FLOW CYTOMETRY STAIN FOR MONOCYTES

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BACKGROUND

This stain is for the detection of CD45 (leukocyte common antigen), CD11b (neutrophils & monocytes), Ly6C (monocyte), CD19 (B-cell) and CD3 (T-cell) surface markers. **LY6C^{hi}** monocytes exit the bone marrow in a CC-chemokine receptor 2 (CCR2)-dependent manner and are recruited to inflamed tissues. Here, they can differentiate into TNF- and iNOS-producing dendritic cells (TIP DCs), inflammatory macrophages or inflammatory DCs, some of which can subsequently migrate to draining lymph nodes. **LY6C^{low}** monocytes patrol the blood vessel lumen by associating with the vascular endothelium. LY6C^{low} monocytes are also recruited to sites of inflammation and possibly contribute to wound healing by differentiating into alternatively activated macrophages.

Note: CCR2+ expressing cells recently left the bone marrow. CCR2- cells are located in the periphery.

MATERIALS

- FACS Wash (0.5% (w/v) BSA, 5mM EDTA (pH 7.4-7.6), 2mM NaN3; for 500mL 2.5g BSA, 5mL of 0.5M EDTA)
- RBC lysis buffer: 1-Step Fix/Lyse Solution (eBioscience 00-5333-57)
- PBS
- 2% Paraformaldehyde (PFA)

PROTOCOL

1. Prepare 3X stain by diluting antibodies below in FACS Wash Buffer

Company	Catalog No.	Antibody	Dilution
eBioscience	48-0451	CD45-AF450	1/75
eBioscience	25-0112	CD11b-PECy7	1/400
eBioscience	45-0114	CD11c-PerCP-Cy5.5	1/250
eBioscience	46-5320	MHCII- PerCP-Cy5.5	1/1000
BD Pharmingen	553104	Ly6C-FITC	1/400

R&D	FAB5538P	CCR2-PE	1/50
eBioscience	17-4801	F4/80-APC	1/250
eBioscience	47-0031	CD3-AF780	1/100
eBioscience	56-0193	CD19-AF700	1/100
eBioscience	56-5941	NK1.1-AF700	1/100

2. Prepare 100uL aliquots of blood samples to be stained in 2mL tubes including aliquots for unstained and isotype controls.
3. To 100uL of blood, add 50uL of 3X stain, 50uL FACS Wash for the unstained or 3X , incubate in dark at 4°C for minimum of 30 minutes in the dark.
4. Add 1X RBC lysis buffer up to 2mL and incubate for 10 minutes with inversion.
5. Spin for 7 minutes at 1500rpm, remove supernatant and wash with 1mL of PBS.
6. Spin for 7 minutes at 1500rpm, remove supernatant and resuspend in 200uL 2% PFA, store at 4°C overnight in a light-tight container.

GATING