WESTERN BLOT

BCH462- Practical

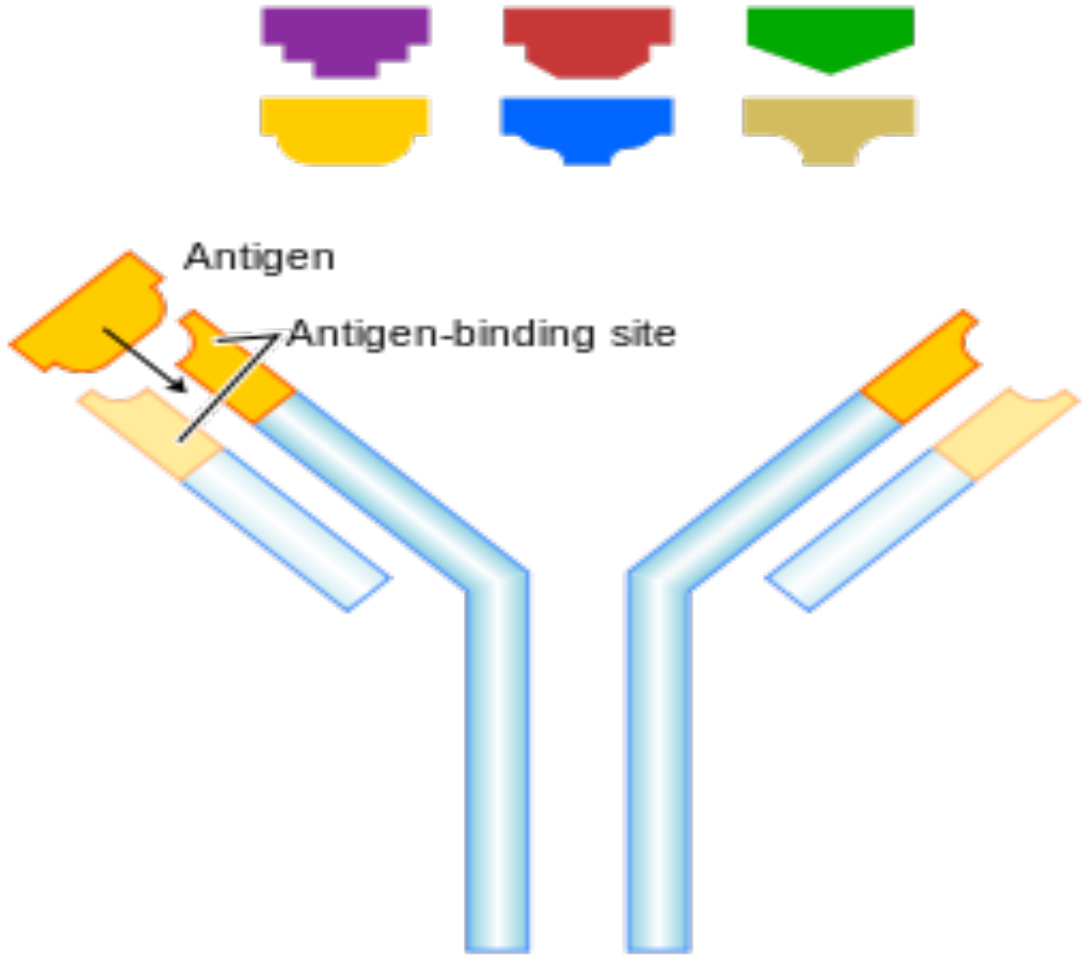
Immunoassay:

- What is Antigen [Ag] ?

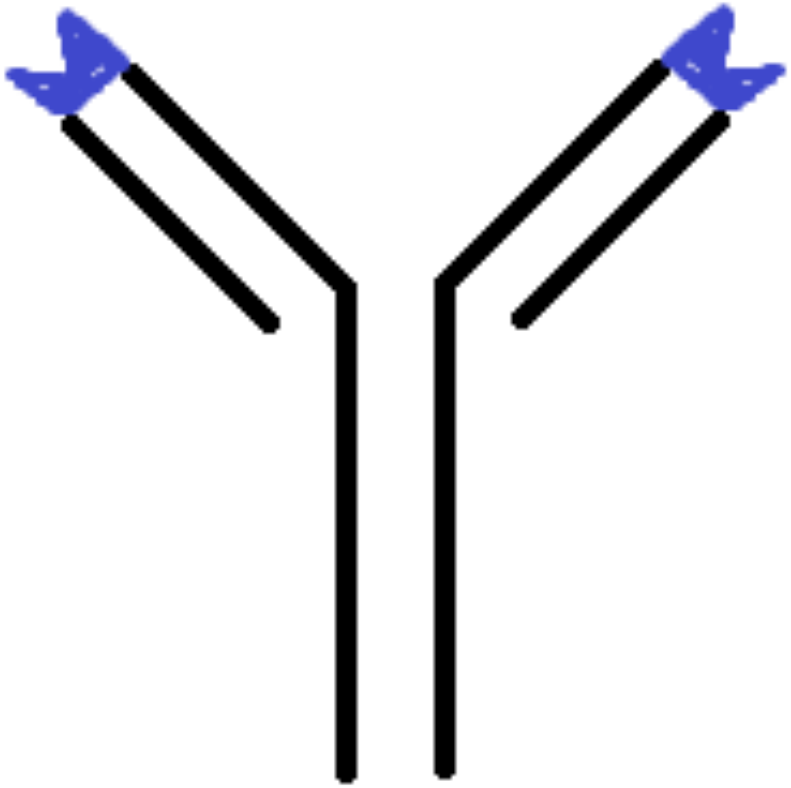
- What is Antibody [Ab] ?

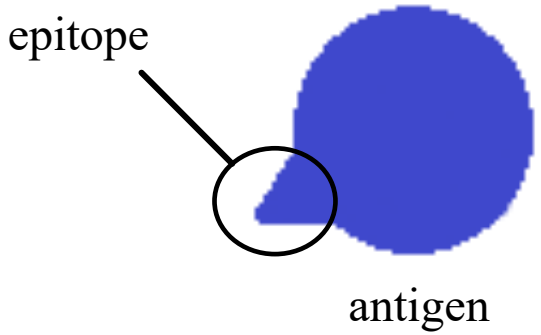
- **Immunoassay:** is a test that uses the highly specific and selective antigen-antibody reactions forming antibody and antigen complexes [immuno-complexes] as a means of generating measurable results

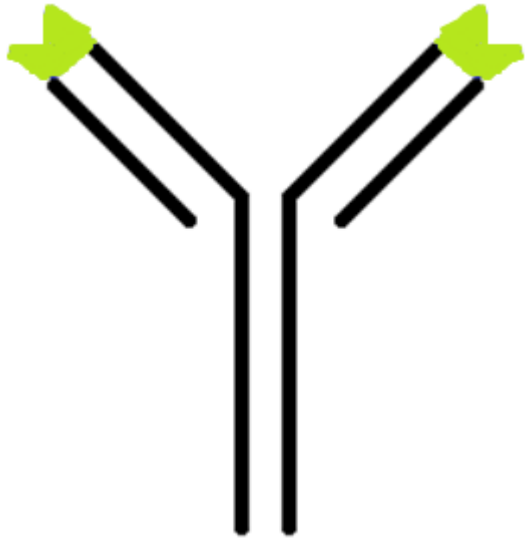
Antigens



Antibody

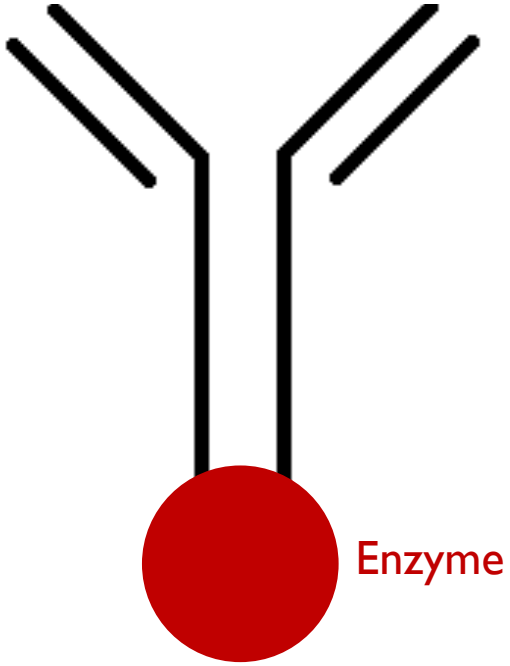

antibody


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 / florescence indicate the presence of this specific protein.
- Application ?

PRACTICAL PART

Aim:

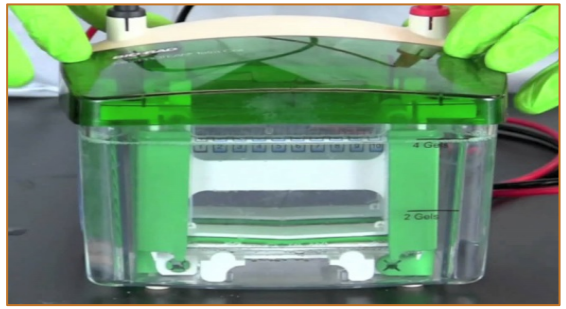
- To understand how proteins (antigens) can be analysed using antibodies raised against these proteins by Immunoblotting technique.
- To preform the steps of western-blot technique to detect the specific protein.

Principle:

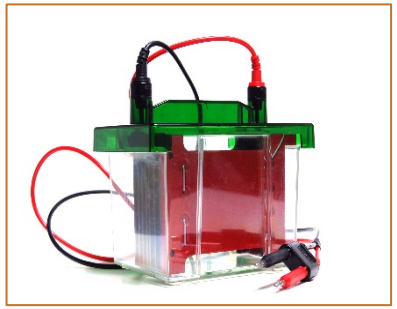
- The mixture of proteins is separated based on **molecular weight**.
- These results are then electro-transferred to solid support producing a band for each protein.
- The transferred protein is detected by incubating the gel **with specific primary antibody** to the protein of interest, **secondary antibody labelled with an enzyme (or Fluorescent-dye) which target the primary antibody**, and substrate which in the end you will get coloured product (or florescence)
- The colour/ florescence 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

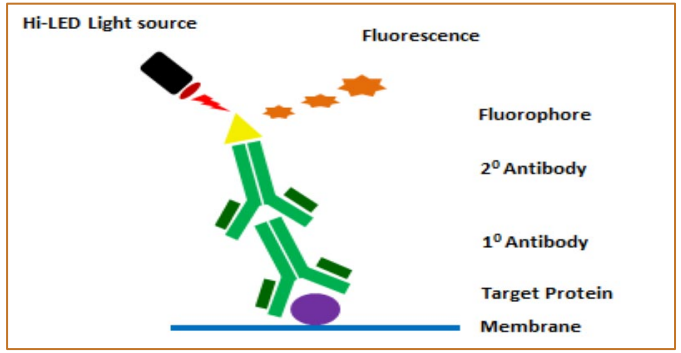
The technique uses three elements to accomplish this task



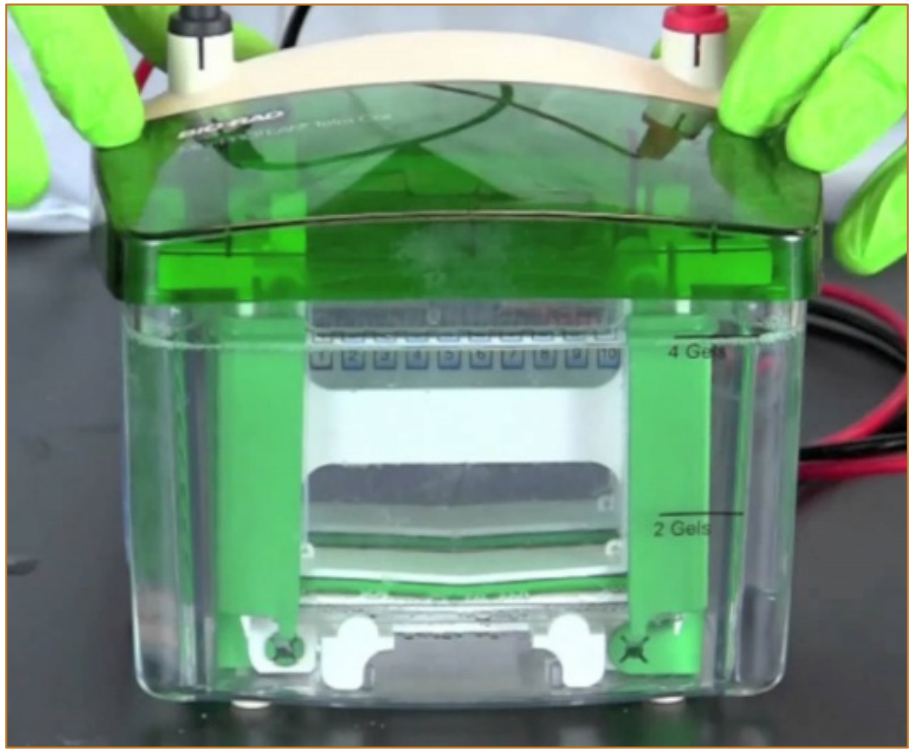
1. Separating the sample mixture by size using SDS-PAGE.



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



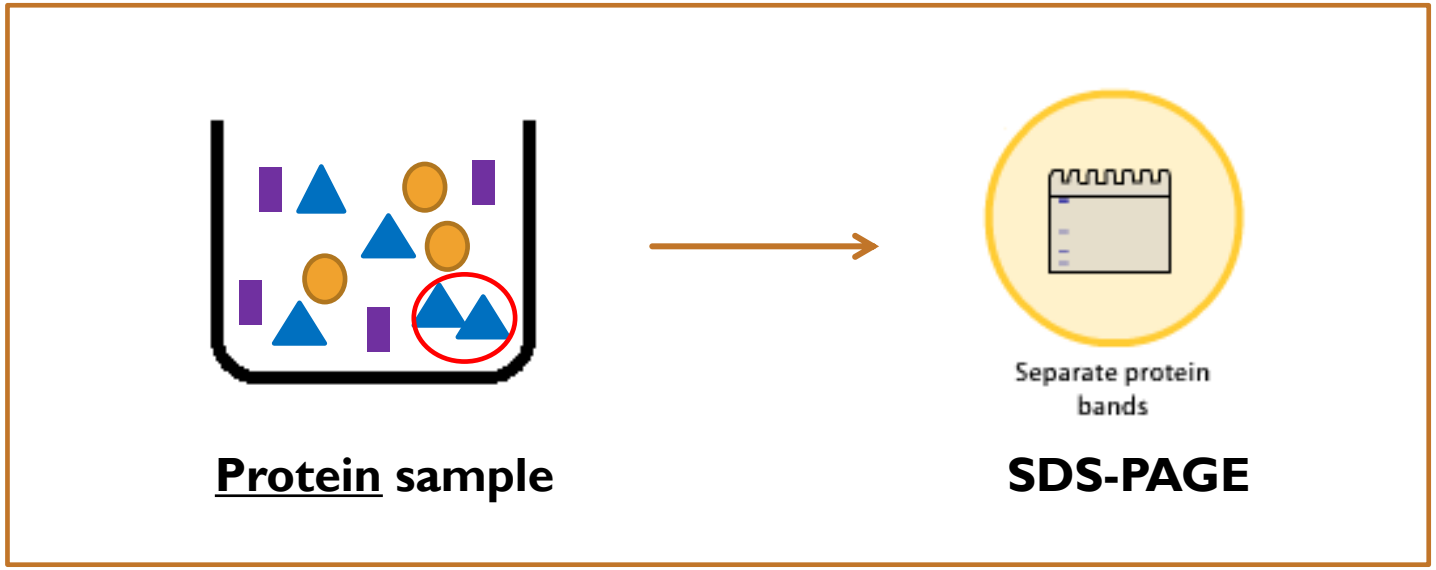
3. Marking target protein using a proper primary and secondary antibody to visualize.



I st Phase: SDS-PAGE

1st Phase: SDS-PAGE

- A protein sample is subjected to polyacrylamide gel electrophoresis.

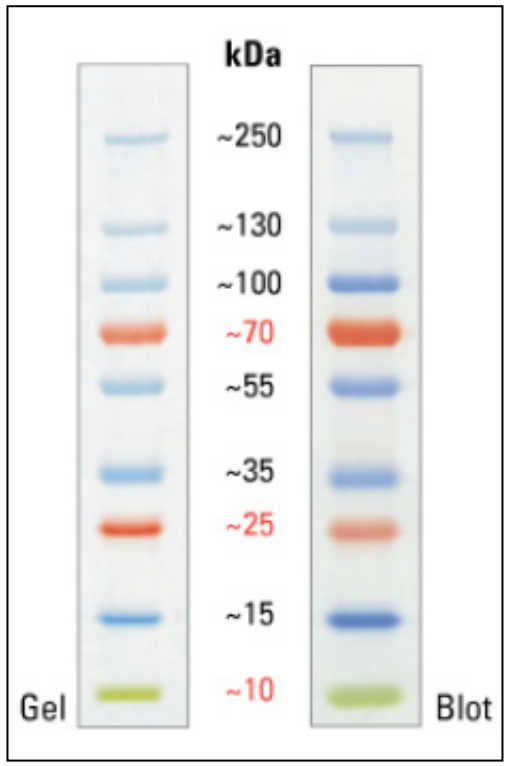


- To confirm the separation of the sample use:

1- Replica of the gel and stain it as usual.

2- Prestained marker.

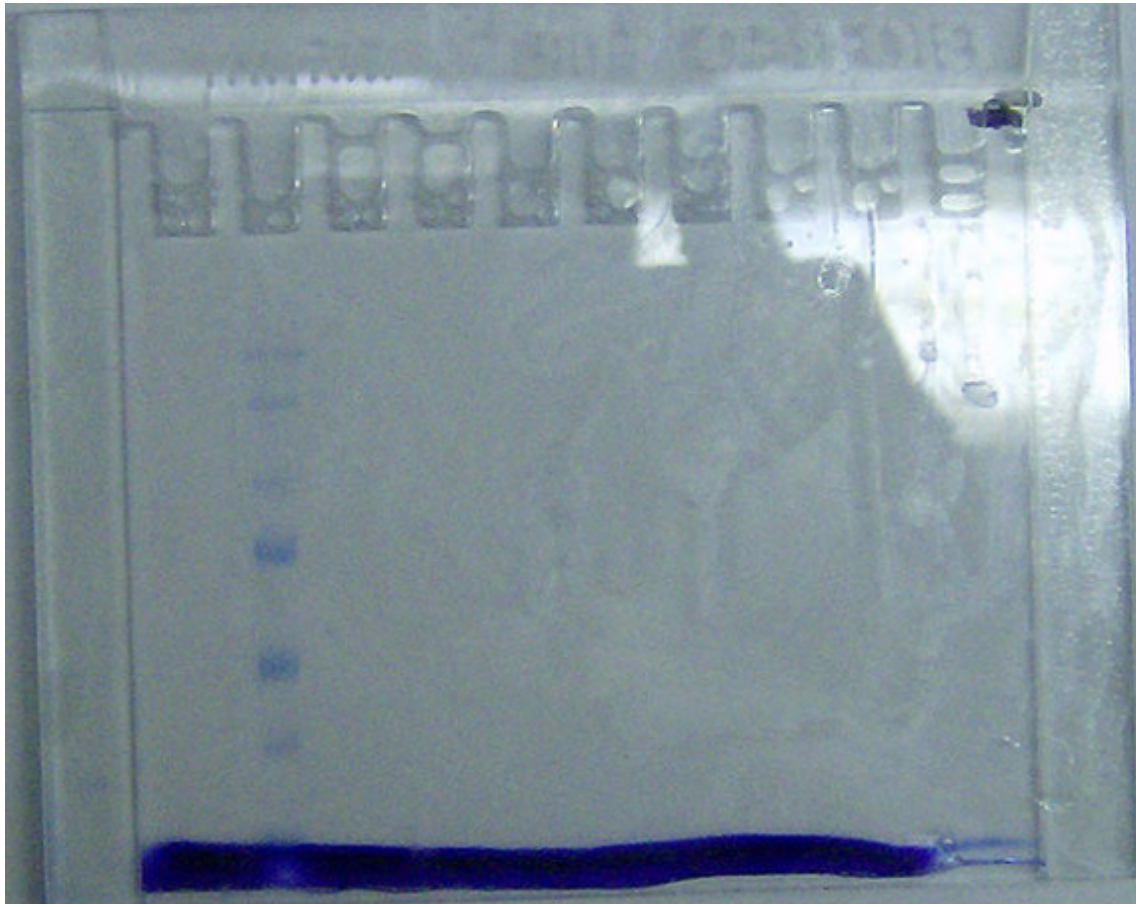
3- Ponceau S.



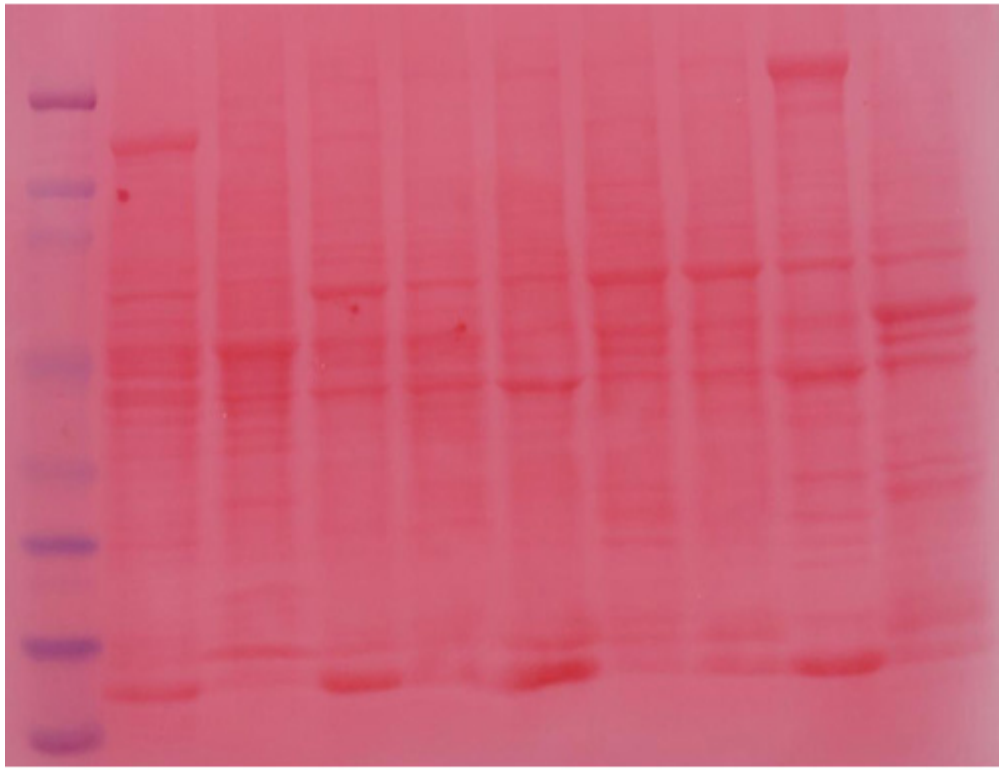
Prestained marker

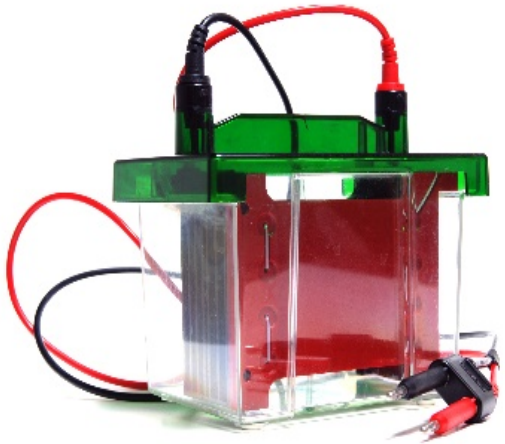
Figure: Protein Ladder is a mixture of nine (9) blue-, orange- and green-stained proteins (10 to 250kDa) for use as size standards in protein electrophoresis (SDS-PAGE) and Western blotting.

Prestained marker



Ponceau S.





2nd Phase: Electroblotting

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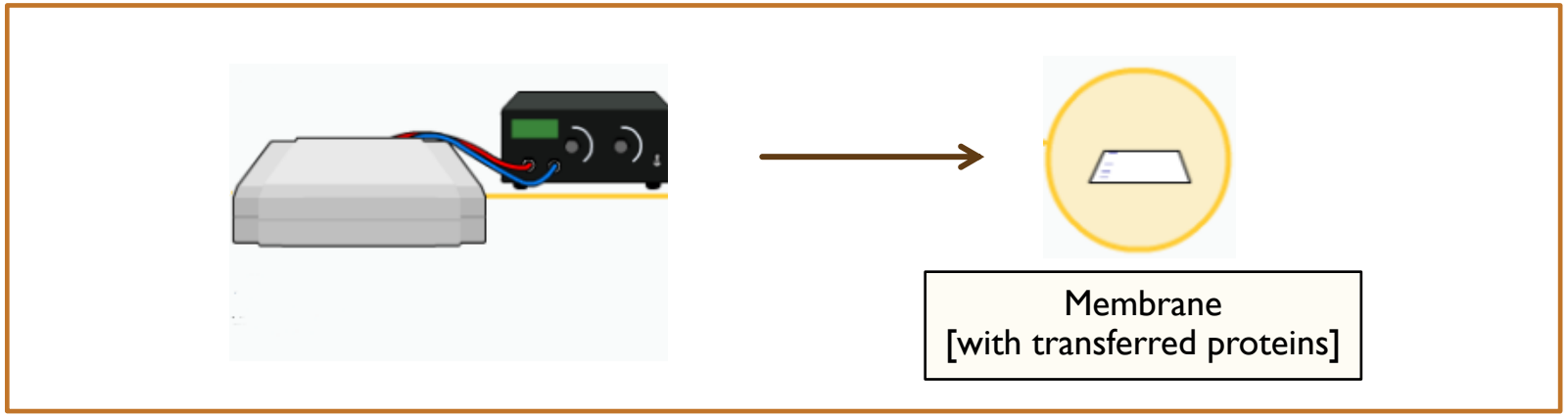
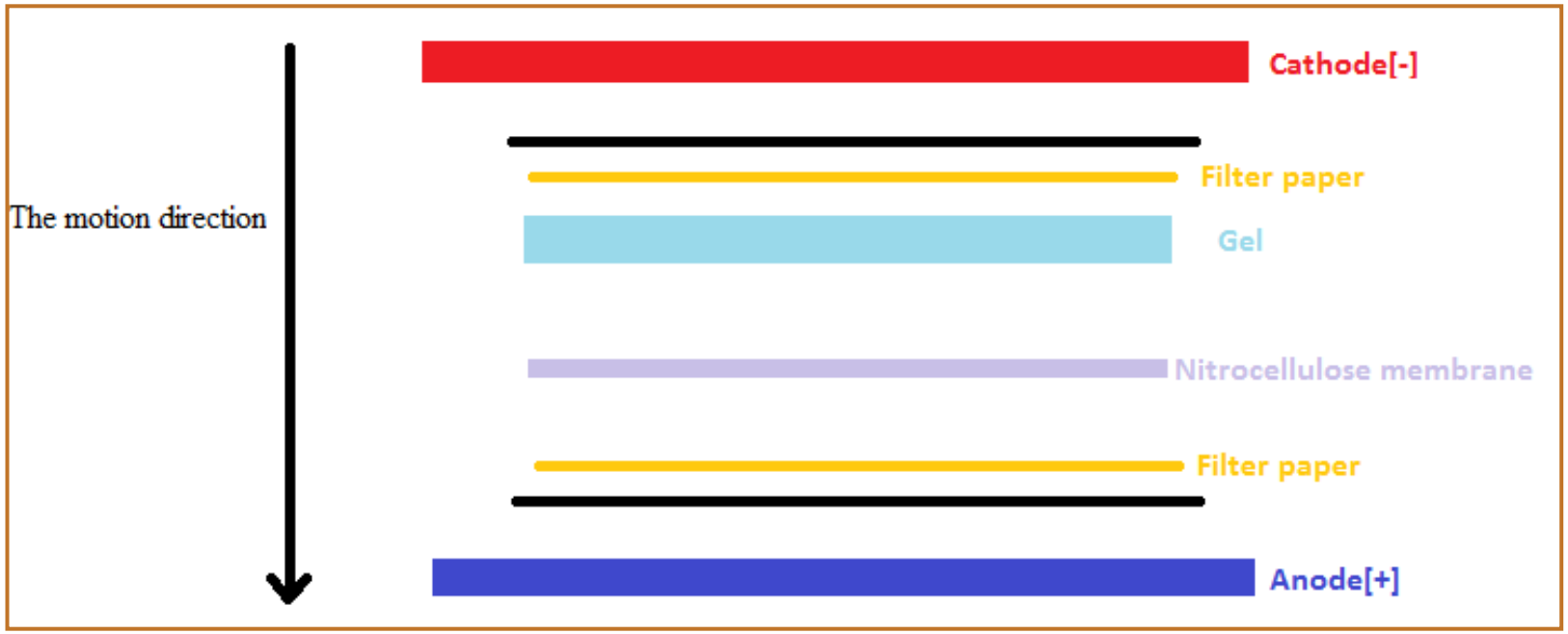
- After that the gel is placed over a sheet of PVDF , the protein in the gel is electrophoretically transferred to the PVDF membrane. "transfer step [Electroblotting]"

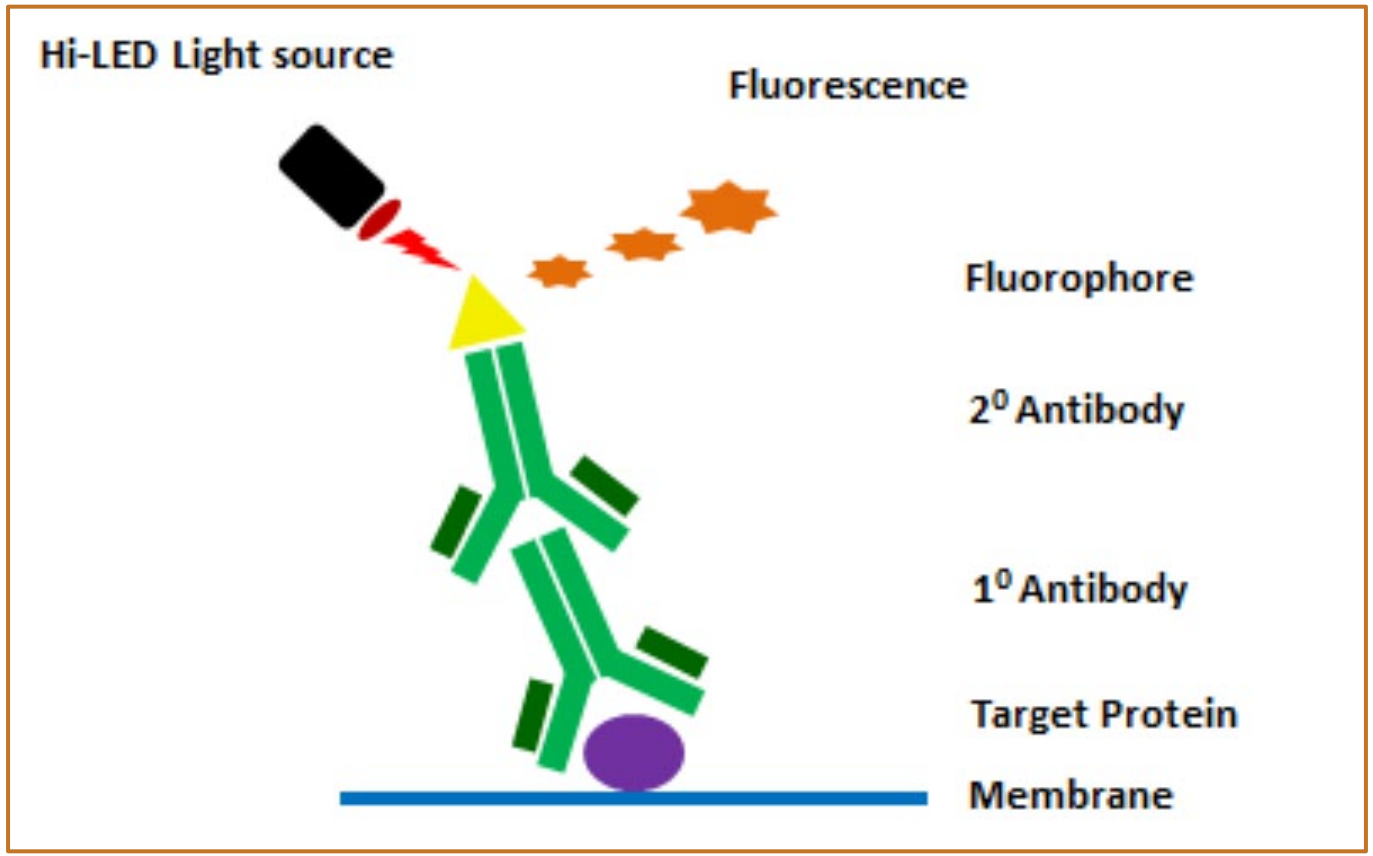


- Methods.

Transfer sandwich

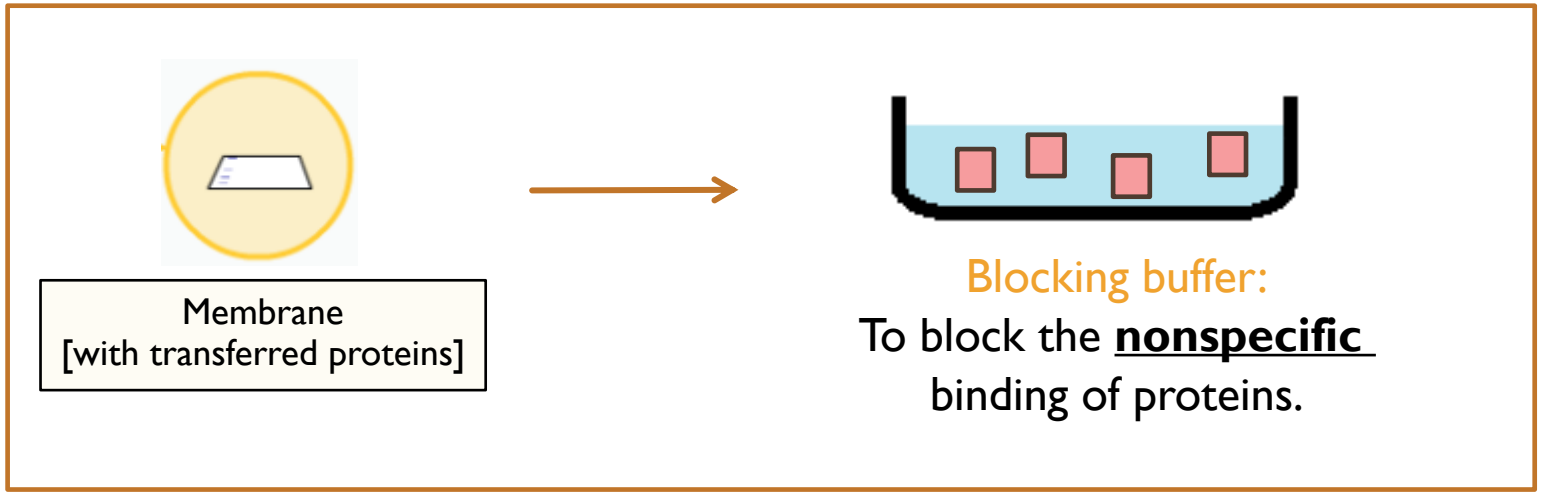


Note: The filter papers, gel and PVDF membrane will soaked in transfer buffer.

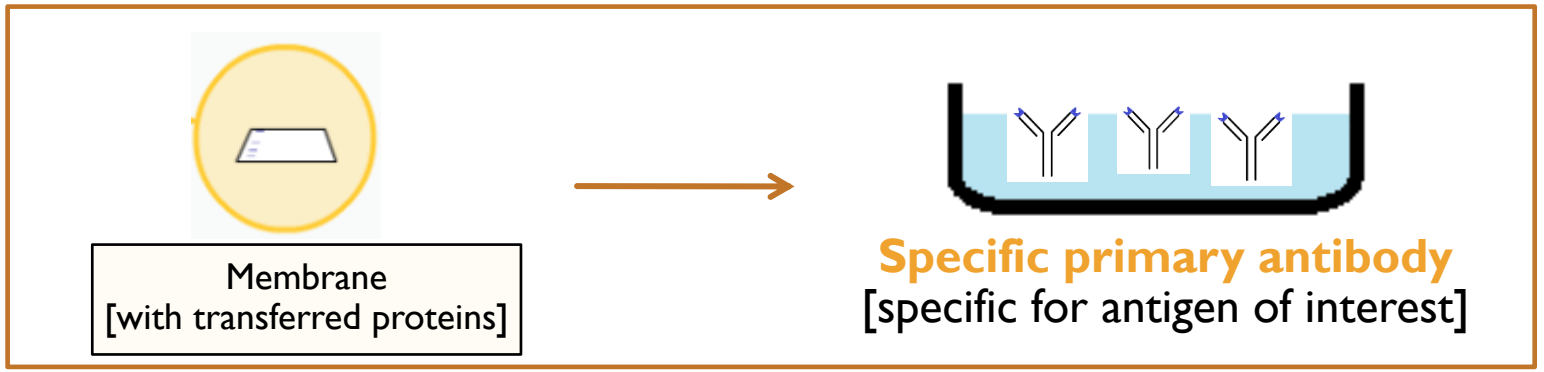


3rd Phase: Marking target protein to visualize

- The PVDF is then soaked in blocking buffer.

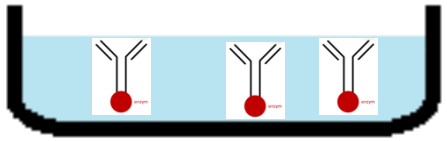


- The PVDF is then incubated with the specific primary antibody for the protein of interest.



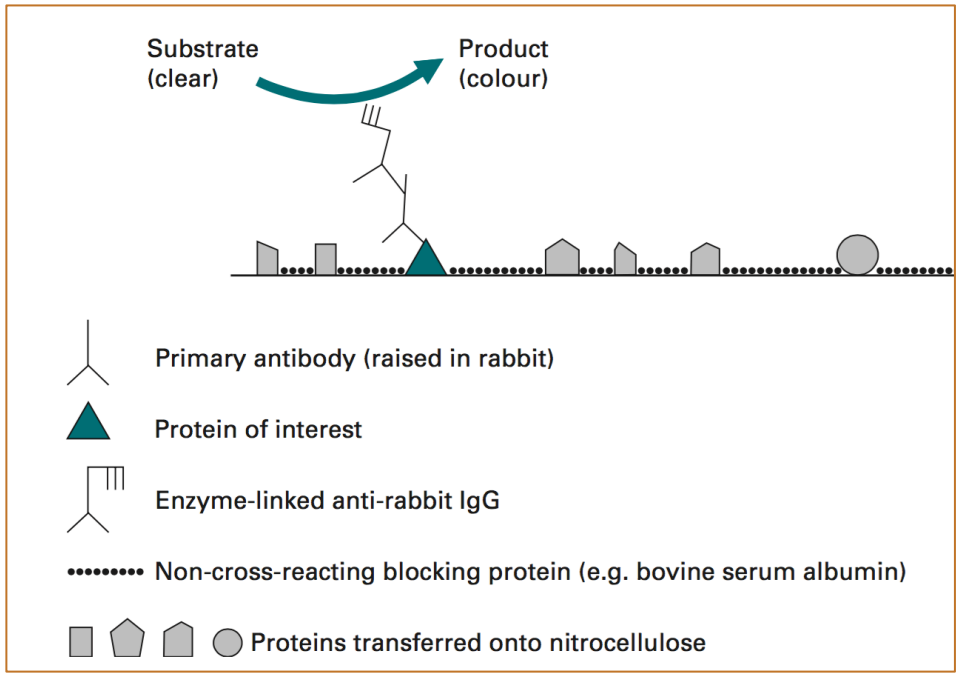
- The PVDF is then washed and incubated with a second antibody, which is specific for the first antibody [primary–antibody].

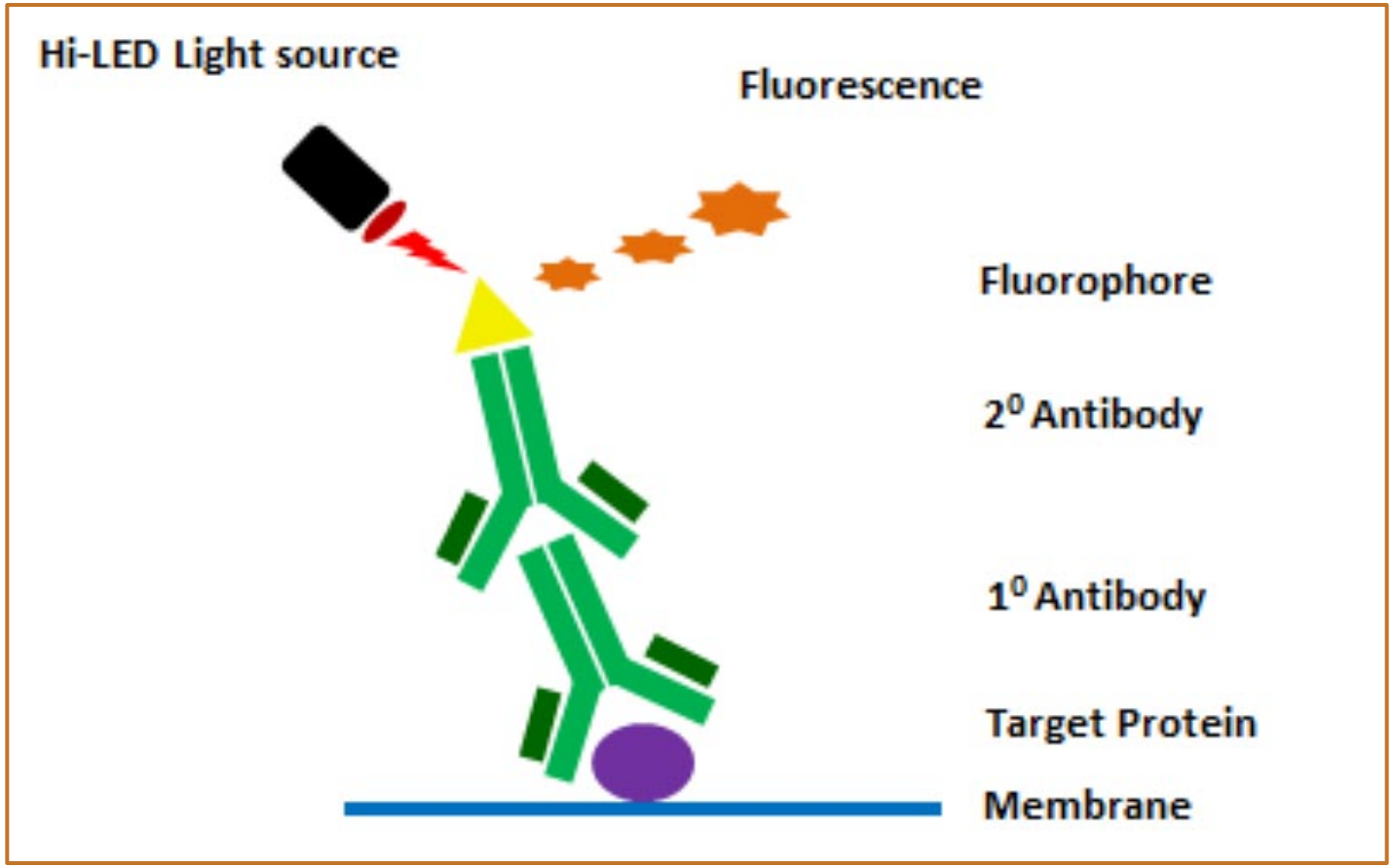
Membrane
[with transferred proteins +primary antibody]



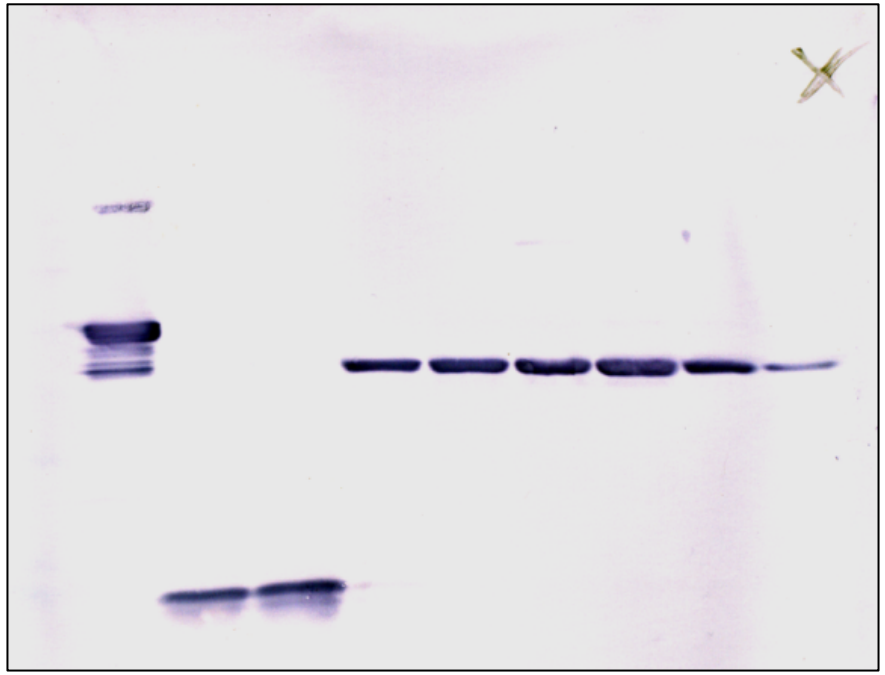
Secondary antibody
[Specific for primary antibody]

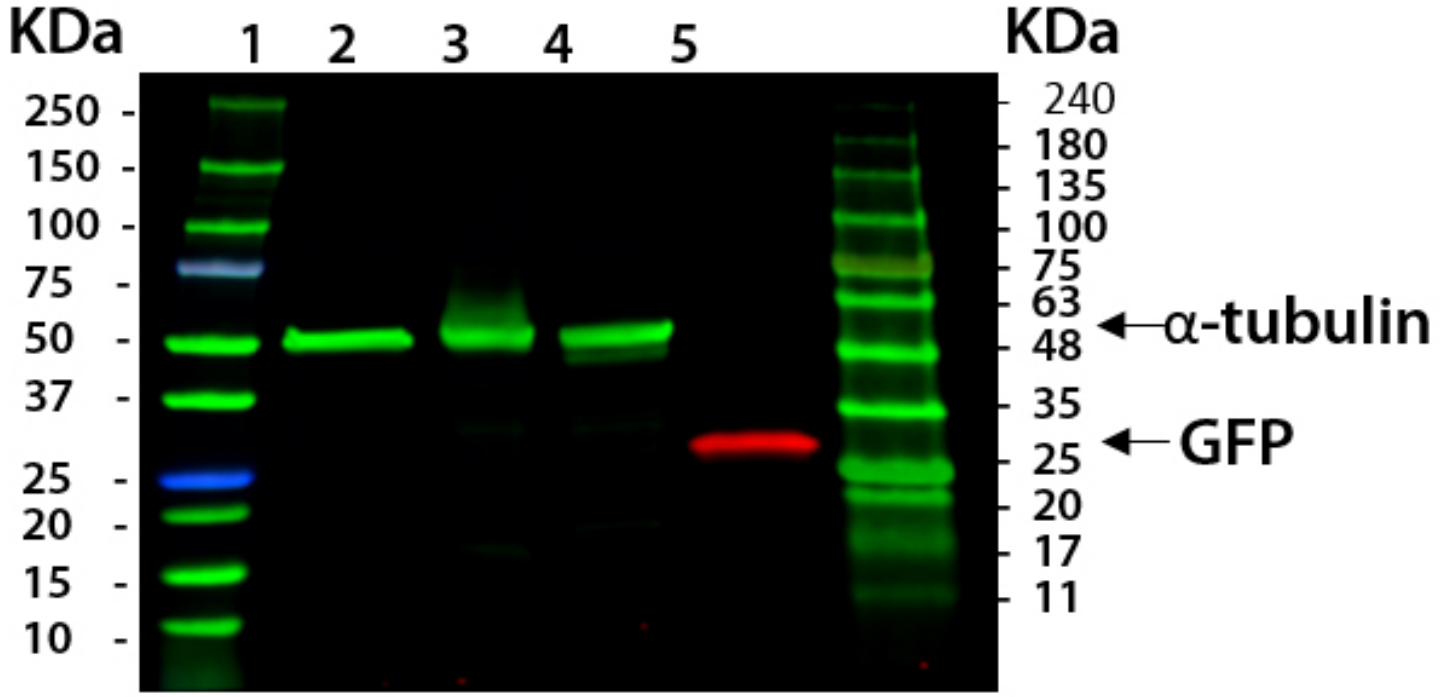
Detection of specific protein using Western bolt





- 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