Protocol: Culture and transfection of iPSC-derived neurons for live-imaging of axonal cargoes

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Key words

iPSC, iNeuron, live-imaging, axon

Abstract

Here, we plate, culture, and transfect human iPSC-derived excitatory glutamatergic neurons for the purpose of observing transport of axonal cargoes under spinning disk confocal microscopy. Protocol is largely as previously described (Boecker et al., 2020, 2021; Fernandopulle et al., 2018). For preceding differentiation of neurons, see "Protocol: Piggybac-mediated stable expression of NGN2 in iPSCs for differentiation into excitatory glutamatergic neurons" and "Protocol: iNeuron differentiation from human iPSCs."

Materials

- 35 mm glass bottom imaging dish (MatTek, Cat# P35G-1.5-20-C)

Reagents

- PLO (CATALOG)
- Borate buffer (CATALOG)
- BrainPhys media (CATALOG)
- NT-3 (CATALOG)
- BDNF (CATALOG)
- B-27 supplement (CATALOG)
- Mouse laminin (CATALOG)
- 5-Fluoro-2′-deoxyuridine
- Uridine

Safety warnings

N/A

Steps

1. In advance, prepare 10x PLO stock (50 mg PLO resuspended in 50 mL 0.1M borate buffer). Store 10x PLO stock at -80 degrees Celsius.

2. The day before plating, coat imaging dishes with 1x PLO solution (10x PLO stock diluted in ddH2O). It is only necessary to fully coat the glass center of the imaging dish.
3. The day of plating, remove PLO solution from imaging dishes and wash twice with ddH2O. Add 2 mL of iNeuron culture media (BrainPhys supplemented with 10 ng/mL BDNF, 10 ng/mL NT-3, 1 µg/mL laminin, and 1x B-27 supplement). Place dishes in cell culture incubator for >30 minutes.
4. Rapidly thaw cryopreserved iNeurons in 37 degree Celsius water bath. Retrieve vial to tissue culture hood when only a small amount of ice remains visible.
5. Centrifuge to remove freezing media and resuspend cell pellet in iNeuron culture media (BrainPhys supplemented with 10 ng/mL BDNF, 10 ng/mL NT-3, 1 µg/mL laminin, and 1x B-27 supplement).
6. Count cells and plate 300k neurons per 35 mm imaging dish. Add cells dropwise to the center area of the dish (so that they sink onto the glass, PLO-coated center).
7. For Piggybac-delivered NGN2 neurons, 10 µM 5-Fluoro-2′-deoxyuridine and 10 µM uridine were included at the time of plating to prevent survival of mitotic cells. These drugs were removed 24 hours after plating.
8. Store neurons in cell culture incubator. Perform partial change of iNeuron media twice per week.
9. On DIV18 (~72 hours prior to imaging), transfect iNeurons for imaging. Transfection conditions may require optimization, but a typical transfection will use 4 µL Lipofectamine Stem and 1 µg of plasmid DNA. (Note: plasmids with the PGK or EF1α promoters express best in iNeurons)

Citations

Boecker, C.A., and Holzbaur, E.L.F. (2021). Hyperactive LRRK2 kinase impairs the trafficking of axonal autophagosomes. Autophagy *00*, 1–3.

Boecker, C.A., Olenick, M.A., Gallagher, E.R., Ward, M.E., and Holzbaur, E.L.F. (2020). ToolBox: Live Imaging of intracellular organelle transport in induced pluripotent stem cell-derived neurons. Traffic *21*, 138–155.

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