

## **General Protocol of FACS Analysis Stack Lab**

1. Detach the cells with Trypsin/EDTA, neutralize with cell culture medium. Resuspend cells in serum free medium (SFM). Seed cells into the appropriate number of vials at 150,000 ~ 200,000 cells/vial.
2. Besides the antigen to be tested, make two extra vials for each cell line: one for cells only(w/o antibody), one for IgG control.
3. Spin down the cells, aspirate the medium, add antibody to each tube in 100ul volume (the concentration depends on antibody, usually 1ug/100ul). Mix gently, incubate at RT for 30 minute.
4. Spin down the cells, wash the cells 3 times with 1ml of PBS each time.
5. Add secondary Ab, 1:1000 dilution in the medium, 100 ul to each tube. Incubate at RT for 20 minutes, KEEP IN DARK.
6. Wash the cells 3 times in 1 ml PBS. Add 700 ul of PBS to each vial. Bring the sample to facility for analysis.