bio-techne®
R&D SYSTEMS

M1 Macrophage

Multi-Color Flow Cytometry Panel

Catalog Number: FMC-P-006

PRODUCT DESCRIPTION

M1 Macrophages are associated with a high level of phagocytosis and secretion of pro-inflammatory cytokines. Their function is critical to host and anti-tumor defense against cancer. This panel contains 7 conjugated antibodies that can be used for the single step staining of M1 Macrophages.

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® 405	100 tests	5 µL/10^6 cells
CD11c	ICRF 3.9	PE	100 tests	10 µL/10^6 cells
CD20	396444	Alexa Fluor® 405	25 tests	5 µL/10^6 cells
CD38	240742	APC	100 tests	10 µL/10^6 cells
CD86	37301	FITC	100 tests	10 µL/10^6 cells
HLA-DR	L203	PerCP	100 tests	10 µL/10^6 cells
NCAM1/CD56	2524C	Alexa Fluor® 700	100 µg	0.25 - 1 µg/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)
- Isotype Control Antibodies (optional)
- 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 surface marker. Vortex tubes.
4. *(Optional)* To a 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 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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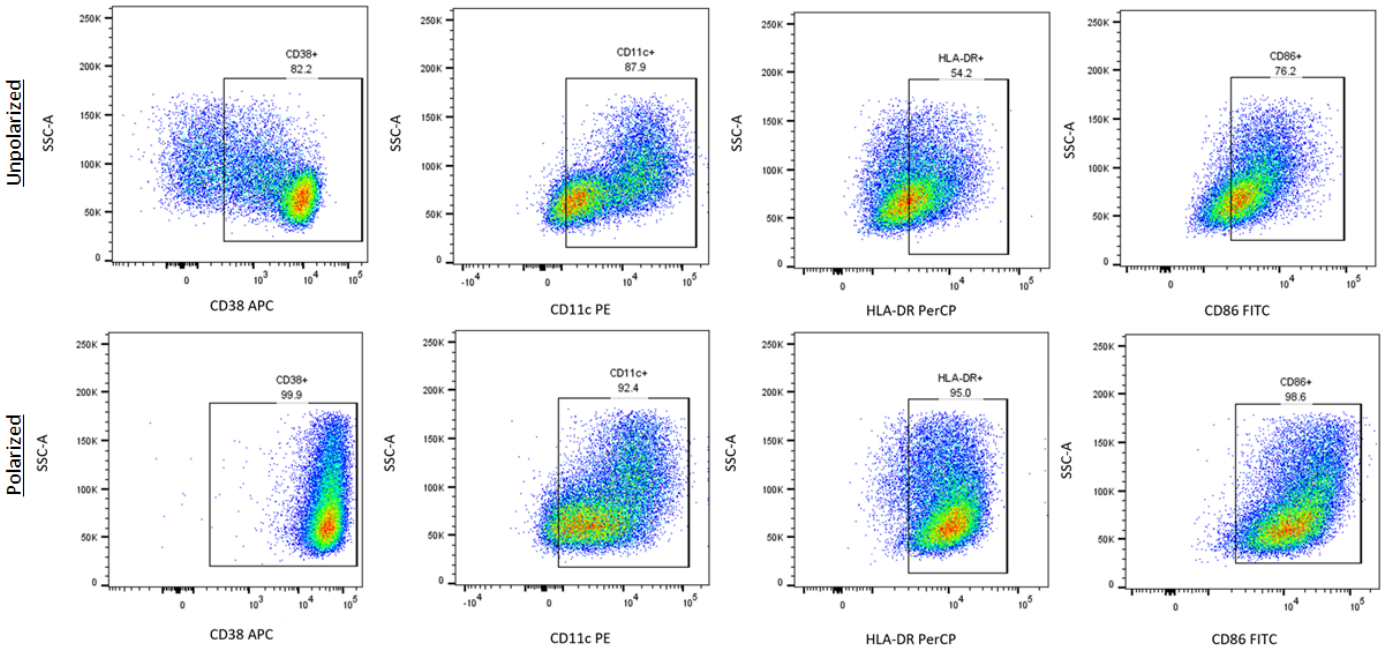
DATA EXAMPLES



Multicolor Flow Cytometry Panel to identify M1 Macrophages. Monocytes were isolated from PBMCs via adherence depletion for 5 hours. Non-adherent cells were removed. Adherent cells were incubated for 6 days in $C3^{+}$ 10% huAB media with 50 ng/mL Recombinant Human GM-CSF (Catalog # 215-GM), media and cytokines are refreshed on day 3. For M1 polarization, 24 hours prior to staining, cells are incubated with 50 ng/mL human GM-CSF (Catalog # 215-GM), 50 ng/mL Recombinant Human IFN-γ (Catalog # 285-IF), and 50 ng/mL LPS. Cells were stained with Anti-human CD3 Alexa Fluor® 405, CD20 Alexa Fluor 405, CD56 Alexa Fluor 700, CD86 FITC, HLA-DR PerCP, CD11c PE, and CD38 APC. Cells were previously gated on Live $CD3^{-}CD20^{-}CD56^{-}$.