Home-made ECL substrate for Western blot imaging

1. Background and purpose of the procedure

Western blot (immunoblot) is a powerful technique to visualize low amount of proteins. Visualizing proteins involve the use of chromogenic substrates such as DAB or enhanced chemiluminescence (ECL). Several ECL substrate kits are commercialized but these kits are expensive and with limited shelf live. This procedure summarizes an alternative to commercially available ECL kits using home-made solutions. This ECL substrate remains very sensitive when compared to commercially available kits.

2. Materials and equipment

- Blot membrane (nitrocellulose or PVDF), probed with HRP-linked antibody
- Luminol stock solution (*see 3. Recipes*)
- p-Coumaric acid stock solution (*see 3. Recipes*)
- 30% Hydrogen peroxide (Fisher Chemical, Certified ACS, H325-500)
- ECL buffer (*see 3. Recipes*)
- ECL working solution (*see 3. Recipes*)
- ddH_2O

- Immunoblot imaging system able to image ECL
- Plastic protective sheet
- Absorbent paper

3. Recipes

a. Luminol stock solution

250 mM Luminol (ACROS Organics, 98% pure, 153850050) *[1 g for 22.6 mL]*
DMSO qsp 22.6 mL (Fisher Bioreagents, BP231-100)

Store at -20˚C in 30 µL aliquot in amber 1.5 mL centrifuge tubes.

b. p-Coumaric acid stock solution

90 mM trans-p-coumaric acid (TCI America, 98.0+%, C039325G) *[500 mg for 33.8 mL]*
DMSO qsp 33.8 mL (Fisher Bioreagents, BP231-100)

Store at -20˚C in 20 µL aliquot in clear 1.5 mL centrifuge tubes.

c. ECL buffer

0.1 M Tris-base (Fisher Bioreagents, Molecular Biology, BP152-500) *[2.42 g for 200 mL]*
ddH_2O qsp 200 mL

Adjust pH to 8.6. This solution can be stored for 6 months at 4˚C.

d. ECL working solution

2.5 mM Luminol *[25 µL of Luminol stock solution for 5 mL]*
197 µM p-coumaric acid *[11 µL of p-coumaric acid stock solution for 5 mL]*
0.01% Hydrogen peroxide (Fisher Chemical, Certified ACS, H325-500) *[1.5 µL for 5 mL]*
ECL buffer qsp 5 mL

Adjust pH to 8.6. This solution can be stored for 6 months at 4˚C.

4. Method

a. Drain the probed membrane of wash buffer
b. Position membrane on plastic protective sheet
c. Pour the ECL working solution onto membrane and allow to stand for 3 min
d. Drain the membrane with absorbent paper
e. Image membrane using ECL imaging system or films
f. Membrane can be rinsed with ddH_2O after imaging
g. Membrane can be used for re-probing after stripping or stored in wash buffer (usually TBS-T) at 4˚C

5. References

Mruk DD, Cheng CY. Enhanced chemiluminescence (ECL) for routine immunoblotting: An inexpensive alternative to commercially available kits. Spermatogenesis. 2011 Apr;1(2):121-122. doi: 10.4161/spmg.1.2.16606. PMID: 22319660.

6. Revision history

Revision #	Date	Prepared by
1.0	**2021-09-25**	**Elie Besserer-Offroy**
Summary of modifications		
Initial version of the protocol		