



OCT 17, 2023

Immunohistochemistry on free-floating cryosections

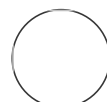
Forked from [Immunohistochemistry on paraffin sections](#)

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ABSTRACT

Immunohistochemistry protocol on rodent brain cryosections

MATERIALS

Reagents :

- TBS 10X : Tris base 121.1g + NaCl 90g in 1L H2O.pH 7.4.
- TBS 1X-Triton 0,5%
- Xilen
- Ethanol : 100%, 95%, 70%
- Unmasking buffer epitopes : Citrate solution 10mM pH6.0
- Blocking Buffer : TBS 1X + 5% NGS
- 1st Ab : Diluted in1X PBS +2%NGS
- 2nd Ab : Diluted in1X PBS +2%NGS
- Endogenous peroxidase blocking solution : TBS 1x + 3% H2O2(30%) + 10% methanol

OPEN ACCESS



DOI:

dx.doi.org/10.17504/protocols.io.rm7vzx7x8gx1/v1

Protocol Citation: Nuriapenuelas 2023.

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<https://dx.doi.org/10.17504/protocols.io.rm7vzx7x8gx1/v1>

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Protocol status: Working
We use this protocol and it's working

Created: Oct 17, 2023

1. Section selection

- 1 Collect the cryosections needed for a caudo-rostral representation of each brain region (every fourth section or every sixth section depending on the section thickness, brain region and animal species) into 24-well-plate (3-4 sections per well).
 - 1.1 Wash 3x5 min in TBS 1X. Put 500ul per well and aspirate the liquid with an air-pump equipment.

2. Blocking endogenous peroxidase

- 2 Incubate sections in endogenous peroxidase blocking solution: TBS 1x + 3% H₂O₂ + 10% methanol for 10 min (500uL/well)
 - 2.1 Wash 3x5 min in TBS 1X.

3. Blocking

- 3 Incubate sections in blocking in TBS 1X + 5% NGS or NDS (500uL/well) 1h at RT

4. Primary antibody

- 4 Incubate sections in TBS 1X + 2% NGS or NDS + primary Ab 24/72h (it depends of the Ab) at 4°C (cold room).

- 4.1** Wash 3x5min in TBS 1X.

5. 2ary antibody

- 5** Incubate sections in 2% NGS or NDS + Secondary Ab 1h at RT.

- 5.1** At this step it is important to prepare ABC solution and let it, at least, 30 min on the shaker

- 5.2** Wash 3x5min in TBS 1X.

6. ABC incubation

- 6** Incubate 1 hour at RT with ABC solution (Ultra-Sensitive or Standard ABC Peroxidase Standard Staining Kit).

- 6.1** Wash 3x5min in TBS 1X.

7. Developing

- 7** In aluminium foil: DAB Standard Kit (1 drop of reagent B in 1 mL of reagent A, gives rise to brown staining), or Vector SG (3 drops Chromogen + 3 drops Hydrogen Peroxide in 5mL PBS, gives rise to blue staining).

- 8** Put 500 uL on each well and put a cardboard box on it to keep darkness for a time ranging from

3-15 minutes depending on the antibody used.

8.1 Remove with an air-pump equipment and clean the material with bleach.

8.2 Wash 3x5min in TBS 1X.

8. Mount sections

9 Mount sections into slides and let it dry overnight.

9. Dehydration

10 Incubate slides in consecutive ethanol solutions (1 min in ethanol 70%-1 min in ethanol 95%-1 min in ethanol 100%).

11 Incubate slides in 2x5 min in Xylene.

10. Mount coverslips

12 Put a line of mounting medium (DPX) by slide. Put the coverslip (washed with ethanol previously) on the slide. Remove bubbles.

13 Let dry the slides in the hood overnight.

