# Applications of WESTERN BLOT

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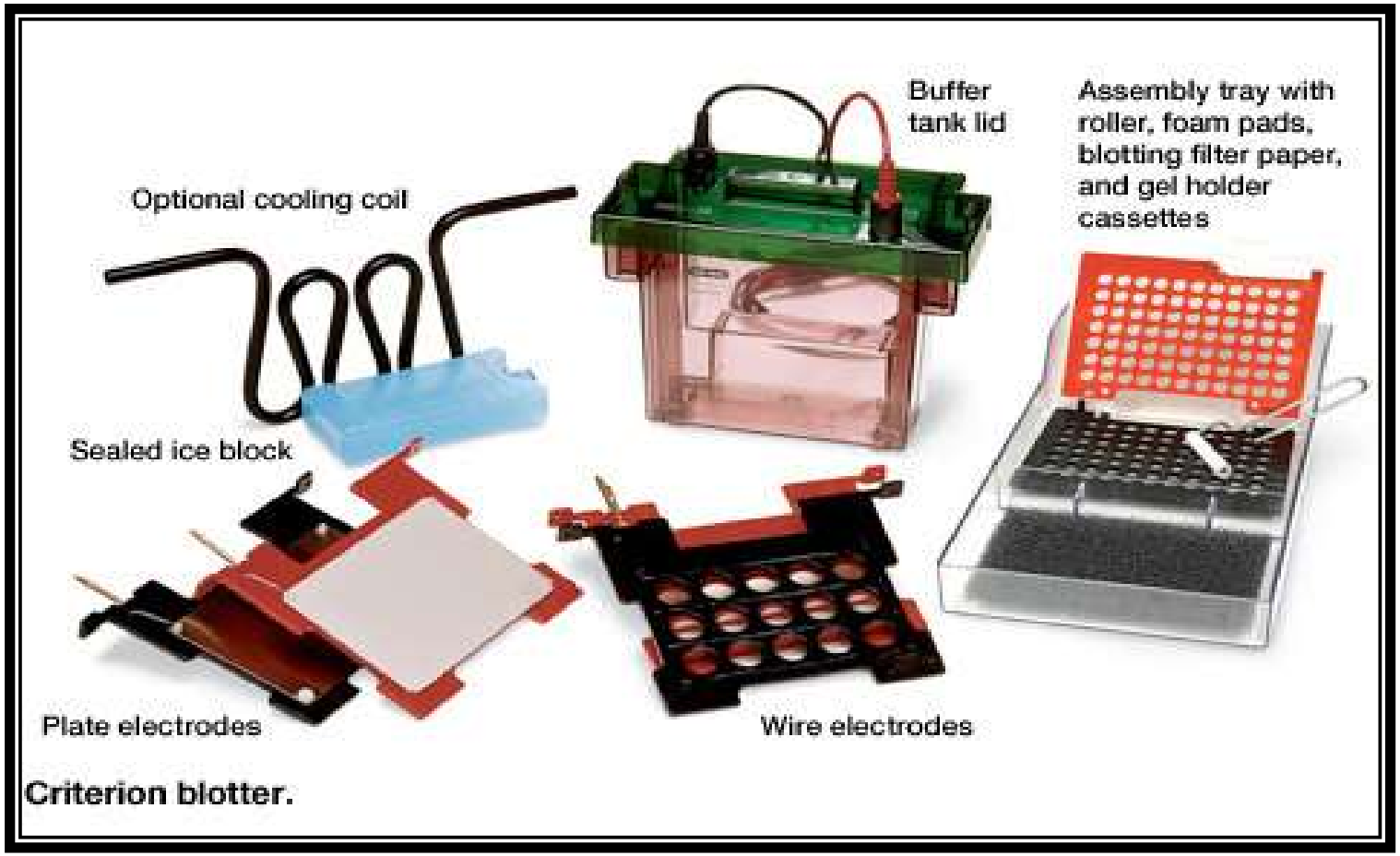

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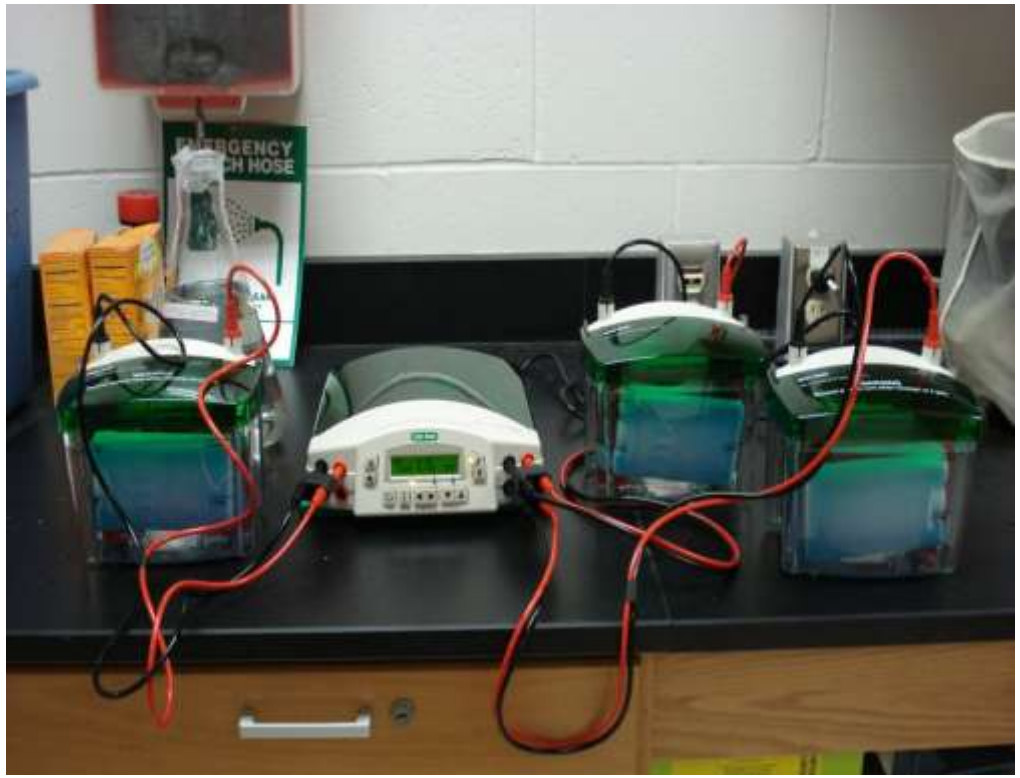
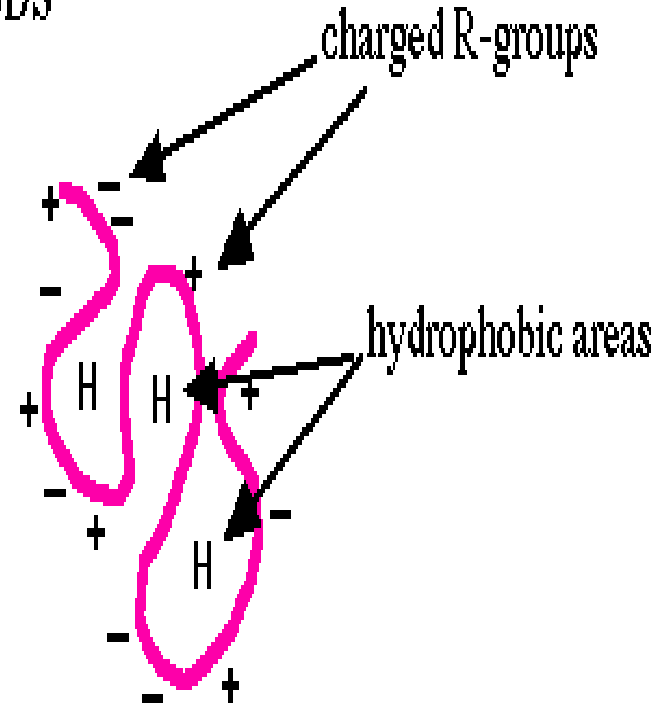


# WESTERN BLOTTING

## Principle

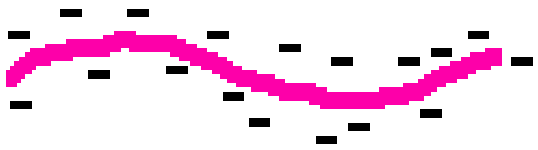
- Western blotting (protein blotting or immunoblotting) is a rapid and sensitive assay for the detection and characterization of proteins.
- It is based on the principle of **immunochromatography**, where proteins are separated onto polyacrylamide gel according to their **molecular weight.**
- The separated proteins are then transferred or electrotransferred onto a nitrocellulose membrane and are detected using a specific primary antibody and a secondary enzyme-labeled antibody and substrate.

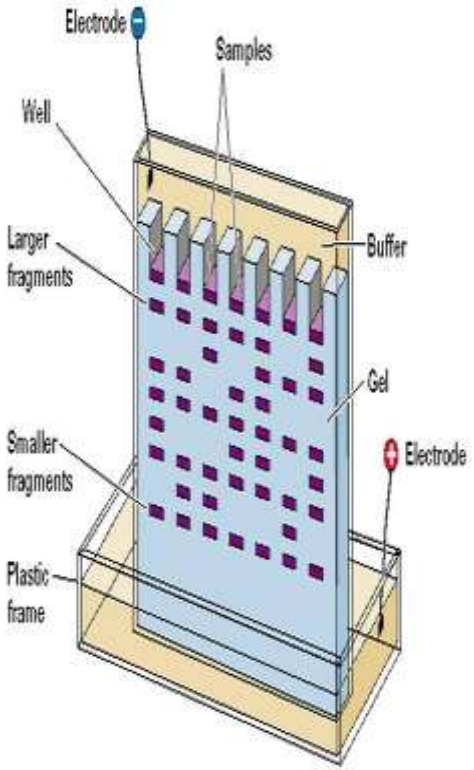
BEFORE SDS




AFTER SDS

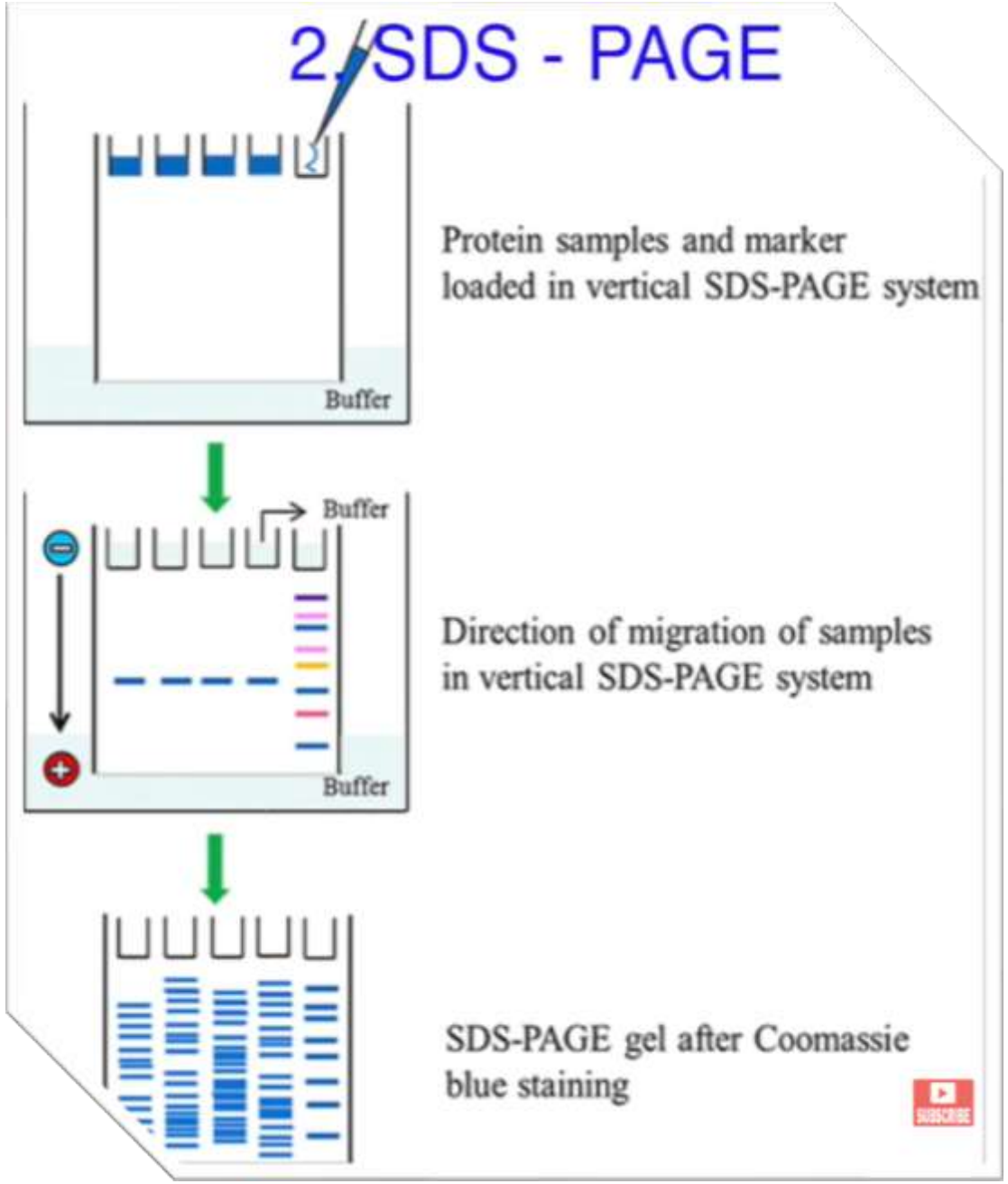
SDS linearizes all proteins and gives net negative charge.





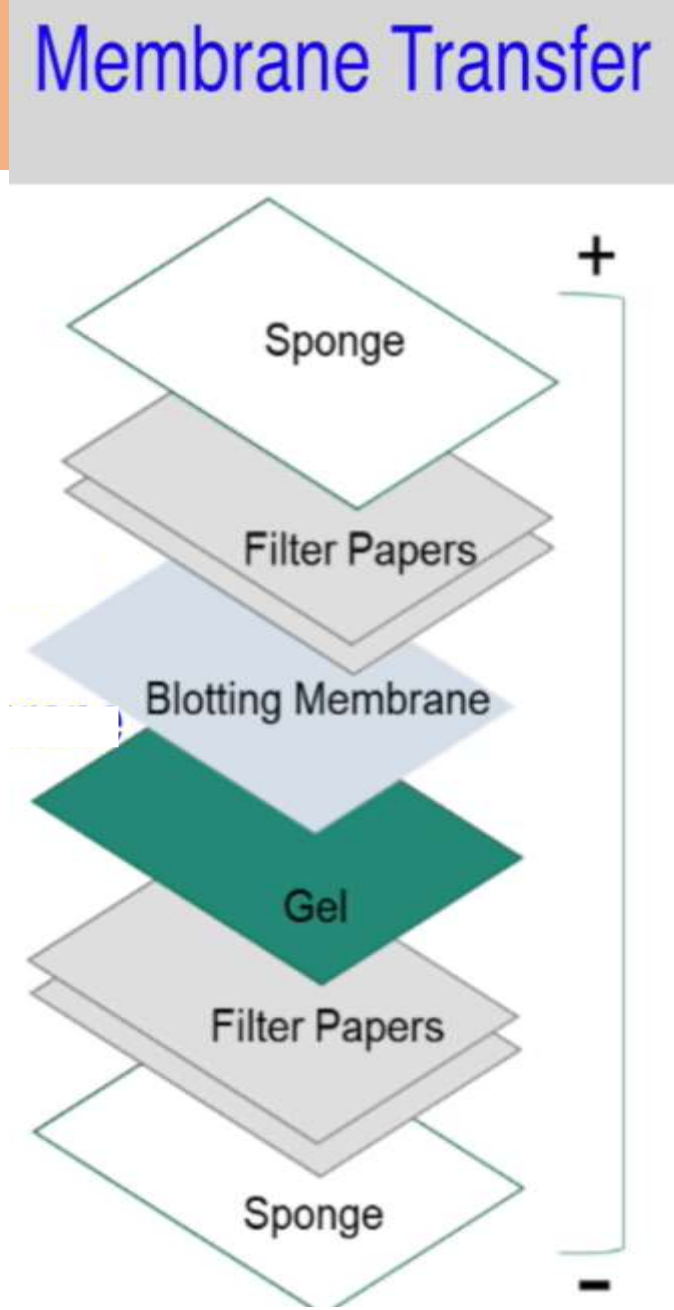
# Step II: Gel electrophoresis

- The sample is loaded in the well of SDS-PAGE Sodium dodecyl sulfate-polyacrylamide gel electrophoresis..
- Proteins are negatively charged, so they move toward the positive (anode) pole as electric current is applied.
- The proteins are separated based on their molecular weight.
- The small size protein moves faster than large size protein.

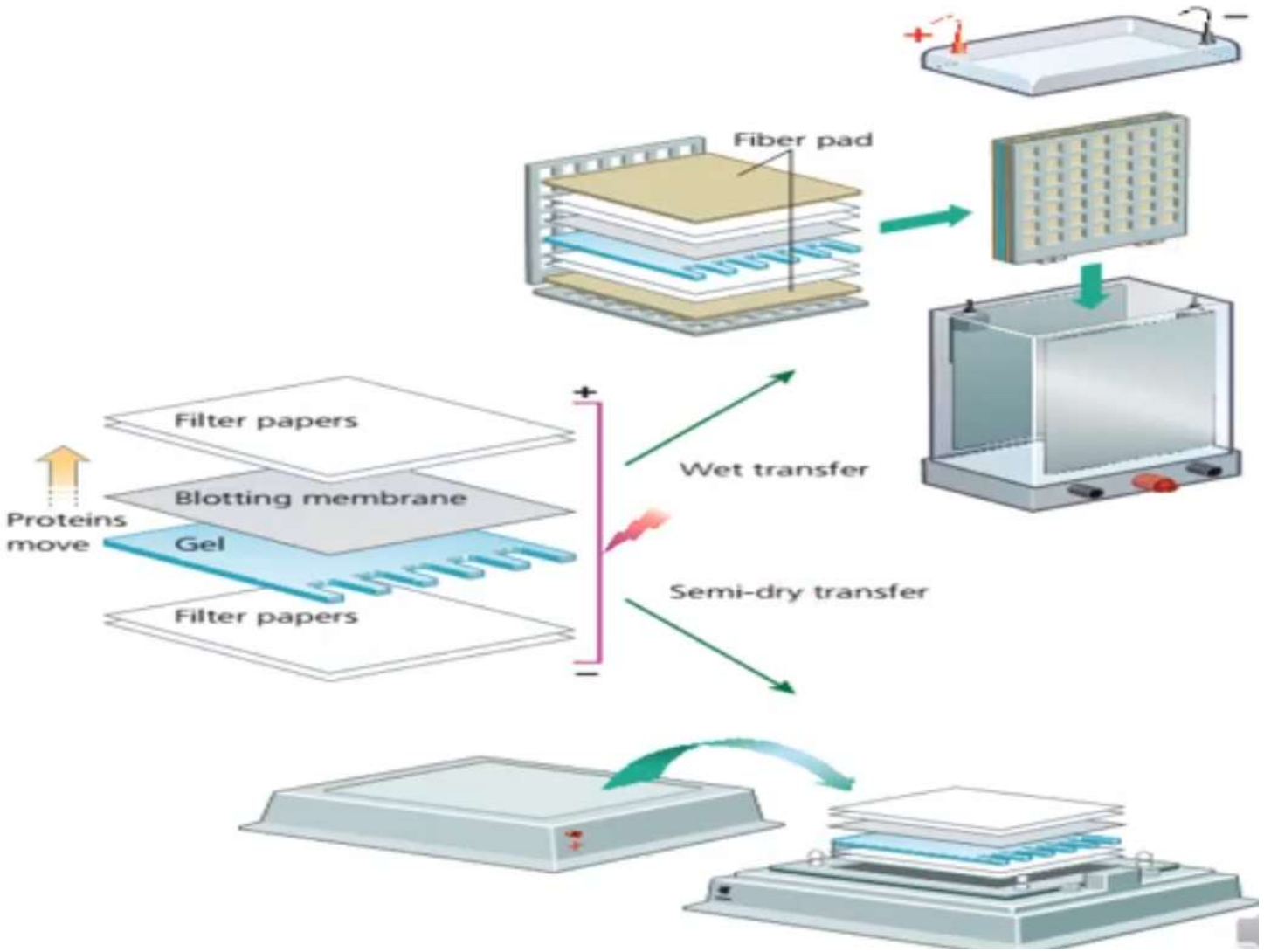


# Step III: Blotting

- The nitrocellulose membrane is placed on the gel.
- The separated protein from gel get transferred to nitrocellulose paper by capillary action.
- This type of blotting is time-consuming and may take 1-2 days
- For a fast and more efficient transfer of targeted protein from the gel to nitrocellulose paper, electro-blotting can be used.
- In electro-blotting nitrocellulose membrane is sandwich between gel and cassette of filter paper and then electric current is passed through the gel causing transfer of protein to the membrane.



# Transfer Methods/Wet and Semi-dry Transfer Method



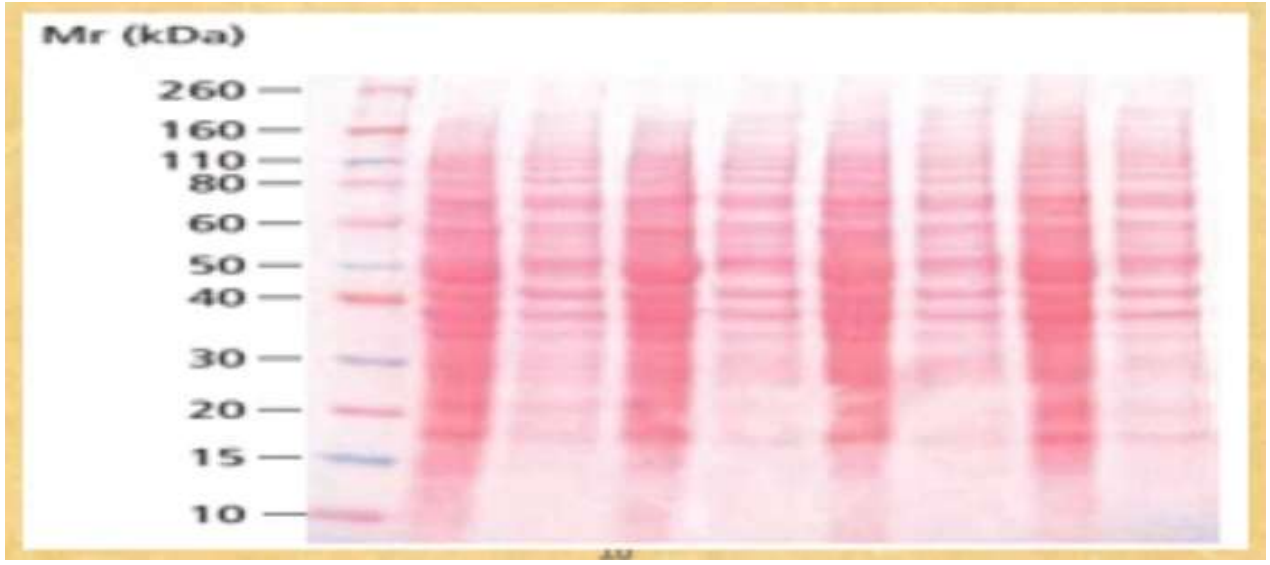

# Transfer Method

- In both types of transfer, the orientation of the construct must be so that the **membrane is on the anode (+) side** of the gel.
- Take care to **avoid wrinkles, folds or air bubbles** between the different layers of the sandwich.

Bubbles will cause **blank spots on the membrane where no protein transfer occurs.**

# Visualized Proteins on the Membrane/Ponceau Red Staining

- **Confirmation of protein transfer to the membrane** can be achieved by **staining the membrane using a total protein stain such as Ponceau Red stain after electrotransfer.**
- This is a **quick and easy** way to visualize proteins transferred to membranes.
- **Ponceau Red** is a **reversible** stain with **poor sensitivity.**

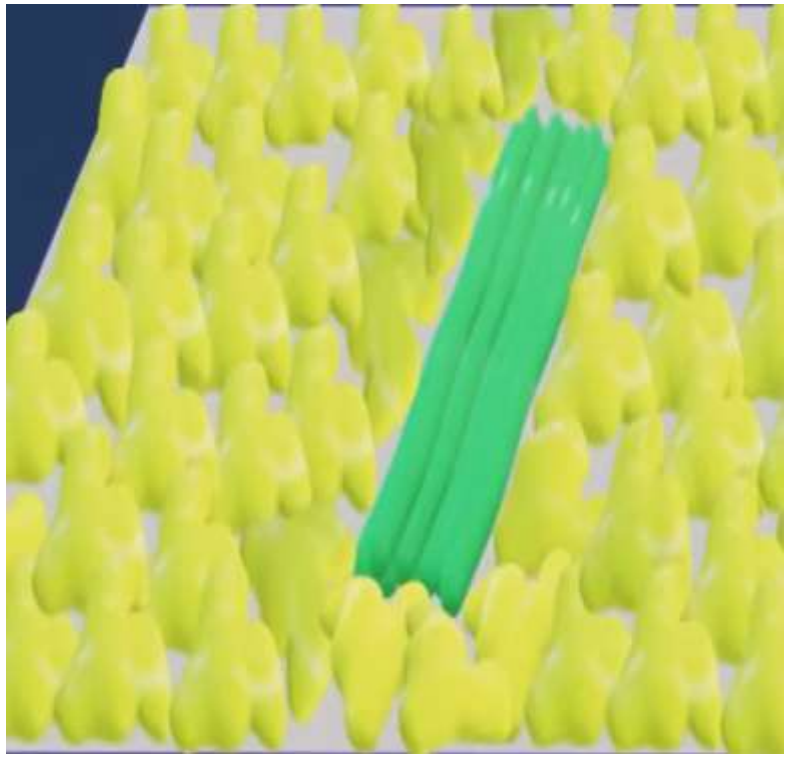


# Step IV: Blocking

Blocking is very important step in western blotting.

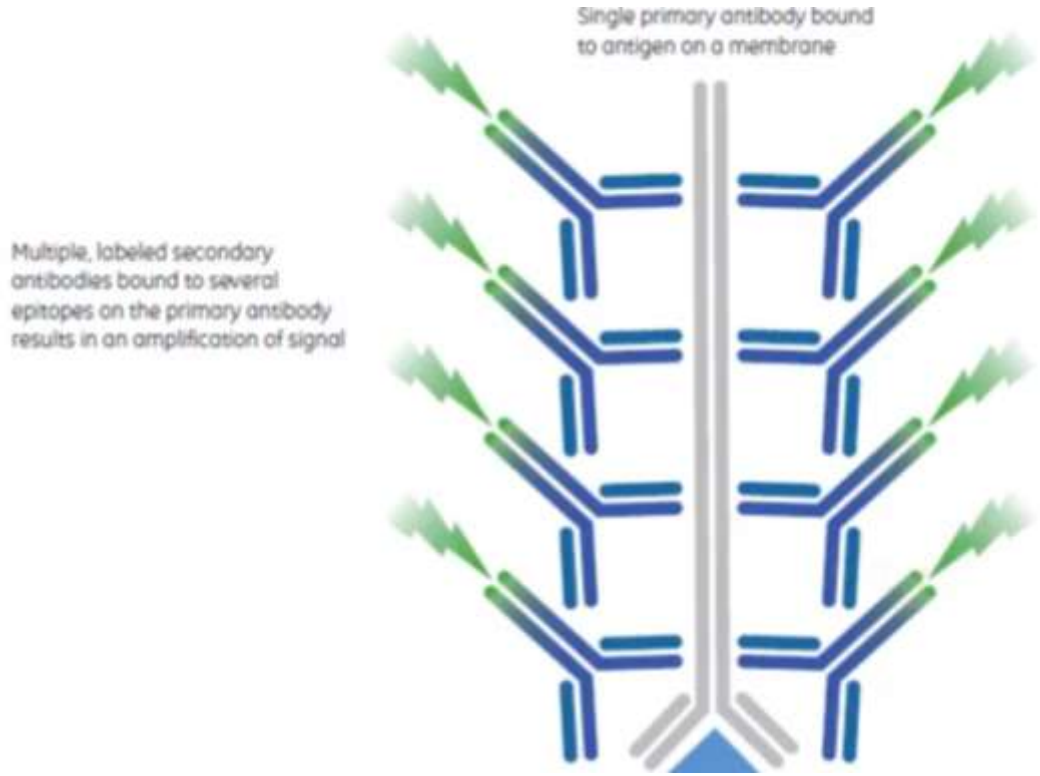
Antibodies are also protein so they are likely to bind the nitrocellulose paper.

So before adding the primary antibody the membrane is non- specifically saturated or masked by using casein or Bovine serum albumin (BSA).



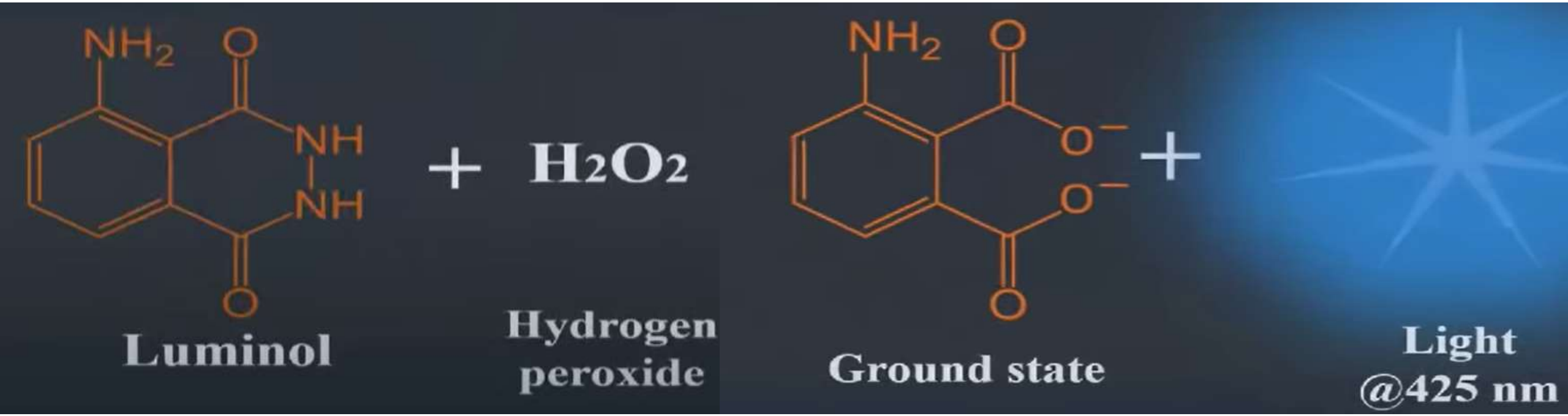
# Step VI: Treatment with secondary antibody

- The secondary antibody is enzyme labelled like alkaline phosphatase or Horseradish peroxidase (HRP) is labelled with the secondary antibody.
- Secondary antibody (2” Ab) is an antibody against the primary antibody (anti-antibody), so it can bind with the Ag-Ab complex.
- Removes unbounded antibodies from the membrane by washing with TBST or PBST buffer



# Step VII: Treatment with suitable substrate

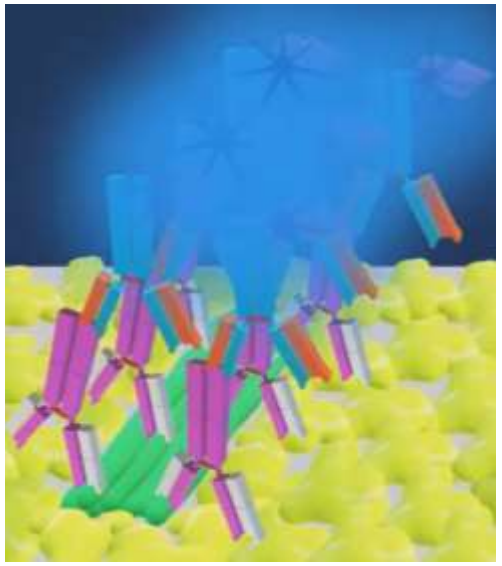
Chemiluminescence detection

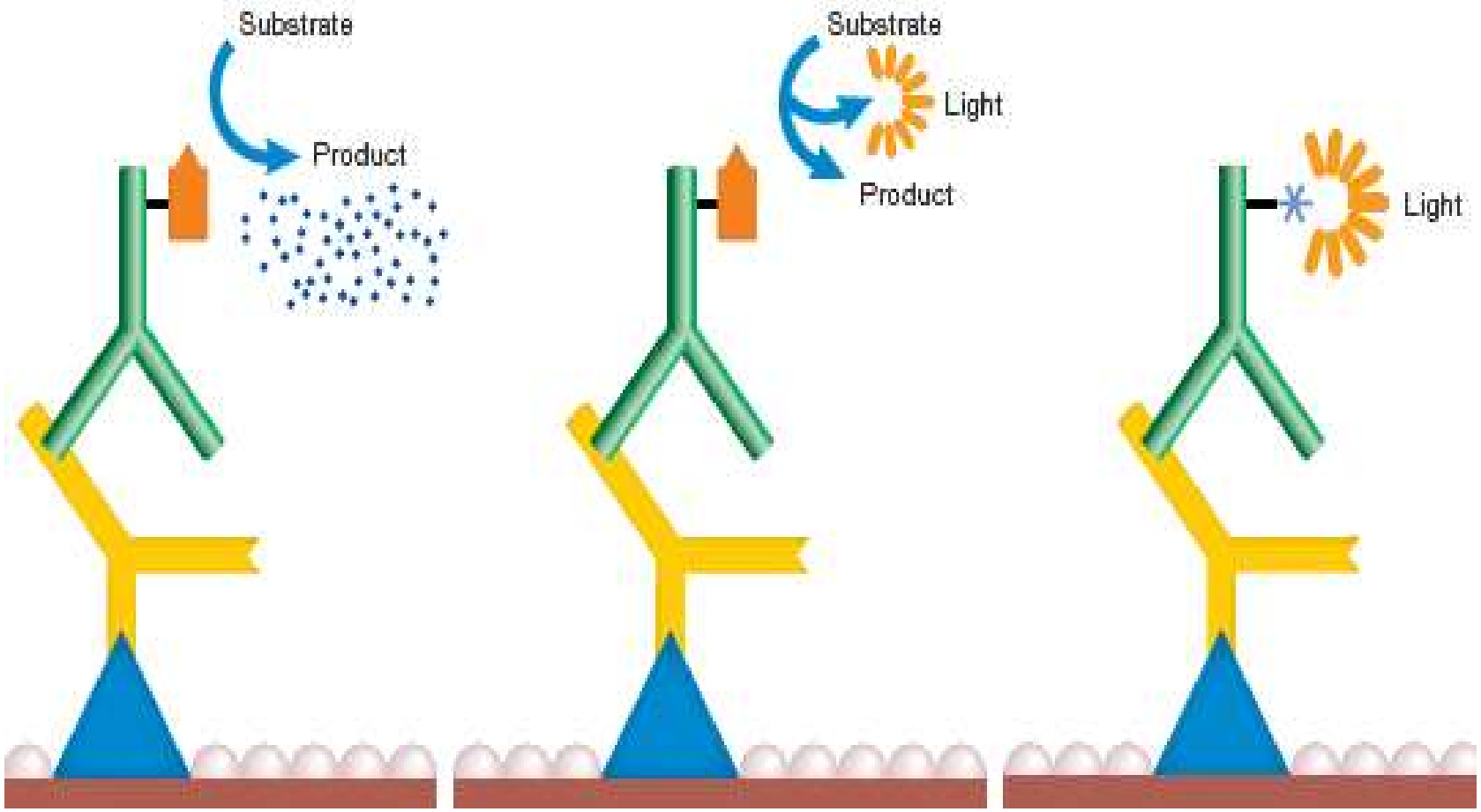


# Step VIII : Protein Detection

Four types of analysis techniques:

- Chromogenic detection
- Chemiluminescence detection
- Fluorescent detection
- radioactive detection





# Applications

- Western blotting is used to detect a particular protein from a mixture of proteins.
- Estimate of protein size and amount in the mixture.
- Thus, WB is a widely used laboratory technique in cancer and disease research for helping Researchers compare protein profiles between normal and cancerous tissues, which helps in identifying the pathogenetic pathway behind developing cancer
- WB is not limited to detecting protein expression in tissue samples, but it is also used to assess the activation and post-translational modifications status of proteins involved in cancer development and progression, which helps in the development of potential therapeutic target


Thank You!