

Typed 30 June 2007

Home-made ECL Reagents:

Stock Solutions:

- 1) 250 mM luminol (Sigma A8511)
- (0.44 g dissolved in 10mL DMSO)
 - 2) 90 mM p-Coumaric acid (Sigma C9008)
- (0.15 g dissolved in 10 mL DMSO)
- Store at 0°C or at -20°C

Both chemicals are on the alphabetical shelf in room 5069.

Solution A:

1 mL Luminol stock solution
0.44 mL p-coumaric acid stock solution
10 mL 1M Tris-HCl, pH 8.5
Make up to 100 mL final volume with H₂O

Store at 4°C

Solution B:

10 mL 1M Tris-HCl pH 8.5
61 µL 30% H₂O₂
Make up to 100 mL final volume with H₂O.

Store at 4°C

Use of Reagents:

To use, mix solns. A and B 1:1, immediately incubate blot for 30 sec., drain off excess reagent and expose to film immediately.

Note: (JLM) I find that for heme-staining, a two-to-three minute exposure will blow out the signal from 6µg of chlorophyll of a -Cu wild-type soluble protein sample. A subsequent 10-12 minute exposure will produce roughly the same signal cyt c6 intensity, but background is much higher. This means that signal decays rapidly with time.