Isolation of monocytes and neutrophils from mouse peripheral blood

1. Harvest peripheral blood from 20 C57BL6 mice via standard eye bleed protocol (non-lethal) using heparinized tubes
2. Pool peripheral blood, bringing volume to ~10 mL with anticoagulant
3. Add 10 mL DMEM+10%FCS (or DMEM+5%FCS) media to 10 mL blood sample
4. Layer onto
5. Centrifuge at 1200cg, 10 min, room temperature
6. Collect cell fractions:
    a. Remove central band of mononuclear cells (PBMNC) to fresh 50 mL tube and proceed to section A
    b. Remove remaining supernatant, keeping red cell + neutrophil pellet, and proceed to section B

----------Section A: Monocyte isolation----------

1. Bring volume to 50 mL with PBS+5%FCS
2. Centrifuge at 1200xg, 5 min, 4°C
3. Remove supernatant and resuspend in 50 mL PBS+5%FCS
4. Repeat wash
5. Resuspend cells in 300 μL PBS+5%FCS
6. Count cells
7. Prepare 3x 5mL FACS <u>control</u> tubes, each with 125 μL PBS+5%FCS and 3 μL of each of the following:
    a. No stain (cells alone)
    b. Mac-1 APC (single stain)
    c. Gr-1 PE (single stain)
8. Add $0.05x10^6$ cells to each control tube
9. Bring volume to 300 μL
10. Prepare 1x5 mL FACS <u>sample</u> tube with 125 μL PBS+5%FCS and 5 μL of each of the following:
    a. Mac-1 APC
    b. Gr-1 PE
11. Add remaining cells to sample tube

12. Incubate on ice, 30 min, protected from light
13. Add 3 mL PBS+5%FCS
14. Centrifuge at 1200xg, 4°C, 5 min
15. Remove supernatant from all 4 tubes and resuspend in 300 μL for analysis

Cell sorting

Monocytes:

$Lin^- Mac1^+ Gr1^-$

----------Section B: Neutrophil isolation----------

1. Resuspend red cell + neutrophil pellet in 50 mL ACK lysis buffer
2. Incubate on ice, 20 minutes
3. Centrifuge at 1200xg, 4°C, 5 min
4. Repeat ACK lysis incubation and centrifugation twice
5. Resuspend in 300 μL PBS+5%FCS
6. Count neutrophils