Oct 31, 2024

SDS-PAGE and immunoblotting to assess whole-cell lysates from cell culture systems

Forked from SDS-PAGE and immunoblotting to assess whole-cell lysates and Endo-IPs and Lyso-IPs in hESCs and iNeurons

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.4r3l295qqv1y/v1

Frances V Hundley[1,2], Miguel A. Gonzalez-Lozano[1,2], Felix Kraus[3], Harper JW[1,2]

[1]Harvard Medical School;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, USA;
[3]Department of Cell Biology, Blavatnik Institute, Harvard Medical School, 240 Longwood Ave, Boston MA 02115, USA

Felix Kraus

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.4r3l295qqv1y/v1

Protocol Citation: Frances V Hundley, Miguel A. Gonzalez-Lozano, Felix Kraus, Harper JW 2024. SDS-PAGE and immunoblotting to assess whole-cell lysates from cell culture systems. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l295qqv1y/v1

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

Created: October 31, 2024

Last Modified: October 31, 2024

Protocol Integer ID: 111343

Keywords: ASAPCRN, cell lysates from cell culture system, cell lysate, immunoblotting, cell culture system, general protocol for western blotting, cell, sd, western blotting, protein

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-025160
Aligning Science Across Parkinson's
Grant ID: ASAP-000282

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

This is a general protocol for Western blotting to detect proteins or interest from whole-cell lysates derived from cell culture systems (HeLa, iNeurons).

Safety warnings

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

SDS-PAGE and immunoblotting

5m

1 For whole-cell lysates, prepare samples following standard protocols, and final samples should be in LDS buffer with DTT or similar. Incubate samples at 80 °C for 00:05:00 . 5m

2 Load samples into a NuPAGE Novex® 4-12% Bis-Tris Midi Protein Gels and separate by electrophoresis in 1xTris/Glycine/SDS buffer.

3 Transfer proteins to PVDF or nitrocellulose membranes by standard wet transfer in 20% methanol Tis/Glycine buffer.

4 Block membrane in blocking buffer (5% non-fat dry milk or 3% BSA in TBST) at Room temperature for 01:00:00 . 1h

5 Incubate membrane in primary antibody solution (blocking solution plus primary antibody at 1:500-1:1,000, depending on the primary antibody) at 4 °C for 12:00:00 to 16:00:00 . 1d 4h

6 Wash membrane six times with TBST for 00:05:00 each wash. 5m

7 Incubate membrane in secondary antibody solution (blocking solution plus secondary antibody conjugated to HRP at 1:5,000-1:10,000) at Room temperature for 01:00:00 . 1h

8 Wash membrane four times with TBST for 00:05:00 each wash. 5m

9 Apply Western Lightning Plus Chemiluminescence substrate (Revvity) to membrane and acquire blot images using a ChemiDoc MP imager.

10 Process raw image files with Image Lab software (Bio-Rad).