SDS-PAGE

Sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis (PAGE)

- Electrophoresis of proteins is generally carried out in gels made up of the cross-linked polymer polyacrylamide. The polyacrylamide gel acts as a molecular sieve, slowing the migration of proteins approximately in proportion to their charge-to-mass ratio.

- Migration may also be affected by protein shape. In electrophoresis, the force moving the macromolecule is the electrical potential, E.

- The electrophoretic mobility of the molecule,is the ratio of the velocity of the particle molecule, V, to the electrical potential. Electrophoretic mobility is also equal to the net charge of the molecule, Z, divided by the frictional coefficient, f, which reflects in part a protein's shape.

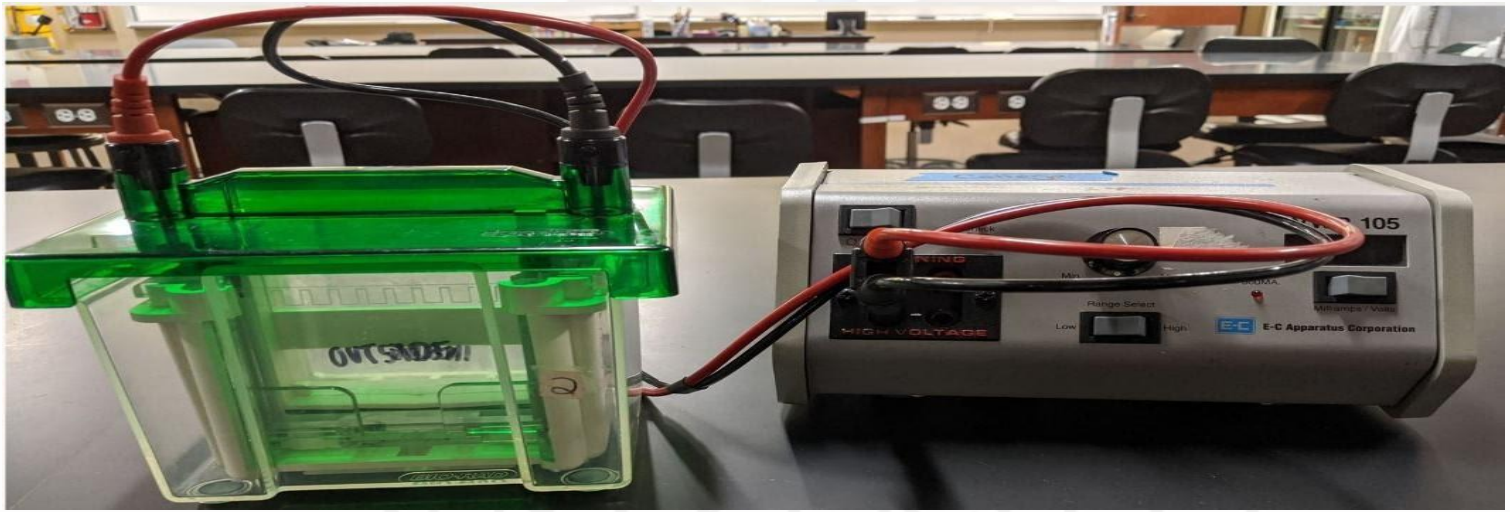
$$\mu = \frac{V}{E} = \frac{Z}{f}$$

- The migration of a protein in a gel during electrophoresis is therefore a function of its size and its shape.

Principle

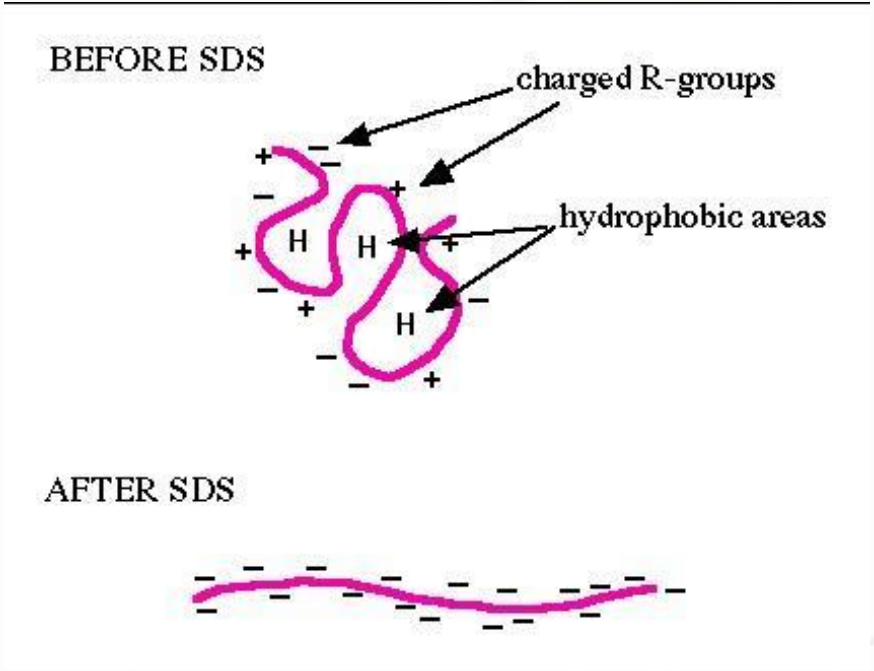
Electrophoresis is a powerful technique for protein separation and separated proteins can be visualized after subsequent staining steps. It is based on the principle of migration of charged proteins in an electric field.

SDS-PAGE is an analytical technique to separate proteins based on their molecular weight



SDS-PAGE Apparatus

- An electrophoretic method commonly employed for estimation of purity and molecular weight makes use o the detergent sodium dodecyl sulfate (SDS).



- SDS binds to most proteins in amounts roughly proportional to the molecular weight of the protein, about one molecule of SDS for every two amino acid residues. The bound SDS contributes a large net negative charge, rendering the intrinsic charge of the protein insignificant and conferring on each protein a similar charge-to-mass ratio.

Material and Reagents required:

1. **Acrylamide solution**: Cross-linked polyacrylamide gels are formed from the polymerisation of acrylamide monomer in the presence of smaller amounts of N,N0-methylene-bisacrylamide (normally referred to as ‘bis’-acrylamide).

2. **10% sodium dodecyl sulfate (SDS)**

3. **10% ammonium persulfate (AP)**: ammonium persulfate provides the free radical necessary for the catalysis of the Polymerization of Acrylamide and Bis-acrylamide

4. **TEMED (N, N, N, N – tetramethylethylenediamine)**: by catalyzing ammounium persulfate to form free radicals, TEMED accelerated the polymerization of acrylamide and bis-acrylamide. Since TEMED only functions in a free base form, the polymerization reaction would be inhibited when the pH is low.

5. **Tris- glycine electrophoresis buffer:** Tris, glycine, and SDS, pH 8.3. Tris is the buffer used for most SDS-PAGE. Its pKa of 8.1 makes it an excellent buffer in the 7-9 pH range. This makes it a good choice for most biological systems. SDS in the buffer helps keep the proteins linear. Glycine is an amino acid whose charge state plays a big role in the stacking gel.

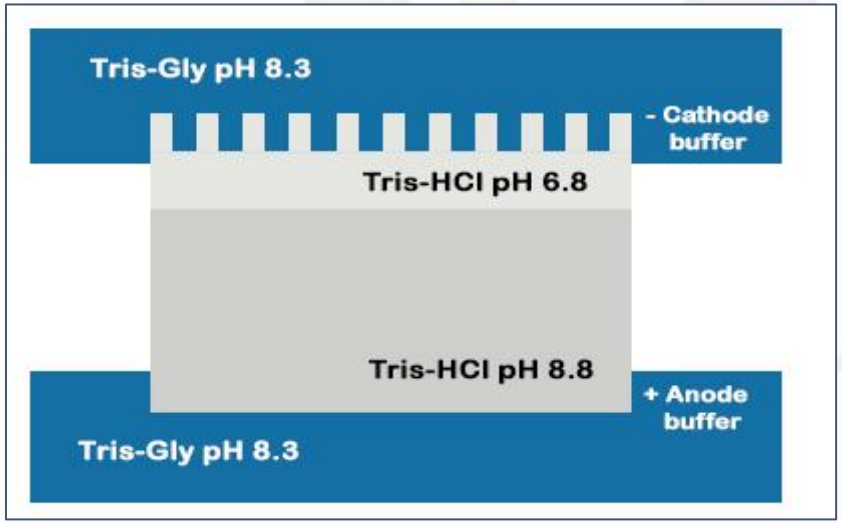
6. sample loading buffer: Tris-HCl, SDS, glycerol, beta mercaptoethanol (BME), Bromophenol Blue.The SDS denatures and linearizes the proteins, coating them in negative charge. BME breaks up disulfide bonds in the proteins to help them enter the gel. Glycerol adds density to the sample, helping it drop to the bottom of the loading wells and to keep it from diffusing out of the well while the rest of the gel is loaded. Bromophenol Blue is a dye that helps visualization of the samples in the wells and their movement through the gel. Sample loading buffer is also known as Laemmli Buffer, named after the Swiss professor who invented it around 1970.

Procedure:

Gel Layers:

1)The stacking layer
2) The resolving layer.

The top (stacking) layer has a lower percentage of acrylamide and a lower pH (6.8) than the bottom (resolving) layer, which has more acrylamide and a higher pH (8.8). SDS PAGE is run in a discontinuous gel system.



A discontinuous gel system

- The purpose of the stacking layer is to get all of the protein samples lined up so they can enter the resolving layer at exactly the same time.

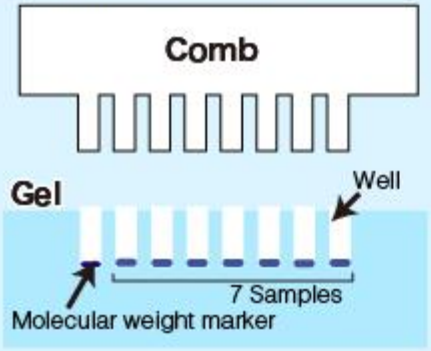
- The resolving layer then separates the proteins based on molecular weight.

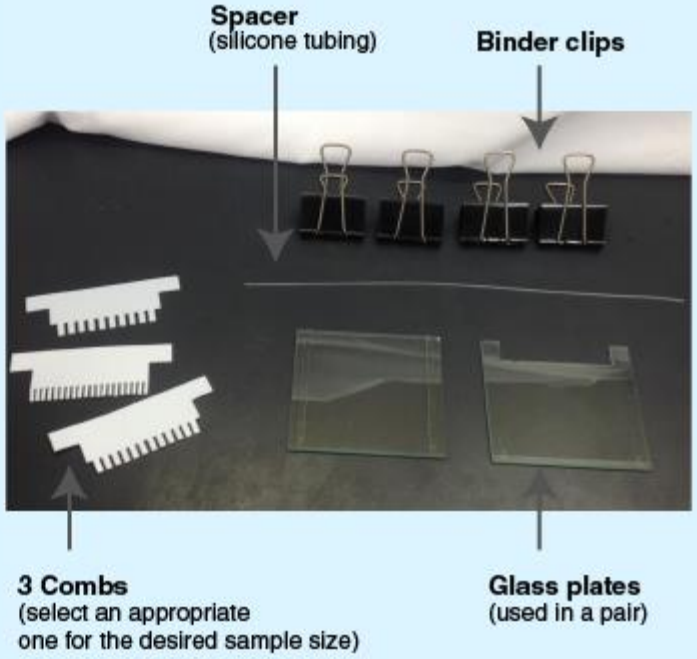
STEP BY STEP PROCEDURE

1

Step-by-step procedure

Gather combs, glass plates, spacer (silicone tubing), and binder clips.
A comb is used to make wells (lanes) to load samples. Use an appropriate comb depending on the sample size.
Example: Use an 8-lane comb for 7 samples and molecular weight markers.





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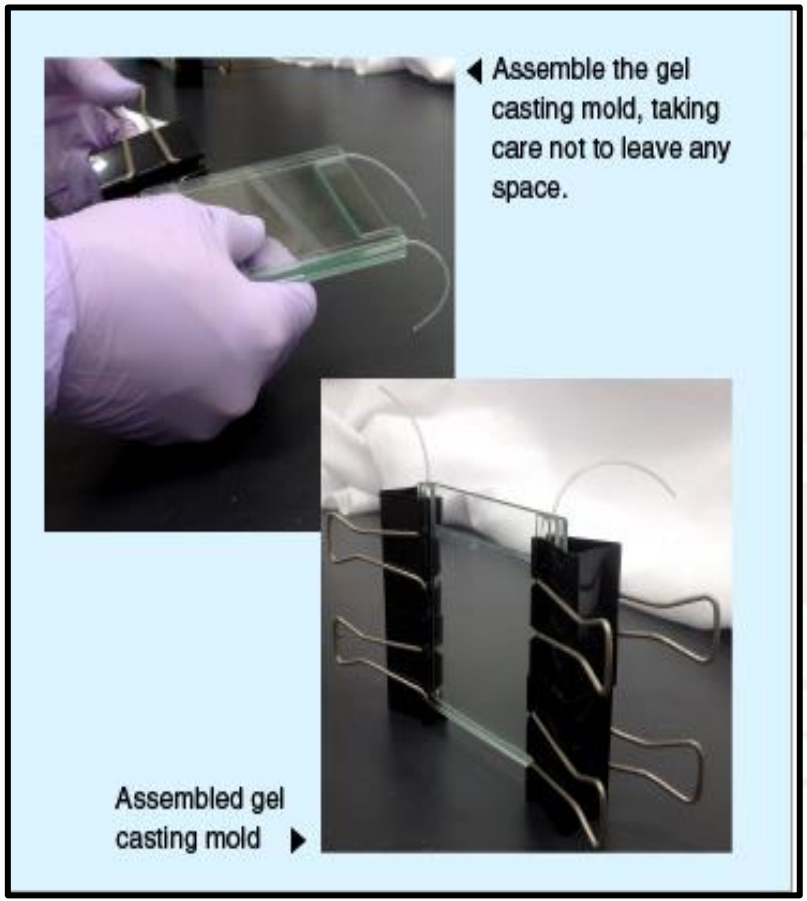

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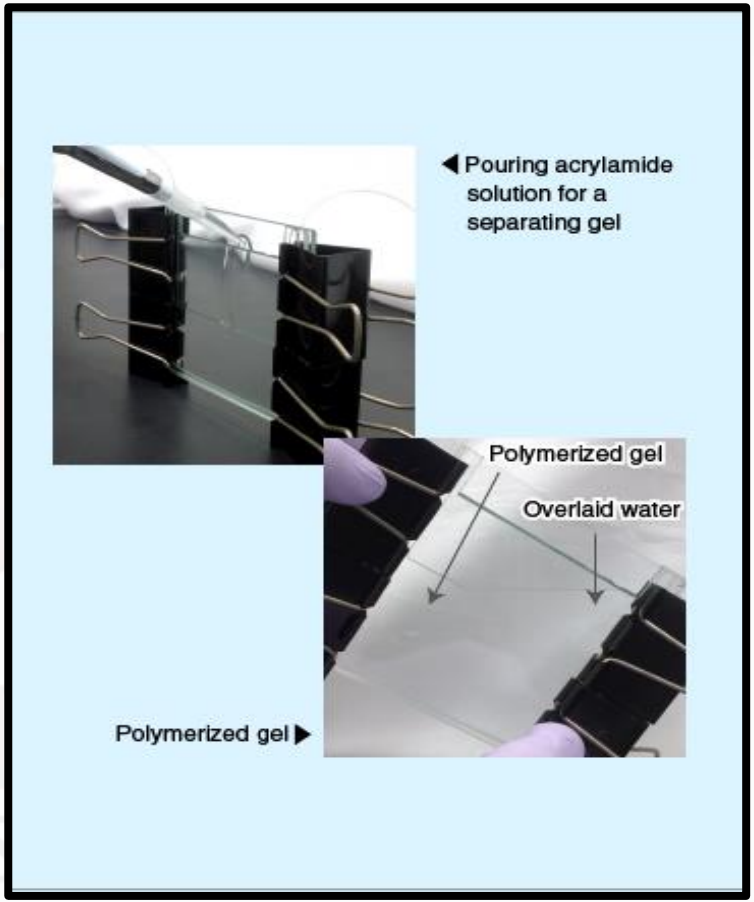

Figures references: The principle and method of polyacrylamide gel electrophoresis (SDS-PAGE),MBL life sciences

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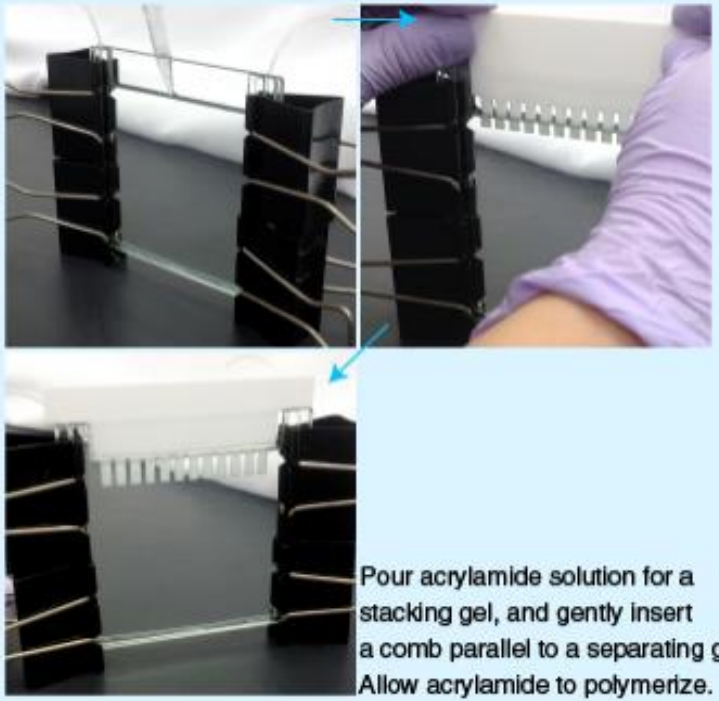

Pour acrylamide solution for a stacking gel, and gently insert a comb parallel to a separating gel. Allow acrylamide to polymerize.

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◀Add sample buffer to the samples and heat.

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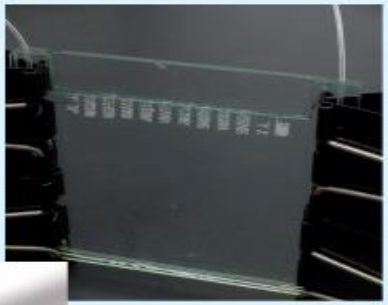
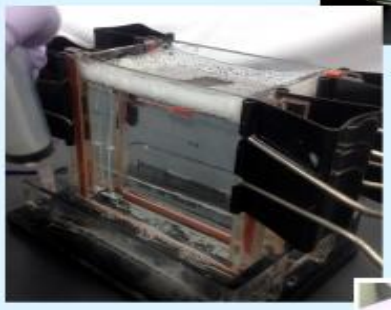
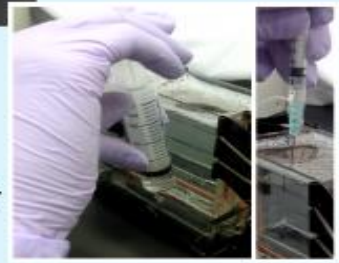
The comb is removed from the gel assembly. ▶



Remove the spacer and binder clips.



◀ The gel is mounted on the electrophoresis apparatus, and running buffer is poured.



Remove air bubbles and small pieces of gel using a syringe. ▶

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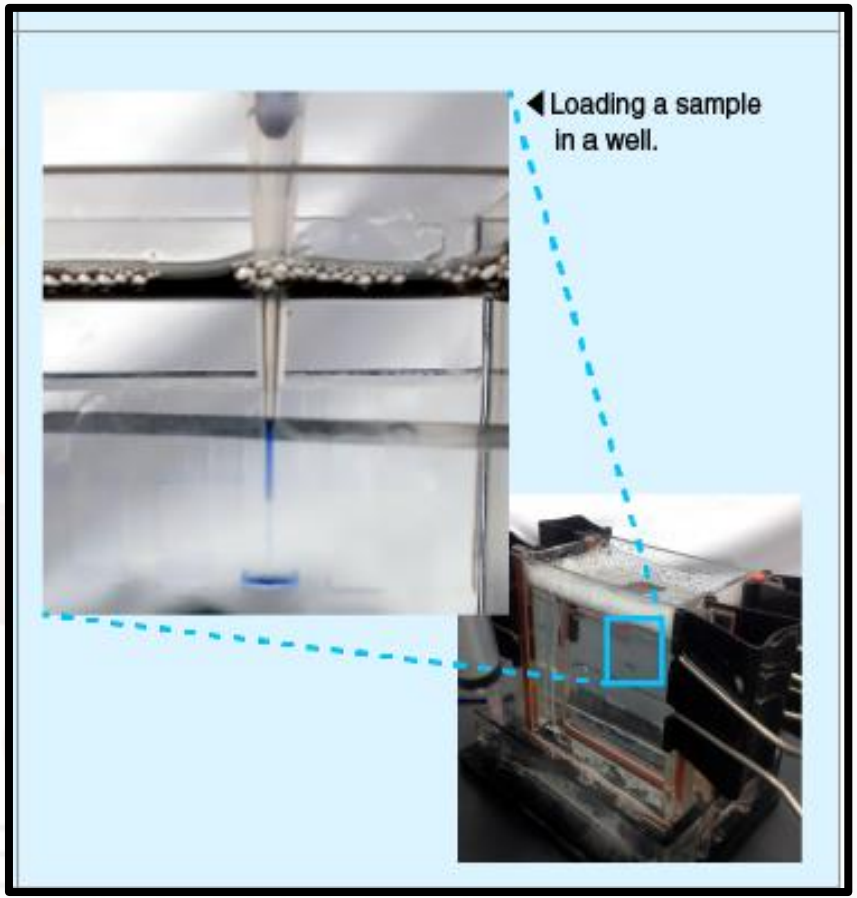

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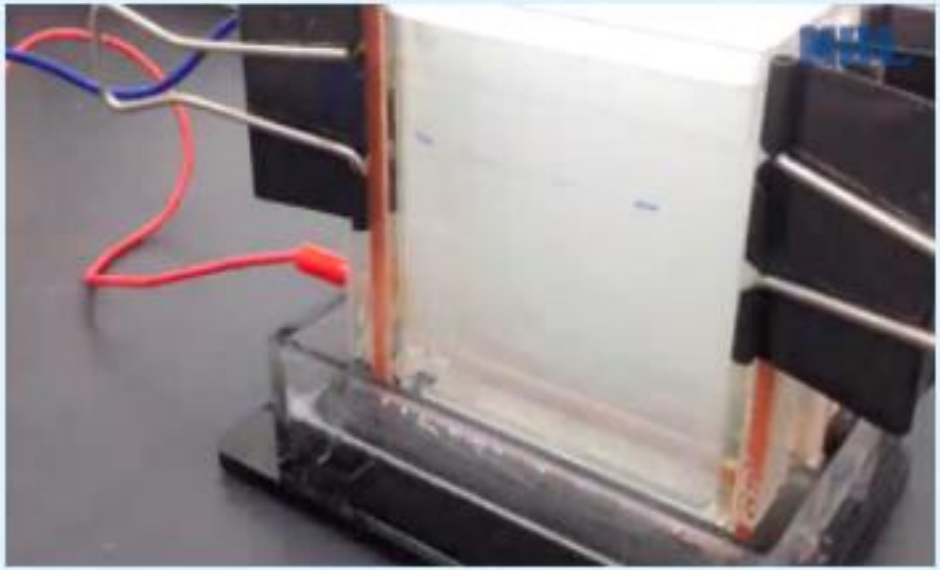
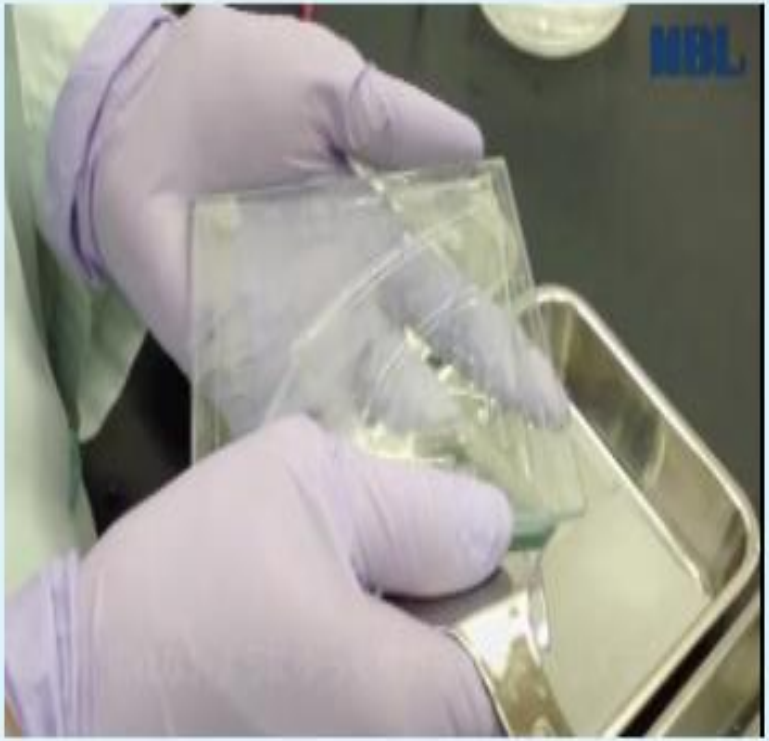
8 **Turn on the power supply, and run the gel until the dye (BPB) in the sample buffer reaches the bottom of the gel.**



9 **Remove the gel assembly from the electrophoresis apparatus. Remove the gel from the glass plates using a spatula,**



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7) The principle and method of polyacrylamide gel electrophoresis (SDS-PAGE),MBL life sciences.


Thank
you!!!