Standard operating procedure for single cell isolation from fresh bovine EDTA blood

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Purpose

Collecting single cells from fresh bovine EDTA blood. Appropriate preparation of required materials and professional training of lab technicians involved cell isolation have to be performed in advance.

Materials

- pipettes, pipette tips
- Strauß canula
- 1X PBS
- beakers
- centrifuge, table centrifuge
- 50 ml falcon tubes
- Biocoll
- AO/PI
- Cellometer Auto2000

Single-cell isolation

1. collect blood by venipuncture of the *Vena jugularis* using a Srauß canula

2. add 1X PBS to the blood according to the calculation: amount of PBS (ml) = 20 * amount of blood (ml) / 12.5

3. add 10 ml Biocoll to a 50 ml falcon (how many falcons are needed depends on the amount of blood-PBS solution)

4. slowly add 40 ml of blood-PBS solution to overlay the Biocoll phase

5. centrifuge falcons for 25 min at 800 g and 20°C with the breaks turned off

6. carefully remove the falcons from the centrifuge, there should be 3 visible phases (plasma phase: contains platelets, interphase: contains PBMC)

7. carefully remove the plasma phase using a pipette

8. carefully collect the interphase without disturbing the phases

9. collected interphases are added to a new 50 ml falcon

10. fill up the 50 ml falcon with 1X PBS and invert falcon

11. centrifuge the falcon for 10 min at 600 g and 20°C (break is set to max)

12. remove the supernatant and resuspend the cell pellet with 1 ml 1X PBS by carefully pipetting up and down

13. cell concentration and viability are measured using AO/PI staining and a Cellometer Auto2000 (Nexcelom)