

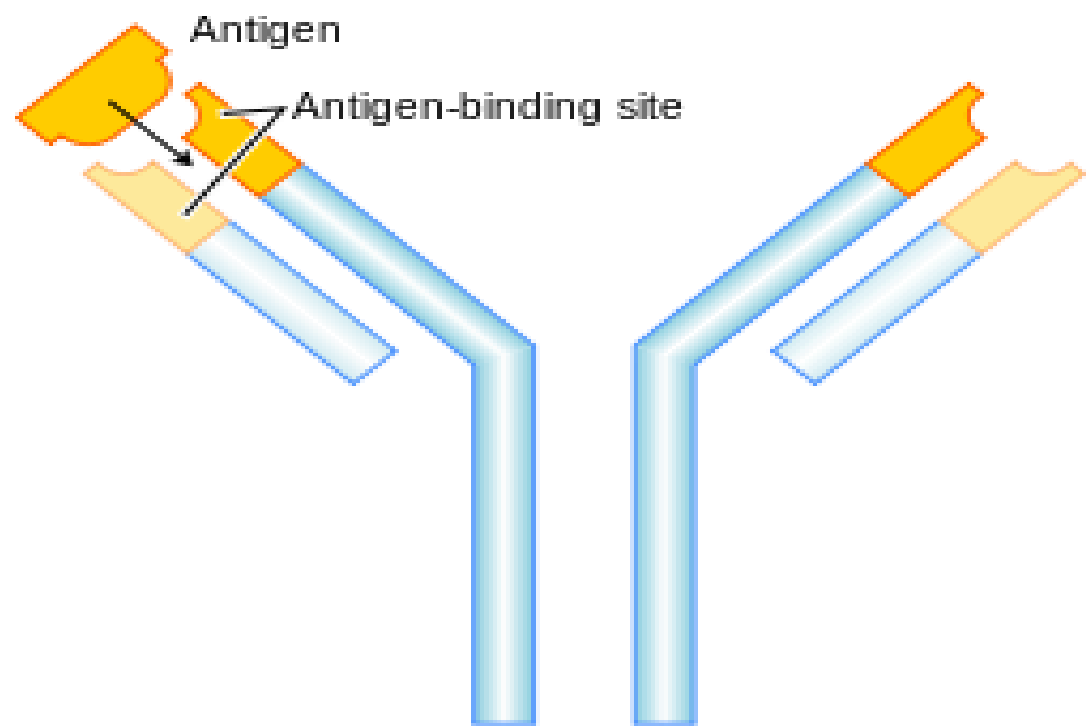
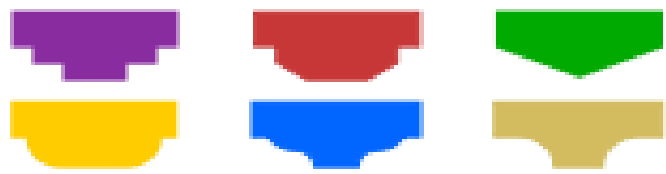


WESTERN BLOT

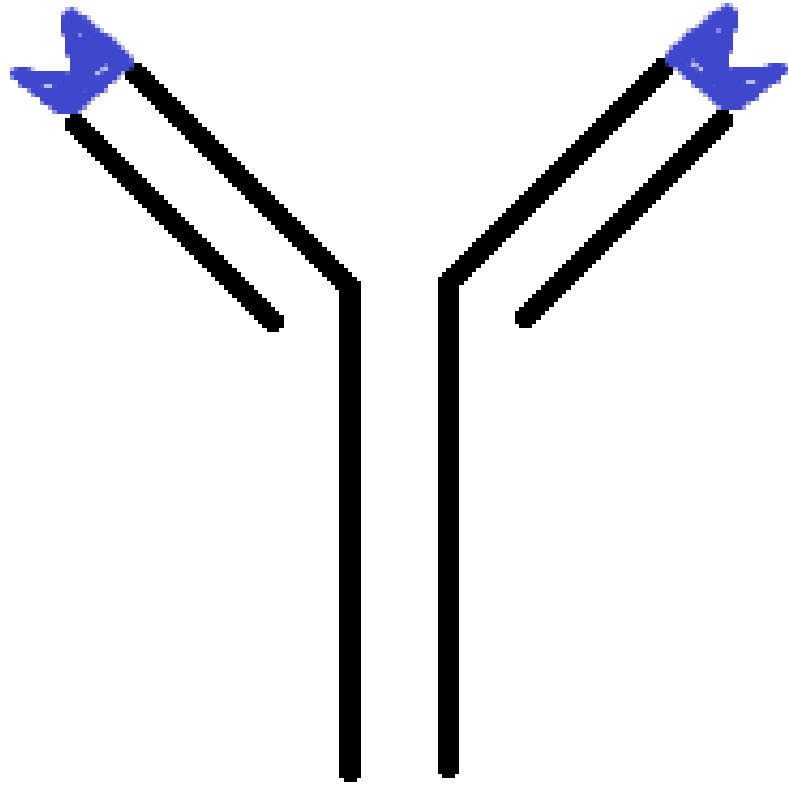
Immunoassay:

- What is Antigen [Ag] ?
- What is Antibody [Ab] ?
- **Immunoassay:** is a test that uses the highly specific and selective antigen-antibody reactions forming antibody and antigen complexes [immuno-complexes] as a means of generating measurable results.

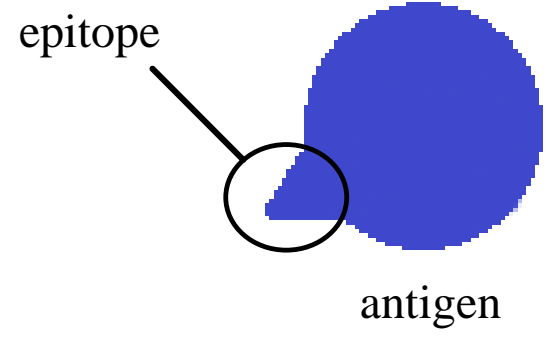
Antigens



Antibody

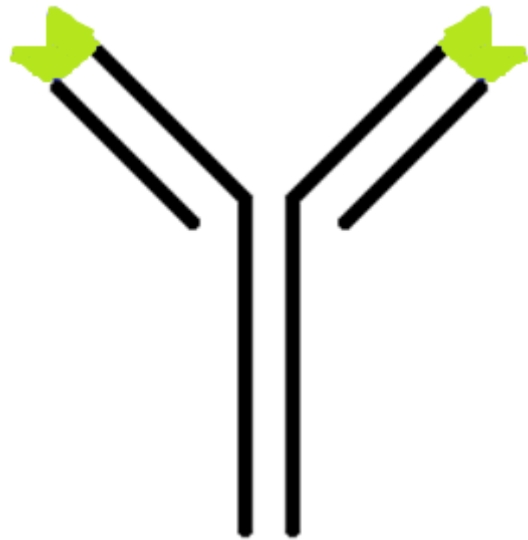


antibody



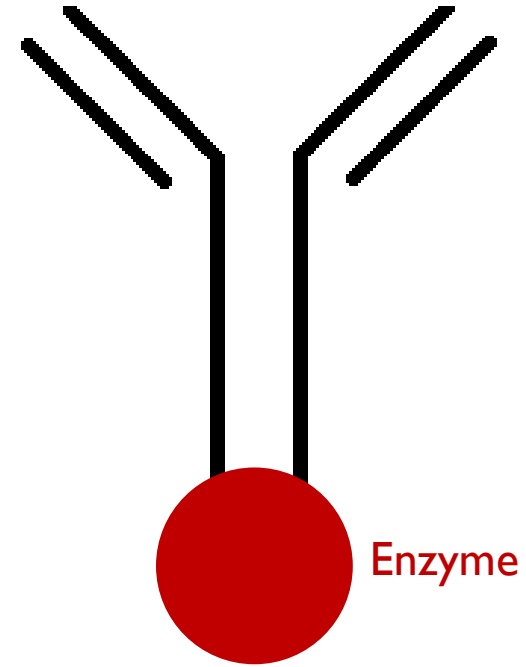
epitope

antigen



Primary antibody

“antibody specified to specific antigen”



Secondary antibody

“antibody specified to Primary antibody”

Western blot:

- Also called protein immunoblot.
- Is a widely used immunoassay technique.
- To identify proteins specific proteins [antigens] in a sample of tissue homogenate or extract, based on their ability [the antigens] to bind to antibodies resulting in colour indicate the presence of this specific protein.
- Application ?



PRACTICAL PART



Aim:

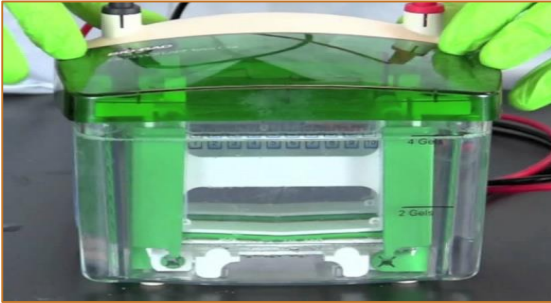
- To understand how proteins (antigens) can be analysed using antibodies raised against these proteins by Immunoblotting technique.
- To understand the steps in the development of Western and antigen-antibody interaction and detection.
- Electroblothing the pre-stained marker.

Principle:

- The mixture of proteins is separated based on **molecular weight**.
- These results are then electro-transferred to solid support producing a band for each protein.
- The transferred protein is detected by incubating the gel **with specific primary antibody** to the protein of interest, **secondary antibody labelled with an enzyme which target the primary antibody**, and substrate which in the end you will get **coloured product**.
- **The colour indicates the presence of the protein of interest.**
- The **thickness of the band corresponds to the amount of protein present.**
- Thus, the molecular weight and amount of the desired protein can be characterized from a complex mixture of proteins by western blotting.

Western blot performing steps

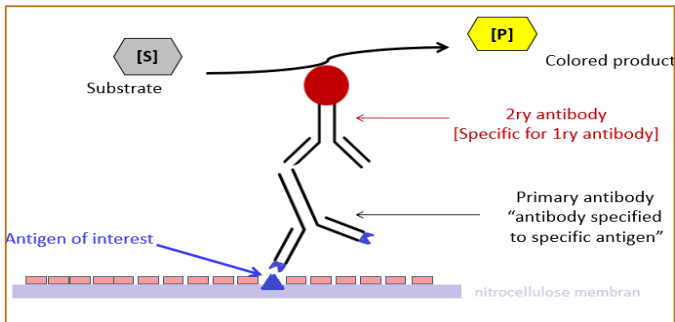
The technique uses three elements to accomplish this task



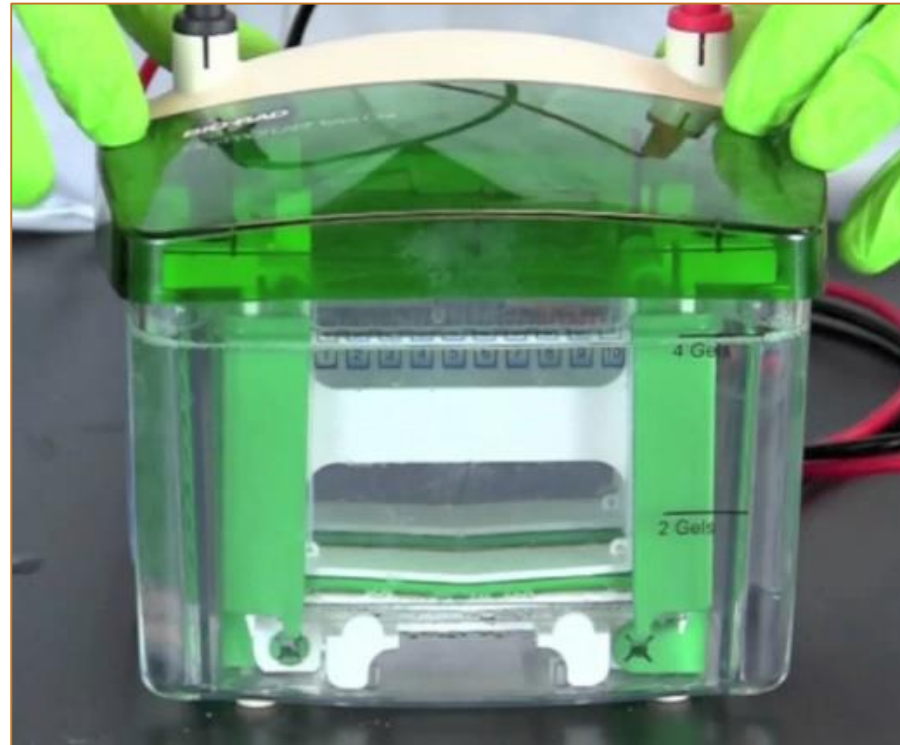
1. Separating the sample mixture by size using SDS-PAGE.



2. Transfer to a solid support (electro-blotting), transfer the proteins bands from the gel to the membrane.



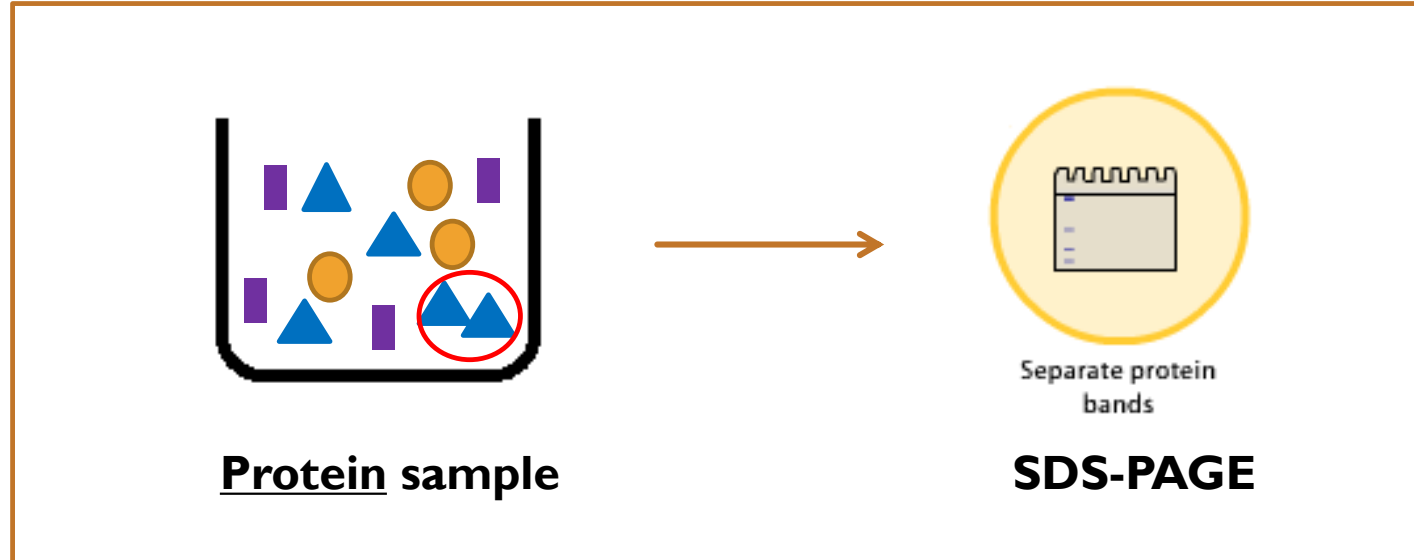
3. Marking target protein using a proper primary and secondary antibody to visualize.



1st Phase: SDS-PAGE

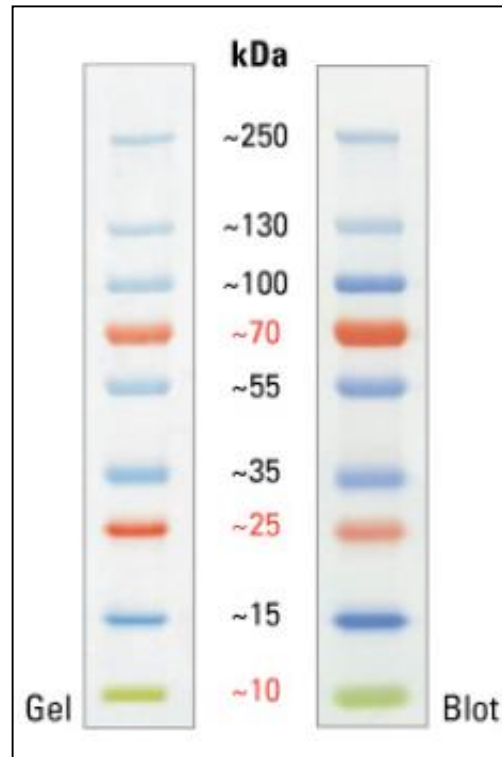
1st Phase: SDS-PAGE

- A protein sample is subjected to polyacrylamide gel electrophoresis.



- To confirm the separation of the sample use:

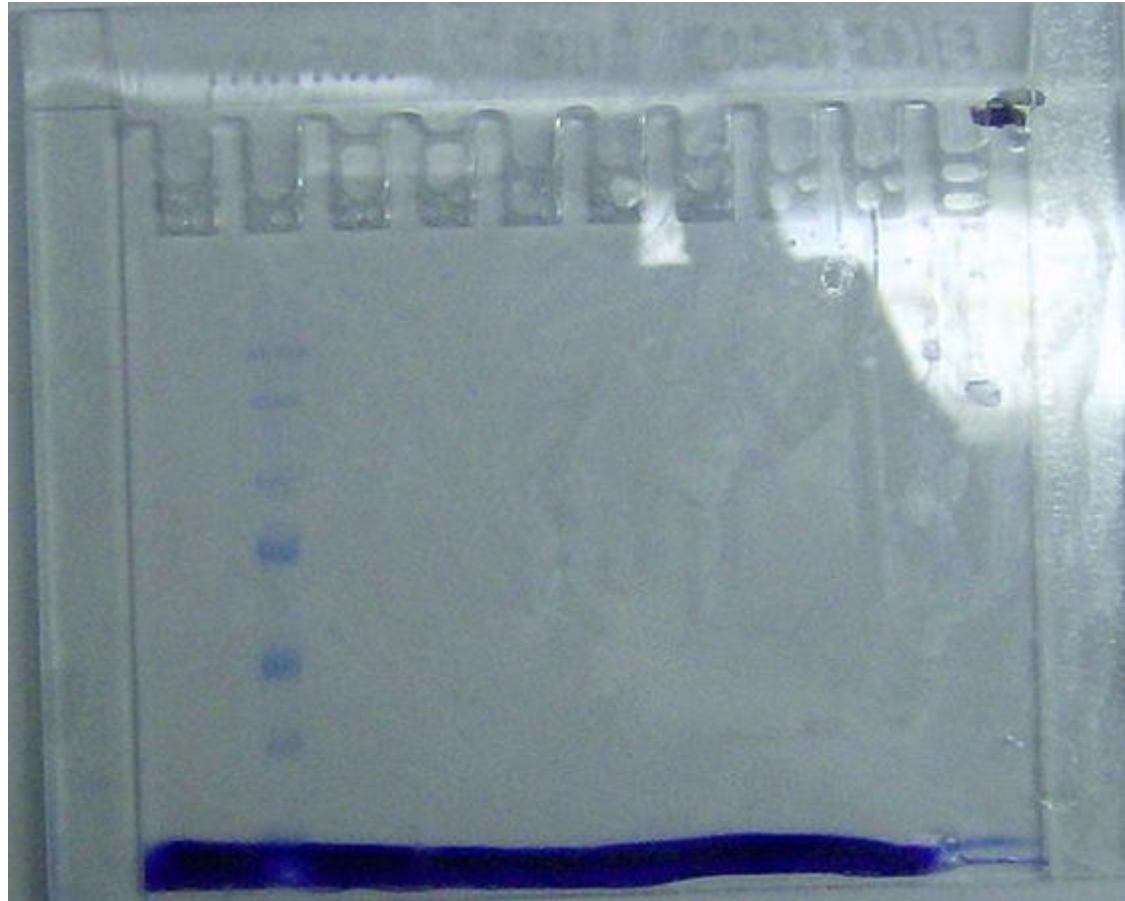
- 1- Replica of the gel and stain it as usual.
- 2- Prestained marker.
- 3- Ponceau S.



Prestained marker

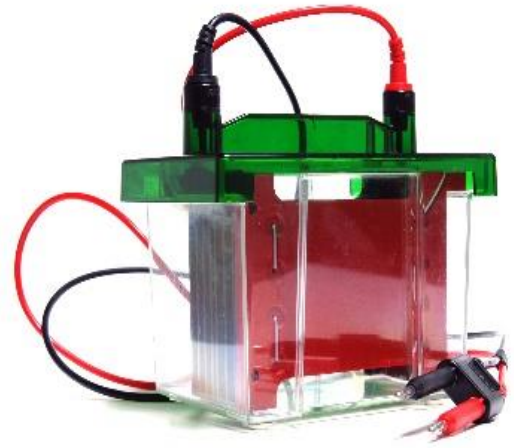
Figure: Protein Ladder is a mixture of nine (9) blue-, orange- and green-stained proteins (10 to 250kDa) for use as size standards in protein electrophoresis (SDS-PAGE) and Western blotting.

Prestained marker



Ponceau S

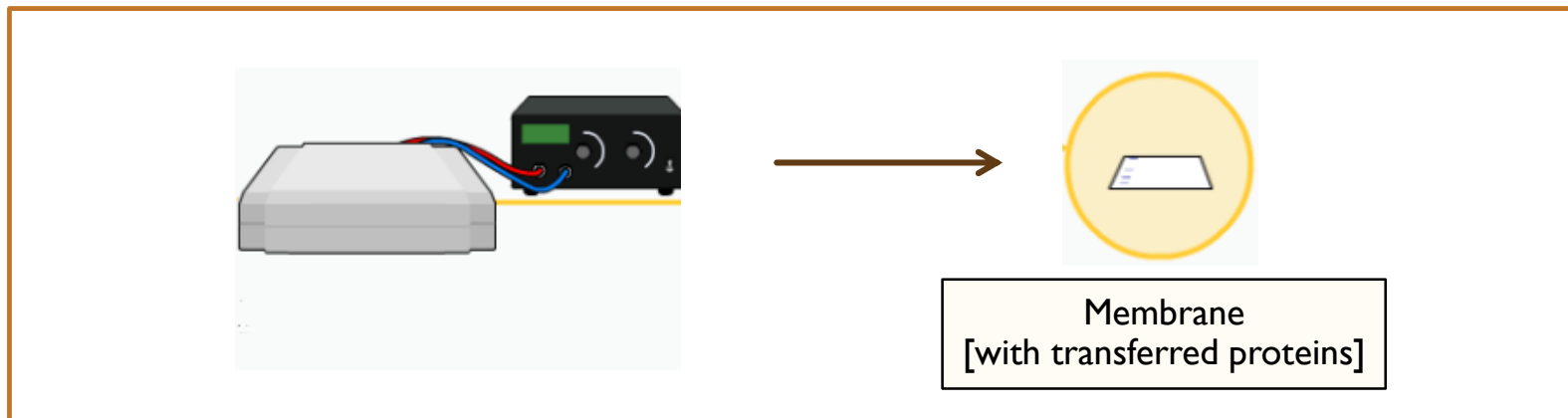




2nd Phase: Electroblotting

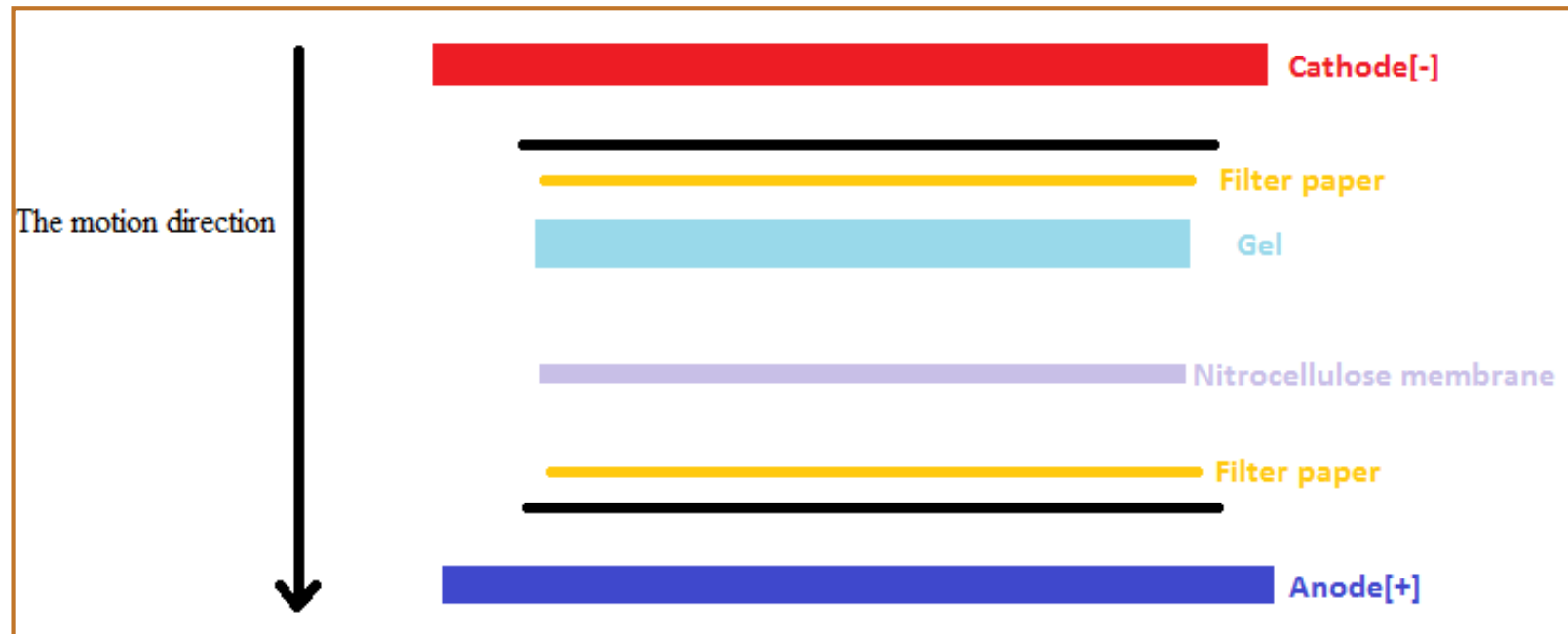
2nd Phase: Electroblotting

- After that the gel is placed over a sheet of nitrocellulose, the protein in the gel is electrophoretically transferred to the nitrocellulose membrane. “transfer step [Electroblotting]”

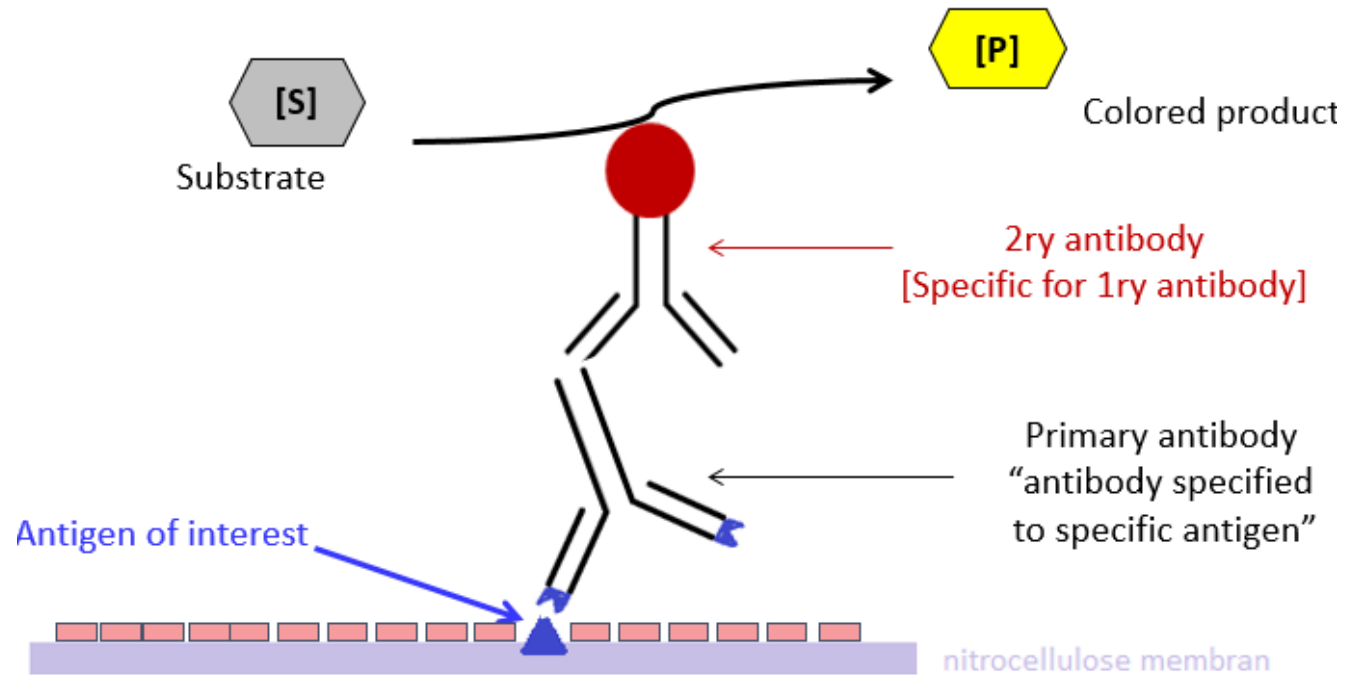


- Methods.

Transfer sandwich

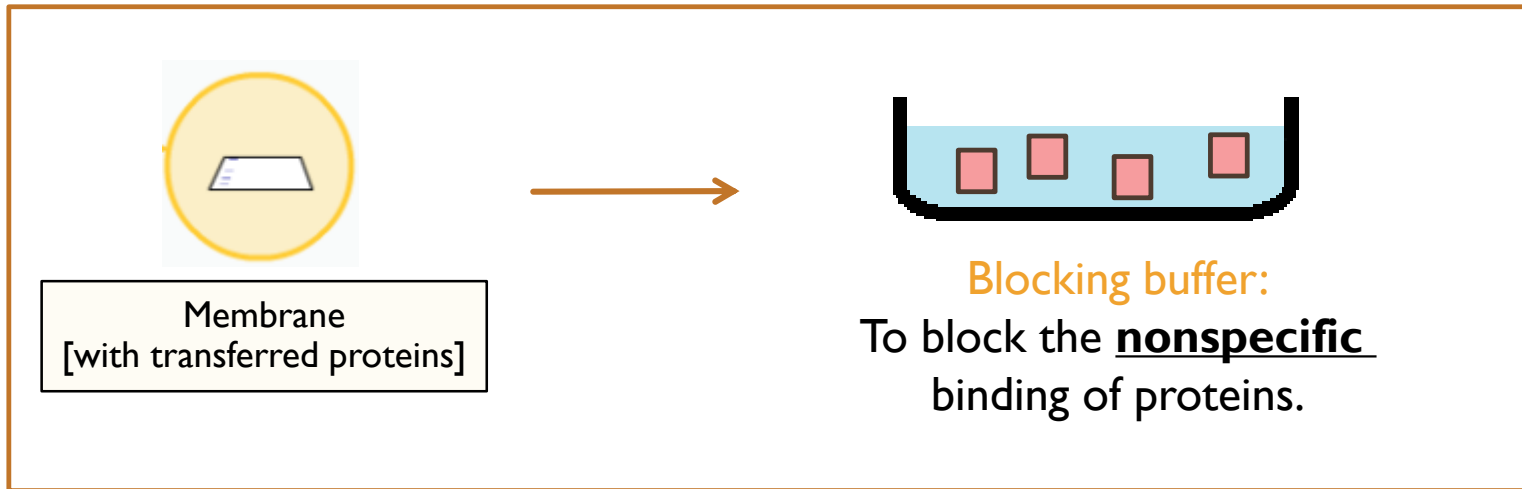


Note: The filter papers, gel and nitrocellulose membrane will be soaked in transfer buffer.

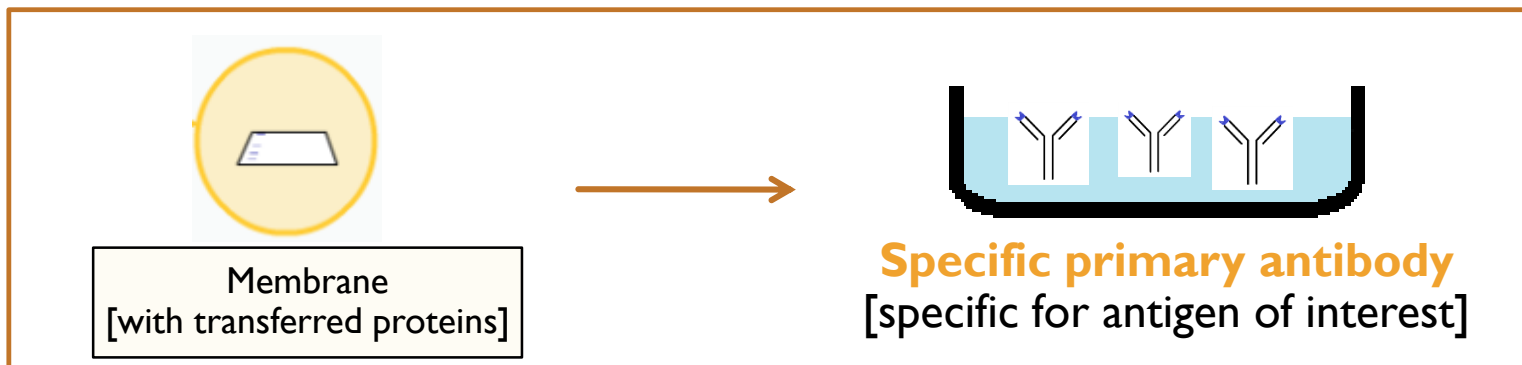


3rd Phase: Marking target protein to visualize

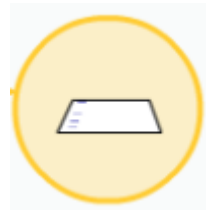
- The nitrocellulose is then soaked in blocking buffer.



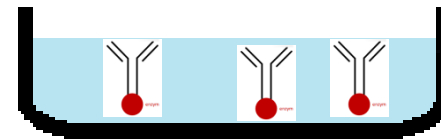
- The nitrocellulose is then incubated with the specific primary antibody for the protein of interest.



- The nitrocellulose is then washed and incubated with a second antibody, which is specific for the first antibody [primary-antibody].

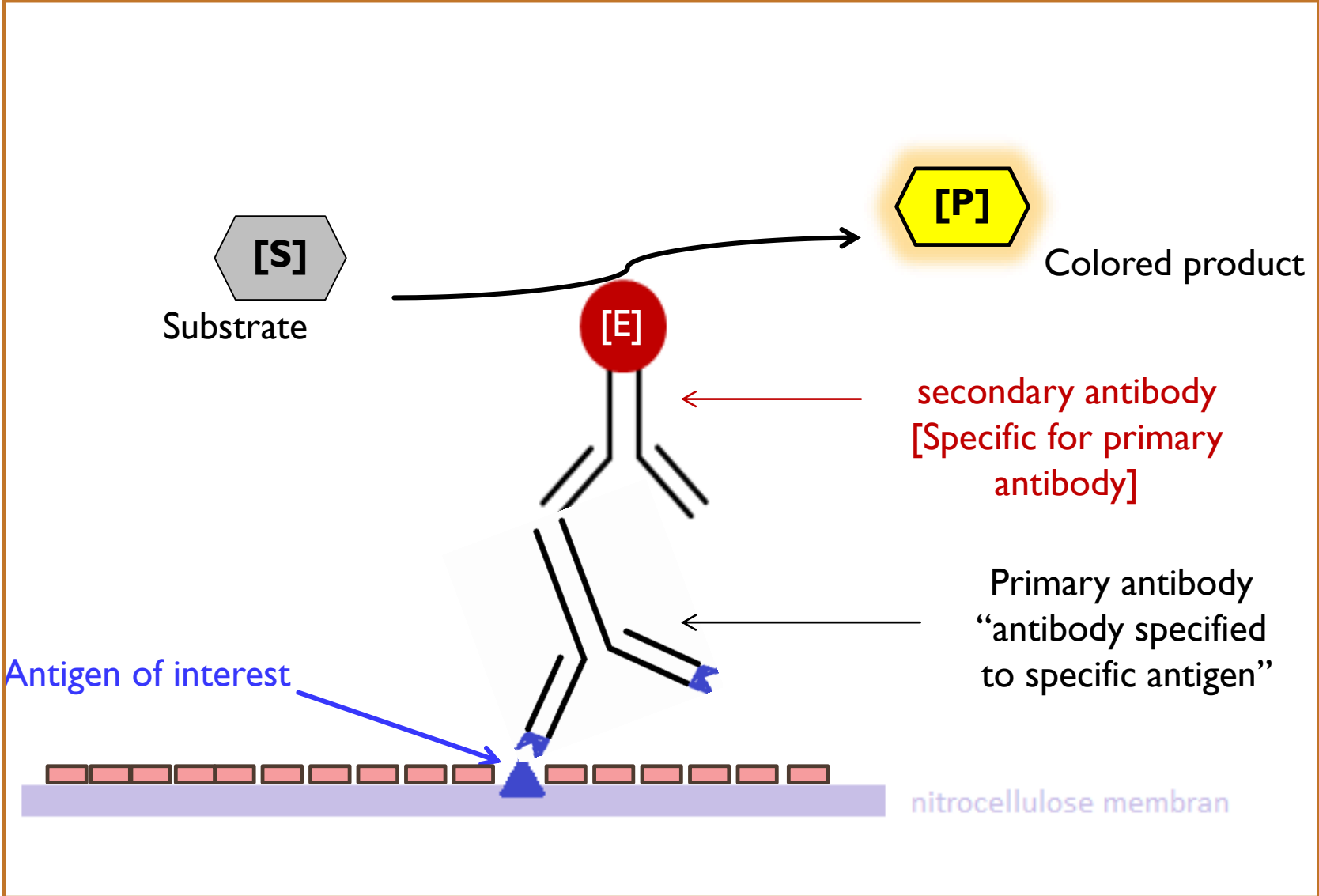


Membrane
[with transferred proteins
+primary antibody]

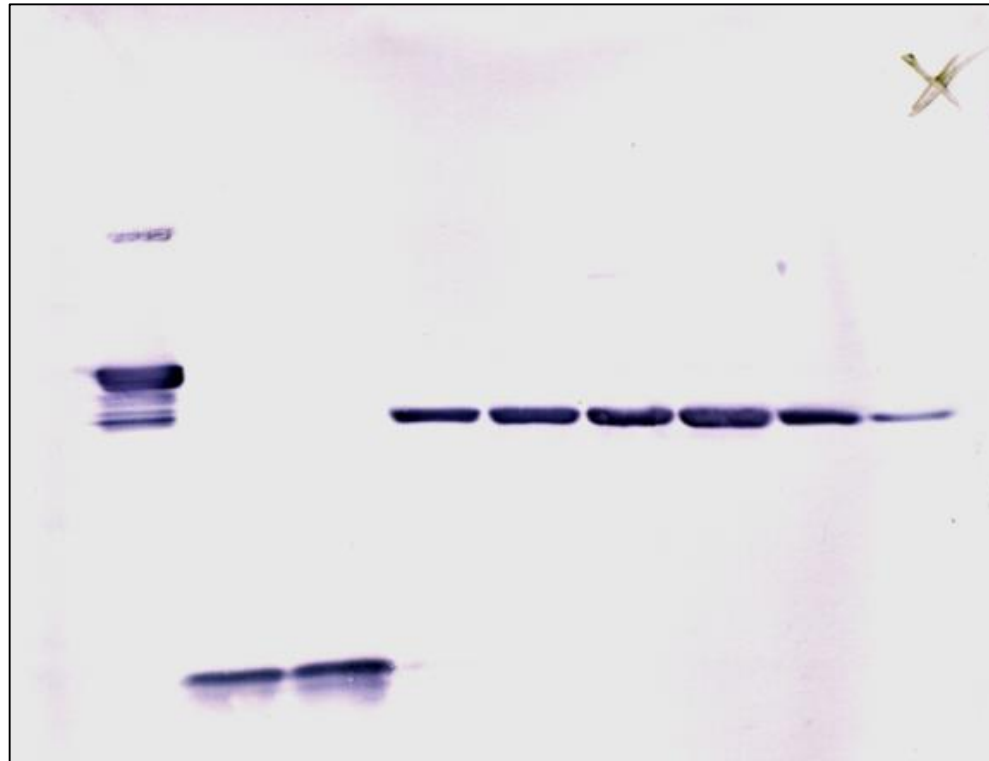


Secondary antibody
[Specific for primary antibody]

Detection of specific protein using Western bolt



- Thus the molecular weight and amount of the desired protein can be characterized from a complex mixture of proteins by western blotting.



Supporting materials:

- **Performing western blot:**

<http://www.youtube.com/watch?v=VgAuZ6dBOfs>

- **Ponceau S Staining:**

http://www.youtube.com/watch?v=Jj_37cDsO7o