

JAN 30, 2024

OPEN ACCESS



DOI:

dx.doi.org/10.17504/protocols.io. 3byl4q4zrvo5/v1

Protocol Citation: mariangela.m assarocenere 2024. Immunohistochemistry freefloating rat brain cryosections . protocols.io

https://dx.doi.org/10.17504/protoc ols.io.3byl4q4zrvo5/v1

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: Jan 25, 2024

(Immunohistochemistry free-floating rat brain cryosections



In 1 collection

mariangela.massarocenere^{1,2,3}

¹Department of Experimental Neuroscience, Santa Lucia Foundation IRCCS, Rome, Italy;

²Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy;

³Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, United States



mariangela.massarocenere

ABSTRACT

Protocol for immunohistochemistry on rat brain cryosections

MATERIALS

Reagents:

- Phosphate buffer solution (PB)1X.
- TritonX-100
- Endogenous peroxidase blocking solution: PB 1x + 3% H₂O₂
- 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)
- Ethanol: 100%, 95%, 70%, 50%

Last Modified: Jan 30, 2024

PROTOCOL integer ID: 94117

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

- Incubate sections with endogenous peroxidase blocking solution: PB1X + 3% H₂O₂ (500 μ l/well) for at RT 10m
 - 2.1 Wash in PB1X 2 times x 5 min (500 ul) at RT

3. Blocking

Incubate with blocking solution (500 μ l/well): PB1X + 10%NGS (serum from the same species as the host of the secondary antibody) + 0.3% TritonX-100, at RT

4. Primary antibody incubation

Remove the blocking solution and incubate sections with PB1X+ 5%NGS+0.3% TritonX-100+ primary Ab (500μ J/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

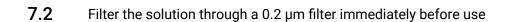
1h 30m

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

7. Substrate preparation

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

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



8. Development

3m

8 Add 500 μ l/ well of substrate solution in the dark

2....

- **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

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

1m

Oct 30 2024



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