

# Extraction of amyloid beta from mouse brain

This protocol is for the extraction of two fractions; soluble (extracted using diethylamine, DEA) and insoluble (extracted with formic acid, FA).

## DEA extraction

DEA extraction isolates non-plaque-associated amyloid beta.

1. Prepare a solution of 0.2% DEA in 50 mM NaCl.
2. Homogenize brain using the 0.2% DEA solution at a concentration of 100 mg tissue/mL on ice.
3. Centrifuge at 100,000 x *g* for 1 h at 4°C (54,000 rpm in 100.3 rotor).
4. Take the supernatant, which contains the soluble fraction, and neutralize by adding 1/10 volume 0.5 M Tris HCl pH 6.8. Vortex gently.
5. Neutralized samples can be analyzed by ELISA without further dilution or can be flash-frozen on dry ice and stored at -70°C.
6. Pellets can be retained and frozen if further extraction is required.
7. The pellet can also be used for western blot of full-length amyloid precursor protein.

## FA extraction

FA extraction isolates deposited plaque-associated amyloid beta.

FA neutralization solution: (1 M Tris base, 0.5 M Na<sub>2</sub>HPO<sub>4</sub>, 0.05% NaN<sub>3</sub>)

- 60.57 g Tris base
  - 35.5 g Na<sub>2</sub>HPO<sub>4</sub>
  - 2.5 mL 10% NaN<sub>3</sub>
  - Add H<sub>2</sub>O to 500 mL; pH is not adjusted; store and use at room temperature
  - Caution: Sodium azide (NaN<sub>3</sub>) is highly toxic
1. Mix 200 µL 10% (w/v) homogenate into 440 µL cold formic acid (minimum 95%, Sigma, 5-0507) in a microcentrifuge tube.
  2. Sonicate each sample individually for 1 min on ice. Immerse the tip of the probe in the sample, then move tube up and down while sonicating.
  3. Spin 400 µL at 135,000 x *g* for 1 h at 4°C (50,000 rpm in a TLA 100.3 rotor).
  4. Dilute 210 µL supernatant into 4 mL of room temperature FA neutralization solution. Mix briefly.
  5. FA neutralization solution is stored and used at room temperature, as a precipitate will form if it is stored at 4°C or placed on ice.
  6. Aliquot and flash freeze on dry ice.
  7. Incubate at 37°C for 5 min prior to loading onto ELISA plates to clarify solution and solubilize precipitate.