

Flow cytometry intracellular staining protocol

Detecting intracellular proteins with flow
cytometry

Fixing and permeabilization

Fix cells before intracellular staining to ensure stability of soluble antigens or antigens with a short half-life (see the special recommendations below for exceptions). This retains the target protein in the original cellular location.

Detecting intracellular antigens requires cell permeabilization before staining. Antibodies should be prepared in permeabilization buffer to ensure the cells remain permeable. When gating on cell populations, the light scatter profiles of the cells on the flow cytometer will change considerably after permeabilization. NB cell surface staining should be performed prior to fixation.

Several methods are available for cell fixation and permeabilization:

Formaldehyde followed by detergent

Fix in 0.01% formaldehyde for 10–15 min, then disrupt membranes using one of the following detergents:

- Triton or NP-40 (0.1–1% in PBS) partially dissolve the nuclear membrane so are suitable for nuclear antigen staining. Loss of cell membrane and cytoplasm will result in decreased light scattering and reduced non-specific fluorescence.
- Tween 20, Saponin, Digitonin and Leucoperm (0.5% v/v in PBS) enable antibodies to go through pores without dissolving plasma membrane. They are suitable for antigens in the cytoplasm or the cytoplasmic face of the plasma membrane and soluble nuclear antigens.

Formaldehyde (0.01%) followed by methanol

Methanol followed by detergent

- Add 1 mL ice cold methanol to each sample
- Mix gently and place at -20°C for 10 min
- Centrifuge and wash twice in PBS 1% BSA

Acetone fixation and permeabilization

- Add 1 mL ice cold acetone to each sample
- Mix gently and place at -20°C for 5–10 min
- Centrifuge and wash twice in PBS 1% BSA

Plastic tubes are not suitable for use with acetone.

Special recommendations

Antigens close to the plasma membrane and soluble cytoplasmic antigens will require mild cell permeabilization without fixation.

Cytoskeletal, viral and some enzyme antigens usually give optimal results when fixed with a high concentration of acetone, alcohol or formaldehyde.

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

Intracellular staining procedure

1. Fix tissue according to the instructions above
2. Add 100 μ L detergent-based permeabilizing agent and incubate in the dark at room temperature for 15 min
3. Wash the cells with 2 mL of PBS (containing 0.1% triton or other permeabilizing detergent), centrifuge at 300 x *g* (2,000 rpm) for 5 min, discard supernatant and resuspend the pellet in the remaining volume
4. Follow antibody staining procedure in our direct and indirect protocols

Prepare antibodies in permeabilization buffer to ensure the cells remain permeable.

Detection of secreted proteins

Detecting secreted proteins can be challenging because they may degrade rapidly. Use Brefeldin A or other compounds that prevent protein release from the Golgi apparatus, enabling the detection of cells expressing the protein.