WESTERN BLOTTING PROTOCOL

Equipment

- PAGE tanks, powerpacks, PAGE running buffer and gels
- PVDF (or nitrocellulose membrane)
- Blotting transfer apparatus
- Shaker table
- For ECL detection: Imager capable of detecting chemiluminescence
- For colour staining using hydrogen peroxidase or alkaline phosphatase conjugated secondary antibody detection a digital camera or imager is desirable.

Reagents and buffers

- Pre-stained molecular weight markers
- Methanol
- Dehydrated milk protein (Marvel from the supermarket, or Dried bovine milk powder (M7409 SigmaAldrich)
- Tris buffered saline (TBS) : 20mM Tris, 0.15M NaCl, pH7.5
- Block solution: 5% milk protein in Tris buffered saline
- Wash solution: 0.5% milk protein, 0.05% Tween20 in Tris buffered saline
- Primary and secondary antibodies
- For ECL detection
    - ECL reagent kit (e.g 32209 or 34577 from Thermo Scientific)
- For 2^{o} antibody conjugated with hydrogen peroxidase
    - Chloronapthol tablets (C6788 Sigma Aldrich or 34011 Thermo Scientific)
    - Ethanol
    - 30% Hydrogen peroxide solution
- For 2^{o} antibody conjugated with alkaline phosphatase
    - 1-Step™ NBT/BCIP Substrate Solution (34042 Thermo Scientific)

Method

1. Run PAGE as required
2. Transfer gel into deionised or MilliQ water (do not stain)
3. If using PVDF membrane pre-wet with methanol (if using nitrocellulose, should be kept dry)
4. Set up blotting transfer apparatus as per manufacturers instructions
5. Remove PVDF or nitrocellulose membrane and confirm successful transfer by observing that the pre-stained molecular weight markers have transferred to the membrane.
6. Block step: To block non specific sites on the membrane, cover membrane with block solution and Incubate for 1 hour at ambient temperature with gentle shaking.
7. Wash step 1: Wash membrane with wash solution. 10 minutes at ambient temperature with gentle shaking.
8. Primary antibody: Make up a solution of primary antibody at recommended dilution in wash solution. Usual dilutions are between 1:1000 and 1:10,000.
9. Cover membrane with primary antibody solution and Incubate for 1 hour at ambient temperature with gentle shaking.
10. Wash step 2: Wash membrane three times with wash solution. 10 minutes each at ambient temperature with gentle shaking.
11. Secondary antibody: Make up a solution of secondary antibody at recommended dilution in wash solution. Usual dilutions are between 1:1000 and 1:10,000.
12. Cover membrane with secondary antibody solution and Incubate for 1 hour at ambient temperature with gentle shaking.
13. Wash step 3: Wash membrane three times with wash solution. 10 minutes each at ambient temperature with gentle shaking.

Options for developing the blot

Either 14. Develop blot: ECL protocol

a. Wash blot briefly with deionised or MilliQ water
b. Follow ECL kit manufacturers protocol to incubate membrane with development solutions.
c. Image gel using chemiluminescence settings on imager

Or 14. Develop blot: Colourimetric detection with hydrogen peroxidase conjugated 2° Ab

a. Dissolve 1 chloronapthol tablet in 20mL of ice cold 100% ethanol
b. Add to 50mL of TBS just prior to use

c. Add 100uL of H_2O_2
d. Cover membrane in completed developing solution and incubate at ambient temperature with gentle shaking until colour develops
e. Image / photograph membrane when blot is developed.

Or 14. Develop blot: Colourimetric detection with alkaline phosphatase conjugated 2° Ab

a. Cover membrane in NBT/BCIP substrate solution as per manufactures instructions and incubate at ambient temperature with gentle shaking until colour develops
b. Image / photograph membrane when blot is developed.