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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 termperature 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 µL Prolong Gold (Thermo Fisher). Using forceps, apply coverglass.