FLOW CYTOMETRY: EXTRACELLULAR STAINING (ECS) FOR SURFACE ANTIGENS

I. Materials Required

1. 1x phosphate saline buffer (PBS), Corning, 21-031-CM
2. Bovine serum albumin (BSA), Gemini, 700-101P
3. Sodium azide, Fosher Scientific, S227-500
4. FACS buffer (1xPBS, 10g/L BSA, 1g/L sodium azide)
5. RPMI, Corning, 10-040-CM
6. Fetal calf serum (FCS), Gemini, 900-108
7. Penicillin/streptomycin, 10,000U/ml penicillin, 10,000µg/ml streptomycin, Lonza, 17-602E
8. "R10" medium (RPMI, 10% FCS, 1% penicillin/streptomycin)
9. DNAseI, Roche, 04716728001
10. Live/Dead fixable Aqua Dead Cell Stain kit, Invitrogen, L34966, diluted 1:60 in 1x PBS (prepare fresh each day from DMSO stock)
11. Paraformaldehyde (PFA), EMS, 15712-S, diluted to 1% in PBS
12. Fluorophore-labelled antibodies of choice

II. Procedure

A. Thawing

1. Thaw samples and rest overnight at a concentration of $2x10^6$/ml in R10 medium + 1µl/ml DNAseI in the incubator at 37°C, 5% CO2.
2. On the next day, resuspend cells and transfer to 15ml or 50ml conical tube.
3. Count the cells and spin at 500g for 5 minutes at room temperature (RT)
4. Resuspend cells in PBS to $1\text{-}5x10^6$/ml and transfer to 5ml FACS tubes

B. Viability and extracellular staining (ECS)

1. spin tubes at 500g for 5 minutes at RT
2. discard supernatant and blot tube over paper towel
3. loosen cell pellet by tapping the tube and add 5µl of 1:60 Aqua viability dye directly to cell pellet
4. incubate for 10 minutes at RT in the dark
5. prepare antibody cocktail master mix with fluorophore-labelled antibodies for extracellular markers and adjust volume to 50µl per test with FACS buffer
6. add 50µl of ECS antibody cocktail to cells and incubate for 20 minutes at RT in the dark (prepare fixation buffer in the meantime)
7. wash cells with 3ml FACS buffer
8. spin tubes at 500g for 5 minutes at RT

9. Discard supernatant and fix cells with 250µl of 1% PFA.
10. Run samples on flow cytometer.