Monocyte and Dendritic Cell Subsets

Multi-Color Flow Cytometry Panel

Catalog Number: FMC-P-008

PRODUCT DESCRIPTION

Monocytes and dendritic cells of the innate immune system are critical mediators of the early host immune response. This panel contains 9 conjugated antibodies for the single step staining of monocyte and dendritic cell subsets.

INTENDED USE

This multicolor flow cytometry panel was validated on human peripheral blood mononuclear cells (PBMCs).

MATERIALS PROVIDED & STORAGE

Store the unopened kit at 2-8 °C. Do not use past expiration date.

MARKER	CLONE	FLUOROCHROME	SIZE	RECOMMENDED CONCENTRATION
CD3	UCHT1	Alexa Fluor® 700	100 tests	5 µL/10^6 cells
CD11c	ICRF 3.9	PE	100 tests	10 µL/10^6 cells
CD14	134620	Alexa Fluor® 750	100 tests	5 µL/10^6 cells
CD16	245536	APC	100 tests	10 µL/10^6 cells
CD20	396444	Alexa Fluor® 700	25 tests	5 µL/10^6 cells
CD56	2524C	Alexa Fluor® 700	100 µg	0.25-1 µg/10^6 cells
CD86	37301	FITC	100 tests	10 µL/10^6 cells
CD123	32703	PerCP	100 tests	10 µL/10^6 cells
HLA-DR	L203	Alexa Fluor® 405	100 tests	5 µL/10^6 cells

Note: Recommended concentrations are given as reference point for antibody titration. Optimal concentrations should be determined by each laboratory for their experimental conditions.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

OTHER SUPPLIES REQUIRED

- PBS
- Flow Cytometry Staining Buffer (R&D Systems®, Catalog # FC001)
- Fc-block (blocking IgG)
- (Optional; Isotype Control Antibodies)
- 5 mL Flow cytometry tubes

PROTOCOL

1. Wash human PBMCs (1 x 10^6 cells per sample) with 2 mL of Staining Buffer (1X) (R&D Systems®, Catalog # FC001) or other BSA-containing buffer, by spinning at 300 x g for 5 minutes, using 5 mL flow cytometry tubes. Decant/aspirate supernatant.
2. Fc-block cells with blocking IgG (1 µg IgG/10^6 cells) for 10 minutes at room temperature.
3. Add previously titrated amount of each of the fluorochrome conjugated antibodies. Vortex tubes.
4. *(optional)* To separate tube, add 5 µL of each of the isotype control antibodies. Vortex tubes.
5. Incubate the mixtures for 30-45 minutes at room temperature **in the dark**.
6. Wash with 2 mL of Staining Buffer (1X), by spinning at 300 x g for 5 minutes at the end of incubation. Decant/aspirate supernatant.
7. Resuspend the cells in 0.2 - 0.5 mL Staining Buffer (1X) and acquire on a Flow Cytometer.

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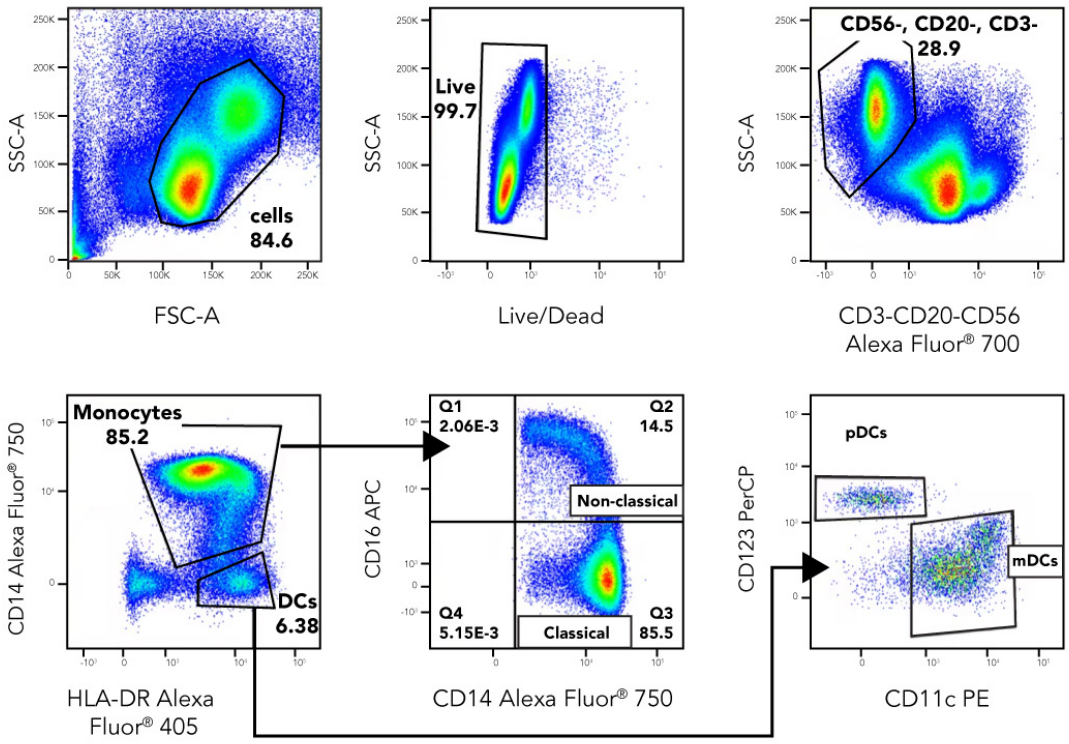
DATA EXAMPLES



Multicolor Flow Cytometry Panel to identify monocyte and dendritic cell subsets. Monocyte and dendritic cell subsets were determined first based on expression of CD14 and HLA-DR. $CD14^+$ monocytes were then gated into $CD16^-$ Classical Monocytes and $CD16^+$ Non-Classical Monocytes. $CD14^-HLA^-DR^+$ Dendritic Cells were then gated into $CD11c^+$ $CD123^-$ monocyte-derived dendritic cells (mDCs) and $CD11c^-CD123^+$ plasmacytoid dendritic cells (pDCs). Cells were stained with Anti-Human HLA-DR Alexa Fluor® 405, CD8 FITC, CD11c PE, CD123 PerCP, CD16 APC, CD20 Alexa Fluor 700, CD3 Alexa Fluor 700, CD56 Alexa Fluor 700, and CD14 Alexa Fluor 750.