Western Blot Protocol

All steps are carried out at room temperature unless otherwise indicated.

SDS-PAGE

1. Prepare an SDS-PAGE gel according to the molecular weight (MW) of your target protein(s).

2. Prepare samples in microfuge tubes. Add 4X SDS sample buffer so the total protein amount is 30-50 µg per sample (according to the protein amount measured by Bradford or BCA protein assay).

3. Flick microfuge tubes to mix samples, and then heat to 95-100°C for 5 minutes.

4. Set up electrophoresis apparatus and immerse in 1× electrophoretic buffer. Remove gel combs and cleanse wells of any residual stacking gel.

5. Load samples and protein markers onto the gel using gel loading tips. Set electrophoresis power pack to 80V (through the stacking gel), before increasing it to 120V when the protein front reaches the separation gel.

Protein Transfer

1. PVDF membranes are strongly recommended. Soak membranes in methanol for 30 seconds before moving to transfer buffer. Soak the filter papers and sponges in transfer buffer as well.

2. Sequentially assemble the transfer constituents and ensure no bubbles lie between any of the layers. Apply semi-dry or wet transfer systems according to the manufacturer's instructions.

Immunoblotting

1. After transfer, wash the membrane twice with distilled water, and using a pencil, mark bands of the MW ladder on the membrane. If desired, stain the membrane with commercial Ponceaured solution for 1 min to visualize protein bands, then wash any Ponceaured staining with copious amounts of 1XTBST.

2. Block with 1X TBST containing (2-5%) nonfat dry milk (or 1-5% BSA for the detection of phospho-epitope antibodies) with constant rocking for 1 hour or overnight at 4°C.

3. Dilute primary antibody in blocking solution with a starting dilution ratio of 1:1000. (Optimal dilutions could be turned to the manual.) Incubate the membrane with primary antibody for 1 hour, or overnight at 4°C, on a bench-top rocker.

4. Wash membrane three times with 1X TBST for 10 minutes each.

5. Incubate the membrane with a suitable HRP-conjugated secondary antibody (recognizing the host species of the primary antibody), diluted at 1:5000-1:50000 in blocking solution. Incubate for 1 hour with constant rocking.

6. Wash membrane three times with 1X TBST for 10 minutes each.

Signal Detection

1. Prepare ECL substrate according to the manufacturer's instructions.

2. Incubate the membrane completely with substrate for 1-5 minutes (adjust time for more sensitive ECL substrates.

3. Expose the membrane to autoradiography film in a dark room or read using a chemiluminescence imaging system.