Flow cytometry protocols and staining procedures vary depending on whether the antigen to be detected is located on the cell surface or intracellularly. Detection of cell surface proteins, such as CD markers, is relatively straightforward and apart from blocking of FcR receptors requires no extra protocol steps. However, when staining intracellular proteins, such as transcription factors, both fixation and permeabilization steps are required prior to antibody staining (Fig. 1).

These steps are essential to preserve the cellular morphology (e.g. by fixing cells in paraformaldehyde or methanol) and to ensure that the antibody is able to penetrate the plasma membrane (e.g. by permeabilization with detergents such as saponin).

For staining of intracellular antigens, Bio-Rad offers Leucoperm™ buffer set (BUF09), which contains fixative and permeabilization reagents that have been optimized to guarantee maximal staining without altering the cellular morphology.

In addition to carefully choosing the most suitable fixative and permeabilization reagents (depending on whether mild or strong membrane permeabilization is required; strong permeabilization with alcohols is commonly used to detect nuclear proteins), the following factors should be considered when designing intracellular flow experiments:

- Not every intracellular antigen staining protocol is the same. Different staining procedures should be used depending on whether the protein to be detected is a cytokine, a transcription factor or a phosphorylated protein.

- For staining of secreted proteins, such as cytokines, a protein transport inhibitor such as, Monensin or Brefeldin A, should be added prior to fixation/permeabilization in order to trap the cytokines inside the cells and enable intracellular staining. Different inhibitors are recommended for different types of cytokines and for the detection in different species (e.g. human versus murine cells).

- When simultaneous detection of surface markers and transcription factors is required in a flow cytometry panel, specific transcription factor buffers should be used. The reason for this being that fixation/permeabilization steps can change/weakens cell surface marker staining. Transcription factor buffer sets ensure the detection of intracellular antigens without having adverse effects on the staining of surface markers.

- Phosphorylation is a highly transient process and counteracted by numerous phosphatases. In order to detect phosphorylation after stimulation, cells should be immediately fixed and permeabilized.

- The brightness of tandem dyes might be reduced by a fixation or permeabilization step. If tandem dye conjugated antibodies are part of your intracellular flow cytometry panel, fixation and permeabilization steps should be as mild as possible and the duration should be as short as possible.

Fig 1: 0.1% Triton used in conjunction with phalloidin, which recognizes and binds to filamentous actin. A, before treatment of cells; B, after fixation and permeabilization. Notice how distinctive the positive dataset becomes. FL, fluorescence.