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## Protein Lysate Preparation Protocol (Tissue)

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

This protocol details the preparation of protein lysate of the tissue sample.

## Attachments



Western Blot and Lys...

19KB



## Protein Lysate Preparation (Tissue)

4h 20m

- 1 If tissue stored at -80 °C , place in -20 °C for at least 04:00:00 or Overnight prior to homogenization. 4h
- 2 Weigh tissue out in mg.
- 3 Add in 10 volumes of 1X TNE (e.g. 10  $\mu$ L of TNE per 1 mg of tissue).
- 4 Either by mechanical (Dounce) or homogenizer machine, homogenize tissue gently and On ice .
- 5 This is TNE crude lysate (no detergents).
- 6 Add equal volume of 1xTNE +1% SDS, 0.5% NP-40, and 0.5% Doc (Complete TNE).
- 7 Sonicate at 40 °C (3 pulses: 10s On/2s Off at 20% amplitude).
- 8 Boil ( 00:10:00 ). 10m

### Note

**\*STOPPING Point: Sample should be in "Complete TNE". Make sure to sonicate and boil prior storage\*.**

- 9 Centrifuge ( 16000 x g, 4°C, 00:10:00 ). 10m
- 10 Collect supernatant for BCA protein assay.

