

Jul 11, 2024

Protein extraction and Western blotting

DOI

<https://dx.doi.org/10.17504/protocols.io.rm7vzj784lx1/v1>

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Protocol Citation: Shiyi Wang 2024. Protein extraction and Western blotting. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.rm7vzj784lx1/v1>

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Protocol status: Working

We use this protocol and it's working

Created: July 11, 2024

Last Modified: July 11, 2024

Protocol Integer ID: 103174

Keywords: ASAPCRN, blotting protein extraction, protein extraction, extraction, protein

Funders Acknowledgements:

Aligning Science Across Parkinson's (ASAP) initiative

Grant ID: ASAP-020607

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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.