



### Immunoblot Staining

1. Transfer proteins from SDS gel to pure nitrocellulose membrane (0.45  $\mu\text{m}$  pore size, e.g. BioRad Transblot 162-0115)
2. Place blot(s) in 4% non-fat dry milk (blotto\*) in 20 mM Tris-HCl, pH 8.0/ 0.15 M NaCl (TBS). Final pH should be  $\approx 7.5$ . Incubate at RT for 45 min with gentle rocking/agitation.
3. Dilute NeuroMab in TBS-Blotto. NeuroMab dilutions will have to be determined empirically for each combination of target sample, NeuroMab and secondary antibody/detection system, but as a general guide, NeuroMab tissue culture supernatants should be used at 1:2-1:20, purified NeuroMab IgG's from 100 ng/ml to 1  $\mu\text{g}/\text{ml}$ . Place blots in NeuroMab solution, incubate from 45 min at RT (or overnight at 4°C) with gentle rocking/agitation. These incubation conditions can also vary with amount of target antigen on blot, and secondary antibody/detection system. Usually, 45 min at RT is sufficient, but some target sample, NeuroMab and secondary antibody/detection system combinations will need more extensive incubations.
4. After incubation, remove NeuroMab solution (can save at 4°C for repeated reuse after adding sodium azide to 10 mM final) and add a small volume of TBS-Blotto to incubation chamber to rinse out excess antibody. Then add TBS-Blotto, and incubate with gentle rocking/agitation for 10 min. Repeat wash 2X.
5. Add blots to anti-mouse secondary antibody solution. (e.g. Antibodies Inc. 48-146-H, affinity-purified horseradish peroxidase conjugated goat anti-mouse IgG at 1:2000), diluted in TBS-Blotto. Incubate 45 min at RT with gentle rocking/agitation. Can also save and re-use secondary antibody.
6. Rinse blots in TBS or PBS, pH 7.5. Wash 3X 10 min each with gentle rocking/agitation.
7. Develop as per guidelines for your specific detection system (e.g. Renaissance ECL reagent from NEN/Dupont).