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© Cell lysis and immunoblotting for protein and phospho-protein quantification

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Protocol status: Working

We use this protocol and it's working

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Abstract

Here, we describe the procedure by which human iPSC-derived neurons or mouse embryonic fibroblasts (MEFs) were lysed and probed for levels of proteins of interest using Western blot.

Attachments



552-1148.pdf

77KB



Materials

Reagents

RIPA buffer

А	В
Tris-HCI	50 mM
NaCl	150 mM
Triton X-100	0.1%
Deoxycholat e	0.5%
SDS	0.1%

- 🔀 HALT phosphatase and protease inhibitor cocktail (100x) Thermo Fisher Scientific Catalog #78442
- Microcystin-LR Microcystis aeruginosa CAS 101043-37-2 Calbiochem Merck MilliporeSigma (Sigma-Aldrich) Catalog #475815m
- Pierce BCA Protein Assay Kit Thermo Fisher Scientific Catalog #23225

4x Protein Loading Buffer

А	В
Tris-HCI, pH 6.8	125 mM
Glycerol	50%
SDS	4%
Orange G	0.2%

- Acrylamide
- **4x Running buffer**



А	В
Trizma base	48 g
Glycine	230.4 g
NaN3	20 mL
ddH2O	Diluted to 4 L

Running buffer

А	В
4x running buffer	250 mL
ddH2O	750 mL
10% SDS	10 mL

Transfer buffer

А	В
4x running buffer	125 mL
ddH2O	875 mL
10% SDS	500 μL
For RABs add 20%	% Methanol

- **☒** Chameleon® Duo Pre-stained Protein Ladder **LI-COR Catalog #**928-60000
- **⊠** Revert[™] 700 Total Protein Stain for Western Blot Normalization (250 ml) **LI-COR Catalog #**926-11021

Revert Wash Solution



А	В
Acetic acid	6.7%
Methanol	30%
in ddH2O	

Revert Reversal Solution

А	В
NaOH	0.1 M
Methanol	30%
in ddH2O	

- SeveryBlot Blocking Buffer 500 ml Bio-Rad Laboratories Catalog #12010020
- Primary antibodies (see Materials and Methods for specific antibodies used)
- Secondary antibodies (see Materials and Methods for specific antibodies used)

Equipment

ODYSSEY CLx Imaging System (LI-COR)

EquipmentMini-PROTEAN Tetra Vertical Electrophoresis CellNAMEElectrophoresis CellTYPEBio-RadBRAND1658004SKUhttps://www.bio-rad.com/en-in/product/mini-protean-tetra-vertical-electrophoresis-cell?
ID=N3F2UD4VYLIN
K



Equipment

Mini Trans-Blot Electrophoretic Transfer Cell

NAME

Electrophoretic Transfer Cell

TYPE

BRAND

1703930

Bio-Rad

SKU

https://www.bio-rad.com/en-in/sku/1703930-mini-trans-blot-electrophoretic-transfer-cell? ID=1703930-mini-trans-blot-electrophoretic-transfer-cell? ID=1703930-mini-trans-blot-electrophoretic-trans-blot-electrophoret

Troubleshooting

Safety warnings



- Microcystin-LR is an extremely potent hepatotoxin and should be handled with great care.
- Acrylamide is a neurotoxin and should be handled with care.
- Methanol-containing reagents should be handled carefully, as methanol can penetrate single-layer laboratory gloves.



Preparation of cell lysates

1 Quickly wash cells twice with ice-cold PBS. After the second wash, tilt the dish and completely aspirate all residual PBS.



2 Immediately add ice-cold lysis buffer, ensuring that the entire surface is covered by lysis buffer. Place cells 🖁 On ice .



Note

The amount of lysis buffer to use depends on cell confluency / cell number, cell type, and cell culture dish. In most cases, using \perp 100 μ L \mid – \perp 150 μ L \mid lysis buffer per well of a 6-well plate should result in a protein concentration > [M] 1 μ g/ μ L.

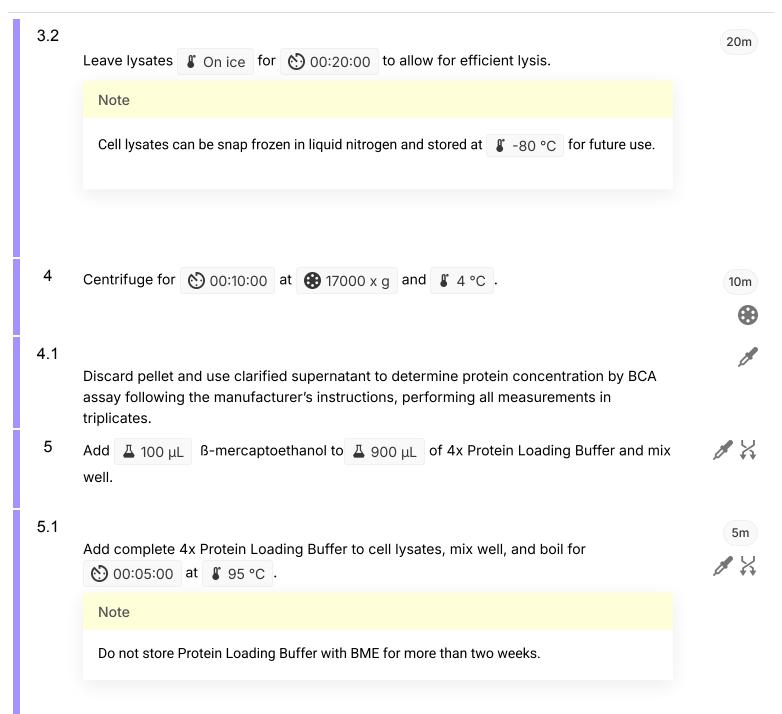
Note

Halt protease and phosphatase inhibitor cocktail and microcystein-LR should be added fresh on the day of use.

3 Scrape cells off the dish using a cell lifter.

3.1





SDS-polyacrylamide gel electrophoresis

6 Load samples onto 8% (for LRRK2 protein) to 15% (for PPM1H and Rab proteins) acrylamide gels alongside Chameleon Duo pre-stained protein ladder (LI-COR).





Note

Carefully rinse wells with running buffer before loading cell lysates.

7 Start electrophoresis at 80 V for 00:20:00 , then increase to 120 V and electrophorese until orange dye runs out.

20m

Protein transfer

8 Activate Immobilion-FL PVDF membrane by submerging in methanol for 00:00:30 -**©** 00:01:00 .

8.1 Wash in ddH₂O and equilibrate in transfer buffer.

1m 30s

8.2 Soak sponges in methanol, wash in ddH₂O and equilibrate in transfer buffer.

8.3 Equilibrate filter paper in transfer buffer.

8.4 Assemble blotting sandwich.

- 8.5 Carefully remove any air bubbles between layers using a roller.
- 9 Fill transfer tank with ice-cold transfer buffer.

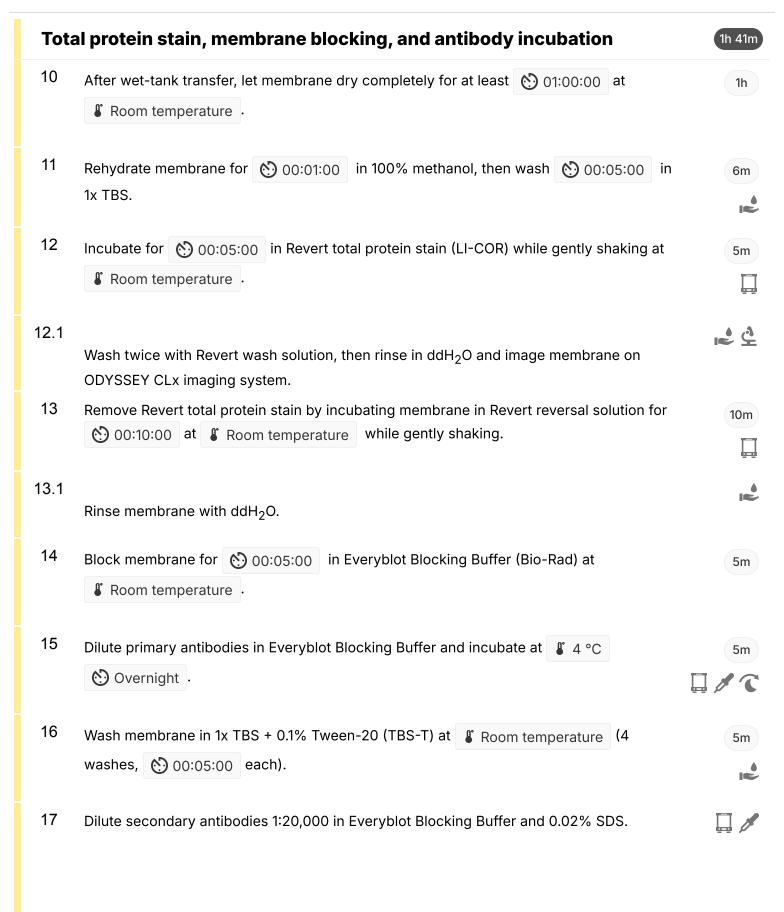
9.1

9.2

1h 15m

Transfer proteins from gel onto PVDV membrane at 100 V for 01:15:00.







Note Incubate membrane in secondary antibodies for 01:00:00 at Room temperature 18 Wash membrane in TBS-T at Room temperature (4 washes, 00:05:00 each). 5m 19 Rinse membrane with TBS (no detergent), then image on ODYSSEY CLx imaging system. Quantify signal intensity using Image Studio Software.