



Sep 23, 2025

Immunocytochemistry for TH-positive neurons

DOI

<https://dx.doi.org/10.17504/protocols.io.8epv5k38jv1b/v1>

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Protocol Citation: Ellen Hertz, Yu Chen, Ellen Sidransky 2025. Immunocytochemistry for TH-positive neurons. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.8epv5k38jv1b/v1>

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Protocol status: Working

We use this protocol and it's working

Created: September 23, 2025



Last Modified: September 23, 2025

Protocol Integer ID: 228005

Keywords: ASAPCRN, positive neurons immunocytochemistry protocol for ipsc, positive neurons immunocytochemistry protocol, derived dopaminergic neuron, dopaminergic neuron, immunocytochemistry for th, immunocytochemistry, ipsc

Funders Acknowledgements:

ASAP

Grant ID: ASAP-000458

Abstract

Immunocytochemistry protocol for iPSC-derived dopaminergic neurons

Protocol materials

⊗ Geneticin™ Selective Antibiotic (G418 Sulfate) (50 mg/mL) **Thermo Fisher Catalog #10131019**

⊗ Paraformaldehyde solution 4% in PBS **Santa Cruz Biotechnology Catalog #sc-281692**

⊗ Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix **Thermo Fisher Catalog #A1413302**

⊗ NucBlue™ Fixed Cell ReadyProbes™ Reagent **Thermo Fisher Catalog #R37606**

⊗ Sheep Tyrosine Hydroxylase **Pel-Freez Catalog #P60101-150**

⊗ Anti-Neomycin Phosphotransferase II Antibody **Merck MilliporeSigma (Sigma-Aldrich) Catalog #06-747**

Cell culture

1 Seed cell in Geltrex



Geltrex[®]; LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix **Thermo Fisher Catalog #A1413302**

coated 8-well imaging chambers (Ibidi Cat# 80807-90) at differentiation day 25 in media according to Kim, T.W., et al., *Biphasic Activation of WNT Signaling Facilitates the Derivation of Midbrain Dopamine Neurons from hESCs for Translational Use*. Cell Stem Cell, 2021. **28**(2): p. 343-355 e5 differentiation scheme.

2 On DIV 27 change media to media including geneticin



Geneticin[®]; Selective Antibiotic (G418 Sulfate) (50 mg/mL) **Thermo Fisher Catalog #10131019**

Immunocytochemistry

3 On day 37; fix cells in



Paraformaldehyde solution 4% in PBS **Santa Cruz Biotechnology Catalog #sc-281692**

for 15 minutes at  Room temperature

4 Wash cells 5 minutes in 200 μ L PBS with gentle rocking. Repeat for a total of 3 washes

5 Add blocking solution (1% BSA, 0.1% saponin in PBS) for 1 hour at



Room temperature

6 Dilute primary antibodies e.g.



Sheep Tyrosine Hydroxylase **Pel-Freez Catalog #P60101-150** 1:400 and/or



Anti-Neomycin Phosphotransferase II Antibody **Merck MilliporeSigma (Sigma-Aldrich) Catalog #06-747**

1:500 in blocking buffer and incubate at  4 °C  Overnight

7 Wash cells 5 minutes in 200 μ L PBS with gentle rocking. Repeat for a total of 3 washes



- 8 Dilute secondary antibodies from Jackson ImmunoResearches matching primary antibodies in blocking buffer and incubate 1 hour at Room temperature
- 9 Wash cells 5 minutes in 200 μ L PBS with gentle rocking. In second wash include NucBlue[®]; Fixed Cell Ready Probes[®]; Reagent **Thermo**
Fisher Catalog #R37606
- 10 Image cells in PBS