Flow cytometry intracellular staining protocol for primary human isolates, PBMCs or Whole Blood.

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:

Here is one example:

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.

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. **If you are using whole blood, AMREPFlow strongly recommend using a Red Blood Cell (RBC) Lysis Buffer (either hypotonic, ammonium chloride or any commercial lysis buffers will suffice). RBCs can and will overshadow (or swamp) your population of Leukocytes, making them hard to clearly indentify**.

2. Fix tissue according to the instructions above
3. Add 100 µL detergent-based permeabilizing agent and incubate in the dark at room temperature for 15 min

4. 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
5. 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 Brefaldin A or other compounds that prevent protein release from the Golgi apparatus, enabling the detection of cells expressing the protein.