**ENHANCED CHEMILUMINESCENCE (ECL) WESTERN**

### Reagents
- Blotto (blocking reagent)
- 2.5mg/ml BSA/1xPBS
- 1xPBS/0.1% Tween 20
- primary antibody
- secondary antibody
- ECL (Amersham RPN 2106)

### Materials
- nutator or platform shaker
- Kodak XAR-5 Xray film
- Exposure cassette
- Xomat film developer

1. Immediately after transfer, place nitrocellulose filter in a small container.
2. Block filter in Blotto for 1 hr on shaker at room temperature (or O/N at 4°C). Add enough Blotto to cover filter completely.
3. Pour off Blotto and rinse filter in 1xPBS/0.1% Tween-20 3x 5 min (on shaker).
4. Dilute the primary antibody to the recommended dilution in 1xPBS/2.5 mg/ml BSA. Incubate the filter with primary antibody for 1 hour at room temp. Save primary antibody by pouring back in conical tube. Rinse filter in 1xPBS/0.1% Tween-20 3x 5 min at room temp.
5. Add the secondary antibody. Dilute the horse radish peroxidase (HRP)-conjugated secondary antibody to the recommended dilution in Blotto. Incubate with secondary antibody for 30’min-1hr. at room temp on the shaker.
6. Discard secondary antibody and rinse filter in 1xPBS/0.1% Tween-20 3x 5 min at room temp. The tween concentration is very important. Insufficient washing results in very high backgrounds. In addition, different antibodies have different washing requirements, but the ones given here are good as a general rule of thumb.
7. Mix ECL reagent #1 with an equal volume of reagent #2 using a sufficient amount such that the blot is completely covered. Incubate for 1 min. Remove blot, dab dry, wrap in Saran wrap, and expose to x-ray film. A variety of exposures may be necessary to get the optimal exposures. If the background is high, try rewashing with 1xPBS/0.3% tween.

### Blotto Blocking solution
- 500ml:
  - 12.5g non-fat dried milk
  - 25ml 20xPBS(pH 7.4)
  - 475 ml dH2O
  - -store at 4°C for ~2 weeks

### 1xPBS/2.5mg/ml BSA
- 500ml:
  - 1.25 g BSA (Sigma A-8022)
  - 25ml 20xPBS(pH7.4)
  - 475 ml dH2O
  - -filter sterilize and store at 4°C.
  - -It is useful to add NaN3 to 0.02% to keep from contamination.

### 1xPBS/0.1% Tween 20
- 500ml:
  - 0.5 ml Tween-20
  - 25ml 20xPBS (pH7.4)
  - 475 ml dH2O
  - store at room temp.
Stripping and Reprobing Blots

1. Wash blots 2 x 5 min with PBS/0.1% Tween.
2. Incubate for 30 min at 50°C in blot stripping solution: 2% SDS, 100mM β-mercaptoethanol and 63 mM tris pH 6.8.
3. Wash 3 x 5 min with PBS/0.1% Tween
4. Repeat procedure above.