جامعة الملك سعود
King Saud University

Central Laboratory

And

Prince Naif Health Research

Center

Medical Studies and Scientific Sections

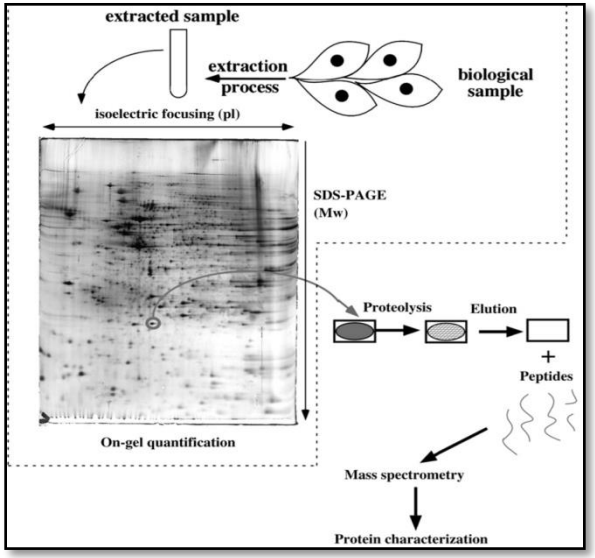
Proteomic Unit

Proteins perform most activities in living cells.

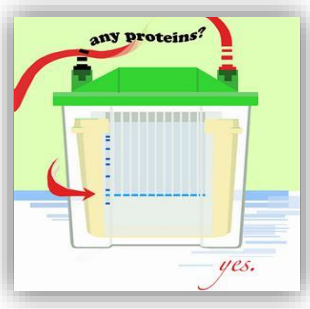
To study all the proteins expressed by the genome of an organism, it is necessary to analyze the proteome.

Proteomics, the analysis of the complete complement of proteins in a cell, tissue, or organism (the proteome), involves the detection of the presence or absence of proteins and the direct measurement of relative protein abundances.

Currently, two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), which is capable of resolving thousands of proteins in a single run, is the primary tool of proteomics research



2-D Electrophoresis



Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) of proteins is a robust and reproducible technique.

It is the most widely used separation tool in proteomics.

It is a powerful, multi-step procedure based on 2 dimensions of separation. Each dimension of separation is based on a property of the protein.

First-Dimension Separation (Isoelectric Focusing)

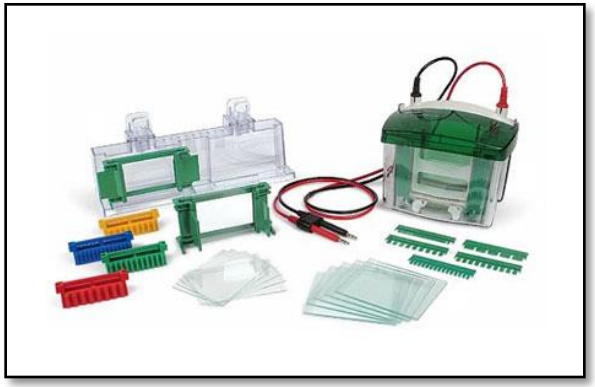
Proteins are first separated on the basis of pI, the pH at which a protein carries no net charge and thus will not migrate in an electrical field.

The technique called isoelectric focusing (IEF).

For 2-D PAGE, IEF best performed in an immobilized pH gradient (IPG) gel strip, which can subsequently be directly applied onto a PAGE gel for second-dimension separation.

Second-Dimension Separation

The second dimension of 2DE separates proteins on the basis of their apparent molecular weights in polyacrylamide gels in the presence of SDS.



Proteins separated on 2D gels are visualized by staining with Coomassie blue dye, silver stains, or fluorescent dyes.

The choice of staining method is determined by several factors including desired sensitivity, linear range, ease of use, expense, and the type of imaging equipment available.

2-D Gel Imaging

Imaging with Calibrated Densitometer Imaging.

Why would researchers want to use 2-D Electrophoresis?

To separate a complex mixture of proteins

Protein Profiling
- Comparison of control vs. treated
- Biomarker Discovery

Protein Identification
- Spot cutting to identify by MS analysis
- Western blotting