F. Sanchez-Madrid, April 1983

IMMUNOBLOTTING ON NITROCELLULOSE PAPER

GENERAL:

This method allows the identification of polypeptides bearing an antigenic determinant recognized by MAb or polyclonal antiserum in a complex mixture of proteins without requirement of antigen radioactive labeling. It is also useful for the localization of the polypeptide subunit that possesses the antigenic determinant in a multimeric protein structure.

The technique followed is the one described by Towbin et al., PNAS (1979), 76: 4350-4354.

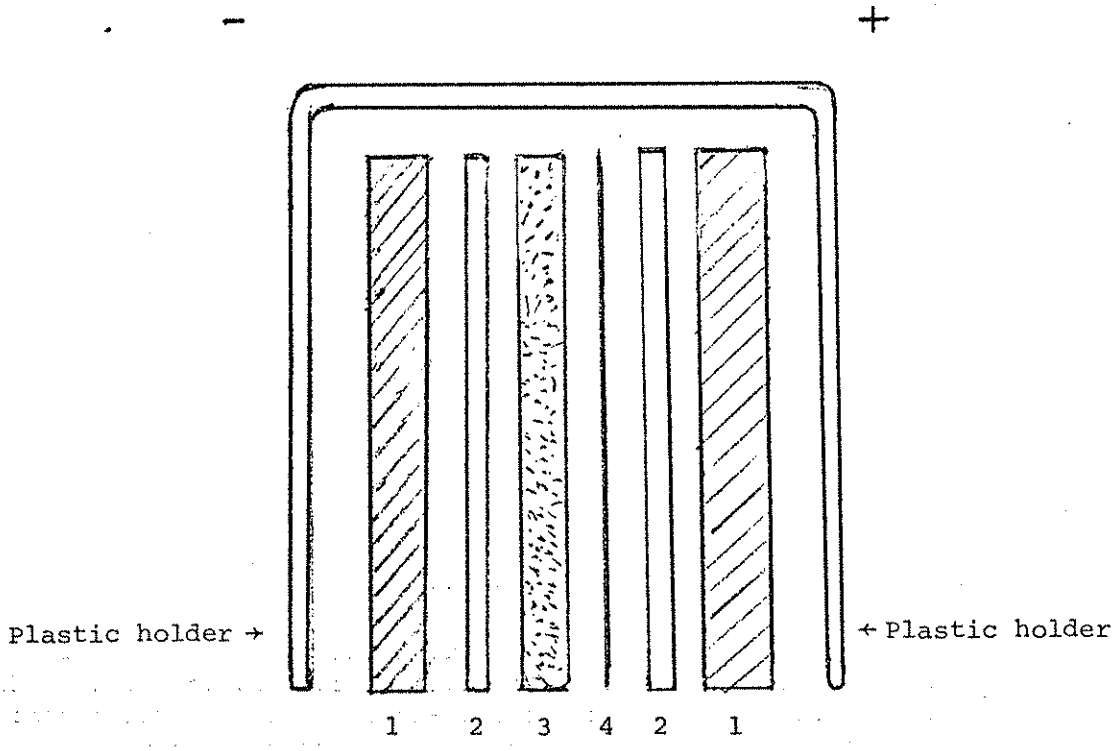
Unlabeled cell lysates or immunoabsorbent purified antigens are subjected to SDS-PAGE to separate by M_r and transferred to nitrocellulose paper to obtain a replica with identical profile of polypeptide bands as in the gel. Then the paper is incubated with the antibody(ies) (MAb or polyclonal antisera) and ^{125}I-labeled second antibody (rat anti-mouse Kappa or mouse anti-rat Kappa MAb) or ^{125}I-protein A. Autoradiography of the dried nitrocellulose paper detects the M_r of the polypeptide band which bear the antigenic determinant recognized by the antibody used.

METHOD:

Transfer: Unlabeled cell lysates (40 µl per well of 5 x 10^7 cells/ml of lysis buffer) or immunoabsorbent purified antigens are separated by SDS-PAGE. The concentration of polyacrylamide used depends on the estimated M_r of the molecules studied. For antigens such as LFA-1 and Mac-1, 8% PAGE; for human LFA-2, 10% or 12% PAGE can be used. Immunoprecipitates containing labeled ^{125}I-antigens or radioactive labeled standard proteins are included and run side by side as positive controls for transfer and to localize M_r positions in immunoblots. The nitrocellulose should be cut exactly to the size of the gel and wet in distilled water for 10-15 min. before use. The nitrocellulose paper should be tightly adhered to the gel to prevent air bubble formation. To facilitate this adherence, the nitrocellulose paper is pressed against the gel while rolling a clean glass tube over the nitrocellulose paper. A schematic drawing of such a transfer sandwich is illustrated below.

The proteins were transferred at room temperature to nitrocellulose membranes (Millipore) in an Electroblot apparatus (BioRad) with water circulator cooling. The time and voltage of transfer should be determined empirically using transfer of the ^{125}I-labeled antigen to be studied. For proteins lower than 95,000 M_r separated in 1.6 mm thick gels 4-5 hours at 200 mA is enough. For proteins of 180,000 or 200,000 M_r overnight transfer ($\simeq$ 15 h) is required. Of course, the polyacrylamide concentration and thickness of the gel also affect transfer time. After transfer, the nitrocellulose blots are then saturated with 3% BSA; 1% HoS in phosphate buffer (PBS) plus 0.02% (w/v) sodium azide, at either room temperature overnight or 37 ^{0}C for one hour.

Immunodetection: Blots are cut into strips and incubated with 5 ml of 1:10 to 1:100 diluted MAb or 1:1000 to 1:10,000 dilutions of polyclonal antisera in plastic bags. Blots are incubated at 4 °C or room temperature for 2 to 5 hours with gentle shaking, then washed for 30 min. with six changes of PBS, 1% BSA, 0.3% HoS. Strips are then shaken, without sticking to one another, at room temperature for 2 hours with ^{125}I-MAb anti-rat or mouse Kappa chain (0.8-1 x 10^5 cpm/ml) in 10 to 100 ml of 3% BSA in PBS, and washed for 30 min. with four changes of PBS, 1% NBSA, 0.03% HoS and two changes of PBS. The blots are dried with a hair-dryer and autoradiographed with intensifying screens.



1. Scotch Brite Pad
2. Whatman paper 3MM
3. Gel
4. Nitrocellulose Paper