

## Western stains using home-made ECL chemiluminescent reagents

### Reagents:

1 M Tris pH 8.5

p-Coumaric acid, MW 164.2, Sigma cat # C-9008, 25 g for \$29.00

3-Aminophthalhydrazide, MW 177.16, Fluka cat # 09253, 25 g for \$70.00 (luminol)

H<sub>2</sub>O<sub>2</sub>, c.g. Fluka cat # 95300, 500 ml (30% solution)

HRP conjugated anti-mouse or anti-rabbit; or prot-A-HRP fro IP-westerns

### Stock solutions:

250 mM luminol (0.44 g / 10 ml) in DMSO, make 0.2 ml aliquots and store at -20°C

90 mM coumaric acid (0.15g/10 ml) in DMSO, 0.088 ml aliquots and store at -20°C

Prepare solutions 1 and 2 fresh, and mix 20 ml + 20 ml just before detection:

#### Solution 1 (20 ml)

17.7 ml	H <sub>2</sub> O		11.6
200 μl	250 mM luminol		133.3
88 μl	90 mM p-coumaric acid		58.7
2 ml	1 M tris pH 8.5		1.33

#### Solution 2 (20 ml):

18 ml	H <sub>2</sub> O		12
12 μl	30% H <sub>2</sub> O <sub>2</sub>		8
2 ml	1 M tris pH 8.5		1.33

Incubate blot (washed after 2nd antibody) in 20-40 ml of freshly mixed solns 1+2, incubate for 30 sec to 1 min, blot dry on 3 MM paper, wrap in saran wrap (careful to limit bubbles) and expose (for several lengths of time)