UC Davis/NIH NeuroMab Facility
Department of Physiology and Membrane Biology, UC Davis, Davis CA 95616
http://neuromab.ucdavis.edu neuromab@ucdavis.edu

Immunoblot Labeling

1) Blot preparation: transfer proteins from SDS gel to membrane as per manufacturer's instructions (*e.g.*, 0.45 µm pore size pure nitrocellulose, BioRad Transblot catalog # 162-0115).

2) Block: place blot in Blotto (recipe below) and incubate with gentle rocking/agitation at room temperature (RT) for 45 min.

3) Primary antibody incubation: dilute NeuroMab in Blotto, place blot into NeuroMab solution and incubate with gentle rocking/agitation at RT for 45 min 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.

4) Wash: remove NeuroMab solution, add Blotto and incubate with gentle rocking/agitation at RT for 10 min. Repeat washes a total of three times.

5) Secondary antibody incubation: dilute anti-mouse secondary antibody in Blotto as per manufacturer's recommendations (*e.g.*, 0.1 µg/mL of horseradish peroxidase-labeled anti-mouse IgG H+L antibody, KPL catalog # 474-1806), place blot into solution and incubate with gentle rocking/agitation at RT for 45 min.

6) Wash: remove solution, add TBS or PBS pH 7.5 and incubate with gentle rocking/agitation at RT for 10 min. Repeat washes a total of three times.

7) Develop: follow manufacturer's guidelines for selected detection system (*e.g.*, Western Lightning Plus-ECL Substrate, Perkin Elmer catalog # NEL105001EA, and X-ray film).

Recipes:

Tris-buffered saline (TBS): 20 mM Tris-HCl, pH 8.0
0.15 M NaCl
final pH should be ≈7.5

Blotto: 4% non-fat dry milk in TBS pH 7.5 (*e.g.*, 4 g in 100 mL TBS)

Reference: Trimmer 1991 PNAS (http://www.ncbi.nlm.nih.gov/pubmed/1961744)

last updated 20Sept2016 JT