How Can you Perform Blotting With The Use of Western Blot Gel Electrophoresis?

Western Blot Gel Electrophoresis is a method for analyzing proteins, or fragments of proteins, on a gel made of agarose or acrylamide. Western blot gel electrophoresis uses an electric current to migrate protein through an SDS-PAGE gel containing nitrocellulose membranes at the bottom to catch any protein that doesn't reach the top of the gel. As a result, the high molecular weight proteins move more slowly than small ones, allowing you to identify abnormal proteins by size. Western blotting is used at many levels in biology ranging from research to industrial applications to forensic analysis. They are destaining the gel using solvents to remove unbound proteins from the bottom and ensure that proteins suspended in SDS migrate at their molecular weight.

What is Western Blotting?

Western blotting, also known as immunoblotting or immunodetection, is a biochemical technique used to detect specific proteins in a sample of tissue homogenate, cell culture supernatant, or extracts from cells, and one of the most reliable platforms offering this service is Kendrick Laboratories. A protein analyzer can prepare tissue samples for the Western blotting technique. This analyzer uses a gel filtration method to separate the proteins by their molecular weights using gel electrophoresis.

The most common type of gel electrophoresis today is SDS-polyacrylamide gel electrophoresis, commonly called 'SDS-PAGE.' Quantitation of the protein concentration for each band can be performed using a densitometer. Protein identification and Western blotting are easy using commercially available kits such as the Pierce Subtotal Protein Blot Detection Kit, which has been used by laboratories worldwide since its introduction in 1976.

Working Principle of Western Blot Gel Electrophoresis:

The proteins are separated by varying their charge and mass. In Western Blot Gel Electrophoresis, the amount of charge on a protein is determined by amino acids on the outer edges of the proteins, positively charged lysine and arginine, negatively charged glutamine and asparagine, or uncharged glycine or proline. The net charge equals zero when they are located in the middle of the protein, called the isoelectric point (pI).

In the middle of the proteins, the direction of charge is 'random,' but it depends on pI. For example, suppose a charged amino acid is on the outer edge of a protein. In that case, it has a certain amount of negative charge in its z direction (relative to the -z-direction in which charged amino acid sits), which pushes proteins away from each other. It also pulls protein molecules together to stick to each other and repel each other's way. The net effect is that proteins move into different zones with different electric charges at different rates along the gel.

How to Use?

First, collect polyclonal antibodies to your protein of interest. Place samples in wells and mount the wells on a plate. Make sure you use the same amount of antibodies and scrapers/labels. Since polyclonal antibodies give different band sizes, run all samples simultaneously and place the exact amounts in each well experimentally. Next, place all washes, electric current, and blank in a beaker with all new media (Hank's solution) that you'll be using and mix everything up with an electric mixer until it is all uniform. Use a standard washing procedure, starting with 20 minutes of washing buffer and then changing to PBS. If you have more aggressive enzymes in your western blot kit, use 10 minutes wash instead.

What about protein transfer or blotting?

At the top of the gel, place a nitrocellulose membrane (ounce to lb) and lay blotting paper over the nitrocellulose membrane. Then place agarose gel on top of the nitrocellulose membrane and blotting paper. Run an electric current between the top and bottom part of the gel through the agarose. To transfer proteins out of an agarose gel onto nitrocellulose membranes, pour fresh transfer buffer over the middle layer (gel/nitrocellulose) and then use methanol to dissolve away everything except proteins that were transferred out. Agarose is a plant extract that acts as a gel, holding proteins in place, so they form a band when electrophoresis is run through them.

The gel stays solid since agarose forms double-stranded DNA when wet, producing strong hydrogen bonds. The gel is placed on plastic plates with wells where samples are placed to perform a western blot. Hanks's solution is used for washing antibodies, and the amount of time determines the intensity of the protein bands on your nitrocellulose membrane. Since most antibodies are monoclonal, it gives higher intensity than polyclonal antibodies.

Protein detection & analysis:

The nitrocellulose membrane is placed on an x-ray film and left to dry. Protein bands are detected by staining with Ponceau S, which is red and binds only to proteins, not DNA or RNA. The protein stain will bind only to the proteins on the nitrocellulose membrane that have not been washed away so that you can visualize the protein bands. Image analysis is done by counting the number of pixels within each band on your nitrocellulose membrane, which indicates its intensity (pI).

Conclusion:

Now you can use the data to determine if your protein is usually expressed and compare it to your controls. Using trypsin, you can also determine how active your protein is (pI). Trypsin

digests proteins at their pI, leaving behind a smear on the bottom of the nitrocellulose membrane, and a negative control (trypsin without your protein) would look the same as this. If you want to learn about Western Blot Gel Electrophoresis, **click here**.