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

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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."

ATTACHMENTS

550-1146.pdf

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

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Keywords: iPSC, iNeuron, live-imaging, axon

MATERIALS

Materials

Equipment

35 mm Dish | No. 1.5 Coverslip | 20 mm Glass Diameter | Uncoated	NAME
Coverslip	TYPE
Mattek	BRAND
p35g-1-5-20-c	SKU
https://www.mattek.com/store/p35g-1-5-20-c-case/	LINK

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

Culture and transfection of iPSCderived neurons for live-im...

30m

1 In advance, prepare 10x PLO stock.

	A	B
	PLO	50 mg
	0.1M borate buffer	50 mL

Note

Store 10x PLO stock at -80 °C.

2 The day before plating, coat imaging dishes with 1x PLO solution (10x PLO stock diluted in ddH_2O).

Note

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 ddH_2O.

3.1 Add 2 mL of iNeuron culture media.

BrainPhys supplemented with

	A	B
	BDNF	10 ng/mL
	NT-3	10 ng/mL
	Laminin	1 µg/mL
	B-27 supplement	1x

3.2 Place dishes in cell culture incubator for > 00:30:00. 30m

4 Rapidly thaw cryopreserved iNeurons in 37 °C water bath.

Note

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

	A	B
	BDNF	10 ng/mL
	NT-3	10 ng/mL
	Laminin	1 µg/mL
	B-27 supplement	1x

6 Count cells and plate 300k neurons per 35 mm imaging dish.

6.1 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, include [M] 10 micromolar (µM) 5-Fluoro-2'-deoxyuridine and [M] 10 micromolar (µM) uridine at the time of plating to prevent survival of mitotic cells.

Note

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 (~ 72:00:00 prior to imaging), transfect iNeurons for imaging. 3d

Note

Transfection conditions may require optimization, but a typical transfection will use 4 µL Lipofectamine Stem and 1 µg of plasmid DNA. Plasmids with the PGK or EF1α promoters express best in iNeurons.