NEUROMICS

General Western Blot Protocol

1. Polyacrylamide gel electrophoresis and blotting

   a. Add an appropriate amount of electrophoresis sample buffer (1X = 125mM Tris-HCl pH 6.8, 2% SDS, 5% glycerol, 0.003% bromophenol blue, and 1% beta-mercaptoethanol) to all samples.

   b. Heat to 95^{o} C for 3-5 minutes.

   c. Load 5-100 ug total protein in a volume that is appropriate for the size of the wells.

   d. Electrophorese proteins for the appropriate time according to the manufacturers' instructions.

   e. Transfer proteins from the gel to a suitable membrane (e.g. nitrocellulose, PVDF, etc.) following the manufacturers protocol for transfer.

   f. High molecular weight proteins (>120 kDa) can be wet transferred more efficiently if transfer time is increased and 0.05% SDS is included in the transfer buffer.

2. Blocking

   a. Remove the filter from the transfer apparatus and rinse in PBST/TBST to remove loose acrylamide.

   b. Transferred proteins can be visualized by staining the membrane for 15-30 minutes with Ponceau S.

   c. Remove stain from filter by washing with PBST/TBST.

   d. Place filter into blocking solution.

   e. Block for 30 minutes at 37^{o} C, 1 hour at room temperature, or overnight at 4^{o} C.

3. Incubation with primary antibody

   a. Decant the blocking buffer and add the antibody, diluted in blocking buffer as suggested in the product description sheet.

   b. Incubate with agitation for 30 minutes at 37^{o} C, 1 hour at room temperature, or overnight at 4^{o} C.

4. Incubation with secondary antibody

   a. Wash for 30 minutes with agitation in wash buffer (TBS or PBS with 0.1% Tween 20), changing the wash buffer every 5 minutes.

   b. Decant the wash solution and add HRP-conjugated secondary antibody, diluted in 5% non-fat dry milk in wash buffer.

   c. Incubate for 30 minutes at 37^{o} C, 1 hour at room temperature, or overnight at 4^{o} C.

   d. Decant the antibody conjugate and wash for 30 minutes with agitation in wash buffer (TBS or PBS with 0.1% Tween 20), changing the wash buffer every 5 minutes.

5. Substrate incubation (ECL)

   a. Decant washing buffer and place the blot in a plastic bag or clean tray containing the development working solution (0.125 ml/cm^{2}) for 1-5 minutes.

   b. Agitate bag or tray to cover the surface of the membrane.

   c. Remove the blot from the bag or tray and place it between two pieces of write-on transparency film.

   d. Smooth over the covered blot to remove air bubbles and excess substrate.

   e. Expose to X-ray film or any sensitive screen. (Check manufacturer's instructions for specific ECL reagents and procedures.)