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## Flow cytometry for TH-positive iPSC derived neurons

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**Protocol status:** Working

**We use this protocol and it's working**

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## 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+