



Immunofluorescence Immunocytochemistry of Cultured Cells

- 1) Cell preparation: rinse cells cultured on coverslips/chamberslides/plates/etc. three times with ice-cold PBS/Ca/Mg (recipe below).
- 2) Cell fixation: add freshly prepared ice-cold 3% formaldehyde fixative (recipe below) and incubate with gentle rocking/agitation at 4°C for 30 min.
- 3) Rinse: remove fixative, rinse three times with PBS-T (recipe below) at room temperature (RT) and collect all solutions containing fixative for proper disposal as per institutional guidelines.
- 4) Block: add Blotto-T (recipe below) and incubate with gentle rocking/agitation at RT for 45 min.
- 5) Primary antibody incubation: add NeuroMab(s) diluted in Blotto-T and incubate with gentle rocking/agitation at RT for 45 min.

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.

- 6) Wash: remove NeuroMab solution, add Blotto-T and incubate with gentle rocking/agitation at RT for 5 min. Repeat washes a total of three times.
- 7) Secondary antibody incubation: add fluorescently-conjugated anti-mouse antibodies diluted in Blotto-T 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 RT for 45 min.

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

- 8) Wash: remove solution, add PBS-T and incubate with gentle rocking/agitation at RT for 5 min. Repeat washes a total of five times.
- 9) Imaging: mount coverslips/chamberslides as appropriate in anti-fade medium (e.g. ProLong Gold Antifade Mountant, Thermo Fisher Scientific catalog # P36930) onto microscope slides, seal with nail polish and visualize under fluorescence microscopy.

Recipes (for plasma membrane surface labeling, omit Triton X-100 from all solutions):

PBS/Ca/Mg: 150 mM NaCl, 1 mM MgCl₂, and 1 mM CaCl₂ in 10 mM Phosphate buffer pH 7.4

Fixative: 3% paraformaldehyde powder and 0.1% Triton X-100 in PBS/Ca/Mg

PBS-T: 150 mM NaCl and 0.1% Triton X-100 in 10 mM Phosphate buffer pH 7.4

TBS-T: 150 mM NaCl and 0.1% Triton X-100 in 20 mM Tris-HCl pH 8.0

Blotto-T: 4% non-fat dry milk in TBS-T