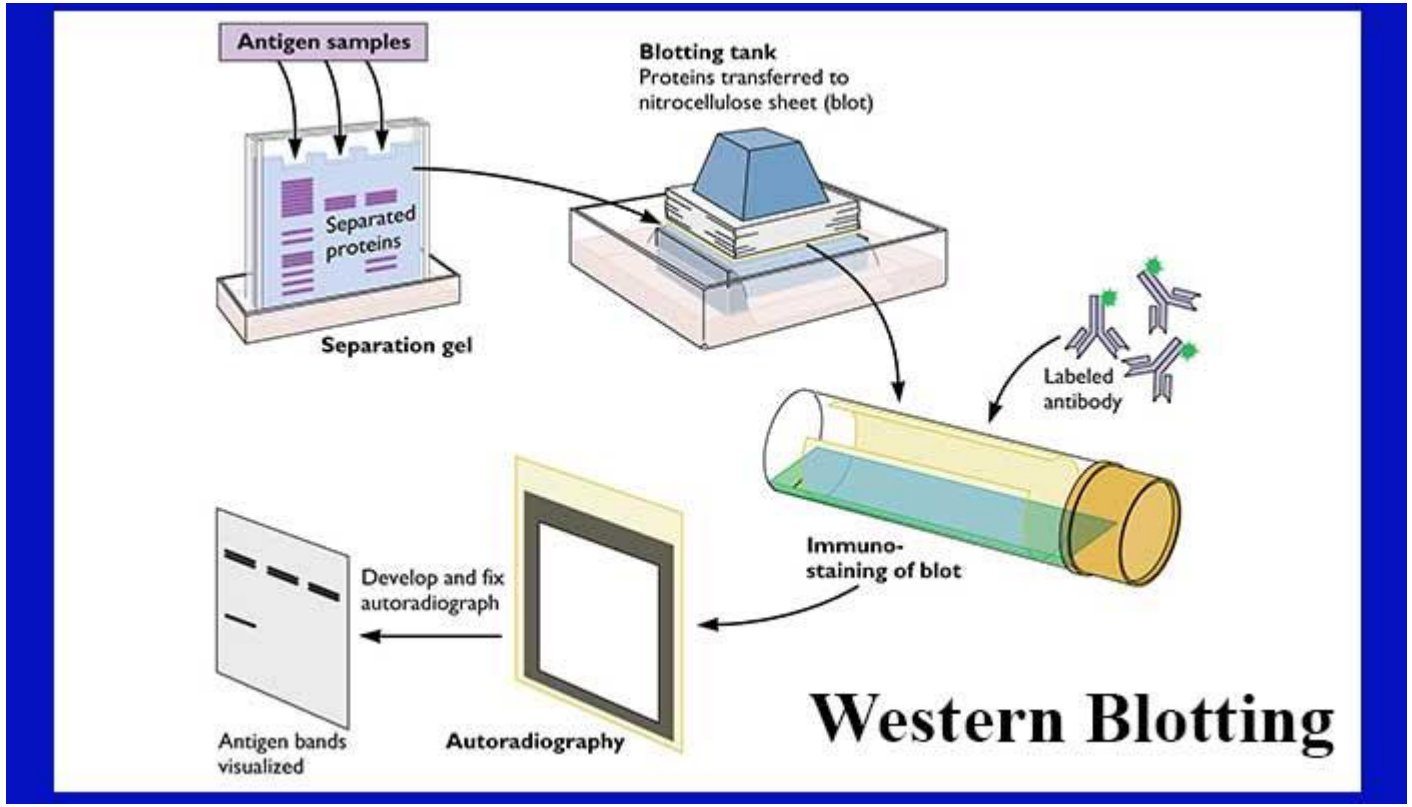
Western blotting or immunoblotting
Introduction, Principle and Applications

Introduction

Western blot is the analytical technique used in molecular biology, immunogenetics and other molecular biology to detect specific proteins in a sample of tissue homogenate or extract. Western blotting is called so as the procedure is similar to Southern blotting. While Southern blotting is done to detect DNA, Western blotting is done for the detection of proteins. Western blotting is also called protein immunoblotting because an antibody is used to specifically detect its antigen.



Principle of Western blotting

The technique consists of three major processes:

1.Separation of proteins by size (Electrophoresis).
2.Transfer to a solid support (Blotting)
3.Marking target protein using a proper primary and secondary antibody to visualize (Detection).

Electrophoresis used to separate proteins according to their electrophoretic mobility which depends on charge, size of protein molecule and structure of the proteins. Proteins are moved from within the gel onto a membrane made of Nitrocellulose (NC) or Polyvinylidene difluoride (PVDF). Without pre-activation, proteins combine with nitrocellulose membrane based on hydrophobic interaction (**Blotting**). For detection of the proteins primary antibody and enzyme conjugated secondary antibody are used. On addition of substrate, a substrate reacts with the enzyme that is bound to the secondary antibody to generate colored substance, namely, visible protein bands.
In this technique a mixture of proteins is separated based on molecular weight, and thus by type, through gel electrophoresis. These results are then transferred to a membrane producing a band for each protein. The membrane is then incubated with labels antibodies specific to the protein of interest. The unbound antibody is washed off leaving only the bound antibody to the protein of interest. The bound antibodies are then detected by developing the film. As the antibodies only bind to the protein of interest, only one band should be visible. The thickness of the band corresponds to the amount of protein present; thus doing a standard can indicate the amount of protein present. Western blotting is usually done on a tissue homogenate or extract.

Principle of Western blotting

It utilizes **SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis)**, a type of gel electrophoresis to first separate various proteins in a mixture on the basis of their shape and size. The protein bands thus obtained are transferred onto a **nitrocellulose or nylon membrane** where they are "probed" with antibodies specific to the protein to be detected. The antigen–antibody complexes that form on the band containing the protein recognized by the antibody can be visualized in a variety of ways.

If the protein of interest is bound by a radioactive antibody, its position on the blot can be determined by exposing the membrane to a sheet of X-ray film, a procedure called **autoradiography**. However, the most generally used detection procedures employ enzyme-linked antibodies against the protein. After binding of the enzyme–antibody conjugate, addition of a chromogenic substrate that produces a highly colored and insoluble product causes the appearance of a colored band at the site of the target antigen. The site of the protein of interest can be determined with much higher sensitivity if a chemiluminescent compound along with suitable enhancing agents is used to produce light at the antigen site.

Applications of Western blotting

1. Identification of a specific protein in a complex mixture of proteins. In this method, known antigens of well-defined molecular weight are separated by SDS-PAGE and blotted onto nitrocellulose. The separated bands of known antigens are then probed with the sample suspected of containing antibody specific for one or more of these antigens. Reaction of an antibody with a band is detected by using either radiolabeled or enzyme-linked secondary antibody that is specific for the species of the antibodies in the test sample.

2. Estimation of the size of the protein as well as the amount of protein present in the mixture.

3. It is most widely used as a confirmatory test for diagnosis of HIV, where this procedure is used to determine whether the patient has antibodies that react with one or more viral proteins or not.

4. Demonstration of specific antibodies in the serum for diagnosis of neurocysticercosis and tubercular meningitis.