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Protein extraction and Western blotting

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Shiyi Wang[1]

[1]Duke University

Shiyi Wang
Duke University

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We use this protocol and it's working

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Disclaimer

Abstract

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1 **Prepare Membrane Solubilization Buffer** - 25 mM Tris (pH 7.4) - 150 mM NaCl - 1 mM CaCl2 - 1 mM MgCl2 - 0.5% NP-40 - Protease inhibitors

2 **Wash Cultured Astrocytes** - Wash cultured rat astrocytes twice with ice-cold TBS containing 1 mM CaCl2 and 1 mM MgCl2.

3 **Extract Protein** - Incubate astrocytes on ice in membrane solubilization buffer for 20 minutes with occasional agitation.

4 **Collect Cell Lysates** - Vortex briefly. - Centrifuge at 4°C at high speed for 10 minutes to pellet non-solubilized material.

5 **Store Supernatant** - Collect the supernatant and store at −80°C.

6 **Determine Protein Concentration** - Use the Pierce BSA Protein Assay Kit (Thermo Fisher).

7 **Prepare Protein Samples** - Mix lysates with 4x Pierce™ Reducing Sample Buffer (Thermo Scientific). - Incubate at 45°C for 45 minutes to denature proteins.

8 **Load Protein into Gels** - Load 7-10 µg of protein (cultured astrocyte lysates) into Bolt™ 4–12% Bis-Tris Plus gels (Thermo Scientific). - Run at 150 V for 1 hour.

9 **Transfer Proteins to Membrane** - Transfer proteins at 100 V to PVDF membrane (Millipore) for 1.5 hours.

10 **Block Membrane** - Block 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.

11 **Incubate with Primary Antibodies** - Incubate overnight at 4°C with the following primary antibodies: - Anti-LRRK2 (Rabbit, 1:500; ab133474, Abcam) - GAPDH (Mouse, 1:5000; ab8245, Abcam) - β-actin (Mouse, 1:5000; A5441, Millipore Sigma)

12 **Wash Membranes** - Wash membranes with TBST.

13 **Incubate with Secondary Antibodies** - Incubate in HRP secondary antibodies (Thermo Fisher Scientific) for 2 hours.

14 **Wash and Image Membranes** - Wash membranes in TBST. - Image on a Biorad Gel Doc imaging system.

15 **Quantify Protein Levels** - Quantify protein levels using FIJI software.