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Western blot

In 1 collection

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We use this protocol and it's working

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Abstract

Western blot

For cell experiments

1 Extract proteins using 0.5% NP40–PBS protein extraction buffer containing protease and phosphatase inhibitors (Roche, Switzerland) on ice.

2 Centrifuge the suspension at 14,000 rpm for 15 minutes at 4°C

For mouse tissue experiments

3 Extract and clean tissue with PBS.

4 Dissect the cortex quickly to prevent protein degradation.

5 Add ice-cold homogenization buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1 mM EDTA, and protease and phosphatase inhibitors) at approximately 1 mL per gram of tissue.

6 Homogenize the tissue manually with a pestle for 10–20 passes on ice.

7 Centrifuge the homogenate at 14,000 rpm for 20 minutes at 4°C.

After centrifugation for both cell and mouse tissue experiments

8 Measure protein concentration in the supernatant using the Pierce™ BCA Protein Assay Kit (Cat. number 23225, Thermo Fisher Scientific).

9 Load 20–50 µg of protein lysate onto a polyacrylamide gel (SurePAGE™, Bis-Tris, 10×8, 4%–12%, GenScript).

10 Separate proteins via electrophoresis and transfer them onto a 0.45 µm polyvinylidene fluoride (PVDF) membrane (Millipore).

11 Block the membrane with either 5% milk powder or 5% bovine serum albumin (BSA) in TBS containing 0.1% Tween 20 (TBST).

12 Incubate the membrane overnight at 4°C with primary antibodies diluted in the chosen blocking solution.

13 Incubate the membrane with horseradish peroxidase (HRP)-conjugated secondary antibodies (Sigma–Aldrich) for 1 hour at room temperature.

14 Visualize the proteins using ECL Western Blotting Detection Reagent (Pierce, Thermo Fisher Scientific) in one of the following imaging systems:
- Odyssey DLx Imager (LI-COR),
- Stella imaging system (Raytest), or
- ChemiDoc MP imaging system (Bio-Rad).

15 Perform densitometric analysis of protein expression using ImageJ software.