**LEE LAB RESEARCH LABORATORY**

**TX-100 FRACTIONATION PROTOCOL**

**Purpose**: to isolate insoluble and soluble proteins from dissected brain regions frozen for biochemical analysis

**Thaw Tissue**
1. If tissue stored at -80C, place in -20C for at least 4 hours or O/N to thaw prior to

homogenization

**Prepare 1X TNE**

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**Homogenize Tissue**

1. Weigh tissue out in mg
2. Add in 10 volumes of 1X TNE

a. 10 uL of TNE per 1mg of tissue

b. Ex: 50mg tissue = 500 uL of 1X TNE

1. Either by mechanical (Dounce) or homogenizer machine, homogenize tissue gently and

on ice

1. This is TNE crude lysate (no detergents)

**Prepare for Soluble v Insoluble**
1. Take specific volume of tissue in TNE and add in equal volume of 1X TNE w/ 2% Triton X-

100 (Tx100)
 a. Ex. 150 uL of TNE tissue + 150 uL 1X TNE+2% Tx100

1. Sonicate @ 4C
a. 3 pulses:10 sec ON / 2 sec OFF
2. Spin down
	* 1. Option 1: 16,000 g for 15 min at 4C
		2. Option 2: 20,000 g for 60 min at 4C
3. After spin:
	* 1. **Supernatant = soluble.** Save supernatant and add equal volume of complete

 TNE (cTNE)
 i. Sonicate @ 4C: 3 pluses: 10 sec ON / 2 sec OFF

 ii. Boil: 10 min at 95C

 iii. Spin down: 16,000 g for 15 min at 4C

 iv. Supernatant from this is the **soluble fraction**

 b. **Pellet = insoluble**

1. Wash pellet in 150 uL of 1X TNE+1% Tx100 (same volume that was used above from TNE)

a. Resuspend pellet via pipette

b. Spin down

* + 1. Option 1: 16,000 g for 15 min at 4C
		2. Option 2: 20,000 g for 60 min at 4C (25,000 g)
1. Resuspend pellet in 75-100 uL of cTNE
2. Sonicate @ 4C: 3 pluses: 10 sec ON / 2 sec OFF
3. Boil: 10 min at 95C
4. Spin down: 16,000 g for 15 min at 4C
5. Supernatant from this is the **insoluble fraction**

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| **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 |

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| **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 |