

Western Blotting:

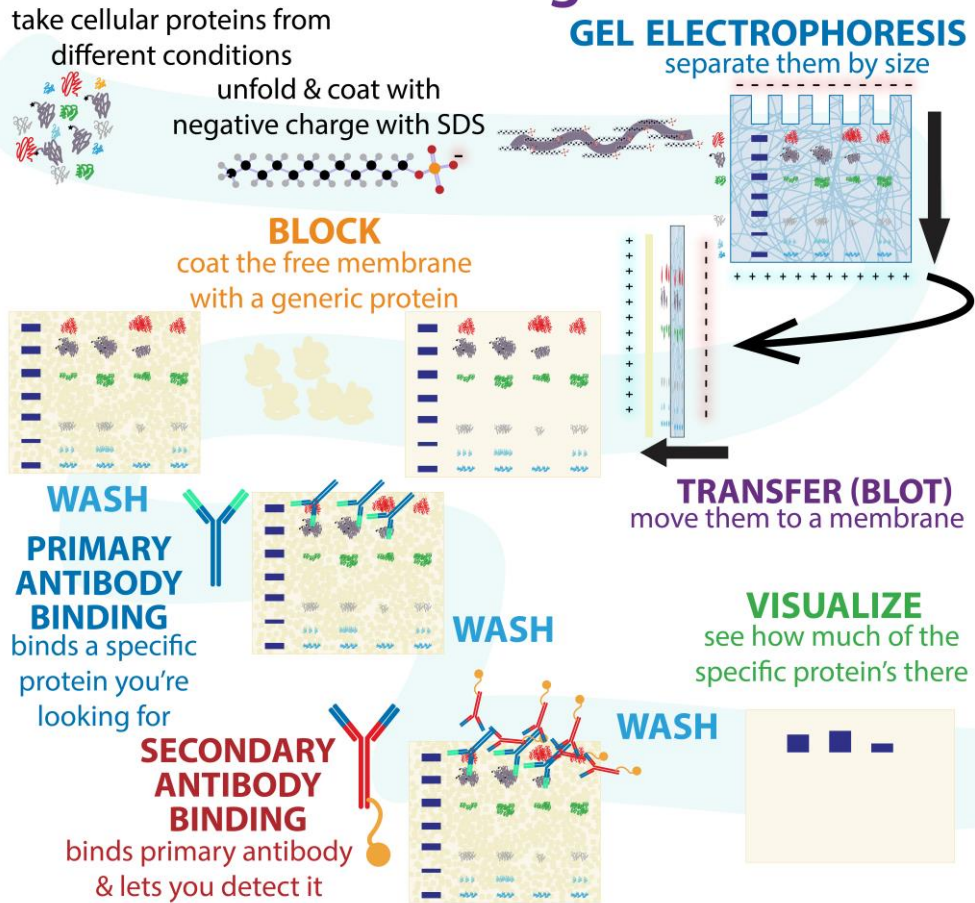
Principle: It is used to identify a particular protein from a mixture of proteins. Labelled antibodies against a particular protein is used to identify that protein. It is a very specific identification technique also known as immunoblotting

Procedure:

- Cell lysate is obtained and protein is extracted. The concentration of protein is determined by spectroscopy and when sufficient amount is available, it is mixed with loading buffer containing glycerol. It helps in sinking of sample in the well.
- Tracking dye (bromophenol blue) is added to monitor the movement of proteins.
- Sample is loaded in wells and run through SDS-PAGE.
- Proteins are separated on the basis of molecular weight.
- Proteins move towards anode as they are negatively charged.
- When proteins are separated on gel, a nitrocellulose membrane is placed on top of it.
- Proteins get transferred to nitrocellulose paper by capillary action or with the help of current depending on the procedure used for transfer.
- The membrane is non-specifically saturated/masked using casein or Bovine serum albumin (BSA).
- This step is called blocking. It is done before adding antibodies so that antibodies do not bind to free nitrocellulose membrane and give false results. This is possible because nitrocellulose membrane binds proteins and antibodies are also proteins.
- Primary antibodies specific to desired protein are added and form Ag-Ab complex.
- Secondary antibodies which are enzyme labelled (alkaline phosphatase or Horseradish peroxidase) are added.
- A substrate is added to the reaction mixture and allowed to incubate.
- Visible coloured product is formed as a precipitate and the protein of interest can be identified and isolated.

The workflow for the Western blot is given in the figure below.

western blotting workflow



Applications:

1. To determine size and amount of protein in given sample.
2. Helps in disease diagnosis.
3. It is a confirmatory test for HIV.
4. It is useful to detect defective proteins.
5. Identify any protein if antibody against it is available.