

## Reference Protocol for Anti-Galectin-3 (HS-477 103) Western 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-rabbit secondary antibody
- Substrat 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 H<sub>2</sub>O.

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.