GEL ELECTROPHORESIS

Gel electrophoresis is a separation technique commonly used in modern laboratories to separate macromolecules such as DNA or proteins. As the name suggests, gel electrophoresis utilizes an electrical current to separate target molecules based on size, charge and other physical properties, through a porous gel matrix. Several alternative types of gel electrophoresis exist and can be classed as one-dimensional such as polyacrylamide gel electrophoresis (native or SDS-PAGE) and Isoelectricfocusing (IEF) or two dimensional such as 2D-PAGE. Gel electrophoresis can also be carried out using narrow gauge capillaries.

POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

PAGE, is one of the most widely used electrophoresis techniques and separates proteins through a polyacrylamide gel matrix. Two types of PAGE can be carried out – native PAGE, in which electrophoresis is carried out under non-denaturing conditions and separation is based on the protein's charge and hydrodynamic size or SDS PAGE in which proteins are denatured prior to electrophoresis and separation is based on a protein's mass or molecular weight. The chemical SDS (sodium dodecyl sulphate) is an anionic detergent which, in combination with DTT (dithiothreitol) or β-mercaptoethanol, breaks intramolecular bonds in the protein destroying any secondary, tertiary or quaternary structure. This leaves only the linear primary amino acid structure of the protein which will contain an overall negative charge proportional to its mass, therefore allowing the proteins to be separated solely on the basis of their molecular mass.

SDS-PAGE gels are generally composed of two different sub-gels, a stacking and a resolving gel, each cast at a different pH and acrylamide concentration. Once samples have been loaded into the wells of the stacker gel, a current is then applied creating a positive and negative charge across the gel. The now negatively charged protein molecules are strongly attracted to the anode in the electrical field. As the proteins enter the resolving gel, the polyacrylamide slows the larger molecules from migrating as fast as smaller molecules so creating separation based on mass.

Protein separation by SDS-PAGE can be used to estimate relative molecular mass, to determine the relative abundance of major proteins in a sample, and to determine the distribution of proteins among fractions. The purity of protein samples can be assessed and the progress of a fractionation or purification procedure can be followed. Once separated, gels are usually stained with coomassie blue to highlight the proteins of interest, however coomassie blue may not stain some proteins, especially those with high carbohydrate content. Stains such as periodic acid-Schiff (PAS), fast green, or Kodak's stain-all are normally employed. Silver staining is generally used when detection of low amounts of protein is required (i.e. >100ng)

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ISOELECTRIC FOCUSING

Isoelectric focusing (IEF) or electrofocusing is a method employed to separate proteins mixtures by electophoresis in a pH gradient based on their isoelectric point (pI). The pH at the anode is lower than the cathode so creating a pH gradient, with the pH range chosen so that the proteins to be separated have their isoelectric points within this range. At a pH other than their isoelectric point, proteins will be charged. If they are positively charged they will move towards the more negative end of the gel and if they are negatively charged they will move towards the more positive end of the gel. At its isoelectric point, the protein will no longer carry a charge and will no longer migrate in the gel. Therefore proteins become focused into sharp stationary bands with each protein positioned at a point in the pH gradient corresponding to its pI. The technique is capable of extremely high resolution with proteins differing by a single charge being fractionated into separate bands. IEF is mainly used as a technique to assess the complexity or purity of protein samples and unlike SDS-PAGE will not yield information about the molecular weight of the separated proteins.

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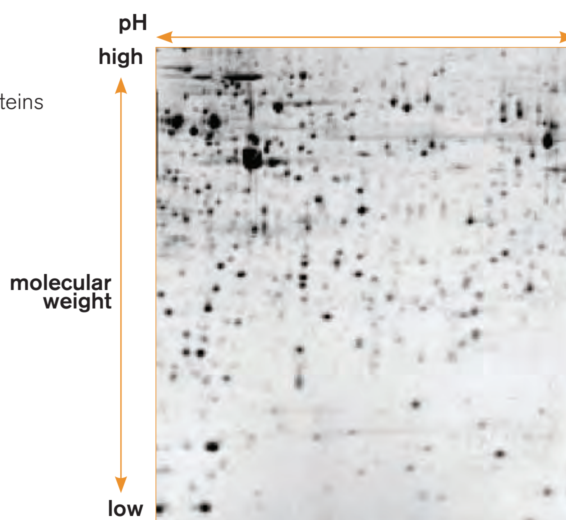
2-DIMENSIONAL GEL ELECTROPHORESIS

Using this technique, proteins are separated by two different properties. Initially proteins or polypeptides are separated on the basis of their net charges by isoelectric focusing. Gels are then turned 90° and separation continues based on their molecular weight. Because it is unlikely that two molecules will be similar in both properties, molecules are more effectively separated in 2-D electrophoresis than in 1-D electrophoresis gels.

In 2-D electrophoresis, proteins are initially separated by isoelectric focusing. This isoelectric focusing occurs across a pH gradient. The protein band will stop moving across the gel at its isoelectric point when the charge associated with the different amino groups is nullified by the pH. The gel is then turned 90° and the second electrophoresis occurs using SDS to separate the proteins by molecular weight. This second dimension of focusing gives a series of spots across the gel and each spot is a specific protein.

Typical representation of a 2-D SDS-PAGE with proteins separated by charge and molecular weight.



BLOTTING TECHNIQUES

Blotting refers to the technique, where molecules that have been separated by electrophoresis are transferred or blotted onto a specific type of paper usually nitrocellulose or PVDF by the application of an electrical current (electroblotting). There are three types of blotting techniques in current use - Southern, northern and western and are used to detect DNA, messenger RNA (mRNA) and protein, respectively.

WESTERN BLOTTING

Once protein samples have been separated, based on molecular mass, charge, pI or a combination of these factors, they are then transferred, usually by electroblotting, from the gel to a nitrocellulose or PVDF membrane. As a result of this process, the proteins are bound to the membrane surface which allows detection by an antibody specific to the protein of interest. However, since the membranes used have a high protein binding capacity, it is essential to block all non-specific protein. This is normally carried out by incubation of the membrane with BSA , non-fat milk powder or casein. Once non-specific sites have been blocked, an antibody specific to the protein of interest can be added. This antibody can be tagged with a label that will allow subsequent detection e.g. horseradish peroxidose or fluorescein. If the primary antibody does not contain a label, a secondary labelled antibody is employed.

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