

INTRACELLULAR STAINING

FIXING AND PERMEABILISATION:

For intracellular staining, cells can be fixed first to ensure stability of soluble antigens or antigens with short half life (see below for important exceptions). This should retain the target protein in the original cellular location.

Detection of intracellular antigens requires a cell permeabilization step prior to staining. Antibodies should be prepared in permeabilisation 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.

N/B Cell surface staining should be performed prior to fixation.

There are several methods available:

1. Formaldehyde followed by detergent:

Fixation in 0.01% formaldehyde for 10-15 minutes (this will stabilise proteins), followed by disruption of membrane by detergent.

Detergents:

Triton or NP-40 (0.1 to 1% in PBS). These will also partially dissolve the nuclear membrane and are therefore very suitable for nuclear antigen staining. It should be noted that loss of cell membrane and cytoplasm will result in decreased light scattering and also in reduced non-specific fluorescence.

Tween 20, Saponin, Digitonin and Leucoperm are mild membrane solubilisers. Use at 0.5% in PBS. These 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.

2. Formaldehyde (0.01%) followed by methanol (SEE 3)

3. Methanol followed by detergent.

Add 1ml ice cold methanol to each sample.
Mix gently. Place at -20°C for 10 minutes.
Centrifuge, wash twice in PBS 1% BSA

4. Acetone fixation and permeabilisation:

Add 1ml ice cold acetone to each sample.
Mix gently. Place at -20°C for 5 to 10 minutes.
Centrifuge, wash twice in PBS 1% BSA

N/B Polystyrene/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 permeabilisation without fixation.

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

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

INTRACELLULAR STAINING PROCEDURE;

1. Add 100ul of fixative. Incubate for 10 minutes at required temperature (see above).
2. Add 100µl detergent based permeabilising agent and incubate in the dark at room temperature for 15 minutes.
3. Wash the cells by adding 2ml of PBS (containing 0.1% triton or other permeabilising detergent), centrifuge at 300g (2000 rpm) for 5 minutes, discard supernatant and re-suspend the pellet in the volume remaining.
- 4 . Follow antibody staining procedure as indicated in our 'direct' and 'indirect' protocols.

Antibodies should be prepared in permeabilisation buffer to ensure the cells remain permeable.



DETECTION OF SECRETED PROTEINS:

Detection of secreted proteins is difficult as the protein will be released from the cell before detection, or may degrade rapidly. Brefaldin A and other compounds are often used as a Golgi-Block. Cells are incubated with Brefaldin A which prevents proteins being released from the golgi. Any cells expressing the protein can then be detected.