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Immunohistochemistry for Tyrosine Hydroxylase (TH) Detection in Brain Sections V.1

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We use this protocol and it's working

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Abstract

This protocol describes the immunohistochemical staining procedure for detecting Tyrosine Hydroxylase (TH) in brain sections using primary antibodies and fluorescent-conjugated secondary antibodies. The protocol ensures optimal signal specificity and preservation of tissue morphology.

Materials

Solutions and Buffers:
- 4% Paraformaldehyde (PFA) in PBS (for washing)
- Blocking Buffer: 10% donkey serum, 0.3% Triton-X in PBS
- Primary Antibody Dilution Buffer: 2.5% donkey serum, 0.3% Triton-X in PBS
- Phosphate-Buffered Saline (PBS)
- Mowiol Mounting Medium

Primary Antibodies:
- Chicken anti-TH (Cat# ab76442, Abcam)

Secondary Antibodies:
- Fluorescent-conjugated secondary antibodies against chicken IgG

Equipment:
- Shaker or rotator
- Leica DM6000 B microscope (Leica Microsystems)
- Coated slides and coverslips
- Pipettes and tips
- Humidity chamber (optional for incubation steps)

Section Washing

1 Wash brain sections once with 4% PFA in PBS.

2 Ensure complete removal of fixative by gentle shaking or rotating for 5 minutes.

3 Rinse briefly with PBS to remove any residual PFA.

Blocking Step

4 Prepare the blocking buffer (10% donkey serum, 0.3% Triton-X in PBS).

5 Incubate sections in the blocking buffer for 1 hour at room temperature (RT) to minimize nonspecific binding.

6 Place the sections on a shaker or rotator to ensure even exposure to the blocking solution.

Primary Antibody Incubation

7 Prepare the primary antibody solution using the following:

7.1 Chicken anti-TH (Cat# ab76442, Abcam)

7.2 Dilute 1:200 in 2.5% donkey serum and 0.3% Triton-X in PBS.

8 Incubate sections with the primary antibodies for 1 hour at RT.

9 Optional: Incubation can be performed in a humidity chamber to prevent evaporation.

Washing After Primary Antibody Incubation

10 Wash the sections three times with PBS for 5 minutes each.

11 Ensure gentle shaking or rotation during washing steps.

Secondary Antibody Incubation

12 Incubate sections with fluorescent-conjugated secondary antibodies specific to chicken IgG dilution 1:1000 for 1 hour at RT.

13 Protect from light to preserve fluorescence.

Final Washing

14 Wash sections three times with PBS for 5 minutes each.

15 Perform the washes in the dark to maintain fluorescence integrity.

Mounting

16 Mount the sections on coated slides.

17 Cover the sections with coverslips using Mowiol mounting medium.

18 Allow the slides to dry in the dark for at least 30 minutes before imaging.

Imaging

19 Visualize the sections using a Leica DM6000 B microscope with a 10× objective.

20 Use appropriate fluorescence filter sets to detect the secondary antibody signals.

21 Adjust exposure settings to optimize signal-to-noise ratio.