Oct 14, 2024

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.q26g71eq9gwz/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.q26g71eq9gwz/v1

External link: https://doi.org/10.1038/s41586-025-09059-y

Protocol Citation: Frances V Hundley, Miguel A. Gonzalez-Lozano, Harper JW 2024. SDS-PAGE and immunoblotting to assess whole-cell lysates and Endo-IPs and Lyso-IPs in hESCs and iNeurons. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g71eq9gwz/v1

Manuscript citation:
Gonzalez-Lozano, M.A., Schmid, E.W., Miguel Whelan, E. *et al.* EndoMAP.v1 charts the structural landscape of human early endosome complexes. *Nature* **643**, 252–261 (2025). https://doi.org/10.1038/s41586-025-09059-y

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

Created: October 02, 2024

Last Modified: October 14, 2024

Protocol Integer ID: 108855

Keywords: ASAPCRN, negative control markers from other organelle, endolysosomal protein, cell lysate, other organelle, negative control marker, ineuron, ips in hesc, protein, ips from hesc, general protocol for western blotting

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

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Abstract

We present a general protocol for Western blotting to detect endolysosomal proteins and negative control markers from other organelles to assess whole-cell lysates and Endo-IPs and Lyso-IPs from hESCs and iNeurons.

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. For Endo-IP and/or Lyso-IP from hESCs and/or iNeurons, post-nuclear supernatant (PNS), flow through, and eluate 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 4-20% Criterion TGX Stain-free precast gel (Bio-Rad) and separate by electrophoresis in 1xTris/Glycine/SDS buffer.

3 Scan gel for total protein using a ChemiDoc MP imager (Bio-Rad).

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

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

6 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

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

8 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

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

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

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

Protocol references

Frances V. Hundley, Miguel A. Gonzalez-Lozano, Lena M. Gottschalk, Aslan N. K. Cook, Jiuchun Zhang, Joao A. Paulo, J. Wade Harper. Endo-IP and Lyso-IP Toolkit for Endolysosomal Profiling of Human Induced Neurons bioRxiv 2024.09.24.614704; doi: https://doi.org/10.1101/2024.09.24.614704