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Immunostaining of iPSC-derived neurons

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Protocol status: Working

We use this protocol and it's working

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Abstract

Here, we fix, permeabilize, and stain human iPSC-derived neurons for the purpose of observing and quantifying somal and axonal abundance of proteins of interest. For preceding culture of neurons, see "Protocol: Culture and transfection of iPSC-derived neurons for live-imaging of axonal cargoes."

Materials

Bouin's solution (Sigma, HT10132)

BrainPhys Neuronal Medium (Stemcell Technologies, Cat# 05790)

Methanol, Optima LC/MS grade (Thermo Fisher, A456-4)

35 mm imaging dish, 20 mm glass diameter (Mattek, p35g-1-5-20-c)

18mm circular coverglass (Electron Microscopy Sciences, Cat# 72222-01)

Prolong Gold Antifade Mountant (Thermo Fisher, P36930)



- 1 Replace culture media in dish with 1 mL room temperature BrainPhys culture media (Stemcell Technologies)
- 2 Add 1 mL Bouin's solution (Sigma). Incubate at room temperature for 30 minutes.
- 3 Wash at least 5 times with PBS. Yellow color should be absent.
- 4 Permeabilize in ice-cold Optima methanol (Thermo Fisher) at -20 degrees C for 8 minutes.
- 5 Wash 3 times in PBS.
- 6 Block for one hour (5% goat serum and 1% BSA in PBS).
- 7 Incubate in primary antibody in blocking solution for 1 hour at room temperature.
- 8 Wash 3 times in PBS.
- 9 Incubate in secondary antibody in blocking solution for 1 hour at room temperature.
- 10 Wash 3 times in PBS.
- 11 Remove PBS and add 40 μ L Prolong Gold (Thermo Fisher). Using forceps, apply coverglass.