DNA concentration)

Kit Components

Item	Part #	Amount	Storage
1. Dewaxil	K5011450-1	28 ml	Room Temp
2. Proteinase K	K5011450-2	1.1 ml	4°C,-20°C after receive
3. FFPE Lysis Buffer	K5011450-3	10 ml	Room Temp
4. Binding Buffer	K5011450-4	11 ml	Room Temp
5. Wash Buffer 1	K5011450-5	1 bottle	Room Temp
6. Wash Buffer 2	K5011450-6	1 bottle	Room Temp
7. Elution Buffer	K5011450-7	2.8 ml	Room Temp
8. Beads Particles	K5011450-8	0.7 ml	4°C
9. Actin control primer	K5011450-9	1 tube	-20°C

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).