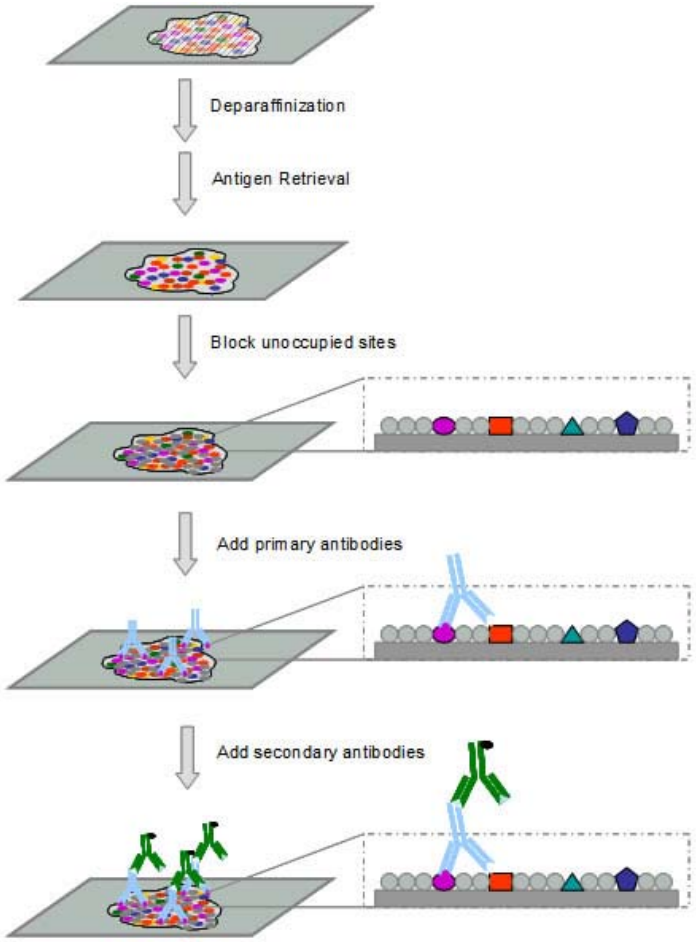
Immunohistochemistry (IHC)

IHC theory overview

In contrast to western blotting and ELISA, immunohistochemistry (IHC) allows the study of the distribution and localization of specific cellular components in cells or tissues. However, IHC still uses labeled antibody to trace antigen-antibody interactions and the antibody can be detected by several methods. Most commonly, a secondary antibody conjugated to an enzyme (horseradish peroxidase or alkaline phosphatase) is used; this binds to the primary antibody and indirectly stains the antigen of interest. A similar technique involving fluorophore-conjugated secondary antibodies is called immunofluorescence.



Procedures

A, Sample preparation Prompt and adequate tissue preparation is crucial in immunohistochemistry. Although there is no single universal method for tissue fixation, many antigens can be successfully detected in formalin-fixed paraffin-embedded tissue sections. Alternatively, sections may be prepared by snap freezing in liquid nitrogen and sectioned with a cryostat, followed by fixation with cold acetone or alcohol. However, there are several disadvantages associated with frozen sections, including poor morphology, poor resolution at higher magnifications, special storage is needed, limited retrospective studies possible and cutting difficulty. Paraffin-embedded sections overcome most of these problems and are the most widely used preparation method.

B, Antigen retrieval Pre-treatment with the antigen retrieval reagent is needed to break the protein cross-links formed by formalin fixation in order to

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uncover hidden antigenic sites. The methods used include the application of heat (HIER: Heat Induced Epitope Retrieval), use of digestive enzymes (PIER: Proteolytic Induced Epitope Retrieval) or a combination of the two. While heat-mediated antigen retrieval is commonly performed by microwave, pressure cooker or autoclave, enzyme-mediated antigen retrieval uses a combination of digestive enzymes such as proteinase K, trypsin, chymotrypsin, pepsin and pronase. However, the use of enzyme digestion could inconsistently destroy some epitopes and tissue morphology, leading to false negative results.

C, Schematic steps in immunohistochemical .

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