Indian Journal of Experimental Biology
Volume 31, March 1993, pp. 301-302

A simple and inexpensive "Western-blotting" apparatus

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Received 29 July 1992

A simple and inexpensive "Western-blotting" apparatus consisting essentially of two rectangular (8 × 20 × 0.8 cm) graphite plates acting as anode and cathode, respectively, is described for efficient semi-dry transfer of polypeptides from SDS-polyacrylamide gels to nitrocellulose membrane.

Transfer of proteins/polypeptides after polyacrylamide gel electrophoresis (PAGE) to nitrocellulose or other membranes is being increasingly used in various cell and molecular biological studies. This "Western-blotting"[1] of polypeptides separated by SDS-PAGE is most commonly done by electrophoretic transfer from gel to membrane. A variety of apparatus for electrophoretic transfer of proteins from gel to the membrane are commercially available but these are rather expensive. We describe here a very simple apparatus for semi-dry transfer of proteins to nitrocellulose membrane after PAGE which can be easily fabricated in any laboratory. Our "Western-blot" apparatus consists essentially of two rectangular (8 × 20 × 0.8 cm) graphite plates each provided with a banana plug (female) at one end (Fig. 1) for connecting wire leads to power supply. The graphite plates were obtained from manufacturers of carbonbrushes in the local market and their surfaces made flat and smooth by rubbing with a fine sand paper in laboratory. The banana plug was fixed to a brass strip which in turn was riveted to the graphite plate in workshop. The rivets were coated with araldite for permanency and insulation. The outer surfaces and all four edges of both the graphite plates were covered with a plastic tape to prevent leakage of current. An acrylic mold to hold the anode plate was prepared by fixing (with chloroform) two parallel strips (2 × 20 cm) of acrylic 8.1 cm apart along the edges of the two long sides of a rectangular acrylic plate (12.1 × 20 cm). To assemble the blotting apparatus, one of the graphite electrodes (anode) is slid into the 8.1 cm slot of this mold with the exposed carbon surface facing up. A stack of 6 Whatman 1 mm papers is placed on the anode, followed by the nitrocellulose membrane, the gel and another stack of 6 Whatman 1 mm papers in that

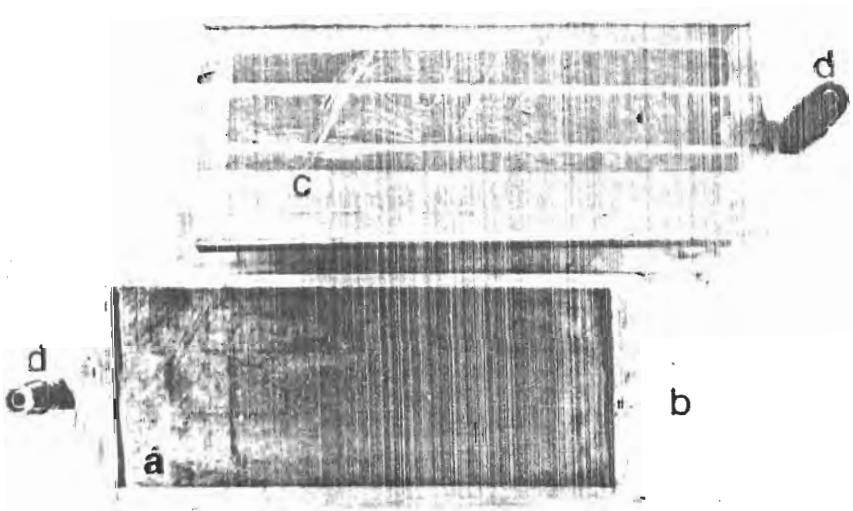

Fig. 1 Photograph of the "Western-blotting" apparatus : the anode (a) slides into the acrylic mold (b); the cathode (c) is placed on the anode with sandwich of filter papers, nitrocellulose membrane, gel etc in between (not shown). A female banana plug (d) is attached through a brass strip on one end of each electrode. The anode is shown partly pulled out of the mold with its electrode face up while the cathode is shown with insulation tape coated face up

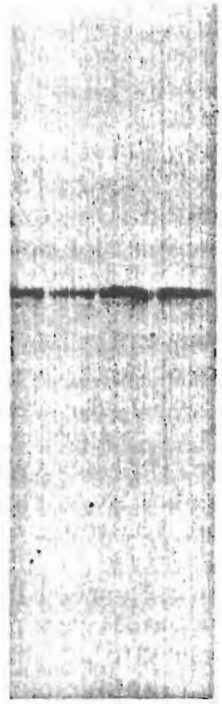

Fig. 2—A typical western blot of proteins from different samples of Malpighian tubules of late third instar larvae of *Drosophila melanogaster* separated by SDS-PAGE, transferred to nitrocellulose membrane using the apparatus described in the text and probed with an antibody that recognizes the 58 kd heat shock polypeptide (homologous to the groEL protein of *E. coli*, details to be published elsewhere)

order. The dimensions of Whatman papers and the nitrocellulose sheet are determined by size of the gel. Finally, the cathode is aligned on top with its exposed carbon surface facing the Whatman paper. A weight of about 500-1000g (e.g., a bottle) is placed over the whole assembly to ensure uniform contact. A strip of cling-film (or Saranwrap) is wrapped around all sides of the electrode assembly so that an air-sealed chamber is created between the anode and the cathode. The banana plug leads are connected to any electrophoresis power supply that can supply 0.8 mamp/cm^2 of the membrane.

For blotting, the filter papers, the nitrocellulose sheet and the gel (immediately after the electrophoretic run) are briefly soaked in a buffer[2] containing 39 m*M* glycine, 48 m*M* Tris base, 0.037% SDS and 20% methanol, *p*H 8.3 and assembled as stated above. The assembly is placed in a refrigerator with the electrode wire leads connected to power supply kept outside. Desired current (0.8 mamp/cm^2) is applied for 2.5 to 3 hr. Sealing the apparatus with plastic film prevents drying of filter papers while keeping in refrigerator during electro-blotting ensures adequate cooling. Using this inexpensive apparatus, we routinely get >60% transfer of proteins from SDS-polyacrylamide (12.5%) gels onto the nitrocellulose membrane in 3 hr. A typical example of a "Western-blot" using this apparatus is shown in Fig. 2.

Thanks are due to Mr. Jagat Narayan Singh, for his skillful technical help in fabricating the "Western-blotting" apparatus.

References

1 Burnette, W H, *Anal Biochem*, 112 (1981) 195.

2 Sambrook J, Fritsch E F & Maniatis T, *Molecular cloning*, 2nd edn (Cold Spring Harbor Laboratory Press, New York) 1989, 18.3.