



Jun 26, 2023

Immunocytochemistry of primary neurons

DOI

<https://dx.doi.org/10.17504/protocols.io.eq2lyjdkmlx9/v1>

giulia.tombesi¹

¹Northwestern University, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815



Giulia Tombesi

Northwestern University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.eq2lyjdkmlx9/v1>

Protocol Citation: giulia.tombesi 2023. Immunocytochemistry of primary neurons. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.eq2lyjdkmlx9/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: June 26, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 84047

Keywords: staining, primary neurons, cell culture, immunocytochemistry, ASAPCRN, immunocytochemistry of primary neuron, great staining of primary neuron, primary neuron, immunocytochemistry, neuron, great staining

Funders Acknowledgements:

Aligning Science Across Parkinson's [ASAP-020600] through the Michael J. Fox Foundation for Parkinson's Research (MJFF)
Grant ID: ASAP-020600

Abstract

This protocol describes the steps to obtain a great staining of primary neurons.

Materials

Blocking Solution: 1% BSA , 50mM Glycine , 2% FBS/Goat Normal Serum/ Donkey Normal Serum, 0,1% TRITON X-100 in PBS1X.

Working Solution:  10 mL Blocking Solution in  40 mL PBS1X



Cells Fixation

30m

- 1 Wash cells with PBS1X/HBSS
- 2 Fix cells with paraformaldehyde (PFA) 4% for ⌚ 00:30:00 at room temperature
- 3 Wash cells with PBS1X for ⌚ 00:05:00 for three times at room temperature

Cells permeabilization and saturation

45m

- 4 Permeabilize cells with 0.3% Triton X-100 (in PBS1X) for ⌚ 00:05:00 at room temperature
- 5 Wash cells with PBS1X for ⌚ 00:05:00 for three times at room temperature
- 6 Saturate cells in **blocking solution** for at least ⌚ 00:30:00
- 7 Wash cells with PBS1X for ⌚ 00:05:00 for three times at room temperature

Incubation with primary antibody

1h 35m

- 8 Incubate with primary antibody in **working solution** for ⌚ 01:30:00 at room temperature
- 9 Wash cells in **working solution** for ⌚ 00:05:00 for three times at room temperature

Incubation with secondary antibody

1h 6m

- 10 Incubate with secondary antibody in **working solution** for ⌚ 01:00:00 at room temperature in the dark



11 Wash cells in **working solution** for  00:05:00 for three times at room temperature

5m

12 Wash cells in PBS1X for  00:01:00 for three times at room temperature

1m

Mounting

13 Rinse coverslips in dH₂O

14 Mount the coverslips on slides with mounting medium