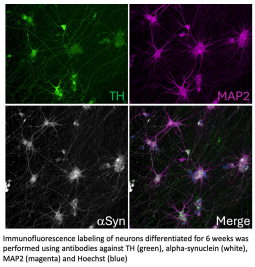
May 01, 2025

# Immunofluorescence Labeling of TH, Alpha-Synuclein, and MAP2 in iPSC-Derived Dopaminergic Neurons



Immunofluorescence labeling of neurons differentiated for 6 weeks was performed using antibodies against TH (green), alpha-synuclein (white), MAP2 (magenta) and Hoechst (blue)

DOI
https://dx.doi.org/10.17504/protocols.io.36wgqdo9yvk5/v1

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**Protocol Citation:** Roxanne Larivière, Edward A. Fon 2025. Immunofluorescence Labeling of TH, Alpha-Synuclein, and MAP2 in iPSC-Derived Dopaminergic Neurons . **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqdo9yvk5/v1

**Protocol status:** Working
**We use this protocol and it is working**

**Created:** December 19, 2024

**Last Modified:** May 01, 2025

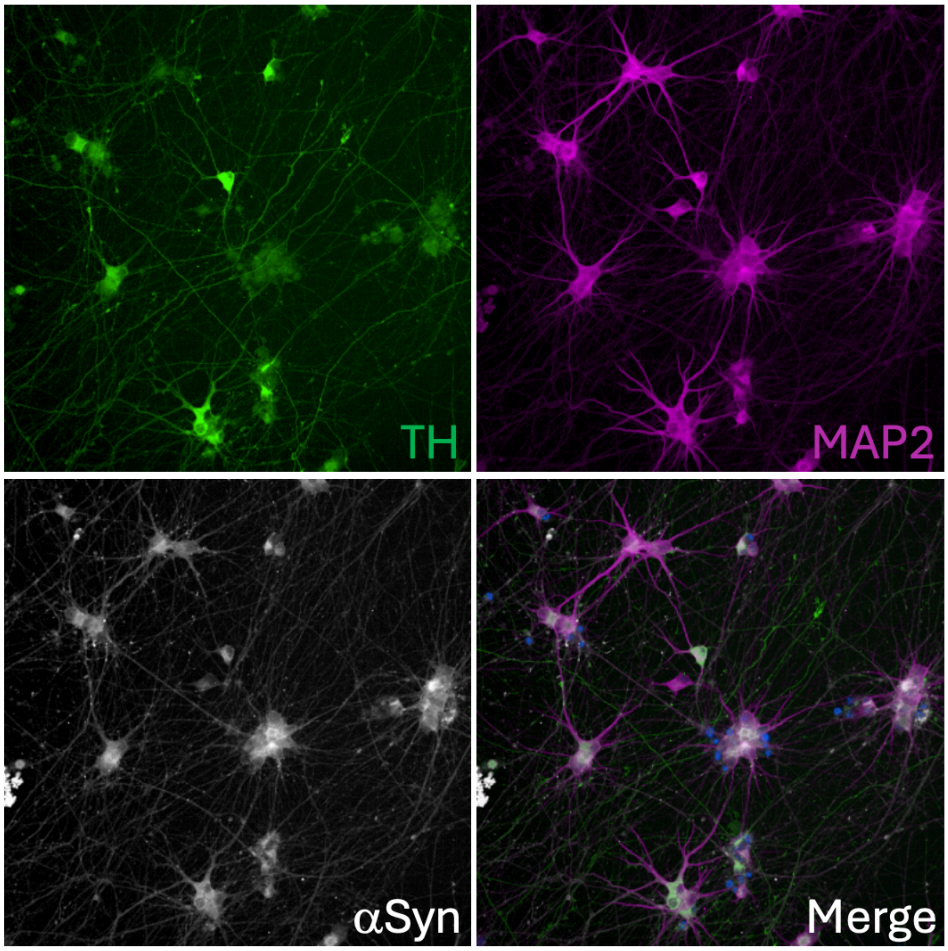
**Protocol Integer ID:** 116390

**Keywords:** IF, immunofluorescence, TH, alpha-synuclein, MAP2 , iPSC, dopaminergic neurons, synuclein, derived dopaminergic neuron, dopaminergic neuron, induced pluripotent stem cell, map2 in ipsc, labeling tyrosine hydroxylase, immunofluorescence labeling, pluripotent stem cell, immunofluorescence labeling of th, tyrosine hydroxylase, using immunofluorescence

**Funders Acknowledgements:**
**Michael J. Fox Foundation for Parkinson's research**
Grant ID: MJFF-020696

## Abstract

This protocol outlines the procedure for labeling tyrosine hydroxylase (TH), alpha-synuclein (α-syn), and microtubule-associated protein 2 (MAP2) in induced pluripotent stem cell (iPSC)-derived dopaminergic neurons using immunofluorescence.




Immunofluorescence labeling of neurons differentiated for 6 weeks was performed using antibodies against TH (green), alpha-synuclein (white), MAP2 (magenta) and Hoechst (blue)

## Materials

| Reagents | Company | Cat. Number |
|---|---|---|
| Rabbit Tyrosine hydroxylase antibody | Pel-Freez | P40101 |
| Mouse alpha-synuclein antibody | BD Biosciences | 610787 |
| Chicken MAP2 antibody | Encor Biotech | CPCA-MAP2 |
| Goat anti-Chicken IgY (H+L) Secondary Antibody, Alexa Fluor 555 | ThermoFisher Scientific | A21437 |
| Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 | ThermoFisher Scientific | A21235 |
| Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | ThermoFisher Scientific | A11008 |
| Hoechst 33342 | SIGMA | H3570 |
| 16% Formaldehyde solution (w/v) Methanol-free | ThermoScientific | 28908 |
| Normal goat serum | SIGMA | G9023 |
| Triton X-100 | SIGMA | X-100 |

## Before start

iPSC-derived dopaminergic neurons are cultured on 96-well plates for up to 4 to 6 weeks of differentiation. We use black Costar 96-well assay plates (Corning cat. no. 3904), which are compatible with imaging using the Opera Phenix high-content screening system.

Be extremely gentle when dispensing into the wells, as dopaminergic neurons are prone to detaching with forceful pipetting.

## Fixation of dopaminergic neurons in 96 well plates

40m

1 - Leave 50 µL of media and gently add 50 µL of 8% PFA per well. 40m
- Incubate at Room temperature 00:20:00 .
- Remove PFA and wash 3 times with 1X PBS, 75 µL per well, 00:05:00 each

## Permeabilization

25m

2 - Prepare permeabilization solution: 0.2% TX-100; 1X PBS. 25m
- Incubate in permeabilization solution 00:10:00 , 60 µL per well
- Remove permeabilization solution and wash 3 times with 1X PBS, 75 µL per well, 00:05:00 each

## Blocking

1h

3 - Prepare blocking buffer: 5% normal goat serum; 0.02% TX-100; 1X PBS 1h
- Incubate in blocking solution 01:00:00 , 60 µL per well

## Incubation with primary antibodies

16h

4 - Prepare primary antibody mix in blocking buffer, 60 µL per well 16h

- rabbit TH antibody 1:500
- mouse alpha-synuclein antibody 1:500
- chicken MAP2 antibody 1:1500

- Incubate Overnight at 4 °C with gentle shaking

## Washes

20m

5 - Wash 4 times with 75 µL 1X PBS, 00:05:00 each 20m

## Incubation with secondary antibodies

2h

6 - Prepare secondary antibody mix in 1% normal goat serum; 1X PBS. Dispense 60 µL per well.

2h

- anti-Rabbit Alexa Fluor 488 1:1000
- anti-mouse Alexa Fluor 647 1:1000
- anti-Chicken Alexa Fluor 555 1:1000
- Hoechst 33342 1:5000

- Incubate 02:00:00 at Room temperature with gentle shaking

## Washes

20m

7 - Wash 4 times with 75 µL 1X PBS, 00:05:00 each

20m

- Leave 100 µL of 1X PBS into wells

## Imaging

8 - Samples are ready for imaging