Flow cytometry (FACS) staining protocol (Cell surface staining)

1. Harvest, wash the cells (single cell suspension) and adjust cell number to a concentration of 1-5x106 cells/ml in ice cold FACS Buffer (PBS, 0.5-1% BSA or 5-10% FBS, 0.1% NaN3 sodium azide*).

   *Do not add sodium azide to buffers if you are concerned with recovering cell function e.g. if cells are to be collected for functional assays. It inhibits metabolic activity.

   Cells are usually stained in polystyrene round-bottom 12 x 75 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. It is always useful to check the viability of the cells which should be around 95% but not less than 90%.

   We recommend staining with ice cold reagents/solutions and at 4 ° C, since 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 100 µl of cell suspension to each tube.
3. The blocking antibody step 3 is optional but should be included if cells express high levels of Fc receptors which will contribute to non-specific binding and background fluorescence.

   Add 100 µl of Fc block to each sample (Fc block diluted in FACS buffer at 1:50 ratio). Incubate on ice for 20 min. Centrifuge at 1500 rpm for 5 min at 4 ° C. Discard supernatant.

4. Add 0.1-10 µg/ml of the primary labeled antibody. Dilutions, if necessary, should be made in FACS buffer(3% BSA/PBS). Propidium iodide can also be added at this point for dead cell exclusion.
5. Incubate for at least 30 min at room temperature or 4°C in the dark.

   This step will require optimization.
6. Wash the cells 3 times by centrifugation at 1500 rpm for 5 minutes and resuspend them in 200 µl to 1ml of ice cold FACS buffer. Keep the cells in the dark on ice or at 4°C in a fridge until your scheduled time for analysis.

   If you use primary unlabeled antibody after completing step 5 do the following: Dilute the fluorochrome-labeled secondary antibody in FACS buffer at the optimal dilution (according to the manufacturer's instructions), resuspend cells in this solution and incubate for at least 20-30 minutes at room temperature or 4oC in the dark. Wash the cells 3 times by centrifugation at 1500 rpm for 5 minutes and resuspend them in 200 µl to 1ml of ice cold FACS buffer. Keep the cells in the dark on ice or at 4°C in a fridge until your scheduled time for analysis.
7. If you need to preserve cells for several days or are analyzing human, infectious materials or bacteria, after completing step 5 instead of resuspending cells in 200 µl to 1ml of ice cold FACS buffer, add 100 µl 1-4% paraformaldehyde and incubate for 10-15 min at room temperature. Centrifuge your samples at 1500 rpm for 5 min and resuspend them in 200 µl to 1 ml of ice cold PBS. Fixation will inactivate most biohazardous agents, minimize deterioration and help to maintain the integrity of your samples. The amount of fixative needed for different sample types will require

optimization by the user.

8. 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 1-4% paraformaldehyde to prevent deterioration.