Home-made ECL Reagents:

Stock Solutions:

1) 250 mM luminol (Sigma A8511)  
   - (0.44 g dissolved in 10 mL DMSO)
2) 90 mM p-Coumaric acid (Sigma C9008)  
   - (0.15 g dissolved in 10 mL DMSO)

Store at 0ºC or at -20ºC

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 4ºC

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 4ºC

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.