Surface Staining

1. Harvest the cells
2. Spin 5min at 4°C, 1500rpm and discard the supernatant
3. Resuspend the cell pellet in 1ml of staining buffer with a P1000 mixing carefully the cells, then add 9ml of staining buffer.
4. Spin 5min at 4°C, 1500rpm and discard the supernatant
5. Resuspend the pellet in 1ml of staining buffer and count the cells using trypan blue to determine viability
6. Resuspend the cells in staining buffer at a concentration 10x106/ml
7. Distribute in epperdorf tubes 100µl of cell suspension equivalent to 1x106 cells, spin the cells and remove the supernatant.
8. Resuspend the cell pellet in 100µl of antibody mix and incubate 20 minutes on ice, protecting the samples from light with foil.
9. Add 1ml of staining buffer
10. Spin 5min at 4°C, 1500rpm and discard the supernatant.
    If your antibody is directly conjugated with a fluorochrome continue to step 13.
11. If a secondary antibody/streptavin is required: Resuspend the cell pellet in 100µl of antibody dilution and incubate 20 minutes on ice, protecting the samples from light with foil.
12. Add 1ml of staining buffer and spin
13. Resuspend the cell pellet in 200µl of PBS 1X, transfer the cell suspension to a facs tube suitable for acquisition in the cytometer. For the LSRFortessa use: Polystyrene Round-Bottom tubes, Ref. 352058 Falcon.
14. Acquire the samples: add a viability dye just before acquisition, vortex the cells and acquire.

Staining Buffer: PBS 1X-2% FCS-2mM EDTA
1000 ml PBS 1X
Take out 24 ml and add:
20ml FCS
4ml EDTA 0.5M

Antibody Mix:

In order to avoid precipitates from the antibodies, prepare your antibody mix in a 1.5ml eppendorf tube and spin during 10min at 4°C, 13.000rpm. Recover the supernatant and use it for staining.