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Immunocytochemistry of primary neurons

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We use this protocol and it's working

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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 — 30m

3 Wash cells with PBS1X for 00:05:00 for three times at room temperature — 5m

Cells permeabilization and saturation

45m

4 Permeabilize cells with 0.3% Triton X-100 (in PBS1X) for 00:05:00 at room temperature — 5m

5 Wash cells with PBS1X for 00:05:00 for three times at room temperature — 5m

6 Saturate cells in **blocking solution** for at least 00:30:00 — 30m

7 Wash cells with PBS1X for 00:05:00 for three times at room temperature — 5m

Incubation with primary antibody

1h 35m

8 Incubate with primary antibody in **working solution** for 01:30:00 at room temperature — 1h 30m

9 Wash cells in **working solution** for 00:05:00 for three times at room temperature — 5m

Incubation with secondary antibody

1h 6m

10 Incubate with secondary antibody in **working solution** for 01:00:00 at room temperature in the dark — 1h

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 dH_2O

14 Mount the coverslips on slides with mounting medium