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We use this protocol and it's working

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Abstract

Preparation of brain and Neuro2A lysates

Troubleshooting



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 $10,000 \times g$ 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 $10,000 \times g$ 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.