Immunofluorescence free-floating rat brain cryosections

In 1 collection

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ABSTRACT

Protocol for immunofluorescence on rat brain cryosections

MATERIALS

Reagents:

- Phosphate buffer solution (PB)1X.
- TritonX-100
- Blocking Buffer: 1X PB + 10% NDS
- 1st Ab : Diluted in1X PBS +5% NDS+ 0.3% TX
- 2nd Ab : Diluted in1X PBS +5% NDS+ 0.3% TX
- 4',6-diamidin-2-fenilindolo (DAPI): Diluted in 1X PB

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1. Sections selection

1 Collect the cryosections needed for a caudo-rostral representation of each brain region (every fifth or sixth section depending on the section's thickness, brain region, and animal species) into a 24-well-plate (3-4 sections per well)

1.1 Wash in PB1X 3 times x 5 min (500 ul) at RT

2. Blocking

1h

2 Incubate with blocking solution (500 µl/well) : PB1X + 10%NDS (serum from the same species as the host o 1h the secondary antibody) + 0.3% TritonX-100, at RT

3. Primary antibody incubation

3 Remove the blocking solution and incubate sections with PB1X+ 5%NdS+0.3% TritonX-100+ primary Ab (500 µl/well) for 24/72 h, depending on the Ab, at +4°C

3.1 Wash in PB1X 3 times x 5 min (500 ul) at RT

4. Secondary antibody incubation

4 Incubate sections with adequate secondary fluorescent Ab diluted in PB1X+ 0.3% TritonX-100, at RT 2h

4.1 Wash in PB1X 2 times x 5 min (500 ul) at RT

5 Incubate with DAPI diluted in PB1X, to stain nuclei 10m

5.1 Wash in PB1X 2 times x 5 min (500 ul) at RT

5. Section Mounting

6 Mount sections on slides and let it dry

6. Mount Coverslips

7 Coverslip slides with aqueous mounting medium, remove bubbles if any, and let dry