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Immunostaining of iPSC-derived neurons for quantification of synaptic proteins

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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 proteins of interest. For preceding culture of neurons, see "Protocol: Culture and transfection of iPSC-derived neurons for live-imaging of axonal cargoes."

Materials

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)

Troubleshooting



- 1 At DIV14, fix human iNeurons in 4% paraformaldehyde supplemented with 4% sucrose for 15 minutes at 37 degrees C
- 2 Wash four times with PBS
- 3 Permeabilize for 15 minutes in 0.2% Triton-X in PBS
- 4 Block for 1 hour with 5% goat serum and 1% BSA in PBS
- 5 Incubate in primary antibody diluted in blocking solution at room temperature for 1 hour
- 6 Wash three times with PBS
- 7 Incubate in secondary antibody diluted in blocking solution for 1 hour at room temperature
- 8 Wash three times with PBS
- 9 Remove PBS and add 40 μ L Prolong Gold (Thermo Fisher). Using forceps, apply coverglass.