Protocol for extracellular staining of adherent cells

1. Vacuum media from plate and rinse cells with 1000µl PBS, mix gently.

2. Add 500µl Trypsin solution (a volume large enough to cover the bottom of the flask), mix well and incubate for 4-5min.

3. on the bench, add 1500µl FACS buffers and pipet up and down the cells to gently remove the adherent cells.

4. Following cell detachment, check under a light microscope to make sure the cells are not in clumps (if the cells are in clumps pipet up and down three times to break up any clumps).

5. count cells, calculate the concentration and take the desirable amount of cells into Eppendorf tubes.

6. Centrifuge at 500g for 5min. discard supernatant (use pipetor).

* if necessary, Centrifuge again at 500g for 5min

7. Resuspend the cells in **Flow Cytometry staining buffer** (the volume is changing depending on the amount of cells) in eppendorf tube, vortex gently.

8. Incubate for 30 min in dark on ice.

9. add 1ml PBS(1x) to 100µl cells and centrifuge at 500g for 5min, discard supernatant. resuspend them in 200µl of ice cold PBS(1x). Keep the cells in the dark on ice.

10. analyze the cells on the flow cytometry as soon as possible.