Electrophoresis and Western blot

I. Electrophoresis

Recipe of making SDS-PAGE

SDS-PAGE		10%	12%	15%	18%
Running Gel 20 ml For 4x1mm plates	4x buffer pH8.8 (ml)	5	5	5	5
	40% Acr-Bis (ml)	5	6	7.5	9
	ddH_2O (ml)	10	9	7.5	6
	10% APS (μl)	100	100	100	100
	TEMED (μl)	20	20	20	20
		4%			
Stacking Gel 10 ml For 6-8 Plates	4x buffer pH6.8 (ml)	2.5			
	40% Acr-Bis (ml)	1			
	ddH_2O (ml)	6.5			
	10% APS (μl)	100			
	TEMED (μl)	20			

	Voltage	**Time**	**Buffer**	**Volume**
Electrophoresis	150V/200V (15/30 mA)	1 h (for 10%)	Tris/Glycine/SDS	> 300 ml tank
Semi-Dry Transfer	5V (40-60 mA/gel)	1-2 h	20% Methanol 1x SDS running buffer	Make 500 ml for 4 gels
Agarose	100V	30min-1h	10 μl EB to 100 ml agarose/TAE	350 ml tank

1x Transfer Buffer

(20% Methanol; 25 mM Tris, 192 mM glycine, pH8.3; with or without 0.1% SDS)

500 ml
50 ml of 10x Transfer buffer (without SDS) or 10x SDS-PAGE running buffer (w/ SDS)
100 ml of Methanol (final **20% methanol**)
350 ml ddH2O

1x TBS-T (1x)

(150 mM NaCl, 10 mM Tris pH8.0; 0.1% Tween 20)

1L
100 ml 10x TBS
10 ml 10% Tween20 (final 0.1% v/v)
890 ml ddH2O

Blocking buffer

(5% Nonfat milk in TBS-T)
5g milk in 100 ml TBST

II. Transfer

1. While eletrophoresis, Cut the membrane to the dimensions of the gel. Pre-wet PVDF membrane should in 100% Methanol for few second, and equilibrate in transfer buffer for 10 minutes before blotting. Completely saturate the filter paper by soaking in transfer buffer.
2. After electrophresis, equilibrate gel in 1x Transfer buffer for 10 -15 min.
3. Setup the transfer sandwich 3 pre-soaked filter paper, PVDF membrane, Gel, and 3 filter papers. Roll a pipet or test tube over the surface of the filter paper to exclude all air bubbles.
4. Set up the transfer condition: 20V for 1 hr (or 5-10V 2 hr, no more than 25V) with SDS in transfer buffer.

III. Blot

5. Following transfer, rinse the membrane with TBST. Block in 100 ml 5% non-fat dried milk in TBST for 30 min – 2 h at R.T. (or O/N at 4^{o}C).
6. Briefly rinse the membrane. Dilute the primary antibody in 10 ml TBST (for 1-2 membrane). Shake at R.T for 1-3 hr. (or O/N at 4^{o}C.)
7. Briefly rinse the membrane with TBST, and then wash the membrane for 3 × 10 min (or 4x 15 min) at room temperature.
8. Dilute the HRP labeled secondary antibody in 15 ml. Incubate the membrane for 30 min at room temperature on an orbital shaker.
9. Rinse, and wash for 3x10 min or 4x15 min.

IV. Detection

10. Mix an equal volume of detection solution 1 with detection solution 2 (1 ml each solution for each mini membrane. The final volume required is 0.125 ml/cm2 membrane.)
11. Drain the excess wash buffer from the washed membranes and place them, protein side up, on a sheet of SaranWrap™ or other suitable clean surface.
12. Pipette the mixed detection reagent on to the membrane. Incubate for 1 minute at room temperature.
13. Drain off excess detection reagent by holding the membrane gently with forceps and touching the edge against a tissue. Place the blots protein side down on to a fresh piece of SaranWrap, wrap up the blots and gently smooth out any air bubbles.
14. Place the wrapped blots, protein side up, in an X-ray film cassette.
15. Exposure for 5-10sec, 30 sec, 1 min, 5-0 min each.

Secondary antibody:
Donkey anti-Goat (Jackson) 1:20,000-25,000