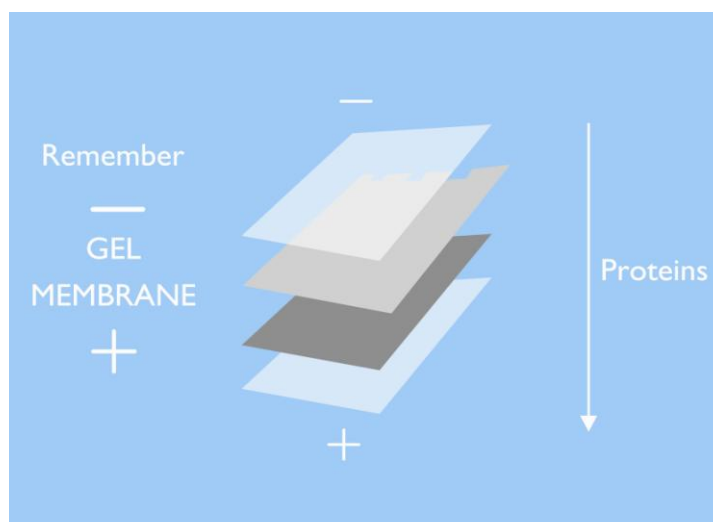
A. Solutions and Reagents

1. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red.
2. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5).
3. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
4. **Nonfat Dry Milk:** (weight to volume [w/v]).
5. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
6. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T).

B. SDS-PAGE



1. Make an appropriate percentage SDS-PAGE gel, based on the molecular weight of the protein you are trying to detect.
2. Add 5x SDS loading buffer to the protein sample solutions to make a final concentration of 1x SDS loading buffer. Mix and boil for 5 min.
3. Load samples onto the SDS-PAGE gel. Loading 60-100 µg of total lysate proteins per lane is suggested.
4. Run the gel at 100V initially and increase the voltage to 120 V when the dye front migrates to the resolving gel.
5. Stop the voltage when the dye front reaches the bottom of the gel.

C Transferring proteins to a membrane

1 PVDF membrane preparation: Wet PVDF membrane (or PSQ membrane for proteins of molecular weight less than 35 kDa) in 100% methanol for 1-2 min and then move into transfer buffer.

2 Soak filter papers in transfer buffer.

3 Assemble semi-dry transfer units according to the figure above.

4 Make sure to avoid any air bubbles in the transfer units.

5 A semi-dry transfer should run at 60 mA per gel for 1-1.5 h.

D Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 8.3 cm x7.8 cm of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash PVDF membrane with TBS for 5 minutes at room temperature.
2. Soak PVDF membrane in blocking buffer to block unoccupied protein binding sites for 1 h at room temperature or overnight at 4°C.
3. Wash three times for 5 minutes each with TBS/T.

II. Primary Antibody Incubation

1. Reconstitute with 0.1 ml of sterile distilled water per 0.1 mg of lyophilized antibody.
2. Make the working solution: 1:500 dilution, the concentration of the working solution is 2 μg/ml.
3. Incubate membrane and primary antibody (6 ml working solution, 2 μg/ml) with gentle shaking for 2 hours at room temperature.
4. Wash three times for 5 minutes each with TBS/T.

5. Incubate membrane with the AP-conjugated secondary antibody (Goat Anti-Rabbit IgG , SIGMA, Cat# A0168, dilution: 1:10000) in blocking buffer with gentle agitation for one hour at room temperature.
6. Wash three times for 5 minutes each with TBS/T.
7. Proceed to detection step in section D.

E. Detection of Proteins

1. Incubate membrane with HRP Chemiluminescent Substrate Reagent kit (Invitrogen (Catalog#WP20005)) with gentle agitation for a few minutes at room temperature.

2. Incubate opportune time at room temperature in the dark prior to scanning.