Standard Protocol for Western Blots with Alkaline Phosphatase Detection

Important: Some proteins have special requirements for good separation (e.g. unboiled samples or special gel systems). Please refer to the **remarks** sections for western blotting on the respective data sheet.

Solutions needed

- **Ponceau S staining solution:** 5% acetic acid, 0.1% Ponceau S
- **5% skimmed milk-TBST:** 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5% (w/v) skimmed milk powder, 0.02% sodium azide, 0.1% Tween 20
- **Substrat buffer for alkaline phosphatase:** 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$
- **BCIP staining solution:** 20 mg/ml in 100% di-methyl formamide
- **NBT staining solution:** 50 mg/ml in 70% di-methyl formamide
- **Staining solution complete:** Substrate buffer containing 80 µl BCIP solution and 60 µl NBT solution per 10 ml. Prepare this solution shortly before use.

Procedure

Separate the protein sample to be examined and a molecular weight standard using SDS-PAGE and transfer to a nitrocellulose membrane by electro-blotting. Follow the manufacturer's instructions for your SDS-PAGE and blotting device.

1. Stain the membrane with **Ponceau S staining solution** for several minutes at room temperature to check the efficiency of transfer.
2. Rinse the membrane in water to remove the Ponceau S staining solution and incubate in **5% skimmed milk-TBST** for 30 min on a lab shaker at RT.
3. Incubate in fresh **5% skimmed milk-TBST containing the primary antibody** at the appropriate dilution for at least 2 h on a lab shaker at RT or over-night at 4°C.
4. Wash 3 - 4 times with **5% skimmed milk-TBST** for 10 min each time.
5. Incubate with fresh **5% skimmed milk-TBST** containing the recommended AP-conjugated secondary antibody (anti-mouse IgG, anti-rabbit IgG, resp.) at the appropriate dilution for at least 1 h on a lab shaker.
6. Wash 3 times with **5% skimmed milk-TBST** for 10 min each time.
7. Wash with **substrate buffer** and equilibrate for 5 min.
8. Replace with fresh **staining solution complete** and develop for 15 - 30 min. Time can be shortened or extended if signals are extremely strong or weak, resp.
9. Stop staining reaction by washing 3 times with H_2O.

Note: *The SYSY standard protocol generates good results in the SYSY labs and may be used as a reference. However, to achieve the highest specific signal and lowest non-specific background signal, the best antibody concentration, incubation temperature, and incubation time must be determined individually.*