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Protein extraction and Western blotting

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Abstract

Protein extraction and Western blotting

1. Protein was extracted from cultured rat astrocytes using membrane solubilization buffer (25 mM Tris pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.5% NP-40, and protease inhibitors).

2. Cultured astrocytes were washed twice with ice-cold TBS containing 1 mM $CaCl_2$and 1 mM $MgCl_2$and incubated on ice in membrane solubilization buffer for 20 minutes with occasional agitation.

3. Cell lysates were collected, vortexed briefly, and centrifuged at 4°C at high speed for 10 minutes to pellet non-solubilized material. The supernatant was collected and stored at −80°C.

4. Pierce BSA Protein Assay Kit (Thermo Fisher) was used to determine protein concentration, and lysates were mixed with 4× Pierce™ Reducing Sample Buffer (Thermo Scientific) and incubated at 45°C for 45 minutes to denature proteins.

5. 7-10 µg (cultured astrocyte lysates) of protein was loaded into Bolt™ 4–12% Bus-Tris Plus gels (Thermo Scientific) and run at 150 V for 1 hour.

6. Proteins were transferred at 100 V to PVDF membrane (Millipore) for 1.5 hours, blocked in 5% BSA in TBST (137 mM NaCl, 2.68 mM KCl, 24.7 mM Tris-Base, 0.1% (w/v) Tween 20) for 1 hour and incubated in primary antibodies overnight at 4°C.

7. Primary antibodies used were: anti-LRRK2 (Rabbit, 1:500; ab133474, abcam), GAPDH (mouse, 1:5000; ab8245, abcam), β-actin (mouse, 1:5000; A5441, Millipore Sigma), phospho-ERM (Rabbit, 1:500; #3141, Cell Signaling), ERM (Rabbit, 1:500; #3142, Cell Signaling), phospho-Rab10 (Rabbit, 1:500; ab230261, abcam), Rab10 (Rabbit, 1:500; ab237703, abcam).

8. The next day, membranes were washed with TBST, incubated in HRP secondary antibodies (Thermo Fisher Scientific) for 2 hours, washed in TBST, and imaged on a Biorad Gel Doc imaging system.

9. Protein levels were quantified using FIJI.