bio-techne®
R&D SYSTEMS

M2 Macrophages

Multi-Color Flow Cytometry Panel

Catalog Number: FMC-P-007

PRODUCT DESCRIPTION

M2 Macrophages, often referred to as tumor associated macrophages (TAMs), are more likely to express inhibitory ligands and secrete anti-inflammatory cytokines. M2 macrophages can inhibit NK Cell cytotoxicity by expressing non-classical MHC I molecules. This panel contains 7 conjugated antibodies that can be used for phenotyping M2 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.

SURFACE/INTRACELLULAR	MARKER	CLONE	FLUOROCHROME	SIZE	RECOMMENDED CONCENTRATION
Surface	CD3	UCHT1	Alexa Fluor® 405	100 tests	5 µL/10^6 cells
	CD20	396444	Alexa Fluor® 405	25 tests	5 µL/10^6 cells
	CD163	215927	PerCP	100 tests	10 µL/10^6 cells
	MMR/CD206	685641	Alexa Fluor® 488	100 tests	10 µL/10^6 cells
	NCAM-1/CD56	2524C	Alexa Fluor® 700	100 µg	0.25 - 1 µg/10^6 cells
Intracellular	SR-AI/MSR/CD204	351615	PE	100 tests	10 µL/10^6 cells
	VEGF	23410	APC	100 tests	10 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)
- Flow Cytometry Fixation Buffer (R&D Systems, Catalog # FC004)
- -20 °C Methanol
- Fc-block (blocking IgG)
- (Optional; Isotype Control Antibodies)
- 5 mL Flow cytometry tubes

PROTOCOL

Surface Staining

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 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.

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PROTOCOL *continued*

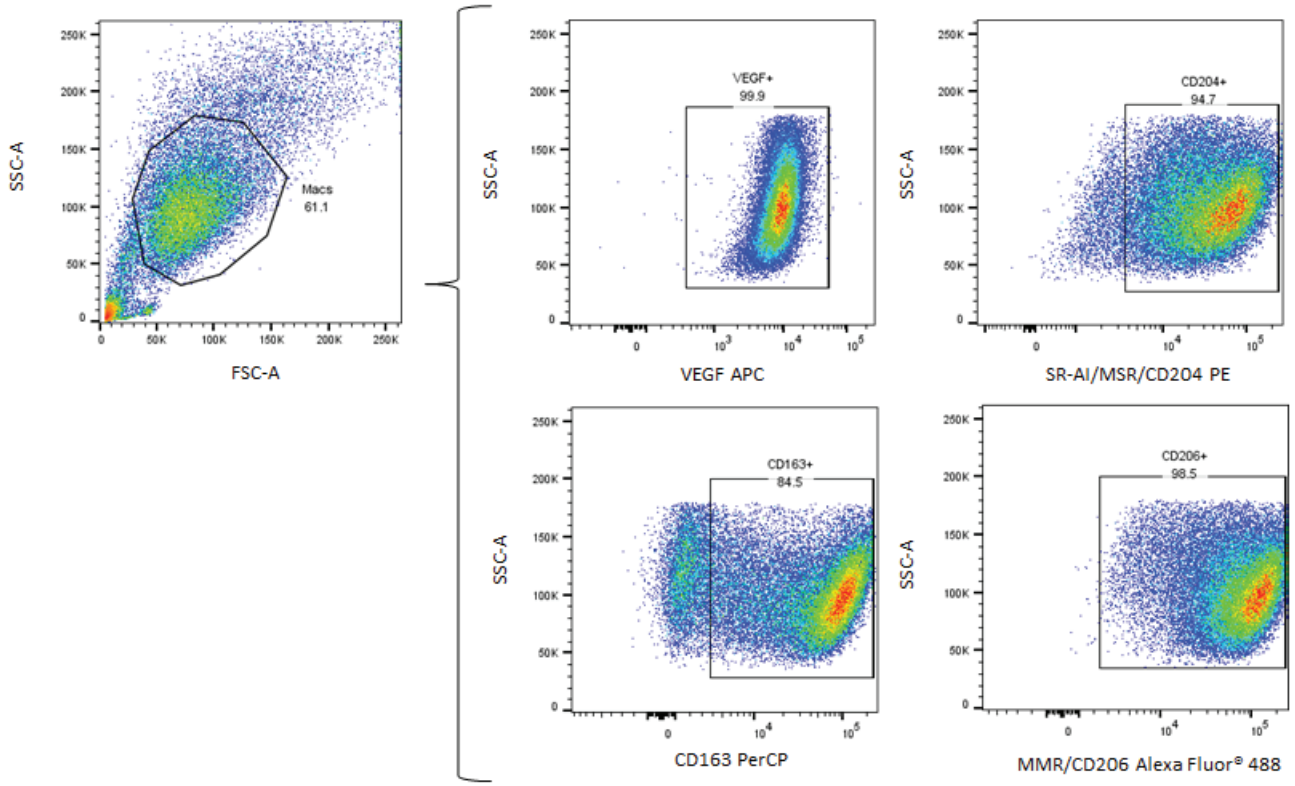
Intracellular Stain (with detergent permeabilization)

1. Add 0.5 mL of cold Flow Cytometry Fixation Buffer (R&D Systems®, Catalog # FC004) and vortex. Incubate at room temperature for 10 minutes. Vortex cells intermittently in order to maintain a single cell suspension.
2. Centrifuge cells 300 - 500 x g for 5 minutes. Decant the Fixation Buffer.
3. Wash cells PBS (or HBSS) by adding 2 mL of PBS (or HBSS), centrifuge at 350-500 x g for 5 minutes, and decant buffer from pelleted cells. Repeat (2 total washes).
4. Resuspend cells in 900 µL of ≤ -20 °C methanol. Incubate for 30 minutes at 2-8 °C.
5. Centrifuge cells for 5 minutes at 350 - 500 x g. Remove and discard the supernatant. Wash 2 times with PBS (or HBSS) as described in Step 1.
6. Add previously titrated amount of conjugated antibody to intracellular activation markers Vortex tubes.
7. Wash cells 2 times with PBS (or HBSS) as described in Step 3.

Note: *If an unconjugated primary antibody is used, incubation with an appropriate secondary antibody should occur now. Dilute the secondary antibody in PBS (or HBSS), starting with the concentration suggested in the product datasheet. Incubate for 20-30 minutes* ***in the dark*** *and wash as in Step 3.*

8. Resuspend cell pellet in 200 - 400 µL of PBS for flow cytometric analysis.

DATA EXAMPLES



Multicolor Flow Cytometry Panel to identify M2 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 M-CSF (Catalog # 216-MC), media and cytokines are refreshed on day 3. For M2 polarization, 24 hours prior to staining, cells are incubated with 50 ng/mL human M-CSF (Catalog # 216-MC), 20 ng/mL Recombinant Human IL-4 (Catalog #204-IL), and 20 ng/mL Recombinant Human IL-13 (Catalog # 213-ILB). Cells were stained with Anti-Human CD3 Alexa Fluor® 405, CD20 Alexa Fluor 405, CD56 Alexa Fluor 700, CD206 Alexa Fluor 488, CD163 PerCP, CD204 PE, and VEGF APC. Cells were previously gated on Live $CD3^-CD20^-CD56^-$.