Experiment (4)
Western Blot

Materials:

Transfer buffer

(25mM Tris, 190 mM glycine, 20% methanol, 0.1% SDS) Adjust the pH to 8.3

Block buffer

（10% milk with 0.5% Tween 20) Or 5% BSA (with fluorescent system)

Washing buffer (TBST)

(25mM Tris, 0.15M NaCl, 0.05% Tween-20) Adjust the pH to 7.5

10X PBS

(NaCl, KCl, Na2HPO4, KH2PO4) Adjust the pH to 7.4

Protocol:

I. Crude extraction of protein from animal tissue

1. Weight the sample, wash it with normal saline, and cut it into small pieces.
2. For each 1g of the sample add 5ml of the extraction buffer (0.1 M Tris-HCl, pH 7.4) and homogenized it in the blender.
3. Transfer the homogenized tissue/buffer mixture into centrifuge tubes.
4. Centrifuge your homogenate for 15 minutes at 3000 xg.
5. Measure the volume of the supernatant.

II. Separation of the protein by SDS-PAGE

1. Prepare 12% of separating gel and 7% of stacking gel.
2. Mix the sample with 10X loading buffer (1:1), then heat them at boiling for 5-10 min.
3. Load 20µl of prepared samples into wells and in different well load 10µl of pre-stained protein marker
4. Run at 120 volts for 60 – 90 minutes

According to: http://www.assay-protocol.com/molecular- biology/electrophoresis/denaturing-page.html

III. Electro-blotting of separated protein (Semi-dry blotter)

1. Wet the filter papers with transfer buffer.
2. The prewetted nitrocellulose / PVDF membrane is put on top the filter. (i.e PVDF.
3. membrane need to be activated with 100% methanol.
4. The gel is put on top of the membrane.
5. Ensure that no air bubbles are anywhere in this stack of membranes.
6. Then wetted filter papers should be placed on top of the gel.
7. Again, remove any bubbles.
8. Put it onto the apparatus.
9. apply a continuous voltage of 25V for 15-20 minutes

IV. Visualization of target protein

1. Block the membrane for 2 hours at room temperature or overnight at 4°C using blocking buffer.
2. Wash the membrane three times using TBST, 5 min each. (Optional)
3. Incubate the membrane with 1:1000 dilutions of primary antibody, prepared in 1XPBS for 2 hours at room temperature or overnight incubation at 4°C.
4. Wash the membrane three times using TBST, 5 min each.
5. Incubate the membrane with the 1:10000 dilution of secondary antibody, prepared in 1XPBS at room temperature for 1 hour.
6. Wash the membrane in three times using TBST, 5 min each.
7. Put the membrane in 1XPBS and visualize it in ODYSSET CLx device.