Western Blot Protocol

MATERIALS

• ImmunoBlot
• Filter Paper (Whatman)
• Tweezers
• X-ray film
• X-ray film processor
• Pipette tips
• Pipettor, small volumes

BUFFERS

Blocking Buffer

• 5% non-fat dry milk
• TBST

Wash Buffer (TBST)

• 125 mM NaCl
• 25 mM Tris pH 8.0
• 0.1% Tween-20

Rehydration

1. Soak the blot in blocking buffer for 30 minutes prior to use.

Blocking

2. Incubate the blot with blocking buffer overnight at 4°C or 2 hours at room temperature with gentle agitation.
3. Remove blot from blocking solution.

Primary Antibody Incubation

4. Dilute antibody to the recommended dilution in 10mL of blocking buffer.
5. Incubate the blot with the primary antibody for one (1) hour at room temperature or overnight at 4°C.
6. Wash the blot three (3) times 10 minutes each in washing buffer with gentle agitation.

Secondary Antibody Incubation

7. Dilute 1µL anti-rabbit IgG-HRP conjugated secondary (or other appropriate secondary) in 10mL of blocking buffer to make a 1:10000 dilution
a. *Note*: working dilution of secondary can vary from 1:2000 to 1:10000.

8. Incubate blot with secondary antibody for one (1) hour at room temperature.
9. Wash three (3) times for 10 minutes each in washing buffer with gentle agitation.

Development

10. Drain wash buffer
11. Add ECL solution (Amersham) per manufacturer directions and develop for 1 minute.

12. Drain the fluid.
13. Cover the blot in plastic wrap.
14. Expose the blot to X-ray film for 1 minute in a dark room.
a. If there is no banding, expose the film for 5 minutes, then 30 minutes and up to overnight if the signal is weak.
b. If the signal is strong, expose the film for 30 seconds or less.
15. Develop the film in an X-ray processor

Notes

16. Optimal dilutions should be determined by each laboratory for each antibody.

Reprobing

17. Incubate blot for 10 minutes at room temperature in 100mM Glycine, pH 2.5.
18. Wash for 10 minutes in DI H_2O.
19. Redo protocol above.