WESTERN BLOTTING ANALYSES USING ALKALINE PHOSPHATASE DETECTION

All steps should be done at room temperature with agitation.

1) Block filter for 1 hour in PBS + 5 mg/ml BSA -or- PBS + 2.5 % dry milk/0.2 % TWEEN-20.

2) Incubate with 1° antibody diluted in PBS/BSA -or- 1mg/ml BSA for 1 hour.

3) Wash 3 x 10 min with PBS + .2% TWEEN 20 (or 0.05% NP-40).

4) Incubate 30 min. - 1 hr. with 2° Ab (for Sigma goat anti-mouse or anti-rabbit Abs, dilutions should be 1:1000-1:2000 in PBS + 5 mg/ml BSA or 2.5 % dry milk.

5) Wash 3 x 10 min with PBS + 0.05% TWEEN (or NP-40).

6) **Detect proteins by adding alkaline phosphatase buffer (100 mM tris pH 9.5; 100 mM NaCl; 5 mM $MgCl_2$) containing substrate (for BioRad premixed substrates, use 6.6 µl NBT/ml and 3.3 µl BCIP/ml). HSP70 bands should show up within a couple minutes. Color reactions can be stopped by rinsing with dH_2O.

   **Proteins can also be detected using peroxidase conjugated secondary antibodies. The drawback to this method is that the bands tend to fade over time. If using a peroxidase-conjugated 2°Ab instead of AP conjugated 2°: After post-incubation washes with 2°Ab, develop in 0.05% 45 chloro-1-naphthol, 0.01% H_2O_2 in PBS. Once fully developed, stop with an H_2O wash. Prepare solutions just before use: 10 ml PBS, 2 ml .3% chlororaphtol in MeOH, 4 µl 30% H_2O_2.