Spleen, MLN and bone marrow Immunophenotyping

Staining protocol

Reagents & Buffers

1. FACS buffer (PBS (-Ca^{2+}/-Mg^{2+}), 0.5% FCS, 2 mM EDTA, 10 mM HEPES)
2. 1x PBS
3. FC Block 1:100 (BD, 553142)
4. ZiR live/dead dye 1:2000 (BioLegend, 423106)

Materials

1. Dispensing troughs for multichannel pipetting
2. 96-well 350 µl polypropylene V-bottom plates (BD falcon, 353263)
3. Low evaporation lids (BD, 353836)

Equipment

1. Centrifuge

Samples are in single cell suspension in FACS buffer in 96 well V-bottom plates.

1. Centrifuge plates for 1 minute at 800×g at 8°C.
2. Resuspend in 50 μl FC block. Incubate for 10 minutes at room temperature.
3. Top plates up with 150 μl PBS.
4. Centrifuge plates for 1 minute at 800×g at 8°C.
5. Resuspend in 200 μl PBS.
6. Centrifuge plates for 1 minute at 800×g at 8°C.
7. Resuspend in 100 μl ZiR. Incubate for 10 minutes at room temperature in the dark.
8. Top plates up with 150 μl FACS buffer.
9. Centrifuge plates for 1 minute at 800×g at 8°C.
10. Resuspend in 200 μl FACS buffer.
11. Centrifuge plates for 1 minute at 800×g at 8°C.
12. Resuspend in 50 μl antibody cocktail.
13. Incubate for 20 minutes in the dark at 4°C.
14. Top plates up with 150 μl FACS buffer.
15. Centrifuge plates for 1 minute at 800×g at 8°C.
16. Resuspend in 200 μl FACS buffer.
17. Centrifuge plates for 1 minute at 800×g at 8°C.
18. Resuspend in 200 μl FACS buffer.
19. Cells are now ready for analysis.