Beta- mercaptoethanol and dithiothreitol (DTT): This reagent contains a soluble thiol (-SH) group to reduce any inter- or intra-molecular disulfide bonds. . Beta-mercaptoethanol eliminates disulfide bonds in proteins by reducing them (adding hydrogen atoms). βmercaptoethanol, a reducing agent.

TEMED and ainitiator (APS)

Acrylamide gel are formed by polymerizing acrylamide with a cross linker (bis acrylamide) in the presence of catalyst (TEMED) and ainitiator (APS) with the presence of suitable gel buffer(tris). Solutions are normally degassed prior to polymerization. Oxygen molecules inhibit polymerization. The rate at which gel polymerize can be controlled by varying the concentration of TEMED and APS. When APS generated free radicals are activates acrylamide and react with successive acrylamide molecule to produce a long polymer chain. TEMED is an agent used with ammonium persulfate to catalyze the polymerization reaction of acrylamide

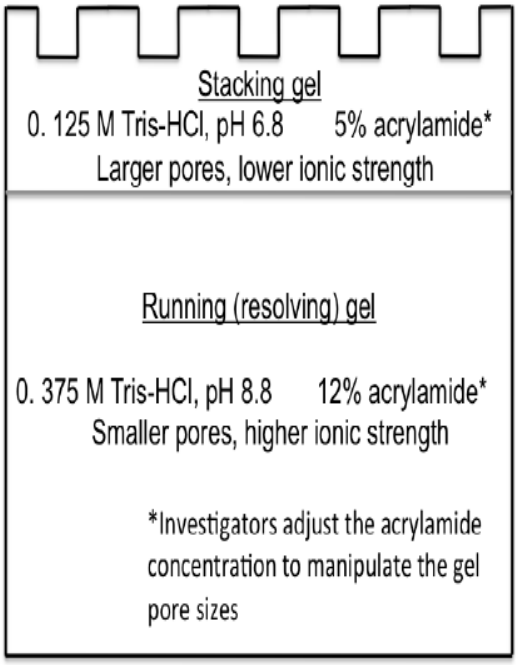
Stacking gel: After loading the sample on to the well of gel, the protein molecules present in the sample are in dispersed state and in anionic form. When electric field is applied on the gel, the glycine-chloride buffer ions and sample move in to the stacking gel which has pH of 6.9. In this pH, the glycine ion is in the form of zwit-terion with net charge zero and no electrophoretic mobility. But the chloride ion and sample are in anionic form at pH 6.9 and act as mobile ions. The sample will tend to accumulate and form a thin, concentrated band sandwiched be-tween chloride and glycinate. The chloride ion and protein carry the most of the current.

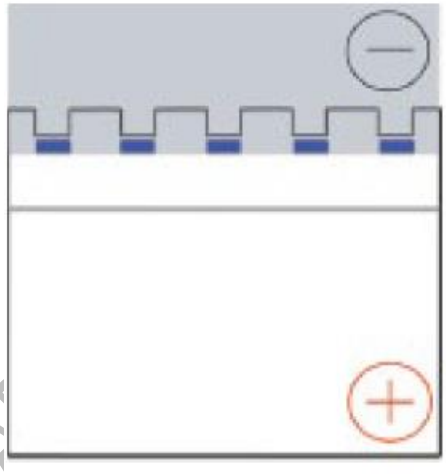
very little glycine has a negative charge in the chamber buffer or stacking gel, and significant ionization does not occur until the glycine enters the more alkaline pH 8.8 environment of the running gel.

BASIC PRINCIPLE:

Once a voltage is applied, the chloride ions in the sample buffer and stacking gel move rapidly toward the positive pole, forming the leading edge of a moving ion front. Glycine molecules have very little charge in the stacking gel, so they migrate at the rear of the moving ion front. This difference in chloride and glycine mobility sets up a steep voltage gradient in the stacking gel that sweeps along the negatively charged protein-SDS complexes. The large pores of the stacking gel present very little resistance to the movement of protein-SDS complexes, which then “stack up” into a very concentrated region at the interface between the running and stacking gels (right). Protein-SDS complexes remain concentrated at the interface until the slowly migrating glycine molecules reach the boundary between the two gels.

Dramatic changes occur as the glycine ions enter the running gel. The pH of the running gel is closer to the pKa of the glycine amino groups, so a significant fraction of the glycine molecules assume a negative charge. Negatively charged glycine molecules begin to move at the same rate as the chloride ions, thereby eliminating the voltage difference that controlled protein mobility through the stacking gel. The pores in the running gel are much smaller than those of the stacking gel, so the pores present frictional resistance to the migration of proteins. Proteins begin to migrate at different rates, because of the sieving properties of the gel. Smaller protein-SDS complexes migrate more quickly than larger proteinSDS complexes (right). Within a certain range determined by the porosity of the gel, the migration rate of a protein in the running gel is inversely proportional to the logarithm of its MW.





An electric filed is established with the positive pole (red plus) at the far end and the negative pole (black minus) at the closer end. Since all the proteins have strong negative charges, they will all move in the direction negative pole

Reagent	Resolving gel	Stacking gel
Deionized water	3.5 mL	2.1 mL
30% acrylamide:bis-acrylamide (29:1)	4.0 mL	0.63 mL
1.5 M Tris-HCl, 0.4% SDS, pH 8.8	2.5 mL	------
0.5 M Tris-HCl, 0.4% SDS, pH 6.8	------	1.0 mL
10% ammonium persulfate (catalyst)	100 µL	30 µL
TEMED (catalyst)	10 µL	7.5 µL

Materials Required

1. **Acrylamide (30% stock):-** dissolved 29.2 g acrylamide and 0.8 bis-acrylamide in distilled water and make upto 100ml. Store under dark in amber colour bottle at 4°c(can use upto 3 month)

2. **Resolving gel/ separating gel buffer pH 8.8, 1.5M tris-HCl:** - dissolved 18.17g tris in 75 ml distilled water. Adjust to pH 8.8 with 6 N HCl. Adjust the total volume to 100ml with distilled water and store at 4°c.

3. **Stacking gel buffer, pH-6.8, and 1.0Mtris-HCl:-** dissolved 3g tris in 40 ml distilled water, adjust to pH 6.8 with 6N HCl. Adjust the total volume to 50ml with distilled water. Store at 4°C.

4. **Electrophoresis buffer pH 8.**3:- dissolved 3g tris, 14.4 g glycine and 1g SDS in 100ml of distilled water. Store at 4°C.

5. **Ammonium per sulphate-initiator 10%:-** dissolved 0.1 g APS in 1 ml distilled water.

6. **TEMED(NNN'N' Tetramethylenediamine):**- catalyst.

7. **Sample buffer:-** 7.25 ml distilled water + 1.25 ml stacking gel buffer + 1ml glycerol + 0.5 ml β-mercaptoethanol + 150 mg SDS and a pinch ofbromophenol blue.

8. **Staining solution:** -dissolved 200mgcoomassie brilliant blue R 250 in 50ml methanol/ethanol, 7ml acetic acid and 43ml distilled water & filter it.

9. **De-staining solution:**- add 7ml acetic acid to 30ml methanol/ethanol and 63ml distilled water

10. **Vertical slab-gel electrophoresis equipment.**

11. **Acrylamide mixture (10%) for 25 ml of resolving gel:-9.9 ml**, distilled water + 8.3ml, 0% acrylamide + 6.3ml, 0.5 M tris-HCl + .25ml, 10% SDS + .25ml, 10% APS +.01 ml, TEMED.

12. **5% stacking gel for 5 ml:-** 3.04ml, distilled water + 0.83ml, 30% acrylamide + .63ml, 0.5 M Tris – HCl(pH-6.8) + 0.05ml, 1% SDS +0.05ml, APS + 0.005ml, TEMED.

Tip: gel buffer and self-prepared acrylamide/bisacrylamide stock solution should be filtered, degassed and stored at 4°C.

Sample Preparation

1. Take 1ml of culture solution of E.coli strain DH5α, in 1.5 ml of eppendorf's tube.
2. Then centrifuge at 12000 rpm for 5 min.
3. Then discard the supernatant.
4. Then add 500 µl of TE in the tube and dissolve the tube by gentle shaking.
5. Then add same volume of sample buffer.
6. Finally heat the sample on boiling bath for 5 minutes and then immediately keep on ice.

The sample to analyze is optionally mixed with a chemical denaturant if so desired, usually SDS for proteins or urea for nucleic acids. SDS is an anionic detergent that denatures secondary and non–disulphide linked tertiary structures, and additionally applies a negative charge to each protein in proportion to its mass. Urea breaks the hydrogen bonds between the base pairs of the nucleic acid, causing the constituent strands to separate. Heating the

samples to at least 60 °C further promotes denaturation