REAGENTS AND MATERIALS

1) Biological Samples

- Fresh human whole blood

2) Room and Equipment

- Biosafety cabinet: Herasafe KS18 GS (TÜV Nord)
- Flow cytometer
- Pipettes: 10-100 µl, 100-1000 µl, 50-200 µl, 1-10 µl & 0.5-2.5 µl (Research, Eppendorf)
- Vortex
- Centrifuges

3) Plasticware

- Falcon 5ml polystyrene tubes with caps, (Cat.No. 352235)
- BD TruCount tubes (Cat.No. 340334)
- 1.5ml eppendorf tubes
- Tips : ART10 (2140-05), ART100 (2065 E-05), ART200 (2069-05) and ART1000 (2079)

4) Solutions

- 1xPBS, no Ca no Mg (Gibco, Cat.No. 14190094)
- Red Blood Cell Lysis solution: BD FACS Lysing solution; BD (Cat.No.23-3204-04) (10x) diluted with milliQ water

5) Abbreviations

Ab – antibody
SOP – standard operating procedure
RT – room temperature: 20 to 22°C
RBC - red blood cell
N - number of sample

STAINING PROTOCOL FOR CYTOMETRY

Prior to sample reception, prepare:

1) Antibodies / premixes

Prepare premix of antibodies for each panel calculating the quantities according to the number of samples, and calibration controls (+1) that will be analyzed that day:

- Label 1.5ml Eppendorf tube with the name of corresponding panel
- Prepare each premix according to dilutions required for each antibody
- Store premixes at +4°C or on ice, protected from light, until use

2) Plasticware

For washing the blood:

- Labelled 5ml tubes for each sample
- 1x PBS (at RT)

3) Reagents:

- Prepare 12ml of 1x RBC lysing solution: Dilute FACS lysing solution at 1/10 in H_2O
- Prepare 5ml of 1x Viability dye solution: Dilute at 1/1000 in sterile 1x PBS
- Keep Viability dye solution at 4°C or on ice, protected from light.
- Keep RBC lysing solution at room temperature for at least 30min before use.
- UltraComp eBeads ThermoFisher (Cat. No 01-2222-41)
- Fixable Viability Dyes (FVD, Termofisher): eight single-channel colors available for 355, 405, 488, 532, 561, or 633 nm lasers in three packaging sizes to match your experiments.
  Live and dead cells are clearly differentiated, even after fixation, allowing easy exclusion of dead cells that can impact the accuracy of the results and fit into almost any staining and immunophenotyping protocol.

4) Cytometer:

- Turn on cytometers, log on user session and select acquisition mode according to the SOP
- Wash cells prior to the staining
- Centrifuge at 500 G for 5min
- Discard supernatant
- Add 1x PBS (RT) up to 4ml

SINGLE STAINED CONTROLS

1) add 1 drop of UltraComp eBeads per 5ml FACS tube
2) add the required amount of an antibody
3) incubate in the dark at RT for 20min
4) add 500µL of 1xPBS
5) Keep at 4°C protected from light until acquisition

CELL STAINING

Note: Incubations can be performed under the hood or on the bench at RT without shaking.

1) Prepare one 5ml FACS tube per panel and per sample (remember to include a tube for unstained control and a tube for live dead FVD single-stained)
2) Transfer 100µl of washed whole blood or cell suspension into the corresponding 5ml tube
3) Mix, by pipeting, all Ab premixes prior to using them
4) Spin shortly (about 20s) all premixes
5) Add each premix into the corresponding tube
6) Mix shortly by vortex
7) Incubate 20min in the dark at RT

<u>If you have a live dead solution</u>

8) Add 1ml 1x viability dye solution
9) Incubate 30min in the dark at 4°C

10) Add 1ml cold PBS (4°C)
11) Centrifuge 5min at 500g and discard supernatant
12) Resuspend in 2ml 1xRBC lysing solution and vortex shortly
13) Incubate 15min at RT protected from light
14) Centrifuge 5min at 500 G, discard supernatant
15) Resuspend in 500µl PBS

<u>If you need absolute count</u>

Transfer the cell suspension into appropriately labeled BD Trucount Absolute Counting Tubes (Cat no 340334)

16) Acquire the entire sample on cytometer