



FlowCollect™ Human NK Cell Characterization Kit
25 Tests

Cat. No. FCIM025164

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

Application

Natural Killer (NK) cells are a subset of lymphocytes that serve in innate immunity (1). These cells are defined phenotypically by the presence of surface neural cell adhesion molecule (NCAM) or CD56, and the absence of the T-cell coreceptor CD3, with a large percentage of these cells also expressing the Fc gamma receptor molecule or CD16. Natural Killer cells are larger in size than their B and T-lymphocyte counterparts.

The activation of NK cells is a two-signal mechanism, consisting of the interplay between a positive and negative signal. The positive signal takes the form of an activation receptor which, when bound by corresponding ligands on the target cells, will trigger activity, while the presence of the major histocompatibility complex I (MHC I) molecule on the surface of the target cell serves as the negative signal. During exposure to bacterial and viral pathogens, the downregulation of surface MHC I on infected cells causes the removal of the inhibitory block on NK function, which when combined with ligation of the activation receptor, results in activation of NK effector functions.

Natural Killer cells can perform two main immune functions upon activation. The more common one is the direct lysing of target cells through the release of pro-apoptotic molecules perforin and granzyme. Meanwhile, a small subset of NK cells can also serve in an immunoregulatory role, through the secretion of various cytokines such as interferon gamma (IFN-g) to activate the function of other innate immunity cells such as macrophages (2).

Due to their importance as a first line of defense against bacteria and viral infections, NK cells are well studied by researchers not only in immunology, but also in cancer biology, and developmental biology. Among the popular topics of research are the modulation of NK cell activity in various healthy and diseased states (3), the study of the kinetics of NK-mediated target cell killing (4), and the genetics of NK cell development from lymphoid progenitors (5).

When characterizing NK cells in flow cytometry in biological samples such as human whole blood and peripheral blood mononuclear cells (PBMCs), it is crucial to properly distinguish NK cells from other immune cell types, such as B and T lymphocytes as well as macrophages and monocytes. The FlowCelect Human NK cell characterization kit is an optimized, easy-to-use tool for scientists to accomplish this, by providing validated, fluorescently labeled monoclonal antibodies against CD3, CD56, and CD16 to enable accurate and quick identification of CD3- CD56+ NK cells, as well as CD16+ and CD16- subsets within this population.

All FlowCelect kits are optimized on guava[®] bench top flow cytometers. FlowCelect kits can be used on any flow cytometer following the same protocol providing researchers a reliable and fully validated solution to study human NK cells in the comfort of their own lab. All antibodies provided in the kit are carefully titrated and optimized together to ensure maximal performance when run in multiplex, alleviating the need for additional optimization.

Test Principle

Millipore's FlowCelect™ Human NK cell characterization kit includes three directly conjugated antibodies, Anti-CD3-APC, Anti-CD56-PE, and Anti-CD16 FITC. This three color kit is designed to characterize NK within mixed cell populations. The antibodies provided have been carefully titrated to ensure the ability to accurately measure the expression of all markers simultaneously. Sufficient reagents are provided to perform 25 three-color tests. Detailed assay instructions are included to assist in analysis and to ensure the correct cell concentration is obtained during acquisition of sample data.

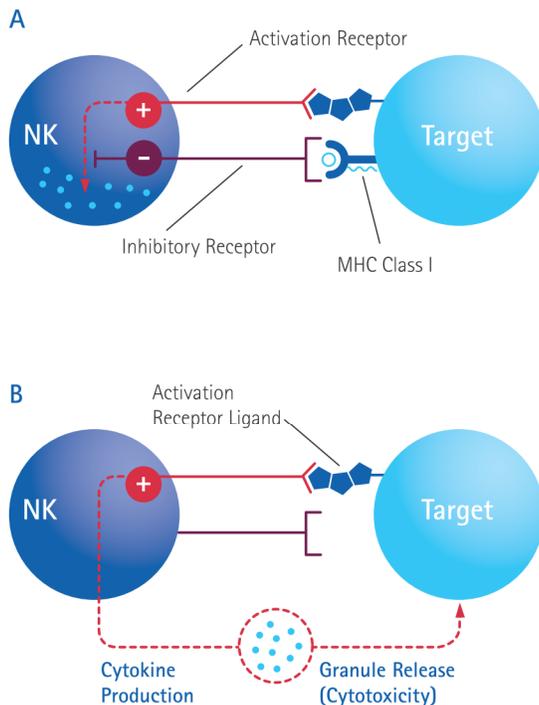


Fig. 1 Mechanism of Human Natural Killer Cell Function

The above figure depicts the events leading up to the activation of NK cells. In panel (a), the activation receptor of the NK cell is bound by its ligand on the target cell to produce a positive signal, which is however inhibited by the ligation of the MHC I molecule on the target cell, which produces a negative signal. In panel (b), the downregulation of surface MHC I on the target cell causes the NK cell activation signal to remain uninhibited, leading to the release of pro-apoptotic molecules granzymes and perforin, which first puncture the target cell membrane and then activate apoptotic pathways within.

Kit Components

FCIM025164 (2-8 °C)

- 20X Anti-CD3-APC: (Part No. CS206532) One vial containing 150 µL.
- 20X Anti-CD16-FITC: (Part No. CS206533) One vial containing 150 µL.
- 20X Anti-CD56-PE: (Part No. CS206534) One vial containing 150 µL.
- Fixation Buffer: (Part No. CS202122) One bottle containing 13 mL.
- 10X Wash Buffer: (Part No. CS202123) One bottle containing 13 mL.
- 5X Assay Buffer: (Part No. CS202124) One bottle containing 55 mL.

Materials Not Supplied

1. easyCyte HT System (guava® easyCyte 8HT or easyCyte 6HT-2L) with guavaSoft™ Software or equivalent flow cytometry system with ability to detect green, yellow, red1, and red2 fluorescence
2. 96-well microplate plates, round bottom (Falcon Cat. Nos. 353910 or 353918) or flat bottom (Falcon Cat. No. 353075 or 353915), or equivalent. Refer to the appropriate Guava System user's guide for other compatible microplates/Tissue culture reagents, i.e. HBSS, PBS w/o Ca²⁺ or Mg²⁺, cell dislodging buffers, etc.
3. Some assay components included in the kit may be harmful. Kit contains a fixation solution containing paraformaldehyde. Please refer to the MSDS sheet for specific information on hazardous materials (MSDS forms can be found by contacting Millipore technical services).
4. Pipettors with corresponding tips capable of accurately measuring 0.1 – 1000 µL
5. Tabletop centrifuge capable of achieving 400 x g
6. Deionized water
7. Human peripheral blood mononuclear cells (PBMCs), human peripheral whole blood, or cells of interest

Precautions

- The instructions provided have been designed to optimize the kit's performance. Deviation from the kit's instructions may result in suboptimal performance and may produce inaccurate data.
- Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling or using this product.
- All fluorochrome conjugated antibodies and dyes are light sensitive and must be stored in the dark at 2-8°C.
- During storage and shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For maximum recovery of product, centrifuge vial briefly prior to removing cap.
- Avoid microbial contamination of the solution, which may cause erroneous results.
- Do not use reagents beyond their expiration date.

Storage

- This kit must be stored at 2 - 8°C
- The 10X Wash Buffer can be stored at either 2 - 8°C or at room temperature upon receipt.
- **Caution:** The fluorochrome conjugated antibodies should always be stored at 2 - 8°C and stored in the dark.

All kit components are stable up to six (4) months from date of receipt if stored and handled correctly. **Please avoid repeated changes in temperature as this will affect the integrity of the product.**

Preparation of Reagents

1. **Wash Buffer** is supplied at 10X concentration and should be diluted to 1X with deionized water prior to use. Prepared 1X Wash Buffer is stable up to one year. Store at 2 - 8°C.
2. **Assay Buffer** is supplied at 5X concentration and should be diluted to 1X with deionized water prior to use. Prepared 1X Assay Buffer is stable up to one year. Store at 2 - 8°C.

Assay Instructions

Flow Cytometry Staining Protocol 1: Human Peripheral Blood Mononuclear Cells (PBMCs) or Human PBMC-derived cells:

Note: This assay protocol has been optimized for human peripheral blood mononuclear cells (PBMCs). During all steps in the assay procedure, keep all reagents on ice. Follow the guidelines listed to ensure proper cell staining for optimal analysis.

Note: This protocol can be performed in bulk (e.g. 2 million cells in 1 mL of 1X Wash Buffer) in a 1.5 mL tube or in individual samples (200,000 cells in 100 μ L of 1X Wash Buffer) in a 96-well plate. If staining in 1.5 mL tube, samples must be aliquoted into 96-well plate before analysis can begin.

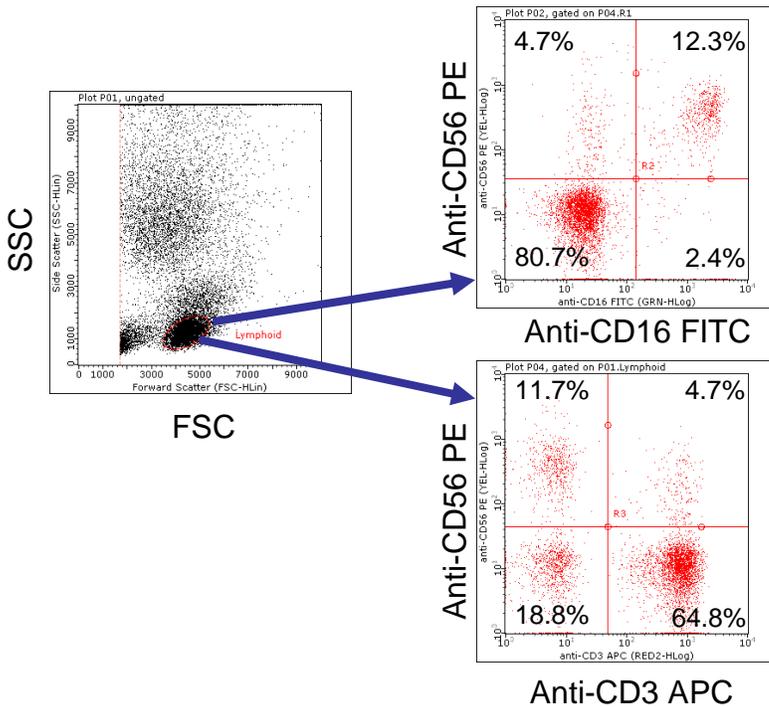
1. Aliquot 200,000 cells and wash with 1 mL of 1X Wash Buffer twice. Spin at 400 x g for 5 minutes and Remove supernatant.
2. Resuspend cells with 85 μ L of 1X Assay Buffer.
3. Add 5 μ L each of anti-CD3 APC, anti-CD16 FITC, and anti-CD56 PE to the sample. Mix gently by pipetting and incubate at room temperature for 30minutes in the dark to stain.
4. After staining, wash with 200 μ L of 1x Assay Buffer to the sample, mix briefly, and centrifuge at 400 x g for 5 minutes. Remove supernatant.
5. Resuspend pellet with 250 μ L of 1x Assay Buffer and centrifuge again for 400 x g for 5 minutes. Remove supernatant.
6. Resuspend pellet with 250 μ L of 1x Assay Buffer and perform flow cytometry analysis
Note: It is recommended to acquire at least 10,000 total events per sample.

Flow Cytometry Staining Protocol 2: Human Peripheral Whole Blood:

Note: This assay protocol has been optimized for staining of human whole blood. Immediate staining of freshly obtained blood is highly recommended. During all steps in the assay procedure, keep all reagents on ice. Follow the guidelines listed to ensure proper cell staining for optimal analysis.

1. Add 5 μ L each of anti-CD3 APC, anti-CD16 FITC, and anti-CD56 PE to 10 μ L of fresh human whole blood in the same well of a 96-well plate.
2. Mix gently, and incubate in the dark at room temperature for 20 minutes.
3. Perform red blood cell lysis by adding 180 μ L of Guava 1x Lysing Solution to sample containing a 1:40 dilution of Fixation Solution. Mix by pipetting and incubate at room temperature in the dark for 30 minutes.
4. After completion of lysis, centrifuge sample at 400 x g for 5 minutes. Remove supernatant.
5. Wash cell pellet with 250 μ L of 1x Assay Buffer. Centrifuge at 400 x g for 5 minutes. Remove supernatant.
6. Repeat step 5.
7. Resuspend pellet with 250 μ L of 1x Assay Buffer and perform flow cytometry analysis
Note: It is recommended to acquire at least 10,000 total events per sample.

Sample Data



Gating Strategy:

Draw an elliptical marker on the FSC vs SSC plot to generate the lymphoid gate. Apply this gate to two dot plots, one for anti-CD16 FITC vs anti-CD56 PE, and the second for anti-CD3 APC and anti-CD56 PE. Create quadstat markers on each plot and move them as needed to distinguish different cell populations.

Data Analysis:

Create a new stat for each of the two quadstat markers to obtain percentages of individual quadrants. Alternatively, one can also create stats to measure MFI of individual fluorescent channels.

Figure 3: Representative flow cytometric data of human peripheral whole blood. The lymphoid gate is further applied to two dot plots, one showing CD16 FITC vs. CD56 PE staining, and the other showing CD3 APC vs. CD56 staining.

Technical Hints

- If minor precipitate is detected in the 10X Wash Buffer place the bottle in a warm water bath for 30 minutes, followed by mixing the contents on a mechanical vortex.
- For cellular staining and analysis to be most effective, make sure that test cells have good viability prior to use.
- For certain cell cultures cell pellets may become hazy or transparent following the fixation step, making it difficult to see. If sampling a small collection of cells for flow analysis, it is recommended that all steps be performed in a smaller collection tube (e.g. centrifuge tube)
- Do not mix or interchange reagents from various kit lots.

Troubleshooting

Assay Step	Potential Problem	Experimental Suggestions
Reagent Preparation	Precipitation found in 10X Wash buffer	<ul style="list-style-type: none"> If storing at -20°C, precipitate can form in the 10X wash buffer. Prior to use, place bottle in a 37°C water bath, swirling the contents occasionally. If this does not remove the precipitate completely, allow 10X Wash Buffer to sit at room temperature overnight.
Cell Preparation	Incomplete Red Blood Cell Lysis	<ul style="list-style-type: none"> If red blood cell lysis appears incomplete (cell pellet is still visibly red), try repeating lysis step with higher volume of lysis buffer
Acquisition	Acquisition rate decreases dramatically	<p>This usually indicates that the fluid pathway on the instrument may be blocked. This can be alleviated by the following:</p> <ul style="list-style-type: none"> Decreasing number of cells for analysis. Guava flow cytometers have the capacity of analyzing a steady stream of 300 – 500 cells per microliter. Any cell densities in excess can essentially block the normal flow, causing disruption during the assay. Decrease the number of cells being analyzed by diluting the sample to approximately 0.5 million cells per milliliter. Adherent or sticky cells can result in cellular clumping. Use a more aggressive enzyme for dissociation such as trypsin during cell harvesting should help keep cells in single suspension. Alternatively, using a cell strainer can help disrupt cell clumping if needed (Catalog No. SCNY00060; 60 µM) After many uses, it is possible that the fluid system on any standard flow cytometer will require cleaning. Run standard cleaning procedures to clean the fluid system during or after an assay. This will prevent any material from forming where the steady flow stream takes place.
Cellular Analysis	A loss or lack of signal	<ul style="list-style-type: none"> Cell numbers may need to increase. Cell loss is common during washing steps in the assay procedure. A substantial decrease in cell numbers can lead to a loss of signal. Make sure that cell density remains at approximately 0.5 million cells per milliliter during analysis. Although the assay procedure has been optimized to function utilizing many different cell types, further antibody titrations may be necessary for some cell types to capture the ideal staining concentration. A lack of signal may indicate that excess antibody will need to be used during the staining procedure.
Cellular Analysis	Background and/or non-specific staining of cells	<ul style="list-style-type: none"> Although the assay procedure has been optimized to function utilizing many different cell types, further antibody titrations may be necessary for some cell types to capture the ideal staining concentration. Non-specific staining and background may indicate that less antibody will need to be used during the staining procedure.
Cellular Analysis	Variability in day to day experiments	<ul style="list-style-type: none"> When using the guava easyCyte™ Plus instrument for flow analysis, make sure that a quality check on the instrument (e.g. calibration) is performed on a daily basis prior to use. (*See Analytical Sensitivity and Detection Limits Section for Guava Check standards)

**For further support, please contact Millipore's Technical services at +1(800) 437-7500*

References

1. Cooper, MA, et al. The biology of human natural killer-cell subsets. *TRENDS in Immunology*. 22: 633 (2001).
2. Reefman, et al. Cytokine secretion is distinct from secretion of cytotoxic granules in NK cells. *The Journal of Immunology*. 184: 4852 (2010).
3. He, et al. T cell-dependent production of IFN- γ by NK cells in response to influenza A virus. *The Journal of Clinical Investigation*. 114: 1812 (2004).
4. Zamai, et al. Kinetics of in vitro natural killer activity against K562 cells as detected by flow cytometry. 32: 280 (1998).
5. Hackett, et al. Transplantable progenitors of natural killer cells are distinct from those of T and B lymphocytes. *Proceedings of the National Academy of Sciences USA*. 83: 3427 (1986).

Related Products

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FCIM025125	FlowCelect™ Mouse Th17 Intracellular Cytokine Kit
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