Miltenyi Biotec

CD56$^+$CD16$^-$ NK Cell Isolation Kit
human

Order no. 130-092-661

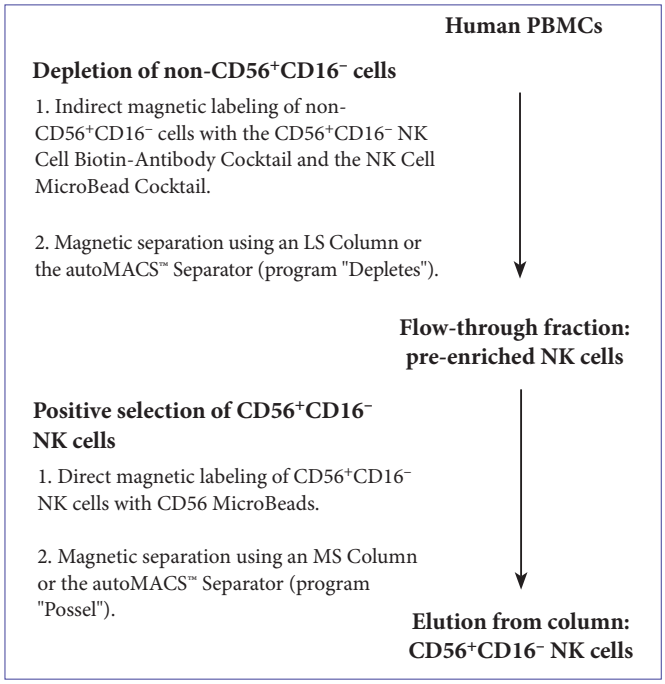
Index

1. Description

Components	**2 mL CD56$^+$CD16$^-$ NK Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal anti-human antibodies. **2 × 2 mL NK Cell MicroBead Cocktail, human:** Cocktail of MicroBeads conjugated to monoclonal antibodies. **2 mL CD56 MicroBeads, human:** MicroBeads conjugated to a monoclonal CD56 antibody (isotype: mouse IgG1).
Size	For 2×10^9 total cells, up to 20 separations.
Product format	All components are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of MACS® Separation

The isolation of CD56$^+$CD16$^-$ NK cells is performed in a two-step procedure. First, non-CD56$^+$CD16$^-$ cells, i.e. T cells, B cells, dendritic cells, stem cells, monocytes, granulocytes, erythroid cells and CD56$^+$CD16$^+$ NK cells are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies against lineage-specific antigens and a cocktail of MicroBeads. Upon subsequent magnetic separation of the cells over a MACS® Column that is placed in a magnetic field of a MACS Separator, the magnetically labeled cells are retained within the column, while the unlabeled cells run through. In the second step, the pre-enriched NK cells are directly labeled with CD56 MicroBeads. Upon subsequent magnetic separation, the CD56$^+$CD16$^-$ NK cells are eluted after removing the column from the magnetic field.



1.2 Background and product applications

NK cells are not a homogeneous cell population, but can be subdivided into several subsets according to functional and phenotypic differences. The CD56$^+$CD16$^-$ NK Isolation Kit was developed to enrich an NK cell population that is CD56^{bright} and CD16$^-$, and lack expression of killer cell immunoglobulin-like receptors (KIRs). Accordingly, they neither show antibody-dependent cell-mediated cytotoxicity (ADCC) nor strong natural cell-mediated cytotoxicity. CD56$^+$CD16$^-$ NK cells are phenotypically characterized by being almost free of granules.

CD56$^+$CD16$^-$ NK cells represent about 1–2% (range: 0.5–4%) of all PBMCs. Unlike CD56$^+$CD16$^+$ NK cells, which only secrete low levels of cytokines, they have a high cytokine production capacity and are thus suggested to have an immunoregulatory role. They predominantly secrete INF-γ[1] which aids in reciprocal interactions with other cells of the innate and adaptive immunity, mainly dendritic cells and T cells.[2,3] Gene expression profiling on freshly isolated CD56$^+$CD16$^-$ NK cells versus CD56$^+$CD16$^+$ NK cells has revealed several differentially expressed genes.

Example applications

- Specific isolation of CD56$^+$CD16$^-$ NK cells from peripheral blood for phenotypical and functional characterization.
- Characterization of NK subset-specific receptor expression or cytokine secretion patterns.
- Analysis of cell-cell or cytokine-mediated interactions with other cells of the innate and adaptive immunity.
- Studies on cell-mediated cytotoxcity and the involved signal transduction pathways.
- Analysis of NK cell differentiation and maturation.

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- Studies on function and benefits of using a distinct NK cell subset for anti-cancer treatment.
- Studies on the function of NK cell subsets in distinct diseases, e.g. sepsis, septic shock, multiple-organ dysfunction, HIV, and Hepatitis B infection, as well as during pregnancy.

1.3 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS) pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °C). Degas buffer before use, as air bubbles could block the column

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as gelatine, human serum, or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Depletion of non-$CD56^+CD16^-$ cells is performed on an LS Column. The subsequent positive selection of $CD56^+CD16^-$ NK cells is performed on an MS Column. Depletion and positive selection can also be performed by using the autoMACS™ Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
Depletion and positive selection			
autoMACS	2×10^8	4×10^9	autoMACS

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibody for flow cytometric analysis, e.g. CD3-FITC (130-080-401), CD3-PE (# 130-091-374), CD3-APC (# 130-091-373), CD56-PE (# 130-090-755) or CD56-APC (# 130-090-843); CD16-FITC (# 130-091-244), CD16-PE (# 130-091-245), or CD16-APC (# 130-091-246); and Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856).

  ▲ **Note:** Fluorescent labeling with CD56 fluorochrome has to be performed after magnetic separation.

- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the labeling and discrimination of dead cells by flow cytometry.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, e.g. Ficoll-Paque™. For details see section General Protocols in the User Manuals or visit www.miltenyibiotec.com.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

2.2 Magnetic labeling of non-$CD56^+CD16^-$ cells

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^8 cells. When working with fewer than 10^8 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^8 cells use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column.

▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 400 µL of buffer per 10^8 cells.
4. Add 100 µL of **$CD56^+CD16^-$ NK Cell Biotin-Antibody Cocktail** per 10^8 cells.
5. Mix well and refrigerate for 10 minutes (4–8 °C).
6. Wash cells by adding 5–10 mL of buffer and centrifuge at 300×g for 10 minutes at 4–8 °C. Aspirate supernatant completely.
7. Resuspend cell pellet in 800 µL of buffer per 10^8 cells.
8. Add 200 µL of **NK Cell MicroBead Cocktail** per 10^8 cells.
9. Mix well and refrigerate for additional 15 minutes (4–8 °C).
10. Wash cells by adding 10–20 mL of buffer and centrifuge at 300×g for 10 minutes at 4–8 °C. Aspirate supernatant completely.
11. Resuspend up to 10^8 cells in 500 µL of buffer.

    ▲ **Note:** For larger cell numbers, scale up buffer volume accordingly.

12. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of non-CD56$^+$CD16$^-$ cells

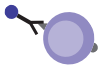
Depletion with an LS Column

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 3×3 mL of buffer. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty. Collect total effluent; this contains the pre-enriched NK cell fraction.
5. Proceed to 2.4 for the isolation of CD56$^+$CD16$^-$ NK cells.

Depletion with the autoMACS™ Separator

▲ Refer to the autoMACS™ User Manual for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose separation program "Depletes".
3. Collect unlabeled fraction from outlet port neg1. This is the pre-enriched NK cell fraction.
4. Proceed to 2.4 for the isolation of CD56$^+$CD16$^-$ NK cells.



2.4 Magnetic labeling of CD56$^+$CD16$^-$ NK cells

▲ Volumes for magnetic labeling given below are for an **initial** starting cell number of up to 10^8 cells. For larger initial cell numbers, scale up volumes accordingly.

1. Centrifuge cells at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 400 µL of buffer.
3. Add 100 µL of **CD56 MicroBeads**.
4. Mix well and refrigerate for 15 minutes (4–8 °C).
   ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
5. Wash cells by adding 5–10 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend cells in 500 µL of buffer.
7. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of CD56$^+$CD16$^-$ NK cells

Positive selection with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 3×500 µL of buffer. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells (CD56$^+$CD16$^-$ NK cells) by firmly pushing the plunger into the column.
   ▲ **Note:** To increase the purity of the magnetically labeled fraction pass the cells over a new, freshly prepared column.

Positive selection with the autoMACS Separator

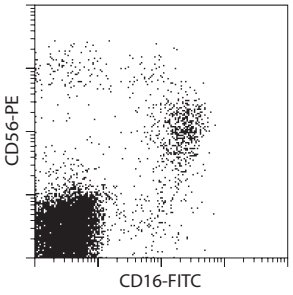
▲ Refer to the autoMACS User Manual for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose separation program "Possel".
3. Collect positive fraction from outlet port pos1. This is the enriched CD56$^+$CD16$^-$ NK cell fraction.

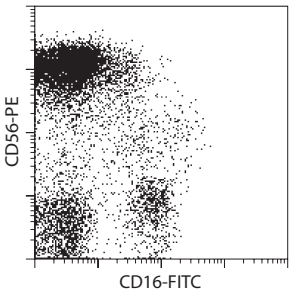
3. Example of a separation using the CD56$^+$CD16$^-$ NK Cell Isolation Kit

CD56$^+$CD16$^-$ NK cells were isolated from human PBMCs using the CD56$^+$CD16$^-$ NK Cell Isolation Kit, an LS and an MS Column, a MidiMACS™ and a MiniMACS™ Separator. Cells are fluorescently stained with CD16-FITC, CD56-PE, and CD3-PE-Cy5 for detection of NK/T cells. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.

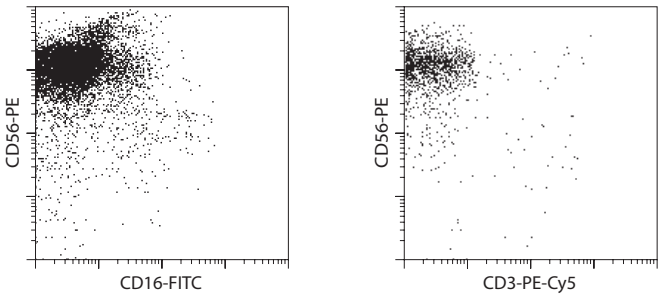
PBMCs before separation



Pre-enriched NK cells after depletion of non-CD56$^+$CD16$^-$ cells



Isolated CD56$^+$CD16$^-$ NK cells



4. References

1. Deniz, G. *et al.* (2002) Human NK1 and NK2 subsets determined by purification of IFN-γ-secreting and IFN-γ-nonsecreting NK cells. Eur. J. Immunol. 32: 879–884.
2. Gerosa, F. *et al.* (2005) The reciprocal interaction of NK cells with plasmacytoid or myeloid dendritic cells profoundly affects innate resistance functions. J. Immunol. 174: 727–734.
3. Romagnani, C. *et al.* (2005) Activation of human NK cells by plasmacytoid dendritic cells and its modulation by CD4$^+$ T helper cells and CD4$^+$ CD25^{hi} T regulatory cells. Eur. J. Immunol. 35: 2452–2458.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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