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

Created: Oct 17, 2023

Immunohistochemistry on free-floating cryosections

Forked from Immunochemistry on paraffin sections

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ABSTRACT

Immunochemistry 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

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1. Section selection

1 Collect the cryosections needed for a caudo-rostral representation of each brain region (every fourth section or every sixth section dependeing on the section thinknes, 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. 1ary antibody

4 Incubate sections inTBS 1X + 2% NGS or NDS + primary Ab 24/72h (it depends of the Ab) at 4ºC (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. Dehydratation

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.