Flow Cytometry Protocol

(Service provided by Shikhar Biotech)

1. Harvest cells, centrifuge and discard the supernatant.
2. Re-suspend cells in 5 ml cold PBS and transfer to a 15 ml centrifuge tube.
3. Centrifuge at 1500 rpm for 5 minutes. Discard supernatant and re-suspend the pellet in 1ml cold PBS.
4. Aliquot 100µl cell suspension in each tube (0.5-2 million cells per tube).
5. Fix cells before intracellular staining **(note: fixing is not required for surface staining)**. Fix cells with 4% PFA, mix well and incubate for 15 minutes at room temperature in dark.
6. Centrifuge and Pipette out PFA.
7. Permeabilize cells with 0.5% Triton X for 30 minutes at room temperature **(note: permeabilization is not required for surface staining).**
8. Block with 10% serum (serum of secondary antibody host).
9. Centrifuge and discard supernatant.
10. Add primary antibody (please refer to product datasheet for recommended concentration), dilute in 1% BSA in PBST-if required and incubate for 1 hour at room temperature in dark.
11. Wash with PBST 2 x 5 minutes.
12. Add secondary antibody (got to optimize) in 1% BSA in PBS for 1 hour at room temperature in dark.
13. Wash with PBST 2 x 5 minutes.
14. Dissolve the pellet in 200 µl PBS and analyze on flow cytometer.