

The server returned a "500 Internal Server Error".

Something is broken. Please let us know what you were doing when this error occurred. We will fix it as soon as possible. Sorry for any inconvenience caused.

Blots with Alkaline Phosphatase Detection

Materials and Reagents

- **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
- **Secondary antibody:** AP-conjugated anti-Guinea pig secondary antibody
- **Substrate buffer for alkaline phosphatase:** 100 mM Tris-HCl, pH 9.5; 100 mM NaCl; 5 mM MgCl₂
- **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. Always prepare this solution **fresh** shortly before use.

Procedure

The protein sample to be examined and a molecular weight standard are separated by SDS PAGE and transferred 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 transfer.
2. Rinse the membrane in water to remove the Ponceau staining and incubate in **5% skimmed milk-TBST** for 30 min on a lab shaker.
3. Replace with fresh **5% skimmed milk-TBST containing the primary antibody** at a 1:1000 dilution and incubate for at least 2 h at RT on a lab shaker or over-night at 4°C.
4. Wash 3 - 4 times with **5% skimmed milk-TBST** for 10 min each time.
5. Replace with fresh **5% skimmed milk-TBST containing AP-conjugated secondary antibody** diluted to the manufacturer's recommended concentration and incubate 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 let 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, respectively.
9. Stop staining reaction by washing 3 times with H₂O.

Note: The SYSY standard protocol generates good staining 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 for each antibody must be individually determined. Please also refer to our general protocols.

Oops! An Error Occurred

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