OCT 02, 2023

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Biochemical assays and evaluation

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ABSTRACT

Preparation of brain and Neuro2A lysates

DOI:
dx.doi.org/10.17504/protocols.io.5jyl8pey7g2w/v1

Protocol Citation: lparra 2023. Biochemical assays and evaluation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8pey7g2w/v1

Protocol status: Working
We use this protocol and it's working

Created: Oct 02, 2023

Last Modified: Oct 02, 2023

Preparation of Brain and Neuro2A Lysates

1

Whole mouse brains were homogenized with a Dounce tissue grinder (20 times) in neuronal protein extraction reagent (N-PER) (Thermo Scientific) containing protease/phosphatase inhibitors (Cell Signaling #5872).

2

Triton X-100 (Sigma #X100-100ML) was added at a final concentration of 1%, and the samples were incubated with rotation for 1 hour at 4 °C.

3

Samples were centrifuged at 10,000 × *g* for 10 min at 4 °C, and the supernatant was collected.

4

For Neuro2A lysates, cells were washed with 1X PBS 3 times and incubated for 5 min on ice in the presence of N-PER reagent supplemented with protease inhibitors.

5

Samples were centrifuged at 10,000 × *g* for 10 min at 4 °C to remove cellular debris.

6

Total protein
concentration was measured in brain and Neuro2A lysates (DC™ Protein Assay Kit II, Biorad), and samples were
used in subsequent experiments.