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Immunohistochemistry for brain sections

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We use this protocol and it's working

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Abstract

Protocol for processing 30um mouse brain sections for immunolabeling.



Materials

Solutions:

TBS-T

For **2l**:

50mM Tris pH 7.5 – 100 ml of 1M stock

150 mM NaCl – 17.53 g

Triton 0.2 % - 4 ml

DW – 1.9 l

ANTI-FREEZE SOLUTION

For **1l**:

400 ml PBS/TBS (no TritonX-100!)

300 ml ethylene glycol

300 ml glycerine

STIR SOLUTION!!

Blocking solution

Prepare the solution in falcon tubes:

10 % goat, donkey or horse serum in TBS-T

Attn.: Do not shake too much (mix by inverting only). Proteins generate bubbles. Between experiments keep the blocking solution in the refrigerator at  4 °C . During experiment keep  On ice .



Tissue Preparation

- 1 **Sections at cryostat:**
 - Thickness 30 μm
 - Sections picked up with a wet brush (not too wet) and moved to the TBS-T wells (usually serial sections). Store section in anti-freeze solution in $-20\text{ }^{\circ}\text{C}$ if not immediately staining.
- 2 **Transfer the sections** into the net-wells.

Day 1: Primary Antibody Incubation

- 3 **Washes:** 3-step wash, 00:10:00 each, on a shaker, in TBS-T. (Between washes, just move the inserts from one tray to another.) 10m
- 4 **Blocking step:** add blocking solution into a 6-well plate. Transfer the sections with a clean brush from the net-inserts into the 6-well plate. Incubate on the shaker for 01:00:00 at Room temperature . 1h
- 5 **Primary antibody:** Transfer the sections into a 6-well plate containing the primary antibody solution (1 ml per well).
- 6 **Incubation:** on a shaker **in the cold room** Overnight. 1h

Day 2: Secondary Antibody Incubation

- 7 **Washes:** a 3-step wash, 00:10:00 each, in TBS-T. 10m
- 8 **Secondary antibody:** Transfer the sections into a 12-well plate containing the secondary antibody. The secondary antibody will be incubated at Room temperature .
- 9 **Incubation:** on a shaker, at Room temperature , for 1-2 hours.



- 10 **Washes:** 3-4 times, for ⌚ 00:10:00 , in TBS-T. 10m
- 11 **DAPI:** incubate with DAPI (1:10,000) shaking for ⌚ 00:10:00 at 🌡 Room temperature . 10m
- 12 **Washes:** a 2-step wash, on a shaker, at 🌡 Room temperature , ⌚ 00:10:00 each. 10m
- 13 **Mounting:** Mount sections and let them dry for ⌚ 00:10:00 at 🌡 Room temperature . 10m
- 14 **Coverslip:** Let the slides dry at least ⌚ Overnight at 🌡 Room temperature . 10m