TX-100 FRACTIONATION PROTOCOL

Svermily [1]

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dx.doi.org/10.17504/protocols.io.5qpvob21zl4o/v1

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ABSTRACT

To isolate insoluble and soluble proteins from dissected brain regions frozen for biochemical analysis.

ATTACHMENTS

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MATERIALS TEXT

Materials

- phosphatase
- protease inhibitors

A	B
1x TNE (TNE) + Inhibitors	**1 mL**
2x TNE	500 µL
100x HALT inhibitor	10 µL
100x HALT EDTA	10 µL
H2O to final	480 µL

A	B
Complete 2x TNE (2x CTNE) + Inhibitors	**1 mL**
2x TNE	500 µL
1% SDS	100 µL
0.5% NP-40	100 µL
0.5% DOC	100 µL
100x HALT inhibitor	10 µL
100x HALT EDTA	10 µL
H2O to final	180 µL

Thaw Tissue 8h

1 8h

If tissue stored at **-80 °C** , place in **-20 °C** for at least **04:00:00** or **Overnight** to thaw prior to homogenization.

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Prepare 1X TNE

2 Prepare 1X TNE with phosphatase and protease inhibitors.

Homogenize Tissue

3 Weigh tissue out in mg.

4 Add in 10 volumes of 1X TNE.

1. **10 µL** of TNE per **1 mg** of tissue.
2. Ex: **50 mg** tissue = **500 µL** of 1X TNE.

5 Either by mechanical (Dounce) or homogenizer machine, homogenize tissue gently and **On ice** .

6 This is TNE crude lysate (no detergents).

Prepare for Soluble v Insoluble 3h 20m 36s

7 Take specific volume of tissue in TNE and add in equal volume of 1X TNE w/ 2% Triton X-100 (Tx100).

- Ex. **150 µL** of TNE tissue + **150 µL** 1X TNE+2% Tx100.

8 Sonicate @ **4 °C** .

8.1 3 pulses: **00:00:10** ON / **00:00:02** OFF. 12s

9 

Spin down.

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9.1 **Option 1**: **16000 x g, 4°C, 00:15:00** . 15m

9.2 **Option 2**: **20000 x g, 4°C, 01:00:00** . 1h

10 After spin:

10.1 **Supernatant** = **soluble**. Save supernatant and add equal volume of complete TNE (cTNE). 25m 12s

1. Sonicate @ **4 °C** : 3 pluses: **00:00:10** ON / **00:00:02** OFF.
2. Boil: **00:10:00** at **95 °C** .
3. Spin down: **16000 x g, 4°C, 00:15:00** .
4. Supernatant from this is the **soluble fraction**.

10.2 **Pellet = insoluble**.

11

Wash pellet in **150 µL** of 1X TNE+1% Tx100.

Note: Same volume that was used above from TNE.

11.1

Resuspend pellet via pipette.

11.2 1h 15m

Spin down.

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1. **Option 1**: **16000 x g, 4°C, 00:15:00** .
2. **Option 2**: **20000 x g, 4°C, 01:00:00** (**25000 x g**) .

12 Resuspend pellet in **75 µL** to **100 µL** of cTNE.

13 Sonicate @ **4 °C** : 3 pluses: **00:00:10** ON / **00:00:02** OFF. 12s

14 Boil: **00:10:00** at **95 °C** . 10m

15  15m

Spin down: **16000 x g, 4°C, 00:15:00** .

16 Supernatant from this is the **insoluble fraction**.

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