WHOLE BLOOD T CELL SENESCENCE-FLOW CYTOMETRY (MOUSE)

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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 (3.14 µL and antibody + 46.86 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)
CD45	eF450	1/147	0.34
CD3	AF700	1/250	0.2
NK1.1	PE	1/500	0.1
CD4	BV510	1/63	0.8
CD8	APC	1/250	0.2
CD44	BV650	1/250	0.2
CD62L	AF488	1/500	0.1
CD49d	PerCPCy5.5	1/100	0.5
CD122	PE-Cy7	1/100	0.5
CD183	PE-Dazzle594	1/250	0.2
			Ab=3.14 µL FACS = 46.86 µL

2. Collect blood (100 µL/stain) from mice. Blood is usually collected retro-orbitally by heparinized capillary tube under isoflurane anaesthesia. Unstained (minimum of one sample per stain experiment) also require aliquots of blood.

3. For each sample, aliquot 100 µL of blood into the tubes and mix by pipetting. Incubate the samples in the dark (i.e. cover with aluminium foil or place in drawer) at room temperature for 30 minutes.

4. 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.*

5. Spin down at 2000rpm for 10 minutes at 4°C

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

7. 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)*

8. Filter cells through 0.45 μm mesh to ensure single cell suspension and add 5 μL of count beads to each sample if counts are needed. (*Note: when setting up the cytometer, Set FSC and SSC slightly higher to spread out the lymphocyte population across the plot. This will allow for easier separation while gating*)

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

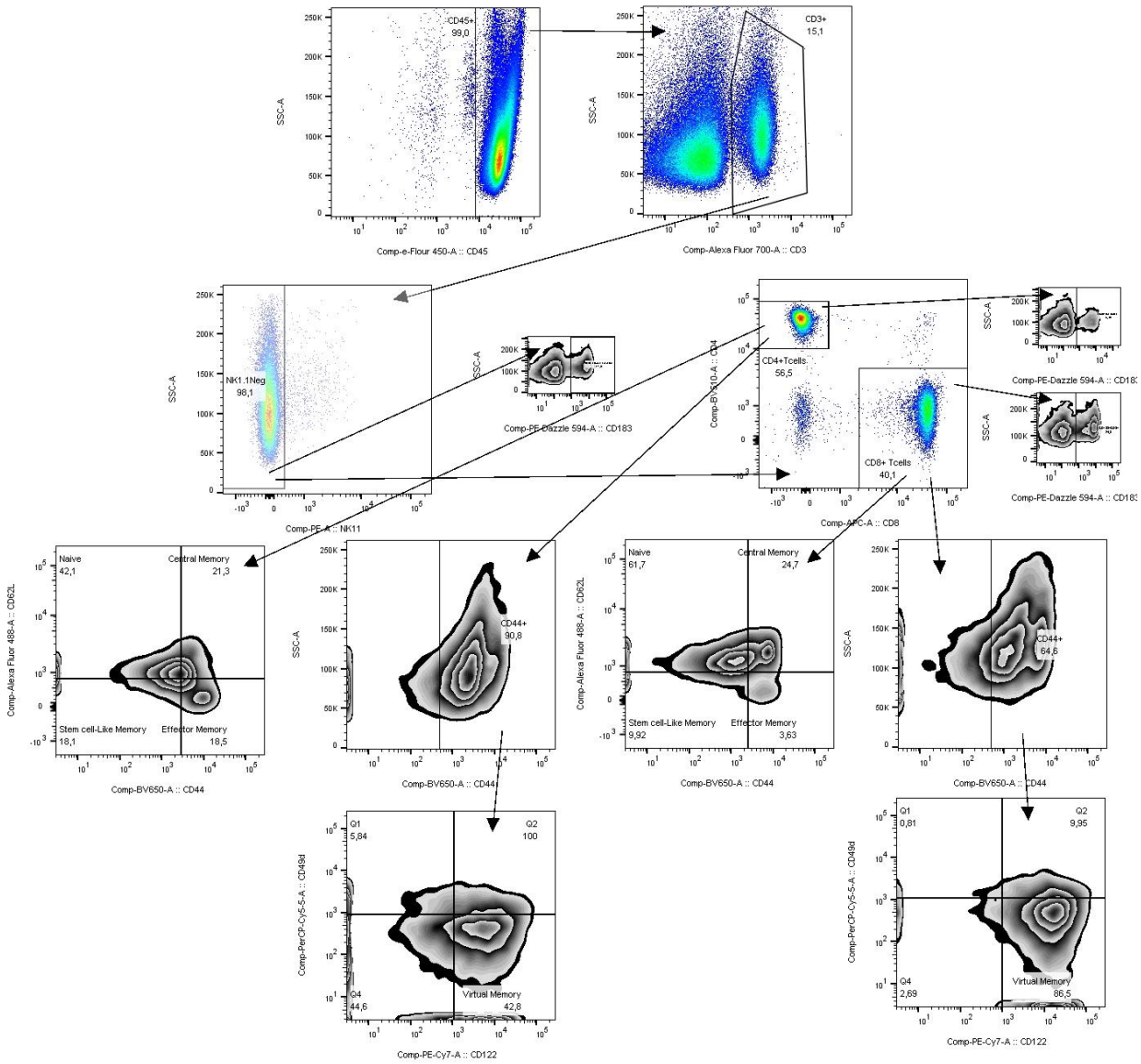
1. In a 5mL polystyrene tube, dilute 2 drops of OneComp eBeads to 2.5mL with FACs Wash and vortex. *Be sure to vortex OneComp eBeads before use.*

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 :



Antibody List

Marker	Flurochrome	Concentration	Clone	Manufacturer	Ref/Cat #
CD45	eF450	1/147	30-F11	Invitrogen	48-0451-82
CD3	AF700	1/250	17A2	Invitrogen	56-0032-82
NK1.1	PE	1/500	PK136	Invitrogen	12-5941-81
CD4	BV510	1/63	RM4-5	BioLegend	100559
CD8	APC	1/250	53-6.7	EBioscience	17-0081-81
CD44	BV650	1/250	1M7	BioLegend	103049
CD62L	AF488	1/500	MEL-14	BioLegend	104419
CD49d	PerCPCy5.5	1/100	R1-2	BioLegend	103619
CD122	PE-Cy7	1/100	TM-/B1	BioLegend	123215
CD183	PE-Dazzle594	1/250	CXCR3-173	BioLegend	126533