Hippocampal Protein Extraction

1. Purpose

To isolate protein from adult mouse hippocampal tissue for biochemical analysis.

2. Procedure

Prepare fresh DOC lysis buffer (Materials) and chill on ice. To protect activity of protease and phosphatase inhibitors make up fresh solution every time and keep on ice. Require 1 mL DOC lysis buffer per mouse.

Prepare 1.5 mL tubes to collect tail sample and “rest of brain” sample for each mouse. Prepare 1.5 mL tube with 200 uL DOC lysis buffer to collect both hippocampii from each mouse. Chill tubes on ice.

Prepare platform from the lid of a 6-well tissue culture plate and put on ice. Cover the platform with 3MM blotting paper (Materials) and wet with ice-cold PBS (Materials).

Euthanase mouse by cervical dislocation. Remove head, scalp and remove brain as fast as possible. Place brain on platform, peel back cortex from middle brain. The hippocampus (whiter than cortex) can then be seen. Cut attachments at either side of hippocampus so it just pops out. Dissection of hippocampii from 8 mice (4 wildtype, 4 mutant) takes approximately 1 hr.

Homogenise both hippocampii from each animal in 200 uL of DOC lysis buffer with electric homogeniser (Materials) for approximately 1 min until tissue appears broken and taking care to avoid making bubbles. Add 800 uL DOC lysis buffer to bring total homogenate volume to 1 mL. Keep homogenate on ice while processing remaining samples.

Cut tail sample for re-genotyping and keep on ice. Collect “rest of brain” (ROB) and immediately snap freeze in liquid nitrogen.

Transfer homogenates to 1.5 mL ultracentrifuge tubes (Materials). Weigh tubes and balance. Centrifuge homogenates at 25000 rpm for 15 min at 4°C in an ultracentrifuge (Materials). Collect supernatant from each tube into 1.5 mL tube on ice. Aliquot supernatant into 5 x 170 μl in 1.5 mL tubes and keep leftover small aliquot for protein quantitation.

Snap freeze protein extracts and tail samples in liquid nitrogen then store at -80°C with ROB samples.

3. Materials

3.1 Chemical reagents

- To make 10mL DOC lysis buffer (added in the following order);
    - 1 M Tris HCl pH 9 — 500 µL
    - Milli-Q H_2O — 6.75 mL
    - 0.5 M sodium fluoride (Sigma S7920) — 1 mL
    - 200 mM zinc chloride (Sigma Z0173) — 1 µL
    - 20 mg/ml PMSF (in EtOH) (i) — 250 µL
    - 200 mM sodium orthovanadate (ii) — 50 µL
    - 25x Complete EDTA-free (iii) — 450 µL
    - 10% (v/v) sodium deoxycholate (DOC) (iv) — 1 mL

      10 mL

  Notes:

  i) The half-life of PMSF (Sigma P7626) activity in aqueous solution is limited, so prepare stock in ethanol and store at -20 ^{o}C.

  ii) Sodium orthovanadate (Sigma S6508) is prepared as single use aliquots and stored at -20 ^{o}C.

  iii) 1 tablet Complete EDTA-free (Roche 11 873 580 001) is dissolved in 2 mL Milli Q H_20 and kept as a frozen 25x stock at -20 ^{o}C.

  iv) Add sodium deoxycholate (Sigma D6750) (DOC) after zinc chloride. 10% DOC solution is slow to dissolve.

- PBS - Phosphate buffered saline
- Liquid nitrogen

3.2 Equipment

- 3MM CHR paper (Whatman 3030-931)
- 6-well tissue culture plate lid
- Dissection instruments:-
    - Large 12 cm straight scissors (FST 14001-18)
    - Delicate scissors (FST 14090-09)
    - Fine point angled serrated stainless steel forceps (FST 11080-02)
    - Chattaway micro nickel 150 mm spatula (Fisher FB65057)
    - 2 Double flat end spatulas 200 mm (Jencons-PLS 679-015)
- Homogeniser - Kontes Pellet Pestle Motor (Sigma Z359971)
- Homogeniser - Autoclavable Pestles (Sigma Z359963)
- Ultracentrifuge tubes (Beckman 343778 11x34 mm PC tubes)
- Ultracentrifuge rotor (Beckman MLA-130)
- Optima MAX Ultracentrifuge (Beckman)

4. Quality control

Tissues are dissected as quickly as possible and all dissections are performed by the same experimenter for consistency.

5. Example data

6. Supporting information

7. Document history

This document was created by Rachel T. Uren on 22 February 2008.