WESTERN BLOTTING (Western Transfer)

The method of transferring proteins from Gel (Polyacrylamide gel) onto the membrane (nitrocellulose) originated from the laboratory of George Stark at Stanford. The name western blot was given by W. Neal Burnette. The western blot is a method of detecting specific proteins in a given sample of tissue homogenate or extract separated by gel electrophoresis. This method involves transfer of electrophoresed protein bands from a polyacrylamide onto nitrocellulose membrane to which they bind strongly. The bound proteins are then available for specific protein – ligand interaction.

Western blotting involves transfer of proteins from gel onto the membrane under electric field. Negatively charged protein molecules tend to move towards anode. But due to the presence of membrane in their path, these molecules having a size bigger than the pore size in membrane fail to reach the respective electrode and get bound to the membrane, which is placed towards anode. The membrane with its transferred proteins is referred to as a blot. once transferred onto membrane, the separated proteins can be examined further by probing the blot usually by using an antibody (Primary) to detect a specific protein.

WESTERN BLOT APPARATUS – * Gel holder, Sponge and Transfer tank
* Power pack and electric lead * Waterbath at 80°c
* Nitrocellulose sheet cut to the size of the gel.
* Whatman (3 mm) paper cut to the size of the gel.
* Polyacrylamide gel on to which proteins been separated after electrophoresis [redacted].

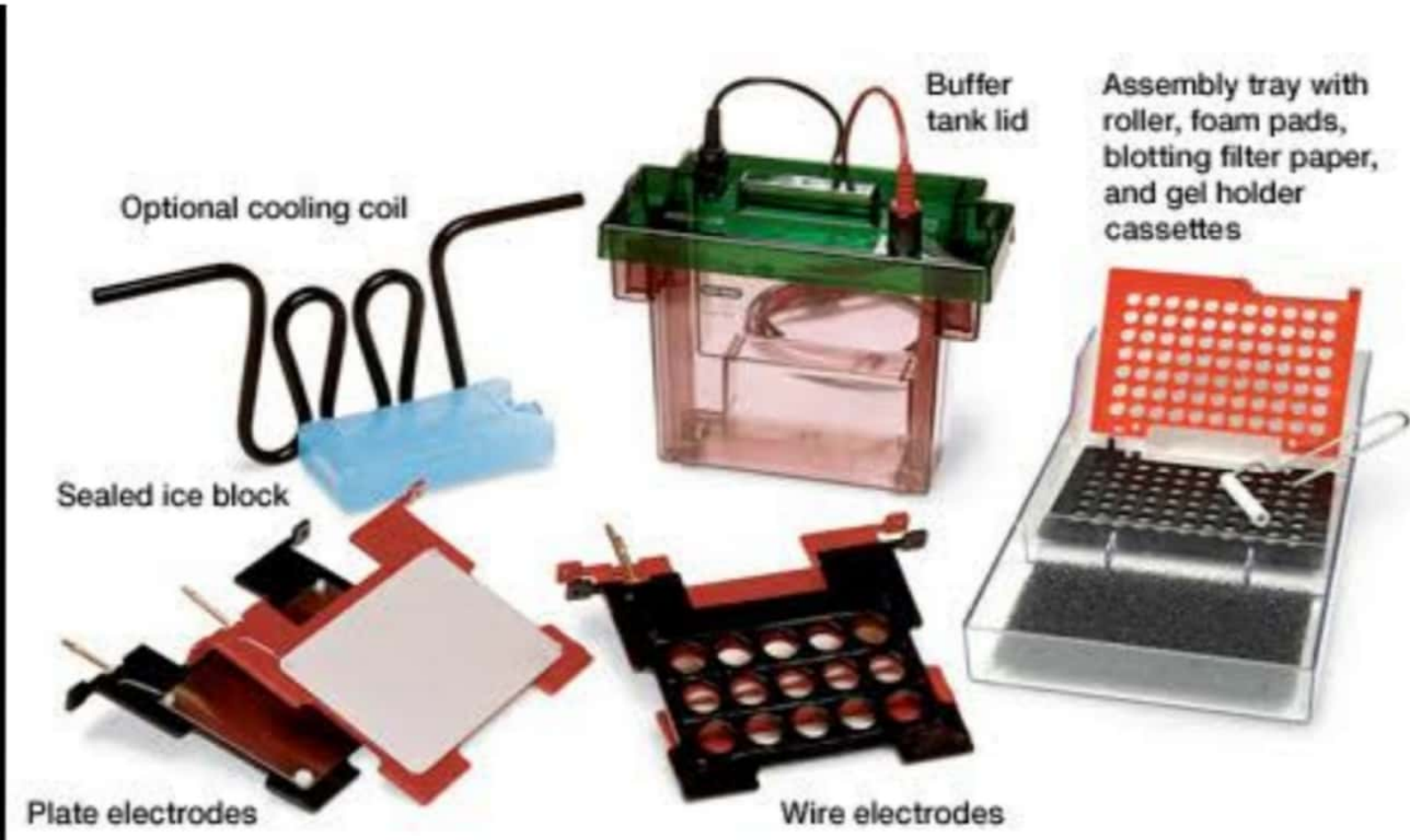
Western transfer buffer

Glycine	14gm
Tris Base	3gm
SDS	0.75gm
Methanol	100 ml

Add distilled H_2O to make it 1 liter.

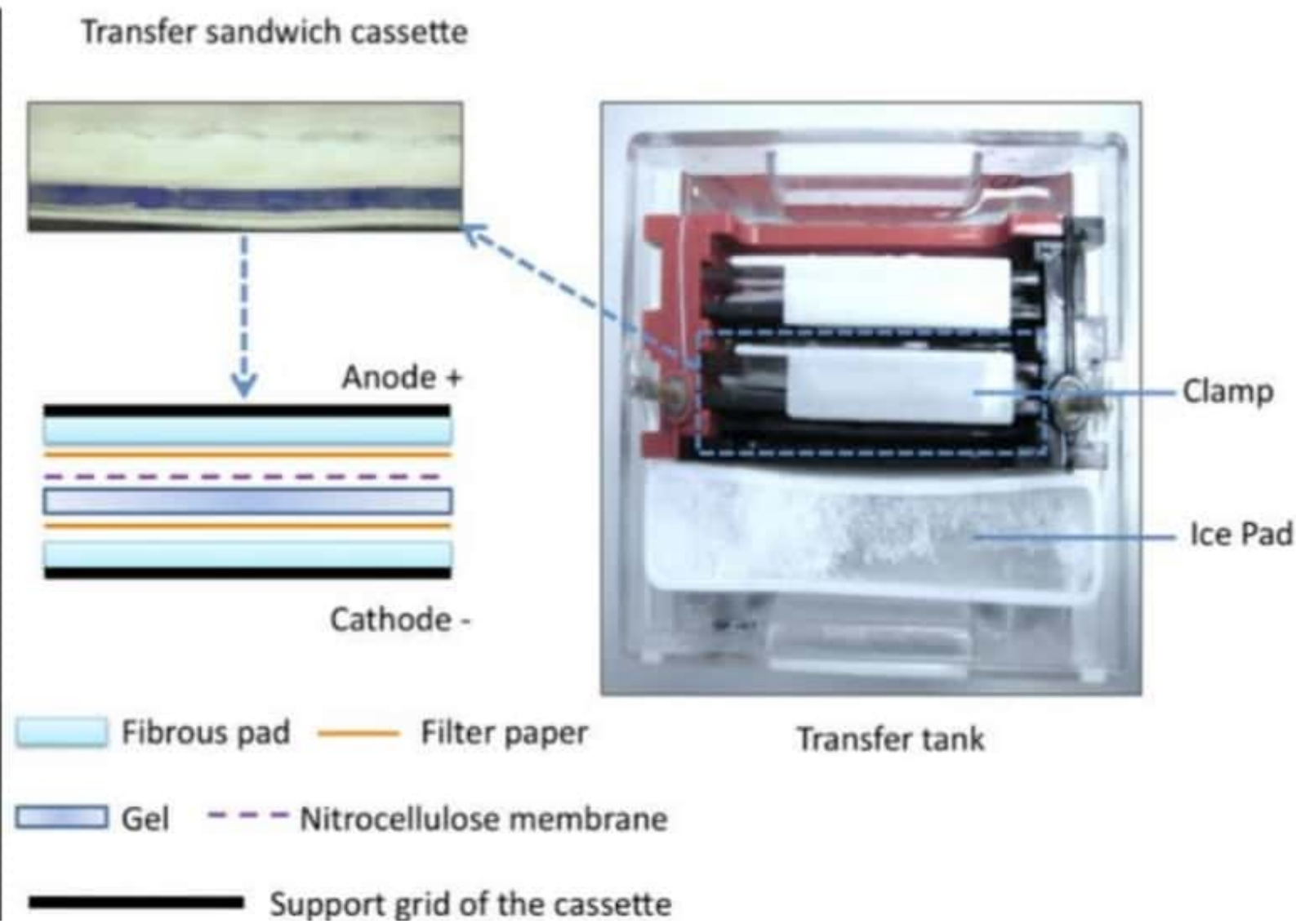
Protocol for Western blotting

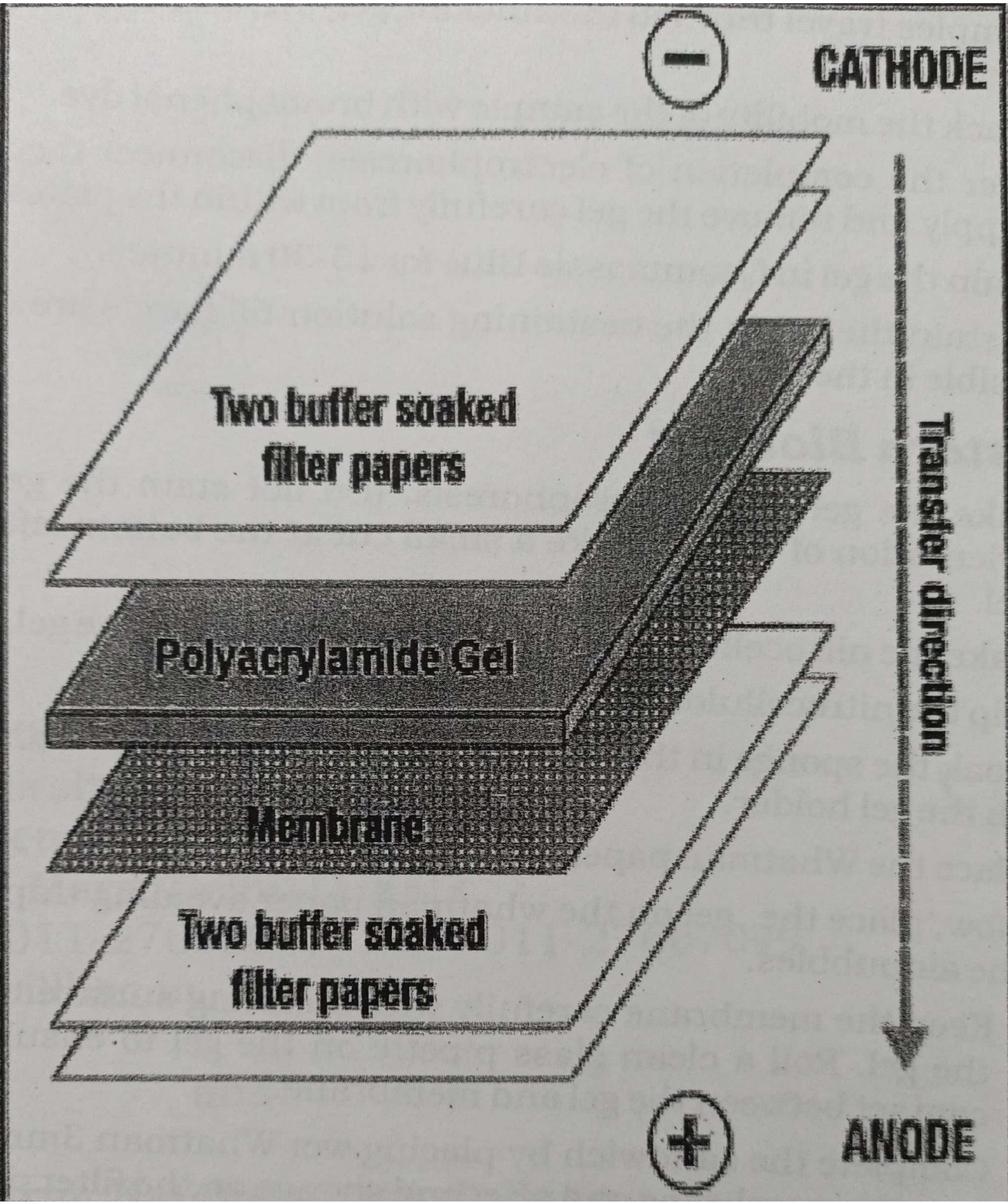
1. Take the gel after electrophoresis. (Donot stain the gel). For orientation of the gel make a small cut at the bottom left of gel.
2. Take the nitrocellulose sheet and cut to the size of the gel.
3. Dip the nitrocellulose sheet into the transfer buffer.
4. Soak the sponge in the transfer buffer and place the wet sponge on the gel holder.
5. Place the whatman paper on the sponge.
6. Now, place the gel on the whatman paper avoiding trapping of the air bubbles.
7. Keep the membrane carefully with shinning surface towards the gel. Roll a clean glass pipette on the gel to ensure good contact between the gel and membrane.
8. Complete the sandwich by placing wet whatman 3mm paper over the membrane and a second sponge on the filter paper
9. Place the assembly in a transfer tank containing sufficient transfer buffer to completely cover the blot
10. Put the assembly in the case with the gel facing the cathode and the membrane towards the anode.
11. Connect the apparatus to the power supply and run for 4 hrs. at 36 volts.



Criterion blotter.

Mini Trans-Blot cell.





12. Lift the membrane the gel once the transfer is complete.
13. Check the uniformity and overall effectiveness of transfer of protein from the gel to the membrane by staining the membrane with staining solution like - Ponceau stain.

Discussion

In order to make proteins accessible to antibody detection, they are moved from the gel onto a nitrocellulose membrane. Protein binding is based upon the hydrophobic interactions, as well as the charged interactions between the membrane and proteins. Once the the protein get transfered they can be detected by immunocytochemical or radioactive or Chemiluminiscent probes. Prior to that, they are stained with Ponceau S staining solution so as to confirm the success of transfer of the proteins from the gel onto the membrane.

Applications of Western Blotting - Medical Diagnostics { Genetic disorders. Infective diseases.

The Confirmatory test for viral infection such as HIV, Coronavirus etc. employs a western blot to detect antiviral antibodies in human serum sample. Proteins from known virally infected cells are separated and blotted on a membrane. Then, the serum to be tested is applied in the primary antibody incubation step. Free antibody is washed away, and a secondary anti-human antibody linked to an enzyme signal is added. Then stained bands then indicate the proteins to which the patient's serum contains antibody.