®•ZmTech FFPE Tissue RNA Extraction Kit (Cat. FFPE-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 zmtech 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. Zmtech 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
Zmtech FFPE Tissue Lysis Buffer A	20.0 mL	–20°C
Zmtech FFPE Tissue Lysis Buffer B	2.0 mL	–20°C
Zmtech RNA extraction reagent C	2.0 mL	–20°C
Zmtech RNA 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 95ºC 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.