

Immuno blotting techniques

Immunoblotting (Western Blotting)

Introduction

- Western blotting is a technique used to detect specific proteins in a sample based on antigen-antibody interactions.

Steps in Immunoblotting

1. Protein Separation (Electrophoresis)

- Proteins are separated by size and charge using polyacrylamide gel electrophoresis (PAGE).

2. Transfer to Membrane

- Separated proteins are transferred onto a nitrocellulose or PVDF membrane for analysis.

3. Antibody Binding

- A primary antibody binds to the target protein.
- It may be labeled with a detection molecule (e.g., fluorescent dye, enzyme, or radioactive isotope).

4. Signal Detection

- The detection molecule produces a signal visible via chemiluminescence, fluorescence, or radioactive imaging.
- Signal intensity correlates with the protein amount.

5. Signal Amplification (Optional)

- A secondary antibody binds to the primary antibody, enhancing detection sensitivity.

Enzyme-Linked Immunosorbent Assay (ELISA)

Definition:

- ELISA is a widely used immunological technique that detects and quantifies specific molecules, such as proteins, peptides, antibodies, and hormones, in biological samples.

Principle:

- ELISA relies on antigen-antibody interactions.
- A specific antigen or antibody is immobilized onto a solid support and subsequently detected using a labeled antibody or enzyme.

Types of ELISA:

- **Direct ELISA:** Uses a single, enzyme-labeled antibody.
- **Indirect ELISA:** Uses a primary antibody and an enzyme-labeled secondary antibody.
- **Sandwich ELISA:** Uses two antibodies, one for capturing and the other for detection.
- **Competitive ELISA:** Measures antigen concentration by competing with a labeled antigen for antibody binding.

Steps of ELISA:

1. **Coating:** The antigen or antibody is immobilized on a solid surface (typically a 96-well plate).
2. **Blocking:** Unoccupied sites are blocked with a non-specific protein to prevent non-specific binding.
3. **Incubation with Primary Antibody:** A specific antibody binds to the target antigen.
4. **Secondary Antibody Addition:** A secondary antibody conjugated with an enzyme (e.g., HRP or alkaline phosphatase) binds to the primary antibody.
5. **Substrate Addition:** A substrate is added, which reacts with the enzyme to produce a detectable signal (colorimetric or fluorescent).
6. **Measurement:** The intensity of the color change is measured using a spectrophotometer.

Applications of ELISA:

1. Detection of viral and bacterial infections.
2. Detection of autoimmune diseases.
3. Drug discovery and development.
4. Environmental monitoring.

Western Blotting (Immunoblotting)

- Western blotting is a widely used technique for detecting and analyzing specific proteins in a sample.

Principle

- Western blotting is based on antigen-antibody interaction, where a specific antibody binds to the target protein for detection.

Steps of Western Blotting:

1. **Sample Preparation:** Proteins are extracted from cells or tissues and denatured using SDS (Sodium Dodecyl Sulfate).
2. **Gel Electrophoresis (SDS-PAGE):** Proteins are separated based on their molecular weight in a polyacrylamide gel.
3. **Transfer to Membrane:** The proteins are transferred from the gel to a membrane (nitrocellulose or PVDF) using an electric field.

4. **Blocking:** The membrane is blocked with a protein solution (e.g., BSA or milk) to prevent non-specific antibody binding.
5. **Incubation with Primary Antibody:** A specific antibody binds to the target protein.
6. **Incubation with Secondary Antibody:** A secondary antibody conjugated with an enzyme (e.g., HRP) binds to the primary antibody.
7. **Detection:** A chemiluminescent or colorimetric substrate is added, and the signal is visualized using X-ray film or a digital imaging system.

Types of Western Blotting

- Two-dimensional (2D) Western blotting
- Multiplex Western blotting

Applications of Western Blotting

1. Protein expression analysis
2. Protein-protein interaction analysis
3. Post-translational modification analysis
4. Disease diagnosis

Southern Blotting

- Southern blotting is a molecular biology technique used to detect the presence of a specific DNA sequence in a sample.
- It was developed by Edwin Southern and is widely used in research and clinical laboratories for studying gene expression, mutations, and DNA polymorphisms. Additionally, it is valuable in forensic analysis and paternity testing.

Principle

- Southern blotting is based on hybridization, where a labeled probe binds to its complementary DNA sequence of interest for detection.

Steps of Southern Blotting:

1. **DNA Extraction:** DNA is isolated from the sample.
2. **Restriction Digestion:** DNA is cut into fragments using restriction enzymes.
3. **Gel Electrophoresis:** DNA fragments are separated on an agarose gel based on size.
4. **Denaturation:** DNA is denatured into single strands using an alkaline solution.
5. **Transfer to Membrane:** The DNA fragments are transferred onto a nitrocellulose or nylon membrane.
6. **Hybridization:** A labeled complementary DNA probe (radioactive or fluorescent) binds to the target DNA sequence.



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