



## ZmTech® FFPE Tissue DNA Extraction Kit (Cat. FFPE-02D)

### Description:

- This kit is designed for extracting DNA 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 cloning, PCR, qPCR and other DNA assays.
- Key features:
  1. Obtain highest yield and purity of total nucleic acid within 1.5 hour.
  2. No filter column or toxic organic solvents required.

### Kit contains:

Optimization of zmtech FFPE tissue fast lysis buffers (A&B), including the tissue digestion reagents and a set of complete proteinase enzymes, able to rapidly lysate FFPE tissues and decrease the formalin-induced cross-linking in the sample. Zmtech DNA extraction reagent C, including the DNA precipitation reagents, DNase I inhibitors and PCR reaction enhancers, able to DNA clean-up and enhance 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 DNA extraction reagent C	20.0 mL	-20°C

**Additional Reagents required but not provided:** Absolute ethanol and 75% ethanol

### Protocol:

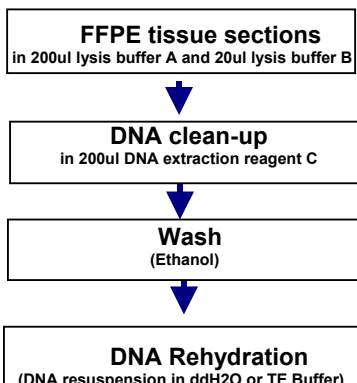
1. Prepare the FFPE sections approximately three or four 20um thick (or 10mg-50mg fresh/frozen paraformaldehyde fixed tissues) in a 1.5 ml clean microcentrifuge tube.
2. Add 200ul of Lysis Buffer A and 20ul Lysis buffer B into the sample tube. Briefly mix by vortexing and incubate at 60°C for 20 minutes. (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 10 minutes.
4. Immediately centrifuge the heated sample tube at maximum speed for 3 minutes at 4°C.
5. Pipette the lysate solution into a new sterile/DNase-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 lysate solution at 10,000xg for 5 minutes at 4°C. Transfer the supernatant into a new clear 1.5ml tube without disturbing the pellets (the remained cell debris, insoluble tissue fragments, RNA and polysaccharides) at the bottom.
7. Add 200ul DNA extraction reagent C, mix thoroughly by pipetting up and down.  
(This mixture may be used for running PCR reactions: Pipette 5-10 ul lysates into a 25ul PCR Master Mixture and run PCR/Real-Time qPCR at thermal cyclers.)
8. Add 100ul absolute Ethanol and place the tube on ice for 10 minutes.
9. Centrifuge at 14,000 xg for 10 minutes at 4°C. Carefully remove the supernatant, as the DNA pellet might translucent at the bottom of the tube.
10. Slowly add 500ul 75% ethanol into tube without resuspending the DNA pellet.



11. Centrifuge at 14,000 xg for 5 minutes at 4°C. Carefully remove the supernatant, as the DNA pellet might be loose.
12. Air dry the DNA pellet for 5-10 minutes and add 20ul sterile/DNase-free ddH<sub>2</sub>O (or TE Buffer) for dissolving DNA. Determine the DNA concentration and purification by measure A260 / A280 with a spectrophotometer. (Store all the DNA extracts at -80°C or -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  $\beta$ -mercaptoethanol into lysis buffer A or longer incubation at step 2. may completely digest the tissue samples and no loss of efficacy.
3. If no pellet is visible at step 9, add 1/10 volume 3M NaOAc, pH5.2 into the solution, mix well and chill at -20°C for overnight. Then, centrifuge one more time.
4. The DNA extractions using this kit are suitable to directly use for ligating and cloning without any further desalted process since no phenol/chloroform and other organic solvents are involved.
5. DNA pellets may be heated at 37°C for 30-60 minutes (or 55 °C for 10 minutes) to completely dissolved in water or TE buffer (10 mM Tris-Cl, pH 7.5; 1 mM EDTA).
6. Schematic of Zmtech FFPE tissue DNA extraction kit (FFPE-002D)

**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.

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