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Immunostaining of Brain Sections (100µm and 40µm)

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We use this protocol and it's working

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Abstract

General Immunohistochemistry protocol

Prepare Solutions

1 Make TBST (0.2% Triton): 49ml 1x TBS + 1ml 10% TritonX (note: make solution fresh to begin your staining. Make sure that Triton bottle has not been open for more than 2-4 weeks)

2 Blocking and antibody solution: 10% goat serum in TBST

3 Note: All incubation and washing steps done with shaking.

Blocking and Primaries

4 Wash sections 3× 10 min in 1x TBS. 1ml of solution per well is sufficient for 4-6 sections per well in a 24 well plate.

5 Incubate in blocking solution (10% goat serum in TBST) for 1hr at room temperature.

6 Prepare primary antibody solution by diluting antibodies in 10% goat serum TBST solution. Vortex briefly to mix. Spin antibodies down at 5 mins, max speed. (This step is done to pellet any insoluble antibody aggregates that might cause background staining.)

7 Incubate in primary antibody solution at 4°C on shaker for at least 2 nights. Can incubate up to 3 nights.

Secondaries and Mounting

8 Incubate in secondary antibodies for 3hr at room temperature.

9 Wash in TBST 2 × 10 mins, 3rd rinse for 30 min.

10 Prepare secondary antibody solutions by diluting 1:200 in 10% goat serum TBST and spinning down as described with primary antibodies.

11 Wash in TBST 3× 10 mins.

12 One by one, transfer sections first to a container filled with 2/3 TBS, 1/3 ddH2O. Mount the sections (3 per slide) with the help of a clean brush on glass slides. Allow sections to dry for approximately 5-10 mins.

13 Use 1 drop of VectaShield mounting media (with DAPI) per section. Coverslip and then seal with nail polish.

14 Important! Wait at least 2hrs before imaging. This time is required for the sections to settle and achieve accurate z-stack imaging.