

## Western Blot – Immunoblotting

Western Blotting is a technique used to detect specific proteins in a sample. It involves the separation of proteins by SDS-PAGE, followed by transfer to a membrane and detection with specific antibodies. The process is highly sensitive and can be used to study protein expression, localization, and function.

- **Sample Preparation:** Proteins are extracted from cells or tissues and denatured using SDS and reducing agents. The denatured proteins are then separated by SDS-PAGE.
- **Transfer:** The separated proteins are transferred from the gel to a membrane (usually nitrocellulose or PVDF). The transfer is facilitated by a buffer containing SDS and other components.
- **Blocking:** The membrane is blocked with a blocking agent (e.g., BSA or casein) to prevent non-specific binding of antibodies.
- **Primary Antibody Incubation:** The membrane is incubated with a primary antibody that specifically binds to the target protein.
- **Secondary Antibody Incubation:** The membrane is incubated with a secondary antibody that binds to the primary antibody. The secondary antibody is conjugated with a reporter molecule (e.g., horseradish peroxidase) for detection.
- **Detection:** The reporter molecule is used to visualize the protein bands. Common detection methods include colorimetric development, chemiluminescence, and fluorescence.

Western Blotting is a powerful tool for studying protein expression and function. It allows researchers to identify and quantify specific proteins in a complex sample, providing valuable insights into cellular processes and disease mechanisms.