WHOLE BLOOD MONOCYTE STAIN-FLOW CYTOMETRY

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EQUIPMENT/MATERIALS

- eBioscience 1-Step fix/lyse solution (10X) (00-5333-57)
- Antibodies (See page 3)
- 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 (01-1111-41)
- CountBright™ Absolute Counting Beads (C36950)
- FlowJo software

PROTOCOL

Buffer Preparation

1. Prepare a 1x working solution of 1-Step Fix/Lyse buffer by mixing 1 part of 10x Fix/Lyse solution with 9 parts of distilled water.

Cell Staining

1. Prepare lymphocyte stain in a 2mL microcentrifuge tube. Stain for 1 sample is made up to 50 µL in FACs Wash (8.25 µL and antibody + 41.75 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)
CD45	BV510	1/100	0.5
CD16	PE-Cy7	1/100	0.5
CD14	BV421	1/100	0.5
CCR2	PE	1/50	1.0
CD11b	APC	1/50	1.0
HLA-DR	PerCPCy5.5	1/100	0.5
CD15	BV650	1/200	0.25
CX3CR1	FITC	1/50	1.0
CD3	AF700	1/50	1.0
CD56	AF700	1/50	1.0
CD19	AF700	1/50	1.0

Note: for each sample, four stains are required. One unstained control (50μL of FACS wash). One CD16 FMO stain (all antibodies in the cocktail except for CD16-PE-Cy7). One CCR2 ISOtype control (all antibodies in the cocktail and replace CCR2-PE with an ISOtype antibody) 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. Spin down at 2000rpm for 5 minutes at 4°C.

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 CD45 antibody into tube 1, 0.5μL of CD16 antibody into tube 2, etc) leaving one tube unstained. Vortex tubes. *Only one compensation tube needed for AF700.*

4. Keep tubes away from light until needed.

Antibody List

Antibody	Fluorophore	Company	Clone	Catalog#
CD45	BV510	BioLegend	HI30	304036
CD16	PE-Cy7	eBioscience	eBioCB16	25-0168-42
CD14	BV421	BioLegend	M5E2	301830
CD11b	APC	BD Pharmige	ICRF44	561015
CCR2	PE	BioLegend	K036C2	357206
HLADR	PerCPCy5.5	eBioscience	LN3	45-9956-42
CD15	BV650	BioLegend	W6D3	323034
CX3CR1	FITC	BioLegend	2A9-1	D070-4
CD3	AF700	BD Bioscienc	UCHT1	557943
CD19	AF700	eBioscience	HIB19	56-0199-42
CD56	AF700	BioLegend	HCD56	318316

Monocyte Gating Strategy