MAR 09, 2023

OPEN ACCESS

**DOI:**
dx.doi.org/10.17504/protocols.io.e6nvwj4kdlmk/v1

**Protocol Citation:** Dan Dou, Erika L.F. Holzbaur 2023. Immunostaining of iPSC-derived neurons. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwj4kdlmk/v1



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

**Created:** Mar 09, 2023

**Last Modified:** Mar 09, 2023

**PROTOCOL integer ID:**
78438

**Keywords:** ASAPCRN

# Immunostaining of iPSC-derived neurons

Dan Dou[1,2], Erika L.F. Holzbaur[1,2]

[1]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, USA

Dan Dou

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