ECL-Detection

Ingredient	ECL Solution I	ECL Solution II
1 M Tris-HCl pH 8.5	5 ml	5 ml
250 mM Luminol in DMSO	500 µl	–
90 mM *p*-Coumaric acid	220 µl	–
30 % H_2O_2	–	30.5 µl
Final	50 ml	50 ml

Ingredient	10 x PBS	PBS-T	PBS-TM
NaCl	80 g	100 ml 10 x PBS	100 ml PBS-T
KCl	2 g		
Na_2HPO_4	14.6 g		
KH_2PO_4	2 g		
20 % Tween-20	–	5 ml	–
Milk Powder	–	–	3 g
H_2O	1 l	895 ml	–

1. Blocking: Incubate the membrane in a plastic container with 15 ml PBS-TM; shake gently and ensure that the membrane is always covered with solution.
2. Remove blocking solution.
3. Primary antibody: Depending on the antibody titer and the abundance of the target protein, dilute primary antibody by 1:500 to 1:20.000 in 15 ml PBS-TM and incubate the membrane with the solution for 1 h with gentle shaking. (We will use HA-antibody: 1:10.000
4. Collect the primary antibody solution in a Falkon tube.
5. Rinse membrane twice with a little PBS-T and wash membrane 3 x 5 min with 15 ml TBS-T.
6. Secondary antibody: Dilute secondary antibody (usually goat anti-rabbit coupled to horse radish peroxidase) at a 1:10.000 dilution in 15 ml PBS-TM and incubate the membrane with this solution for 1 h with gentle shaking.
7. Discard the secondary antibody solution.
8. Rinse membrane twice with a little PBS-T and wash membrane 3 x 5 min with 15 ml TBS-T.
9. Cut Whatman paper to a size a little larger than the membrane and place it onto the FUSION detection tray.
10. For each mini-gel blot mix 2 ml of ECL solution I and 2 ml of ECL solution II in a test tube and vortex well (careful: do not cross-contaminate ECL solutions, this will destroy them). Work with gloves.

11. Distribute the mixed ECL solutions evenly across the Whatman filter paper; squeeze out bubbles with a test tube.
12. Place membranes with the protein side up onto the Whatman filter paper.
13. Detect the chemiluminescence signal with the FUSION device.