ZmTech Scientifique: Innovation and Development of Laboratory Accessories and Bio-Technologies

® •ZmTech FFPE Tissue Protein Extraction Kit (Cat. FFPE001)

Description:

- **This kit is designed for extracting proteins from formalin-fixed, paraffin-embedded (FFPE) tissue samples (Fresh/frozen tissues, glass slides and blocks), prepared for 1D and 2D electrophoresis, Western Blotting, and other protein/DNA assays.**

Kit contains:

1. **Optimization of zmtech FFPE tissue lysis buffer, including optimized buffer stabilizer, complete protease inhibitors and phosphatase inhibitors.**
2. **1M DTT**

Kit storage:

Component	Quantity (100 extracts)	Storage
Zmtech FFPE Tissue Lysis Buffer	10.0 mL	–20°C
DTT, 1M	30.0 μL	–20°C
Zmtech Protease/Phosphatase Inhibitors (I208052)	250.0 μL	–20°C

Additional Reagents Required but not provided:

- **Absolute ethanol and Ethanol 70%**
- **Xylene or Xylene substitutes**
- **SDS, 20%**

Protocol: (Extracting proteins from 5mm² FFPE fresh/frozen sections or 10mg fresh/frozen tissues)

P.S* for preparing working reagents prior to proceeding.

1. **FFPE sections 50 uM thick 5mm² (or 10mg fresh frozen tissues) were cut and place in a 1.5 ml reaction tube.**
2. **Add 1 ml Xylene (or Xylene substitutes) into tube and incubate at 65°C for 5minutes.**
3. **Vortex at highest speed for 5 seconds and centrifuge 14,000 xg for 5 minutes, discard the supernatant.**
4. **Repeat step.2 and step 3.**
5. **Add 800ul ethanol Abs. vortex twice for 5 seconds.**
6. **Centrifuge 14,000 xg for 5 minutes, discard the supernatant.**
7. **Add 800ul ethanol 70%. vortex twice for 5 seconds.**
8. **Centrifuge 14,000 xg for 5 minutes, discard the supernatant.**
9. **Heat at 100°C for 5minutes to dry pellets.**
10. **Add 100 ul Zmtech FFPE tissue lysis buffer and continue incubate at 100°C for 20 minutes, followed by incubation at 65°C for 1 hour. Vortex twice vigorously for 10 seconds during incubation.**

11. **Centrifuge at 14,000 xg for 20 minutes at 4°C and transfer the supernatant (protein fractions) into a clean pre-chilled micocentrifuge tube and place the tube on ice.**
12. **Determine the protein concentration by Bradford or BCA Assay.**
    - **store all the extracts aliquots at -80°C**

Protocol: (Extracting proteins from 5mm² FFPE samples on glass slides)
P.S* for preparing working reagents prior to proceeding.

1. **Place slides into a Xylene (or Xylene substitutes) bath and incubate for 5 minutes.**
2. **Transfer slides into a new Xylene (or Xylene substitutes) bath and incubate for another 5 minutes.**
3. **Transfer slides into an ethanol Abs. bath and incubate for 10 minutes.**
4. **Transfer slides into a 70% ethanol bath and incubate for 10 minutes.**
5. **Scratch samples form slides into a 1.5 ml reaction tube.**
6. **Heat at 100°C for 5minutes to dry pellets.**
7. **Add 100 ul Zmtech FFPE tissue lysis buffer and continue incubate at 100°C for 20 minutes, followed by incubation at 65°C for 1 hour. Vortex twice vigorously for 10 seconds during incubation.**
8. **Centrifuge at 14,000 xg for 20 minutes at 4°C and transfer the supernatant (protein fractions) into a clean pre-chilled micocentrifuge tube and place the tube on ice.**
9. **Determine the protein concentration by Bradford or BCA Assay.**

. Store all the extracts aliquots at -80°C

P.S*
Prepare working reagents prior to performing extraction procedure:

For 10 Extractions:
Add 1ul DTT (1M),
100ul SDS (20%),
20ul Zmtech protease/ phosphatase Inhibitors,
into 1ml Zmtech FFPE Tissue Lysis Buffer.

For 20 Extractions:
Add 2ul DTT (1M),
200ul SDS (20%),
40ul Zmtech protease/ phosphatase Inhibitors,
into 2ml Zmtech FFPE Tissue Lysis Buffer.

For 100 Extractions:
Add 10ul DTT (1M),
1000ul SDS (20%),
200ul Zmtech protease/ phosphatase Inhibitors,
into 10 ml Zmtech FFPE Tissue Lysis Buffer.

P.S:**

The Zmtech protease/phosphatase Inhibitors (I208052) cocktail (250ul) supplied in DMSO, contains optimized AEBSF, Aprotinin, E64, Leupeptin, Pepstatin A, Sodium fluoride, Sodium Orthovanadate and Sodium pyrophosphate.