In the name of Allah
the most beneficent, the most merciful

SDS-PAGE

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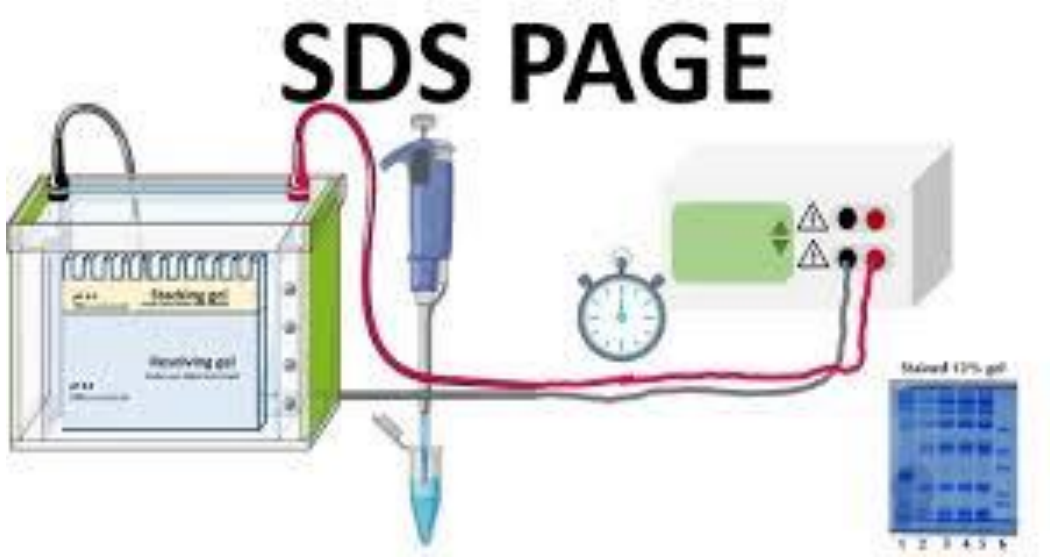
Over view

1. Introduction
2. Sample and gel preparation
3. SDS-PAGE procedure
4. Staining and visualization of protein bands
5. Analysis and interpretation of results
6. Applications
7. Advantages and limitations
8. Safety tips and common Errors

Introduction

SDS-PAGE(Sodium dodecyl sulfate polyacrylamide gel electrophoresis) is a widely used technique in biochemistry and molecular biology to **separate proteins** based on their **molecular weight.**



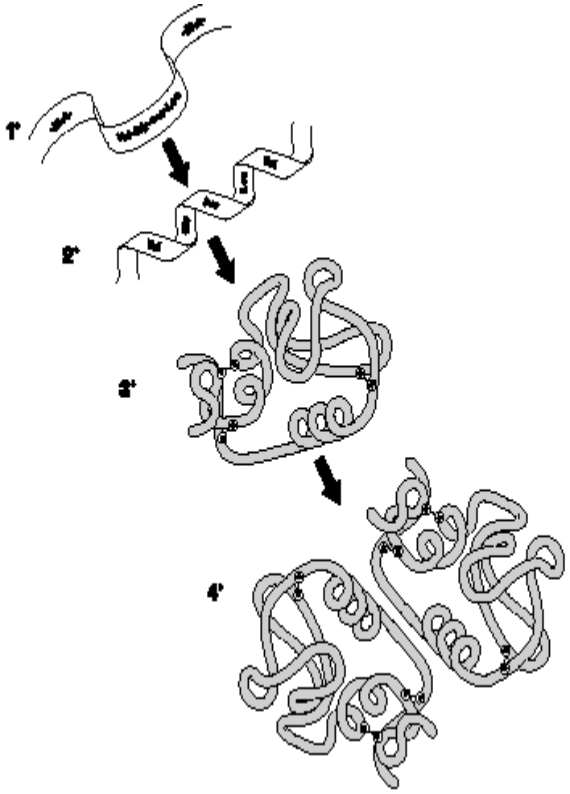
Proteins: characteristics

1. Primary structure = linear chain of amino acids

2. Secondary structure = domains of repeating structures, such as β sheets and α-helices

3. Tertiary structure = 3-dimensional shape of a folded polypeptide, maintained by disulfide bonds, electrostatic interactions, hydrophobic effects

4. Quaternary structure = several polypeptide chains associated together to form a functional protein



Protein Size

- Size measured in daltons (Da) or kilodaltons (kDa)
- **Dalton** (atomic mass unit) = corresponds to mass of hydrogen molecule (1.66×10^{-24} gram)

= defined also as 1/16 of the mass of an atom of oxygen

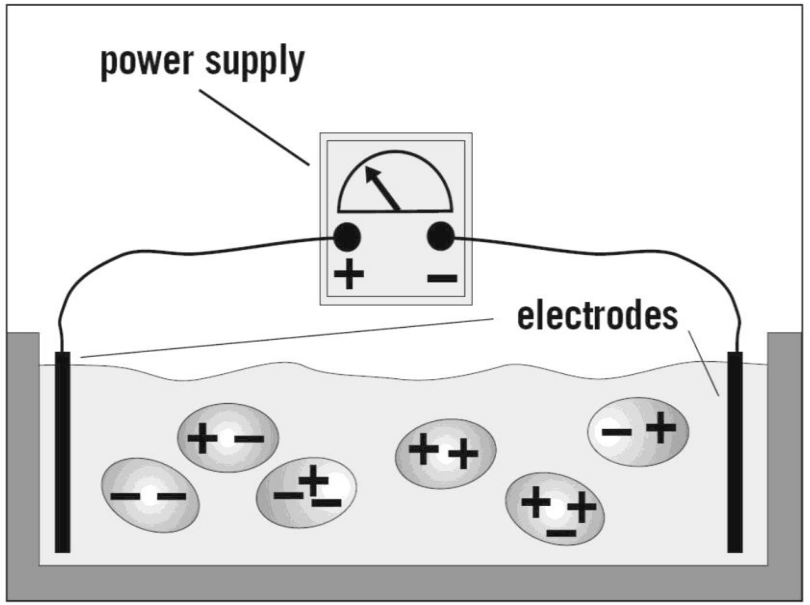
Average amino acid = 110 Da

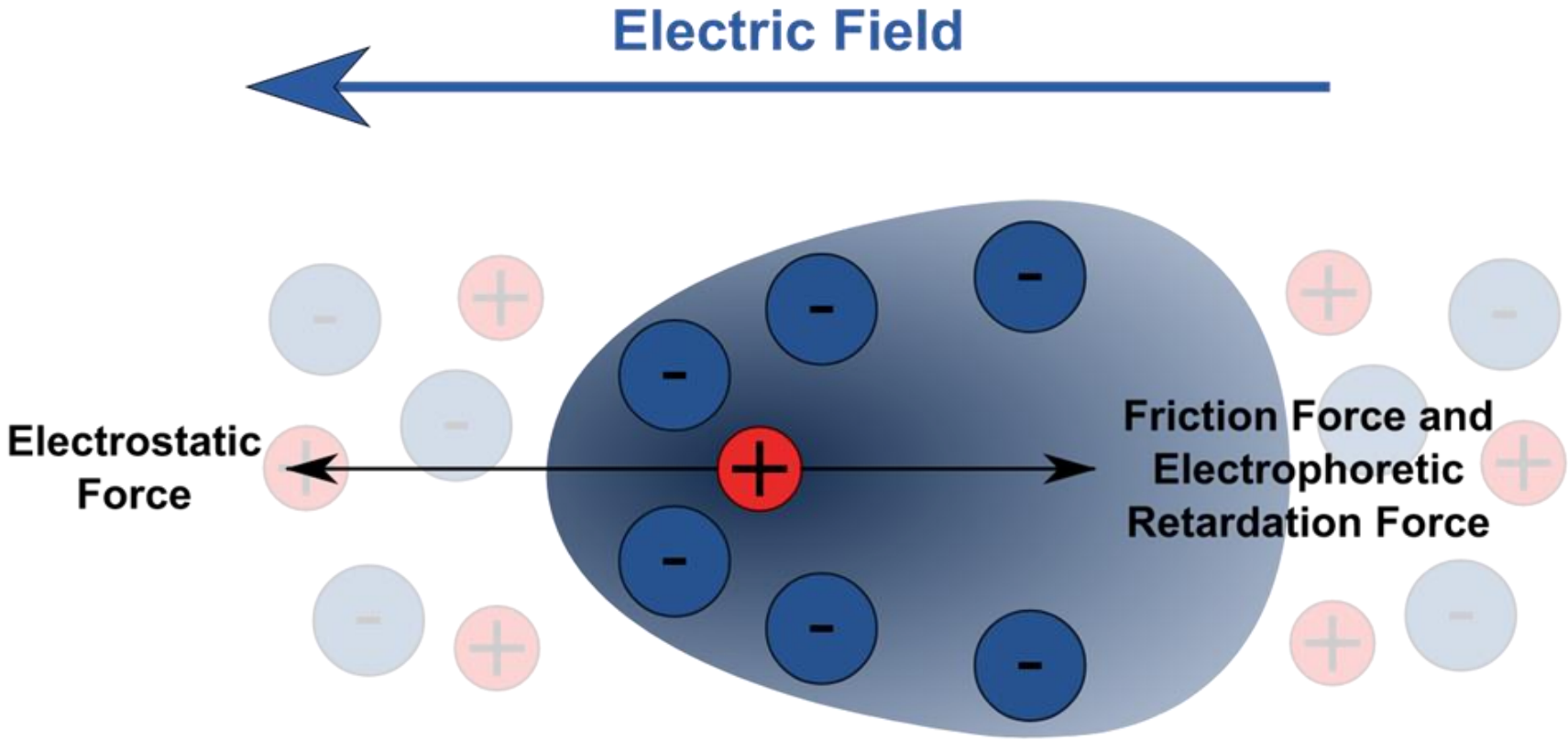
Average nucleotide pair = 650 Da

Electrophoretic procedures

- Gel electrophoresis is a technique in which charged molecules are separated according to physical properties such as **charge** or **mass** as they are forced through a sieving gel matrix by an electrical current.

➢ Proteins are commonly separated in this manner using polyacrylamide gel electrophoresis (PAGE) to identify individual proteins in complex samples or to examine multiple proteins within a single sample.




Electric Field
Electrostatic Force
Friction Force and Electrophoretic Retardation Force

Types of Gels

- The most common types of gels are:

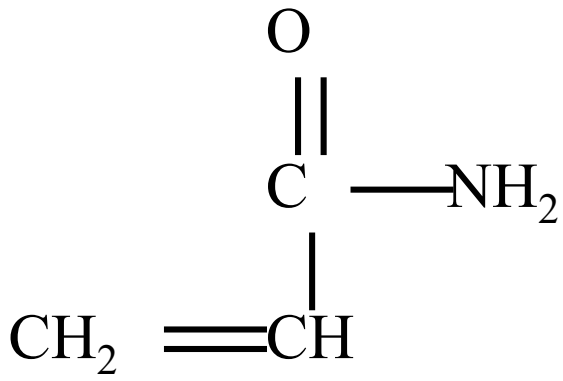
Starch gels: seldom used nowadays

Agarose gels: for separation of nucleic acids and large proteins

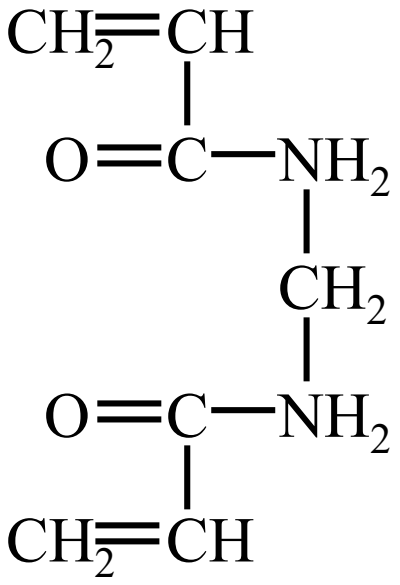
Polyacrylamide gels: for separation of most proteins and small nucleic acids

Polymerization

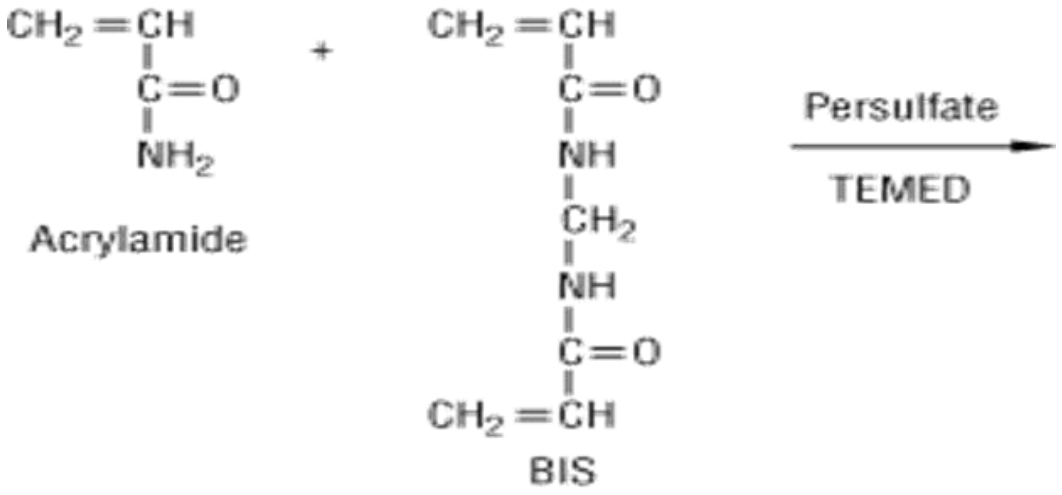
- Polyacrilamide is a polymer made of acrylamide (C_3H_5NO) and bis-acrilamide (N,N'-methylene-bis-acrylamide $C_7H_{10}N_2O_2$)

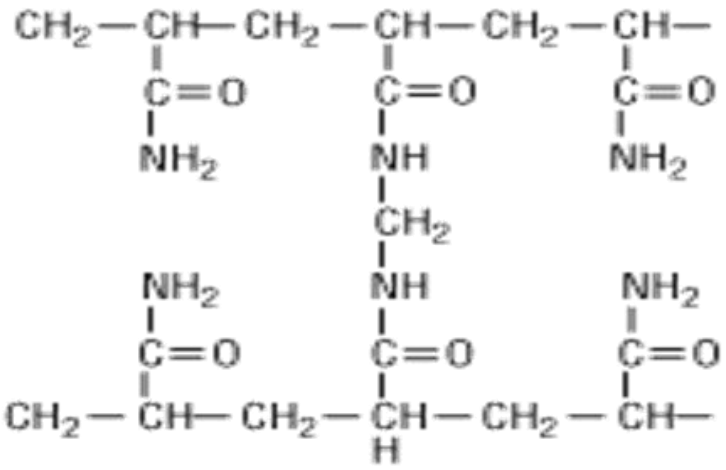


Acrylamide



bis-Acrylamide





Polyacrylamide

Polyacrylamide Gel Electrophoresis (PAGE)

PAGE can be classified according the separation conditions into:

1- Native-PAGE:

- Separation is based upon **charge**, **size**, and **shape** of macromolecules.
- Useful for separation and/or purification of mixture of proteins
- This was the original mode of electrophoresis (introduced in 1930s).

2- Denatured-PAGE or SDS-PAGE

- Separation is based upon the **molecular weight** of proteins.
- The most common method for determining MW of proteins
- Very useful for checking purity of protein samples

Role of SDS

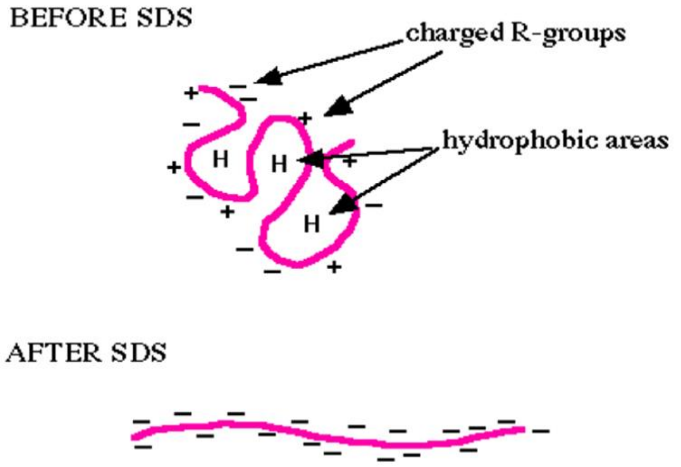
- SDS is an **anionic detergent** that denatures **proteins** and imparts a uniform negative charge, allowing separation by **size**.

- Migration of proteins:

*directly proportional to the overall charge of proteins

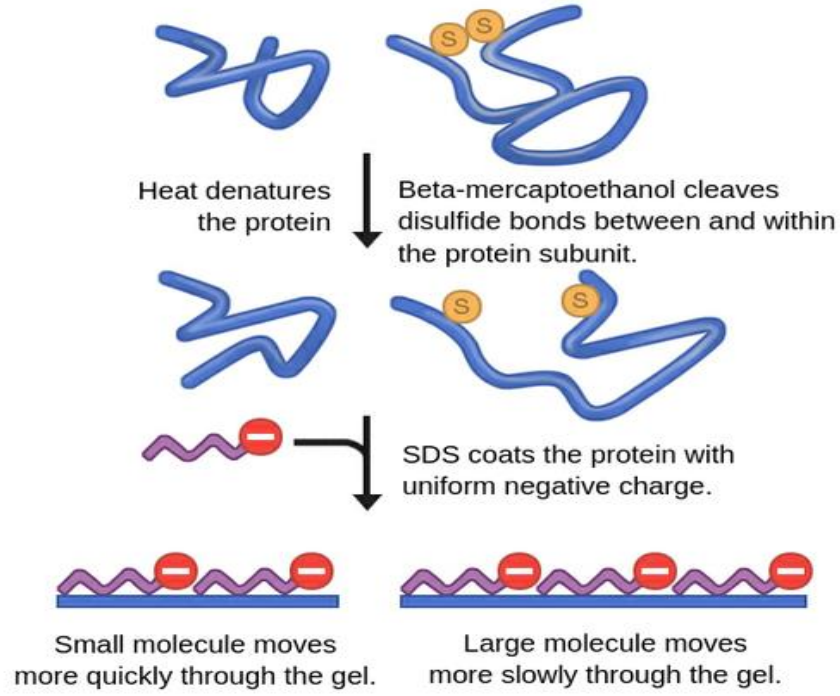
*inversely proportional to protein size (molecular weight)

✓Without SDS, different proteins with similar molecular weights would migrate differently due to differences in mass charge ratio.



Reducing Agents

- **β -mercaptoethanol** or **Dithiothreitol (DTT)** break disulfide bonds, ensuring proteins are fully denatured and linear.



Sample Buffer Preparation

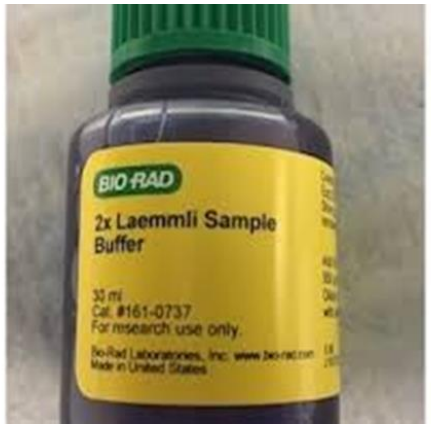
1. Tris buffer to provide appropriate pH

2. SDS (sodium dodecyl sulphate) detergent to dissolve proteins and give them a negative charge

3. *β-mercaptoethanol*, a reducing agent: eliminates ***disulfide bonds*** in proteins by ***reducing*** them (adding hydrogen atoms).

4. Glycerol to make samples sink into wells

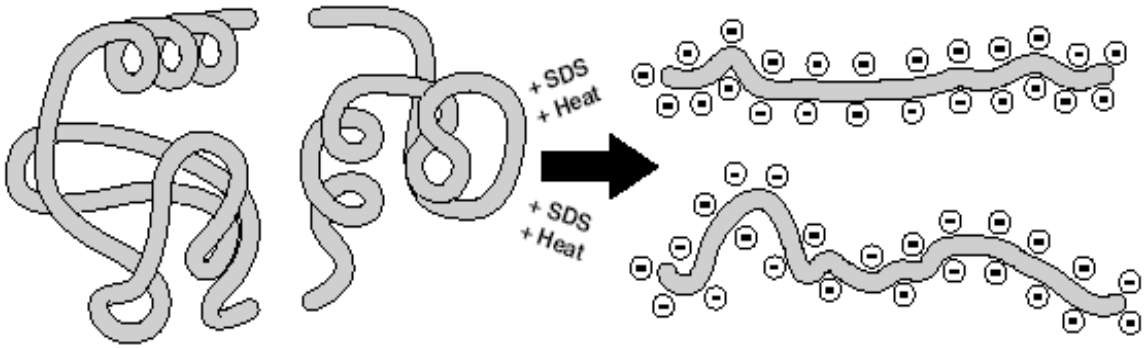
5. Bromophenol Blue dye to visualize samples



Completion of Denaturation

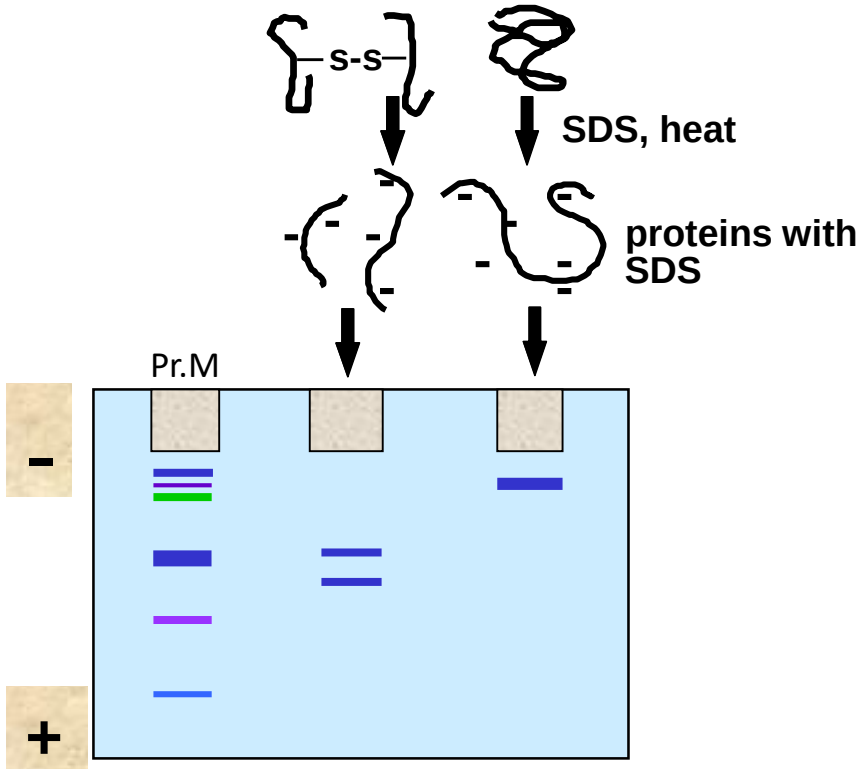
- Proteins denatured by heating (**95°C** for **5-10 minutes**) them in a sample buffer containing sodium dodecyl sulphate (SDS): the proteins no longer have any secondary, tertiary or quaternary structure.

- Resultant proteins take on a rod-like shape and a uniform negative charge-to-mass ratio proportional to their molecular weights.



How does an SDS-PAGE gel work?

- Negatively charged proteins move to positive electrode
- Smaller proteins move faster
- Proteins separate by size



Gel Composition

- Polyacrylamide gel consists of **stacking** and **separating** layers with different acrylamide concentrations and pH.

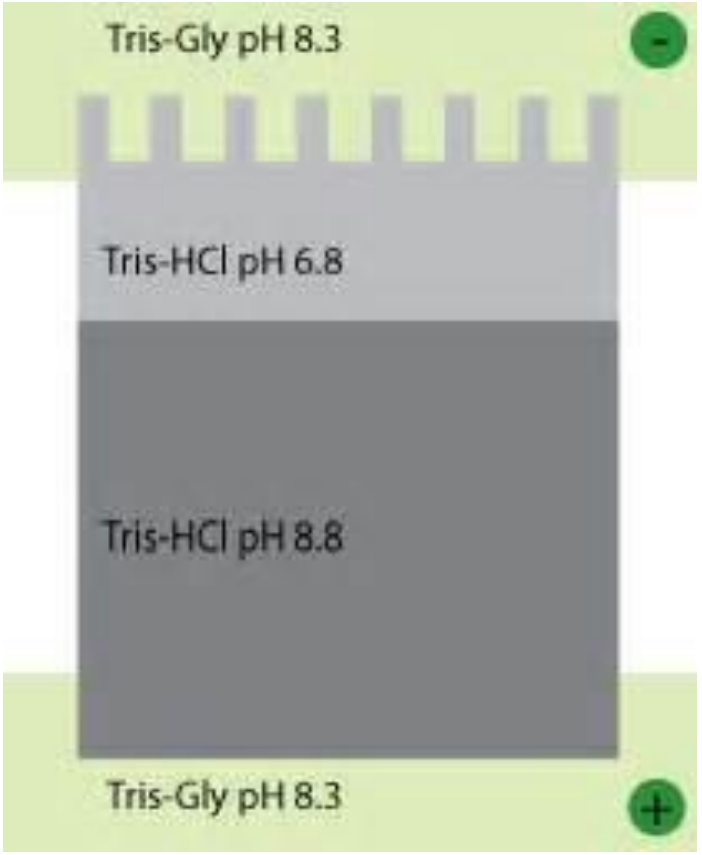
1. Stacking (concentrating) gel
   - 4% acrylamide (36.5:1, acryl/bis)
   - 125 mM Tris-H^+Cl^-, pH 6.8, 0.1% SDS

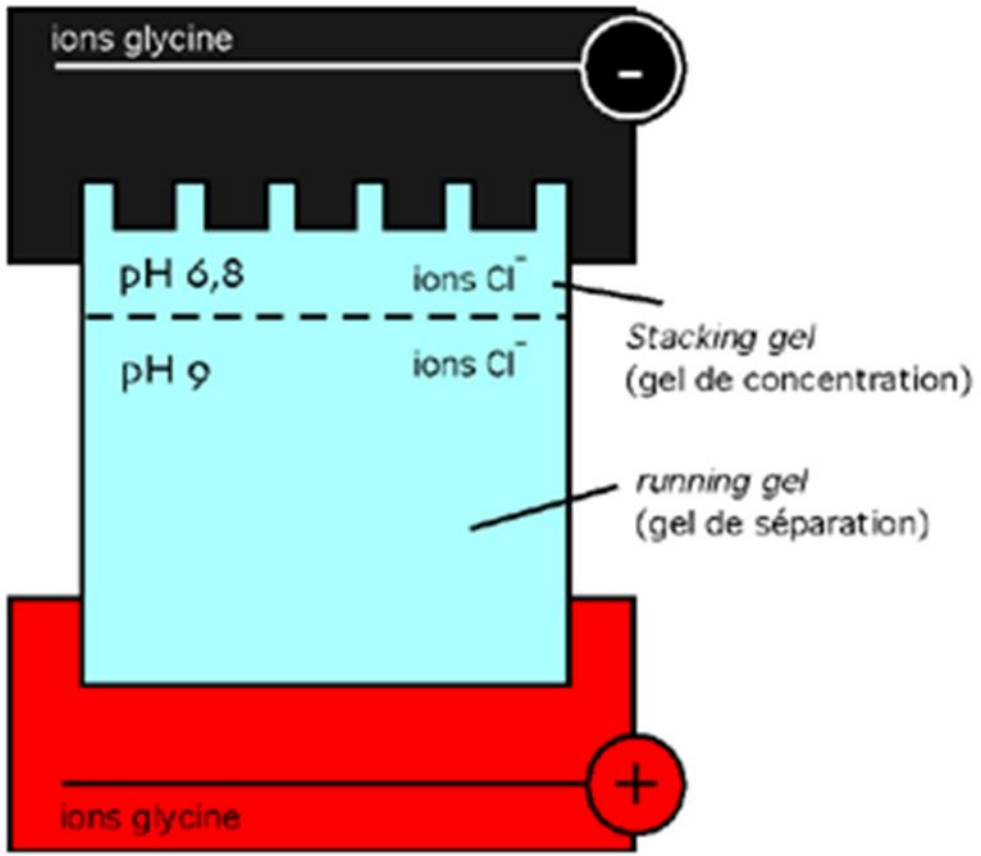
2. Resolving (separating) gel
   - 10% acrylamide (36.5:1, acryl/bis)
   - 425 mM Tris-H^+Cl^-, pH 8.8, 0.1% SDS

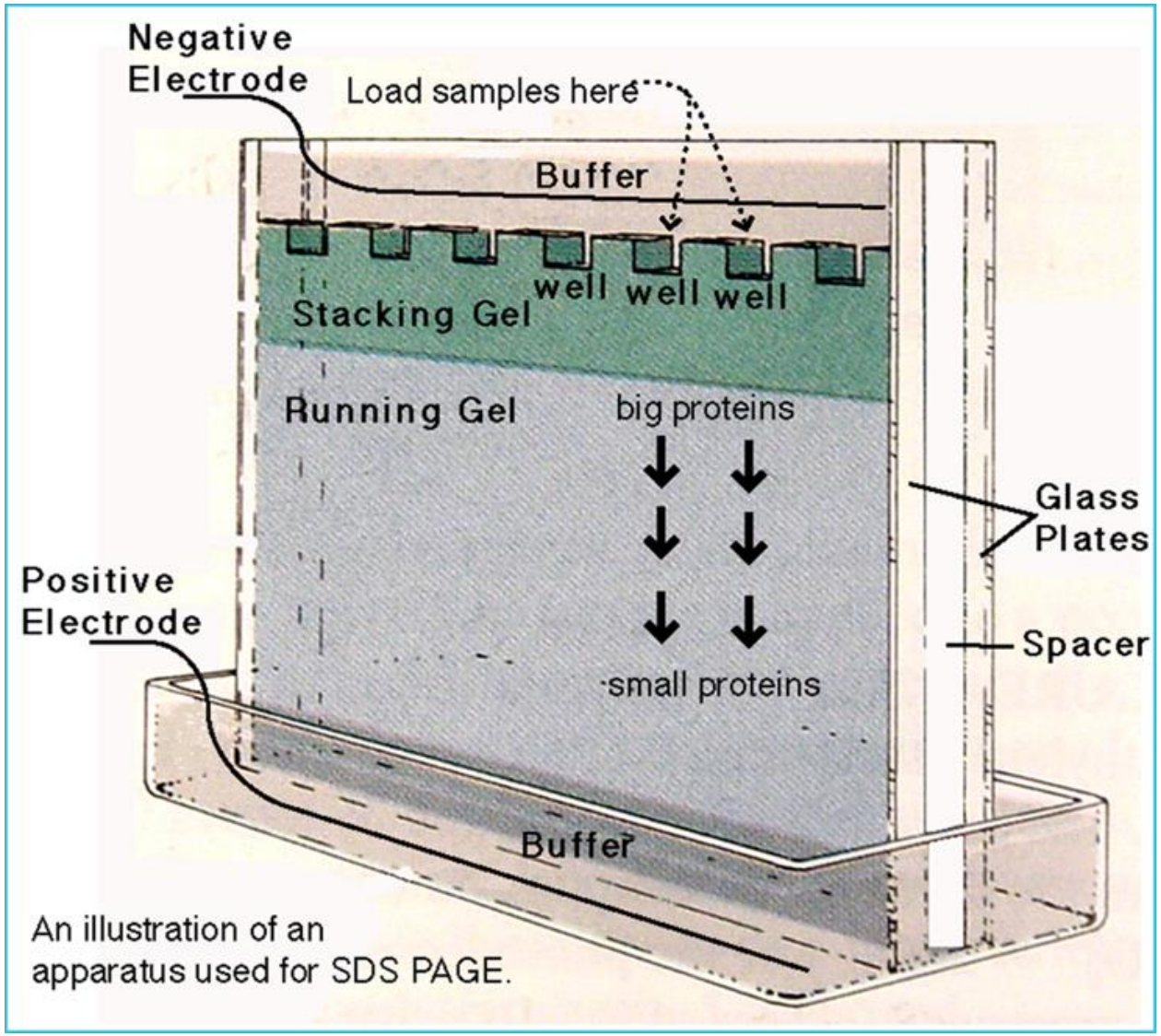
Discontinuous System

Running buffer

- 25 mM Tris base; 192 mM glycine, pH 8.3; 1% SDS




ions glycine
pH 6,8
ions Cl⁻
pH 9
ions Cl⁻
Stacking gel
(gel de concentration)
running gel
(gel de séparation)
ions glycine

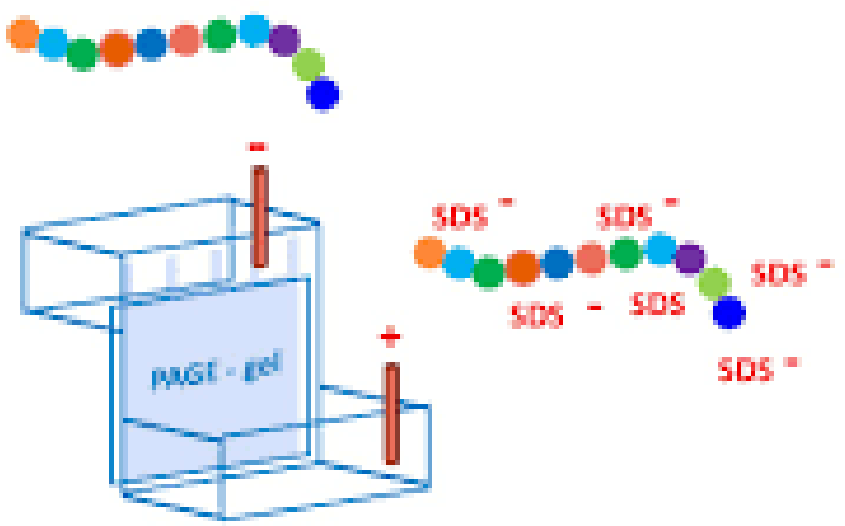


An illustration of an apparatus used for SDS PAGE.

Quickly Understand

SDS – PAGE

Stacking vs Resolving

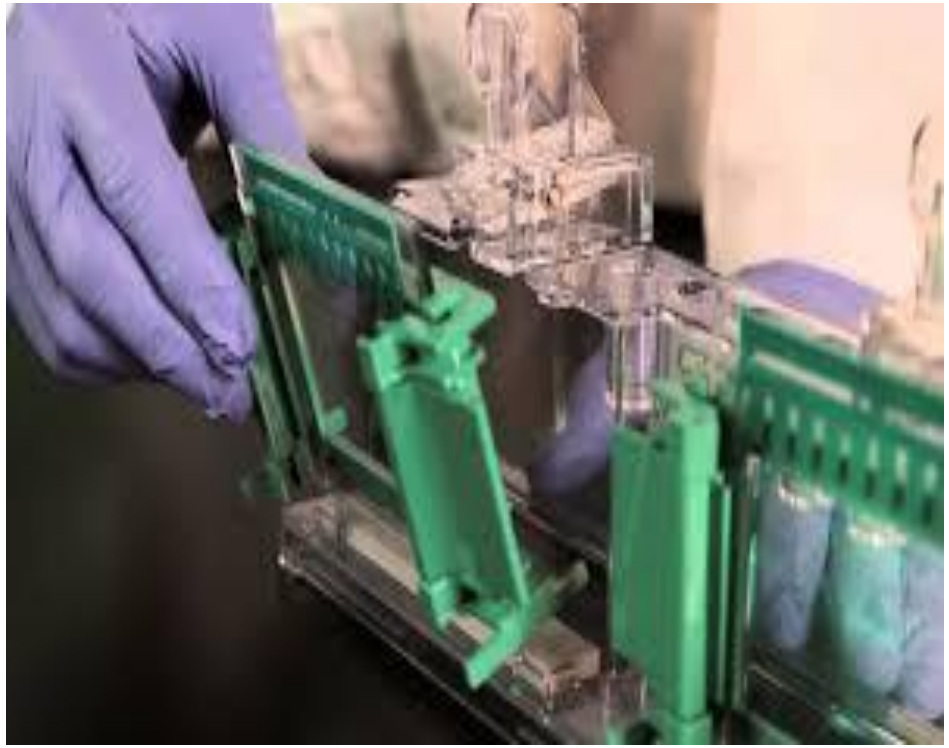


Casting the poly acrylamide Gel

1) Casting the resolving gel: mix acrylamide, Tris-HCL, SDS, APS, and TEMED (Tetramethylethylenediamine), and pour into the gel cassette.

2) Overlay with Isopropanol: to prevent oxygen interference during polymerization.

3) Casting the Stacking gel: after removing isopropanol, pour the stacking gel and insert the comb to form wells.

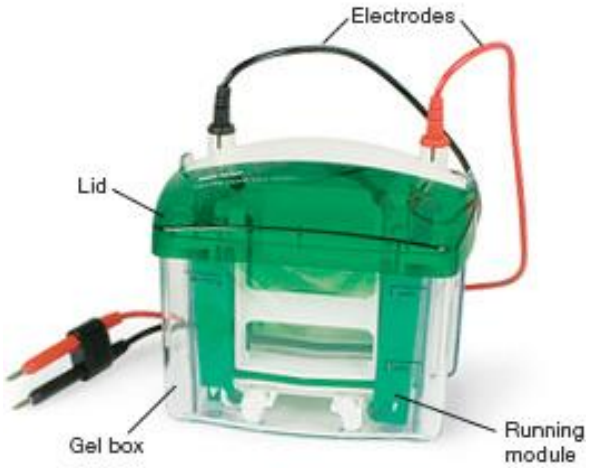


Inserting the Comb

- Creates wells for loading protein samples.

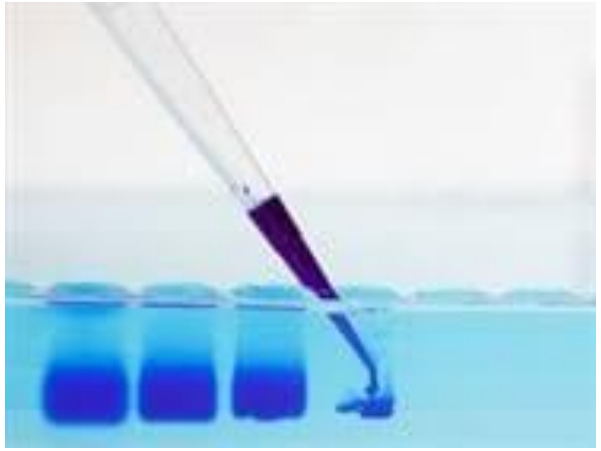
Setting Up the System

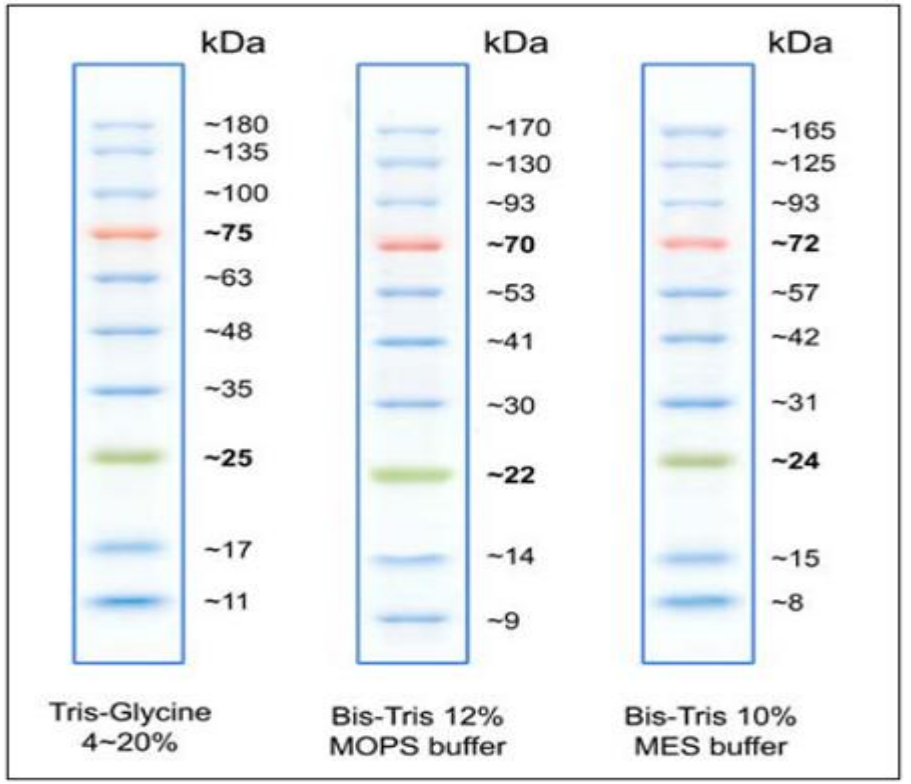
- Insert the gel into the electrophoresis tank and fill with **running buffer**.
- Running buffer Contains **Tris**, **glycine**, and **SDS** to facilitate current and maintain pH.



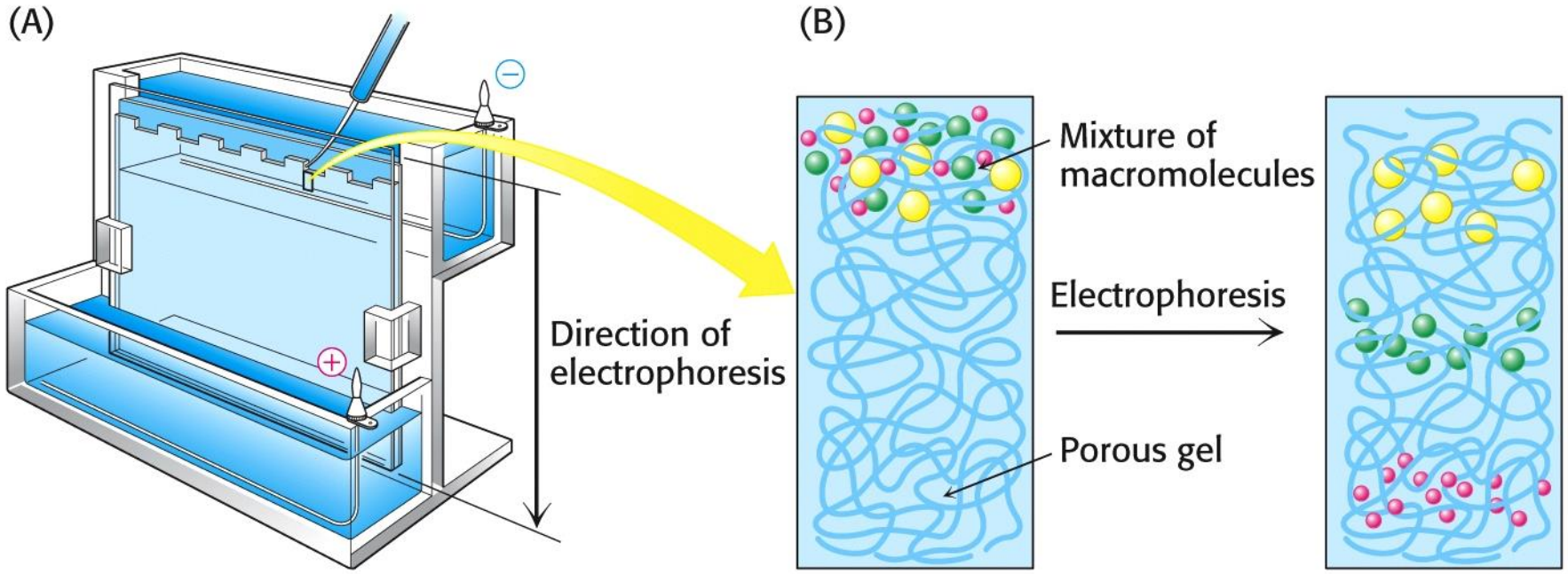
Sample Loading

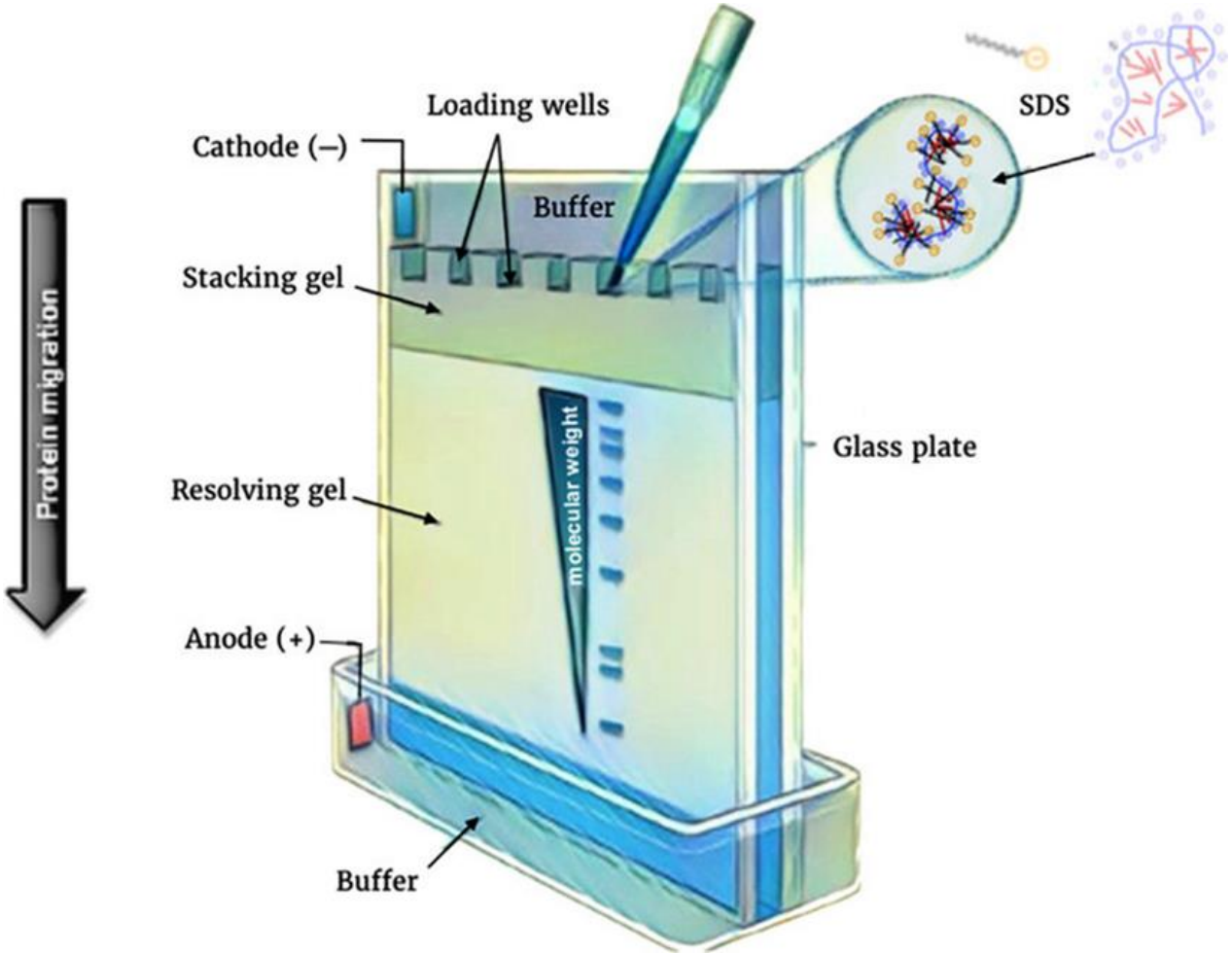
- Carefully load protein samples and molecular weight markers into the wells.





Overall process:




Loading wells
Cathode (–)
Buffer
Stacking gel
Protein migration
Resolving gel
molecular weight
Anode (+)
Buffer
SDS
Glass plate

Running the Gel

- Apply voltage: start low for stacking, increase for separation (**typically 70-120V**).

Electrophoresis Completion

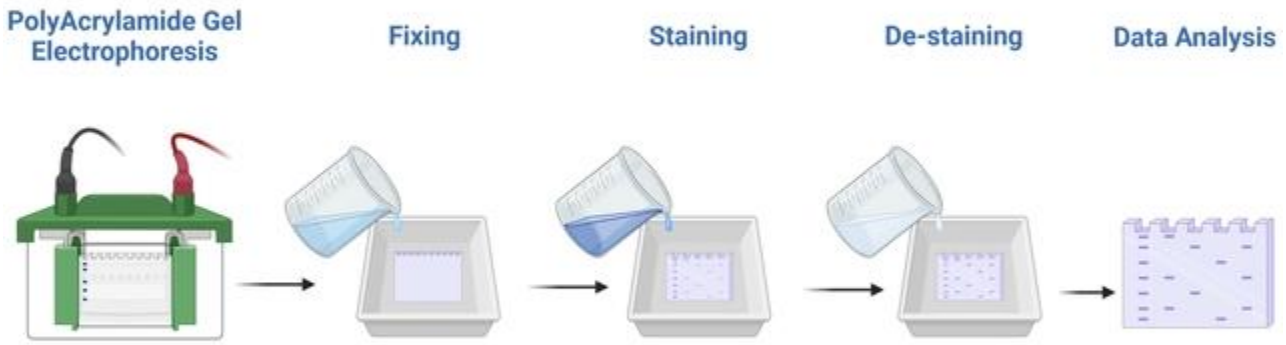
- Run for 1-2 hours until **tracking dye** reaches the bottom of the gel.

Gel Staining

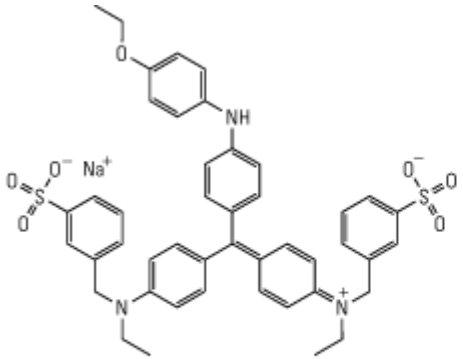
- Remove the gel and proceed to staining to visualize protein bands.

Coomassie Blue Staining

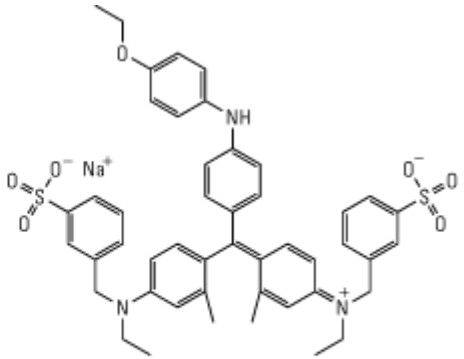
- Stain the gel in Coomassie solution followed by destaining to reveal bands.



Coomassie blue: G250 and R250



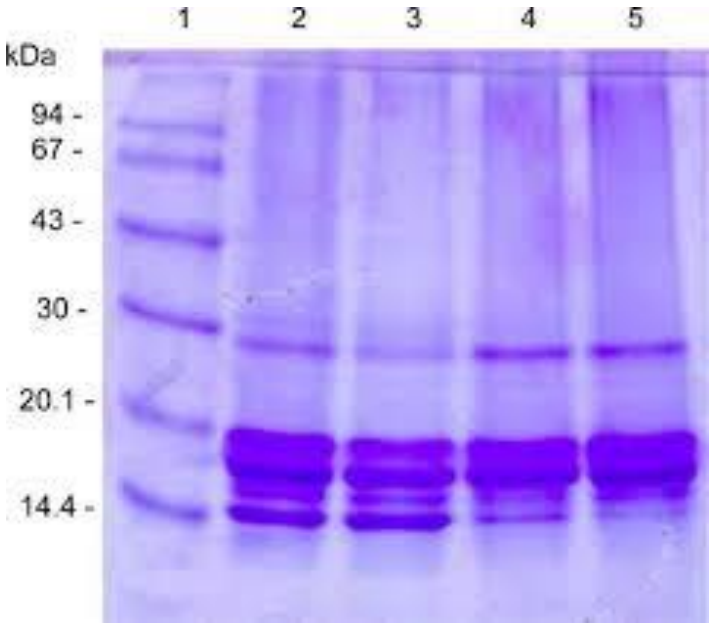
Coomassie Brilliant Blue R-250 Dye
$C_{45}H_{44}N_3NaO_7S_2$
MW 825.972

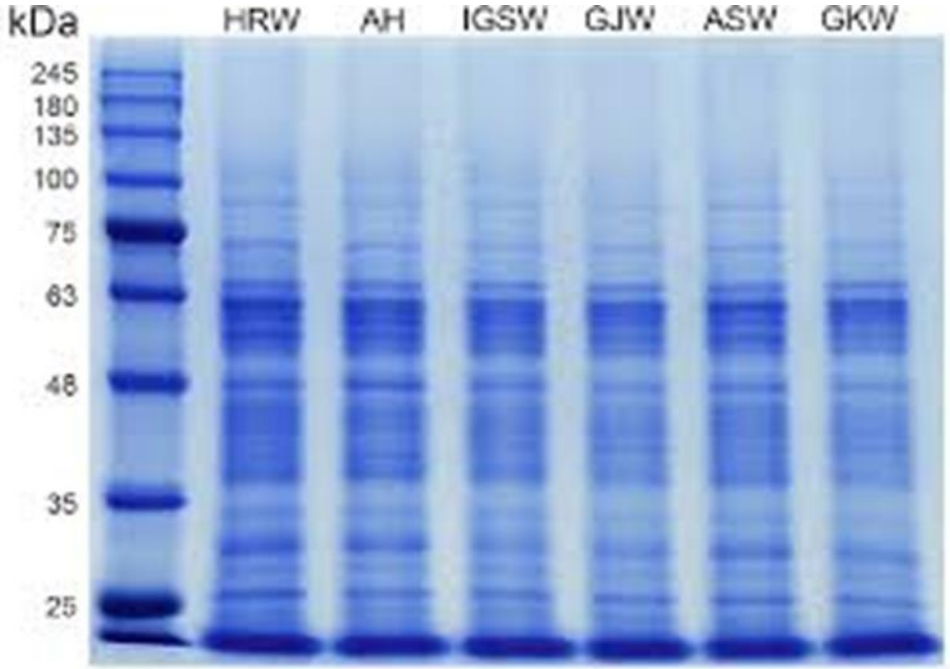


Coomassie Brilliant Blue G-250 Dye
$C_{47}H_{48}N_3NaO_7S_2$
MW 854.02

Advantages of Coomassie

- Simple, cost-effective, and suitable for most routine analyses.
- The CBB staining can detect about 0.5 µg of protein in a normal band.





Silver Staining

- Offers higher sensitivity; ideal for detecting low-abundance proteins.
- The silver stain system are about 100 times more sensitive, detecting about 10 ng of the protein.

Silver Staining Steps

1. Fixation
2. Sensitization (sodium thiosulfate)
3. Silver impregnation
4. Development (sodium carbonate+formaldehyde)
5. Stopping

Applications

- Protein molecular weight estimation
- Purity assessment of protein samples
- Monitoring protein expression and purification steps
- Separation of complex protein mixtures prior to downstream analysis (e.g., Western blotting, mass spectrometry)
- Detection of protein degradation or aggregation
- Comparison of protein profiles across different samples

Advantages

- High resolution and reproducibility for protein separation
- Proteins are separated strictly by molecular weight (due to denaturation and uniform charge-to-mass ratio)
- Relatively simple, rapid, and inexpensive technique
- Compatible with a wide range of staining methods (Coomassie, silver, fluorescent)
- Small sample requirement (typically a few micrograms)
- Simultaneous analysis of multiple samples on a single gel

Limitations

- Proteins are fully denatured; no information on native structure, subunit interactions, or biological activity can be obtained
- Hydrophobic or membrane proteins may aggregate or precipitate during sample preparation
- Very large (>500 kDa) or very small (<5 kDa) proteins may not resolve well or may run off the gel

Safety Tips

- Unpolymerized acrylamide is a neurotoxin; weigh powder and handle liquid in a fume hood with nitrile gloves.
- Wear nitrile gloves and eye protection when preparing gels and working with staining/destaining solutions.
- TEMED and ammonium persulfate are irritants; avoid skin contact and inhalation.
- Connect the electrophoresis tank correctly; never touch electrodes or buffer while the power is running (electric shock hazard).
- Keep the lid closed while running the gel.
- Dispose of chemical waste (contaminated tips, staining waste) according to institutional safety guidelines.

Common Errors

- Incorrect gel percentage: using a resolving gel concentration that is too high or too low for the target molecular weight range, leading to poor separation.
- Leaking gel cassettes: improper assembly causing the gel solution to leak before polymerization.
- Bubbles in the gel or between the comb and gel surface, distorting wells.
- Under- or over-boiling samples: insufficient denaturation or excessive heating can cause aggregation or degradation.
- Running the dye front off the bottom of the gel, losing small proteins.
- Using aged or improperly stored TEMED or ammonium persulfate, resulting in incomplete polymerization.
- Insufficient destaining, leaving a high background that obscures weak bands.

Thanks for your attention

Any comment welcome!