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# Immunohistochemistry for brain sections

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**Protocol status:** Working
**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 °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. 1h
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.

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 1h

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