

Enhanced ECL formulation is taken from C. Haan, I. Behrmann. (2007) *J Immunol Methods* **318**:11-19.

1. Per 1 ml 100mM Tris pH 8.8 add:
 - 5 μ l 250mM luminol (dissolve in DMSO and store in a foil-wrapped tube at room temperature)
 - 22.2 μ l 90mM 4-iodophenylboronic acid (dissolve in DMSO and store in a foil-wrapped tube at room temperature)
 - 0.54 μ l 30% H₂O₂ (add last, right before use)
2. Add ECL to the PVDF membrane and expose immediately

Notes:

- The luminol and 4IPBA stocks are stable for long periods of time at room temperature.
- 4IPBA is a weaker, but more controlled, enhancer of chemiluminescence than p-coumaric acid. Longer exposures may be required, but background chemiluminescence is substantially reduced, which leads to crisper blots and greater sensitivity for detecting quantitative differences in band intensity.
- On our CCD camera (Alpha Innotech), 5 min is the maximum exposure before camera noise starts to overtake the chemiluminescence signal