

Video Article

Immunoblot Analysis

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Abstract

Immunoblotting (western blotting) is a rapid and sensitive assay for the detection and characterization of proteins that works by exploiting the specificity inherent in antigen-antibody recognition. It involves the solubilization and electrophoretic separation of proteins, glycoproteins, or lipopolysaccharides by gel electrophoresis, followed by quantitative transfer and irreversible binding to nitrocellulose, PVDF, or nylon. The immunoblotting technique has been useful in identifying specific antigens recognized by polyclonal or monoclonal antibodies and is highly sensitive (1 ng of antigen can be detected). This unit provides protocols for protein separation, blotting proteins onto membranes, immunoprobing, and visualization using chromogenic or chemiluminescent substrates.

Protocol

The complete text protocol for this experimental approach is available in Current Protocols in Molecular Biology

References