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Protein Extraction for Amyloid Beta Fractionation

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We use this protocol and it's working

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Abstract

This protocol can be used to isolate protein from murine brain tissue (volumes listed are for hippocampal samples) by solubility. It creates three protein fractions: tris soluble, triton soluble, and formic acid soluble. This protocol can be helpful when evaluating amyloid beta pathology as different amyloid beta structures can be found within each fraction. For example, insoluble amyloid beta associated with amyloid plaques will be found within the formic acid soluble fraction, whereas more soluble non-plaque associated amyloid beta will be found within the other two fractions.

Preparation

1 **5x Homogenization Buffer (store 4°C):**
125 ml of 1.0 M Tris
30 ml of 0.5 M $MgCl_2$
25 ml of 0.1 M EDTA (pH to 7.2 and store at 4°C for up to 6 months)
20ml of MiliQ Water

2 **1x Homogenization Buffer (dilute day of):**
Dilute 5x Homogenization buffer to 1x in MiliQ Water and add 1 tablet protease inhibitor (cat#11697498001 Roche) per 10 mL

3 **1x Homogenization Buffer with 1% Triton X (dilute day of):**
Add 1% Triton X-100 to 1x Homogenization buffer made above

4 **70% Formic Acid (store RT)** **Caution Acidic**
7mL formic acid
3mL water

5 **1.5x Homogenization Buffer pH 14 (store 4°C)** **Caution Corrosive**
Dilute 5x Homogenization buffer to 1.5x in MiliQ Water and pH to 14

Tissue Processing

6 Weigh frozen tissue sample and record for later normalization

7 Homogenize the tissue on ice using quick bursts of a sonicator in 400µl 1x Homogenization buffer

8 Centrifuge maximum speed at 4°C for 1hr

9 Collect supernatant as tris soluble fraction and store -80°C for later use

10 Homogenize the pellet on ice using quick bursts of a sonicator in 200µl 1x Homogenization Buffer with 1% Triton X-100

11 Centrifuge maximum speed at 4°C for 1hr

12 Collect supernatant as triton soluble fraction and store -80°C for later use

13 Homogenize the pellet on ice using quick bursts of a sonicator in 50µl 70% formic acid **Be careful with acid**

14 Centrifuge maximum speed at 4°C for 1hr

15 Collect supernatant as formic acid soluble fraction and neutralize with 1.5x Homogenization buffer pH14 on ice *Be careful with acid and base**. Store -80°C for later use