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Immunophenotyping of the peripheral blood immune cells by flow cytometry

In 1 collection

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ABSTRACT

Protocol for immunophenotyping peripheral blood immune cells

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1 Deeply anesthetize the animal. Check for no reflexes and slower breathing

2 Cut the animal's thorax and collect peripheral blood from the left ventricle

3 Lyse the whole blood with the RBC (1X) solution for 10 minutes at 37°C.

3.1 Upon lysis, wash cells and resuspend in cold PBS for immunophenotyping

4 Stain cells surface with FITC-conjugated anti-CD4(1:100, Biolegend), anti-agranulocytes (1:100, Miltenyi biotec), PerCP5.5-conjugated anti-CD8 (1:100, Biolegend) or anti-CD45 (1:100, Biolegend), APC-conjugated anti-CD25 (1:80, Biolegend), PE-conjugated anti-CD45RA (1:100, Biolegend), APC-conjugated anti- CD161 (1:100, Biolegend), PE-Vio770 conjugated anti-CD3 (1:100, REAffinity Miltenyi biotec) and APC-Vio770-conjugated anti-CD11b/c (1:100, REAffinity Miltenyi biotec.) for 15 minutes at 4°C in the dark

4.1 Wash 5 minutes in cold PBS

5 Incubate with a fixing solution (1:3) for 15 minutes at room temperature in the dark

5.1 Wash 5 minutes in cold PBS

6 Permeabilize cells with Cytofix/Cytoperm (1:10, Biolegend) and then stain them intracellularly with PE-CF594-conjugated anti-FoxP3 (1:50, BD Biosciences) in Cytoperm at room temperature for 30 min

7 Acquire cells with an appropriate flow cytometer

8 Gate cells on CD45 to identify total leucocytes. Inside this gate, identify granulocytes with their specific marker, monocytes, and myeloid cells as CD11b/c+, T-lymphocytes as CD3+, B-lymphocytes as CD45RA+, and NK cells as CD161+ cells.

9 For each analysis, acquire at least 0.5x10^6 live by gating on aqua Live/Dead negative cells and then analyze using Flowjo software (Tree Star)