Immunoblotting and dot blotting

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David Ian Stott
Department of Bacteriology and Immunology, University of Glasgow, Western Infirmary, Glasgow Gll 6NT, Scotland, U.K.

Introduction

A variety of methods has been available for many years for the analytical separation of mixtures of proteins into their component parts by electrophoresis in a gel, usually agarose or polyacrylamide. Popular techniques are: zonal electrophoresis in agarose gel or on cellulose acetate membranes; discontinuous electrophoresis in polyacrylamide gel (PAGE); SDS-polyacrylamide gel electrophoresis (SDS-PAGE); isoelectric focusing (IEF); and two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), which is capable of resolving complex mixtures of proteins containing hundreds or even thousands of components. All of these methods require some means of identifying particular proteins of interest. In some cases it is possible to do this simply on the basis of mobility, molecular weight (MW) (SDS-PAGE) or by using selective stains, e.g., for enzyme activity. These methods of identification are severely limited in applicability and, for this reason, antibodies have been used as highly specific probes for electrophoretically separated proteins, ever since the invention of immunoelectrophoresis. Direct overlay of the gel with antibody (immunofixation) has also been used to identify antigens of interest but such methods suffer from the disadvantages of prolonged incubation times resulting in diffusion of the bands and consequent loss of resolution. Immunofixation is also primarily limited to agarose systems since antibody molecules cannot readily penetrate polyacrylamide gels owing to their small pore size.

Keywords: Immunoblotting Dot blotting Slot blotting Dot immunobinding Electrophoresis Western Blotting (Methods)(Review)

Reference:

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