Tissue Lysate Preparation: Acid-Extraction of BDNF for Western Blotting Analysis

BDNF is bound to its receptors and chaperons within many tissues, which may interfere with detection of the monomeric form (14 kDa) of BDNF by Western Blotting. Acid extraction protocols have been developed to release this bound BDNF, which not only release the bound BDNF, but also precipitates the receptors.

The following sample preparation protocol can be used as an alternative to standard RIPA-based homogenization procedures, to detect BDNF protein in acid-treated samples by Western Blotting. This protocol is based on Kolbeck *et al.* (1999) as published by researchers from Professor Y.-A. Barde's laboratory.

Acid-Extraction Buffer

50 mmol/L sodium acetate (820 mg / 200 mL), 1 mol/L NaCl (11.7 g / 200 mL), 0.1% Triton X100 (200 µL / 200 mL, add acetic acid until pH 4.0 is reached. Before use add one "Complete" or "Complete Mini" protease inhibitors cocktail tablet (Roche, cat. no. 11697498001 or 11836153001), to be used as recommended by the manufacturer.

Protocol

1. Dissect brain structures, weigh tissue fragments and then freeze them rapidly in liquid nitrogen
2. For long term storage, transfer the frozen tissue samples to -80°C
3. Re-suspend brain tissues in approximately 10 weight/volume-ratio of extraction buffer (for example, 100 µL extraction buffer for 10 mg tissue)
4. Sonicate the suspension to homogeneity on ice with a probe sonicator (e.g. Misonix Ultrasonic Liquid Processor); alternatively, homogenize tissue with a bead homogenizer
5. Sonicate in short bursts (5-7 sec) to avoid excessive sample heating
6. Keep homogenates on ice for 30 min, then repeat sonication as well as incubation on ice; when using a bead homogenizer, repeating the extraction is not required
7. Centrifuge homogenates for 30 minutes at 10,000 – 20,000 x g and 4°C, then transfer clear supernatants into clean tubes and discard pellets
8. Measure total protein concentration (eg., BCA or Bradford protein assay)
9. These supernatants may be stored at -80°C and must be centrifuged again for 30 min at 10,000-20,000 x g and 4°C immediately after thawing and before being used for Western Blotting.

Reference

Kolbeck R *et al.* 1999, *Brain-derived neurotrophic factor levels in the nervous system of wild-type and neurotrophin gene mutant mice.* J Neurochem. May;72(5):1930-8.