



MEASUREMENT OF GFAP LEVELS IN MOUSE BRAIN EXTRACT SAMPLES

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/ CONTEXT

Glial Fibrillary Acidic Protein (GFAP) is the principal 8-9 nm intermediate filament in mature astrocytes of the Central Nervous System (CNS). GFAP is mainly located in the brain and is not found outside the CNS. GFAP is often used as an astrocytic marker.

The findings showed that GFAP is quickly released after a traumatic brain injury and is related to brain injuries severity and outcome. In the CNS, following injury, either as a result of trauma, disease, genetic disorders, or chemical damage, astrocytes become reactive. Their response is called astrogliosis which is characterized by rapid synthesis of GFAP.

To study brain injuries, it is important to be able to measure GFAP in mouse brains with an accurate and reproducible method.

/ MATERIALS

- 7mL CK28 Precellys lysing tube (cat# P000935-LYSK0-A.0)
- GFAP (Human) ELISA kit (cat# A05188)
- BCA Protein Quantification kit (cat# D05077)
- Tissue: mouse brain / store collected tissue samples at -70°C up to 3 months

/ PROTOCOL

TISSUE EXTRACTION

1/ Precellys® Evolution extraction:

- Place 300 mg of brain tissue in a 7mL CK28 Precellys lysing tube containing 3 ml of TBS.
- Homogenize the brain tissue with Precellys® Evolution + Cryolys® Evolution with the following protocol: 6500rpm, 3x20sec, 15sec break at 4°C.

2/ After homogenization, centrifuge the samples at 3000xg and 4°C for 10 minutes.

3/ Transfer and store supernatant (=tissue extract samples) in aliquots at -70°C.

DETERMINATION OF PROTEIN CONCENTRATION IN TISSUE EXTRACT SAMPLES

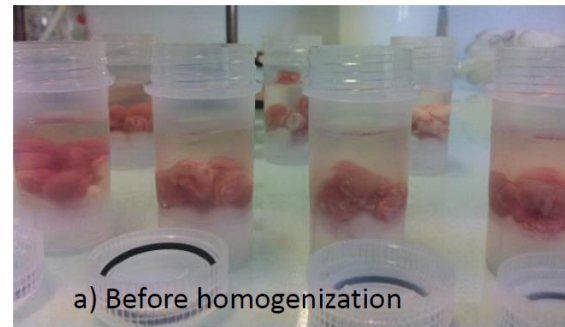
Determine total protein concentration in tissue extract samples using the BCA Protein Quantification kit

ASSAY PROCEDURE

1/ Dilute tissue extract samples all to the same concentration:

- take the aliquot of extracted samples from known protein concentration and dilute it down to the level of 6µg of total protein/ml with a 0.9% NaCl solution
- subsequently dilute them 3x in a EIA Buffer from the A05188 kit (to get the final protein concentration of 2 µg/ml).

2/ Carry out the test according to the instructions in the technical booklet: using 100 µl of diluted samples (at 2 µg of total protein/ml) per well.



b) After homogenization

/ CONCLUSION

Precellys® Evolution is a high-throughput homogenizer that can process up to 36 mouse brain samples in less than two minutes. Moreover, the Precellys® Evolution avoids the issue of cross-contamination between samples with its single-use tubes (0.5mL, 2mL, 7mL and 15mL).

When using the Precellys in combination with Bertin Bioreagent's GFAP ELISA kit (cat# A05188), researchers can measure accurately and reproducibly GFAP levels in mouse brains.