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

Staining

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

Following addition of viability dye, samples should be kept in the dark as much as possible.

Work on ice at all times unless otherwise stated.

1. Prepare 1.5mL Eppendorf tubes for controls as necessary. Transfer cell populations/compensation beads to tubes. For cells, transfer 10^6 cells in 1mL volume. Follow supplier-provided protocol for compensation beads.
    a. Unstained controls
    b. Fluorescence-minus-one (FMO) controls
    c. Compensation controls (CC)
        i. For abundant and clearly defined markers, collected cells may be used for CC. For low abundance/low signal markers, compensation beads are preferred.
2. Add 1µL of viability stain to appropriate samples. Incubate 15-30 minutes on ice in the dark.
    a. For cells, this should be 1µL in 1mL of cell suspension.
    b. Ensure that cells are suspended in PBS only. Amine-reactive viability stains can bind to proteins in FBS/BSA/other supplements, leading to drastic increases in background signal.
3. Equilibrate volumes in all tubes stained for viability. Centrifuge, discard supernatant, and wash with PBS for a total of 2 washes.
4. Discard PBS supernant. For cells, replace with 0.1mL of 1% BSA in PBS.

   a. For stained compensation beads (i.e. viability), repace with 200-500µL PBS and set aside.
   b. For unstained compensation beads, replace with 0.1mL PBS.

5. Add 0.5µL FcX to every sample containing cells. Incubate 5 minutes.
   a. FcX = anti-CD16/32 (FcR) to block nonspecific binding.
6. Add necessary concentrations of fluorophore-conjugated antibodies per supplier recommendations/optimization data to tubes containing 0.1mL cell suspensions + FcX. Incubate 15-30 minutes on ice in the dark.
7. Top up tubes with PBS-BSA (cells) or PBS (beads) to 1mL volume. Centrifuge & wash in appropriate buffers 2x times.
   a. If using primary-secondary antibody system, repeat step 6-7 using secondary antibodies.
8. *Optional:* resuspend cells in 0.5mL 4% PFA and incubate at room temperature for 10 minutes. Centrifuge & wash twice using PBS-BSA
   a. Do so if not analyzing samples immediately.
   b. If stored appropriately (+4C & in the dark), fixed samples are generally stable for 2-3 days and reported to be stable up to 7 days.
9. Resuspend samples in 200µL PBS-BSA (PBS for beads) into 5mL Falcon flow cytometry tubes with 25µm strainer caps (caps not needed for beads). Analyze immediately unless fixed.