Dec 16, 2024

Western Blotting for Neuronal Proteins

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.3byl4wqdjvo5/v1

Mukesh Kumar[1], Timothy A. Ryan[1,2]

[1]Department of Biochemistry, Weill Cornell Medicine, New York, NY 10065;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, Maryland 20815, USA

Mukesh Kumar
Weill Cornell Medical College

DOI: https://dx.doi.org/10.17504/protocols.io.3byl4wqdjvo5/v1

Protocol Citation: Mukesh Kumar, Timothy A. Ryan 2024. Western Blotting for Neuronal Proteins. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl4wqdjvo5/v1

Protocol status: Working
We use this protocol and it's working

Created: November 26, 2024

Last Modified: December 16, 2024

Protocol Integer ID: 114389

Keywords: ASAPCRN, western blotting for neuronal protein, detailed procedure for neuronal protein extraction, neuronal protein extraction, neuronal protein, western blot analysis, quantification of target protein, protein extraction, western blotting, protein, target protein

Funders Acknowledgements:
NIH
Grant ID: NS036942
NIH
Grant ID: NS11739
ASAP
Grant ID: ASAP-024404

Abstract

This protocol outlines a detailed procedure for neuronal protein extraction and western blot analysis, enabling precise detection and quantification of target proteins.

Guidelines

The protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Materials

- Neurons cultured from neonatal P0-1 rodent brain
- Poly-D-lysine: (SigmaAldrich, P7886)
- Lentivirus (produced as per lentivirus production protocol)
- RIPA lysis buffer (common reagents procured from Sigma):

	A	B
	Tris-HCl, pH 7.6	25 mM
	NaCl	150 mM
	NP-40	1%
	sodium deoxycholate	1%
	SDS	0.1%
	Complete protease inhibitor	(Roche, 11697498001)

- cOmplete™ Protease Inhibitor Cocktail **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**11697498001
- Pierce BCA Protein Assay Kit **Thermo Fisher Scientific Catalog #**23225
- 4x Laemmli Sample Buffer **Bio-Rad Laboratories Catalog #**1610747
- Immobilon®-FL PVDF Membrane **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**Ipfl00010
- Li-Cor Intercept (TBS) blocking buffer (Li-Cor, P/N: 927-60001) **LI-COR Catalog #**P/N: 927-60001
- Anti-CPT2 antibody [EPR13626] - C-terminal **Abcam Catalog #**ab181114
- GAPDH (D16H11) XP® Rabbit mAb #5174 **Cell Signaling Technology Catalog #**5174S
- HRP-conjugated secondary antibodies:

1. Invitrogen, 31430 (anti-mouse),
2. Invitrogen, 31460 (anti-rabbit)

- Immobilon Forte Western HRP substrate, 100 mL **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**WBLUF0100

Equipments:

- Tissue culture incubator
- Centrifuge
- BioRad SDS-PAGE apparatus
- BioRad Western blot transfer system
- LiCor Odyssey Fc system.

Neuronal Culture and Lentivirus Infection

3d

1 Isolate neurons from rat pups (P0-1 days) and plate them on 35 mm dishes coated with 50 µL poly-D-lysine.

2 Culture the neurons under standard conditions (37 °C , 5% CO_2) and allow them to adhere.

3 At 2 days in vitro (DIV-2), introduce lentivirus into the culture media at an appropriate multiplicity of infection (MOI) tested previously for individual virus prepared.

4 Incubate the neurons with lentivirus for 48:00:00 - 72:00:00 to ensure efficient transduction.

3d

Protein Extraction

55m

5 Remove the culture media and rinse neurons gently with ice-cold PBS.

6 Add RIPA lysis buffer (supplemented with complete protease inhibitor) to the dish.

7 Scrape the neurons into the lysis buffer using a cell scraper.

8 Transfer the lysate to a microcentrifuge tube and incubate On ice for 00:20:00 .

40m

9 Centrifuge the lysate at 14000 rpm, 4°C, 00:15:00 to remove debris.

15m

10 Transfer the supernatant (cytosolic protein fraction) to a new tube.

Protein Quantification

11 Measure protein concentration using the BCA Protein Assay Kit according to the manufacturer's instructions.

12 Normalize the protein concentration of all samples.

SDS-PAGE and Transfer to PVDF membrane

5m

13 Mix equal amounts of protein with SDS sample buffer (2X) and heat at 95 °C for 00:05:00 to denature the proteins.

5m

14 Load the samples onto an SDS-PAGE gel and run electrophoresis at the appropriate voltage.

15 Transfer the separated proteins onto a PVDF membrane using a wet transfer system.

Membrane Blocking

1h

16 Block the PVDF membrane in LiCor blocking buffer for 01:00:00 at Room temperature with gentle agitation.

1h

Primary Antibody Incubation

1h

17 Dilute the primary antibodies in LiCor blocking buffer according to the manufacturer's instructions.

18 Incubate the membrane with the primary antibody Overnight at 4 °C with gentle rocking.

1h

Secondary Antibody Incubation

1h 30m

19 Wash the membranes 3 times with TBST (Tris-buffered saline with 0.1% Tween-20), 10 minutes per wash.

19.1 Wash the membranes with TBST (Tris-buffered saline with 0.1% Tween-20) for 00:10:00 (1/3). 10m

19.2 Wash the membranes with TBST (Tris-buffered saline with 0.1% Tween-20) for 00:10:00 (2/3). 10m

19.3 Wash the membranes with TBST (Tris-buffered saline with 0.1% Tween-20) for 00:10:00 (3/3). 10m

20 Dilute HRP-conjugated secondary antibody in LiCor blocking buffer and incubate the membrane for 01:00:00 at Room temperature . 1h

Signal Detection 30m

21 Wash the membrane 3 times with TBST, 10 minutes per wash.

21.1 Wash the membrane with TBST for 00:10:00 (1/3). 10m

21.2 Wash the membrane with TBST for 00:10:00 (2/3). 10m

21.3 Wash the membrane with TBST for 00:10:00 (3/3). 10m

22 Apply ECL reagent to the PVDF membrane.

23 Capture chemiluminescent signals using the LiCor Odyssey Fc system.

Note

- Ensure all steps are performed on ice during protein extraction to prevent protein degradation.
- Optimize antibody dilutions and incubation times for specific targets.
- Blotting-Grade Blocker (Bio-Rad, 1706404) can be used in place of LiCor blocking buffer.
- Handle all reagents and equipment following standard laboratory safety protocols.