Aim 56

Western Blotting of Proteins from SDS-Polyacrylamide Gel

Introduction

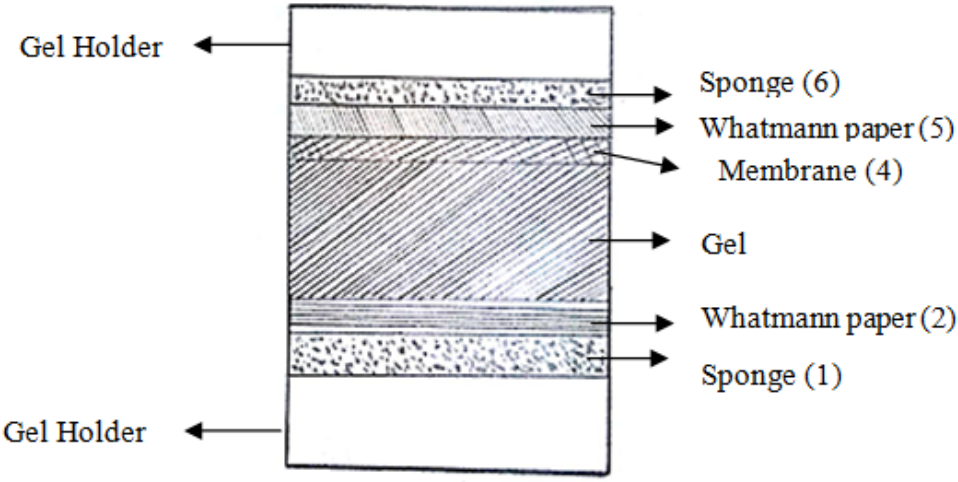
Proteins are those charged particles which have negative charge in the presence of SDS. In the presence of electric field these negatively charged particles moves from cathode to anode. Proteins are taken up by nitrocellulose which is placed on gel and the proteins are immobilized onto sheet. This technique of transferring of protein from gel to nitrocellulose membrane is western blotting.

Requirements

1. SDS –polyacrylamide slab gel
2. Whatman's 3 mm filter paper
3. Western blot apparatus with power pack
4. Transfer buffer
   a. Glycine 14.7 g
   b. Tris 3.0 g
   c. Methanol 200 ml
   d. pH 8.3
   e. Final volume 1 L
5. Nitro cellulose sheet

Procedure

1. Put the gel in transfer buffer for equilibrium for 30 min.
2. Take nylon membrane/nitrocellulose membrane and by cutting make it to size equal to gel. Now dip it in transfer buffer for 30 min.
3. Now do the packing in the order shown in diagram.



a. Firstly, soak the sponge in transfer buffer and put it on gel holder.
b. Now take Whatman's paper and cut it to the size of the gel, dip it in transfer buffer and keep it on the sponge.
c. Now on Whatman's paper, place the gel. Care should be taken to avoid entrapping of air bubble
d. Next step is to place the nitrocellulose membrane on to the gel with shining side towards the gel, to remove the air bubble, between the membrane and gel use sterile pipette. Place the Whatman's filter paper over the membrane and on top wet sponge is set.

4. The gel holder is closed and put it in the transfer tank.
5. Add transfer buffer in sufficient quantity to dip the prepared packing.
6. Western blot apparatus is connected to power supply and run it at 60 V for 3 – 4 hours.

7. Disconnect the power supply and membrane is taken out from packing gently.
8. The gel is stained with coomassie brilliant blue stain after sometime destain it. Now see the pattern of blue bands.
9. An unstained membrane can be treated with the antibodies against this particular protein to check the presence of that protein. When it is treated with secondary antibodies which are conjugated with enzyme, then it converts the chromogenic substrate into colourful product. This coloured reaction helps in the identification of desired protein.

Precautions

1. During handling of the nitrocellulose sheet glove should be worn.
2. During packing avoid the entrapment of air bubble.