Transfer and Western Blotting

Reagents:
1x Transfer Buffer… See the recipe.
TBS-T…TBS with 0.2 % Tween 20
Blotting Buffer… 5 % milk in TBS-T

1) While you are running SDS-PAGE, prepare a membrane (PVDF). (Cut the membrane for the gel size. Soak it in Ethanol, and then soak it in 1x Transfer Buffer and shake the container slowly for 10 to 30 min . Prepare Bio-Rad paper, 2 pieces for 1 gel. (Or, 6 pieces of 3MM paper for 1 gel.)

2) Take the gel sandwich out of Bio-Rad Mini-PROTEAN 3 Cell system. Take apart the gel sandwich. Remove the Upper Gel. Soak the Lower Gel in 1x Transfer Buffer.

3) Prepare Transfer Sandwich in a transfer cassette.

(-) Minus (Black)

Sponge
3MM Paper
Gel
Membrane
3MM Paper
Sponge

(+) Plus (Clear)

When you place the gel on top of the membrane, mark the Marker bands with black ballpoint pen. Remove all bubbles between the membrane and the gel. Set the cassette to Bio-Rad Mini Trans-Blot system. Place an ice chamber inside. Fill the transfer tank with 1x Transfer Buffer.

4) Put the lid on. Start transferring.
100V 0.35A for 1-2 hour
*Transfer can be performed with lower voltage for O/N.

5) Blocking
Take the membrane out. Wash it with TBS shortly. Soak the membrane in the blotting buffer for 30 min at RT (room temperature). Do not dry the membrane from now on.
*Blocking can be performed in the cold room for more than 1 hour to O/N.

6) Put the membrane in a container with the 1st antibody in Blotting Buffer with 0.02% Sodium Azide for O/N on the shaker at 4˚C. Each 1st antibody has different optimal concentration. Check the anti-body you are going to use.
*The 1st antibody reaction can be done at RT for 2-3 hour but 4˚C-O/N is recommended.

7) Wash the membrane in TBS-T shortly. And then wash the membrane in TBS-T for 10 min x3 times.

8) Put the membrane in a container with the 2nd anti-body (1/5000) in Blotting Buffer for 30 to 60 min on the shaker at RT.

9) Wash the membrane in TBS-T for 5 min x6 times.

10) Prepare Super Signal mix solution. Mix two kinds of solution at 1:1.

11) Soak the membrane into Super Signal mix solution for about 1 min. Place the membrane in the development clear file. Place the file in the film cassette. Take it to the dark room with X-ray film.

12) Turn the light off, and then open the X-ray film box and take one. Put a film on top of the development clear file. Close the cassette. Expose your protein to the film. Develop the film with developer. Never open the X-ray film box under the light.

Common antibodies:

1st anti-body	1st anti-body conc.	2nd anti-body	2nd anti-body conc.
α myc (9E10)	1/1000	α mouse IgG	1/5000
α FLAG (M2)	1/5000	α mouse IgG	1/5000
PAP	1/1000	α rabbit IgG	1/5000