Western Blotting: Protein Detection Technique

Western blotting is a laboratory technique used to detect **specific proteins** in a sample. Unlike **Southern blotting (for DNA)** and **Northern blotting (for RNA)**, **Western blotting is used for proteins** and involves **antibody-based hybridization** instead of nucleic acid hybridization.

Steps of Western Blotting

1. Sample Preparation

- Extract **proteins** from cells or tissues.
- Use **detergents** (like SDS) to break open the cells and release proteins.

2. SDS-PAGE (Protein Separation by Electrophoresis)

- Proteins are mixed with **SDS (sodium dodecyl sulfate)**, which unfolds them and gives them a **uniform negative charge**.
- The sample is loaded onto a **polyacrylamide gel** and run using an **electric field**.
- Proteins separate based on **size** (smaller proteins move faster).

3. Transfer to a Membrane (Blotting)

- The separated proteins are transferred onto a **nitrocellulose or PVDF membrane** using **electroblotting**.
- This makes the proteins more accessible for detection.

4. Blocking

- The membrane is treated with a **blocking solution** (like milk or BSA) to prevent **non-specific binding** of antibodies.

5. Antibody Hybridization

- **Primary antibody**: Specifically binds to the target protein.
- **Secondary antibody**: Recognizes the primary antibody and is linked to an **enzyme** (like HRP or alkaline phosphatase) for detection.

6. Detection

- A chemical **substrate** reacts with the enzyme on the secondary antibody to produce **a visible signal** (chemiluminescence, fluorescence, or colorimetric reaction).
- The signal is detected using **X-ray film or specialized imaging systems**.

Applications of Western Blotting

✅ **Medical Diagnostics** – Used to detect **HIV, Lyme disease, and prion diseases**.

✅ **Protein Research** – Studies **protein expression and modifications**.

✅ **Cancer Research** – Identifies **biomarkers in tumors**.