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Immunofluorescence on paraffin sections

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ABSTRACT

Immunofluorescence 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 xylene

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 PBS 1X

IMPORTANT: The buffer used for immunofluorescence is PBS, not TBS as for immunohistochemistry and the endogenous peroxidase blocking step is not required.

Antigen retrieval

7 Add 200 mL/section of citrate buffer 10mM, pH 6

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7.1 Put sections in a boiling water bath for 20 min

8 Let sections cool down for 20 min at room temperature (RT)

Washing

9 Wash 3x5 min in PBS 1X-Triton (0.5%)

Blocking

10 Put wet paper in a black box for immunohistochemistry

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

12 Add 200 uL/section of blocking solution (5% NGS (in PBS 1X) (200uL/section) + 0.1% Triton (in PBS 1X)) 1h at RT

1ary antibody

13 Gently wipe off the water ofthe slides

14 Put 200ul/section of 1ary antibody in 2% NGS + 0.1% Triton overnight at 4ºC (cold room)

Washing

15 Wash 3x5min in PBS 1X-Triton (0.5%)

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2ary antibody

16 Gently wipe off the water ofthe slides

17 Put 200ul/section of 2ary antibody in 2% NGS + 0.1% Triton + DAPI for 1 h at RT

Alexa goat anti-mouse 488 (green color) 1:500 or 1: 1000, Alexa goat anti-rabbit 594 (red color) and 1:5000 DAPI (nuclei staining in blue color)

Washing

18 Wash 3x5min in PBS 1X

Mounting

19 Mount sections with fluorescent mounting medium. Put the coverslip (washed with ethanol previously) on the slide. Remove bubbles

Storage

20 Let dry the slides on the tray in the hood for 5 minutes and store at 4ºC **asap**

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