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Western Blot of human iPSC-derived Dopamine Neurons (DaNs)

Forked from Immunoblots

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We use this protocol and it's working

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Abstract

This protocol describes a standard procedure for protein separation and identification of protein of interest. The steps are similar to other Western Blot procedures found elsewhere with small modifications to prevent aggregation or post-transfer loss of alpha-synuclein.

Materials

Reagents:

- Gel, 4-5%, 18 wells, Stain-free Midi-PROTEAN Precast Gels (Bio-Rad Laboratories Ltd, CAT# 5678084)
- Trans-Blot Turbo Mini 0.2 µm PVDF Transfer Packs (Bio-Rad Laboratories Ltd, CAT# IPVH000101704156)
- Tris base (ThermoFisher Scientific, CAT# BP152-5)
- Glycine (ThermoFisher Scientific, CAT# BP381-5)
- Spectra™ Multicolor Broad Range Protein Ladder (ThermoFisher Scientific, CAT# 26634)
- TBS
- Triton X100 (Sigma-Aldrich, CAT# T8787-250ML)
- Protease inhibitor (complete Mini, EDTA-free, Roche)

Primary Antibodies:

- Rabbit anti-α-synuclein [MJFR1] (Abcam, CAT# ab138501, dilution 1:10000)
- Rabbit anti-DDC (Sigma-Aldrich, CAT# AB1569, dilution 1:1000)
- Rabbit anti-MAO-B (Abcam, CAT# ab137778, dilution 1:1000)
- Rabbit anti-TH (Millipore, CAT# AB152, dilution 1:1000)
- Rabbit anti-Phosphorserine TH (Sigma-Aldrich, CAT# AB5935, dilution 1:1000)
- Rabbit anti-vATPase (Abcam, CAT# Ab179858, dilution 1:1000)
- Rabbit anti-vGLUT2 (Synaptic Systems, CAT# 135403, dilution 1:1000)
- Rabbit anti-VMAT2 (Abcam, Ab70808, dilution 1:1000)

Secondary Antibody:

- Donkey anti-Rabbit IgG Alexa Fluor 647 (Molecular Probes, RRID:AB_2536183)

SOLUTIONS

Preparing RIPA buffer:

- 10 mM Tris-HCl
- 1 mM EDTA
- 1 mM EGTA
- 140 mM NaCl
- 1% Triton X-100
- 0.1% SDS
- 0.1% Sodium deoxycholate pH 7.4

Preparing TBS-T:

- 20 mM Tris pH 7.4
- 150 mM NaCl
- 0.1% Tween-20

Sample Preparation

4h 50m

1 Lyse cells in RIPA buffer (see **Materials**) with protease inhibitor for 30 minutes and spun 10 min 10,000x g to remove cell debris. 45m

2 Mix protein-containing supernatants with SDS sample loading buffer and 50 mM DTT. 10m

Note

We use 10 or 20 µg proteins per lane depending on the target of interest.

Gel Electrophoresis and Membrane Transfer

3 Heat samples at 75°C for 10 min to prevent aggregation.

4 Separate samples on SDS-PAGE gels for 45 mins, 200 V.

5 Image separated proteins for total protein using the stain-free diazo dye.

6 Transfer separated proteins to PVDF membranes using the Trans-Blot Turbo Transfer System.

Fixation, Blocking and Antibody Incubation

30m

7 Fix membranes for 30 min in 4% formaldehyde solution to prevent loss of small proteins. 30m

8 Block membranes with 5% skimmed milk powder in TBS-T (see **Materials**) at room temperature for 1 h with constant agitation.

9 Incubate membranes with the desired Primary Antibody (see Materials) at 4°C overnight.

10 Wash membranes with TBS-T for 3 times (10 mins each) with agitation.

11 Incubate membranes with secondary antibody for 1 h at room temperature.

Detection

12 Use Amersham ECL substrate and image membrane using BioRad ImageLab.