Products  ›   [Immunofluorescence](http://www.eurodiagnostica.com/index.php?langId=1&amp;headId=3&amp;subId=0&amp;pageId=3&amp;catId=10) Immunofluorescence Immunofluorescence

(IF) is a common laboratory technique, which is based on the use of specific antibodies which have been chemically conjugated to fluorescent dyes.

These labeled antibodies bind directly or indirectly to cellular antigens (see below). The technique has a number of different biological applications including evaluation of cells in suspension, cultured cells, tissue, beads and in microarrays.

The fluorescent dye is subjected to short-wavelength, high energy light, which is absorbed and emitted as light of a different wavelength. The emitted fluorescence has a lower energy than the absorbed light, so the wavelength of the emitted light is longer than that of the excitation light. The fluorescence can be visualized using fluorescence microscopy. The IF technique allows for a visualization of the presence as well as the distribution of target molecules in a sample.

Types of immunofluorescence

There are two classes of immunofluorescence techniques, primary (or direct) and secondary (or indirect).

Primary (direct)

Primary, or direct, immunofluorescence uses a single antibody that is chemically linked to a fluorophore. The antibody recognizes the target molecule and binds to it, and the fluorophore it carries can be detected via microscopy. This technique has several advantages over the secondary (or indirect) protocol below because of the direct conjugation of the antibody to the fluorophore. This reduces the number of steps in the staining procedure making the process faster and can reduce background signal by avoiding some issues with antibody cross-reactivity or non-specificity. However, since the number of fluorescent molecules that can be bound to the primary antibody is limited, direct immunofluorescence is less sensitive than indirect immunofluorescence.

Secondary (indirect)

Secondary, or indirect, immunofluorescence uses two antibodies; the unlabeled first (primary) antibody specifically binds the target molecule, and the secondary antibody, which carries the fluorophore, recognises the primary antibody and binds to it. Multiple secondary antibodies can bind a single primary antibody. This provides signal amplification by increasing the number of fluorophore molecules per antigen. This protocol is more complex and time consuming than the primary (or direct) protocol above, but it allows more flexibility because a variety of different secondary antibodies and detection techniques can be used for a given primary antibody.