Immunofluorescence (IF)

IP theory overview

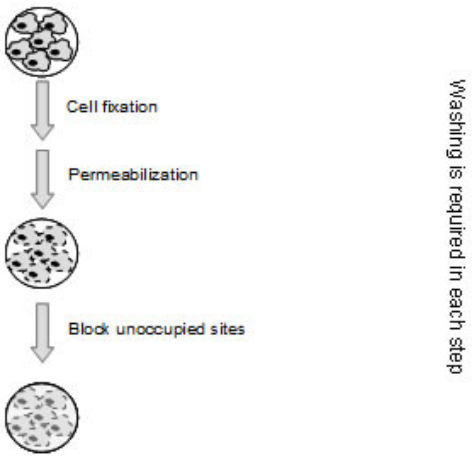
Immunofluorescence uses a fluorophore to locate the specific interactions between antibodies and antigens within tissues or cells. It is similar to immunohistochemistry, in allowing the study of the distribution and localization of specific cellular components in cells or tissues. There are two ways of detecting the antigen, either through direct labeling of the detector antibody or by labeling a secondary antibody (indirect) which specifically recognizes the detector antibody. In most of cases, indirect labeling is favored over direct labeling because it only requires a few sets of fluorophore-conjugated secondary antibodies. However, in some cases, direct labeling is more desirable since it decreases the number of steps in the staining procedure and may reduce cross-reactivity and high background problems.

Sample specimen preparation

Sample preparation includes cell fixation as well as cell permeabilization. Both steps are critical for antibody penetration and binding. There are three main fixation methods: cross-linking, precipitation and cryofixation. Selection of a fixation protocol depends on the size or thickness of a given sample, which limits the use of certain fixatives due to cell permeability. Cross-linking involves treating the samples with reagents such as glutaraldehyde and formaldehyde. These reagents can penetrate into the cells and form covalent cross-links between intracellular components. The precipitation method uses pre-chilled organic solvents such as acetone or methanol to fix samples. While such organic solvents may damage the structural integrity of the samples, precipitation rarely destroys antibody binding sites and has a lower chance of introducing autofluorescence than the cross-linking method. Cryofixation, on the other hand, fixes cells by rapid freezing, followed by treatment with a cross-linking reagent.

Photobleach

The photobleaching of a sample is a problem in fluorescence microscopy. Photobleaching occurs when fluorescent emissions diminish over the time as a result of the fluorophore being covalently modified, losing its ability to fluoresce. Although the exact mechanism of photobleaching is unknown, addition of certain reagents such as N-propyl gallate and p-phenylenediamene (PPD) in mounting media may prolong the lifetime of fluorescently-labeled samples.



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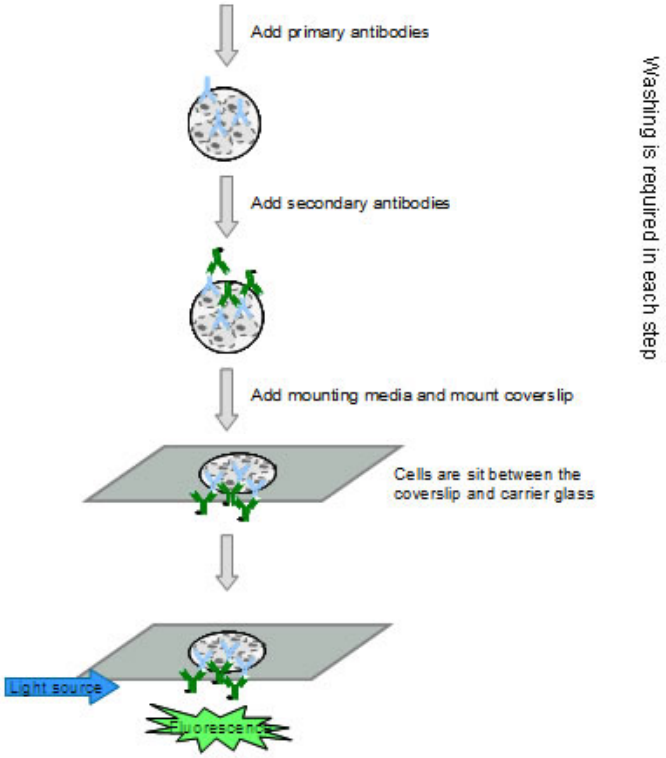

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