Direct flow cytometry protocol

General procedure for flow cytometry using a conjugated primary antibody.

1. Harvest, wash the cells and adjust cell suspension to a concentration of 1-5 x 106 cells/ml in ice cold PBS, 10% FCS, 1% sodium azide. Cells are usually stained in polystyrene round bottom 12 x 75 mm2 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 centrifuged sufficiently so the supernatant fluid can be removed with little loss of cells, but not so the cells are difficult to resuspend.

   We recommend staining 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. Internalization can cause 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 (Propidium 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 optimization.
4. Wash the cells 3 x by centrifugation at 400 g for 5 minutes and resuspend them in 500 µl to 1 ml of ice cold PBS, 10% FCS,1% sodium azide. Keep the cells in the dark on ice or at 4°C in a fridge until your scheduled time for analysis.
5. 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 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 require fixation using the same procedure. Cells should not be fixed if they need to remain viable. There are several methods available, please refer to the fixation section in the indirect staining protocol. The fixation for different antigens will require optimization by the user.