IMMUNOHISTOCHEMISTRY

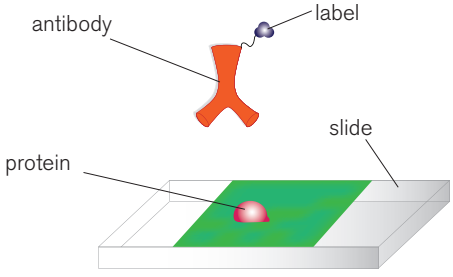
Immunohistochemistry (IHC) is a method of analysing and identifying protein distribution and localization in a tissue sample based on the binding of antibodies to specific cellular targets. The principle of immunohistochemistry has existed since the 1930s, but it was not until 1941 that the first immunohistochemistry study was reported. *Coons et al.* used FITC-labelled antibodies with a fluorescent dye to localize Pneumococcal antigens in infected tissues. With the expansion and development of immunohistochemistry technique, enzyme labels have been introduced such as peroxidase and alkaline phosphatase. The technique is widely used in the analysis of anomalous tissue samples such as tumour biopsies.

For immunohistochemical analysis, it is essential that the morphology of the tissues and cells be retained and that the antigenic sites be accessible. Ideally, it would be preferable to carry out IHC using fresh, rapidly frozen, tissue sections, however, to maintain cell integrity and preserve the cell components, tissue samples are normally fixed by the addition of a chemical compound such as formalin and then embedded into wax. This fixing results in the cross linking of amino acids in the tissue and can disguise the epitope regions thereby inhibiting the action of any protein specific antibodies. Epitope recovery or retrieval is therefore required to expose hidden regions. This is usually carried out by enzymatic digestion or by the use of heat.

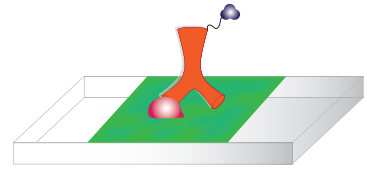
Once tissues have been treated to remove endogenous peroxidase activity and non-specific sites have been blocked, proteins can be detected using the same methods as described for western blotting. This is either directly – using a labelled antibody specific to the protein or indirectly-using an unlabeled primary antibody that has specificity for the protein, followed by the addition of a labelled secondary antibody specific to the primary antibody.

DIRECT

The labelled antibody is specific to the protein



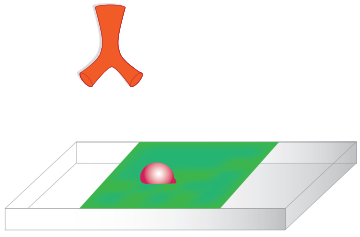
Labelled antibody added to slide



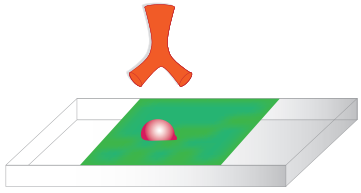
Antibody binds to revealed epitopes on protein in tissue

INDIRECT

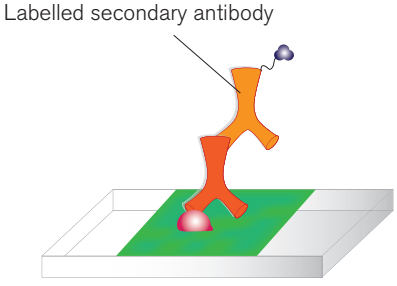
An unlabelled primary antibody is added that has specificity for the protein followed by addition of a labelled secondary antibody specific to the primary antibody.



Unlabelled antibody added to slide



Antibody binds to revealed epitopes on protein in tissue



Labelled secondary antibody binds to primary antibody allowing identification of the protein