



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 $\mu\text{g}/\text{mL}$.

- 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 $\mu\text{g}/\text{mL}$ 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>)

A cooperative venture between the University of California at Davis, the National Institutes of Health and Antibodies Incorporated

last updated 20Sept2016 JT