# Western Blot Protocols

### Western blot analysis

1. Run SDS-PAGE gel, and then Western transfer the protein samples to nitrocellulose (NC) membrane for immunoblot analysis.
2. After transfer, transfer the membrane to western-blot tray, briefly wash the NC membrane with distilled water.
3. (Optional) Visualize the proteins on the membrane by Ponceau's staining.
4. Wash off the red stain with distilled water.
5. Block the membrane with 5-10ml blocking buffer (made by 5% non-fat milk in 1xPBST) for 30 minutes at R/T.
6. Dilute the primary antibody with blocking buffer according to the suggested dilution factor on datasheet.
7. Remove the blocking buffer and add enough diluted primary antibody to cover the membrane.
8. Incubate the membrane with primary antibody for 1hr at R/T. (Note: Or you can do overnight incubation at 4C, make sure you cover the western-blot tray to prevent excessive evaporation). To prevent uneven coverage, the western-blot tray can be rocked on a rocker platform.
9. Collect the primary antibody and store them at 4C for up to two weeks. (If you would like to store them longer, you can freeze the diluted antibody at –20C. Remember frequent freezing and thawing will gradually decrease the antibody titer.)
10. Briefly wash the membrane with 1xPBST once to remove any excessive primary antibody.
11. Add enough 1xPBST to cover the membrane and leave the Western-blotting tray on a rocker platform.
12. Wash the membrane for 15 minutes. (Note: If the background is high, repeat this step for two to three times.), turn on the developer during the wash time.
13. Dilute HRP-conjugated secondary antibody with blocking buffer (1:1000 or higher dilution is usually good for Goat anti-mouse-HRP; TA130003).
14. Incubate the membrane with secondary antibody for 30 minutes to 1hr.
15. Wash the membrane with 1xPBST for 15 minutes, and then 3 times (5 min/time).
16. Prepare the chemiluminescence development substrate mixture by mixing equal amount of solution1 and 2 (TA100016; Normally 1ml will be enough for one membrane).
17. Prepare a plastic saran film, lay the film on a flat surface, and dispense 1ml of substrate mixture for one membrane on the plastic saran film.
18. Use a forceps to take washed the blot from the western-blotting tray, flip it, lay on the substrate mixture, and then incubate for 1 to 5 minutes. (Note: To avoid air bubbles, always lay the blot by touching one edge first.)
19. Remove excess Chemiluminescence Reagent and wrap the membrane in plastic. Place inside X-ray cassette.
20. Expose to film and develop

### Buffer preparation

1xPBS: This buffer is made by dissolving 8g of NaCl, 0.2g of KCl, 1.44g of Na2HPO4 and 0.24g of KH2PO4 into 800ml of distilled water. Then adjust the pH to 7.4 with HCl, and add H2O to 1 liter.

1xPBST: 0.05% Tween 20 in 1xPBS

### Reference

Sambrook, Fritsch, and Maniatis (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, volume 3, apendix B.12