NeuroMab

UC Davis/NIH NeuroMab Facility
Department of Neurobiology, Physiology and Behavior, UC Davis, Davis CA 95616-8519
http://neuromab.ucdavis.edu neuromab@ucdavis.edu (530) 752-8398

Immunofluorescence Labeling of Free-Floating Perfusion-Fixed Brain Sections

Note: protocol can be completed in one day by shortening primary antibody incubation (Step 3).

Day 1.

1) Section preparation: wash sections four times with ice-cold (4ºC) 0.1 M PB (recipe below) with gentle rocking/agitation at room temperature (RT) for 5 min each.

2) Block: incubate sections in vehicle (recipe below) with gentle rocking/agitation at 4°C for 1 h.

3) Primary antibody incubation: transfer sections into ice-cold (4ºC) vehicle containing diluted NeuroMab(s) and incubate with gentle rocking/agitation at RT for 3 h (or at 4°C overnight).

Note: antibody concentrations and incubation conditions should be determined empirically for each combination of target sample, NeuroMab, secondary antibody and detection system but, as a general guide, NeuroMabs should be used between 0.1 and 10 µg/mL.

Day 2.

4) Wash: transfer sections into fresh ice-cold (4ºC) vehicle and wash a total of four times with gentle rocking/agitation at RT for 5 min each.

5) Secondary antibody incubation: transfer sections into ice-cold (4ºC) vehicle containing fluorescently-conjugated anti-mouse secondary antibodies diluted as per manufacturer's recommendations (*e.g.,* 0.5 µg/mL of Alexa Fluor 488 conjugated anti-mouse IgG1 antibody, Thermo Fisher Scientific catalog # A-21121) and incubate with gentle rocking/agitation protected from light at 4°C for 1 h.

Note: use subclass-specific secondary antibodies matched to the NeuroMab IgG subclass (*i.e.,* IgG1, IgG2a or IgG2b) for optimal signal detection and lowest background (Manning et al., PLoS One 7:e38313, 2012, http://www.ncbi.nlm.nih.gov/pubmed/22675541).

6) Wash: transfer sections into 0.1 M PB, wash twice with gentle rocking/agitation at RT for 5 min each and wash once with 0.05 M PB with gentle rocking/agitation at RT for 5 min.

7) Mounting: transfer sections into 15 mL Petri dish containing 0.05 M PB, carefully mount onto gelatin-coated microscope slides and allow to air dry.

8) Sudan Black B treatment (optional, see below).

9) Coverslipping: pipet anti-fade medium (*e.g.,* ProLong Gold Antifade Mountant, Thermo Fisher Scientific catalog # P36930) on each section according to manufacturer's instructions, place

glass coverslip on top, cure for at least 24 hours and seal edges with nail polish.

Note: tissue autofluorescence can be reduced with an optional Sudan Black B treatment (SBB, Oliveira et al., Histol Histopathol 25:1017, 2010, https://www.ncbi.nlm.nih.gov/pubmed/20552552).

1) Completely rehydrate slide-mounted sections with ddH_20
2) Completely submerge slide in a tray containing 0.05% SBB (recipe below).
3) Incubate with gentle rocking/agitation at RT for 5 min. Sections will become blue.
4) Remove slide from tray and rinse well with ddH_20, paying attention to remove any SBB particulates.
5) Air dry and proceed to coverslipping step.

Recipes:

0.1 M PB: Dilute 500 mL of stock 0.4 M PB (see perfusion protocol for recipe) into ≈1400 mL ddH_20, adjust pH to 7.4 and bring to final volume of 2 L.

Vehicle: 10% normal goat serum and 0.3% Triton X-100 in 0.1 M PB

SBB solution:

1) Prepare stock solution of 1% SBB (Electron Microscopy Sciences catalog # 21610) in 100% ethanol.
2) Vortex or place into a sonicating water batch for 1 h.
3) Vacuum filter SBB through 0.45 µM cellulose acetate, low protein binding membrane (Corning catalog # 430314).
4) Prepare working 0.05% SBB solution. Dilute 2.5 mL SBB stock in 47.5 mL 70% ethanol **immediately** prior to each use in order to minimize particulates. If autofluorescence levels are high, then higher SBB concentrations (up to 0.15%) may be justified but increasing SBB concentrations may also reduce immunofluorescence signal.

Note: stock SBB solution can be reused but must be sonicated for at least 1 h prior to preparing working solution.

Refs: Oliveira et al., Histol Histopathol 25:1017, 2010 (https://www.ncbi.nlm.nih.gov/pubmed/20552552); Manning et al., PLoS One 7:e38313, 2012 (https://www.ncbi.nlm.nih.gov/pubmed/22675541); Bishop et al., J Neurosci 35:14922, 2015 (https://www.ncbi.nlm.nih.gov/pubmed/26538660)