SDS-PAGE

Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis

By

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Electrophoresis Types

- Gel electrophoresis
  - Agarose gel
  - Polyacrylamide gel
  - Others.
- Pulsed Field Gel Electrophoresis
- Capillary Electrophoresis
- Isoelectric focusing
- 2D electrophoresis

Gel Electrophoresis

- Use of a gelatinous material.
- The gel acts as a support medium
- Used to separate proteins or nucleic acids.

Gel Types

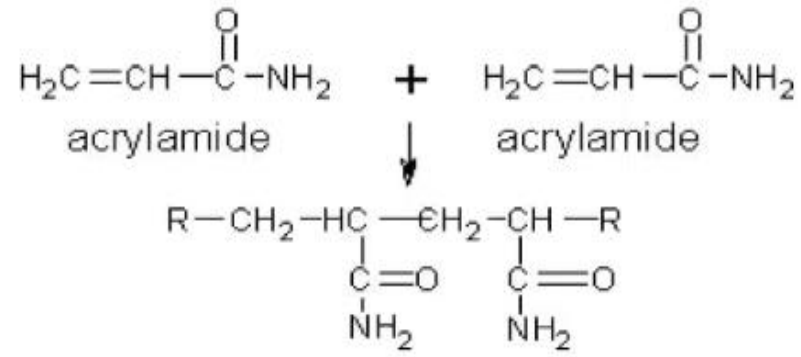
- Starch-Rarely used
- Polyacrylamide-Protein, small nucleic acid fragments
- Agarose-Nucleic acids, large proteins
- Cellulose acetate-Proteins

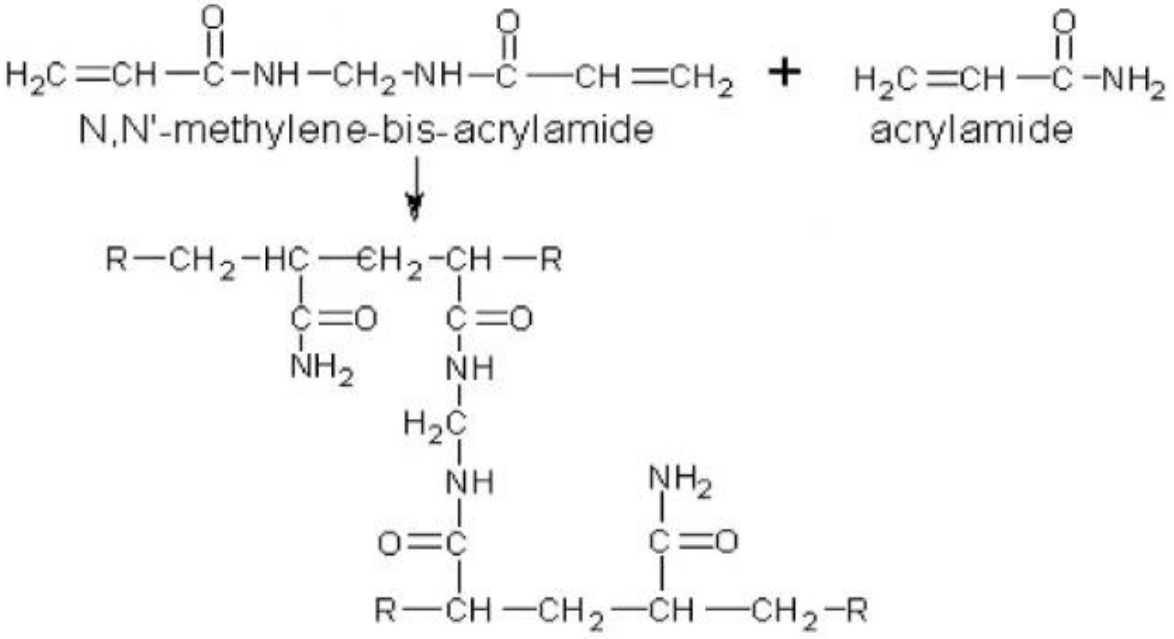
Commonly used

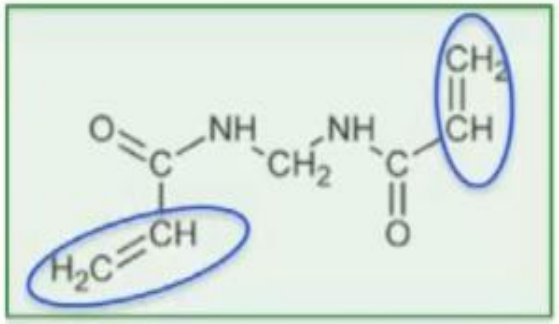
Electrophoresis

- Separates
  - Nucleic acids
  - Proteins
  - Peptides
  - Amino acids
  - Organic acids/bases
  - Drugs
  - Pesticides
  - Inorganic anions/cations.
- Everything that can carry a charge.!

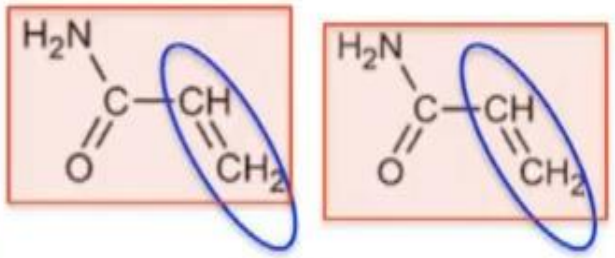
Polyacrylamide Gels





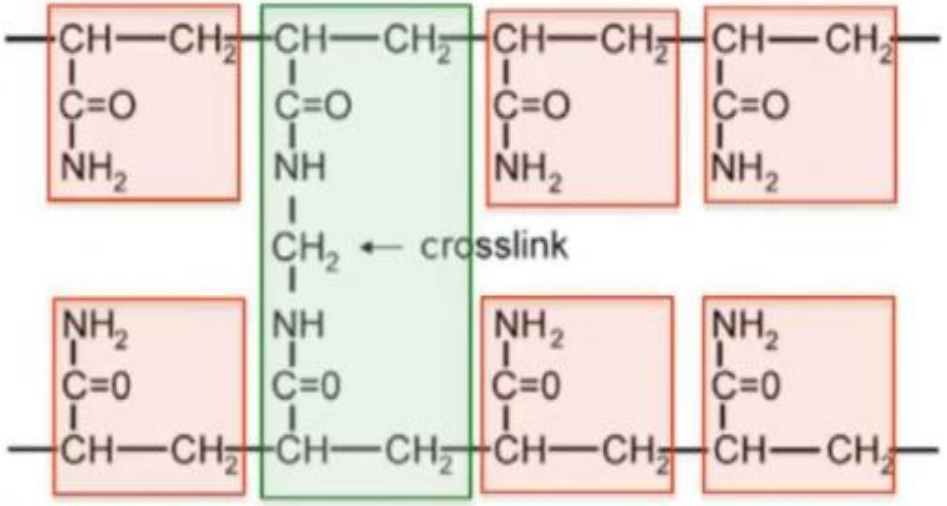


N,N'-methylenebisacrylamide
crosslinking monomer



acrylamide monomer

ammonium persulfate
TEMED

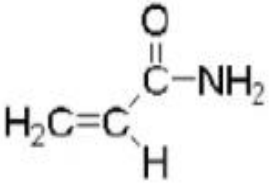


polyacrylamide

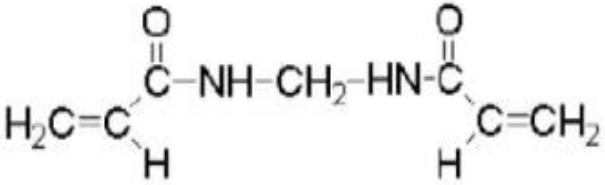
- Ammonium persulfate forms radicals in solution
- These radicals react with acrylamide to form acrylamide radicals
- Acrylamide radicals react with acrylamide to form long polymer chains (No branch)
- The polymers must be crosslinked to form a gel
- This is accomplished by polymerizing in the presence of a "crosslinker"-N,N'-methylene-bis (acrylamide)
- The product is a large network of linked acrylamide chains

- **The Starting Materials**

Acrylamide
: Crosslinking agents

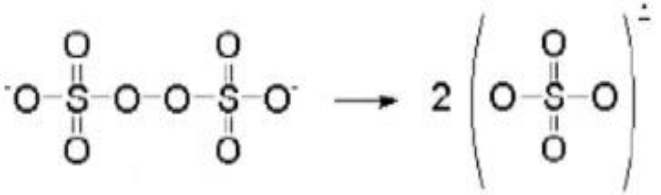


Acrylamide



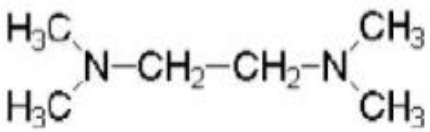
N,N'-Methylene-bis[Acrylamide]

Ammonium persulfate
: "initiator" of the free radical polymerization



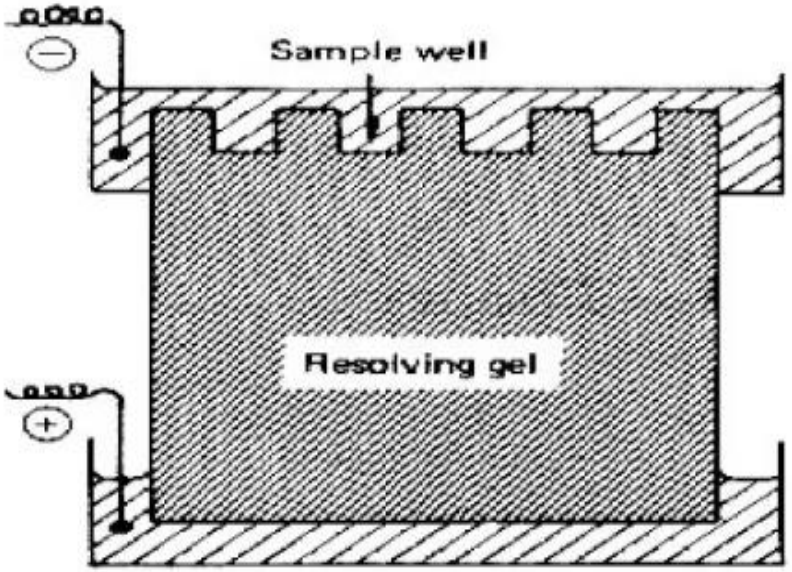
Persulfate free radical formation

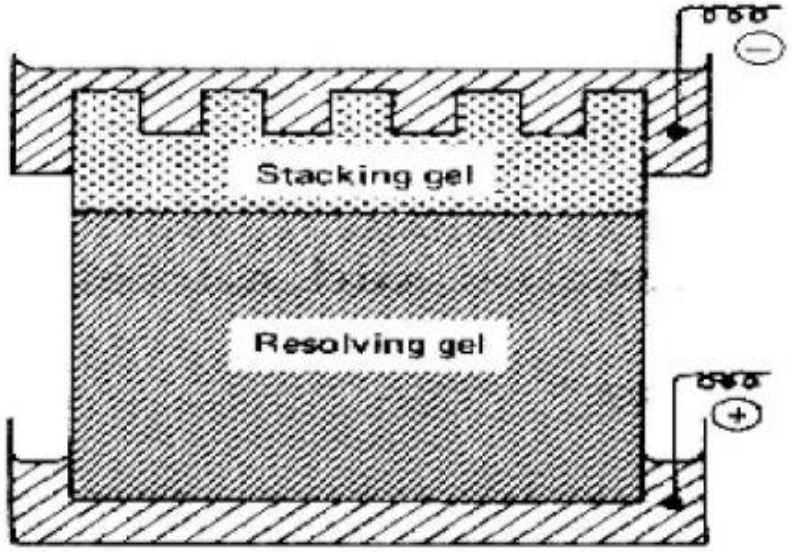
TEMED(tetramethyl-ethylenediamine)
: "catalyst" of the gel formation reaction



Tetramethylethylenediamine (TEMED)

Acrylamide concentration(%)	Protein size (kDa)
15.0	15~45
12.0	15~60
10.0	18~75
7.5	30~100
5.0	60~212





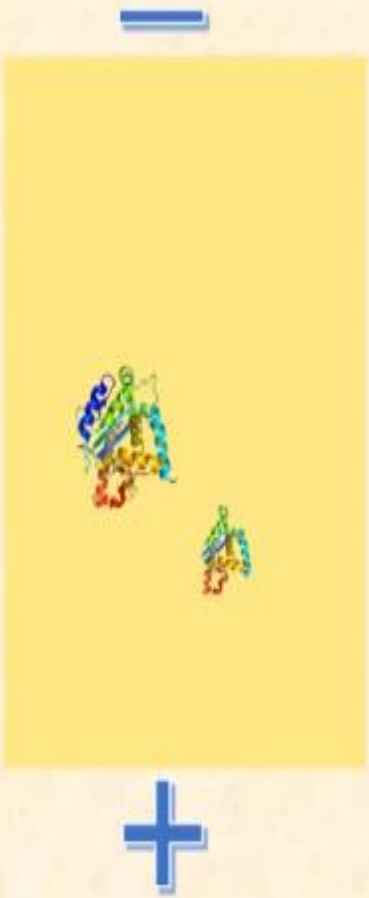
NATIVE-PAGE

Proteins remain in their NATIVE form.

- Separation of proteins depends on…..

Charge density (Charge-to-mass ratio)

Size and **Shape**



PAGE

Modified PAGE Technique

SDS-PAGE

APPLICATIONS OF SDS-PAGE

SDS-PAGE is used mainly for the following purposes:

1. Measuring molecular weight.
2. Peptide mapping.
3. Estimation of protein size.
4. Determination of protein subunits or aggregation structures.
5. Estimation of protein purity.
6. Protein quantitation.
7. Monitoring protein integrity.
8. Comparison of the polypeptide composition of different samples.
9. Analysis of the number and size of polypeptide subunits.
10. Post-electrophoresis applications, such as Western blotting.
11. Staining of Proteins in Gels with Coomassie G-250 without Organic Solvent and Acetic Acid.
12. Pouring and Running a Protein Gel by reusing Commercial Cassettes.
13. Selective Labelling of Cell-surface Proteins using CyDye DIGE Fluor Minimal Dyes.
14. Detection of Protein Ubiquitination.
15. SDS-PAGE/Immunoblot Detection of Aβ Multimers in Human Cortical Tissue Homogenates using Antigen-Epitope Retrieval.

Determination of…

Molecular weights of Proteins in a given sample

Whether the given protein is made up of single subunit or multiple subunits.

1.Protein: Proteins are the biomolecules, composing of amino acid, which forms the basic building blocks of the system and performs most of the biological function of the system.

2.SDS-PAGE: Sodium dodecyl sulphate –Polyacrylamide Gel Electrophoresis is used for the separation of proteins based the molecular weight of the proteins. The cocktail consists of acrylamide, bisacrylamide, TEMED, SDS, Ammonium persulphate.

3.Acrylamide and bisacrylamide: used as a crosslinker for polymerization reaction to forms the gel like polymer.

4. TEMED (Tetramethylethylenediamine): catalysis free radical formation of the ammonium persulphate in the cocktail.

5. Ammonium persulphate
: APS helps in the formation of the free radicals that aid in the polymerization reaction of acrylamide and bis acrylamide.

6. Stacking gel: has a acidic pH of 6.8 and a low acrylamide concentration of 5.5%. Under these conditions proteins separation is poor but form thin, sharply defined bands at the start of resolving gel

7.Resolving gel: has a basic pH of 8.8, and a higher polyacrylamide of 12.5%. As a protein, concentrated into sharp bands by the stacking gel, travels through the separating gel, the pores have a sieving effect, allowing smaller proteins to separate more faster than higher molecular weight proteins.

Buffer and reagent for electrophoresis- The different buffer and reagents with their purpose for vertical gel electrophoresis is as follows-

1. **N, N, N', N'-tetramethylethylenediamine (TEMED)**-it catalyzes the acrylamide polymerization.

2. **Ammonium persulfate (APS)**-it is an initiator for the acrylamide polymerization.

3. **Tris-HCl**- it is the component of running and gel casting buffer.

4. **Glycine**- it is the component of running buffer.

5. **Bromophenol blue**- it is the tracking dye to monitor the progress of gel electrophoresis.

6. **Coomassie brilliant blue R250**-it is used to stain the polyacrylamide gel.

7. **Sodium dodecyl sulphate**-it is used to denature and provde negative charge to the protein.

8. **Acrylamide**- monomeric unit used to prepare the gel.

9. **Bis-acrylamide**- cross linker for polymerization of acrylamide monomer to form gel.

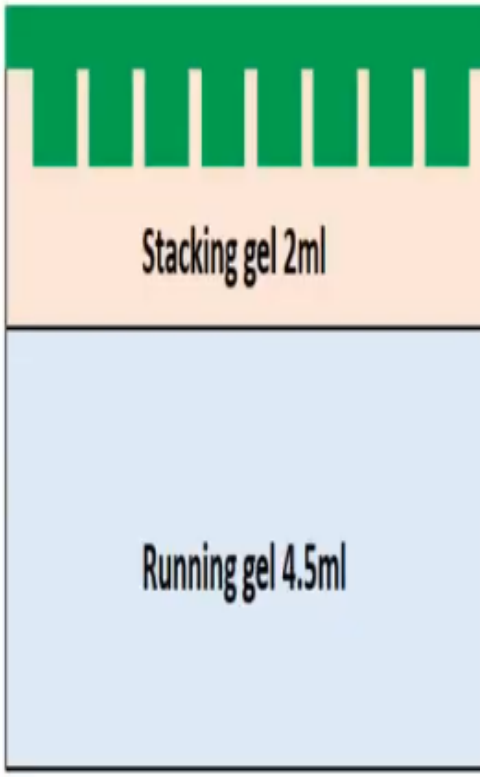
Gel preparation

- Gels used are vertical slabs, because it is more economical and more sample can be compared with each other when run under identical conditions (eg. 20 different samples)
- Gels are prepared in glass containers in which they are to be used. The two glass plates are held together but held apart from each other by plastic spacer, vertical slab gels are run along with the glass plate.
- Choice of percentage of gel to be used depends on the size of the protein sample. Separating gel used may vary from 10 % PAGE to 15 % PAGE. 15 % of gel used for separation of protein having molecular weight 10,000 to 1, 00,000 and 10 % of gel used for separation of protein having molecular weight 1, 50,000.

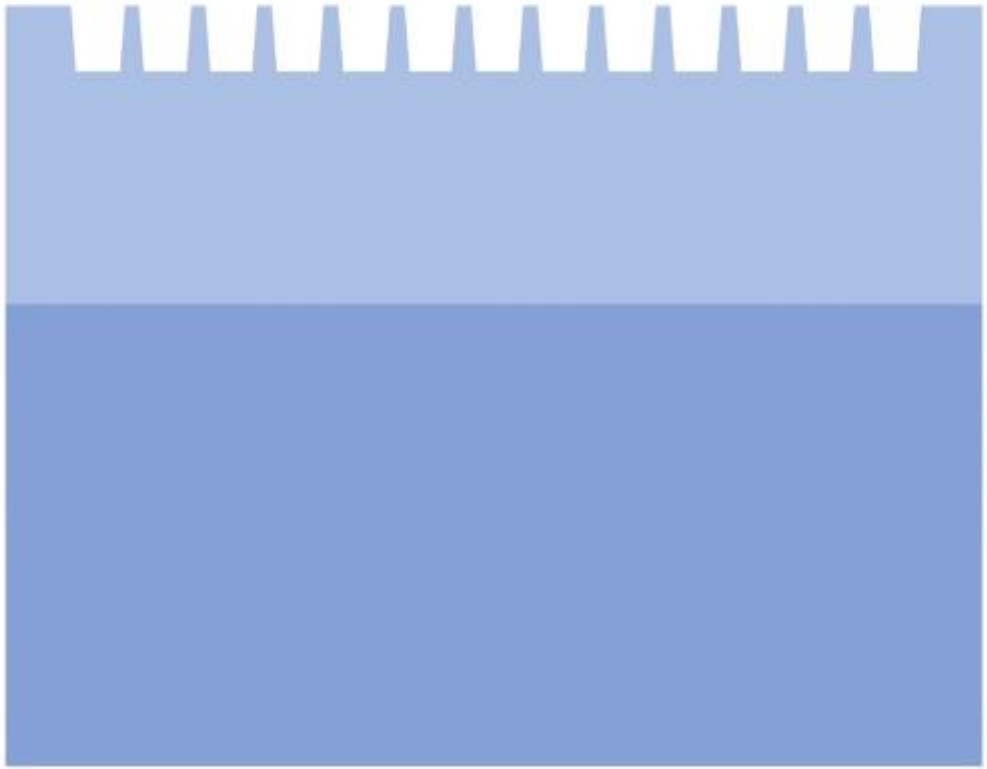
Gel Preparation

- Mix ingredients **GENTLY!** in the order shown above, ensuring no air bubbles form.
- Pour into glass plate assembly CAREFULLY.
- **Overlay gel with isopropanol** to ensure a flat surface and to exclude air.
- Wash off isopropanol with water after gel has set (±15 min).

	Running gel (10%) : 10 ml	Stacking gel (5%) : 4 ml
H_2O	4ml	2.7ml
30% acrylamide/bis mix	3.3ml	0.67ml
1.5M Tris (pH8.8)	2.5ml	-
1M Tris (pH6.8)	-	0.5ml
10% SDS	0.1ml	0.04ml
10% APS	0.1ml	0.04ml
TEMED	0.01ml	0.004ml



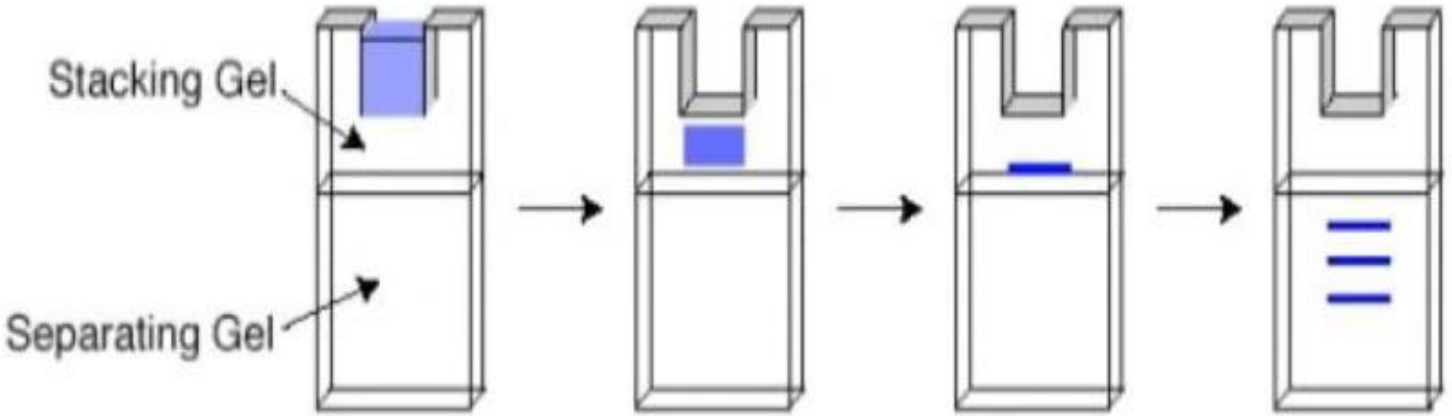
SDS-PAGE



Purpose of using two layers of gel

Stacking gel(5%) needed to concentrate all the proteins is one band, so that they will start migrating in running gel all at the same time

Separating gel (8~15%) or running gel allows to separate the proteins based on their molecular weight



Sample preparation

- The sample is boiled with the buffer containing β-mercaptoethanol and SDS for 5 min. β-mercaptoethanol reduces any disulfide bridges in protein that are holding tertiary structure of protein.
- SDS binds strongly to proteins and denature the protein
- On average one SDS molecule binds to every two amino acids. So the native charge of the molecule is completely swamped by SDS molecule (negative)
- Each protein molecule will be fully denatured, opens in to a rod shaped structure with a series of – ve charged SDS molecule along with polypeptide chain.

PAGE

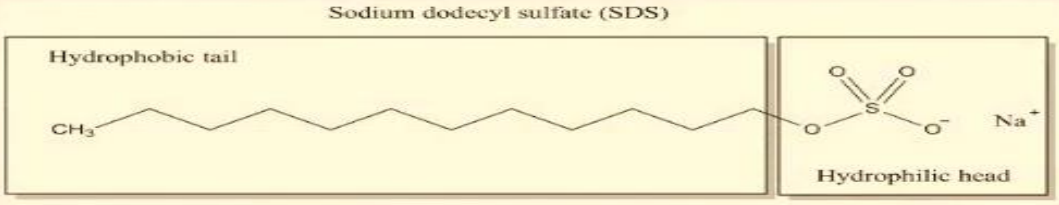
Modified PAGE Technique

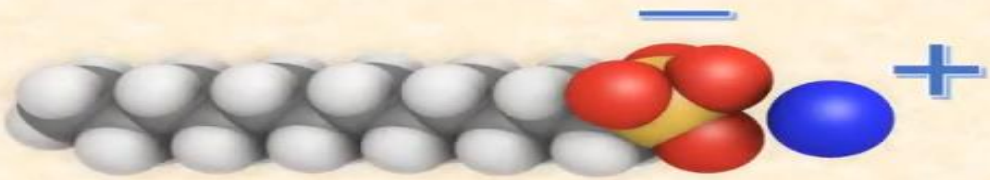
SDS-PAGE

Protein sample is pre-treated with an anionic detergent, SDS

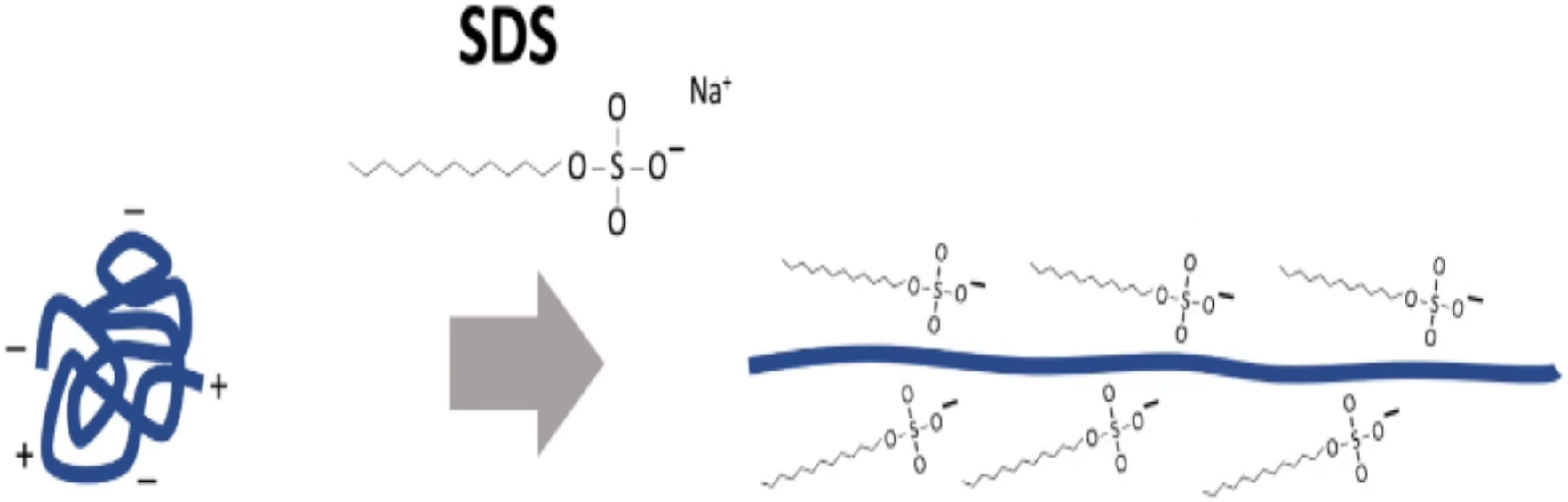
SDS-PAGE

SDS = Sodium Dodecyl Sulfate

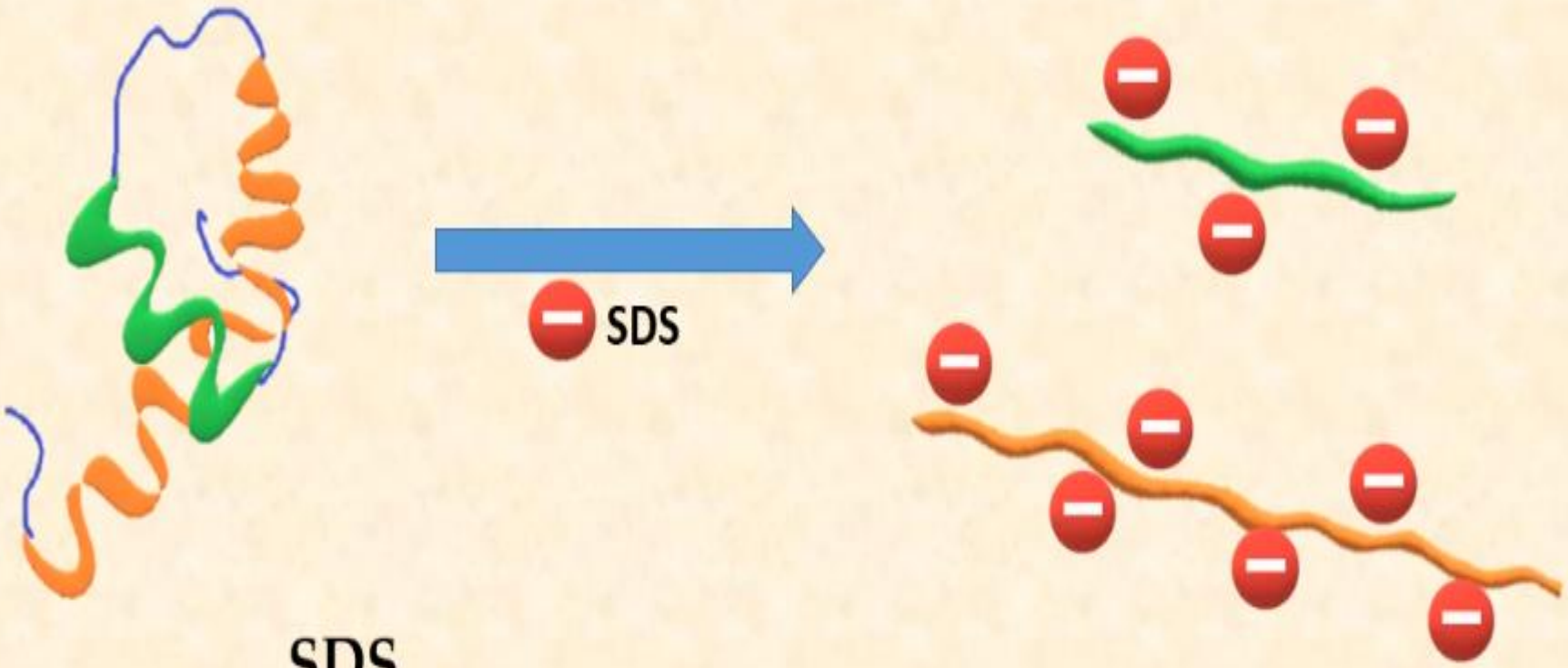




SDS-PAGE



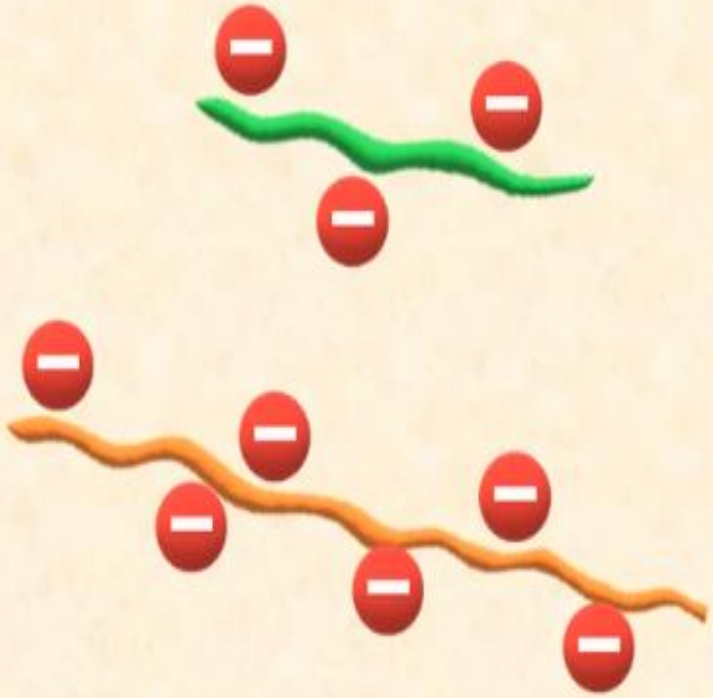
What happens when protein sample is treated with SDS?



SDS

- Denaturation of Protein molecule
- Coats them with negative charges

SDS-PAGE



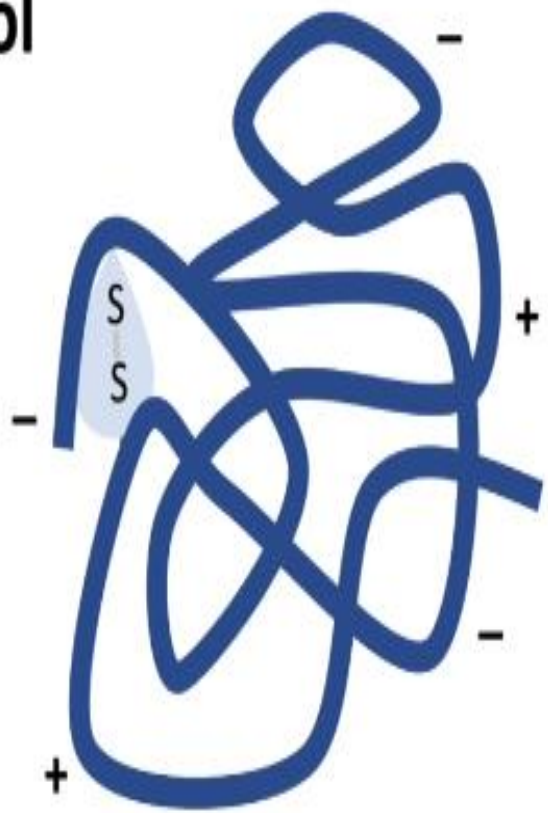
SDS denatured protein molecules

Denatured protein molecules have now approx. SAME Charge density and Shape

The only parameter left for gel electrophoresis is SIZE (or Molecular weight)

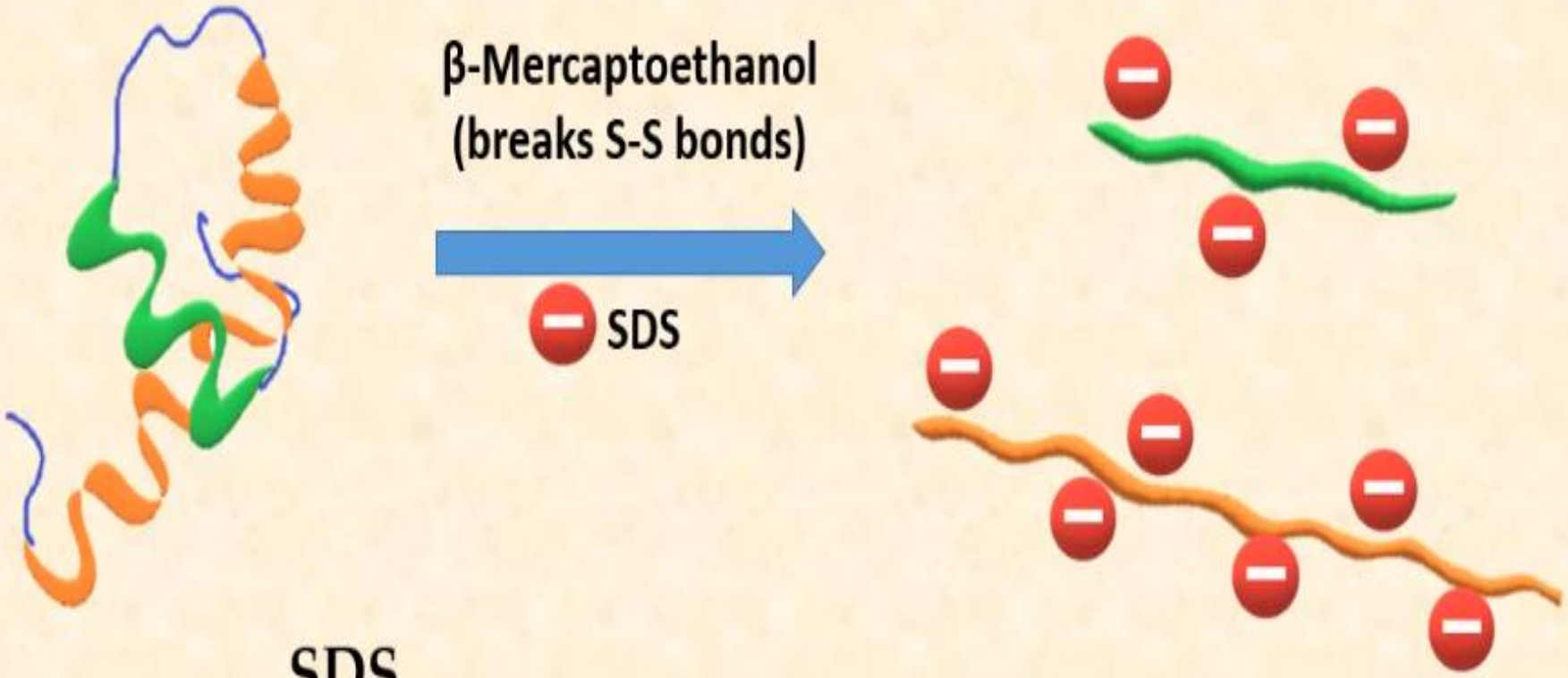
SDS-PAGE

ß-Mercaptoethanol



reduce Disulfide Bonds

What happens when protein sample is treated with SDS?



SDS

- Denaturation of Protein molecule
- Coats them with negative charges

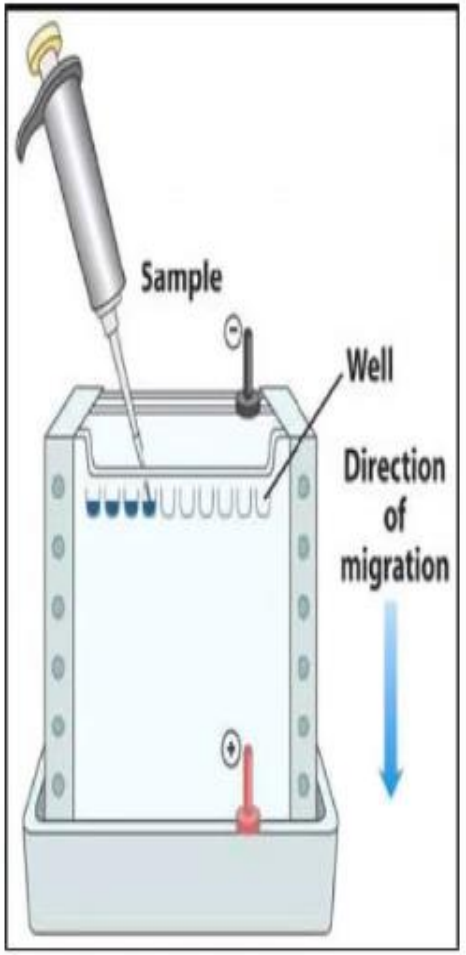
Loading Samples & Running the gel

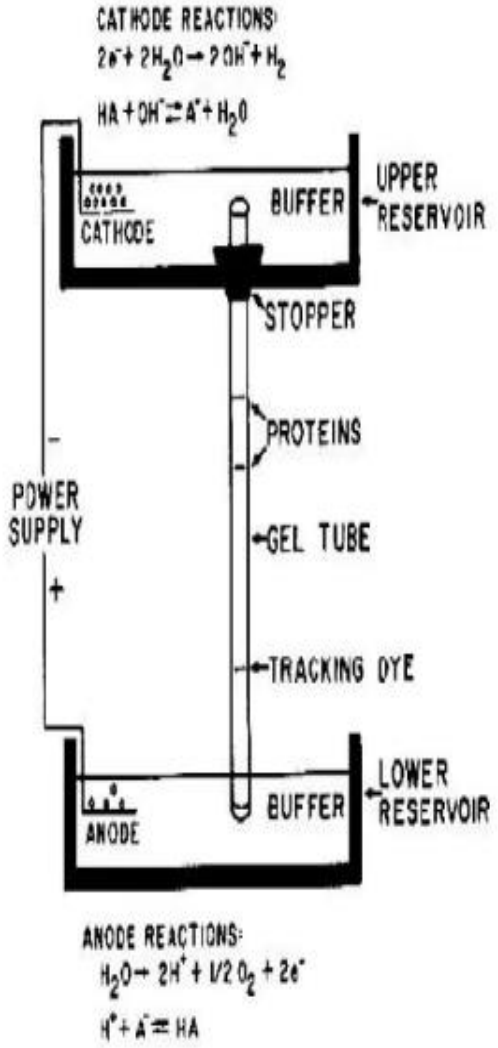
- Run at 200 volts for 30-40 minutes

- **Running Buffer, pH 8.3**

Tris Base	12.0 g
Glycine	57.6 g
SDS	4.0 g

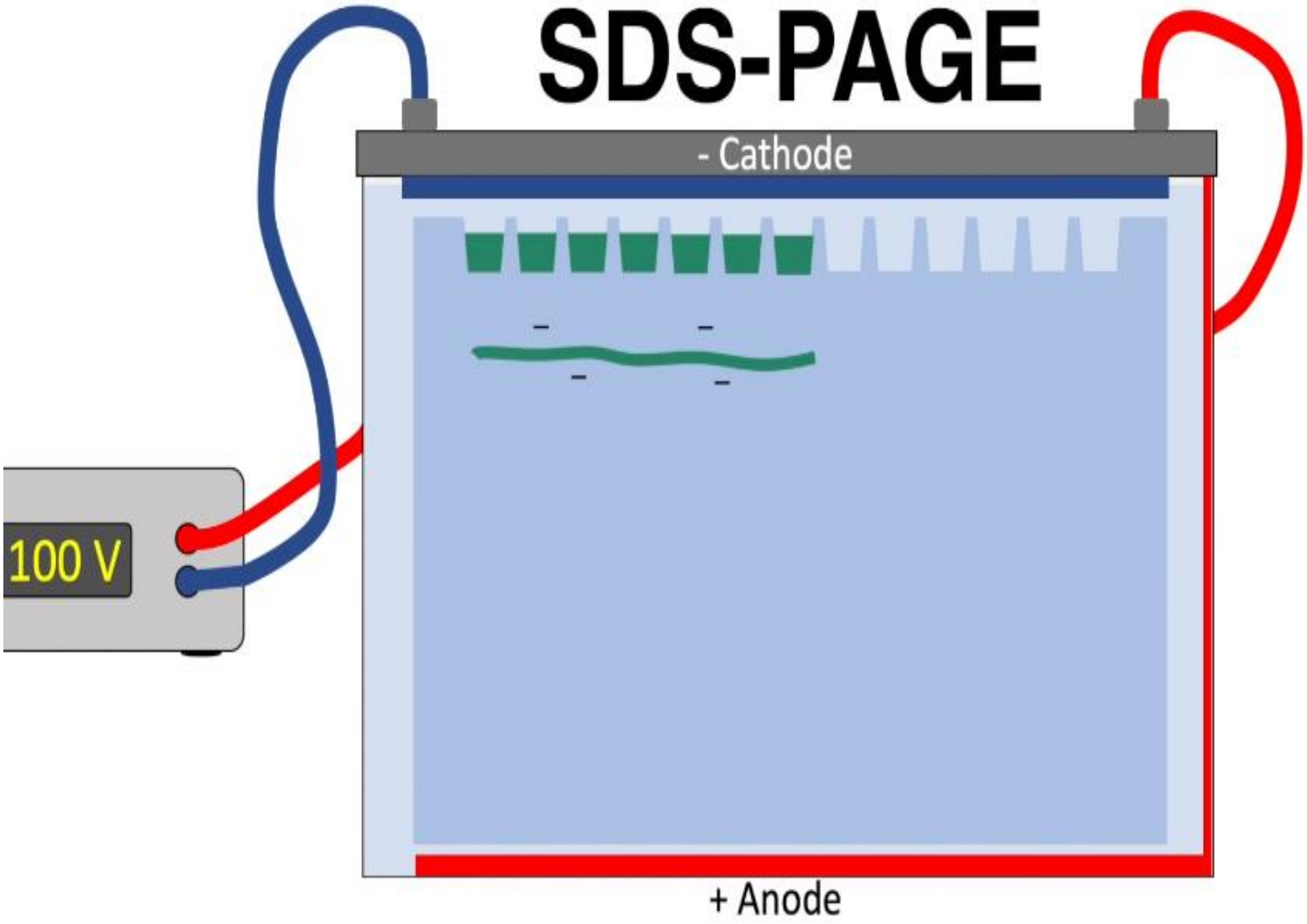
distilled water to 4 liter

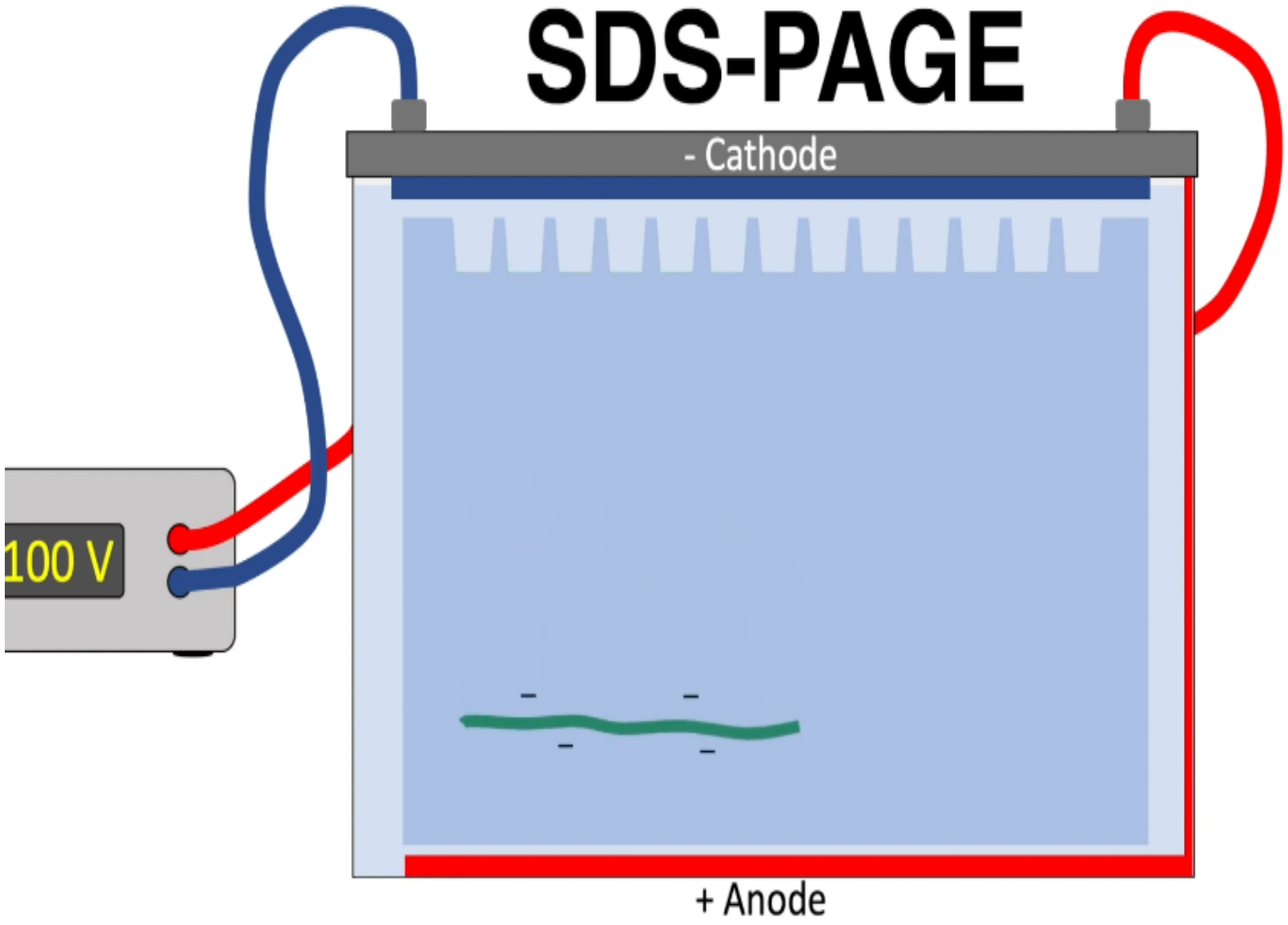




Running the gel

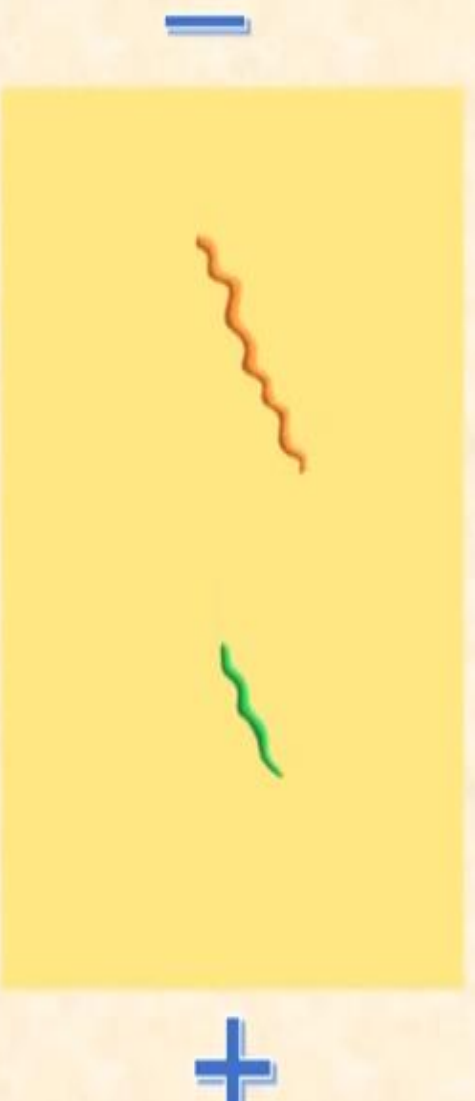
- The gel slab sandwiched in between the glass plate is placed in the lower reservoir with the top of the gel in contact with the buffer in the upper reservoir.
- Thus the gel completes the electrical circuit between the lower and upper compartments. Although the buffer dissipates the heat generated, additional cooling may be needed.
- In sample small protein can more easily pass through the pores and larger proteins are successively retarded by frictional resistance due to sieving effect of the gel.
- Precise voltage and time required for the optimal separation Voltage: 30 mA; Time; 3 hrs


SDS-PAGE
- Cathode
100 V
+ Anode


SDS-PAGE
- Cathode
100 V
+ Anode

SDS-PAGE

SDS-PAGE separates proteins on the basis of their Size or Molecular Weight.



Larger the Size (Higher the Molecular weight),

Slower will be the migration.

Migration Depends on

- Strength of electric fields.
- Temperature
- Features of the molecule
  - Net charge of molecule
  - Size of molecule
  - Shape of molecule
- Features of the Gel
  - Gel type
  - Gel concentration
- Buffer Type/pH.

Voltage

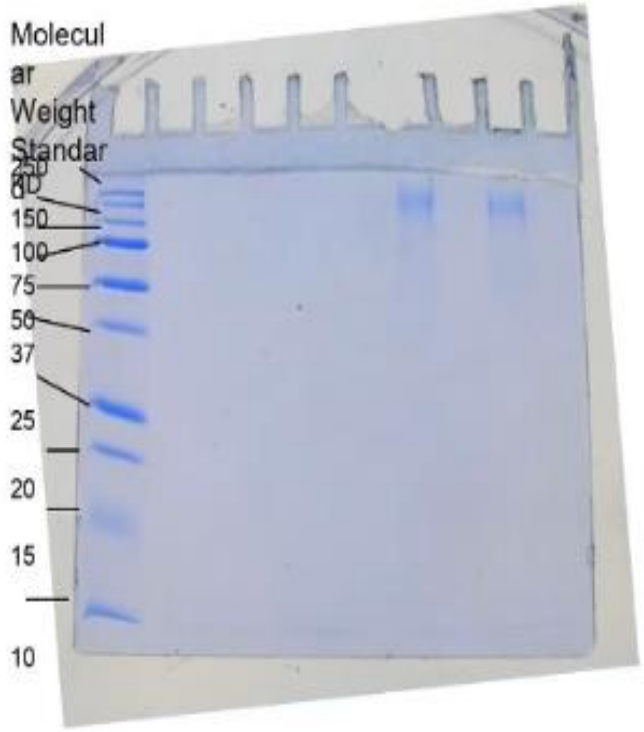
- More voltage, more quick gel runs.
- But,
  - Low resolution.
  - Increase temperature
- As a result, low quality separation.
- <5-8 V/cm of gel length 75mA.(100mA for minigels)
- By trial and error (Emprical approach)

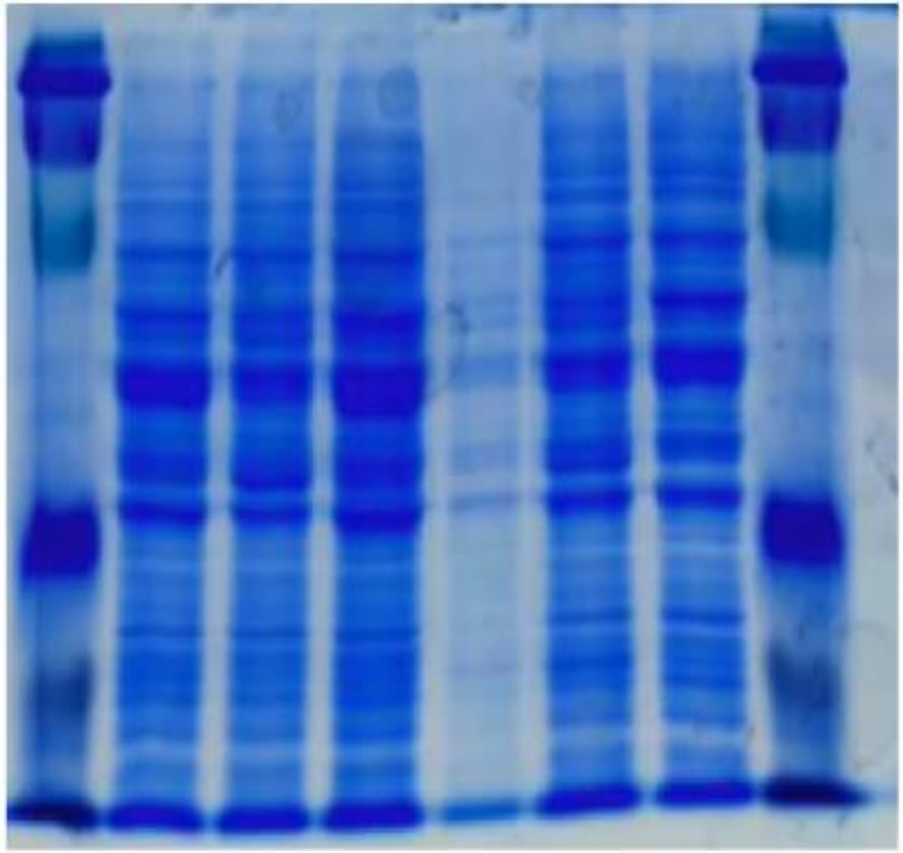
Staining Proteins in Gels

- **Coomassie Brilliant Blue**
  - The CBB staining can detect about 1 µg of protein in a normal band.

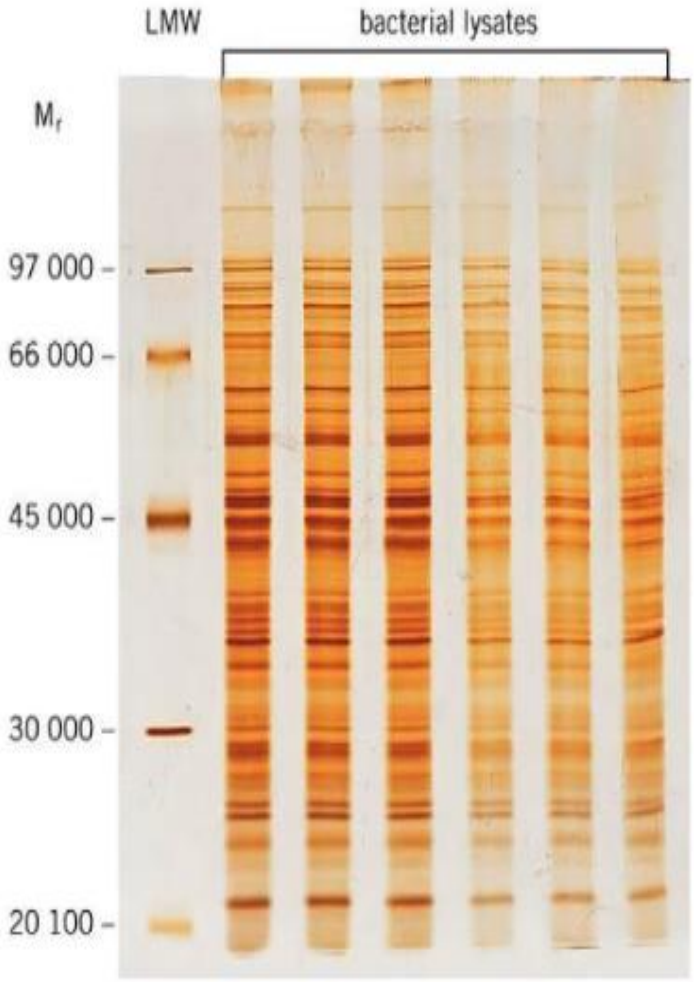
- **Silver Staining**
  - The silver stain system are about 100 times more sensitive, detecting about 10 ng of the protein.

Protein bands observed in SDS-PAGE Coomassie Brilliant Blue





Example of silver stained gel



Silver staining is usually 10-100 times more sensitive than Coomassie Blue staining, but it is more complicated.

Faint but still visible bands on this gel contain less than 0.5 ng of protein!

PAGE

NATIVE-PAGE

Proteins remain in their native form.

Proteins get separated on the basis of their Charge density, Size and Shape.

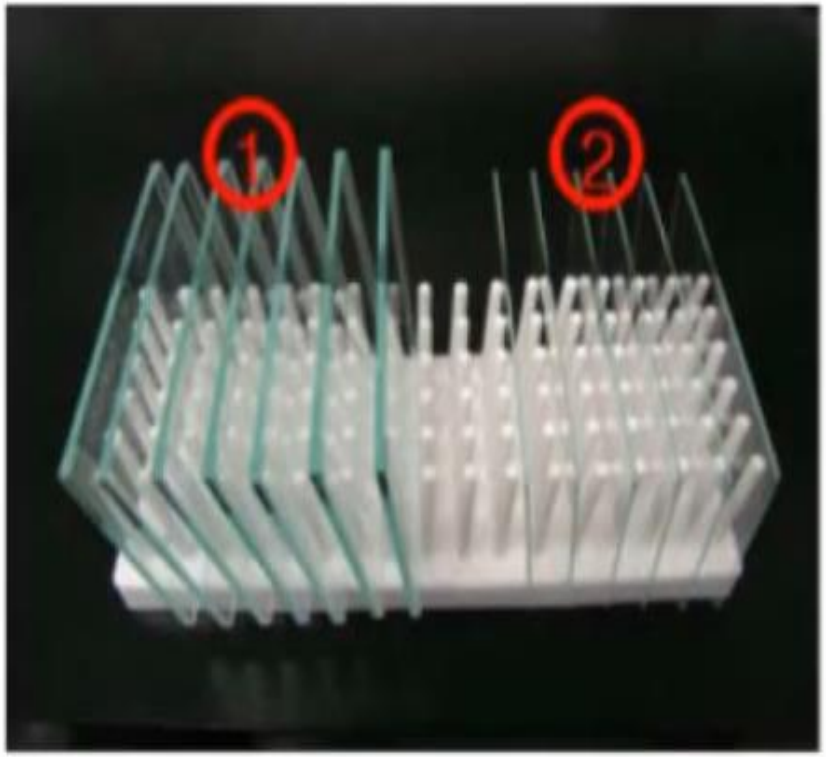
Used for the separation and detection of enzymes.

SDS-PAGE

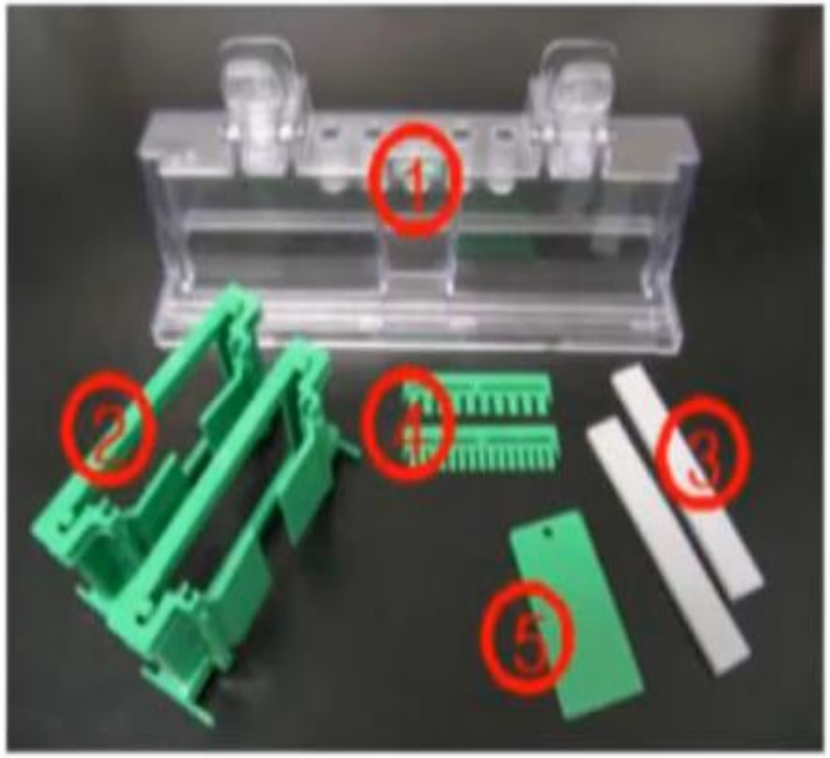
Proteins are first denatured using SDS and reducing agents.

Proteins get separated on the basis of their Size (Molecular Weight).

Used for the determination of molecular weights of proteins.



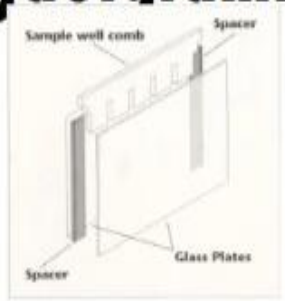
① Spacer plate
② Short plate

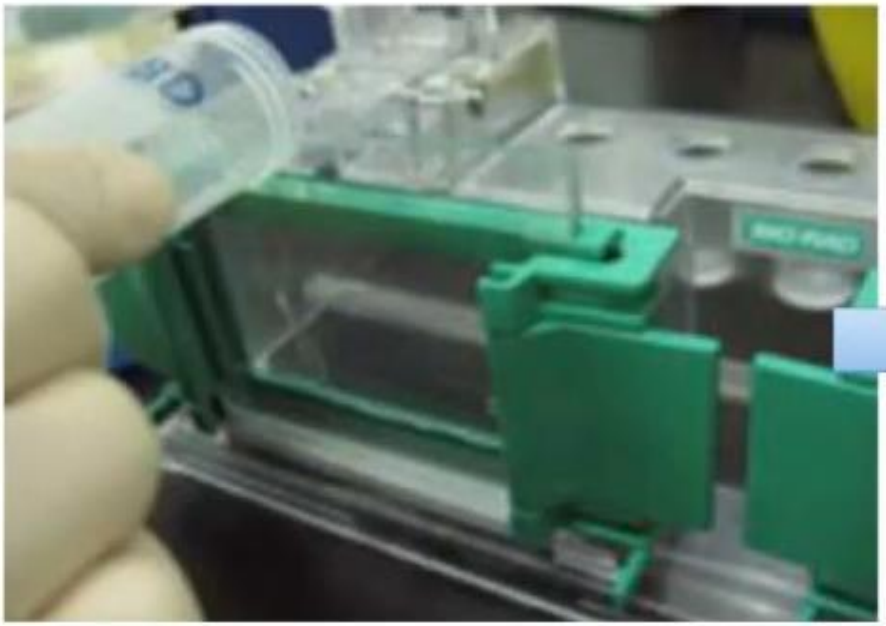


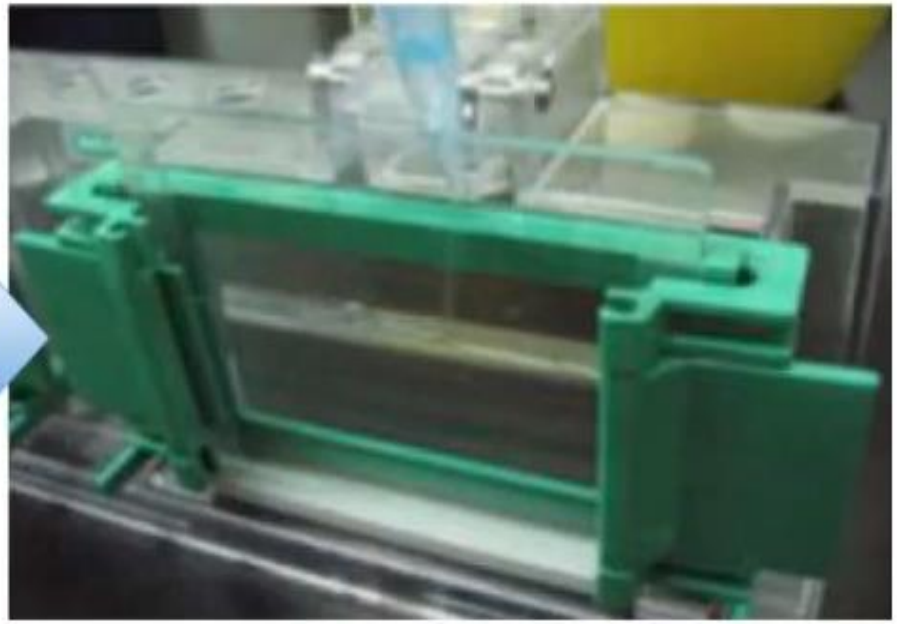
① Casting stand
② Casting frame
③ Casting stand gasket
④ Comb
⑤ Gel releaser

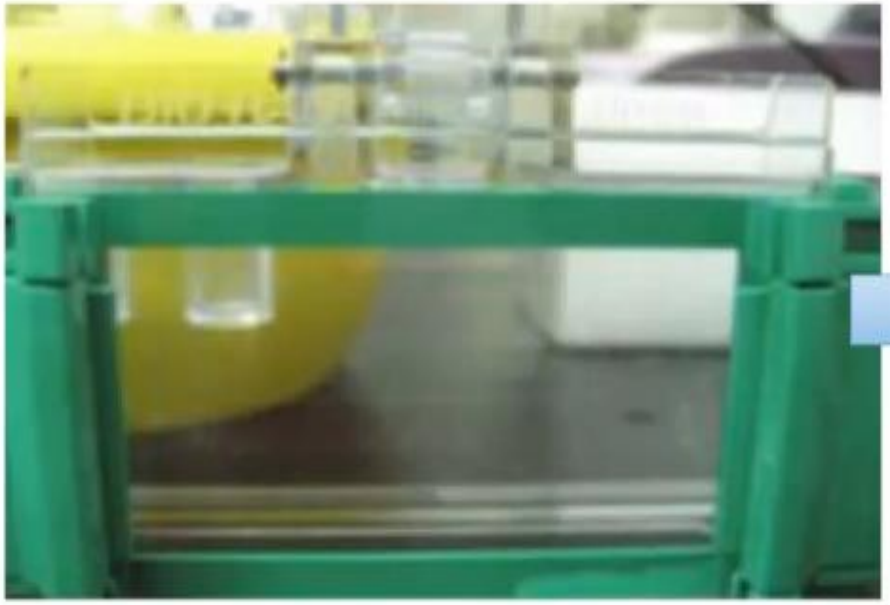
Step by Step Instructions on how to assemble the polyacrylamide gel apparatus

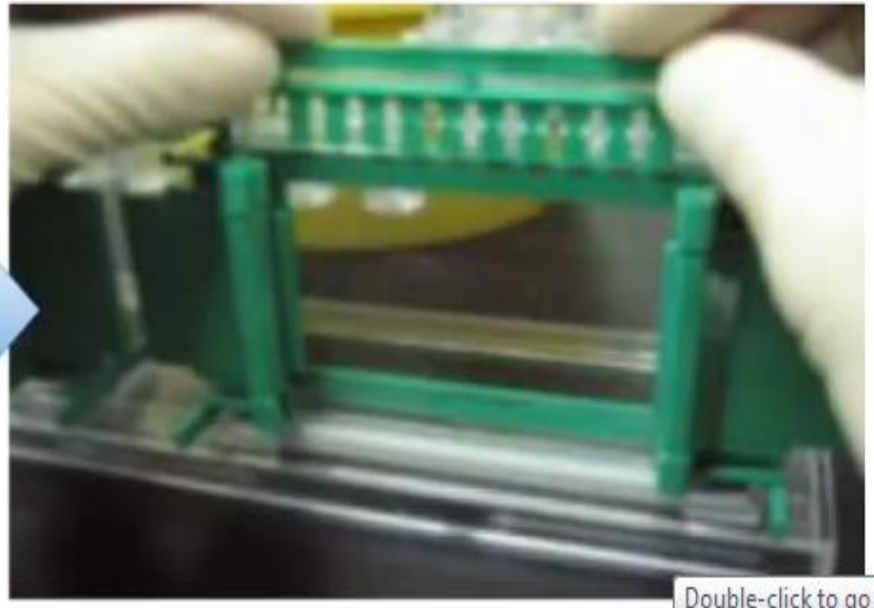
- Prepare polyacrylamide gels
- Add diluted samples to the sample buffer
- Heat to 95°C for 4 minutes
- Load the samples onto polyacrylamide gel
- Run at 200 volts for 30-40 minutes
- Stain

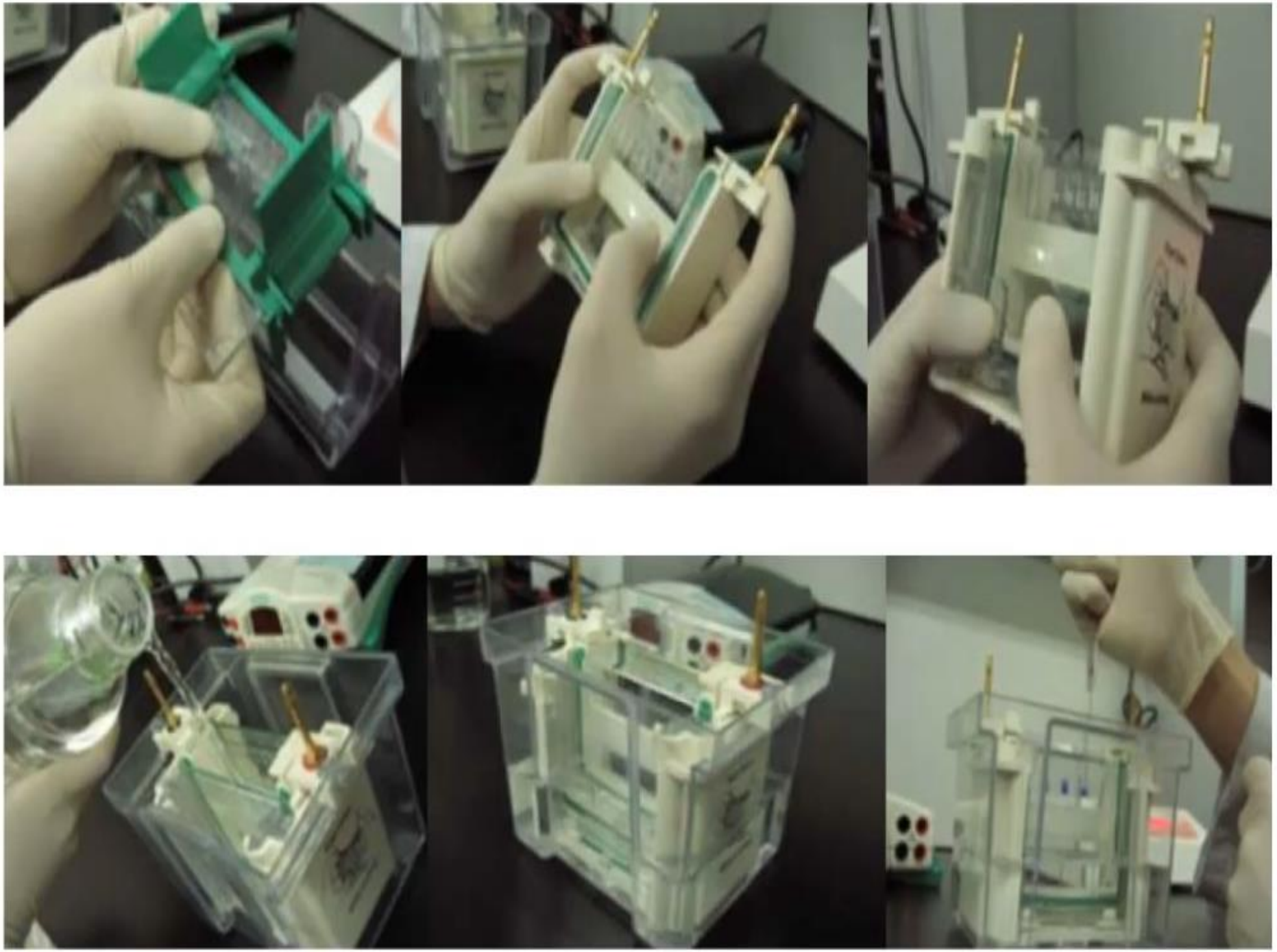


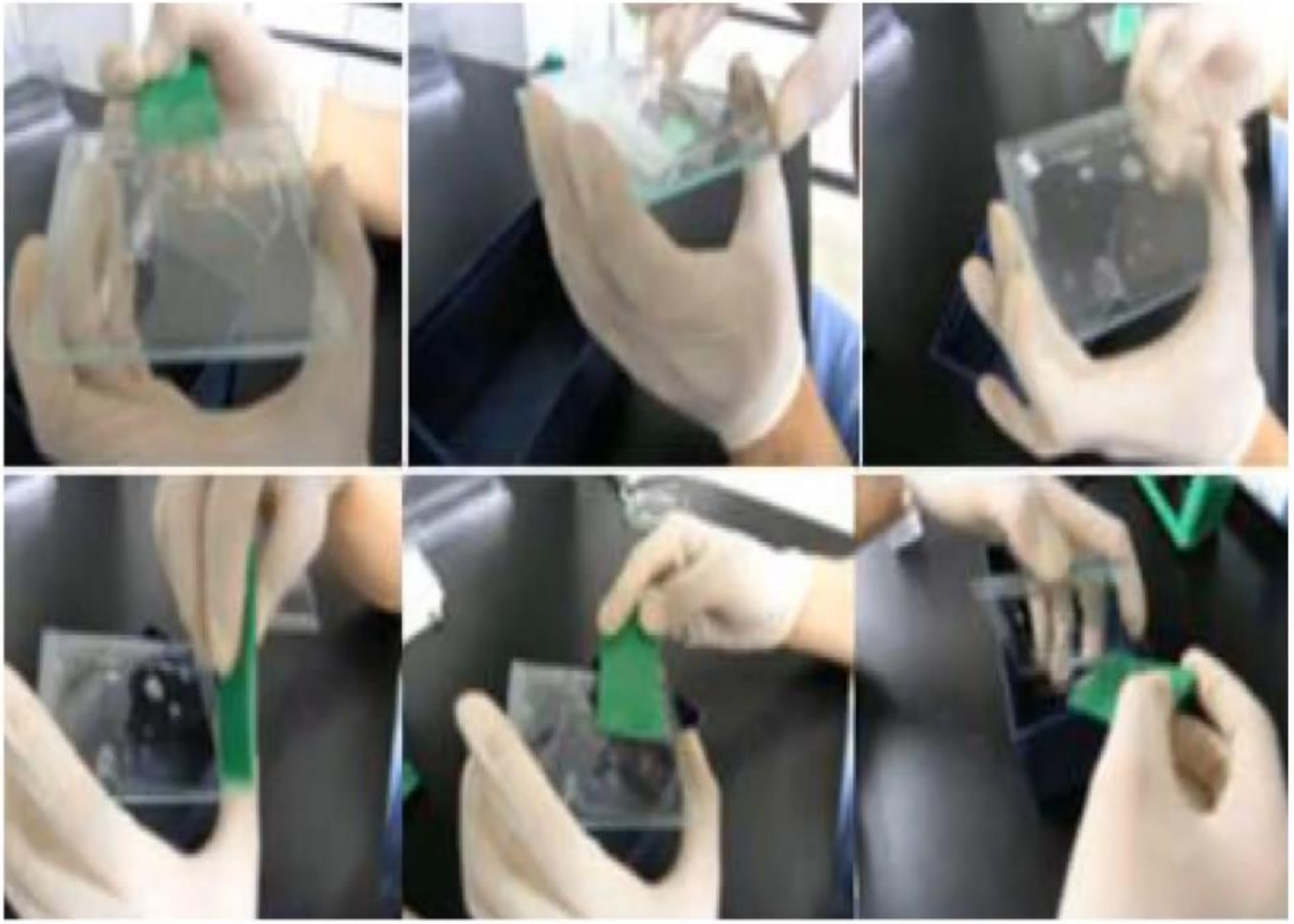




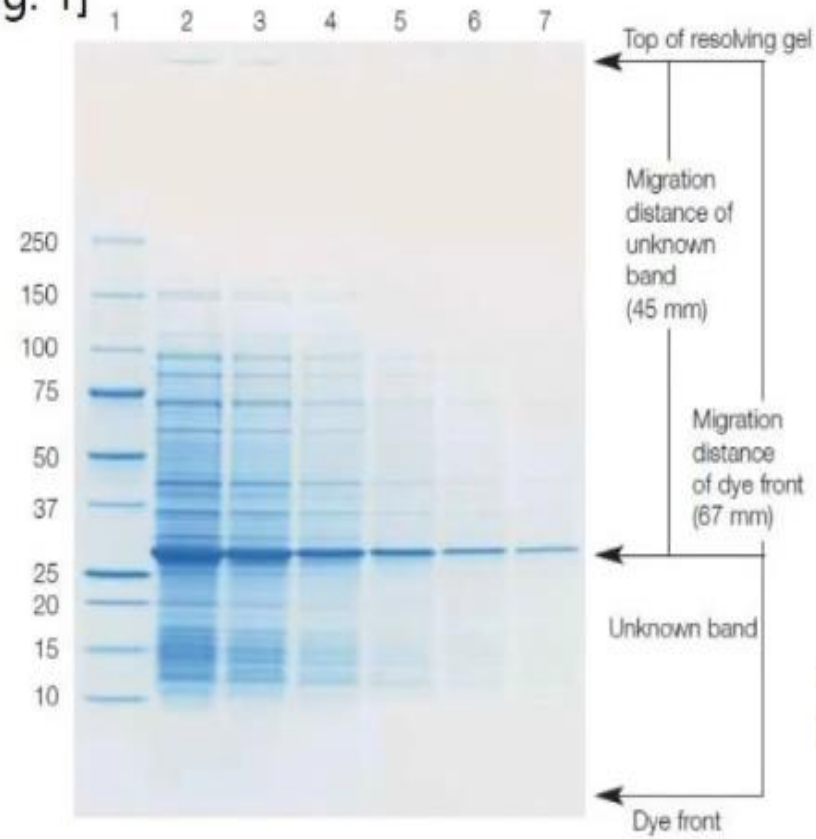




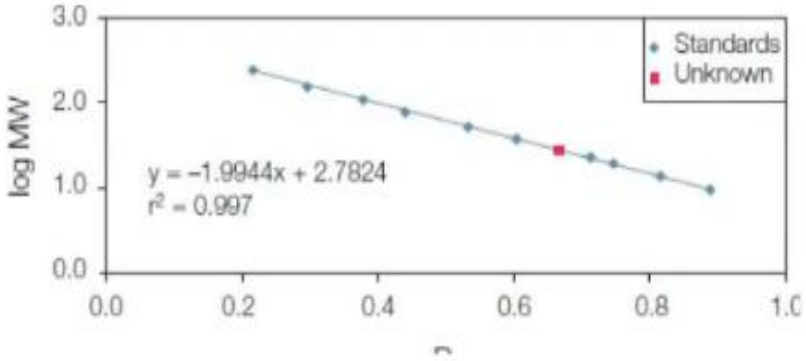




[Fig. 1]



[Fig. 2]



Procedure

Figures 1 and 2 illustrate the procedure.

1. Run the standards and unknown on an SDS-PAGE gel.
2. Process the gel with the desired stain and then destain to visualize the protein bands.
3. Determine the R_f graphically or using Quantity One software (or equivalent).
4. Use a graphing program to plot the R_f versus log MW. From the program, generate the straight line equation $y = mx + b$, and solve for y to determine the MW of the unknown protein.

Example of Calculation

From Figure 1:

Migration distance of unknown protein: 45 mm

Migration distance of dye front: 67 mm

So R_f = 45 mm/67 mm = 0.67

Rf = migration distance of the protein/ migration distance of the dye front

From Figure 2:

$y = -1.9944x + 2.7824$

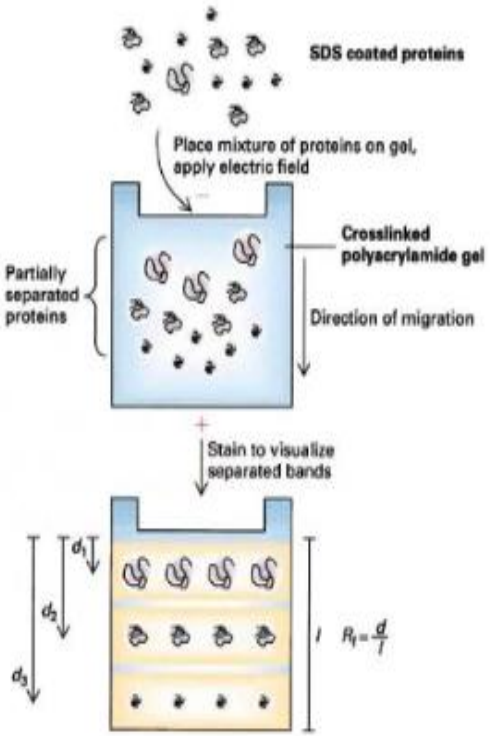
$x = R_f$ of unknown protein = 0.67

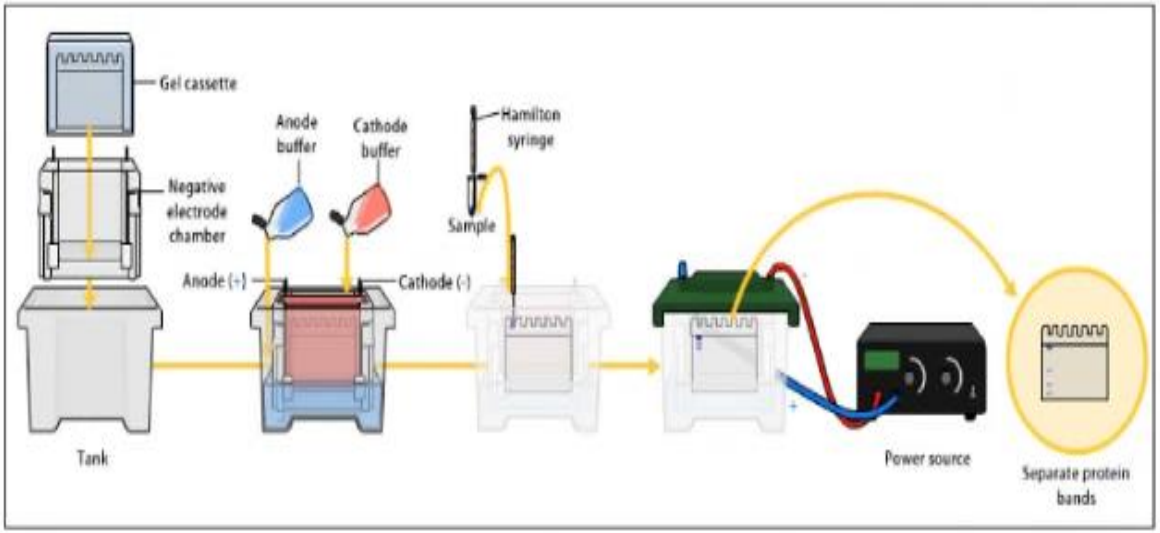
$y = \log MW$

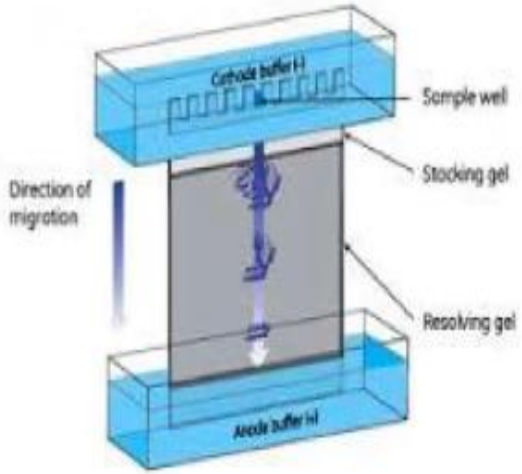
So $MW = 10^y = 10^{-1.9944(0.67) + 2.7824} = 28.1$ kD

Making Proteins

DNA	TAC	GGA	TCG	AGA	TGA
mRNA	AUG	CCU	AGC	UCU	ACU
tRNA	UAC	GGA	UCG	AGA	UGA
Amino Acid	Tyr	Gly	Ser	Arg	STOP







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Thank you