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Lieferung & Zahlungsart

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Zuschläge

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- Trockeneiszuschlag
- Gefahrgutzuschlag
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Protocol

Product: **FFPE Tissue RNA Extraction Kit**

Catalog Number: **K1011100**

Shipping Condition: Shipped in dry ice, store proteinase K bottle at +4°C and actin control primer tube at -20°Cupon arrival

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 due to filtering washes
- Short and robust protocol
- No inhibition on downstream applications

Description
amsbio's FFPE Tissue RNA Extraction Kit allows for facile and efficient ribonucleic acid extraction from FFPE tissues, with potential high throughput capabilities and full compatibility for down-stream applications such as qRT-PCR. Utilizing heat and proteinase K treatment, ambio's FFPE Tissue RNA Extraction Kit is optimized in the removal of paraffin, partial reversal of formalin crosslinking, and release of RNA from fixed tissues, allowing for the retrieval of all RNA molecules longer than 100 base pairs without the need for toxic compounds and lengthy protocols.

Content
All necessary reagents for RNA extractions in FFPE tissue specimens are provided. The kit contains sufficient reagents for 100 FFPE tissue RNA extraction reactions.

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

Storage Condition
Store proteinase K at +4°C upon arrival, and -20°C upon dilution with FFPET Lysis Buffer. Reconstituted Proteinase K solution and actin control primer is stable up to one year in -20°C, and FFPET Lysis Buffer is stable at least one year after delivery after room temperature.

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 scaleable to accommodate larger or smaller amount of input sections.

Recommendations for downstream PCR applications: Due to the highly fragmented nature of the ribonucleic acids obtained from FFPE tissues, care should be taken in the design of primers. PCR amplicons shall be less than 300 bases in length with PCR profiles at 40 amplification cycles to ensure successful amplification. For cDNA synthesis, random or gene-specific primers should be

used instead of oligo-dT primers. Lysates shall be diluted 2 times prior to use in standard reverse transcription or DNA amplification protocols.

Protocol for FFPET RNA Extraction

Prior to initial use:

Resuspend FFPET Proteinase K with 3.5 ml FFPET Lysis Buffer

Aliquot into appropriate amounts and store aliquots at -20°C until use

1. Using scalpel, trim excess paraffin off sample block, and cut sections 5-10 µm thick.
2. Place paraffin sections directly into 1.5 ml microcentrifuge tube
3. Add 170 µl FFPET Lysis Buffer into each microcentrifuge tube
4. Add 30 µl FFPET Proteinase K into each microcentrifuge tube
5. Gently mix and briefly spin down contents, ensure section is completely submerged.
6. Incubate specimen samples at 65°C for 1 hour with intermittent mixing (shaker/rotator preferred)
7. Incubate at 98°C for 2 minutes
8. Briefly spin down and immediately place on ice for 2 minutes
9. Carefully transfer lysate content to fresh new tube, avoiding white paraffin residues
10. Lysate is now ready for RT-PCR downstream applications
11. Store extraction lysate at -80°C immediately or when not in use

Kit Components

Item	Part #	Amount	Storage
1. Proteinase K	K1011100-1	1 bottle	+4°C -20°C after reconstitution
2. FFPET Lysis Buffer	K1011100-2	1 bottle	Room Temp
3. Actin Control Primer	K1011100-3	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).