Western blot

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Principle:

Western blotting (protein blotting or immunoblotting) is a rapid and sensitive assay for detection and characterization of proteins. It is based on the principle of immunochromatography where proteins are separated into polyacrylamide gel according to their molecular weight.

The protein thus separated are then transferred or electrotransferred onto nitrocellulose membrane and are detected using specific primary antibody and secondary enzyme labeled antibody and substrate.

Procedure/Steps:

1. Extraction of protein
2. Gel electrophoresis: SDS PAGE
3. Blotting: electrical or capillary blotting
4. Blocking: BSA
5. Treatment with primary antibody
6. Treatment with secondary antibody(enzyme labelled anti Ab)
7. Treatment with specific substrate; if enzyme is alkaline phosphatase, substrate is p-nitro phenyl phosphate which give color.

Step I: Extraction of Protein

- Cell lysate is most common sample for western blotting.
- Protein is extracted from cell by mechanical or chemical lysis of cell. This step is also known as tissue preparation.
- To prevent denaturing of protein protease inhibitor is used.
- The concentration of protein is determined by spectroscopy.

Step II: Gel electrophoresis

- The sample is loaded in well of SDS-PAGE Sodium dodecyl sulfate- poly-acrylamide gel electrophoresis.

- The proteins are separated on the basis of electric charge, isoelectric point, molecular weight, or combination of these all.
- The small size protein moves faster than large size protein.
- Protein are negatively charged, so they move toward positive (anode) pole as electric current is applied.

Step III: Blotting

- The nitrocellulose membrane is placed on the gel. The separated protein from gel get transferred to nitrocellulose paper by capillary action. This type of blotting is time consuming and may take 1-2 days
- For fast and more efficient transfer of desired protein from the gel to nitrocellulose paper electro-blotting can be used.
- In electro-blotting nitrocellulose membrane is sandwich between gel and cassette of filter paper and then electric current is passed through the gel causing transfer of protein to the membrane.

Step IV: Blocking

- Blocking is very important step in western blotting.
- Antibodies are also protein so they are likely to bind the nitrocellulose paper. So before adding the primary antibody the membrane is non-specifically saturated or masked by using casein or Bovine serum albumin (BSA).

Step V: Treatment with Primary Antibody (Antibody Probing)

- The primary antibody (1° Ab) is specific to desired protein so it form Ag-Ab complex

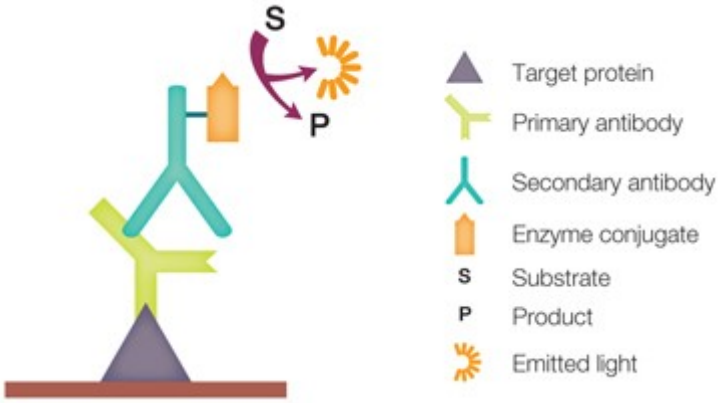
Step VI: Treatment with secondary antibody

- The secondary antibody is enzyme labelled. For eg. alkaline phosphatase or Horseradish peroxidase (HRP) is labelled with secondary antibody.
- Secondary antibody (2° Ab) is antibody against primary antibody (anti-antibody) so it can bind with Ag-Ab complex.

Step VII: Treatment with suitable substrate

- To visualize the enzyme action, the reaction mixture is incubated with specific substrate.
- The enzyme convert the substrate to give visible colored product, so band of color can be visualized in the membrane.

- Western blotting is also a quantitative test to determine the amount of protein in sample.



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.

Western blotting can detect target proteins as low as 1 ng in concentration due to high-resolution capacity of gel electrophoresis and strong sensitivity and specificity of the immunoassay.

Use Of Western Blotting:

Western blotting is an extremely powerful technique, despite its overall simplicity, because it provides additional information not readily gathered from other key immunological laboratory techniques. Since proteins are separated by size during the gel electrophoresis stage, and then detected by a specifically directed antibody, the procedure essentially confirms the identity of a target protein.

Western blots are in wide use across a broad range of scientific and clinical disciplines . Their ability to clearly show the presence of a specific protein both by size and through the binding of an antibody makes them well-suited for evaluating levels of protein expression in cells, and for monitoring fractions during protein purification.

Likewise, they are helpful for comparing expression of a target protein from various tissues, or seeing how a particular protein responds to disease or drug treatment . In many cases, Western blots are used in combination with other key antibody based detection techniques, such as ELISAs or immunohistochemistry.

In these instances, Western blots provide confirmation of results both in research and diagnostic testing. For example, with HIV and prion disease.

It is very important to be aware that the data produced with a western blot is typically considered to be semi-quantitative. This is because it provides a relative comparison of protein levels, but not an absolute measure of quantity.

Contd.

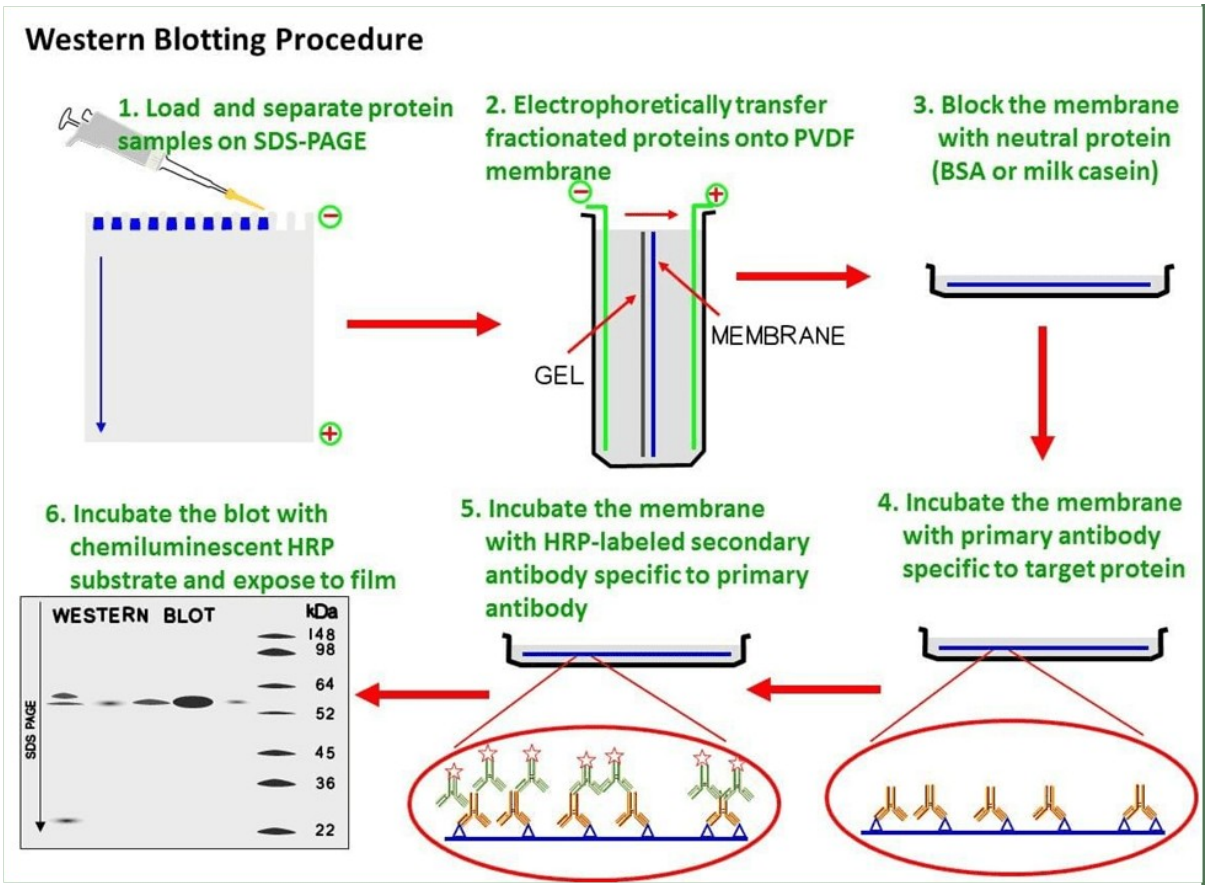


Diagram collected from :

Western Blot Technique: Principle, Procedures and Uses
May 12, 2017 Bina Bhandari Immunology, Lab Diagnosis of Viral Disease