

Principle of PAGE

Polyacrylamide gel electrophoresis (PAGE) is a commonly used technique in molecular biology to separate and analyze biomolecules, such as proteins and nucleic acids, based on their size and charge. The principle of PAGE gel electrophoresis involves the migration of charged biomolecules through a porous polyacrylamide gel matrix under the influence of an electric field. Here is a basic outline of the principle and steps involved:

Gel preparation: Polyacrylamide monomers are polymerized to form a gel matrix. The percentage of acrylamide and the crosslinking agent (usually bisacrylamide) determine the pore size and, therefore, the resolution of the gel. Higher percentages of acrylamide result in smaller pores and better resolution for smaller molecules, while lower percentages allow better separation of larger molecules. The gel is typically cast between two glass plates with spacer wells that create sample-loading wells.

Sample preparation: The biomolecules to be separated are mixed with a loading buffer, which contains a denser substance (such as glycerol or a tracking dye) that allows visualization of the migration front during electrophoresis. The samples are denatured or treated with a reducing agent if necessary, to ensure uniform charge distribution and to disrupt any secondary structures.

Loading and electrophoresis: The samples are loaded into the wells of the polyacrylamide gel. An electric field is applied by connecting the electrodes to the gel apparatus. When the current is applied, the negatively charged biomolecules migrate toward the positively charged electrode. Smaller molecules move more easily through the gel matrix, while larger molecules encounter more resistance and migrate more slowly.

Separation and visualization: During electrophoresis, the biomolecules separate based on their size and charge. After a specific duration of electrophoresis, the gel is removed from the apparatus and stained with appropriate dyes or stains specific to the biomolecule of interest. For proteins, common stains include Coomassie Brilliant Blue or silver stains. For nucleic acids, ethidium bromide or SYBR Green dyes are commonly used. The stained gel is then visualized under UV light or using other imaging systems to observe the separated biomolecules as distinct bands or patterns.

Molecular weight determination: To determine the molecular weight of the separated biomolecules, a set of molecular weight markers, also known as protein or DNA ladders, are run alongside the samples. These markers consist of known-sized biomolecules and help establish a correlation between the migration distance and the molecular weight of the target molecules in the sample.

PAGE gel electrophoresis can provide valuable information about the size, purity, and relative abundance of biomolecules. It is widely used for various applications, including protein characterization, DNA fragment analysis, and nucleic acid sequencing, among others.