NeuroMab

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Immunofluorescence Staining of Free Floating Brain Sections

Day 1.

1) Wash sections 4X with gentle rocking/agitation at 4ºC, 5 min each in ice-cold 0.1 M PB (0.1M Na Phosphate pH 7.4).

2) Block sections in vehicle (10% normal goat serum in 0.1 M PB+ 0.3% Triton X-100) for 1 h with gentle rocking/agitation at 4ºC

3) Incubate sections in NeuroMab diluted in ice-cold vehicle. Dilution factors for NeuroMabs should be determined empirically, but generally NeuroMab tissue culture supernatants should be used at 1:2-1:10, purified NeuroMab IgG from 1-20 μg/ml. Incubate sections overnight at 4ºC with gentle rocking/agitation.

Day 2.

4) Wash sections 4X with gentle rocking/agitation at 4ºC, 5 min each in ice-cold vehicle.

5) Incubate sections for 1 h with gentle rocking/agitation at 4ºC in appropriate fluorescent goat anti-mouse secondary antibody. We typically use Alexa Fluor conjugated goat anti-mouse isotype specific (i.e., anti-IgG1, IgG2a or IgG2b) secondary antibodies, which have a higher signal and lower background than anti-IgG secondary antibodies.

From this step on the sections should be protected from room light.

6) Wash sections with gentle rocking/agitation at 4ºC, 5 min in vehicle.

7) Wash sections with gentle rocking/agitation at 4ºC, 5 min in 0.1 M PB.

8) Wash sections with gentle rocking/agitation at 4ºC, 5 min in 0.05 M PB.

9) Mount on gelatin-coated microscope slides (floating in 0.05 M PB in a 15 ml Petri dish).

10) Air dry

11) Put 15 μl mounting media (e.g., Prolong Gold antifade reagent, Invitrogen P36930) on each section and coverslip.

12) Seal with nail polish.