Biology | Immunology

Immunofluorescence Assay

General Aim

To fluorescently label a target antigen in a tissue, and visualize it with a fluorescent microscope.

Method

Indirect Immunofluorescence

Learning Objectives (ILOs)

- To understand the basic concepts of the Immunofluorescence Assay.
- To apply indirect Immunofluorescence protocol.
- To learn how to perform cell fixation.
- To practice the 'washing technique' skill.

Theoretical Background/Context

Immunofluorescence is an immunological technique designed to visualize antigens in cellular contexts. This is done by using antigen's specific fluorescently tagged antibody.

There are two types of Immunofluorescence techniques:

1. Direct Immunofluorescence: Only one antibody is used. It's called the primary antibody. It is tagged with a fluorescent stain.
2. Indirect Immunofluorescence: Two antibodies are used. The secondary antibody is the one that is fluorescently stained.

Principle of Work

We use the indirect immunofluorescence staining technique to detect an antigen inside cultured cells. To prepare cells, fixation and cell permeabilization is done. Afterwards, a blocking buffer is added prior to the addition of the primary antibody, which is specific to the antigen to be detected. Washing steps are applied to get rid of the excess unbound antibodies. Secondary antibody is then added to label the antigen fluorescently, so that it can be visualized on a fluorescent microscope.