Immunocytochemistry

Immunocytochemistry (ICC) is a method for detection and visualization of proteins, or other antigens, in cells using antibodies specifically recognizing the target of interest. The antibody is directly or indirectly linked to a reporter, such as a fluorophore or enzyme. The reporter gives rise to a signal, such as fluorescence or colour from an enzymatic reaction, which can be detected in a microscope. The type of microscope used depends on the type of reporter.

In ICC, the staining technique is applied on cultured cells or individual cells that have been isolated from eg. tissues, blood samples or mouth swabs. This is in contrast to immunohistochemistry (IHC), where cells are analyzed within intact tissue sections.

Discussion on steps in ICC

Immunocytochemistry is usually performed in four sequential steps.

First, the cells are seeded on a solid support, which is usually a glass slide or a glass-bottom plate. Depending on the type of cells and seeding technique, an incubation time might be necessary before proceeding with immunostaining. In case of seeding adherent cells, the cells will attach to the solid support surface during the incubation, which varies from half an hour to 24 h for the different cell types.

In the second step, the cells are subjected to immunostaining, which involves fixation, permeabilization, and antibody incubation.

- Fixation retains the proteins at their location in the cell and preserves their chemical and structural state at the time of fixation.
- It can be done by crosslinking or by precipitating the proteins using organic solvents. E.g. paraformaldehyde.
- Upon permeabilization, membranes are punctured with the use of solvents or detergents, allowing the relatively large antibodies to cross the cellular membranes. E.g. Triton X-100 detergent.
- The permeabilization requires fixation, and hence limits the technique to studying dead cells.
- During antibody incubation, the antibodies are allowed to bind to target antigens within the cells, after which unbound antibodies are removed by washing.

In the third step, the cells and the locations of antibodies bound to target antigens are visualized using microscopy. Images are acquired using a camera or other detector.

In the final step, the images are analyzed and cellular structures annotated.

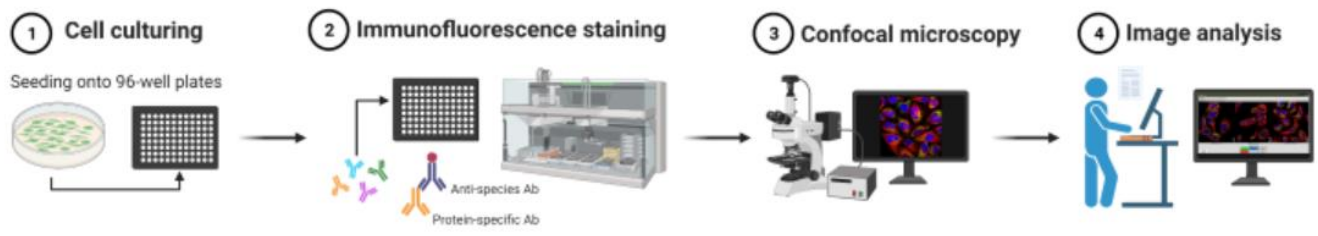

Figure showing work flow in typical ICC using fluorescence reporter

Reporters used in the ICC:

There are different reporter systems available for ICC.

One is the use of enzyme-coupled antibodies. After the addition of a substrate, the enzyme catalyzes a reaction that generates a coloured product at the site where the enzyme-coupled antibody is bound in the cells. For example, the commonly used enzyme horseradish peroxidase (HRP) can convert 3,3'-diaminobenzidine (DAB) into a brown precipitate, which can be detected using light-microscopy.

Another type of reporter is fluorophores. These molecules can be transiently excited to a higher energy state upon absorption of light with a particular wavelength, and thereafter relax to the ground state while emitting light of a longer wavelength.

In this case, a fluorescence microscope is used to excite the fluorophores and to detect their emission. Since different fluorophores are excited by different wavelengths of light and also emit light at different wavelengths, multiple fluorophores with different colours may be combined in the same sample. This enables the acquisition of multicolour images, where each colour represents a specific antigen target. However, the number of fluorophores used in the same sample is limited by the spectral overlap of the excitation and emission profiles of the fluorophores, as the signals from fluorophores with similar spectral properties cannot readily be separated.

In addition to fluorophore-labelled antibodies, there are molecules that are fluorescent by themselves and have an intrinsic ability to bind specifically to other molecules. These molecules may be used together with the fluorophore-labelled antibodies.

One example is 4',6-diamidino-2-phenylindole (DAPI), which binds to DNA and is commonly used to visualize the cell nucleus. DAPI is excited by ultraviolet light and then emits light in the blue spectrum.

Note: When using fluorophores as reporters bleaching will occur when the fluorophores are exposed to light. This causes the brightness of the sample to decrease over time.

Direct vs Indirect ICC:

The detection method for the immunostaining can be either direct or indirect. In the direct method, the molecule of interest is directly targeted by a primary antibody linked to the reporter, giving a rapid and specific method.

However, it is usually not sensitive enough for most proteins as the number of present copies of the protein is too low to yield a strong enough signal. In the indirect method, the molecule of interest is targeted by an un-labelled primary antibody, which is in turn detected using a reporter-coupled secondary antibody that recognizes the primary antibody.

The indirect method is more sensitive due to binding of multiple secondary antibodies to each primary antibody, resulting in signal amplification.

Another advantage is also an increased flexibility because of the possibility to vary the primary and secondary antibody combination. Also, since the secondary antibody is targeting the constant region of the primary antibody, which is species-specific, the same secondary antibody can be used for all primary antibodies raised in a given species.

The disadvantages of the indirect method are the necessity of a more laborious and time-consuming protocol, and the risk of non-specific binding of the secondary antibody.