Protein immunoblotting
(Western blotting)

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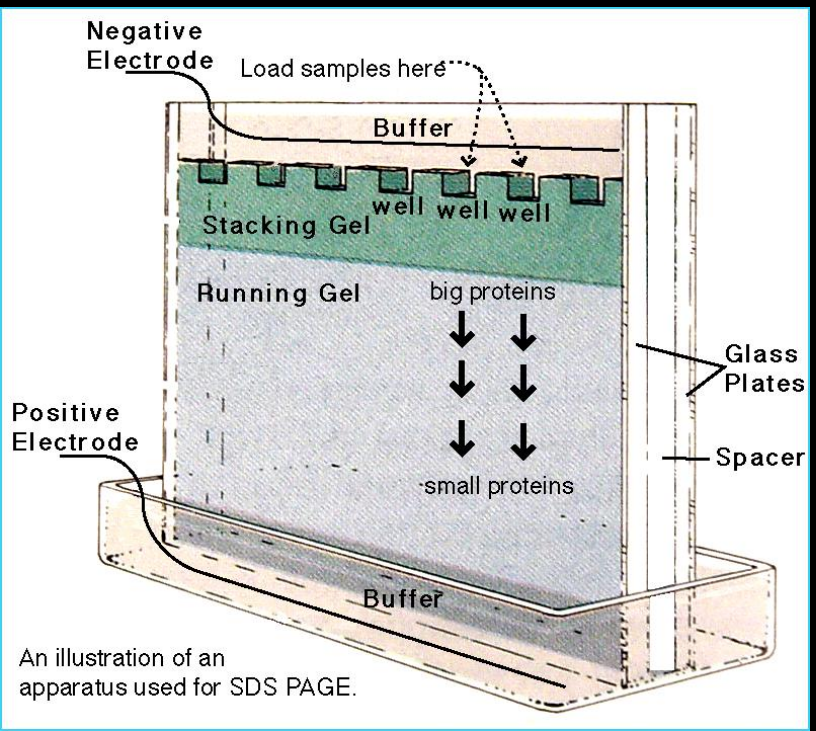
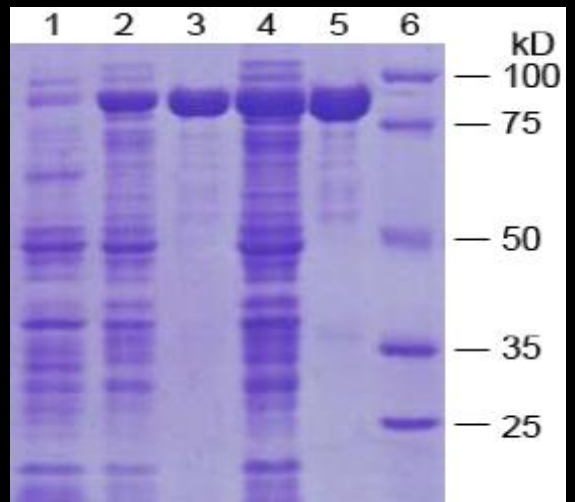
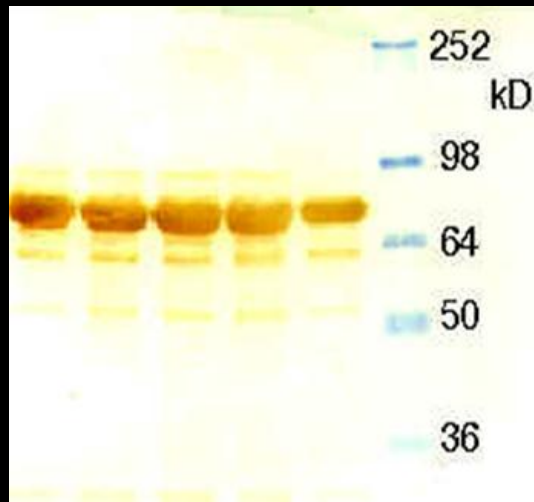


An illustration of an apparatus used for SDS PAGE.





Western blotting

-It is an analytical technique used to detect specific proteins in a cell, tissue, organ, or body fluid. The technique depends on the reaction of an antibody with a protein that is immobilized on a thin membrane.

-This technique can be used to identify a target protein in a complex mixture, and to measure it's expression level.

-The method originated in the laboratory of George Stark at Stanford

-The name *Western blot* was given to the technique by W. Neal Burnette

-Southern blot?
-Northern blot?

-The transfer of DNA from agarose gel onto NC is called Southern blot

- The transfer of RNA from agarose gel onto NC is called Northern blot

Western blotting

-Identification of protein based on two distinguishing properties:-

1- Molecular weight

2- Antibody binding specificity

Western blotting carried out through the following steps:-

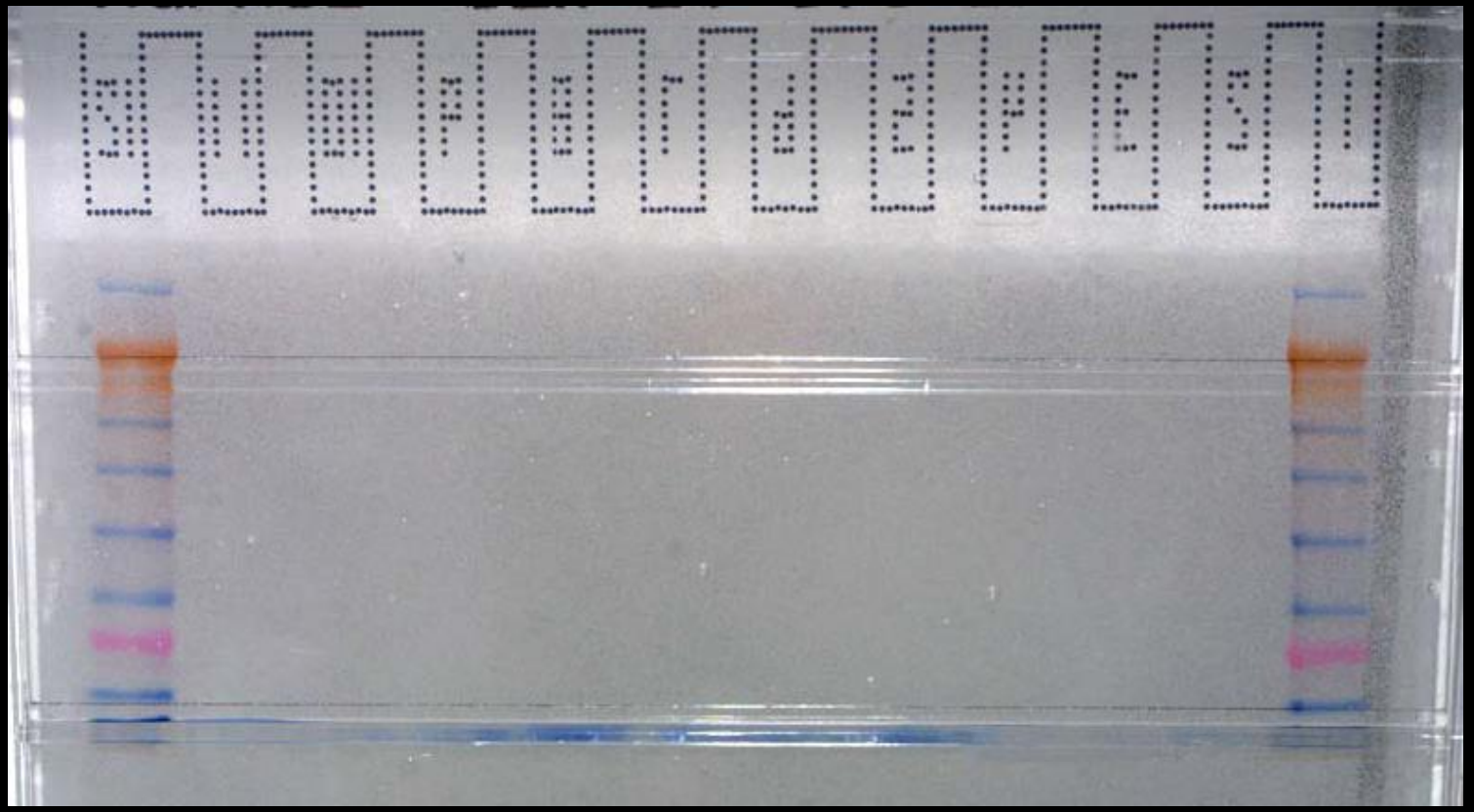
1- Sample preparation

2- SDS-PAGE to separate native proteins

3- Transfer of protein to a membrane (nitrocellulose or PVDF).

4- Detection of target proteins by specific antibodies

Sodium Dodecyl Sulfate-Polacrylamide Gel Electrophoresis (SDS-PAGE)



- Proteins usually have a net positive or negative charge (reflects the mixture of charged amino acids they contain)

-Protein will migrate at a rate that depends on its net charge and on its size and shape

-In the mid-1960s SDS polyacrylamide-gel electrophoresis (SDS-PAGE) was developed

-It uses a highly cross-linked gel of polyacrylamide as the inert matrix through which the proteins migrate

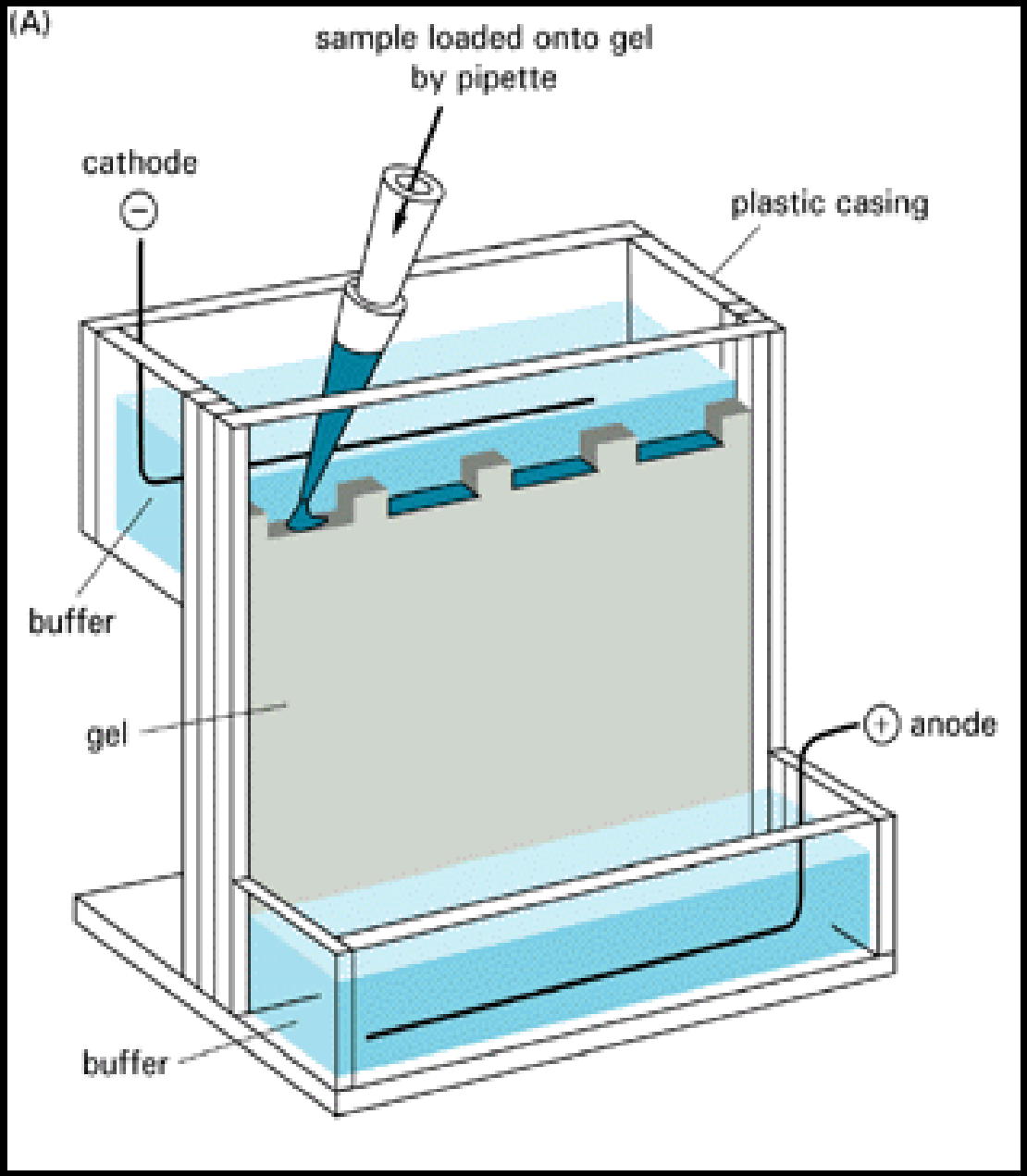
-The gel is usually prepared immediately before use by polymerization from monomers

-The pore size of the gel can be adjusted so that it is small enough to retard the migration of the protein molecules of interest

-The proteins in solution includes a powerful negatively charged detergent, sodium dodecyl sulfate (SDS)

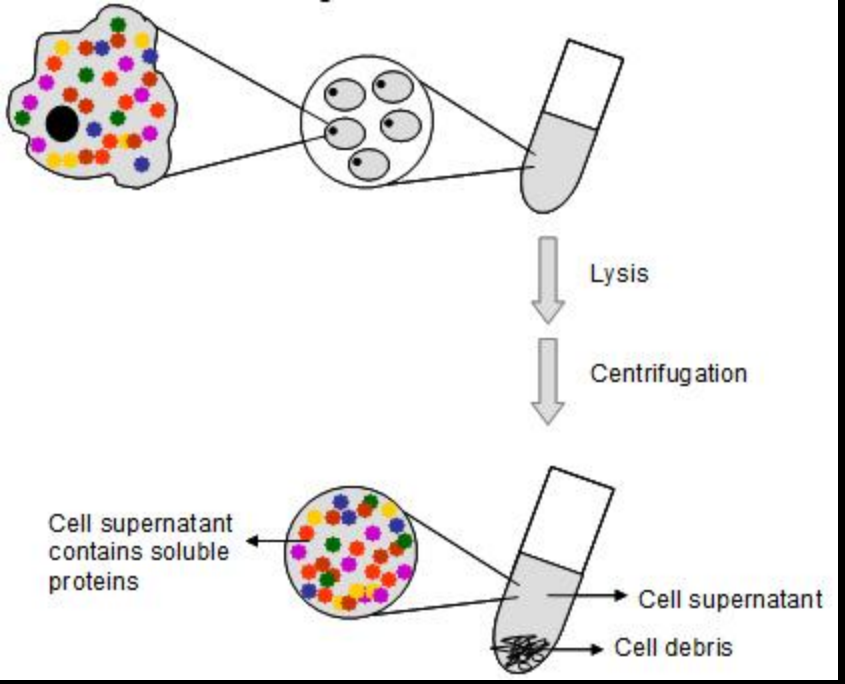
-SDS binds to hydrophobic regions of the protein molecules, causing them to unfold into extended polypeptide chain

-Mercaptoethanol (reducing agent) is usually added to break any S - S linkages in the proteins so that all of the constituent polypeptides in multi-subunit molecules can be analyzed separately

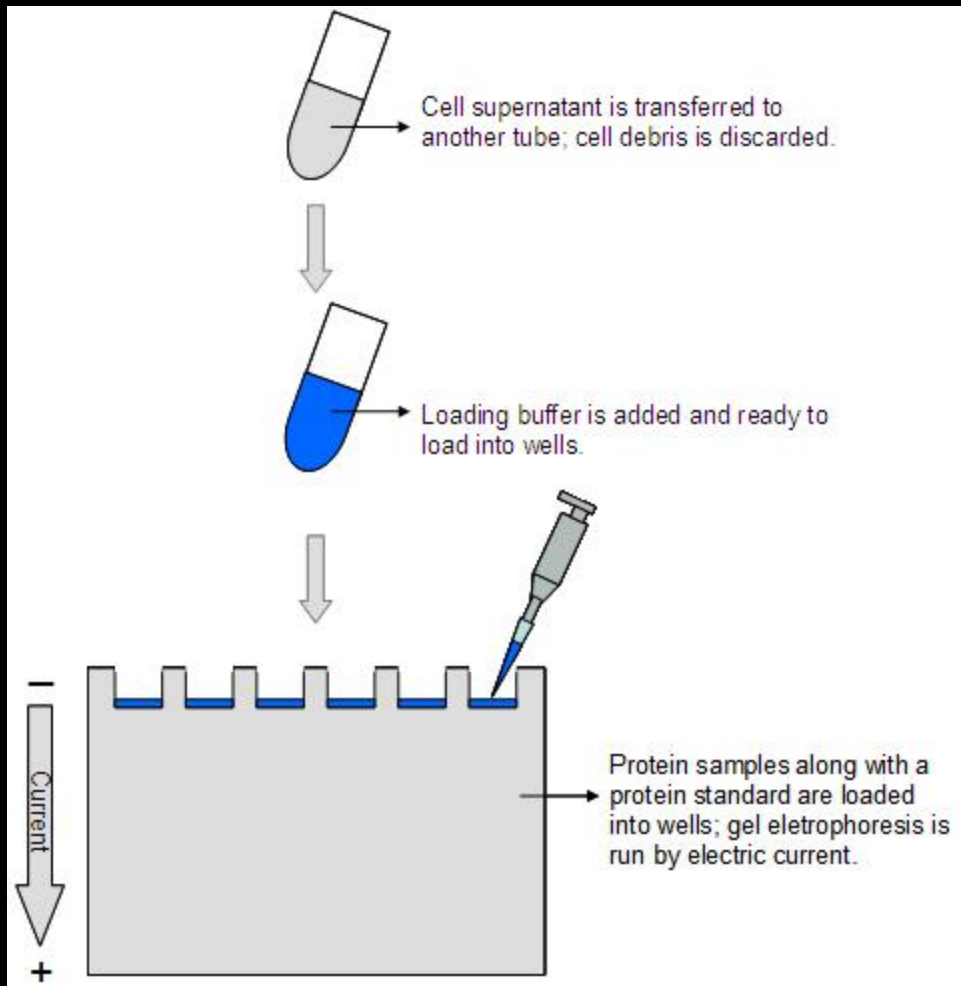


Sample loading onto the SDS-PAGE gel

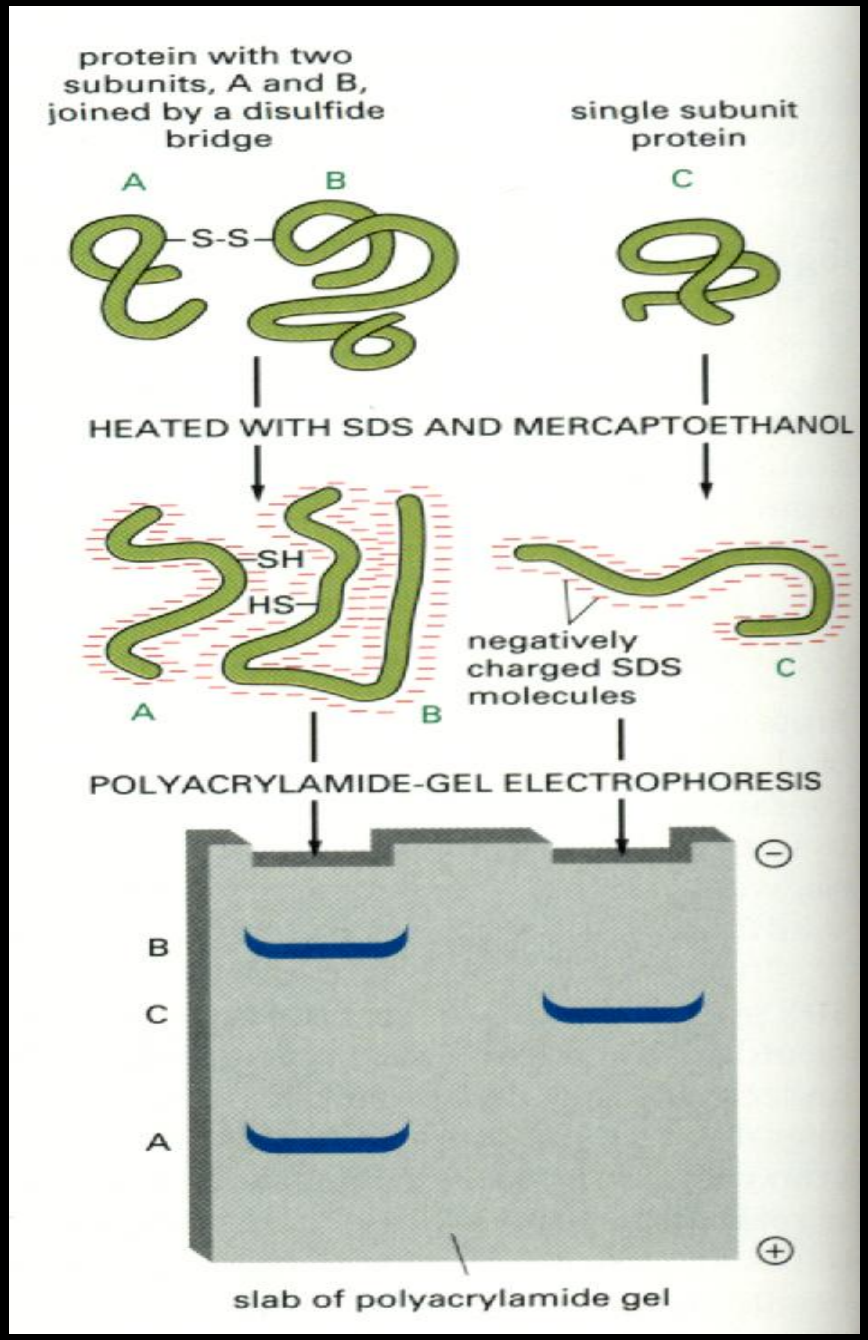
Sample preparation



SDS-PAGE



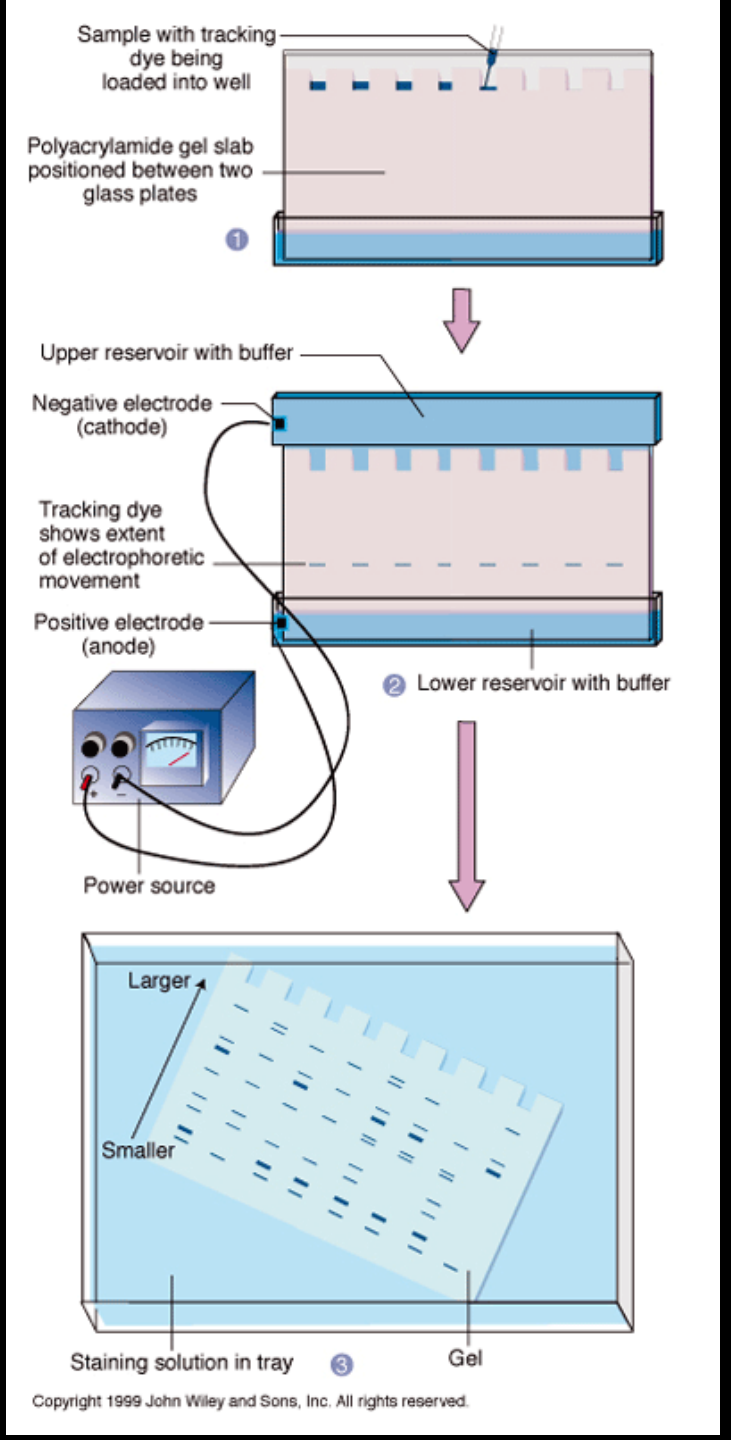
Large proteins are retarded much more severely than small ones.



1-Loading samples onto SDS-PAGE gel

2- Electrophoresis

3-Staining



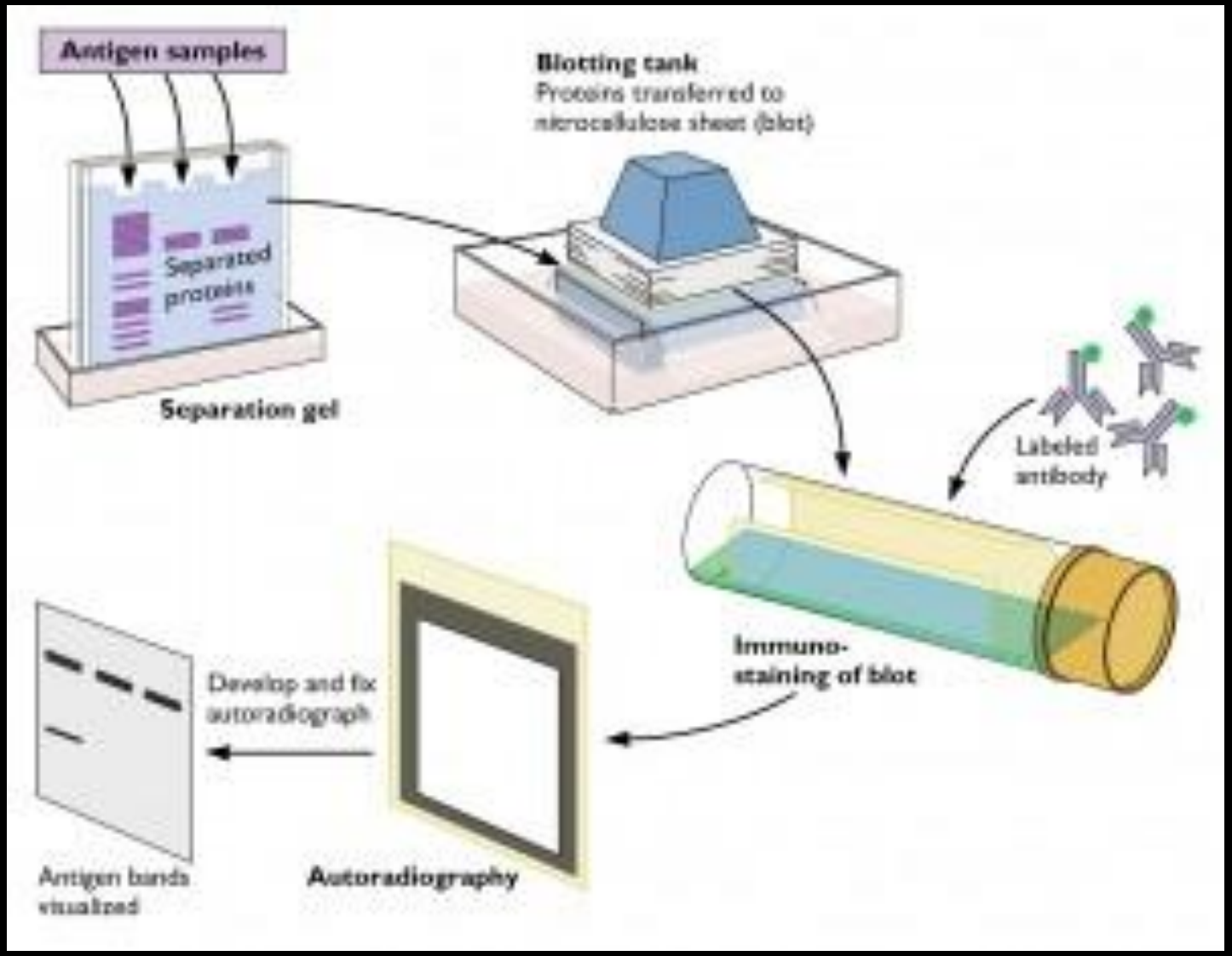
SDS-PAGE is a more powerful method of protein analysis

-Separate all types of proteins, including those that are insoluble in water

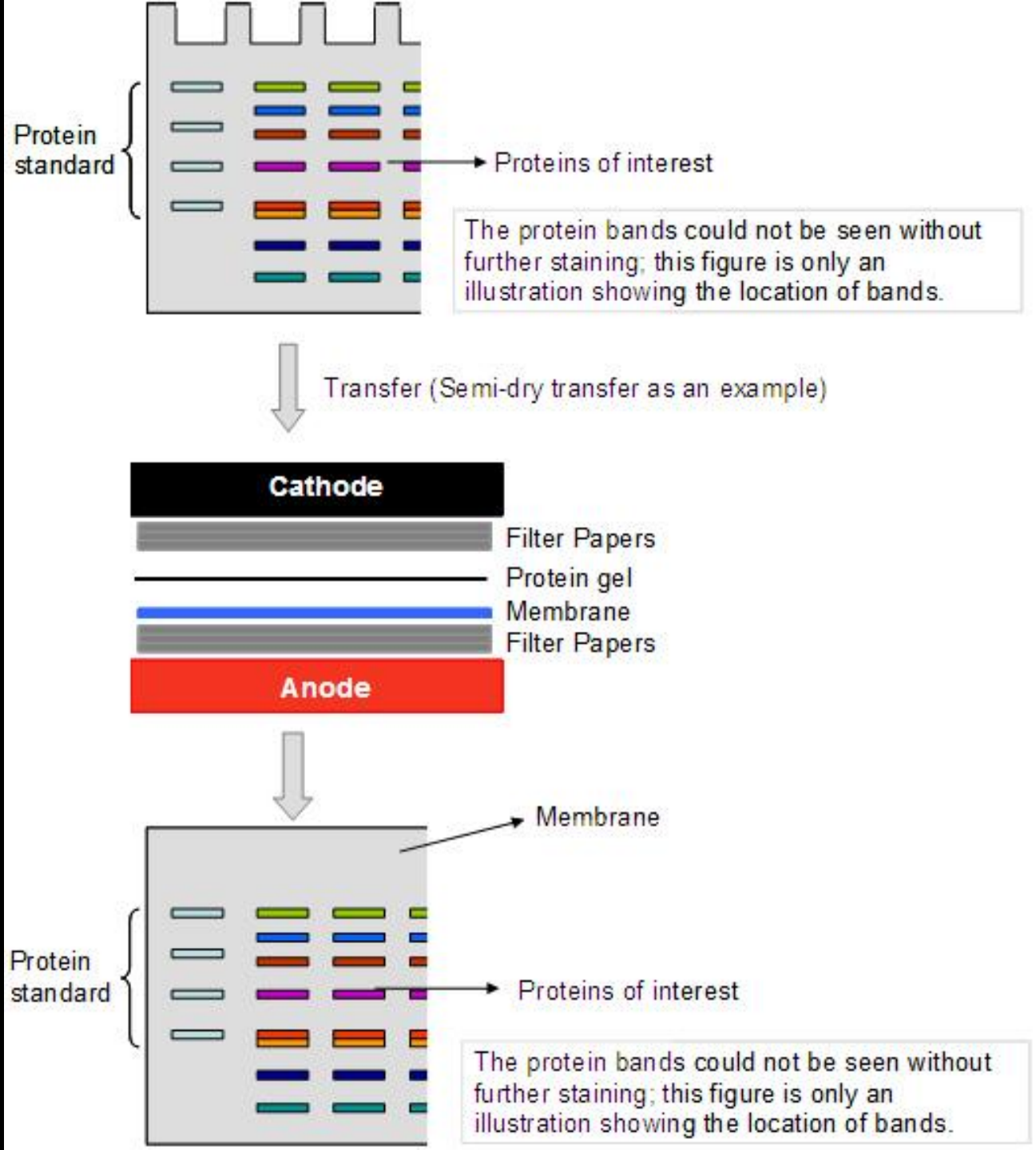
- Membrane proteins, protein components of the cytoskeleton, and that are part of large macromolecular can all be resolved

- SDS-PAGE separates polypeptides according to size (molecular weight and the subunit composition).

The transfer of the proteins onto a membrane



Why not add SDS in the transfer buffer?

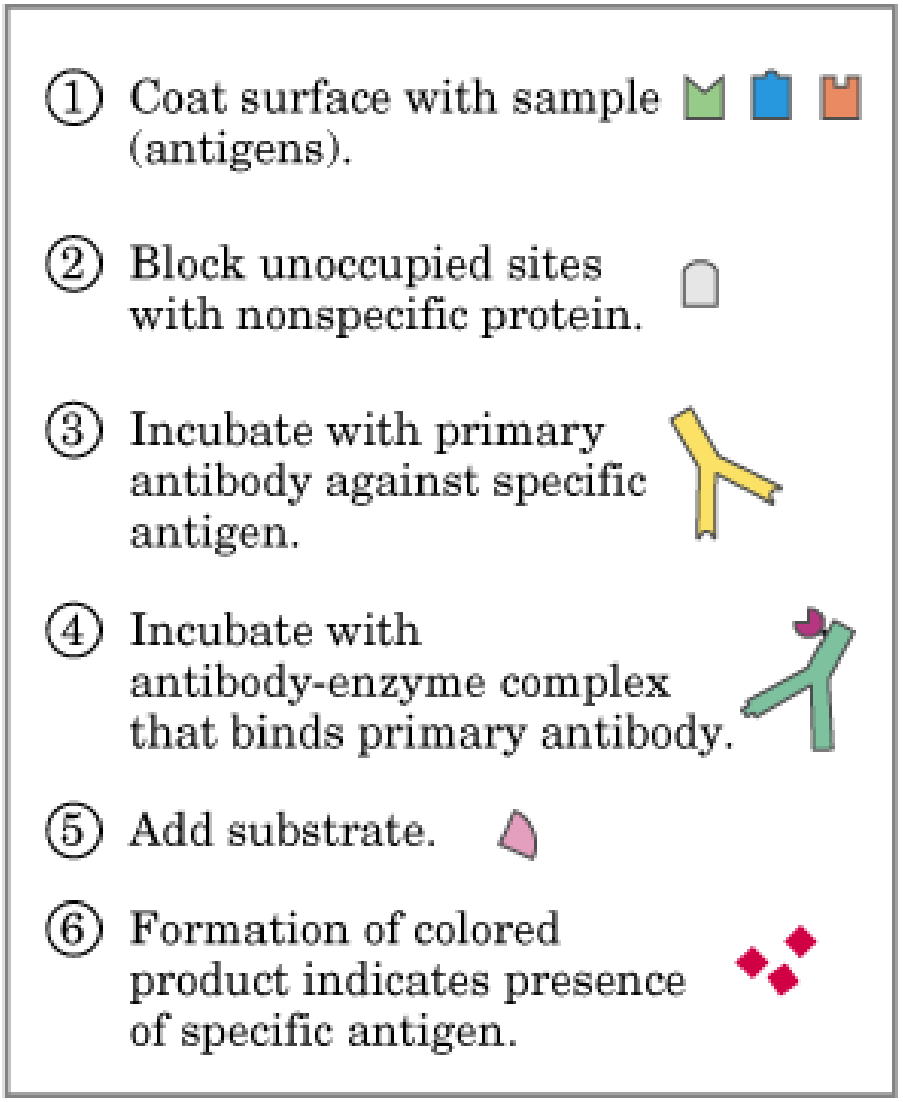

Protein standard
Proteins of interest
The protein bands could not be seen without further staining; this figure is only an illustration showing the location of bands.
Transfer (Semi-dry transfer as an example)
Cathode
Filter Papers
Protein gel
Membrane
Filter Papers
Anode
Membrane
Protein standard
Proteins of interest
The protein bands could not be seen without further staining; this figure is only an illustration showing the location of bands.

Protein detection

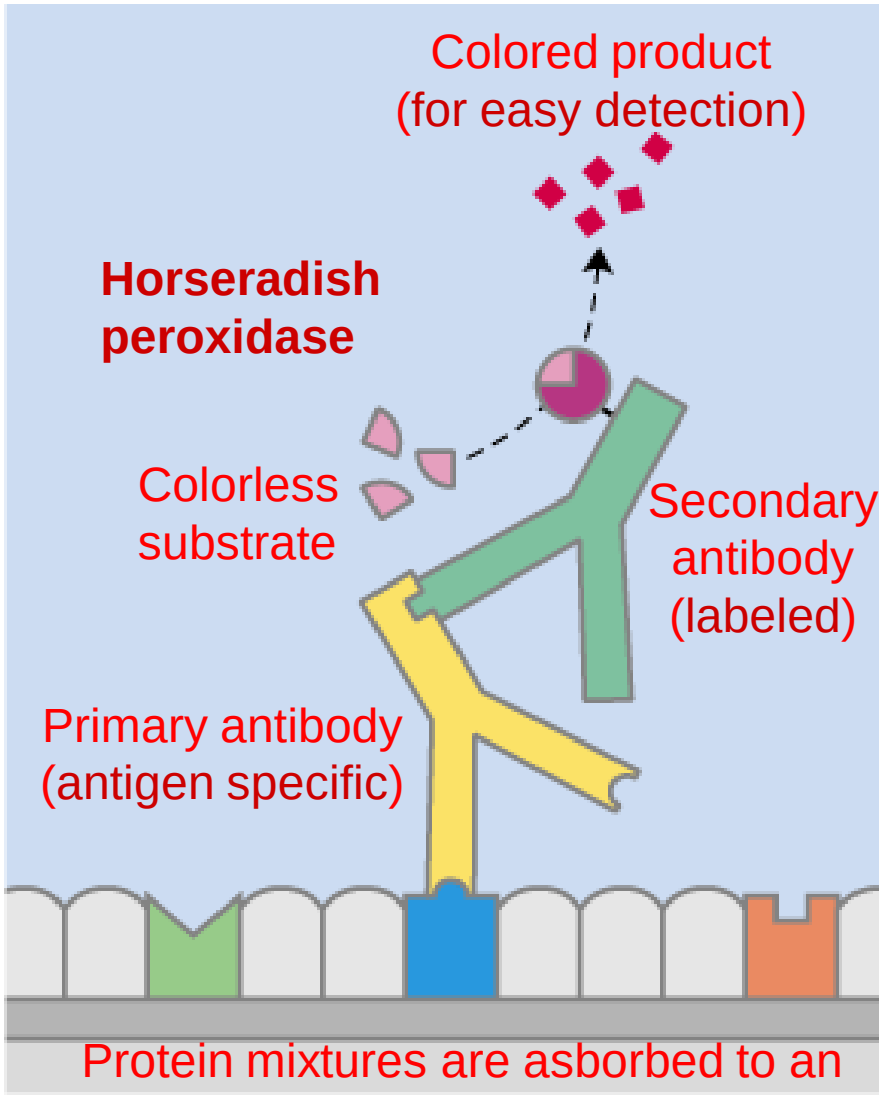
1- Primary antibody incubation step.
The primary antibodies which specifically recognize the proteins of intrest are used.

2- Secondary antibody incubation step.
Use of secondary antibody which recognizes the primary antibody

3- Visualization step
Making the antigen-antibody complex visible (staining).

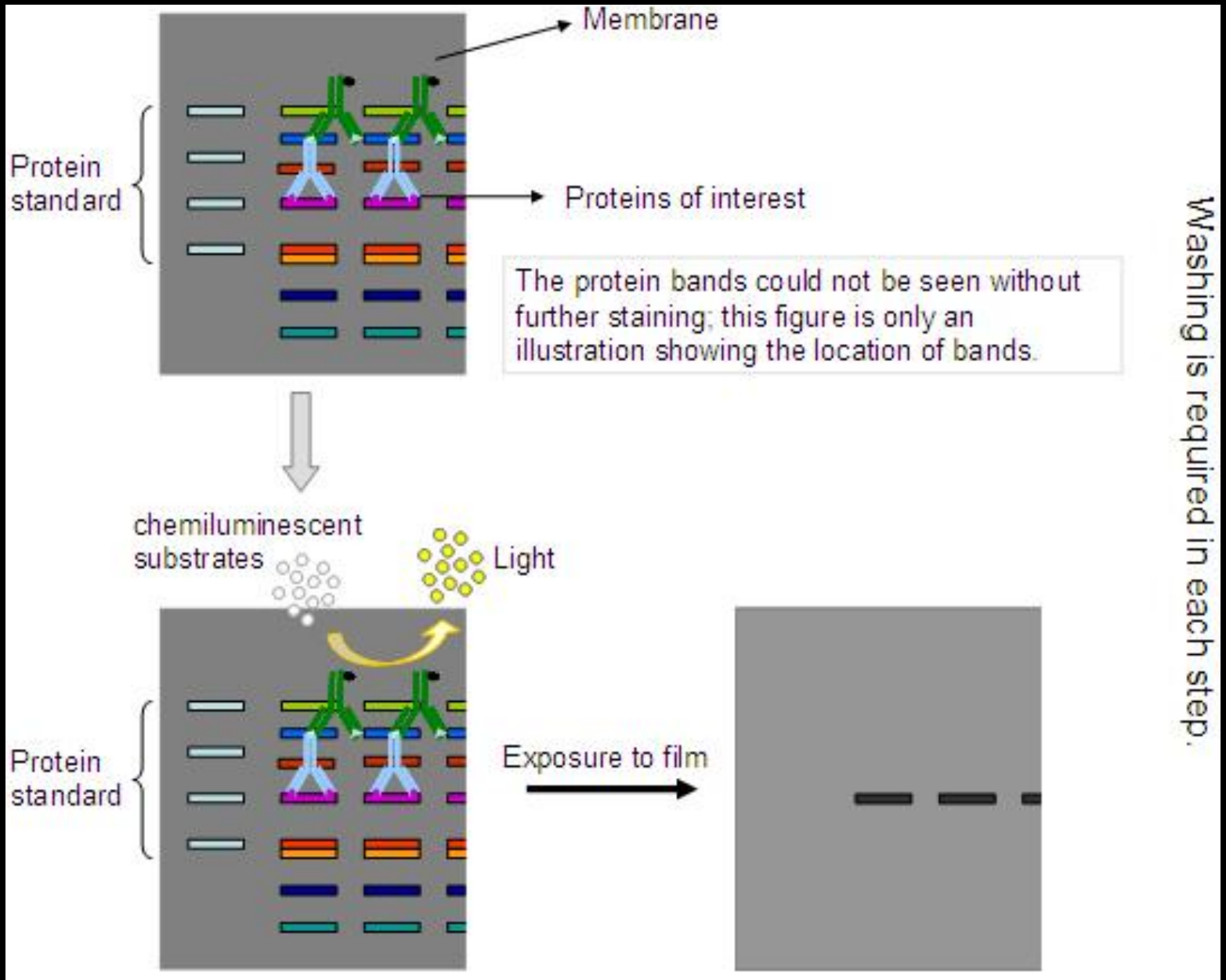


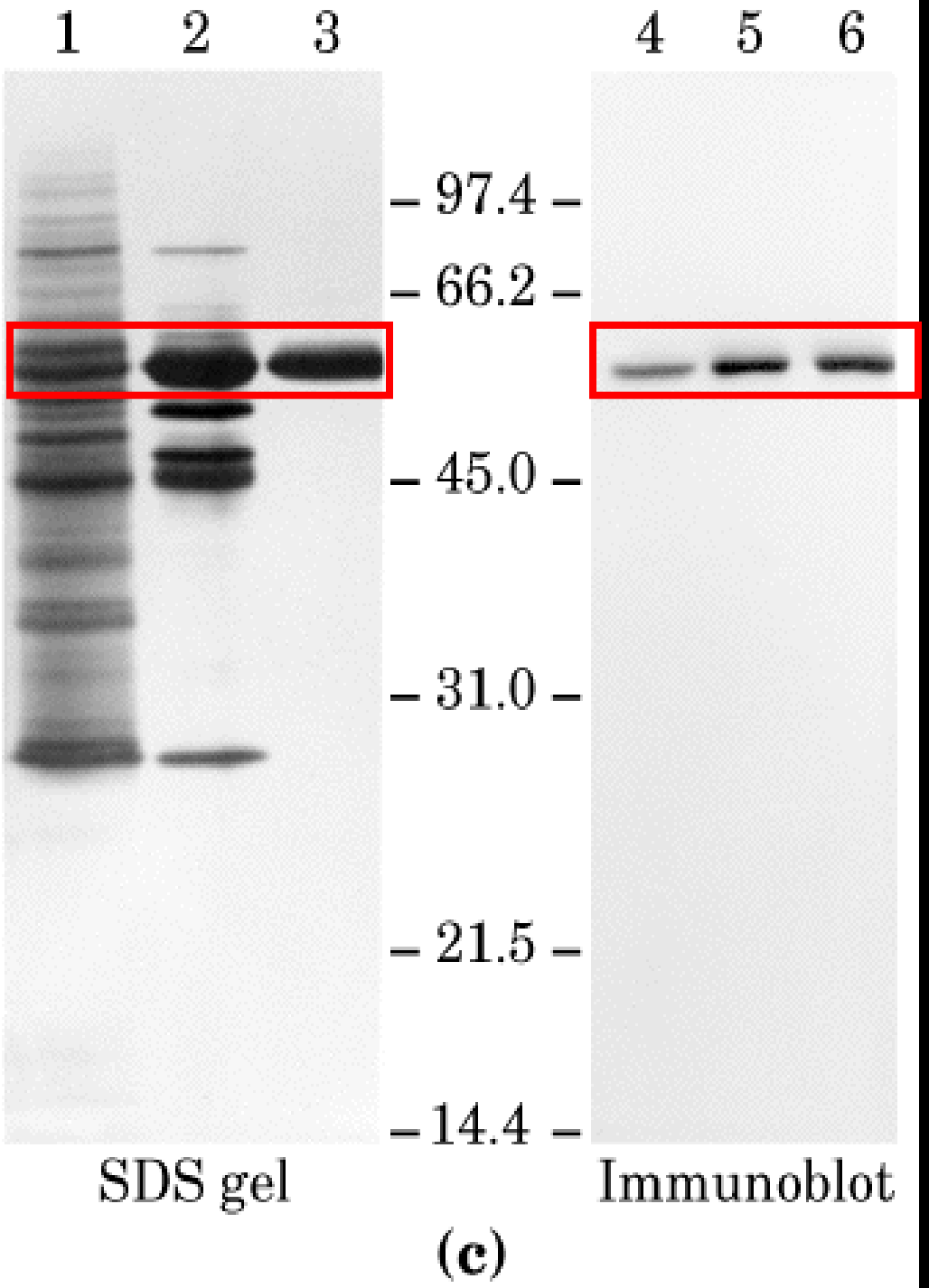
(a)



Protein detection in western blot

Protein detection





Western blotting to detect a specific antigen protein in a protein mixture using a specific antibody.

Gel-separated antigen proteins are transferred onto a nitrocellulose membrane before being probed with antibodies.


Mujahid Awais