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Aβ Extraction from Murine Brain Homogenates

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Abstract

This protocol details beta-amyloid (Aβ) extraction from transgenic murine brain homogenates. Specifically, mechanical homogenization of brain tissue and sequential extraction of both soluble and insoluble proteins are detailed. DEA extracts soluble proteins, such as Aβ isoforms and APP. Formic acid enables extraction of insoluble protein aggregates, such as Aβ isoforms associated with plaques. This procedure produces soluble and insoluble extracts that are amenable to analysis of Aβ species via western blotting and/or enzyme-linked immunosorbent assays (ELISAs), and these results help assess amyloidogenic burden in animals.

Materials and Reagents

1. 5.0 ml open-top polyallomer ultracentrifuge tubes (or tubes capable of undergoing high-speed centrifugation) (Denville Scientific, catalog number: U5022)
2. Diethylamine (DEA) (≥99.5%) (Sigma-Aldrich, catalog number: 471216)
3. 95% formic acid (FA) (AMRESCO, catalog number: 0961)
4. 100 mM NaCl (store at room temperature)
5. Tris base (Thermo Fisher Scientific, catalog numerber: BP152)
6. 0.5 M sodium phosphate dibasic (Na_2HPO_4) (AMRESCO, catalog numerber: 0348)
7. 0.05% sodium azide (NaN_3) (Thermo Fisher Scientific, catalog numerber: S2271)
8. 250 mM sucrose (Thermo Fisher Scientific, catalog numerber: S5)
9. 0.5 mM Ethylenediaminetetraacetic Acid, Disodium Salt Dihydrate (EDTA) (Thermo Fisher Scientific, catalog numerber: S311)
10. 0.5 mM Ethylene glycol-bis(2-aminoethylether)-N, N, N′, N ′-tetraacetic acid (EGTA) (Sigma Aldrich, catalog numerber: 03777)
11. Tris-hydrochloride (Tris-HCl)

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12. 0.4% DEA in 100 mM NaCl (see Recipes)
13. 0.5 M Tris-HCl (pH 6.8) (see Recipes)
14. Formic acid neutralization buffer (see Recipes)
15. Tissue homogenization buffer (THB) (see Recipes)
16. Protease inhibitor cocktail (Sigma-Aldrich, catalog number: P8340) (see Recipes)

Equipment

1. Beckman Coulter Optima L-90K Ultracentrifuge (used with a SW50.1 rotor)
2. Ultrasonic sonicator (see Note 7, below) (Kontes, model: KT50 and catalog number: 12038)

Procedure

Note: The following protocol has been used to extract Aβ from multiple mouse models of Alzheimer's disease [please see Cramer et al. (2012) and Casali et al. (2015)]. The user may need to modify dilutions of the final extracted product depending on the particular application (e.g. ELISA and/or Western blotting). Our lab usually dilutes DEA and FA fractions for Aβ ELISAs at least five-fold to fall within our in-house ELISA detection limits. For western blots of Aβ and modified APP fragments, we recommend 10 to 50 micrograms protein per well, and for more details about Western blotting using the DEA-soluble extraction, please see Morales-Corraliza et al. (2012).

A. Mechanical homogenization

1. Mechanically homogenize brain tissue (see Note 1) in 850 µl cold THB buffer containing fresh protease inhibitor cocktail on ice. If using flash-frozen brains, immediately homogenize. Freshly dissected brains may also be used. Homogenize thoroughly enough such that a homogenous mixture results.
2. Aliquot 250 µl of homogenate into 1.5 ml Eppendorf tubes for DEA/FA extraction on ice (see Note 2). Proceed to DEA/FA extraction below. If not immediately extracting, flash freeze samples on dry ice and store at −80 °C (Note 3).

B. DEA/FA Aβ extraction

1. To 250 µl homogenate, add 250 µl (Note 4) 0.4% DEA and vortex rigorously until mixture appears homogenous.

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[7]Our lab uses a Kontes micro-ultrasonic (20 KHz frequency) cell disrupter rated at 50-Watts power, 120 volts, and 2 amperes.
[1]Our lab uses one brain hemisphere with cerebellum and midbrain removed and flash frozen on dry ice. Our lab does not remove brain meninges upon harvesting of the tissue.
[2]If performing other assays on brain tissue homogenate, aliquot the remaining homogenate accordingly for downstream application (*e.g.*: Western blotting; RNA extraction; *etc*).
[3]Provided storage at −80 °C in a properly functioning freezer and the samples are stored in tight-capped tubes, our lab has routinely used samples 6-months post-collection.

2. Transfer 500 µl of the homogenate/DEA sample to a tube capable of undergoing high-speed centrifugation.

3. Using a swinging-bucket rotor (Note 5), perform a high-speed spin at 135,000 *x g* for 1 h at 4 °C.

4. Remove 425 µl supernatant and neutralize with 42.5 µl 0.5 M Tris-HCl (pH 6.8). Vortex. Divide into 220 µl aliquots and flash-freeze on dry-ice. Store at −80 °C (Note 3). There will be a residual amount of soluble fraction remaining in the tube with the pellet that will not affect the downstream extraction-only remove 425 µl supernatant.

5. Using the homogenate pellet that remains from step B4, add 125 µl cold formic acid (Note 6). Keep tubes on ice.

6. Sonicate each sample on ice for 1 min continuously between output amplitude of 30–50 (Note 7). The pellet should dissolve after this amount of time. If not, sonicate until the pellet dissolves.

7. Perform another high-speed spin at 109,000 *x g* for 1 h at 4 °C.

8. Remove 105 µl sample, and add 1.895 ml of formic-acid neutralization buffer. Vortex and then divide into 2 x 1 ml aliquots and flash-freeze on dry-ice. Store at −80 °C (Note 3).

   Note: For the expected yield of DEA soluble extracts and FA fractions, our lab routinely obtains between 1.0 to 3.0 mg/ml protein and approximately 0.1 to 0.5 mg/ml protein respectively.

Recipes

1. 0.4% DEA solution

   200 µl DEA

   1 ml 5 M NaCl

   ddH_2O to 50 ml

   Stored at room temperature and use within 3 months

2. Formic acid neutralization buffer

   1 M Tris base

   0.5 M Na_2HPO_4

   0.05% NaN_3

   Stored at room temperature and use within 3 months

3. Tissue homogenization buffer (THB)

[4]The amount of DEA to add to homogenate is 1:1 (volume/volume).
[5]Usage of a swinging-bucket, or carriage, is essential in performing efficient extraction.
[6]The formic acid must be cold (*i.e.* chilled to at least 4 °C) in order to precipitate insoluble proteins.

2 mM Tris (pH 7.4)

250 mM sucrose

0.5 mM EDTA

0.5 mM EGTA

q.s. RNase-free H_2O

Stored at 4 °C and use within 3 months

Note: “q.s.” means “quantity required”.

4. 0.5 M Tris-HCl (pH 6.8)

   39.4 g tris-hydrochloride

   q.s. ddH_2O

   Adjust pH to 6.8

   Stored at room temperature and use within three months

5. Protease inhibitor cocktail

   Use at 1:100 dilution and make fresh with each homogenization (if your desired downstream application examines phosphorylated proteins, add phosphatase inhibitors in addition to protease inhibitor cocktail).

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