Oct 24, 2025 Version 2

Immunostaining V.2

DOI
https://dx.doi.org/10.17504/protocols.io.n92ld62d8g5b/v2

Sabina Marciano[1], Jacquelyn Haytayan[2,3,4], Roberta Marongiu[2,3,4]

[1]Weill Cornell medicine; [2]Weill Cornell Medicine; [3]Aligning Science Across Parkinsons; [4]Collaborative Research Network

Jacquelyn Haytayan
Weill Cornell Medicine

DOI: https://dx.doi.org/10.17504/protocols.io.n92ld62d8g5b/v2

Protocol Citation: Sabina Marciano, Jacquelyn Haytayan, Roberta Marongiu 2025. Immunostaining . **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ld62d8g5b/v2Version created by **Jacquelyn Haytayan**

Protocol status: Working
We use this protocol and it's working

Created: October 24, 2025

Last Modified: October 24, 2025

Protocol Integer ID: 230698

Keywords: ASAPCRN, immunostaining , Histology , psyn , Antibody, microglial marker iba1, synuclein aggregate, multiplexed fluorescent detection of dopaminergic neuron, synuclein, microglia, neurodegenerative features in parkinsonian model, study of neuroinflammatory, neurodegenerative feature, neuroinflammatory, pathological markers in aof, dopaminergic neuron, endogenous peroxidase, pathological marker, multiplexed fluorescent detection, pd tissue, immunohistochemistry protocol, parkinsonian model, quenching of endogenous peroxidase, phosphorylated alpha

Funders Acknowledgements:
Aligning Science Across Parkinsons
Collaborative Research Network

Abstract

This immunohistochemistry protocol was developed to visualize pathological markers in AOF-PD tissue, focusing on phosphorylated alpha-synuclein (psyn), tyrosine hydroxylase (TH), and microglial marker Iba1, with quenching of endogenous peroxidases to reduce background. The protocol enables multiplexed fluorescent detection of dopaminergic neurons, microglia, and pathological alpha-synuclein aggregates, facilitating the study of neuroinflammatory and neurodegenerative features in Parkinsonian models.

Materials

Primary Antibodies:

- Goat anti-Iba1 Iba1, WAKO, cat. 01919741 1:1500
- Rabbit anti-TH Abcam, cat. Ab113, 1:1000
- Mouse anti-Myc Cell Signaling, cat. 2276
- Mouse anti-psyn Abcam cat. ab51253 1:1000

Secondary Antibodies:

- Donkey anti-goat 594 1:1000
- Donkey anti-rabbit 647 1:500
- Donkey anti-mouse 488 1:1000
- Neurotrace 455 (1:100)

Solutions:

PBS-T Triton 0.2 % 150mL: 300uL Triton in 150mL PBS

Before start

All washing steps are performed for 10min and on a shaker

Immunostainin

1 **Washes** Wash 3x in PBS-T, 10min each (0.2% Triton in PBS)

2 **Quench** Incubate in 3% H2O2 in PBST for 2hrs at room temperature in the DARK! (x3)

2.1 Can be done the day before and sections can be stored in PBS overnight at 4 degrees C

3 **Washes** Wash 3x in PBS-T, 10min each (0.2% Triton in PBS)

4 **Blocking** Incubate in 3% BSA/PBS-T for 1 hr at room temperature

5 **Primary Antibody** Incubate in primary antibody (diluted in blocking solution) overnight.

5.1 **Goat anti-Iba1 Iba1, WAKO, cat. 01919741 1:1500**
Rabbit anti-TH Abcam, cat. Ab113, 1:1000
Mouse anti-Myc Cell Signaling, cat. 2276
Mouse anti-psyn Abcam cat. ab51253 1:1000

6 **Washes** Wash 3x in PBS-T, 10min each

7 **Secondary Antibody** Incubate in secondary antibody (diluted in blocking solution) 1hr at room temperature

7.1 **Donkey anti-goat 594 1:1000**
Donkey anti-rabbit 647 1:500
Donkey anti-mouse 488 1:1000
Neurotrace 455 (1:100)

8 **1) Washes** Wash 3x in PBS-T, 10min each