Western Blot

1. Thaw sample on the ice.
2. Turn on the heater at 95°C.
3. Make gel with cassette(invitrogen) and prepare samples.

1) Separating gel: 7.5% polymerase gel with 4% shocker. Receipt:

dd-water 4.14 ml
Tris HCL 1.875 ml PH=8.8
20% SDS 37.5 ul
A/Bis 1.405 ml
10% APS 37.5 ul
TEMED 5 ul

Pipette the gel into cassette until it reaches 2nd line.
Add water sol. Bustinol (not too much) to prevent evaporation.
Wait 30 min

2) Receipt: tissue, lysis buffer (stored at -20°C), loading dye (stored at -20°C)
Volume: 25 ul, Protein: 50 ug
Then, add 1/20 volume β-ME
Centrifuge for 1min, heat 5 min or more at 95°C, stored at RT.

3) Discard the Water sol. Bastinol, rinse thoroughly with dd- water, wipe off.
Loading gel: 4% polymerase gel. Receipt:

dd-water 1.92 ml (RT)
Tris HCL 0.75 ml (RT) PH=6.8
20% SDS 15 ul (RT)
A/Bis 0.3 ml (4°C)
10% APS 15 ul (-20 °C)
TEMED 2 ul (RT)

4) Move the comb and rinse thoroughly.

4. Load 1* SDS Elution buffer between the gels until it reaches the first line.
Receipt: 10 * SDS elution buffer stock
30.3 g Tris base (0.25M)
144 g Glycine (1.92M)
1.0 g SDS
Dilute to 1000 ml with dd –water
Stored at 4 °C
1:10 diluted in dd-water when used.
5. Load the marker(ladder) and samples.
6. Load 1* SDS Elution buffer outside the gels until the samples were immersed
7. Run the gel 75V 5~10 min until blue dye pass the first line
120 V 1.5 hr -> 2.5 hr until blue dye is approximately 0.5-1 mm above the bottom.
Note: if the blue dye is not straight, the gel does not perfectly polymerase
8. Transfer the protein to membrane.

Pre-wet the sponge and filter paper, membrane with ½ * Transfer buffer stock.

------------------------- Sponge *2 (blotting pad)
------------------------- Filter paper
------------------------- Transfer membrane

------------------------- Gel
------------------------- Filter paper
-------------------------- Sponge *2 (blotting pad)

Put a sponge between them if transferring two membrane at the same time.
And run overnight at 15 V in cold room.

Receipt: 10 X transfer buffer
30.3 g Tris Base (0.25 M)
144 g Glycine (1.92 M)
Diluted to 1000 ml with dd-water PH=8.2-8.3. stored at 4°C

½ * Transfer buffer
50 ml 10* transfer buffer
50 ml Methanon (RT Hood)
900 ml water Stored at 4°C

9. remove the membrane, rinse with TBST (Tris –buffered Tween-20)

Receipt: TBST
100 ml TBS 10X
900 ml dd-water
500 ul tween-20 (hood)

10X TBS (Tris buffered saline)
24.2 g Trizma (0.2m)
80 g sodium chloride (1.37M)
PH to 7.6 using 6M HCL
Diluted to 1000 ml with dd-water stored at RT

10. Wash membrane in TBST
11. wash membrane in TBSTM 1 hour to block.

Recipe: Tris buffered saline tween -20 w milk
10 g dry milk
20 ml 10X TBS
100 ul Tween- 20
180 ml dd H_2O

12. Incubated in Primary antibody 1 hour
13. wash membrane in TBST 4 times. 5 min/ each
14. Incubated in secondary antibody 2 hours
15. Wash membrane with TBST 4 times. 5min/each
16. Turn on film developer (standby), prepare ECL reagent. (5ml, 5ml)
17. Expose for 2 min – 5 min, and run