PROTOCOL FOR WESTERN BLOTTING

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1. First, run the samples in SDS-PAGE.
2. Fetch and mark the membrane (right size) with pencil; then soak it in MeOH and wash it under tap water.
3. Equilibrate the membrane, pads and transfer foam in transfer buffer (store in 4°C) for 5 minutes.
4. Assemble transfer sandwich like as follows and put it into transfer tank.
5. Run: 250mA and 70 minutes in cold room.
6. Blocking: block the membrane in cold room (rocking) with blocking solution (5-10% milk-TSBT) @ 4°C O.N. or @ RT 2hrs.
7. Trim the membrane as small as possible.
8. Prep first antibody solution: Calculate the total volume needed and prepare it (dilute the primary antibody with blocking solution (1:500-1:1000)).
9. Place the membrane into the 1^{st} antibody solution and rock it for 1--2 hours @ RT.
10. Wash the membrane with TSBT 5 minutes X 3 (rocking).
11. Prepare 2^{nd} antibody solution with TSBT (1:15,000) and put the membrane in it for 20-30 minutes (rocking).
12. Wash it with TSBT 5 minutes X 3.
13. Mix the ECL Dection Reagents 1 and 2 (1:1 volume).
14. In dark room, add the mix directly to the membrane and incubate for 60 seconds and drain off with paper.
15. Wrap the membrane and put the fluorescent marker on the membrane.
16. Place them into a film cassette and expose to X-ray film for 10 or 30 seconds.
17. Let the Development System treat the film.

Or

14a. Let the membrane soak in the solution for 60 seconds and expose it by Imagestation.

TBST (Tris-Buffered Saline-Tween-20):

10mM Tris-HCl (pH8.0)
150mM NaCl
0.05% Tween-20 (after all and water are mixed, add tween-20)

Transfer Buffer:

800ml Methanol
12.12g Tris
57.63g Glycine ----------4L