Western Blot

1. Treat the membrane with 99.5% methanol for 15 seconds.
2. Wash the membrane with deionized water for 5 minutes on shaking table.
3. Block the membrane with 5% NFDM/TBST. Swing slightly for 1 hour at RT on shaking table or at 4°C overnight.
4. Wash the membrane with 1 x TBST for 10 minutes.
5. Dilute the primary antibody with 5% NFDM/TBST. Incubate the membrane for 1 hour at RT on shaking table. Swing slightly.
6. Wash the membrane for 3 times with 1 x TBST, 10 minutes per time.
7. Dilute the HRP conjugated goat anti-rabbit IgG secondary antibody (ab97051, abcam) with 5% NFDM/TBST to 1:20,000 (the dilution could be adjusted according to different testing system). Incubate the membrane for 1 hour at RT on shaking table. Swing slightly.
8. Wash the membrane for 3 times with 1 x TBST, 10 minutes per time.
9. Prepare ECL substrate solution
10. (ab133406, abcam). Incubate the membrane for 2 minutes at RT.
11. Remove excess ECL substrate and place the membrane in transparent plastic wrap.
12. Signal development using darkroom development technique.

Solution preparation

10 x TBS: Dissolve 1314.9g of Sodium chloride and 545.3g of Tris in deionized water, then adjust the pH value to 7.4 by diluted hydrochloric acid. Finally dilute with deionized water to 22.5L.

1 x TBST: Add 2.25L of 10 x TBS and 22.5ml of Tween-20 to deionized water to 22.5L.