WHOLE BLOOD T CELL SENESCENCE-FLOW CYTOMETRY

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EQUIPMENT/MATERIALS

- eBioscience 1-Step fix/lyse solution (10X)
- Antibodies (See page 5)
- 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 (9.25 µL and antibody + 40.75 µL FACs Wash) and is in 3X working concentration. *Concentrations shown below are for 50µL final volume.*

Cell Surface Marker	Fluorophore	Antibody Concentration	Volume (μL)
CD19	eFluor 450	1/50	1.0
CD3	PerCPCy5.5	1/200	0.25
CD4	APC.Cy7	1/200	0.25
CD8	BV510	1/200	0.25
CD45	Alexa Fluor 700	1/100	0.5
CD45RA	FITC	1/100	0.5
CD14	eFluor 450	1/75	1.5
CD15	eFluor 450	1/100	0.5
CCR7	PE-eFluor 610	1/50	1
CD57	PE	1/50	1
CD28	APC	1/50	1
CD56	eFluor 450	1/100	0.5
Zombie Yellow	Qdot585	1/50	1
			Ab=9.25 FACS = 40.75

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*

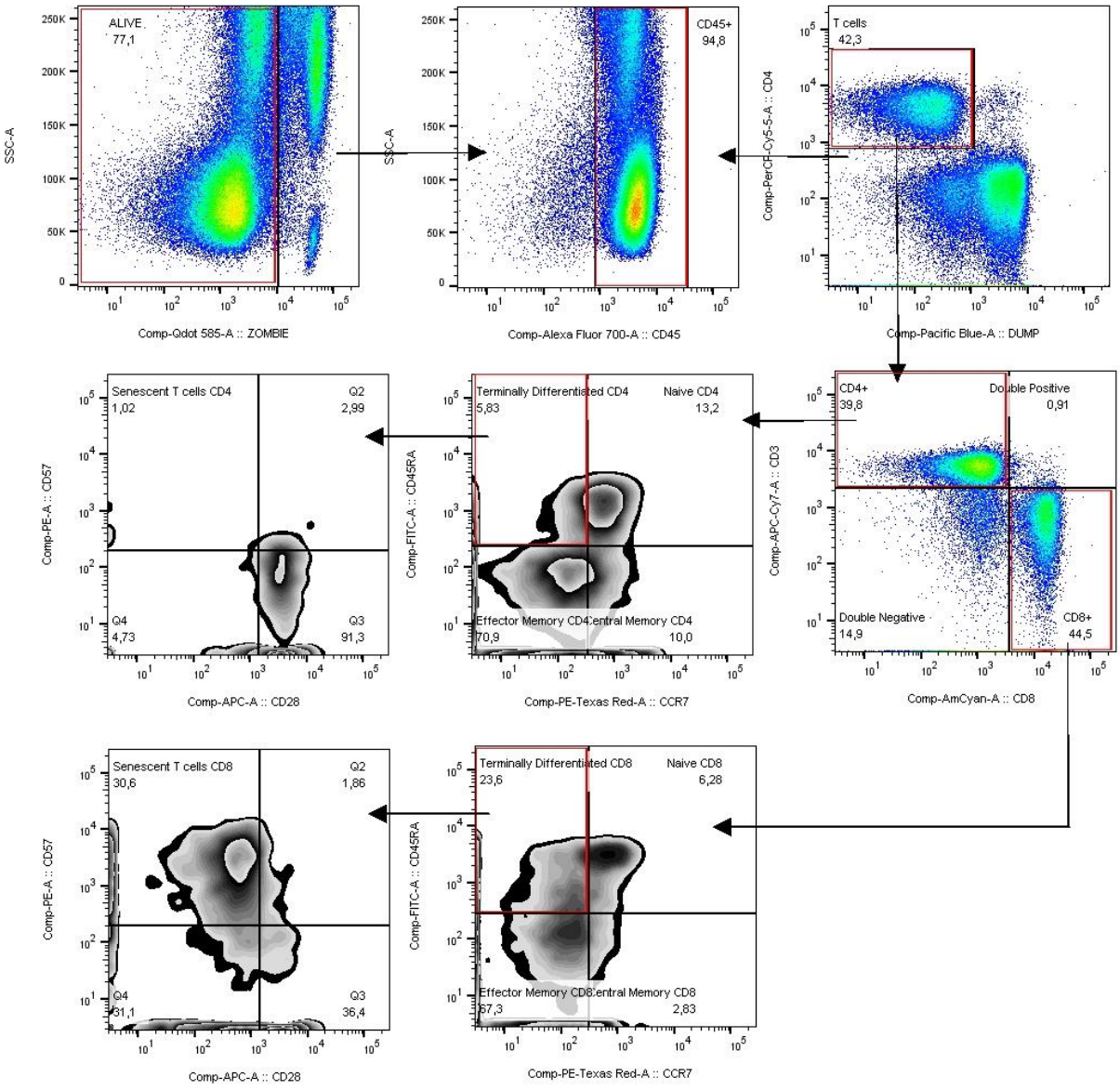
1. In a 5mL polystyrene tube, dilute 2 drops of OneComp eBeads to 2.5mL with FACs Wash and vortex. *Be sure to vortex OneComp eBeads before use.*

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 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 eFluor 450.*

4. Keep tubes away from light until needed.

Senescent T cell Gating (Below example is with Isolated PBMCs):



Antibody List

Marker	Flurochrome	Concentration	Clone	Manufacturer	Ref/Cat #
CD19	eFluor 450	1/50	HIB19	eBioscience	48-0199-41
CD3	PerCPCy5.5	1/200	OKT3	eBioscience	45-0037-41
CD4	APC.Cy7	1/200	OKT4	BioLegend	317417
CD8	BV510	1/200	SK1	BioLegend	344731
CD45	Alexa Fluor 700	1/100	2D1	eBioscience	56-9459-41
CD45RA	FITC	1/100	JS-83	eBioscience	11-9979-41
CD14	eFluor 450	1/75	61D3	eBioscience	48-0149-41
CD15	eFluor 450	1/100	MMA	eBioscience	48-0158-41
CCR7	PE-eFluor 610	1/50	3D12	eBioscience	61-1979-41
CD57	PE	1/50	TB01	Invitrogen	12-0577-41
CD28	APC	1/50	CD28.2	eBioscience	17-0289-41
CD56	eFluor 450	1/100	TULY56	eBioscience	48-0566-41
Zombie Yellow	Qdot585	1/50	--	ThermoFisher	L34967