# Poly-Acrylamide Gel electrophoresis (PAGE)

SDS-PAGE (sodium dodecyl sulphate- Poly-Acrylamide Gel electrophoresis) is the most widely used method for analysing protein mixtures qualitatively. It is particularly useful for monitoring protein purification and because the method is based on the separation of proteins according to size. The method can also be used to determine the relative molecular mass of protein. SDS is an anionic detergent. Samples to be run on SDS-PAGE are firstly boiled for five minutes in sample buffer containing beta-mercapto-ethanol and SDS. The beta-mercapto-ethanol reduces any disulphide bridges present that are holding together the protein tertiary structure and SDS binds strongly to and denatures the protein. Each protein in the mixture is therefore fully denatured by this treatment and opens up into a rod shaped structure and with a series of negatively charged SDS molecules along the polypeptide chain. On average, one SDS molecule binds for every two amino acids residues. The original native charge on the molecule is therefore completely swamped by the negatively charged SDS molecules. The rod-like structure remains, as that tends to fold up the protein chain would result in repulsion between negative charges on different parts of the protein chain, returning the conformation back to the rod shape. The sample buffer also contains an ionisable tracking dye, usually bromophenol blue, that allows the electrophoretic run to be monitored and sucrose or glycerol, which gives the sample solution density thus allowing the sample to settle easily thorough the electrophoresis buffer to the bottom when injected into the loading well. Once the samples are all loaded, an electric current is passed through the gel. The samples are to be separated are not in fact loaded directly in to the main separating gel. When the main separating gel has been poured between the glass plates and allowed to set , a shorter (approximately 1 cm) stacking gel is poured on the top of separating gel and it into this gel that the wells are formed and the proteins loaded. The purpose of the stacking gel is to concentrate the protein sample into a sharp band before it enters the main separating gel.

This is achieved by utilising difference in ionic strength and pH between the electrophoresis buffer and the stacking gel and involves a phenomenon known as *isotachaphoresis* (Explained in the page number 8).

When the dye reaches the bottom of the gel, the current is turned off, and the gel is removed from between the glass plates and shaken in an appropriate stain solution (usually Coomassie Brilliant Blue) for a few hours and then washed in destain solution overnight. The destain solution removes unbound background dye from the gel leaving stained proteins visible as blue bands on a clear back ground.

Staining of the gel is usually done with Coomassie Brilliant Blue R-250 (CBB). Staining is usually carried out

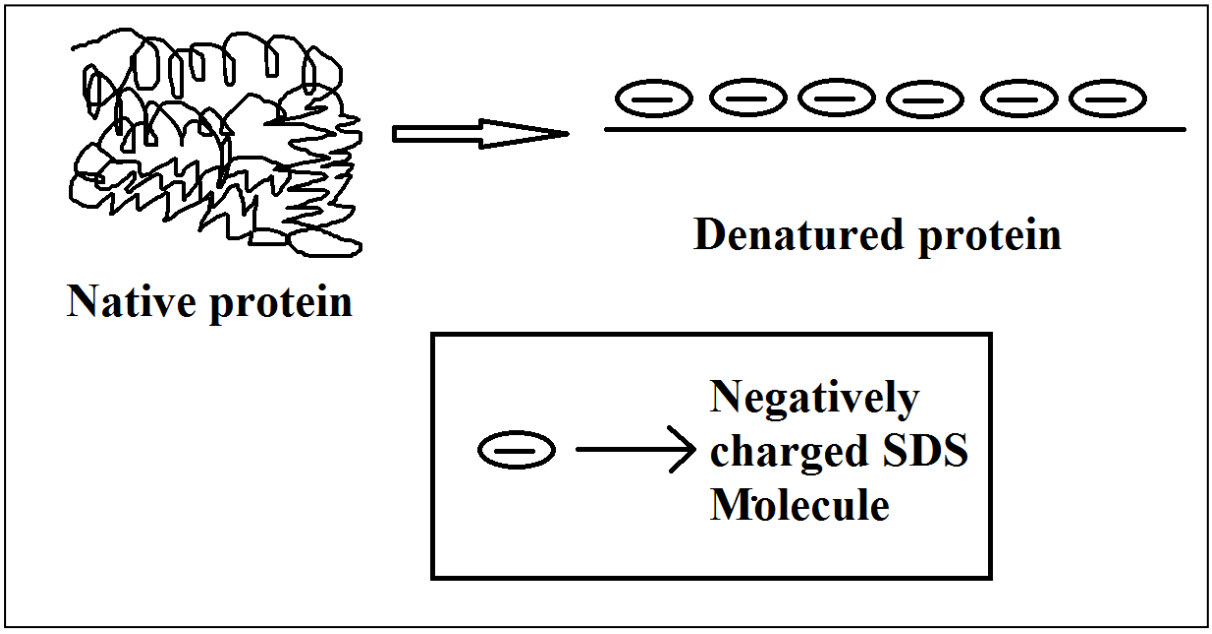


Fig. The native protein is denatured by SDS molecules into straight chain of polypeptides.

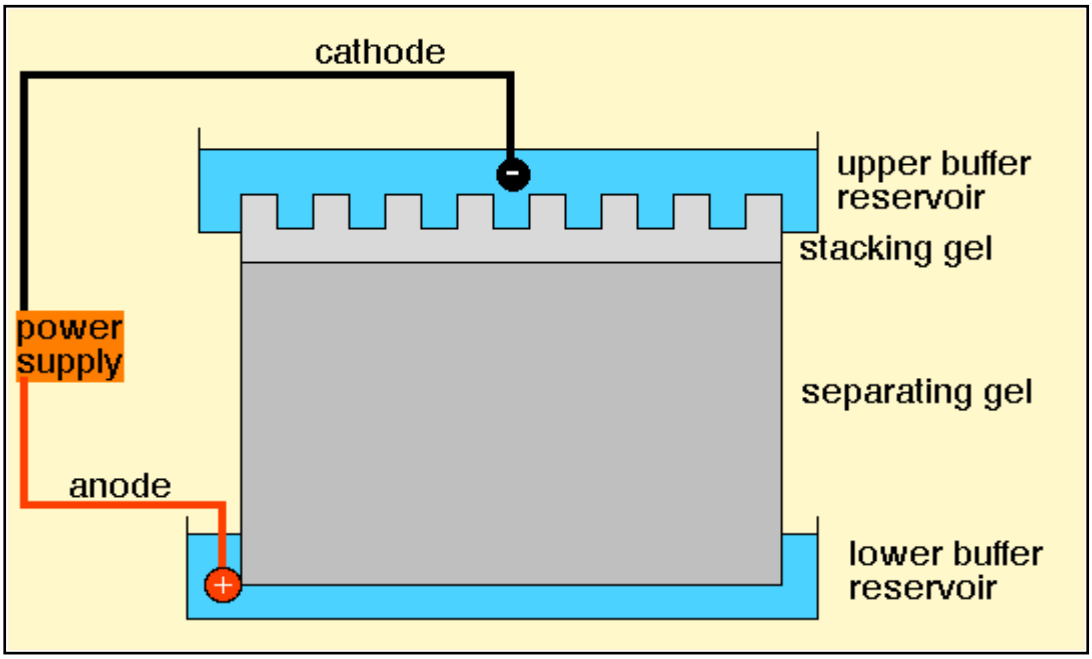


Fig. Schematic diagram of SDS-PAGE assembly.

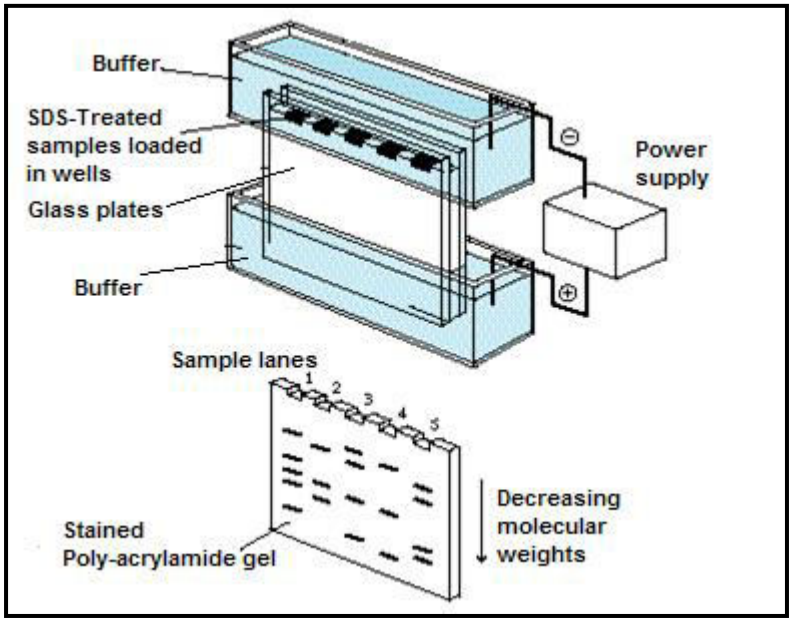


Fig. Complete SDS-PAGE assembly

# SDS PAGE solutions

1. Sample buffer (2X) for 20 ml

| | Volume in ml |
|---|---|
| Stacking gel buffer (pH 6.8) | 0.2 |
| 10% SDS | 8 |
| 1 % Bromophenol Blue | 4 |
| Glycerol | 4 |
| Beta mercaptoethanol | 2 |
| Water | 1.8 |

*Vortex the suspension and keep it in 4 degree Celsius.*

2. Seperating gel buffer

| | |
|---|---|
| Tris base | 1.5 M |
| pH | 8.8 |

Adjust pH by adding conc HCl

3. Stacking gel buffer

| | |
|---|---|
| Tris base | 1.0 M |
| pH | 6.8 |

Adjust pH by adding conc HCl

4. Ammonium per sulfate: 10%

5. Electrode Buffer (5X)

| | |
|---|---|
| Tris | 15.1 g |
| Glycin | 94 g |
| SDS | 5.0 g |
| pH | 8.3 |

Make final volume upto 1000 ml by adding triple glass distilled water
*Prepare 5X stock by dissolving 15.1 g Tris base and 94 g Glycin in 900 ml of deionized water. Then add 5g of electrophoresis grade SDS and adjust the volume to 1000 ml with water.*

6. Staining solution

| | |
|---|---|
| Coomassie Brilliant Blue R-250 | 0.25 g |
| Methanol | 50 ml |
| Acetic Acid | 10 ml |

| | |
|---|---|
| Water | 40 ml |

7. Organic Destaining solution

| | |
|---|---|
| Methanol | 50 ml |
| Acetic Acid | 10 ml |
| Water | 40 ml |

8. Stock Acrylamide solution (30%)

| | |
|---|---|
| Acrylamide | 29 % |
| Bis-Acrylamide | 1 % |

9. Seperating gel (8 ml)

| | 12 % gel | 15% gel |
|---|---|---|
| Water | 2.632 ml | 1.84 ml |
| Acrylamide solution (30%) | 3.2 ml | 4 ml |
| Seperating gel buffer (pH 8.8) | 2 ml | 2 ml |
| 10% SDS | 80 µl | 80 µl |
| 10% APS | 80 µl | 80 µl |
| TEMED | 4 µl | 4 µl |

10. Stacking gel (5ml) 5%

| | |
|---|---|
| Water | 3.4 ml |
| Acrylamide solution (30%) | 830 µl |
| Stacking gel buffer (pH 6.8) | 630 µl |
| 10% SDS | 50 µl |
| 10% APS | 50 µl |
| TEMED | 5 µl |

PROCEDURE FOR THE PREPARATION OF SDS-PAGE GELS

1. Assemble the glass plates of SDS-PAGE assembly.
2. Determine the volume of the gel mold to be prepared.
3. Take a clean and dry beaker and prepare the separating gel as mentioned in the table no. 9. Mix the component in the order shown. The polymerization will begin as soon

as the TEMED has been added. Immediately proceed to the next step after addition of the TEMED.

4. Pour the separating gel solution into the gap between the glass plate. Leave sufficient space for the stacking gel.
5. Overlay the separating gel with the triple-glass distilled water.
6. After the polymerization of separating gel is complete, remove the overlay and wash the top of the separating gel with triple-glass distilled water.
7. Prepare the stacking gel as mentioned in table no. 10.
8. Pour the stacking gel solution immediately after addition of TEMED.
9. Place the comb over the separating gel and alow it to polymerize.

Procedure for the sample for the SDS-PAGE

1. Take the protein solution in a plastic centrifuge tube (1.5 ml).
2. Add equal volume of 2×SDS-PAGE sample buffer and mix it properly.
3. Place the tube in boiling water bath for five minutes.
4. Centrifuge the sample at 10,000×g for five minutes.
5. Take the supernatant in separate tube.

PROCEDURE FOR LOADING THE SAMPLE FOR THE SDS-PAGE

1. Load 15µl of the sample into the well of stacking gel.
2. Run the electrophoresis at the voltage of 8 Volts per cm.
3. After the dye front has reached the separating gel, increase the voltage to 15 Volts per cm.
4. Run the gel until the tracking dye reaches the bottom of the gel.

PROCEDURE FOR STAINING THE SDS-PAGE GEL

1. Prepare the staining solution as mentioned in table no. 6.

2. Remove the gel from the two glass plates carefully and place into the staining solution.
3. Keep the gel in staining for about 3 hours.
4. Prepare the destaining solution as mentioned in table no. 7.
5. Pour off the staining solution and add the destaining solution.
6. Keep the gel in destaining solution for overnight.
7. The gel will show the protein bands after overnight destaining.

## THE PHENOMENON OF ISOTACHAPHORESIS

The stacking gel concentrate the protein sample into a sharp band before it enters the main separating gel. The electrophoretic mobility of glycinate ions, SDS-protein complex and $Cl^-$ ions are not same. Their electrophoretic mobility is in the order of glycinate<SDS-Protein< $Cl^-$. Now if all the chemical species do not move in same speed the electric circuit will break. Thus all three will have to move in same speed that is possible if glycinate ions decrease its concentration than SDS-Protein complex and $Cl^-$ ions. And on the other hand the SDS-Protein complex and $Cl^-$ will have to increase their concentration to decrease the electrophoretic mobility. The electrophoretic mobility of ionic species is inversely proportional to their concentration. This phenomenon is called isotachaphoresis.

$$Electrophoretic\ mobility \propto \frac{1}{Concentration}$$