ENHANCED CHEMILUMINESCENCE (ECL) WESTERN

Reagents	**Materials**
Blotto (blocking reagent)	nutator or platform shaker
2.5mg/ml BSA/1xPBS	
1xPBS/0.1% Tween 20	Kodak XAR-5 Xray film
primary antibody	
secondary antibody	Exposure cassette
ECL (Amersham RPN 2106)	
	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	1xPBS/2.5mg/ml BSA	1xPBS/0.1% Tween 20
500ml:	500ml:	500ml:
12.5g non-fat dried milk	1.25 g BSA (Sigma A-8022)	0.5 ml Tween-20
25ml 20xPBS(pH7.4)	25ml 20xPBS(pH7.4)	25ml 20xPBS (pH7.4)
475 ml dH_20	475 ml dH_20	475 ml dH_20
-store at 4°C for ~2 weeks	-filter sterilize and store at 4°C.	store at room temp.
	-It is useful to add NaN_3 to 0.02% to keep from contamination.	

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 ß-mercaptoethanol and 63 mM tris pH 6.8.
3. Wash 3 x 5 min with PBS/0.1% Tween
4. Repeat procedure above.