

FIXATION AND PERMEABILIZATION IN IHC/ICC

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 permeabilisation method used will depend on the sensitivity of the epitope and antibody 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 will 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 minutes only.
Rinse with PBS or PBS 1% BSA

Note: Fixing in paraformaldehyde for more than 10-15 minutes 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 100-200ul per slide of cooled 95% ethanol, 5% glacial acetic acid for 5-10 minutes. Wash with PBS or PBS 1% BSA

3. Methanol

Add 100-200ul per slide of ice cold methanol.
Place at -20oC for 10 minutes.
Wash with PBS or PBS 1% BSA

Note: methanol will also permeabilize, but not in all cases as some epitopes are very sensitive to this. Can try acetone instead for permeabilization if required.

4. Acetone

Add 100-200ul per slide ice cold acetone. Place at -20oC for 5 to 10 minutes.
Wash with PBS or PBS 1% BSA

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

PERMEABILIZATION

Permeabilization should only be required for intracellular epitopes when the antibody required access to the inside of the cell to detect the protein. However, it will also be required for detection of transmembrane membrane proteins if the epitope is 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 to permeabilize the cells.

Detergents:

1. Triton or NP-40

Use 0.1 to 0.2% in PBS, 10 minutes 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 if they are used at higher concentrations or for longer amounts of time which will affect staining results.

2. Tween 20, Saponin, Digitonin and Leucoperm

Use 0.2 to 0.5% for 10 to 30 minutes.

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

SPECIAL RECOMMENDATIONS:

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

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