HistoSure
A SySy brand

Excellent Antibodies for Histopathology

Reference Protocol for Anti-Nucleocapsid Cov-2 (HS-452 011) 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-mouse secondary antibody
- **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. 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.

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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_2O.

***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.*

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