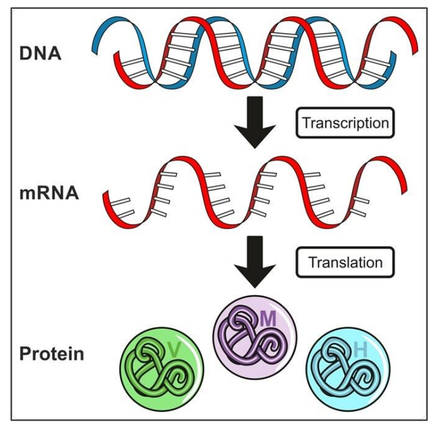
Protein Expression and Separation using SDS-PAGE

Dayana Doss

What is protein expression?

The ability to express functional proteins enables researchers to study them in vitro more easily. Recombinant protein expression refers to the manufacture of proteins derived from recombinant DNA

Protein Expression refers how the proteins are synthesized depending upon the functional need in the cell. The blue prints for proteins are stored in DNA and decoded by highly regulated transcriptional process to produce mRNA. The message code by an mRNA is then translated into a protein. The transcription is the transfer of information from DNA to mRNA, and translation is the synthesis of protein based on a sequence specified by.



Why protein expression?

Protein expression can help scientists identify the molecular basis of phenotypic differences and to select gene expression targets for in-depth study. Such expression differences can lead to potential biomarker discover for a particular disease phenotype and enable further biomarker validation.

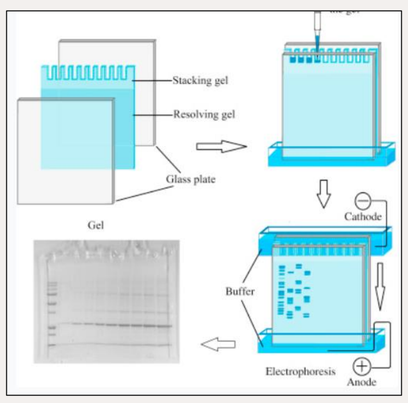


Figure 1. Schematic diagram of polyacrylamide gel electrophoresis

SDS Polyacrylamide Gel Electrophotesis (SDS-PAGE)

The electrophoresis of protein is generally carried out in Polyacrylamide gels that ensure the dissociation of proteins into individual polypeptides. Anionic detergent SDS along with reducing agent is used and heat is supplied for the dissociation of proteins. The denatured polypeptides bind with SDS and becomes negatively charged which is proportional to their mass. Thus the protein migrates to the anode of the gel in an electric field. By using standard markers the molecular weight of our desired protein can be determined. Larger molecules run slower than the smaller molecules due to stronger friction.

Components required

Preparation of Solutions:

- 30% Acrylamide mix: 29% Acrylamide and 1% N, N-methylene bis-acrylamide is dissolved in hot distilled water and stored at 4 ºC
- Separating Gel Buffer: (1.5 M Tris-Cl, pH 8.8)
- Stacking Gel Buffer: (1.0 M Tris-Cl, pH 6.8)
- Running Gel Buffer: (25 mM Tris, 250 mM glycine, 0.1% SDS, pH 8.3)
- 2X SDS Gel-loading Buffer: (100 mM Tris-Cl, pH 6.8, 200 mM β-mercapto ethanol, 4% SDS, 0.2% bromophenol blue, 20% glycerol)

Components for SDS-PAGE

Components	Separating Gel (12% for 10 ml)	Stacking Gel (12% for 5 ml)
Double distilled water	3.3	3.4
30% Acrylamide mix	4.0	0.83
1.5 M Tris-Cl (pH 8.8)	2.5	-
1.0 M Tris-Cl (pH 6.8)	-	0.63
10% SDS	0.1	0.05
14% Ammonium per sulphate	0.1	0.05
TEMED	0.005	0.005

Procedure

The Gel plates were assembled and the Separating Gel was prepared using the components given above and was poured in between the plates. This gel was over layered with butanol to get a straight line. After polymerization was completed, the butanol was rinsed out and Stacking Gel was prepared using the above components. This was then poured between the glass plates and the comb was inserted. After the Stacking Gel has completely polymerized, the comb was removed and the gel was placed in the Electrophoresis chamber and is filled with running gel Buffer. 1X gel loading Buffer was added to the protein samples and heated at 100 ºC and then loaded into the wells of the gel. A power supply of 100 V was provided when the protein migrates in Stacking Gel and 150 V when the protein migrates in separating gel. After the completion of electrophoresis the gels were stained with Coomassie staining solution for 30 minutes on rocker. After Staining the gels were destained (45% Methanol, 10% Acetic acid) until a clear band was visible.

How to Read SDS-PAGE Results?

After electrophoresis, protein separation cannot be directly observed by the naked eye, and subsequent staining techniques are needed. Coomassie brilliant blue staining is a common method for detection and quantification of proteins separated by electrophoresis. After simple processing such as fixation-staining-decolorization, distribution of protein can be clearly observed. the

How to Store SDS-PAGE Gel?

Freshly SDS-PAGE gels are usually prepared before each experiment. However, gels can also be stored in clean water at 4°C for about a week. If the gel cannot be photographed in time after dyeing, it needs to be placed in water to prevent drying and shrinking of the gel. It is advised to photograph the staining results as soon as possible. Band will disperse if the gel is soaked in water for a long time

Advantages of SDS-PAGE

- SDS-PAGE has simple operation
- Good reproducibility in reproduction of protein
- Detection of specific proteins
- Identification of specific species

Recombinant Dehalogenase Expression using SDS-PAGE

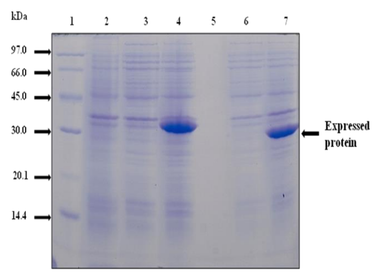


Figure: 2 - Lane 1: Protein marker; Lane 2: vector control; Lane 3: Uninduced clone 4; Lane 4: Induced clone 4; Lane 6 : Uninduced clone 6; Lane 7 : Induced clone 6

Chemicals

Sodium dodecyl sulphate (SDS) - It's a very power ionic detergent that denatures protein from the cell.
30% Acrylamide mix - Polyacrylamide gels are formed by reaction of acrylamide and bis acrylamide that results in highly cross-linked gel matrix. The gel acts a sieve through which proteins move in response to the electric field.
Tris-HCl - Tris-HCl is a pH range of 7-9 and is capable of extracting cytoplasmic proteins.
Tetramethylethylenediamine (TEMED) - TEMED is a free radical stabiliser generally included to promote polymerisation.
Ammonium per sulphate - It is an oxidising agent used with TEMED to catalyse the polymerisation of acrylamide and bis acrylamide.

Reading Scientific Services Ltd
The Reading Science Centre, Whiteknights Campus, Pepper Lane, Reading, Berkshire RG6 6LA • Tel: **+44 (0)118 918 4000** • Email:lifesciencesales@rssl.com • **www.rssl.com**