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Immunostaining V.1

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Protocol status: Working

We use this protocol and it's working



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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

Immunostaining: AOF-PD: psyn, TH, Iba1, quench

- 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