Intracellular NK Cell Activation

Multi-Color Flow Cytometry Panel

Catalog Number: FMC-P-005

PRODUCT DESCRIPTION

Natural Killer (NK) cells are innate lymphocytes that are critical for host defense against viral infection and cancer. This panel contains 6 conjugated antibodies that can be used for investigating expression of intracellular effector molecules.

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
	NCAM1/CD56	2524C	Alexa Fluor® 647	100 µg	0.25-1 µg/10^6 cells
	Fc Gamma R III (CD16)	245536	PerCP	100 tests	10 µL/10^6 cells
Intracellular	Granzyme B	351927	Alexa Fluor® 488	100 tests	5 µL/10^6 cells
	Granzyme K	2471A	Alexa Fluor® 594	100 µg	0.1-1 µg/10^6 cells
	Perforin-1	1031751	Alexa Fluor® 750	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)
- Flow Cytometry Fixation Buffer (R&D Systems, Catalog # FC004)
- Flow Cytometry Permeabilization Buffer/Wash Buffer I (R&D Systems, Catalog # FC005)
- 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 surface marker. 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 end of incubation. Decant/aspirate supernatant.

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PROTOCOL *continued*

Intracellular Stain (with detergent permeabilization)

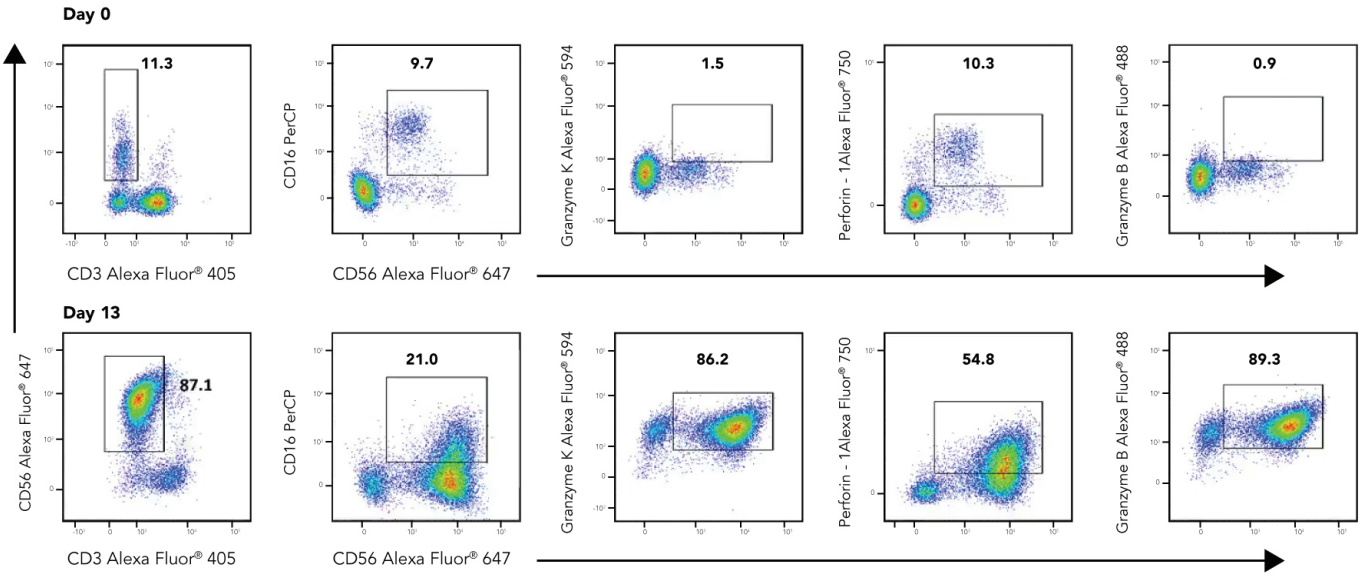
1. Add 0.5 mL of cold Flow Cytometry Fixation Buffer (R&D Systems® Catalog # FC004) and vortex. Incubate at room temperature for 10 minutes. Vortex cells intermittently to maintain a single cell suspension.
2. Centrifuge cells 350 - 500 x g for 5 minutes. Decant the Fixation Buffer.
3. Wash cells PBS (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 the cell pellet in 0.1 - 0.2 mL of Flow Cytometry Permeabilization Buffer/Wash Buffer I (R&D Systems Catalog # FC005)

Note: *Saponin-mediated cell permeabilization is a reversible process, it is important to keep the cells in the presence of Permeabilization Buffer I during intracellular staining.*

Note: *Depending on the specific antibody and cell sample being used, the fixation and permeabilization steps can be performed simultaneously using Flow Cytometry Fixation/Permeabilization Buffer I (R&D Systems, Catalog # FC007).*

5. Add previously titrated amount of conjugated antibody to intracellular targets. Vortex tubes.
6. Incubate cells for 30 minutes at room temperature **in the dark.**
7. Wash cells 2 times with Flow Cytometry Permeabilization Buffer/Wash Buffer I (R&D Systems, Catalog # FC005) as described in Step 3.
8. Resuspend the cells in 0.2 - 0.5 mL PBS (or HBSS) buffer and acquire on a Flow Cytometer.

DATA EXAMPLES



Intracellular NK Cell analysis over a 14-day time course. NK cells were expanded from PBMCs using plate-bound Anti-Human NKp46 Monoclonal Antibody (Catalog # MAB1850) in ExCellerate™ Human NK Cell Expansion Media, Xeno-Free (Catalog # CCM032) plus rhIL-2 (27 ng/mL, Catalog # 202-IL), rhIL-12 (10 ng/mL, Catalog # 219-IL), rhIL-18 (10 ng/mL, Catalog # 9124-IL), rhIL-21 (10 ng/mL, Catalog # 8879-IL) for 13 days. NK cells were assessed on Days 0 and 13 for expression of human CD16 PerCP, Granzyme K Alexa Fluor® 594, Granzyme B Alexa Fluor 488, Perforin Alexa Fluor 750, CD56 Alexa Fluor 647, and CD3 Alexa Fluor 405. Cells were gated on singlets (FSC-A by FSC-H) and live cells.