

Application of protein electrophoresis and western blot

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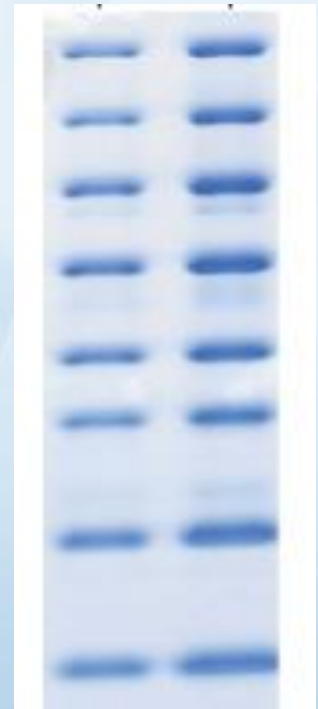
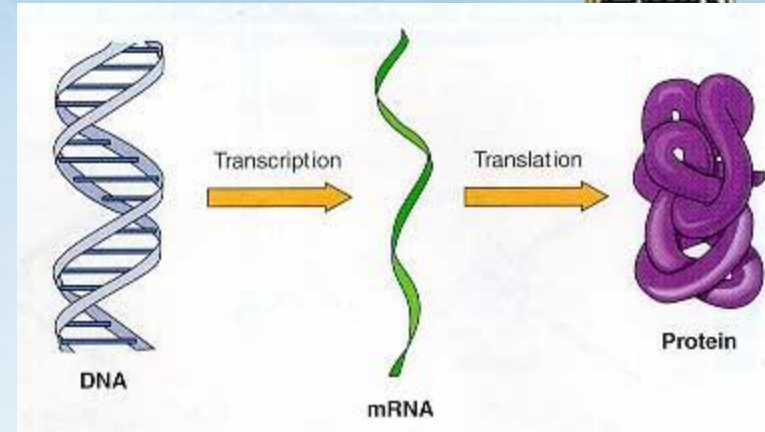
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Applications



- Determine **purity** of protein samples
- Determine **molecular weight** of protein
- **Quantifying** proteins
- Blotting applications
- Patterns of bands that suggest presence of isozymes
- Changes in gene expression during developmental stages or resulting from experimental intervention



Advantages & disadvantages

•Advantages

- Migration is proportional to the molecular weight
- Highly sensitive test, separates 2% difference in mass
- Require small amount of samples

•Disadvantages

- Gel preparation is difficult and require longer time
- proteins are rapidly degraded

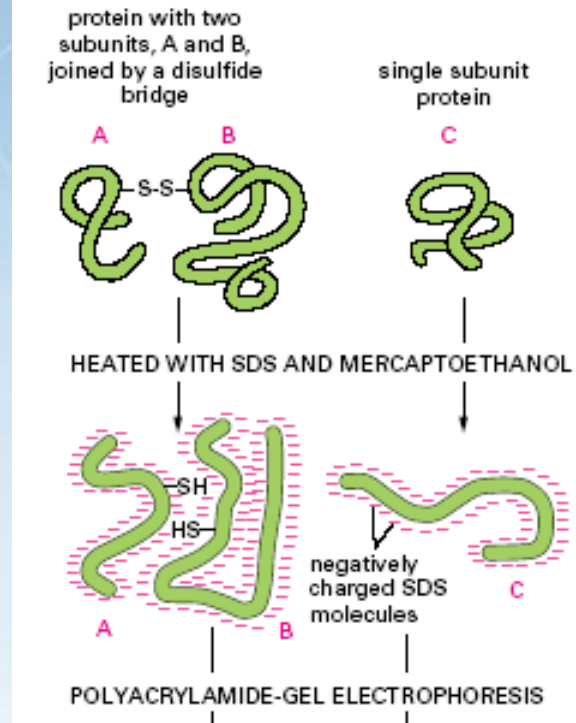
Protein Gel Electrophoresis methods

1. SDS PAGE
2. Native PAGE
3. Native Gradient PAGE
4. IEF
5. 2D PAGE
6. Western Blot

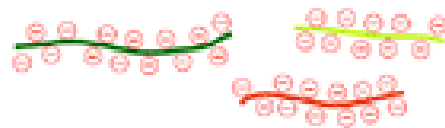
Native PAGE



Non-denatured protein



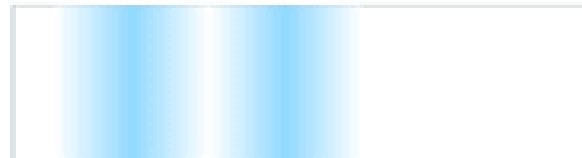
(A) SDS PAGE



(Electrophoretic pattern)

⊖ Cathode Electrophoresis ⊕ Anode

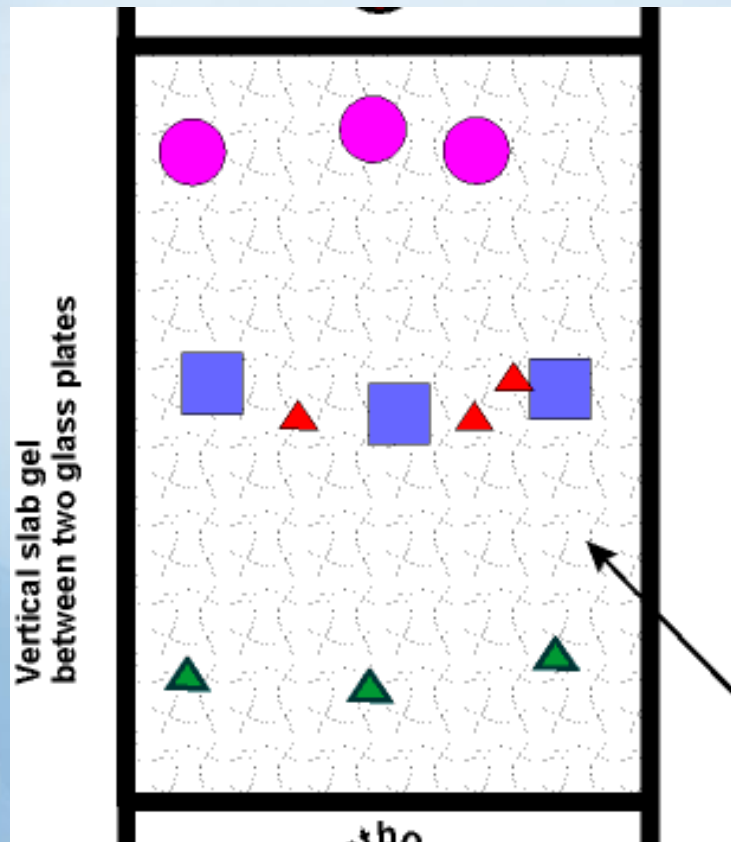
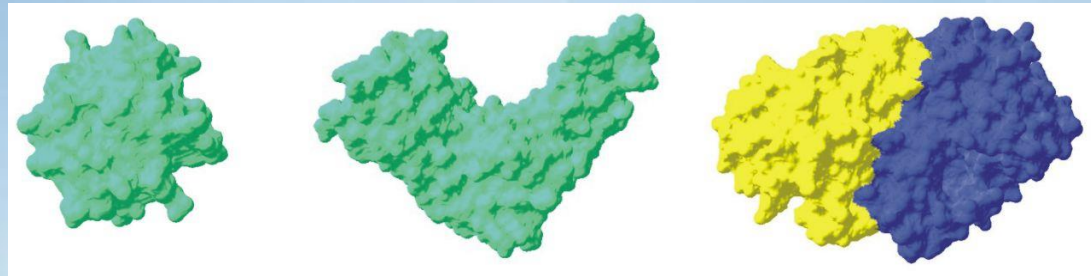
(B) Conventional native PAGE



Native PAGE

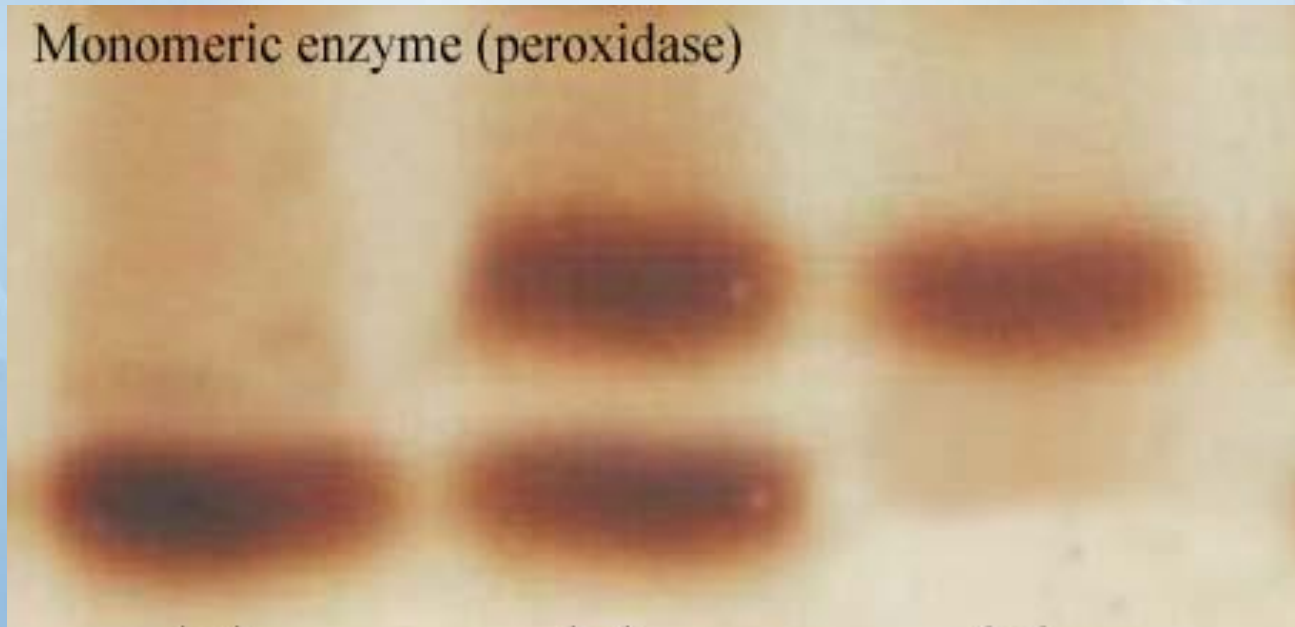
Separates by

- charge
- size
- shape

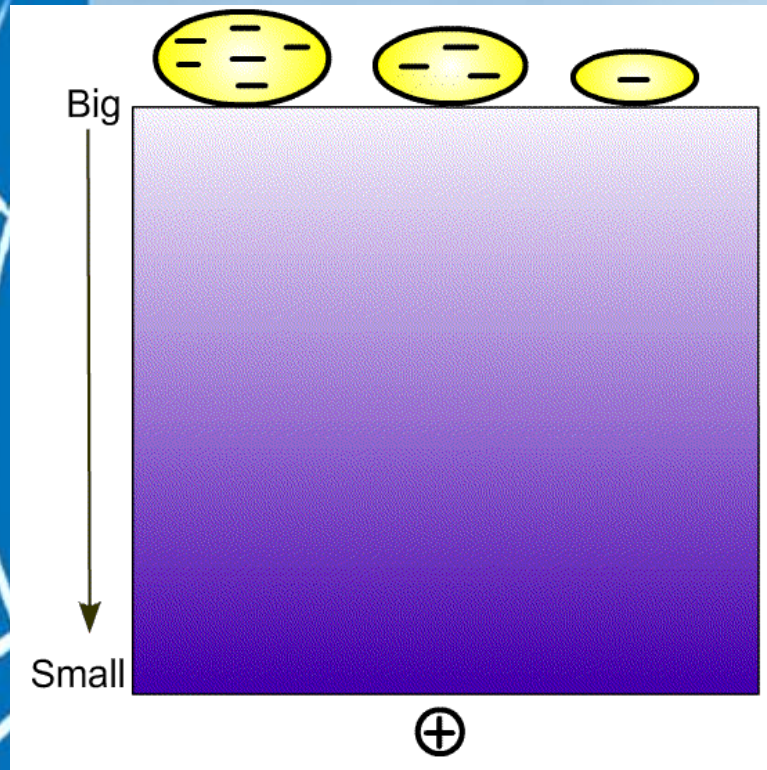


- reaction with specific activity stains (depending on enzyme).
- substrates + cofactors + buffer
- **colored** bands such as Est, Prx, Mdh ...

Monomeric enzyme (peroxidase)

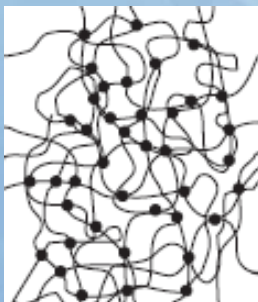


Native gradient PAGE



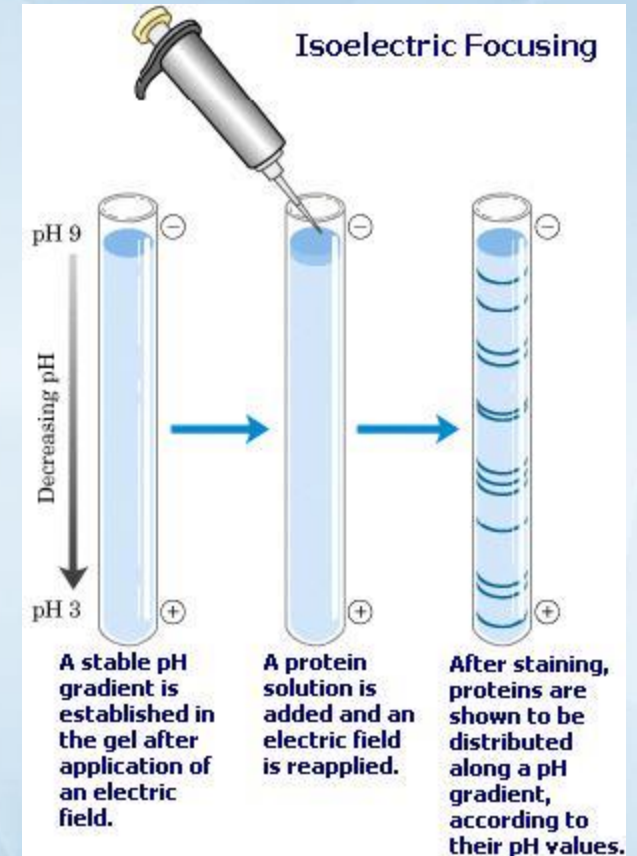
Separate native proteins by size - proteins stop moving when they reach a certain gel density (but this may take a very long time ...)

A great technique to study protein oligomerization!

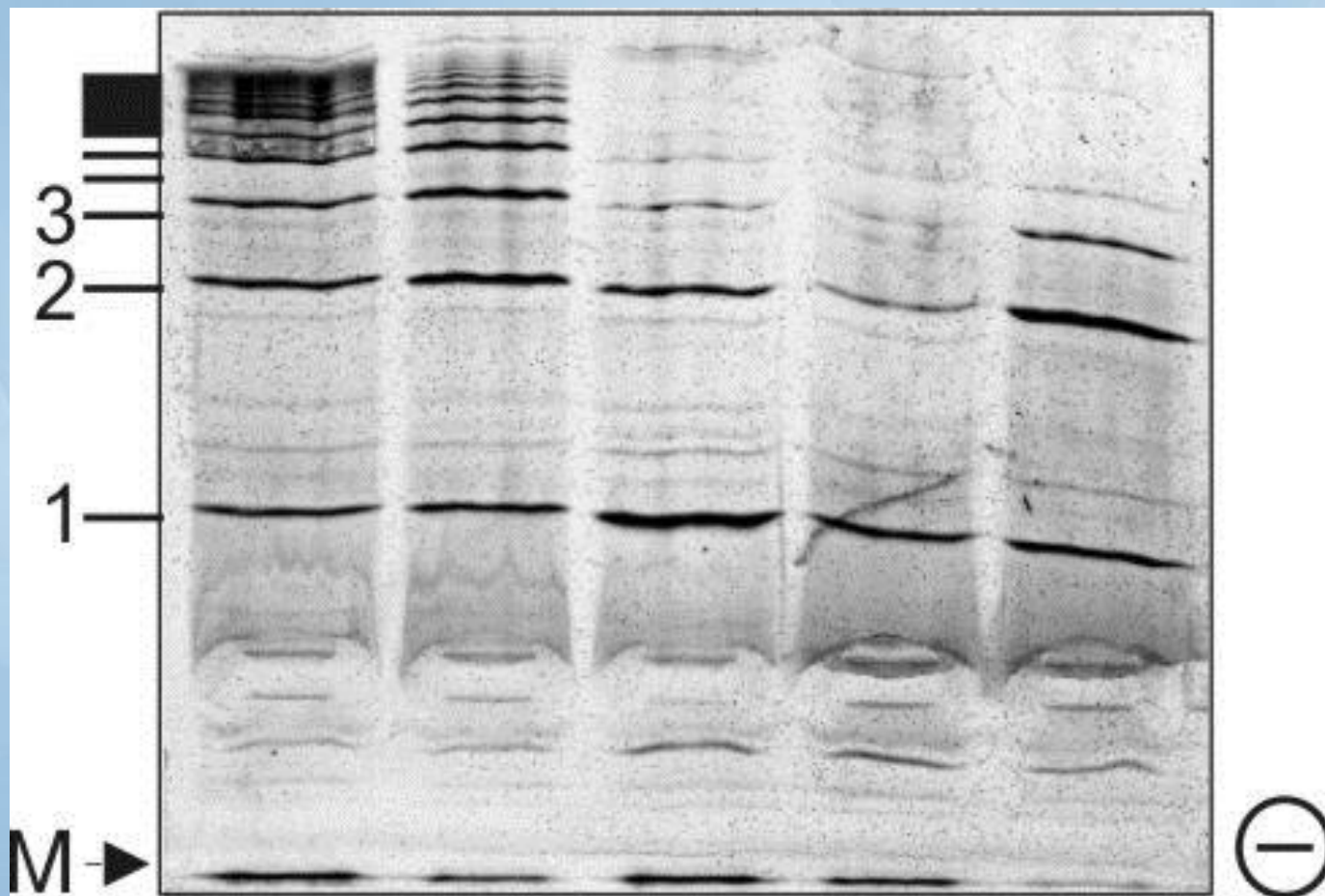


What is Isoelectric focusing?

- Gel is prepared with pH gradient
- Separates proteins by their isoelectric points (pI)
- Each protein has own pI = pH at which the protein has equal amount of positive and negative charges (the net charge is zero)
- Charge on the protein changes as it migrates across pH
- When it gets to pI, has no charge and stops

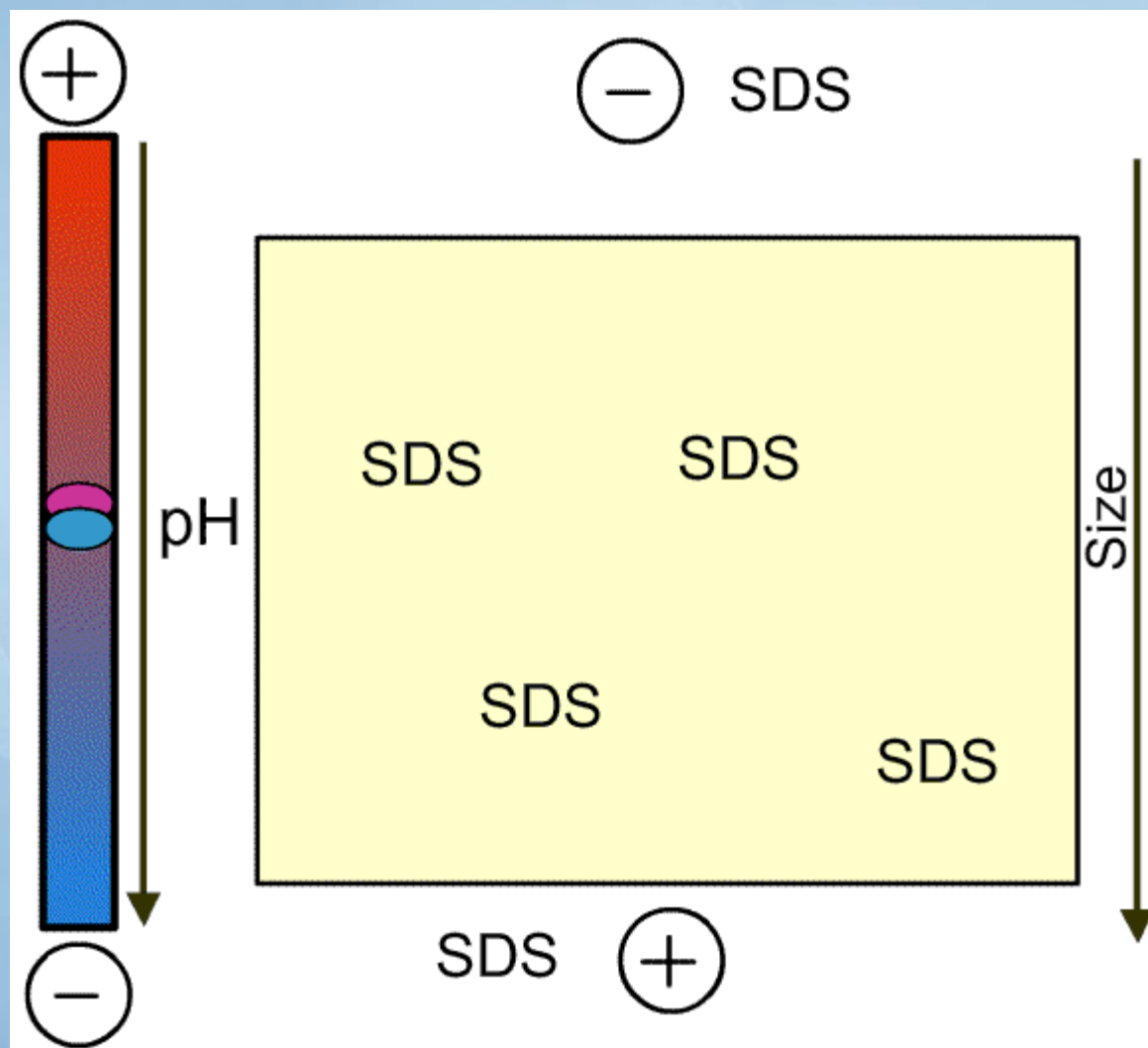


IEF example

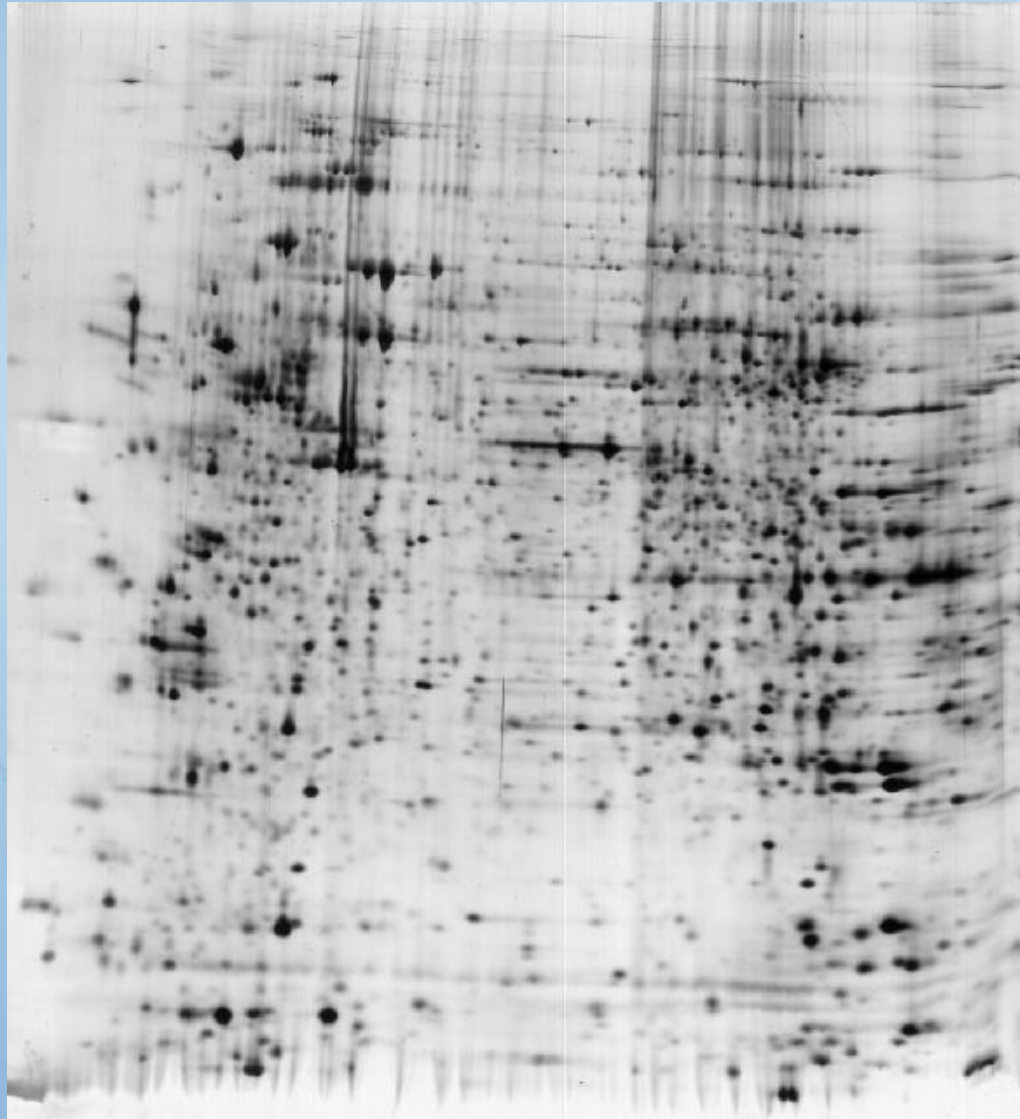


- PI of proteins can be theoretically predicted. Therefore, IEF can also be used for protein identification.

2D PAGE



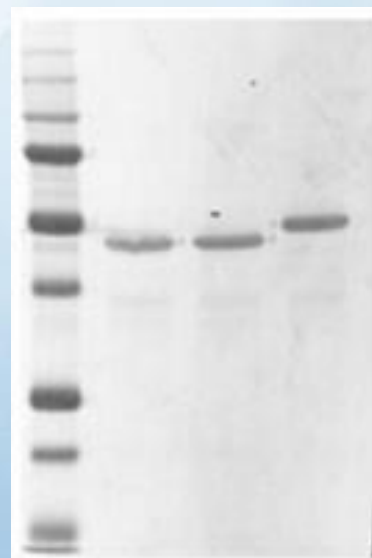
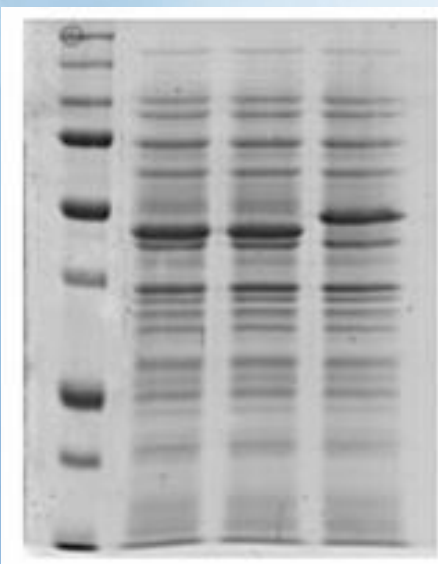
2D PAGE



Western Blot Analysis

Laboratory procedure that allows you to:

1. Verify the expression of a protein
2. Determine the relative amount of a protein present in different samples
3. Analyze protein-protein interactions



Western Blot Analysis

What happens after electrophoresis?

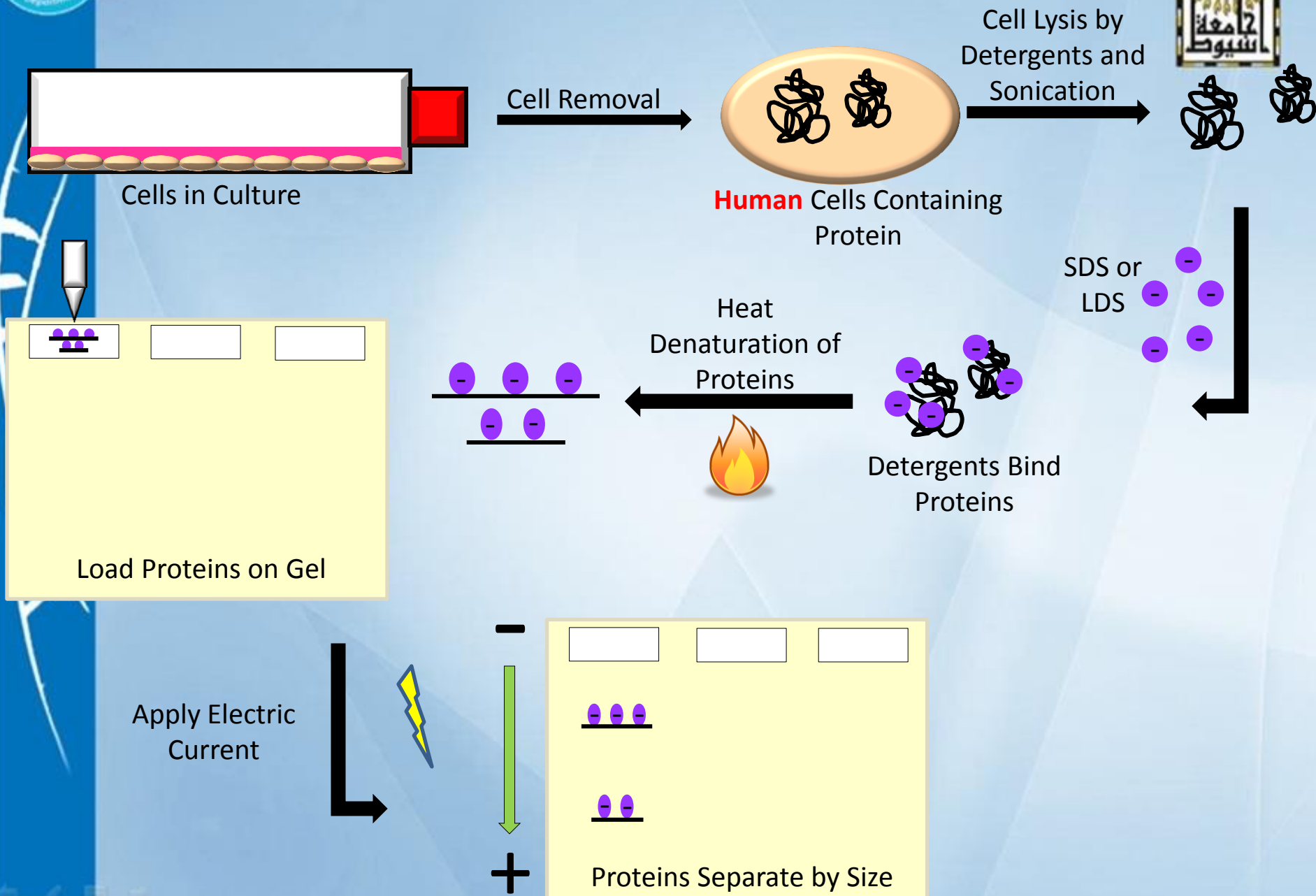
- 1. Fix the proteins in the gel and then stain them.
- 2. Electrophoretic transfer to a membrane and then probe with **antibodies**- (Western blotting) (Refer Western Blot first few slides)

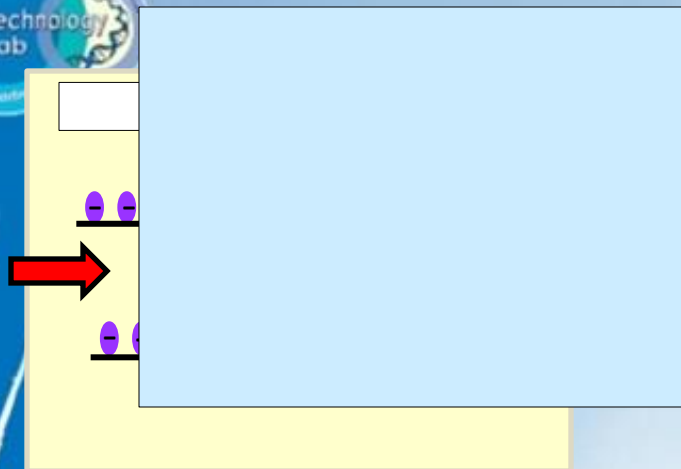
Western Blot : 4 steps

1. Separation of proteins using SDS PAGE
2. Transfer of the proteins onto e.g. a nitrocellulose membrane (blotting)
3. Immune reactions
4. Visualization

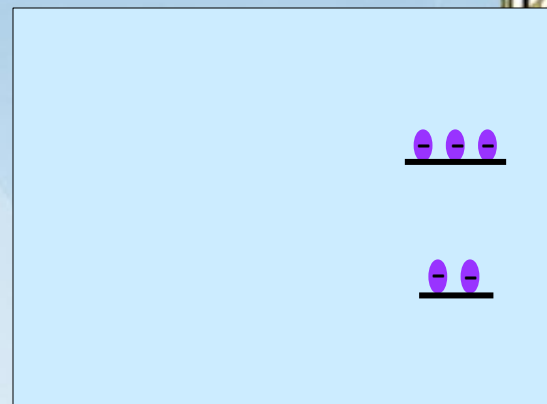


SDS-PAGE Western Blot Method

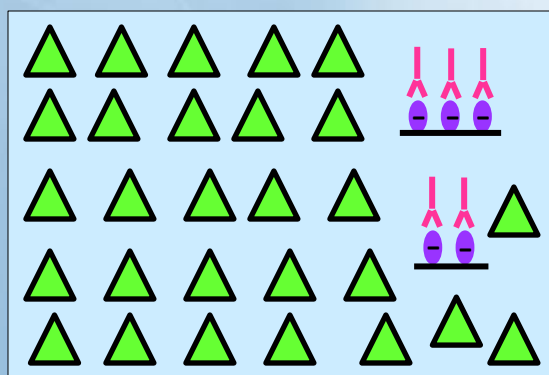




Transfer or Blot Protein
from Gel to
Nitrocellulose and/or
PVDF Membrane



Block Membrane
with Non-
Specific Proteins

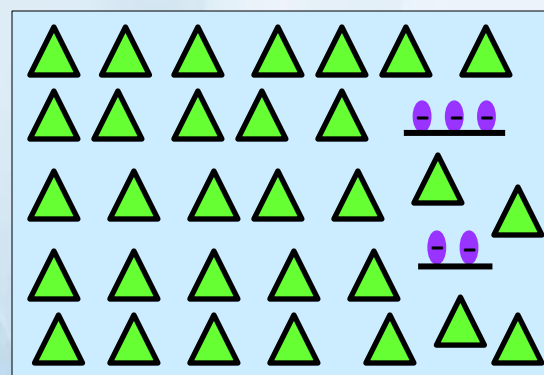


1° Antibody Binds Antigen
(i.e. Protein of Interest)

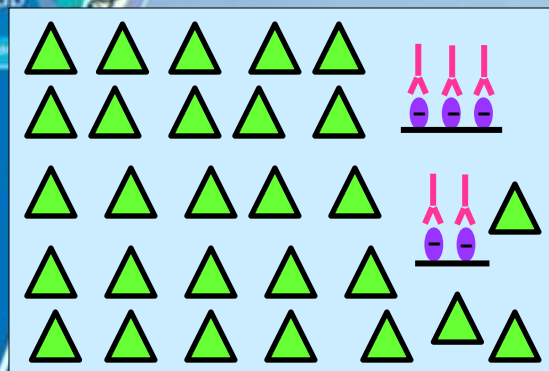
Incubate Membrane with
1° Antibody



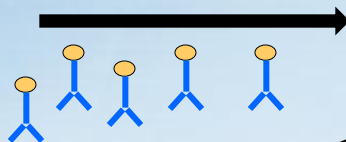
1° Antibody is a Rabbit
Anti-Human β -Actin
Antibody



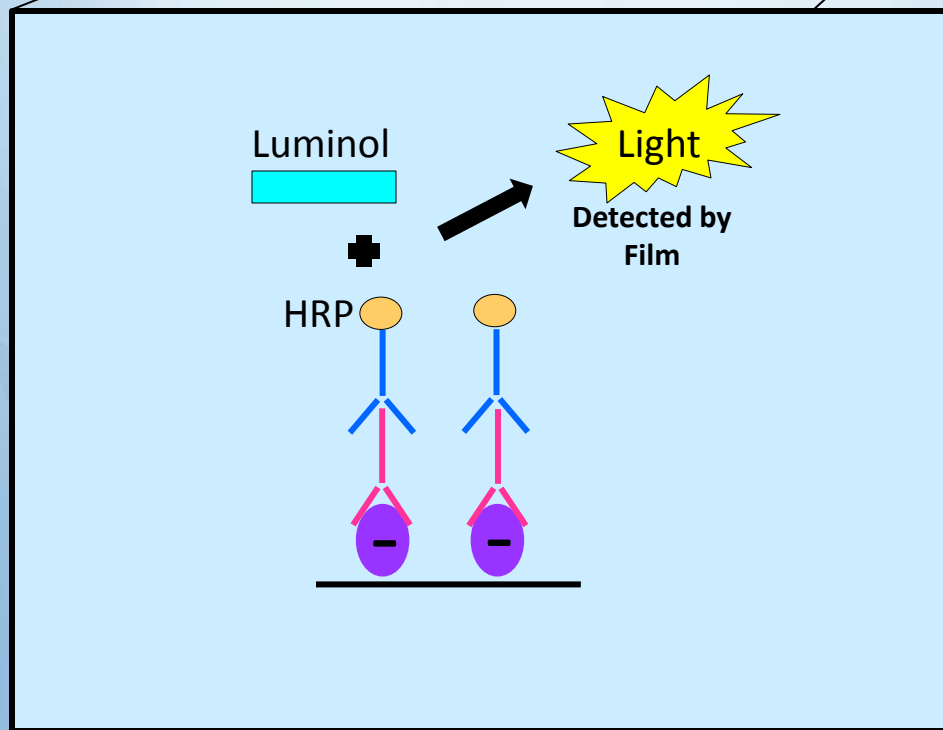
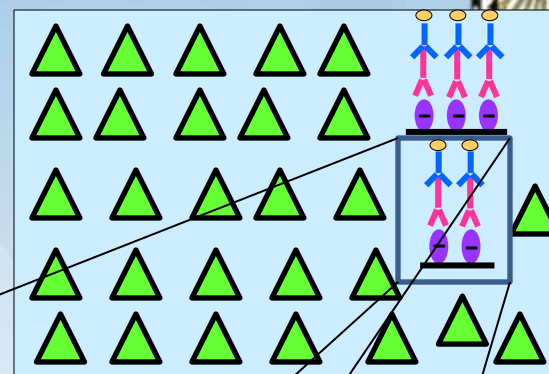
Non-Specific Proteins Bind to
Unbound Regions of
Membrane

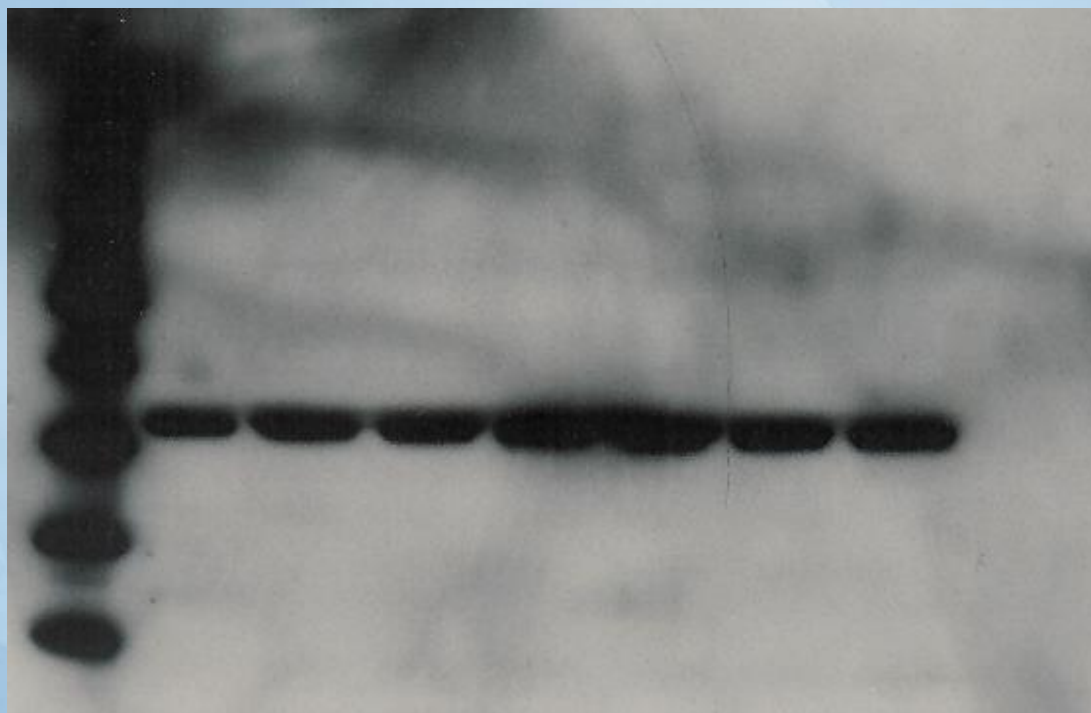


Add HRP-Conjugated 2^o
Antibody



2^o Antibody is a Goat
Anti-Rabbit-HRP-
Conjugated Antibody

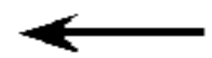




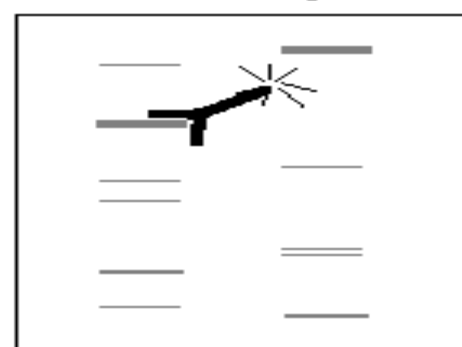
Protein Blot on Nitrocellulose



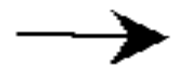
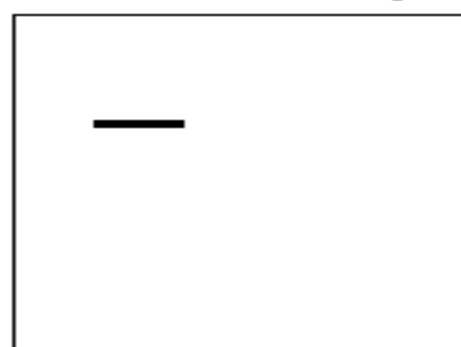
SDS Polyacrylamide Gel Electrophoresis



Label with Specific Antibody

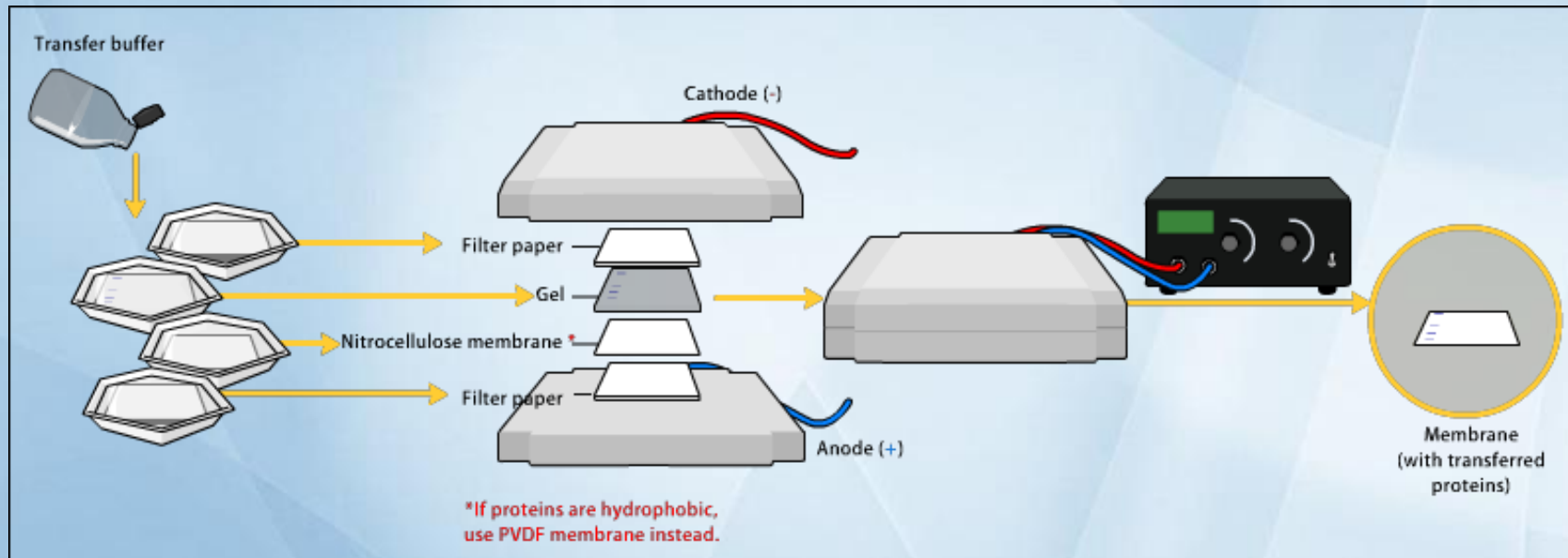


Detect Antibody

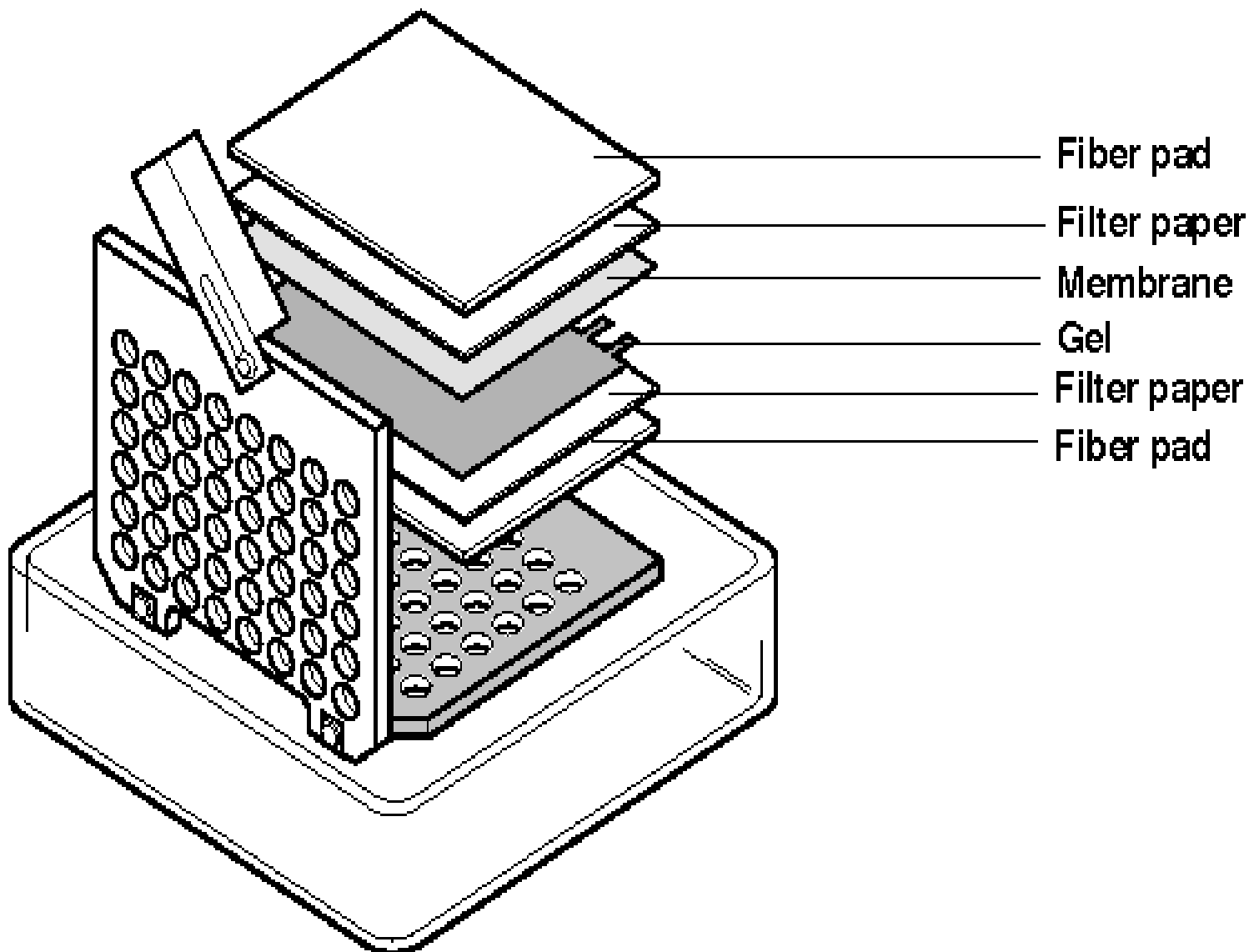


Reveals Protein of Interest

Blotting

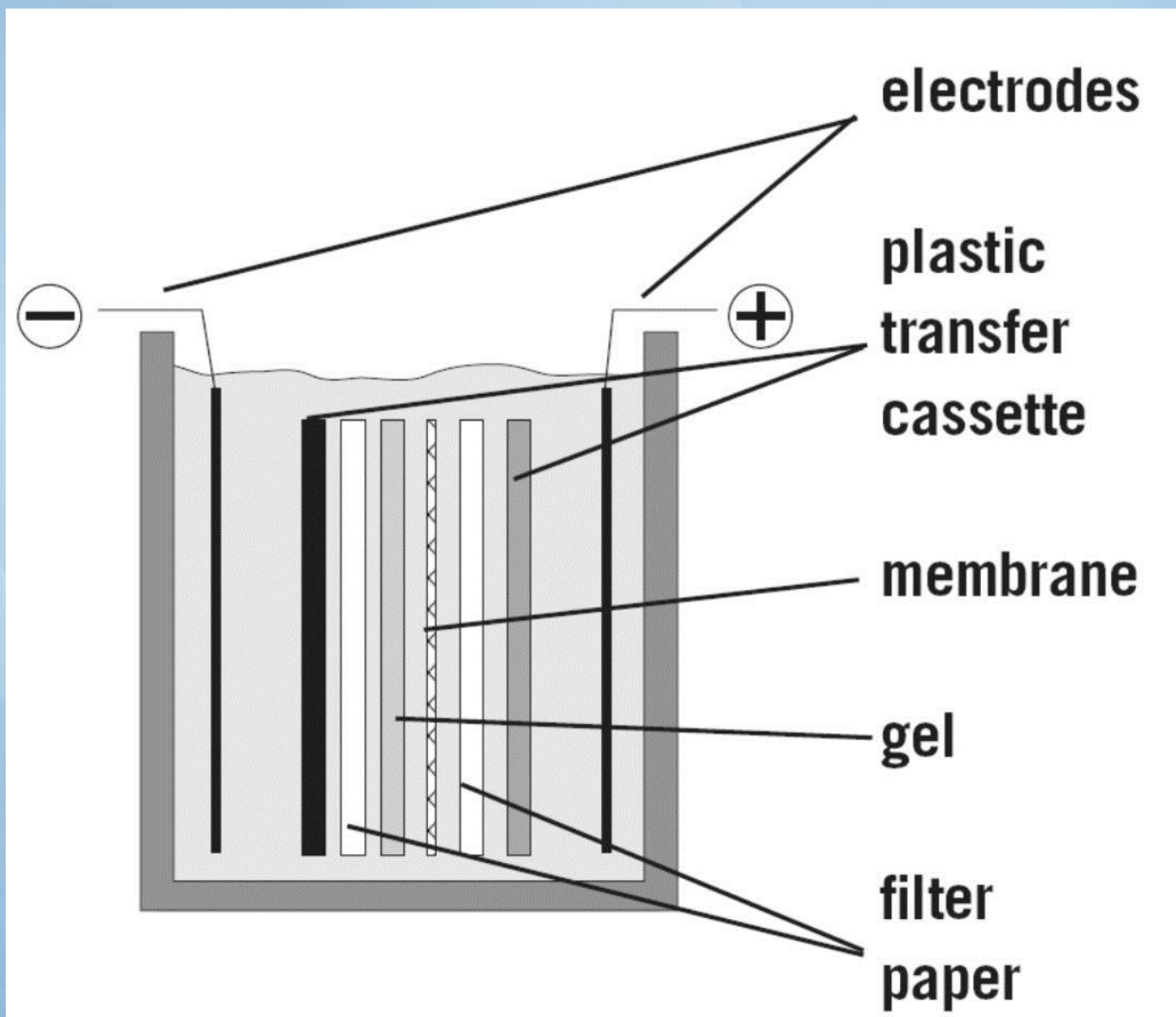


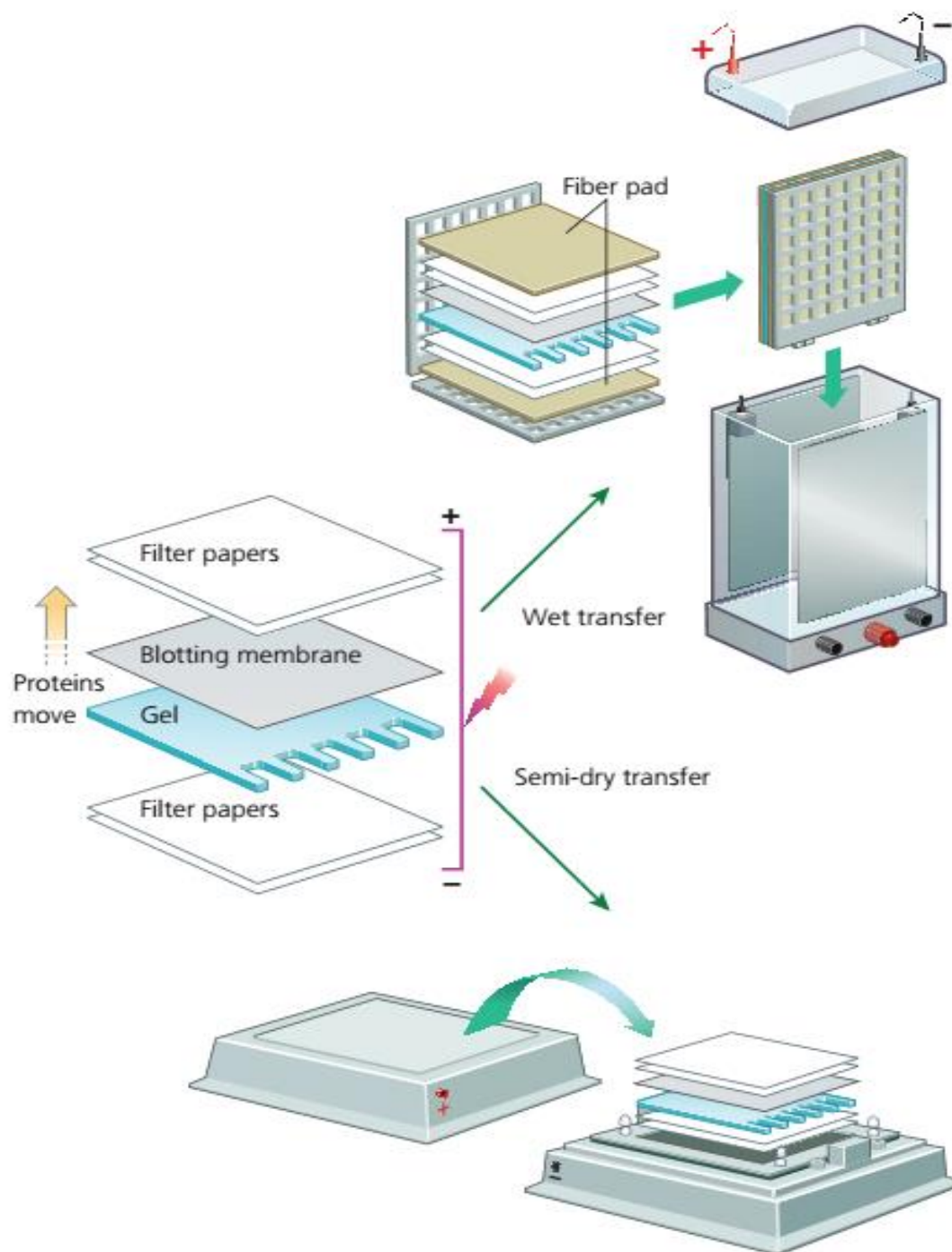
Transfer of proteins to the membrane



Western blotting-wet transfer apparatus







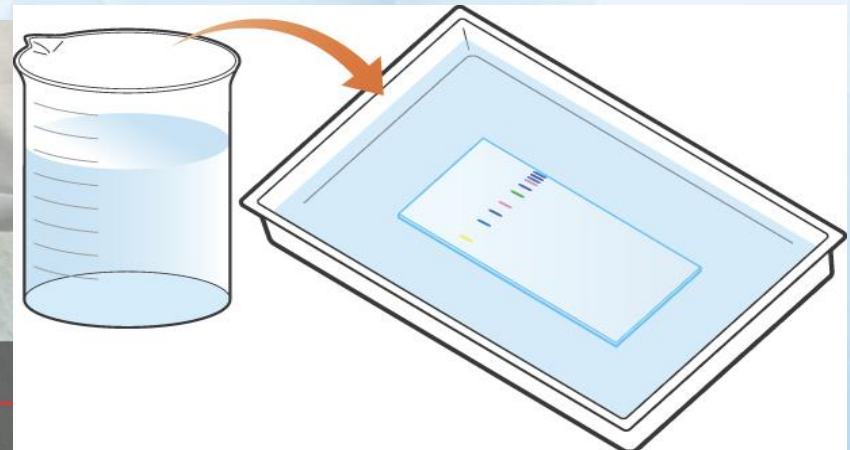
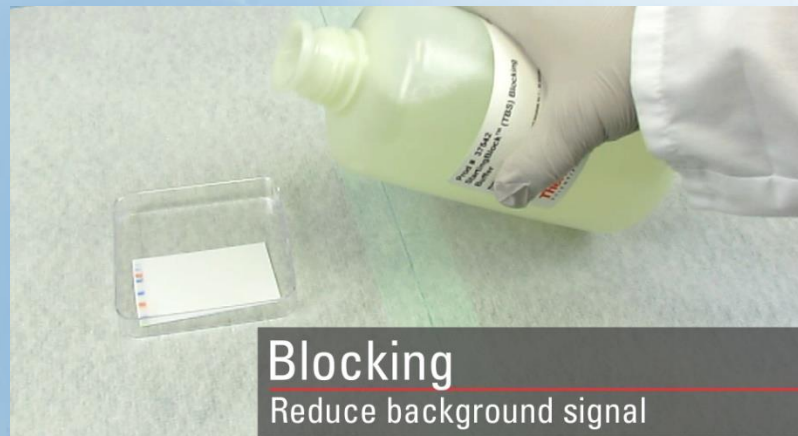
Blocking Buffer

Remove membrane from the blotting sandwich and immerse in 25ml of blocking solution for 15minutes

5% non-fat milk: Prevents the primary antibody from binding randomly to the membrane

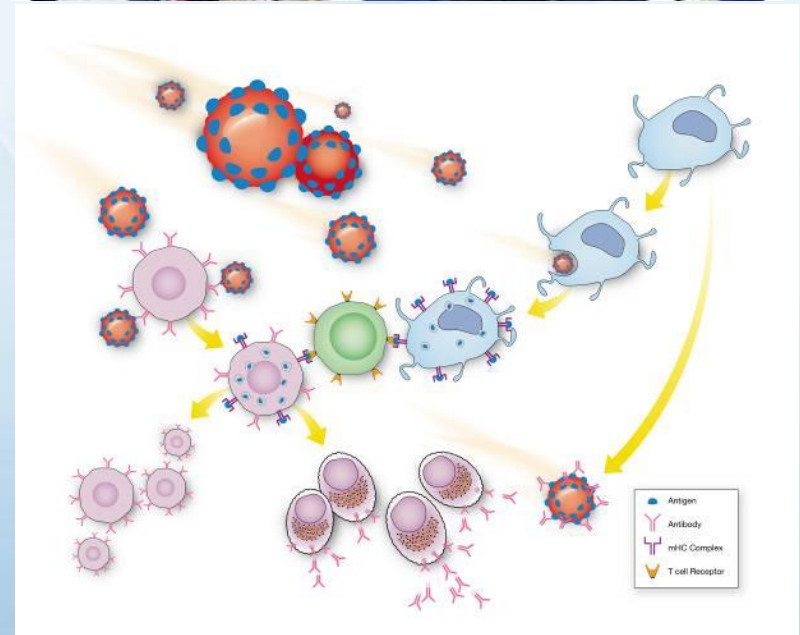
Phosphate buffered saline (PBS): Provides the correct environment (pH, Salt) to maintain protein shape

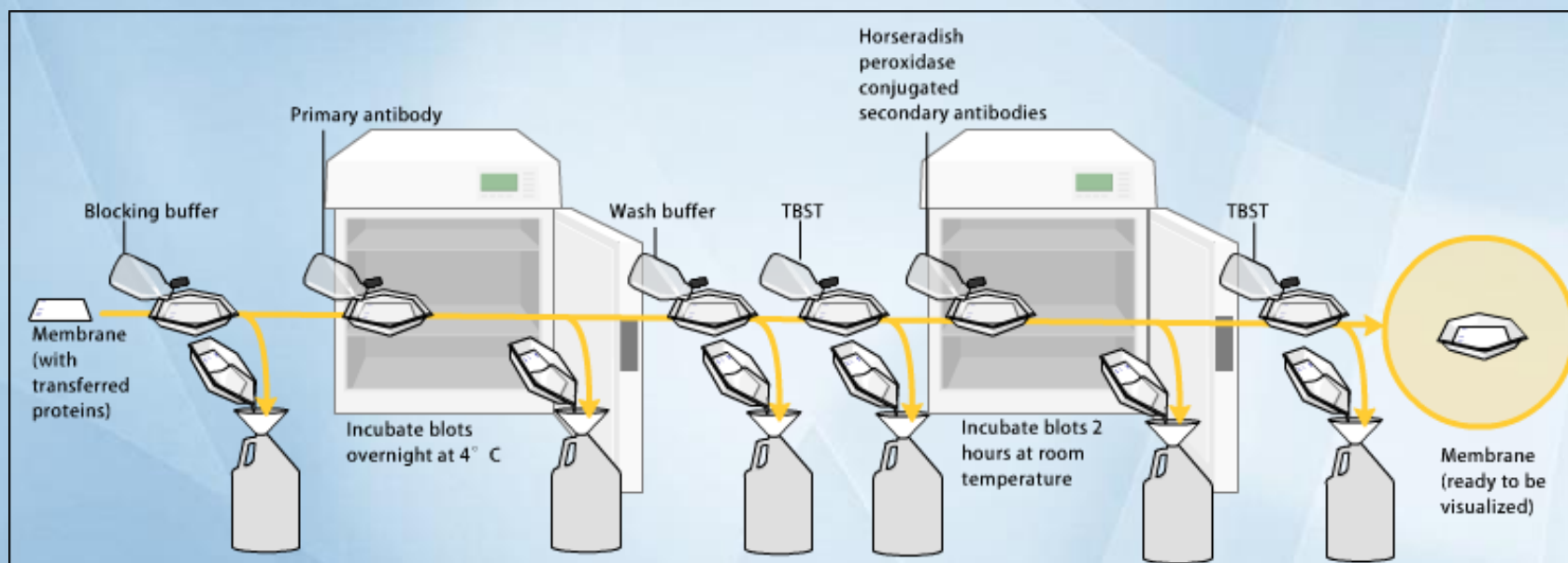
0.025% Tween 20: non-ionic detergent that prevents non-specific binding of antibodies to the membrane



**Using the mammalian
immune system to
produce antibodies**

**These antibodies are
specific for our protein
of interest**





Rinse and Store

- Rinse the developed membrane **twice** with **distilled water** and blot dry
- Air dry for 30min-1hr and store in lab notebook



- <http://www.currentprotocols.com/WileyCDA/CurPro3Video/videoId-694565070.html>

