IMMUNOREACTION WITH WESTERN BLOTS

1. Remove filter from blotting apparatus and incubate in **blocking buffer** for 3 x 10' at room temperature with shaking.

2. Discard last wash and replace with antibody diluted in **blocking buffer**. Normally 10 μl serum is added to 10 ml **blocking buffer**. Incubate shaking for 1 – 24 h. If incubating overnight, shake in cold room.

3. Pour off antibody into a falcon tube and store at –20°C. This solution can be re-used up to 20 times.

4. Wash filter in 3 changes of **blocking buffer** - 10' each.

5. Discard last wash and replace with 2° antibody diluted in **blocking buffer**.
   Normally 5 μl 2° antibody is added to 10 ml buffer. Incubate at room temperature for 1 – 3 h.

6. Pour off antibody and wash filter in 3 changes of **blocking buffer** - 10' each.

7. Discard last wash and replace with **stain solution**.

8. When bands are developed, rinse filter in dH_2O to stop the reaction.

9. Filter can stored dry in saranwrap and used again at a later date.

Notes:
If using a secondary detection system that uses biotin you must use blocking buffer B. All other detection systems are OK with buffer A which is cheaper.
The stain solution used depends on whether horseradish peroxidase or alkaline phosphatase conjugated antibodies are used.

Blocking buffer A (2L)

5% non fat milk	100 g

125 mM NaCl	50 ml 5M
10 mM phosphate buffer pH 7.0	20 ml 1 M

Blocking buffer B (2L)

1mgml^{-1} BSA	2 g
125 mM NaCl	50 ml 5M
10 mM phosphate buffer pH 7.0	20 ml 1 M

Stain solution (HRP) (12 ml)

500 μgml^{-1} 4-chloro-1-naphthol	2 ml of 3 mgml^{-1} in methanol
125 mM NaCl	300 μl 5 M
10 mM phosphate buffer pH 7.0	120 μl 1 M

Add 5 μl 30% H_2O_2 at the last minute.