WESTERN BLOT

(Ray Chan, adapted from CPMB for immunoprobing 10.8.7)
Last modified 5/26/06

1. Run protein samples out on SDS-PAGE. Generally the limit of detection for western blot is around 5-20 ng.

   1X loading buffer: 0.1 M Tris HCl pH 6.8
   2% SDS
   7.5 M Urea
   BpB

2. Remove gel from plates. Trim gel, if necessary, nick the upper corner of the gel over the marker lane.

3. Cut two pieces of Whatman 3MM paper to the exact dimensions of gel and cut one piece of nitrocellulose paper to 2-5 mm larger than the gel in each dimension.

4. Soak gel, sponge pads, blotting paper, and nitrocellulose filter in transfer buffer for 1-15 minutes.

5. Assemble the components in the following order

   pad
   blotting paper
   nitrocellulose
   gel
   blotting paper
   pad

   Remove bubbles in between layers by gently rolling a Pasteur pipet over the nitrocellulose and the last piece of blotting paper.

6. Place the assembled cassette into the transfer unit such that the nitrocellulose is facing the anode (the red terminal). Transfer protein at approximately 20 V overnight for proteins > 100 kD or 90-100 V for .5-1 hour for smaller proteins (at 4 °C)

7. Carefully remove the gel from the nitrocellulose membrane.

8. Stain memberane with Ponceau S (dissolve .3 g Ponceau S in 1 mL glacial acetic acid and add to 100 mL nH_2O) for 1 min. Destain for 2 min. in H_20.

9. Note the lane and the MW marker positions with a pencil. Destain completely in TTBS in about 5 minutes.

10. Block membrane in 5 mL TTBS + 5% dry milk for .5-1 hr. at room temp. Place on rocker.

11. Pour off blocking buffer and add diluted 1° antibodies in TTBS or blocking solution. Blot for 1-3 hr. at room temp or overnight at 4 °C.

If using serum, dilute 1:200-1:500.
If using affinity purified antibodies, use 1:500-1:1000.

12. Wash 4 x in 100 mL TTBS at room temp. for 5-10 min. each time.

13. Blot with 2° antibodies in TTBS or blocking solution for .5-1 hr. at room temp or overnight at 4 °C. Generally HRP-conjugated antibodies are diluted 1:5000.

14. Wash □ 4 x in 100 mL TTBS at room temp. for 5-10 min each time.

15. Mix equal volumes of Amersham ECL solutions 1 and 2. For mini-blots, 700 □L each should be sufficient.

16. Place membrane on solution, protein-side down facing solution. Leave for 1 min, flip over, blot dry with paper towel.

17. Expose to film in the dark room for 15, 30, and 60 seconds initially and do longer exposures if necessary.

Stripping a membrane for re-blotting:

1. Incubate in stripping buffer [100 mM Tris-Cl, pH 6.8; 100 mM □-mercaptoethanol; 2% SDS] for 1 hr at 50 °C; preferably in a rocking water bath.

2. Wash 3 x 10 min. in TTBS. For long term storage, keep TBS + 0.02% NaN_3.

Nitrocellulose membrane: Schleicher & Schuell; 0.45 □m pore size (BA85)
Enhanced chemiluminence: Amersham ECL Kit

Tris buffered saline (TBS): 100 mM Tris-Cl, pH 7.5; 0.9% (0.156 M) NaCl
100 mL 1M Tris pH 7.5
31.2 mL 5M NaCl
$H_2O$to 1L

TTBS: TBS + 0.05% Tween-20 (0.5 mL Tween to 1 L)
Blocking solution: TTBS + 5% dry milk
Transfer buffer: 48 mM Tris base; 39 mM glycine; 0.037% SDS; 20% methanol
1L (make fresh)
5.83 g Tris base
2.91 g glycine
1.85 mL 20% SDS, H_2O to 800 mL
200 mL MeOH

5 x SDS/electrophoresis buffer: 125 mM Tris base; 0.95 M glycine; 0.5% SDS