Acid-Extraction of BDNF From Brain Tissue

Introduction:

BDNF is bound to its receptors and chaperons within many tissues, which prevents its detection in ELISA asssays, and may interfere with the detection of the unbound forms of BDNF in Western Blotting. Acid extraction protocols have therefore been developed to release this bound BDNF, which not only release the bound BDNF, but also precipitates the receptors. We recommend the following sample preparation protocol for measuring total BDNF concentrations in acid-treated samples. This protocol is based on Kolbeck *et al.* (1999) as published by researchers from Professor Y.-A. Barde's laboratory, and can be used as an alternative tissue extraction method to RIPA-based or other extraction methods. Samples prepared following this protocol can be assayed for BDNF content by ELISA (eg., Biosensis Mature BDNF *Rapid*™ ELISA, cat# BEK-2211), and also used in Western Blotting to assess BDNF protein expression.

Required Buffers:

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 glacial 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.

Incubation/Neutralization Buffer (for ELISA assay analysis only)
0.2 mol/L phosphate buffer, pH 7.6. For instance, weigh in the following amounts per 200 mL total buffer volume and adjust pH with concentrated NaOH solution (≥ 5 mol/L):
KH_2PO_4 (MW 136.09 g/mol): 2.72 g
Na_2HPO_4 (MW 141.96 g/mol): 2.84 g

Acid-Extraction Sample Diluent (for ELISA assay analysis only)
Mix acid-extraction buffer and incubation/neutralization buffer at a ratio of 1 to 3 (eg., 1 mL of extraction buffer and 3 mL of incubation/neutralization buffer). Check pH of solution is approximately pH 6.6 to 7.0.

Acid-Extraction Protocol:

1. Dissect brain structures, weigh tissue fragments and then freeze them rapidly in liquid nitrogen. For long term storage transfer the frozen tissue samples to -80°C.

2. Re-suspend brain tissues in approximately 10 weight/volume-ratio of extraction buffer (for example, 100 µL extraction buffer for 10 mg tissue).

3. Sonicate the suspension to homogeneity on ice with a probe sonicator (e.g. Misonix Ultrasonic Liquid Processor); alternatively, a bead homogenizer can be used.

4. Sonicate in short bursts (5-7 sec) to avoid excessive sample heating. Keep homogenates on ice for 30 min, then repeat sonication as well as incubation on ice; if bead homogenization is used, repeated extraction is not required.

5. 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. 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.

6. Measure total protein concentration (eg., BCA or Bradford protein assay)

7. For Western Blotting, these acid-extracted homogenates can be used directly for sample preparation. For ELISA Assay, follow step 8 onwards.

8. Reconstitute the BDNF standard supplied in the ELISA kit with 1 mL of Acid-Extraction Sample Diluent; perform a serial dilution to cover the assay's calibration range.

9. Neutralize acidic samples by diluting 1 part tissue extract with 3 parts of Incubation/Neutralization Buffer (eg., 50 µL tissue extract and 150 µL Incubation/Neutralization buffer); check that pH of sample is near neutral. Note that the neutralization step equals a sample dilution factor of 4, which needs to be considered when calculating BDNF concentrations in samples.

10. The prepared, neutralized samples are now ready for the ELISA assay. Note that freezing of prepared, neutralized, samples is not recommended. Typically, freezing of samples is best when protein concentrations are more concentrated, as in step 5.

11. If the BDNF concentration in the sample is out of the assay range after performing the initial tests, dilute the solution prepared in step 9 further with Acid-Extraction Sample Diluent for best results.

12. Results can then be reported as ng BDNF/mg total soluble protein or g wet weight if tissue sample is large enough

Note: Biosensis recommends evaluating the recovery of BDNF when preparing tissue extracts. In order to conduct a recovery experiment, known amounts of BDNF standard are added to an aliquot of the brain tissue homogenates and assayed. Spike-recovery experiments allow comparison of technique and methods. Recoveries of 80-120% of spiked values are acceptable. Refer to our Technical Note #1 (ELISA Assay Validation) for more details.

Example Data:

Fifty-three milligram of normal rat cerebellum were homogenized in 530 µL acid-extraction buffer (weight/volume ratio of 10:1) according to the procedure outlined above. Tissue was homogenized using a bead homogenizer.

The brain sample was assayed for BDNF content at 1:8 – 1:512 dilution (4-fold dilution series) in acid-extraction sample diluent, using the Biosensis Mature BDNF *Rapid*™ ELISA, cat# BEK-2211. Each diluted sample was spiked with 100 pg/mL mature BDNF standard to assess recovery.

OD values of serial diluted brain extract showed parallelism to the BDNF calibration curve, demonstrating accurate quantification of BDNF in acid-extracted rat brain extracts (Figure 1).

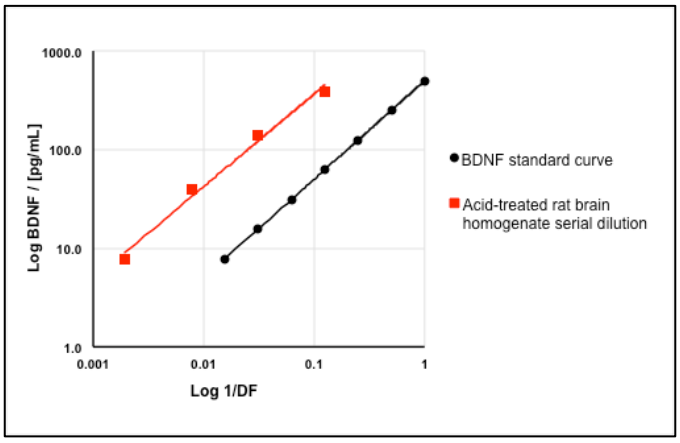

Figure 1: Parallelism of serially diluted rat brain extract and mature BDNF standard curve.

Excellent recovery of spiked BDNF (Table 1) further demonstrated that this ELISA assay yields accurate results in acid-extracted brain tissue.

Table 1: Recovery of spiked BDNF in rat brain extract.

Sample	Dilution	Recovery*
Rat brain extract	1/8	99%
	1/32	119%
	1/128	103%
	1/512	107%

* Mean of 2 assays

Table 2 summarizes BDNF values obtained from acid-extracted rat cerebellum, assayed by ELISA:

Table 2: BDNF content in acid-extracted rat cerebellum

BDNF / [ng/mL]	Total protein* / [mg/mL]	BDNF / [ng / mg extracted protein)	BDNF / [ng / g brain]
3.67	1.30	2.82	36.6

* Determined by Bradford assay

The same rat brain sample was used for assessing BDNF protein expression by Western Blotting (Figure 2).

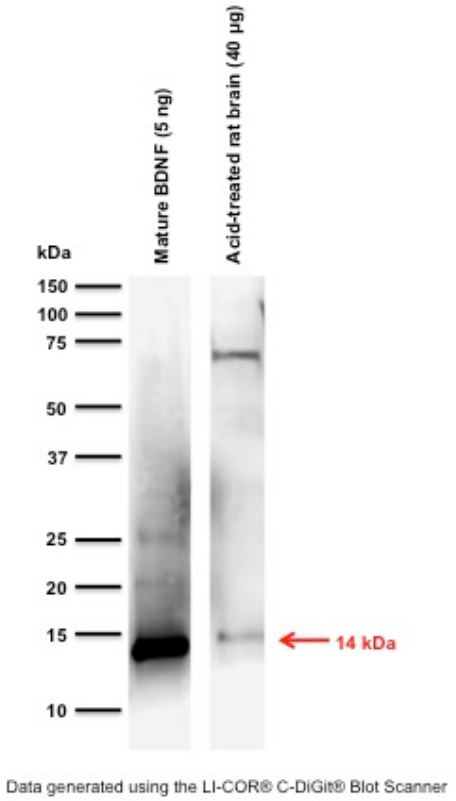

Figure 2: Western blot analysis of BDNF expression in acid-treated rat cerebellum. BDNF expression was assessed with Biosensis' monoclonal BDNF antibody M-1744-50/100 (1 µg/mL). M-1744-50/100 detects BDNF monomer at 14 kDa. An additional higher MW band is present, but has not been characterized as yet.

SDS-PAGE: denaturing and reducing, 12% Bis-Tris gel; Transfer: Tris-Glycine buffer, semi-dry transfer; Membrane: nitrocellulose (0.22 µm); Blocking: 5% skim milk in TBST, 1 hour at RT; Primary antibody: overnight at 4°C; Secondary antibody: anti-mouse-HRP (1/6000), 2 hours at RT; Detection: Chemiluminiscence.

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.