# PBMC Immunophenotyping (Panels 1-5)

PART 1: Staining Protocol (Panels 1-4)

## REAGENTS AND BUFFER

1. 1x DPBS (Invitrogen, Catalogue number 14190)
2. FACS buffer (1X PBS + 2% FCS + 2mM EDTA)
3. BD Cell fix (BD Biosciences, Catalogue number 340181)
4. BD Brilliant violet stain buffer (BD Biosciences, 566385)
5. Live dead dye (BD Biosciences, Catalogue number 565388)

## MATERIALS

1. P10, P20, P200 and P1000 filter tips
2. Beaker for waste

## EQUIPMENT

1. BSL3 facility
2. BSC2 safety cabinet
3. Centrifuge
4. P10, P20, P200 and P1000 pipettes
5. Multichannel pipette
6. Dispensing troughs for multichannel pipetting
7. BD LSR Fortessa X20

## Inside BSL3

Samples are in 96 well V-bottom plates as detailed in '*Sample preparation protocol*'.

Antibody panels can be found in the '*Set up and panels*' document.

1. Centrifuge cells for 5 minutes at 400g at RT.

2. Prepare Live dead (L/D) dye to working concentration (1:4000 dilution of stock prepared according to manufacturer's instructions in 1x PBS)

3. Remove supernatant, and gently resuspend the cell pellets in 100 µl L/D working solution by pipetting up and down. Incubate for 20 minutes at RT, protected from light.

4. After the L/D incubation step is over, centrifuge cells for 5 minutes at 400g at RT.

5. Remove supernatant from cells, and gently resuspend the cell pellet in 200µl FACS buffer by pipetting up and down.

6. Centrifuge cells for 5 minutes at 400g at RT.

7. Remove supernatant from cells, and gently resuspend the cell pellet directly into 100 µl of the antibody master mix prepared in BD Brilliant Violet Stain Buffer and incubate for 30 minutes at RT

8. Add 100 µl of FACS buffer to wells and centrifuge cells for 5 minutes at 400g at RT.
9. Remove supernatant from cells, and gently resuspend the cell pellet in 200µl of FACS buffer by pipetting up and down.

10. Centrifuge cells for 5 minutes at 400g at RT.

11. Fixation: Remove supernatant from cells, and gently resuspend the cell pellet in 100µl of FACS buffer. Add 100µl of fixation buffer and incubate cells for 1 hour protected from light.

12. Centrifuge cells for 5 minutes at 400g at RT.

13. Remove supernatant from cells, and gently resuspend the cell pellet in 200µl of FACS buffer by pipetting up and down.

14. Centrifuge cells for 5 minutes at 400g at RT.

15. Remove supernatant from cells, and gently resuspend the cell pellet in 200µl of FACS buffer by pipetting up and down.

16. Store at 4ºC and acquire the next day on BD LSR Fortessa X20