NeuroMab

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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 $MgCl_2$, and 1 mM $CaCl_2$ 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