



WHOLE BLOOD MONOCYTE MATURITY 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 μ L in FACs Wash (7.5 μ L and antibody + 42.5 μ L FACs Wash) and is in 3X working concentration. *Concentrations shown below are for 50 μ L final volume.*

Cell Surface Marker	Fluorophore	Antibody Concentration	Volume (μ L)
CD45	BV510	1/100	0.5
CD16	PE-Cy7	1/100	0.5
CD14	BV421	1/100	0.5
HLA-DR	PerCPCy5.5	1/100	0.5
CD3	AF700	1/50	1.0
CD56	AF700	1/50	1.0
CD19	AF700	1/50	1.0
CD115	APC	1/50	1.0
CD64	BV605	1/50	1.0
CD13	PE	1/100	0.5

Note: for each sample, two stains are required. One unstained control (50 μ L of FACS wash) and one complete stain as described above. **FMOs are encouraged for CD115, CD64 and CD13 when compensations are altered.

2. Add 100 μ L 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 μ L of FACS Wash. *If running the sample on cytometer immediately, continue to step 7. If not, store samples at 4 °C 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 240 μL of diluted eBeads into 10 polystyrene tubes.
3. Add 0.5 μL of each antibody used in the stain into one tube (i.e. 0.5 μL of CD45 antibody into tube 1, 0.5 μL 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

Marker	Fluorophore	Company	Catalogue #
CD45	BV510	Biolegend	304036
CD14	BV421	Biolegend	301830
CD16	PE-Cy7	eBioscience	25-0168-42
CD115	APC	Biolegend	347306
HLA-DR	PerCPCy5.5	eBioscience	45-9956-42
CD19	AF700	eBioscience	56-0199-42
CD56	AF700	BD	555516
CD3	AF700	BD	557943
CD64	BV605	Biolegend	305034
CD13	PE	Biolegend	301703

Considerations:

We have observed a significant decrease in the CD115+ monocyte population after 4 hours of collection. Collected blood should be processed within four hours to ensure accurate representation of the monocyte populations.

Monocyte Maturity Gating Strategy

