Schwer Lab

Super-ECL Solution
(modified from Kricka et al., Analytical Biochemistry 1996 and Haan and Behrmann, J Immunol Methods 2007)

REAGENTS

250 mM Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) solution
Sigma #A8511-5G
MW 177.16. Store powder at RT in the dark. Stable for years.
Dissolve 1 g Luminol in 22.57 mL DMSO. Vortex. Prepare 0.5-mL aliquots and store at -20°C in the dark.

100 mM 4-iodophenylboronic acid (4IPBA) solution
Sigma # 471933-5G
MW 247.83.
Dissolve 1 g 4IPBA in 40.35 mL DMSO. Vortex. Prepare 1-mL aliquots and store at -20°C in the dark.

30% hydrogen peroxide
Sigma #H1009-100ML

1 M Tris-HCl, pH 8.8

PROTOCOL

Solution S-A [2.5 mM luminol, 4 mM 4IPBA, 100 mM Tris-HCl, pH 8.8]
To 25 mL MilliQ water add:
0.4 mL 250 mM Luminol
1.6 mL 100 mM 4IPBA
4 mL Tris-HCl 1M, pH 8.8
Add MilliQ water to a final volume of 40 mL. Wrap with aluminum foil. Store at 4°C in the dark.

Solution S-B [10.6 mM H₂O₂, 100 mM Tris-HCl, pH 8.8]
To 25 mL MilliQ water add:
43.3 µL 30% (9.8 M) hydrogen peroxide solution
4 mL 1 M Tris-HCl, pH 8.8
Add MilliQ water to a final volume of 40 mL. Wrap with aluminum foil. Store at 4°C in the dark.

Prepare Solution S-A and S-B fresh after 1 month of storage.

How to develop blots: per standard 8 x 10 inch membrane, mix 1 mL Solution S-A with 1 mL Solution S-B in a 15-mL conical tube. Vortex briefly. Cover membrane with 1 mL of S-A/S-B solution. Incubate at RT for 1 min. Blot off excess solution. Expose to film.