

Fixation

Fixation should immobilize antigens while retaining cellular and subcellular structure. It should also allow for access of antibodies to all cells and subcellular compartments. The fixation and permeabilization method used will depend on the sensitivity of the epitope and the antibodies themselves, and may require some optimization.

Fixation can be done using crosslinking reagents, such as paraformaldehyde. These are better at preserving cell structure, but may reduce the antigenicity of some cell components as the crosslinking can obstruct antibody binding. For this reason, antigen retrieval techniques may be required, particularly if there is a long fixation incubation or if a high percentage of crosslinking fixative is used. Another option is to use organic solvents. These remove lipids while dehydrating the cells. They also precipitate proteins on the cellular architecture.

1. 4% Paraformaldehyde

Add 4% paraformaldehyde to slides for 10 mins only.
Wash with PBS or PBS with 1% BSA.

Note: Fixing in paraformaldehyde for more than 10-15 mins will cross link the proteins to the point where antigen retrieval may be required to ensure the antibody has free access to bind and detect the protein.

2. Ethanol

Add 100-200 µl per slide of cooled 95% ethanol, 5% glacial acetic acid for 5-10 mins.
Wash with PBS or PBS with 1% BSA.

3. Methanol

Add 100-200 µl per slide of ice cold methanol.
Place at -20°C for 10 min.
Wash with PBS or PBS with 1% BSA.

Note: Methanol will also permeabilize. Some epitopes are very sensitive to methanol as it can disrupt epitope structure. Can try acetone instead for permeabilization if required.

4. Acetone

Add 100-200 µl per slide ice cold acetone.
Place at -20°C for 5 to 10 min.
Wash with PBS or PBS with 1% BSA.

Note: acetone will also permeabilize. Consequently, no further permeabilization step is required.

Permeabilization

Permeabilization is only required when the antibody needs access to the inside of the cells to detect the protein. These include intracellular proteins and transmembrane proteins whose epitopes are in the cytoplasmic region.

Solvents:

1. Acetone fixation will also permeabilize.
2. Methanol fixation can be used to permeabilize but is not always suitable.

These reagents can be used to fix and permeabilize, or can be used after fixation with a crosslinking agent such as paraformaldehyde.

Detergents:

1. Triton or NP-40
Use 0.1 to 0.2% in PBS for 10 min only.

These will also partially dissolve the nuclear membrane and are therefore very suitable for nuclear antigen staining.

Note: as these are harsh detergents, they will disrupt proteins when used at higher concentrations or for longer amounts of time, affecting staining results.

2. Tween 20, Saponin, Digitonin and Leucoperm
Use 0.2 to 0.5% for 10 to 30 min.

These are much milder membrane solubilizers. They will give large enough pores for antibodies to go through without dissolving the plasma membrane. They are suitable for antigens in the cytoplasm or the cytoplasmic face of the plasma membrane and for soluble nuclear antigens.

Special recommendations:

Cytoskeletal, viral and some enzyme antigens usually give optimal results when fixed with acetone, ethanol or formaldehyde (high concentration).

Antigens in cytoplasmic organelles and granules will require a fixation and permeabilization method depending on the antigen. The epitope needs to remain accessible.