Immunohistochemistry free-floating rat brain cryosections

In 1 collection

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ABSTRACT

Protocol for immunohistochemistry on rat brain cryosections

MATERIALS

Reagents:

- Phosphate buffer solution (PB)1X.
- TritonX-100
- Endogenous peroxidase blocking solution: PB 1x + 3% H_2O_2
- Blocking Buffer: PB 1X + 10% NGS
- 1st Ab : Diluted in1X PBS +5% NGS+ 0.3% TX
- 2nd Ab : Diluted in1X PBS +5% NGS+ 0.3% TX
- 3,30-diaminobenzidine (DAB)
- Xilen
- Ethanol : 100%, 95%, 70%, 50%

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1. Sections selection

1 Collect the cryosections needed for a caudo-rostral representation of each brain region (every fifth or sith sections depending on the section's thickness, brain region, and animal species) into a 24-well-plate (3-4 sections per well)

1.1 Wash in PB1X 3 times x 5 min (500 ul) at RT

2. Inactivation of endogenous peroxidase

10m

2 Incubate sections with endogenous peroxidase blocking solution: PB1X + 3% H_2O_2 (500 µl/well) for at RT 10m

2.1 Wash in PB1X 2 times x 5 min (500 ul) at RT

3. Blocking

3 Incubate with blocking solution (500 µl/well) : PB1X + 10%NGS (serum from the same species as the host o 1h the secondary antibody) + 0.3% TritonX-100, at RT

4. Primary antibody incubation

4 Remove the blocking solution and incubate sections with PB1X+ 5%NGS+0.3% TritonX-100+ primary Ab (500 µl/well) for 24/72 h, depending on the Ab, at +4°C

4.1 Wash in PB1X 3 times x 5 min (500 ul) at RT

5. Secondary biotinylated antibody incubation

2h

5 Incubate sections with adequate secondary biotinylated Ab diluted in PB1X+ 0.3% TritonX-100, at RT 2h

5.1 Wash n PB1X 3 times x 5 min (500 ul) at RT

6. Extravidin-peroxidase reaction

1h 30m

6 Incubate with Extravidin-peroxidase buffer solution diluited in PB1X+0.3% TritonX-100, at RT 1h 30m

6.1 Wash n PB1X 3 times x 5 min (500 ul) at RT

7. Substrate preparation

7 Allow each DAB tablet to reach room temperature before use and Dissolve a DAB tablet in 15 mL of Tris-buffered saline, pH 7.6

7.1 Add 12 µL of fresh 30% hydrogen peroxide prior to use

7.2 Filter the solution through a 0.2 µm filter immediately before use

8. Development

3m

8 Add 500 µl/ well of substrate solution in the dark

3m

8.1 Remove and clean al material with bleach

8.2 Wash 2 times x 5 min in PB1x

9. Section Mounting

9 Mount sections on SuperFrost plus slides and let it dry overnight

10. Dehydratation

1m

10 Dehydrate in consecutive steps of ethanol solutions (50%, 70%, 95%, 100%)

1m

10.1 Incubate slides in 2 times x 5 min in Xylene

11. Mount Coverslips

11 Coverslip slides with mounting medium, remove bubbles if any, and let dry