

Whole Blood Immunophenotyping

Staining Protocol (Panels 6-8)

REAGENTS AND BUFFERS

1. 1x DPBS (Invitrogen, Catalogue number 14190)
2. FACS buffer (1X PBS + 2% FCS + 2mM EDTA)
3. BD Cell fix (BD, Catalogue number 340181)
4. BD FACS™ Lysing Solution 10X Concentrate (BD, Catalogue 349202)
5. BD Brilliant violet stain buffer (BD Biosciences, 566385)

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 U-bottom plates containing respective antibody mastermixes for flow panels 6, 7 and 8 (50 µl of whole blood + 50 µl of antibody mastermix).

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

1. Incubate for 15 minutes at room temp.
2. Add 150µl BD cell fix to each well and mix well. Incubate for 10 minutes at room temp.
3. Transfer all samples to a clean 96 well U-bottom plate
4. Wipe plate exterior with ethanol and place in clean box.
5. Spray box with ethanol and bring outside Cat3 for analysis.

Outside BSL3:

The goal of the panel is to accurately enumerate cell subset numbers in a given blood volume. To achieve this, it's essential that no sample is lost and prior to acquiring, the samples are resuspended in precisely 200µl PBS.

6. Prepare red cell lysis buffer at working concentration (1:10 dilution in dH₂O).
7. Centrifuge the plate at 2000rpm for 1 minute.
8. Remove cell fix inside hood into paper lined plastic bin bag.
9. Add 200µl of RBC lysis buffer to each well.

10. Incubate 15 minutes at RT.
11. Centrifuge the plate at 2000rpm for 1 minute.
12. Aspirate lysis buffer.
13. Add 200 μ l of RBC lysis buffer to each well and resuspend red cell pellet.
14. Incubate 15 minutes at RT.
15. Centrifuge the plate at 2000rpm for 1 minute.
16. Aspirate lysis buffer.
17. Wash plate with 200 μ l PBS twice.
18. Resuspend in exactly 200 μ l PBS.
19. Acquire 100 ul/sample on BD LSR Fortessa X20.