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Immunofluorescence on paraffin sections

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Protocol status: Working

We use this protocol and it's working

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Abstract

Immunofluorescence protocol on paraffin-embedded rodent brain sections

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



1. Deparaffinization and hydration :

- 1 Put the slides 30 min in the incubator at 60°C
- 2 Wash 3×3 min in xylene
- 3 Wash 1×10 min in ethanol 100%
- 4 Wash 1×10 min in ethanol 95%
- 5 Wash 1×5 min in ethanol 70%
- 6 Wash 2×5 min in PBS 1X

Note

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 Incubate slides in citrate buffer 10mM, pH 6 in a boiling water bath (95°C) for 20 min
- 8 Let sections cool down for 20 min at room temperature (RT)

Washing

- 9 Wash 3×5 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 μ L/section of blocking solution (5% NGS (in PBS 1X) (200 μ L/section) + 0.1% Triton (in PBS 1X)) 1h at RT

1ary antibody

- 13 Gently wipe off the water of the slides
- 14 Put 200 μ L/section of 1ary antibody in 2% NGS + 0.1% Triton overnight at 4°C (cold room)

Washing

- 15 Wash 3 $\times$ 5min in PBS 1X-Triton (0.5%)

2ary antibody

- 16 Gently wipe off the water of the slides
- 17 Put 200 μ L/section of 2ary antibody in 2% NGS + 0.1% Triton + Hoechst/DAPI for 1 h at RT

Washing

- 18 Wash 3 $\times$ 5min 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 4°C **asap**