



### **Immunofluorescence Staining of Cultured Cells**

- 1) Rinse cells cultured on cover slips 3X with ice-cold PBS/Ca/Mg (10 mM Phosphate buffer, pH 7.4; 0.15 M NaCl, 1 mM MgCl<sub>2</sub>/ 1 mM CaCl<sub>2</sub>).
- 2) Add freshly prepared ice-cold 3% paraformaldehyde/0.1% Triton X-100/PBS/Ca/Mg. Incubate at 4°C for 30 min with gentle rocking/agitation.
- 3) Rinse 3 times in PBS + 0.1% Triton X-100 at RT.
- 4) Incubate 30-45 min at RT with gentle rocking/agitation in blotto TBS-T (4% nonfat milk powder in 10 mM Tris, pH 8.0/0.15 M NaCl + 0.1% Triton X-100).
- 5) Add NeuroMab diluted in blotto-TBS-T. NeuroMab dilutions will have to be determined empirically for each combination of target sample and NeuroMab, but as a general guide, NeuroMab tissue culture supernatants should be used at 1:2-1:10, purified NeuroMab IgG's from 1-10 µg/ml. Incubate 45 min- 1 hour at RT with gentle rocking/agitation.
- 6) Wash 3 times 5 min each in blotto TBS-T at RT with gentle rocking/agitation.
- 7) Incubate in fluorescent second antibody diluted in at RT with gentle rocking/agitation 45 min-1 hour at RT with gentle rocking/agitation.
- 8) Wash 3X 5 min each in PBS+ 0.1% TX-100 at RT with gentle rocking/agitation.
- 9) Mount on microscope slides in anti-fade mounting medium, seal with nail polish. View under epifluorescence.