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Immunochemistry on paraffin sections

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ABSTRACT

Immunochemistry protocol on paraffin-embedded rat brain sections

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MATERIALS TEXT

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

1. Deparaffinization and hydratation :

1 Put the slides 30 min in the incubator at 60ºC.

2 Wash 3x3 min in Xilen.

3 Wash 1x10 min in ethanol 100%.

4 Wash 1x10 min in ethanol 95%.

5 Wash 1x5 min in ethanol 70%.

6 Wash 2x5 min in TBS 1X.

2. Blocking endogenous peroxidase:

7 Put the slides 10 min in endogenous peroxidase blocking solution: TBS 1x + 3% H2O2 + 10% methanol

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Wahsing

8 Wash 3x5 min in buffer TBS 1X.

Antigen retrieval

9 Put sections in 200 mL of citrate buffer 10mM, pH 6

9.1 Put sections in a boiling water bath for 20 min

10 Let sections cool down for 20 min at RT

Washing

11 Wash 3x5 min in TBS 1X.

Blocking

12 Put wet paper in a black box for immunohistochemistry

13 Gently dry and circle sections with the hydrophobic pen ImmEdge Vector H 4000 without touching the tissue

14 Blocking in TBS 1X + 5% NGS (200uL/slides) 1h at RT

1ary antibody

15 Gently wipe off the water ofthe slides

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16 Put 200ul/slide of TBS 1X + 2% NGS + primary Ab48/72h (it depends of the Ab) at 4ºC (cold room)

Washing

17 Wash 3x5min in TBS 1X Buffer.

2ary antibody

18 Gently wipe off the water ofthe slides

19 Put 200ul/slide TBS 1X + 2% NGS + Secondary Ab 1h at RT.

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

Washing

20 3x5min in TBS 1X

Peroxidase staining

21 Incubate 1 hour at RT with ABC solution (4drops A/B in 10 mL of TBS 1x)

Washing

22 3x5min in TBS 1X

Developing preparation

23 In aluminium foil: DAB Standard Kit: 1 drop of reagent B in 1 mL of reagent A (gives rise to brown staining)

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23.1 In aluminium foil: Vector SG: 3 drops Chromogen + 3 drops Hydrogen Peroxide in 5mL PBS (gives rise to blue staining)

Developing

24 Cover tray plates with aluminium foil

24.1 Put 200 uL on each section and put a cardboard box on it to keep darkness for a time ranging from 3-15 minutes depending on the antibody used

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

Washing

25 3x5min in TBS 1X

Dehydratation

26 1 min in ethanol 70%

27 1 min in ethanol 95%

28 1 min in ethanol 100%.

29 2x5 min in xylene

Mounting

30 Puta line of mounting medium(DPX) byslide.Put the coverslip (washed with ethanol previously) on the slide. Remove bubbles

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31 Let dry the slides on the tray in the hood overnight

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