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Product Information

Western Blot Procedure

Preparation Instructions

1× PBS – 10 mM $NaH_2PO_4 \cdot H_2O$ and 130 mM NaCl, pH 7

1× PBST – 1× Phosphate buffer saline with 0.2% (v/v) TWEEN® 20

Blocking Buffer – Add 5 g of non-fat milk to 100 mL of 1× PBST

Procedure

Perform appropriate SDS-PAGE and transfer protein to a PVDF membrane. Keep the membrane at –20 °C no longer than a week, if the membrane will not be used immediately.

1. Add an adequate volume of Blocking Buffer and leave the membrane at room temperature for 1 hour or overnight at 2–8 °C.
2. Remove the membrane from the Blocking Buffer, add the primary antibody diluted in Blocking Buffer to the membrane, and incubate at 2–8 °C overnight.
3. Wash the membrane with 1× PBST for 10 minutes. Repeat the wash 3 times.
4. Add the secondary antibody-HRP conjugate and the membrane at room temperature for 1 hour.
5. Wash the membrane 1× PBST for 10 minutes. Repeat the wash 4 times.
6. After washing the membrane, place it in a sealable bag and add sufficient freshly prepared chemi-luminescent substrate reagents to coat the entire membrane.
7. Take photographs immediately with CCD camera at 5 seconds, 20 seconds, 1.5 minutes, and 5 minutes to obtain proper image.

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