Procedure

Block nitrocellulose strips or sheets for 30mins in TBST (see later for description) containing 3% milk powder. Milk solution should be filtered through Whatman paper to avoid spots.

Use primary antibody at an appropriate dilution in TBST, again with 3% milk. Gently rock the solution at room temperature for one hour or if overnight at 4C.

Blots are then washed three times for ten minutes in TBST (no milk powder)

The secondary antibody (in our case either anti rabbit or anti mouse antibody conjugated to horse radish peroxidase for ECL used at a dilution of 1:4000) is then incubated in TBST with 3% milk powder and incubated at room temperature with rocking for one hour.

The blots are then washed in TBST three times for ten minutes each.

The blots are then developed using the Amersham ECL Solutions:

Blots are soaked in an equal mix of solutions A and B for one minute.

Then take the solution of A+B and soak a piece of Whatman paper with it. Transfer the blot to the soaked Whatman paper. Cover with saran wrap and then expose to photographic film usually for around 5 secs to 5 min.

Sometimes weak signals can be due to excess of TBST on the blot before adding the solutions A+B. Blot of the excess between Whatman paper (but do not allow to dry!!!).

Low background at this stage requires extensive washing after the 2 antibody, especially when the signal is weak

Solutions

TBST Solution

- 10mM Tris HCl pH8.0
- 150mM NaCl
- 0.05% Tween 20