

Tissue RNA Extraction Kit (Cat. VFFPE-02R)

Description:

- This kit is designed for isolating total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples (fresh/frozen tissues, glass slides and blocks) using a simple, rapid, environmental-friendly process without using vacuum filtration and toxic organic solvents such as xylene, phenol or chloroform, prepared for RT-PCR amplification and other RNA assays.
- Key features:
 1. Obtain the highest yield and integrity of total RNA within 1 hour.
 2. No filter column or toxic organic solvents required, to reduce the loss of RNA during extraction procedures.
 3. Suitable for nucleic acid extractions from the small/tiny tissues including frozen, fresh and FFPE samples.
 4. Able to obtain the highest quantity of total RNA and DNA at the same tissues.

Kit contains:

Optimization of FFPE tissue fast lysis buffers (A&B), including the tissue digestion reagents and a set of optimized proteinase enzymes, able to rapidly lysate FFPE tissues and decrease the formalin-induced cross-linking in the Lysates. RNA extraction reagent C and D, including the protein precipitation reagents, RNase-free DNase I, and PCR reaction enhancers, able to RNA clean-up and enhance RT-PCR reactions.

Kit storage:

Component	Quantity (100 extracts)	Storage	Tissue
Lysis Buffer A	20.0 mL	-20°C	Tissue
Lysis Buffer B	2.0 mL	-20°C	RNA
extraction reagent C	2.0 mL	- 20°C	
extraction reagent D	1.0 mL	-20°C	

Protocol:

1. Prepare the FFPE sections approximately three or four 20um thick (or 10mg-50mg fresh/frozen paraformaldehyde fixed tissues) in a 1.5 ml clean microcentrifuge tube.
2. Add 200ul of lysis buffer A and 20ul lysis buffer B into the sample tube. Briefly mix by vortexing and incubate at 60°C for 20-30 minutes. (Vortex for 5 seconds every 10 minutes).
3. Place the tube in a PCR machine (or water bath or block) and incubate at 95°C for 2 minutes.
4. Immediately centrifuge the heated sample tube at maximum speed for 3 minutes at 4°C.

5. Pipette the lysated solution into a new sterile/RNase-free 1.5ml tube by penetrating the thin paraffin layer at the top and not disturbing the pellets (cell debris) at the bottom.
6. Centrifuge the crude lysates at maximum speed for 10 minutes at 4°C.
7. Pipette the supernatant and divide it into two tubes: one for RNA extraction, another for DNA assays. (The lysates contained cellular DNA may be directly used for running PCR reactions: Pipette 5-10 ul lysates into a 25ul PCR Master Mixture and run PCR/qPCR at thermal cyclers.)
8. Add 20ul RNA extraction reagent C into the RNA extraction tube, mix thoroughly by pipetting up and down, and incubate at 37°C for 10 minutes.
9. Add 10ul RNA extraction reagent D into the RNA extraction tube, mix thoroughly and incubate at 65°C for 10 minutes.
10. Centrifuge at 14,000xg for 10 minutes at 4°C. Transfer the supernatant into a new clear 1.5ml tube without disturbing the pellets at the bottom. (This supernatant containing total RNA is ready for cDNA synthesis: pipette 5-10 ul extracted total RNA into a standard 20ul reverse transcriptase protocol and 1-5ul of cDNA for running PCR/Real-Time qPCR at thermal cyclers.)
11. Store all the RNA extracts at -80°C and the DNA extracts at -20°C

Technical Tips:

1. Ensure the tissue samples are completely submerged in the reagent A and B at step 2. (Use roughly proportional volume of reagents for different sized samples).
2. If necessary, add 2ul β-mercaptoethanol into lysis buffer A or longer incubation at step 2. may completely digest the tissue samples and no loss of efficacy.
3. If necessary, use the following procedure to further purify and concentrate the crude nucleic acid extractions: add 1/10 volume 3M NaOAc, pH5.2 into the solution obtained from step 4. (DNA tube) and step 9. (RNA tube), mix well and chill at -20°C for overnight. And then, add 0.7 volume of pure ethanol to precipitate nucleic acids, spin down nucleic acids at 4°C for 20 minutes, discard supernatant and dissolve DNA/RNA in 50ul TE buffer (10 mM Tris-Cl, pH 7.5; 1 mM EDTA).
4. The kit may be used for other nucleic acid extractions from various animal fresh/frozen tissues and cell lines, prepared for standard PCR, mitochondrial PCR, random amplification of polymorphic DNA (RAPD) PCR and real time PCR with Sybr® green I Dye, universal probes or other similar dye detection.

Precautions and Disclaimer:

This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process.