DIRECT STAINING PROTOCOL
(CELL SURFACE STAINING)

General Procedure:

1.Harvest, wash the cells and adjust cell suspension to a concentration of 1-5x10^6 cells/ml in ice cold PBS, 10%FCS, 1%sodium azide* .

Cells are usually stained in polystyrene round-bottom 12x75 mm Falcon tubes. However, they can be stained in any container for which you have an appropriate centrifuge e.g. test tubes, eppendorf tubes, and 96-well round-bottomed microtiter plates. In general, cells should be spun down hard enough that the supernatant fluid can be removed with little loss of cells, but not so hard that the cells are difficult to resuspend.

We recommend to stain with ice cold reagents/solutions and at 4°C as low temperature and presence of sodium azide prevent the modulation and internalization of surface antigens which can produce a loss of fluorescence intensity.

2. Add 0.1-10 µg/ml of the primary labelled antibody. Dilutions, if necessary, should be made in 3% BSA/PBS (Propridium iodide can also be added at this point for dead cell exclusion).

3. Incubate for at least 30 min at room temperature or 4°C. This step will require optimisation.

4. Wash the cells 3X by centrifugation at 400 g for 5 minutes and resuspend them in 500ul to 1ml of ice cold PBS, 10% FCS,1% sodium azide*.

Keep the cells in the dark on ice or at 4oC in a fridge until your scheduled time for analysis.

5. Analysis. For best results, analyze the cells on the flow cytometer as soon as possible.

We recommend analysis on the same day. For extended storage (16 hr) as well as for greater flexibility in planning time on the cytometer, resuspend cells in 1% paraformaldehyde to prevent deterioration

FIXATION:

If you need to wait longer than for an hour, you may need to fix the cells after step three. This can preserve them for at least several days. (This will stabilize the light scatter and inactivate most biohazardous agents). Controls will required fixation using the same procedure. Cells should not be fixed if they need to remain viable. There are several methods available, please refer to fixation in the Indirect Staining protocol. The fixation for different antigens will require optimisation by the user.