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Immunostaining

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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 tyrosine hydroxylase (TH), microglial marker Iba1, GFAP, and DAT 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

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

Immunostaining: AOF-PD: TH, Iba1, GFAP, DAT

1 **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 RT in the DARK! (x3) (can be done the day before and sections can be stored in PBS ON at 4degrees)

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

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

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

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

5.1 **Goat anti-Iba1 Iba1, WAKO, cat. 01919741 1:1500**

5.2 **Rabbit anti-TH Abcam, cat. Ab113, 1:1000**

5.3 **Mouse anti-GFAP ThermoFisher cat. PA1-10019 1:1500**

5.4 **Rat anti-DAT Millipore Cat #MAB369 1:500**

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

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

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

7.1 **Donkey anti-goat 594 1:1000**

7.2 **Donkey anti-rabbit 488 1:500**

7.3 **Donkey anti-mouse 405 1:1000**

7.4 **Donkey anti-rat 647 1:1000**

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

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