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Immunoblotting of I3 Neurons and dopaminergic neurons

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We use this protocol and it's working

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Abstract

This protocol describes the preparation of cell lysate from and iPSC-derived neurons (I^3 Neurons, dopaminergic) and the immunoblotting procedure.

Attachments

DOCX

Rafiq_Immunoblotting...

17KB

Materials

- **TBS:** [M] 50 millimolar (mM) Tris-Cl, [M] 150 millimolar (mM) NaCl, adjust pH 07.5 .
- **TBST:** TBS with 0.1% TWEEN-20 (Sigma-Aldrich).

Cell culture and treatments

10m

1 Grow I^3 Neurons and dopaminergic (DA) neurons on six-well plates (3-5 × 10^5 cells/well).

2 After differentiation in their respective maturation media, wash the neurons with 1 mL ice-cold PBS.

3 Lyse the cells in 200 µL 1xRIPA lysis buffer (10X RIPA lysis buffer, Sigma-Aldrich) supplemented with complete™ EDTA-free protease inhibitor cocktail (Roche) and PhosSTOP phosphatase inhibitor cocktail (Roche).

4 Centrifuge the cells at 13000 x g for 00:10:00 . 10m

5 Collect the supernatant and store at -20 °C .

6 Determine the protein concentration in sample using Pierce BCA assay (ThermoFisher).

Gel electrophoresis and immunoblotting (Tris-glycine buffer system)

5h 10m

7 Incubate appropriate volume of cell lysate solution at 95 °C for 00:05:00 in SDS sample buffer containing 1% 2-mercaptoethanol (Sigma). 5m

8 Separate the extracted proteins by SDS-PAGE in Mini-PROTEAN TGX precast polyacrylamide gels (Bio-Rad) at 250V and transfer to nitrocellulose membranes (Bio-Rad) at 100 V for 01:00:00 or 75 V for 02:00:00 (for high molecular weight proteins: >150 kDa). 3h

9 Block the nitrocellulose membranes for 01:00:00 with 5% non-fat milk (AmericanBIO) in TBST (tris-buffered saline [TBS] + 0.1% tween 20), then incubate Overnight at 4 °C with primary antibodies. 1h

> Note
>
> Antibody dilutions can be found in Table S1.

10 Wash the blots with TBST, thrice, each 00:05:00 . 5m

11 Incubate the blots with IRDye 680RD or 800CW (LI-COR) secondary antibodies (1:8000) for 01:00:00 at Room temperature in TBST. 1h

12 Image blots using the Gel Doc imaging system (Bio-Rad) using manufacturer's protocols.