WHOLE NEUTROPHIL-FLOW CYTOMETRY

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EQUIPMENT/MATERIALS

- eBioscience 1-Step fix/lyse solution (10X)
- Antibodies (See page 4)
- FACs Wash (0.5% (w/v) BSA, 5mM EDTA (pH 7.4-7.6), for 500mL 2.5g BSA, 5mL of 0.5M EDTA)
- 2mL microcentrifuge tubes
- 5mL polystyrene round bottom tubes
- 5mL polystyrene round bottom tubes with cell-strainer cap (Corning REF:352235)
- eBioscience OneComp eBeads
- CountBright™ Absolute Counting Beads
- FlowJo software

PROTOCOL

Cell Staining

1. Prepare lymphocyte stain in a 2mL microcentrifuge tube. Stain for 1 sample is made up to 50 μL in FACs Wash (10 μL and antibody + 40 μL FACs Wash) and is in 3X working concentration. *Concentrations shown below are for 50μL final volume.*

NEUTROPHIL STAIN			
Marker	**Fluorophore**	**Dilution**	**1rxn(50uL)**
CD15	BV650	1/200	0.25
CD45	BV510	1/100	0.5
CD16	PE-Cy7	1/100	0.5
HLA-DR	PerCPCy5.5	1/100	0.5
CD11b	AF700	1/100	0.5
CD13	PE	1/100	0.5
CD33	FITC	1/100	0.5
TIGIT	BV421	1/50	1
CD62L	APC	1/100	0.5
CD66b	PE/dazzle	1/100	0.5
		Ab	**5.25**
		FACS Wash	**44.75**

Note: for each sample, three stains are required. One unstained control (50µL of FACS wash). One TIGIT and CD62L ISOtype control (all antibodies in the cocktail and replace TIGIT and CD62L with their ISOtype antibodies) and one complete stain as described above.

2. Add 100 µL of whole blood that was collected in a heparinized or EDTA coated blood collection tube. Incubate for 30 minutes at room temperature away from light.

3. Top up 2mL microcentrifuge tube containing stain and blood with 1-Step fix/lyse solution (made to 1X in distilled water). Incubate for 10 minutes at room temp away from light with intermittent inversion. *This step is for RBC lysis as well as fixation of white blood cells.*

4. Spin down at 2000rpm for 5 minutes at 4°C

5. Aspirate supernatant. Wash pellet with 2mL FACs Wash. Repeat step 4.

6. Aspirate supernatant and resuspend in 240 µL of FACs Wash. *If running the sample on cytometer immediately, continue to step 7. If not, store samples at 4℃ away from light for up to 24 hours)*

7. Filter cells through 0.45 µm mesh to ensure single cell suspension and add 10 µL of count beads to each sample if counts are needed.

Compensation Controls- *To be made right before running flow*

1. In a 5mL polystyrene tube, dilute 2 drops of OneComp eBeads to 2.5mL with FACs Wash and vortex. *Be sure to vortex OneComp eBeads before use.*

2. Aliquot 240µL of diluted eBeads into 10 polystyrene tubes.
3. Add 0.5µL of each antibody used in the stain into one tube (i.e. 0.5µL of CD15 antibody into tube 1, 0.5µL of CD45 antibody into tube 2, etc) leaving one tube unstained. Vortex tubes.
4. Keep tubes away from light until needed.

Neutrophil Gating Strategy