Western Blot: Principle and Theory

Western blotting is a technique used to separate and identify specific proteins within cell and tissue samples containing a mixture of various proteins. The three main parts of the Western blot technique are **separation of proteins by size, transfer to a solid membrane, and labeling with protein-specific antibodies.** Western blotting has a few interesting applications, including:

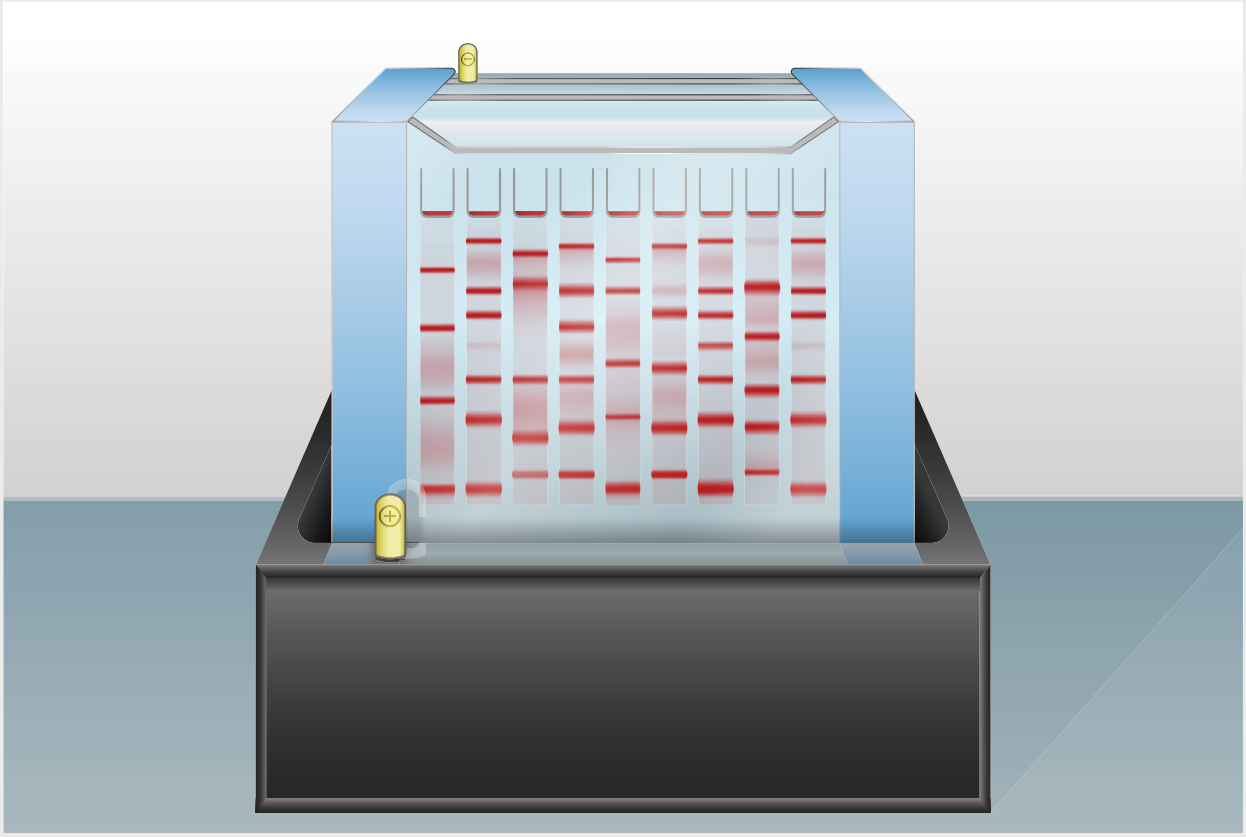
- **Biochemistry:** detection of post-translational modifications in proteins and analysis of protein production
- **Medical diagnostics**: detection of HIV antibodies, Lyme disease, Creutzfeldt-Jakob disease, etc.
- **Enforcing proper practices in the Olympics:** used by the World Anti-Doping Agency to detect blood doping, an illegal technique meant to increase one's red blood cell mass and improve performance

The principle of the Western blot is relatively simple – proteins are first separated by size and then detected using specific antibodies. This technique is an efficient way to confirm protein identity and can be used in conjunction with other detection techniques, such as ELISAs or IHC, to compare protein expression in various tissues or to see how proteins respond to different treatments. Read on to learn more about the main steps involved in this procedure:

- Gel electrophoresis
- Transfer process
- Probing
- Detection

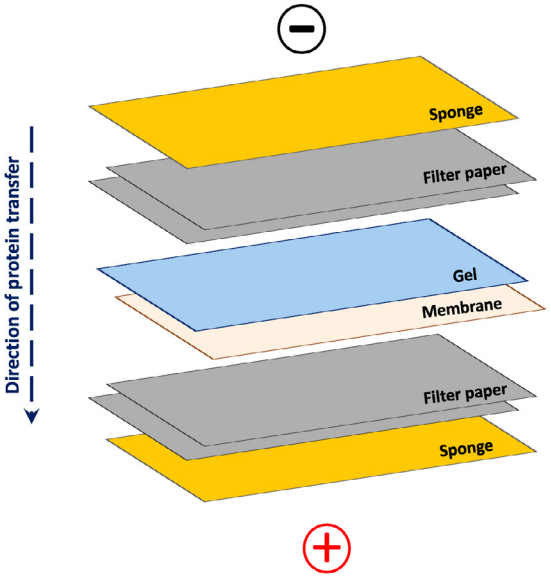
Gel Electrophoresis

This step is where proteins are separated by their size, or rather, their molecular weight. Often, SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel) electrophoresis is used for Western blotting: With SDS-PAGE, sodium dodecyl sulfate is used to denature proteins and to grant them a uniform negative charge. Treated proteins are then loaded into the wells of a polyacrylamide together with a marker ladder containing proteins of known molecular weights for comparison. Then, an electrophoresis chamber is used to apply a an electric field voltage across the gel, causing proteins to move toward the positively charged anode. Proteins of low molecular weight travel faster than those of high molecular weight, resulting in proteins separated by size.



Transfer Process

Once proteins are separated, they are transferred onto a solid membrane. This is usually done by applying an electric field perpendicular to the gel's surface, which draws the proteins out of the gel and onto the membrane. This process commonly called electro-blotting, or just blotting, can be carried out in different ways. While semi-dry and dry blotting systems gain popularity, wet blotting transfer in a liquid tank is still the most widely used technique. To do this, a gel-membrane-fiber "sandwich" is formed. The gel is placed in the middle and the transfer membrane is placed adjacently on what will be the anode side. Very importantly, the gel must maintain close contact with the membrane, with no air bubbles, so that the proteins transfer clearly and uniformly. Then filter paper is places on both sides, followed by sponges, forming a "sandwich" that will be locked in a support grid structure. The setup is shown in the figure below:



The gel-membrane-fiber sandwich contained in the support grid is then placed into a tank containing transfer buffer and cathode and anode on either side. It is important that the membrane is placed between the gel and the anode, so that the negatively charged proteins will migrate towards the anode and thus get transferred onto the membrane. Most commonly used membranes are either nitrocellulose or polyvinylidene difluoride (PVDF). Both membrane material offer good non-specific protein binding, facilitated by both hydrophobic and charged interactions between proteins and membrane material.

Probing

Once proteins have been successfully transferred to the solid membrane, they must undergo a blocking procedure to prevent non-specific binding. This is often done with BSA (bovine serum albumin) or non-fat dried milk in TBST (Tris-Buffered Saline Tween-20). Then, the proteins are detected with either one or two antibodies. If direct detection is performed, a primary antibody is used on its own; this antibody specifically binds to the protein of interest. More commonly, indirect detection is used. In this case, a primary and a secondary antibody are used. The secondary antibody is directed against the primary antibody; several secondary antibodies will bind to the primary antibody, thus allowing for an enhanced signal and making it possible to detect proteins at lower concentrations. In either case, once the primary antibody is added and incubated, the membrane is washed with TBST to reduce background and remove any unbound antibody. Similarly, if used, the secondary antibody is then added, incubated, and washed out.

In order for the proteins to be properly visualized, the primary and secondary antibodies must be obtained from separate hosts. If the primary antibody is from a rabbit host, the secondary antibody must be “anti-rabbit” and come from a non-rabbit host.

Detection

Most commonly, protein detection is performed using secondary antibodies coupled to enzymes. The two main enzymes used for detection in Western blotting are horseradish peroxidase (HRP) and alkaline phosphatase (AP). Chromogenic and chemiluminescent substrates may be added to these enzymes for visualization. Chromogenic substrates react with the enzyme to produce a colored precipitate, yielding results that are visible and readily interpretable to the naked eye. Alternatively, chemiluminescent substrates can be added to induce luminescence, which in turn is detected with the help of photographic film and developers or specialized imaging equipment. Fluorophore-conjugated antibodies may also be used to produce a fluorescent signal that can be detected using specialized equipment.

References:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3456489/

https://www.ruf.rice.edu/~bioslabs/studies/sds-page/gellab2.html