Immunofluorescence (IF) Protocols

-Instruction

Immunofluorescence is a technology that combines both reactions between antibody with antigen and fluorescence imaging to directly observe target protein cell or tissue. This process reveals the localization and relative expression of the target protein by providing image and data at the same time.

-Solution and reagents

4% formaldehyde

0.2% TritonX-100

10% Normal goat serum

Primary antibody

Secondary-fluorescence antibody

DAPI

PBS

-Sample preparation

Preparation for sample of cell

A fluorescence microscope is used to detect the fluorescence signal. To compatible with the device, cells need to be cultured on a support material. Typical support formats include glass-bottom cell culture dishes and glass coverslips. Besides, cell density should not be too large and individual dispersion is appropriate.

Preparation for sample of tissue

Paraffin sections require dewaxing, hydration, and antigen retrieval steps (refer to IHC protocol) before incubation with antibodies for a fluorescence detection.

Frozen section should be balanced at RT for 10-20min at first. And then, the sections need to be fixed for 10-15min by 4% formaldehyde. Permeabilization performs by 0.2% TritonX-100 for 20min as well as sample of cell.

-Fixation and permeabilization

The fixation and permeabilization of samples are key steps that determine the success of your experiments. Aldehyde-based fixatives (such as formaldehyde, formalin and glutaraldehyde) and dehydrating fixatives (such as ethanol) are often used as a fixative. Aldehyde-based fixatives, which cross

the membrane of cells, can also crosslink with cellular proteins, stabilizing and hardening the sample. Compared to the dehydrating fixatives, the aldehyde-based fixatives work better. So we recommend 4% formaldehyde for cell fixation. In order to make antibodies access the target proteins, permeabilization needs to be done. Detergents are often used as a permeabilization reagent. TritonX-100 is recommended. It can destruct membrane structure, and create many pores on cell membrane. The pores provide a passageway for antibodies to get access to the target proteins. Aldehydes cross the plasma membrane and fix soluble proteins better than alcohols, but some targets can lose their antigenicity with aldehyde crosslinking.

1. Wash cells with PBS for three times.
2. Fix the cells with 4% formaldehyde for 10-15min at RT
3. Wash cells with PBS for three times.
4. Permeabilize the cells with 1% Triton X-100 for 20min at RT.
5. Wash cells with PBS for three times.

-Blocking

This process is to reduce the non-specific binding between antibodies and other proteins which are not specific to the antibody. The blocking solution that we often use is the normal goat serum (the species must be consistent with the secondary antibody). The serums can not only block the non-specific binding proteins, but also block the FC receptor in the cells or tissues.

1. Blocking with 10% normal goat serum for 1h at RT.
2. Wipe off the excess liquid and keep the sample moist.

-Antibody incubation

1. Prepare primary antibody solution by referring to the recommend dilution ratio, and keep the sample completely covered in primary antibody solution.
2. Incubate primary antibody at 4℃ overnight.
3. Wash cells with PBS for three times.
4. Prepare secondary antibody solution by referring to the recommend dilution ratio in dark, and keep the sample completely covered in primary antibody solution.
5. Incubate secondary antibody for 1h at RT.
6. Wash cells with PBS for three times.

-Nuclear staining

DAPI is often used as a nuclear reagent to make the cell structure more clear. Dilute the DAPI reagent according to the instructions and incubate with the sample for a proper time. Dyeing time should not be too long.

-Detection

Keep the sample in dark and detect the fluorescence signal as soon as possible by