Biology | Molecular Biology

Protein Electrophoresis

General Aim

To prepare a PolyAcrylamide Gel Electrophoresis (PAGE) set up.

Method

SDS-PAGE

Learning Objectives (ILOs)

- Practice proper sample preparation for SDS-PAGE.
- Prepare polyacrylamide gel properly.
- Visualize the results of a successful protein electrophoresis run.

Theoretical Background/Context

- SDS-PAGE (SDS-polyacrylamide gel electrophoresis) is an electrophoretic technique that separates polypeptide chains according to their molecular weights (Mr). The technique utilizes polyacrylamide gel containing sodium dodecyl sulfate (SDS). SDS in the sample and the gel cancels the effect of intrinsic electrical charge of the sample proteins. All proteins acquire a negatively charged rod like structure, so separation becomes largely dependent on Molecular weight of sample proteins.

Theoretical Background/Context (Cont')

- Applications of protein gel electrophoresis: Once proteins have been separated by gel electrophoresis, they can be utilized for a number of downstream applications including:

1. Determine size and isoelectric point of separated proteins.
2. Enzyme assays.
3. Further purification.
4. Immunoblotting or western blotting.
5. Elution and digestion for mass spectrometric analysis.
6. Serum protein electrophoresis is tested when a patient has an abnormal total protein or albumin blood test, or, if a patient has symptoms of diseases associated with abnormal protein production, such as multiple myeloma or multiple sclerosis.

Principle of Work

The gel used in SDS PAGE can be divided into stacking gel and separating gel. Stacking gel (acrylamide 5%) is poured on top of the separating gel (after solidification) and a gel comb is inserted in the stacking gel. The acrylamide percentage is chosen in accordance with the size of target proteins in the sample. Samples are applied in wells created by the comb. Upon electric current application, proteins migrate according to their Mr, and thus are separated.