

# Validating phospho-specificity of antibodies

1. PVDF membranes (after protein transfer and rehydration of the membrane in 100% methanol followed by water) should be incubated overnight at 37°C in 1% calf intestinal alkaline phosphatase diluted in NE buffer 1 (NaCl 10 mM; Tris 5 mM, pH 7.9; MgCl<sub>2</sub> 1 mM; dithiothreitol 0.1 mM).
  2. Sister membranes should be incubated in the NE buffer only. Rinse three times in TBST.
  3. Block membranes in 5% milk in TBST.
  4. Incubate membranes overnight in primary antibody.
  5. Secondary antibody concentrations and detection protocol should be performed as usual.
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- Pezet S, Spyropoulos A, Williams RJ and McMahon SB (2005). *European Journal of Neuroscience*, 21(7), 1785-97.