General Protocol for Western Blot Development

Pre-preparation: Make available target proteins from cell and tissue lysates separated by SDS PAGE and blotted onto PVDF membrane.

1. Cut and label required strips (35ug protein each)
2. Soak the strips in methanol for 10 seconds.
3. Wash the strips first in TBS for 5 minute, then in TBST for 15 minute. Except for the incubation in methanol, all other incubations should be carried out on a rocking table.
4. Block the strips by blocking buffer (3% skimmed milk in TBST) for 40 minutes.

Development and Detection of the Primary antibodies

5. Incubate the strips with primary antibody (diluted e.g. 1ug/ml in blocking buffer -please refer to datasheet for recommended concentration) for 1 hour at room temperature. Please note: Primary incubation overnight at 4°C requires significant dilution of the primary antibody and non-specific background may result.
6. Wash the strips with TBST for 3 x 10minutes.
7. Incubate the strips with diluted secondary antibody (1:40K) in blocking buffer for 1 hour.
8. Wash the strips for 3 x 10minutes.
9. Incubate strips with chemiluminescence substrate (100ul/strip) for 3 minutes.
10. Develop the bands on x-ray film in dark room under the red light for approx 15 seconds or determine based upon signal in films.
11. Label the detail on the film including molecular weight marking of strips.
12. Scan the film and read the molecular size and strength of bands.

Further Guidelines:

- Tris buffered saline (TBS): 20mM Tris, pH7.4 in 150mM NaCl
- Include a negative control lane – this may be a lysate, or just omission of the Primary antibody.
- Where possible include a positive control lysate (e.g.as per datasheet)