Flow Cytometric Analysis of Murine Decidual Immune Cells (E6.5-12.5)

Tissue Collection

All centrifuge steps performed at +4C, 500rcf for 5 minutes unless otherwise stated.

1. Prepare 1.5mL Eppendorf + 2x 15mL + 1x 50mL conical tubes labeled for each sample that will be collected.
    a. 1.5mL + 1x 15mL tube to be loaded with 500µL and 4.5mL digestion buffer respectively; keep cooled in +4C fridge.
    b. Load remaining 15mL tube with 5mL PBS/HBSS.
2. Euthanize mice per animal facility protocols. Dissect and remove uterine horns & store in 15mL tube containing 5mL PBS. Keep on ice as much as possible.
    a. For optimal viability, dissection of uterine horns (step 4-6) should be completed ASAP, however it is acceptable to delay around 30 minutes to an hour without severe consequences to cell viability (e.g. if intending to complete preparation at alternative site).
3. Add PBS to a petri dish. The volume should be sufficient to cover the bottom of the dish but insufficient to allow the implantation sites to float.
    a. When possible, chill the PBS/petri dish before use.
    b. Ensure the 1.5mL + 15mL tubes loaded with digestion buffer is prepared, on ice, and nearby.
    c. Tissues must be kept on ice as much as possible.
4. Working quickly, transfer 1 set of uterine horns to petri dish. Using #5 splinter forceps, remove the myometrium from the decidua. Transfer collected decidua to 1.5mL tube containing digestion buffer ASAP. Complete dissection of remaining implantation sites.

   a. Depending on the population of interest, the myometrium may be discarded at this point.
   b. When possible, a second operator should complete step 7 simultaneously

5. Discard PBS from petri dish. Rinse and clean dish and tools w/ 70% alcohol, water, and distilled water.
6. Repeat steps 3-5 for all uterine horns.
7. Using dissection scissors, cut/mince collected decidua into chunks of approximately $1mm^3$ in 1.5mL tubes containing digestion buffer.
   a. Enlist a second operator whenever possible as the increased surface area improves O_2 access for cells within tissues and therefore viability.
8. Using a P1000 pipette, transfer the minced decidua in digestion buffer to the corresponding 15mL tube.
   a. Volume within tube should now be approximately 5mL.
   b. Consider cutting off the end of the P1000 tip to improve uptake of tissue chunks.
9. Invert 15mL tubes to mix tissues. Ensure all tissue chunks are submerged/suspended in buffer.
10. Place tube in tissue incubator/agitator. Set incubator to +39C & rotate at 120rpm. Incubate 1-1.5hr. Invert all tubes every 15 minutes.
    a. Skip this step if working w/ uNK's as enzymatic digestion is omitted.
11. Transfer contents of 15mL tubes to their corresponding 50mL tube through a 40µm cell strainer. Add PBS through strainer to ensure all released cells go through the strainer.

12. Centrifuge and discard supernatant. Wash with 10mL cold PBS. Repeat for 2-3 times total.
13. *Optional:* magnetic cell sorting (MACS) can be performed at this time to enrich for immune cells or other populations of interest. Refer to the protocol for the specific MACS kit.
14. Count collected cells using hemacytometer.
15. Aliquot cells into labeled 1.5mL tubes containing 1mL PBS, aiming for 10^6 cells per tube. Keep on ice.