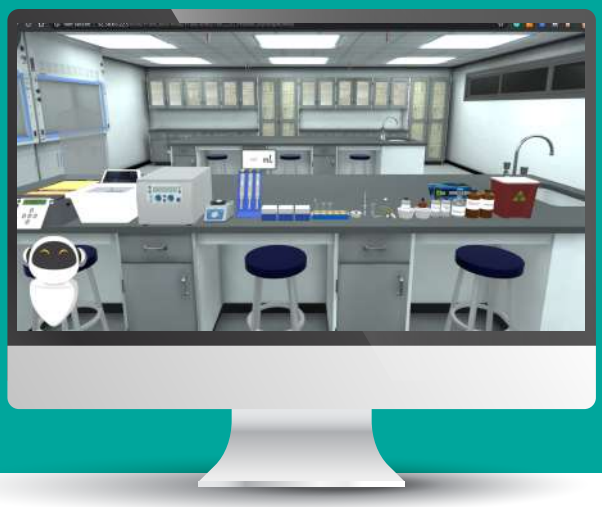
Biology | Molecular Biology

2D Protein Electrophoresis



General Aim

To extract the cellular proteome from a sample, followed by its isoelectric focusing and SDS-polyacrylamide gel electrophoresis.

Method

Protein separation using isoelectric focusing and SDS-PAGE.

Learning Objectives (ILOs)

- To describe the techniques used in proteomics to analyze and separate proteins.
- To apply general guidelines for efficient protein extraction from samples.
- To understand the principle of 2D SDS PAGE protein separation.
- To practice the set up required for successful iso-electric focusing.
- To practice the steps of preparation of SDS PAGE and perform a run.
- To visualize the end results on gel and interprete them.

Theoretical Background/Context

- Within any cell, the genome is constant, but the proteome varies and is dynamic. The proteome is the whole set of proteins produced by an organism in a cell. It varies with time and divergent statuses or stresses subjected to that cell or organism.
- Isoelectric Focusing (IEF) [1st dimension] is a technique for separating molecules according to their isoelectric point (pI). A protein present in a pH region below its pI will carry positive charge; therefore it migrates towards cathode (negatively charged electrode). While it migrates through an increasing pH gradient, the protein's charge decreases until it reaches the pH region that corresponds to its pI. This is where the protein has zero net charge so it stops migration. This is simply how proteins focus into sharp bands.

Theoretical Background/Context (Cont')

- The second dimension depends on SDS-PAGE (SDS-polyacrylamide gel electrophoresis), which is an electrophoretic method for separating polypeptides according to their molecular weights (Mr). this way proteins carrying the same pI can be further separated in a second dimension according to their Mr. The technique is performed in polyacrylamide gels containing sodium dodecyl sulfate (SDS). The intrinsic electrical charge of the sample proteins is not a factor in the separation due to the presence of SDS in the sample and the gel. When proteins are treated with both SDS and a reducing agent, the degree of electrophoretic separation within a polyacrylamide gel depends largely on the molecular weight of the protein.
- In this way, a mixture of thousands of different proteins can be separated and the relative amount of each protein can be determined.

Principle of Work

Cellular proteome can be simultaneously separated using 2D Protein Electrophoresis. This technique separates proteins in two steps, according to two independent properties:

1. First-dimension is isoelectric focusing (IEF), which separates proteins according to their isoelectric points (pI).
2. Second-dimension is SDS-polyacrylamide gel electrophoresis (SDS-PAGE), which separates proteins according to their molecular weights (MW).

   a. Preparing the second-dimension gel.
   b. Equilibrating the IPG strip(s) in SDS buffer.
   c. Placing the equilibrated IPG strip on the SDS gel.
   d. Electrophoresis and staining.