Oct 14, 2024

Immunofluorescence of endolysosomal markers in human iNeurons

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.kqdg32zrpv25/v1

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Protocol Citation: Frances V Hundley, Miguel A. Gonzalez-Lozano, Harper JW 2024. Immunofluorescence of endolysosomal markers in human iNeurons . **protocols.io https://dx.doi.org/10.17504/protocols.io.kqdg32zrpv25/v1**

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

Created: October 02, 2024

Last Modified: October 14, 2024

Protocol Integer ID: 108844

Keywords: ASAPCRN, immunofluorescence of endolysosomal marker, human ineuron, endosome, endolysosomal marker, markers of the endolysosomal system, lysosome, endolysosomal system, ineuron, immunofluorescence, immunofluorescence protocol, using endo

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

Disclaimer

Abstract

Human induced cortical-like neurons (iNeurons) with endogenously tagged 3xFLAG-EEA1 and TMEM192-3xHA can be used to isolate endosomes and lysosomes, using Endo-IP and Lyso-IP, respectively. Here, we present an immunofluorescence protocol to assess the extent of colocalization between tagged and untagged EEA1 and TMEM192 and markers of the endolysosomal system in iNeurons.

Troubleshooting

Prepare glass-bottomed dishes & seed iNeurons

1 Coat 24W glass-bottomed dishes with Matrigel.

2 Seed iNeurons on desired day of differentiation onto Matrigel-coated glass-bottomed dishes.

Fix and stain iNeurons for immunofluorescence

1h 10m

3 Pre-warm 8% paraformaldehyde (PFA) (dilute 16% PFA stock to 8% with PBS) and pre-warm PBS.

4 Fix iNeurons by adding a volume of 8% PFA equal to the volume of media in the well such that the final concentration of PFA is 4%. Incubate at 37 °C for 00:20:00 . 20m

5 Gently remove fixation solution, and dispose of hazardous waste appropriately. Wash cells once with pre-warmed PBS, and likewise dispose of this hazardous waste wash solution appropriately.

6 Permabilize cells by adding 0.5% Triton X-100 in PBS at Room temperature for 00:20:00 to 00:30:00 . 50m

7 Block cells with blocking solution (3% BSA and 0.1% Triton X-100 in PBS) at Room temperature for 01:00:00 . 1h

8 Gently remove blocking solution and add appropriate primary antibody solution (primary antibody at 1:100-1:200, depending on the antibody in blocking solution). Incubate in primary antibody solution at 4 °C for 12:00:00 to 16:00:00 . 1d 4h

9 Gently remove primary antibody solution, and gently wash three times with washing solution (0.02% Tween-20 in PBS), where each wash is at Room temperature for 00:05:00 . 5m

10 Incubate in secondary antibody solution (Alexa Fluor-conjugated secondary antibodies at 1:400 in blocking solution) at Room temperature for 01:00:00 . 1h

11 Gently remove secondary antibody solution and wash once with washing solution.

12 Stain nuclei with DAPI solution (1ug/mL final concentration DAPI in PBS) at Room temperature for 00:10:00 . 10m

13 Gently removed DAPI solution and gently wash three times with washing solution, where each wash is at Room temperature for 00:05:00 . 5m

14 After final wash, store sample in PBS at 4 °C until micrsocopy analysis.

Spinning disk confocal microscopy

15 Image cells on a confocal microscope using a 100x oil objective.

Data analysis

16 Use Fiji/ImageJ software to display z-series as maximum intensity projections, then segment images for neuronal soma using CellProfiler Image Analysis Software.

17 Calculate Mander's correlation coefficients between two channels with BIOP JACoP plugin in Fiji using the segmented images from the previous step and the Yen thresholding.

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