WHOLE BLOOD LYMPHOCYTE STAIN-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 (5.25 μL and antibody + 44.75 μL FACs Wash) and is 3X working concentration. *Concentrations shown below are for 50 μL final volume.*

Cell Surface Marker	Fluorophore	Antibody Concentration	Volume (μL)
CD3	APCef780	1/200	0.25
CD4 (OKT4)	PerCPCy5.5	1/100	0.5
CD4 (RPA-T4)	PerCPCy5.5	1/100	0.5
CD8	PECy7	1/50	1
CD45	BV510	1/100	0.5
CD56	PE	1/50	1
NKp46	PE	1/50	1
CD19	AF700	1/50	1

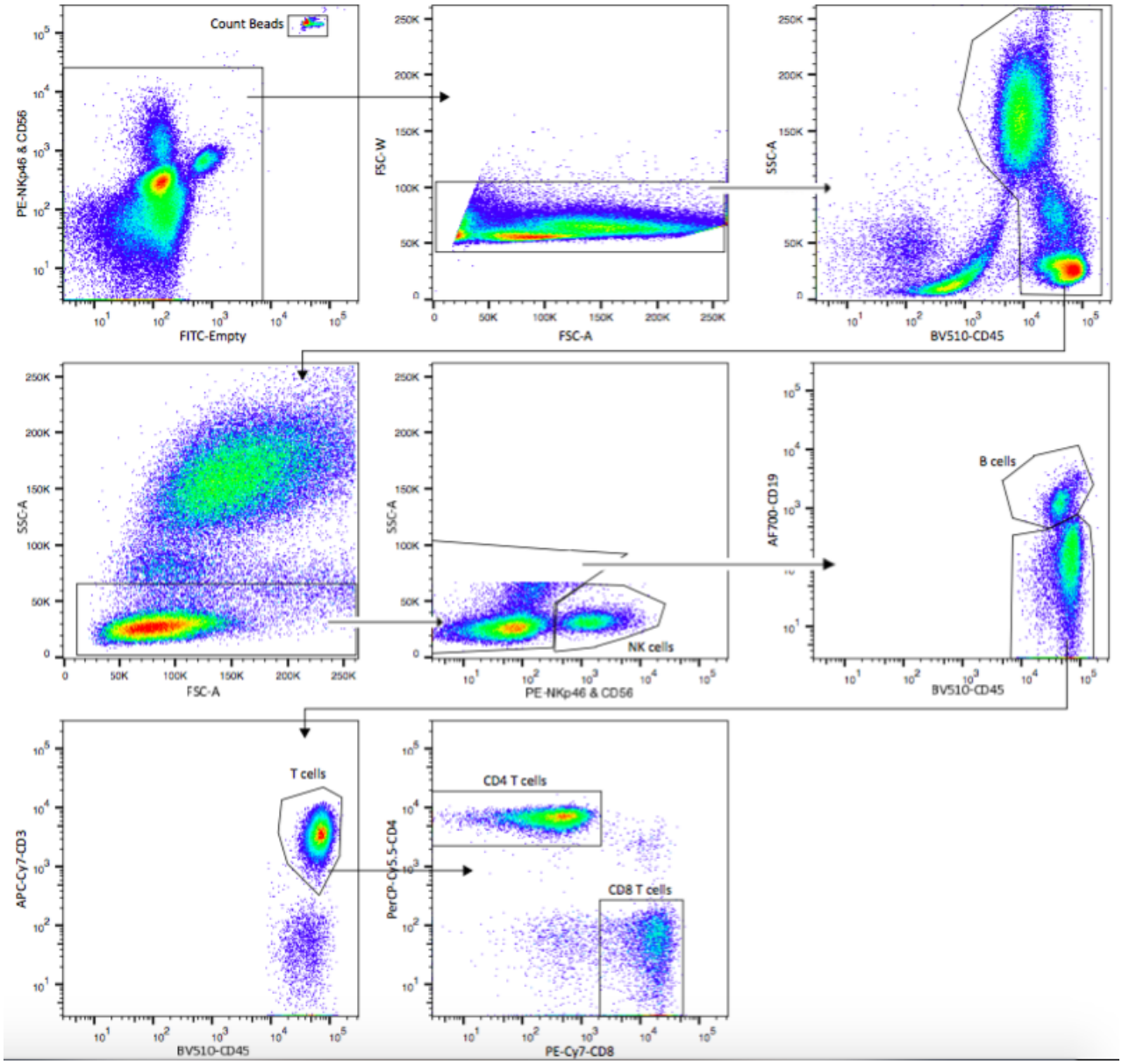
2. Add 100μL of whole blood that was collected in a heparinized or EDTA coated blood collection tube. Incubate for 30 minutes at room temperature away from light.
3. Top up 2mL microcentrifuge tube containing stain and blood with 1-Step fix/lyse solution (made to 1X in distilled water). Incubate for 10 minutes at room temperature 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 the cytometer immediately, continue to step 7. If not, store samples at 4℃ away from light for up to 24 hours)*
7. Filter cells by through 0.45 μm mesh to ensure single cell suspension and add 10μL of count beads to each sample if absolute 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 2mL with FACs Wash and vortex. *Be sure to vortex OneComp eBeads before use.*

2. Aliquot 240µL of diluted eBeads into 7 polystyrene tubes.
3. Add 0.5µL of each antibody used in the stain into one tube (i.e. 0.5µL of CD3 antibody into tube 1, 0.5µL of CD4 antibody into tube 2, etc) leaving one tube unstained. Vortex tubes. *Only one compensation tube needed for PE.*
4. Keep tubes away from light until needed.

Lymphocyte Gating Strategy



Antibody List

Antibody	Fluorophore	Company	Clone	Catalog#
CD3	APCef780	eBioscience	UCHT1	47-0038-42
CD4	PerCPCy5.5	eBioscience	OKT4	45-0048-42
CD4	PerCPCy5.5	eBioscience	RPA-T4	45-0049-41
CD8	PECy7	BD Bioscienc	RPA-T8	557750
CD45	BV510	BioLegend	HI30	304036
CD56	PE	BD Bioscienc	B159	555516
NKp46	PE	BD Bioscienc	9-E2	557991