



Sep 23, 2025

Flow cytometry for TH-positive iPSC derived neurons

DOI

dx.doi.org/10.17504/protocols.io.81wgbxwkqlpk/v1

Ellen Hertz¹, Ellen Hertz¹, Gani Perez¹, Martha Kirby¹, Stacie Anderson¹, Yu Chen¹, Ellen Sidransky¹

¹National Institutes of Health

ASAP Collaborative Res...



Ellen Hertz

National Institutes of Health

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.81wgbxwkqlpk/v1>

Protocol Citation: Ellen Hertz, Ellen Hertz, Gani Perez, Martha Kirby, Stacie Anderson, Yu Chen, Ellen Sidransky 2025. Flow cytometry for TH-positive iPSC derived neurons. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.81wgbxwkqlpk/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: November 21, 2023

Last Modified: September 23, 2025

Protocol Integer ID: 92533

Keywords: ASAPCRN, flow cytometry for th, flow cytometry, flow cytometry procedure, derived neuron, neurons this protocol, positive ipsc, ipsc

Funders Acknowledgements:

ASAP

Grant ID: ASAP-000458

Abstract

This protocol details the flow cytometry procedure.

Attachments



[n4ysbzyvpj.pdf](#)

30KB

Materials

- ⊗ Phosphate buffered saline (PBS) without Ca/Mg **Thermo Fisher Scientific Catalog #14190144**
- ⊗ MemBrite® Fix Cell Surface Staining Kits **Biotium Catalog #30093**
- ⊗ PDS Kit Papain Vial **Worthington Biochemical Corporation Catalog #LK003176**
- ⊗ Deoxyribonuclease I **Worthington Biochemical Corporation Catalog #LS006333**
- ⊗ DMEM, high glucose, GlutaMAX®; Supplement **Thermo Fisher Catalog #10566016**
- ⊗ Paraformaldehyde solution 4% in PBS **Santa Cruz Biotechnology Catalog #sc-281692**
- ⊗ Normal Donkey Serum **Jackson ImmunoResearch Laboratories, Inc. Catalog #017-000-121**
- ⊗ Rabbit Tyrosine Hydroxylase **Pel-Freez Catalog #P40101-150**
- ⊗ Alexa Fluor® 647 AffiniPure Donkey Anti-Rabbit IgG (H L) **Jackson ImmunoResearch Laboratories, Inc. Catalog #711-605-152**
- ⊗ NucBlue®; Fixed Cell ReadyProbes®; Reagent **Thermo Fisher Catalog #R37606**



Flow cytometry dopaminergic fraction

5m

- 1 Wash the neurons in PBS and incubate with Membrane prestain solution, dilute 1:1000 in HBSS, for 00:05:00 at 37 °C .
- 2 Change the prestain solution to staining solution and incubate for another 00:05:00 at 37 °C .
- 3 Dissociate the cells with prewarmed papain with DNase, Y-27632 and Emricasan for 00:10:00 at 37 °C .
- 4 Quench the reaction in DMEM/Glutamax with 10% fetal bovine serum and collect by centrifugation at 400 x g, 00:05:00 .
- 5 Resuspend the pellet in 4% paraformaldehyde and incubate for 00:10:00 at Room temperature on a rolling wheel.
- 6 Resuspend the cells in DMEM with 10% FBS and spin at 500 x g, 00:05:00 after which the cells were incubated in permeabilization buffer (1% BSA, 0.1% saponin in PBS, 10% normal donkey serum for 00:30:00 at Room temperature and thereafter label with tyrosine hydroxylase antibody or isotype controls (1:400, Cat.# P40101-150, Pel-Freez and Cat.# AB-105-C R&D respectively) Overnight at 4 °C .
- 7 Wash the cells twice with 2% BSA and 1.5 millimolar (mM) EDTA in PBS and then incubate with diluted Alexa647-conjugated secondary antibody, for 01:00:00 at Room temperature .
- 8 Wash the cells as described above with NucBlue Fixed Cell ReadyProbes Reagent in the second wash and resuspend in wash buffer for flow cytometry analysis.
- 9 Initial gate: Include Membrane+ cells
Second gate: Include single cell DAPI (exclude duplicates).
Third gate: Alexa647+