ELISA

METHODOLOGY

ELISA can be utilised for different neurotrophic, inflammatory and oxidative stress markers. Below is a brief outline of the methodology for BDNF.

There is evidence of impairment of Brain Derived Neurotrophic Factor (BDNF) in schizophrenia. BDNF can moderate synaptic plasticity, protein synthesis, dendritic growth, NMDA receptor dependent synaptic modulation and cognitive function. It can be determined in different brain regions using ELISA.

Sample preparation

Homogenisation

Regions of extra are dissected from the frozen brain and homogenised on ice in a 10-fold volume (mg:µL) of buffer (10mM Trizma base, Sigma-Aldrich; 320µM sucrose, Sigma-Aldrich; 2mM EDTA, Sigma-Aldrich; and protease- and phosphatase- inhibitor cocktails, Sigma-Aldrich) buffered to a pH of 7.4. Phenylmethylsulfonyl fluoride (PMSF) and sodium orthovanadate are added to the solution at a 1% (v/v) concentration. Samples are centrifuged at 800xg for 15 minutes at 4°C. The supernatant is collected and centrifuged at 11,700xg for 20 minutes, and the resulting supernatant is collected and stored at -20°C until analysis.

Bradford Assay

Protein concentrations of each sample are calculated using a Bradford Assay. Samples are diluted into protein assay dye reagent concentrate (BioRad) and compared to bovine serum albumin (Sigma) of known concentration (1.0-0.1 mg/ml protein). A standard curve is created using a simple linear regression. Standards and samples are run in triplicate.

ELISA (Brain Derived Neurotrophic Factor)

Quantitative measurement of BDNF in brain tissue is conducted using a Rat Brain Derived Neurotrophic Factor (BDNF) ELISA Kit (Cat#EKU02787-96T, BIOMATIK). Briefly samples are diluted to 2.5 mg/mL protein. An 8-point, 2-fold standard curve is created (31.25 – 1000 pg/mL), including a diluent-only control. The standards and samples are pipetted into the pre-coated 96-well plate and incubated for 1 hour at 37°C. The samples are removed from each well without washing. Next, detection reagent A is pipetted into the plate and incubated for one hour at 37°C. The samples are washed eight times with wash buffer, and the remaining wash solution is removed from the plate by inverting the plate onto absorbent paper. Then, detection reagent B is added and incubated for 30 minutes at 37°C. The wash step is repeated. Finally, the substrate solution is added and developed in the dark. Once the stop solution is added, the plate is read on a microplate reader at 450 nm.