

Immuno detection by ECL

Amersham.

1. Block Membrane 6% milk for 1 hour changing once after 10 mins blocking.
2. Wash by rinsing Membrane 2x quickly in TBST and then 3x for 10 mins intervals.
3. Incubate Membrane with antibody in TBST and place rotating for 1 hour at room temperature or overnight at 4°C.
4. Wash by rinsing Membrane 2x quickly in TBST then 3x for 10 mins.
5. Incubate with 2° antibody HRP conjugate use required dilution 1:5000 - 1:10,000. Incubate at R-temp for 45-60 mins.
6. Wash by rinsing 2x quickly in TBST then for 3x more 10 mins intervals.
7. Detection can be done by incubating Membrane in equal volumes of detection reagent 1 and 2 for 1 minute.
8. Drain of detection reagent and wrap Membrane in Saran wrap.
9. Expose to X-Ray Film. (Vary exposure times 5-30 sec)
10. Develop X-Ray Film

TBST
Tris buffered Saline - Tween pH 7.6

2.42g Tris base 20ml
8g NaCl 137ml
pH to 7.6 with HCl
dilute to 1000ml

Add 1ml Tween-20
Final conc = 0.1%

PBS-T
Phosphate buffered Saline - Tween 20

11.5g disodium hydrogen
orthophosphate anhydrous (80ml)
2.96g sodium dihydrogen
orthophosphate (20ml)
5.84g NaCl (100ml)
pH to 7.5 with HCl
dilute to 1000ml

Add 1ml Tween-20
Final conc = 0.1%.