8.4 WESTERN BLOTTING

Western blotting is a widely used technique for the detection and analysis of protein and is also known as immunoblotting.

This name is given because it involves an antibody probe which is utilized for the detection of the target protein on the membrane.

There are 5 main steps which are involved in the western blotting. These are:

Step 1: Protein gel electrophoresis: The first step involves separation and characterization of protein by gel electrophoresis.

The most widely used technique for protein electrophoresis is Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). This technique separates protein according to their molecular weight on the membrane.

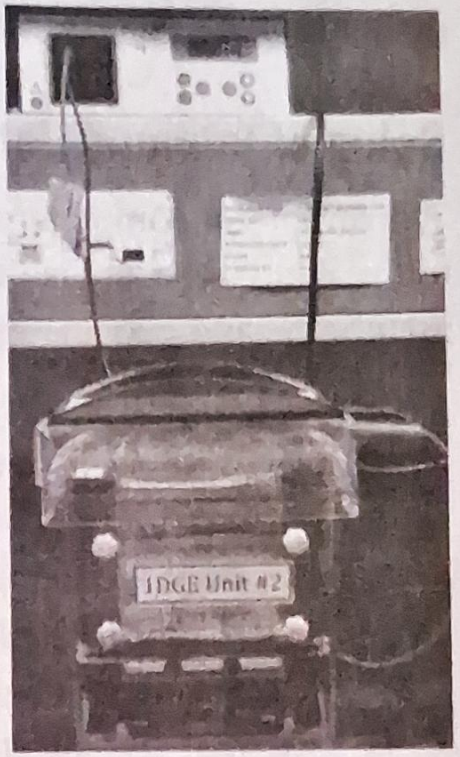

Fig. 8.2. *gel electrophoresis*

Step 2: Protein transfer/ Blotting: The next step to transfer this protein from the gel on to a suitable membrane. The membrane used in western blotting has high affinity for protein such as nitrocellulose and polyvinylidene difluoride (PVDF).

The method used to transfer protein from gel to membrane is known as electrophoresis transfer. In this method electric current is used to elude protein from the gel and transfer them on to the membrane. The both are placed in such a way that gel is on the negative side of electrode whereas membrane is on the positive side of electrode.

The proteins are negatively charged so they move towards positive charge on the electrode.

For transfer of protein a stack or Transfer sandwich of gel is prepared which consist of sponge and filter paper.

The arrangement of sandwich is like this

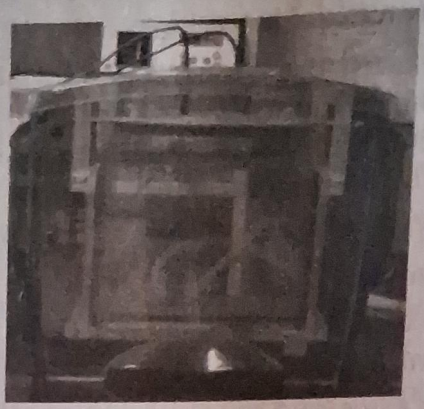

Fig. 8.3. *Arrangement of protein stack or transfer sandwich of gel.*

The filter paper maintains uniform flow of transfer buffer whereas the sponge maintains the proper pressure during the transfer. This sandwich of gel is place inside the cassette which is non-conducting and kept gel on the membrane is closed contact.

This entire set up is kept entirely submerged under transfer buffer within a buffer tank. The placement is such that the gel is at the side of negative electrode and membrane is at the side of positive electrode. When electric current is supplied the negatively charged protein move from gel and travelled towards the membrane which is at the side of positive electrode.

Setup of Immunoblotting (Western Blotting)

The important component of transfer buffer is methanol which increases the binding of proteins to the membrane. It also removes SDS from the protein. All the protein from gel moved to the membrane and become tightly attached to it

Step 1: Blocking: In this step first block the membrane with non-specific protein in order to prevent antibody from binding to the membrane. This nonspecific binding of antibodies to the membrane is detrimental to the specificity and sensitivity of the assay therefore it is essential to block space that are not already occupied by proteins so in this step blocking agent is added. The most common blocking agent used are bovine serum albumin BSA and non-fat milk.

Fig. 8.4. *Western blot.*

These blocking agents would fill all the unoccupied sites on the membrane since these blocking agents specifically bind the membrane. They will not disturb the already bound proteins on the membrane.

Step 2: Antibody probing: The membrane is incubated with primary antibody since this antibody is specific to target protein. It will bind to the protein on the membrane. After this, excess of primary antibodies are removed by washing.

Step 3: Detection: The final step is detection followed by further analysis of protein. The membrane is incubated with labelled secondary antibody and these to the primary antibody that is already bound to the target protein on the membrane. Excess secondary antibodies are also removed by washing. The enzyme [Horseradish peroxidase (HRP)] is most preferred enzyme which is used for detection the presence of the enzyme is detected by adding a suitable chemiluminescent substrate such as 4 -chloro-1- naphthol.

The enzyme HRP binds with the substrate and catalyzes by oxidation of substrate into an insoluble purple product. This purple colour is visible on the blot. Thus the target protein is detected.