Miltenyi Biotec

CD56$^+$CD8$^+$/CD8$^-$ NK Cell Isolation Kit
human

Order no. 130-092-659

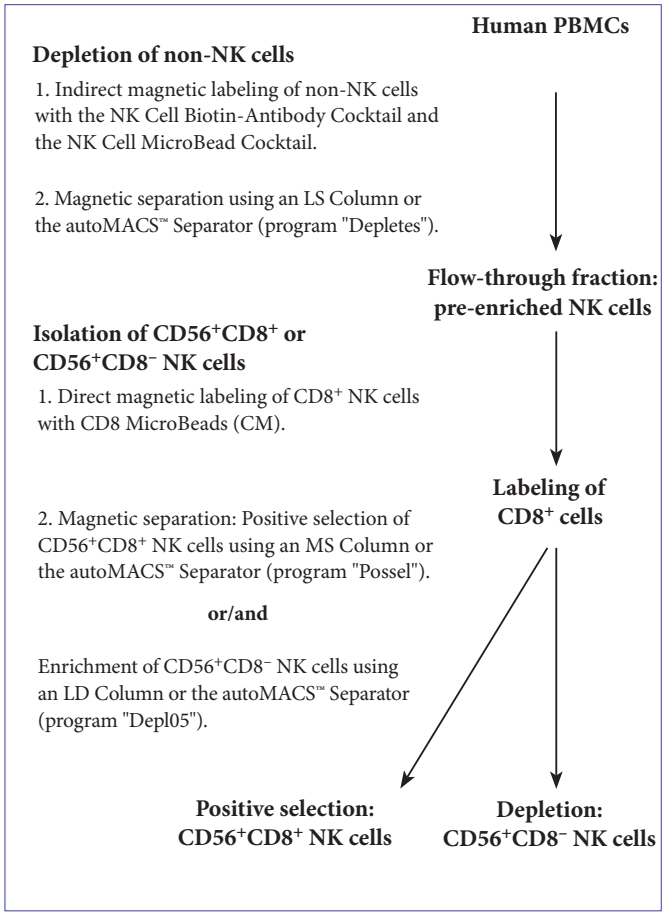
Index

1. Description

Components	**2 mL NK Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal anti-human antibodies against antigens not expressed by NK cells.
	2 × 2 mL NK Cell MicroBead Cocktail, human: Cocktail of MicroBeads conjugated to monoclonal antibodies.
	2 mL CD8 MicroBeads (CM), human: MicroBeads conjugated to a monoclonal CD8 antibody (isotype: mouse IgG2a).
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 both CD56$^+$CD8$^+$ and/or CD56$^+$CD8$^-$ NK cell subsets is performed in a two-step procedure. In both cases, non-NK cells, i.e. T cells, B cells, dendritic cells, stem cells, monocytes, granulocytes, and erythroid cells are first 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 non-NK cells are retained within the column, while the unlabeled NK cells run through. In the second step, the pre-enriched NK cells are directly labeled with CD8 MicroBeads (CM). Upon subsequent magnetic separation, either the non-labeled CD56$^+$CD8$^-$ NK cells are retrieved from the flow-through fraction, or the labeled CD56$^+$CD8$^+$ NK cells are eluted after removing the column from the magnetic field.



Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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$^+$CD8$^+$/CD8$^-$ NK Isolation Kit was developed to enrich two NK cell populations: CD3$^-$CD56$^+$CD8$^+$ and CD3$^-$CD56$^+$CD8$^-$ NK cells. CD3$^-$CD56$^+$CD8$^+$ NK cells represent approx. 3% and CD3$^-$CD56$^+$CD8$^-$ NK cells about 4% of all PBMCs in healthy donors.

Both NK cell populations differ in the presence of homodimeric CD8 formed by two α-chains. Recently, the expression of CD8α/α on CD3$^-$ NK cells revealed to be of fundamental significance, as the CD3$^-$CD56$^+$CD8$^+$ subset was shown to have a stronger cytotoxic function compared to its CD8$^-$ counterpart.[1]

Example applications

- Specific isolation of CD56$^+$CD8$^+$ or CD56$^+$CD8$^-$ 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.
- 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-NK cells is performed on an LS Column. The subsequent positive selection of CD56$^+$CD8$^+$ NK cells is performed on an MS Column. If CD56$^+$CD8$^-$ NK cells are to be enriched, then an LD Column is used for the second cell separation step. If both NK cell populations are needed, first isolate the CD56$^+$CD8$^+$ NK cells over an MS Column. The flow-through fraction of this separation can then be passed over an LD Column to additionally enrich the CD56$^+$CD8$^-$ NK cell population. 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			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, 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) and Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856).

  ▲ **Note:** For staining of CD8, we recommend using antibodies that recognize an other epitope than clone BW135/80.

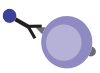
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the labeling and discrimination of dead cells by flow cytometry.

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-NK 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.

140-001-859.03

▲ 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 **NK Cell Biotin-Antibody Cocktail** per 10^8 cells.
5. Mix well and refrigerate for 10 minutes (4–8 °C).
6. Add additional 300 µL of buffer per 10^8 cells.
7. Add 200 µL of **NK Cell MicroBead Cocktail** per 10^8 cells.
8. Mix well and refrigerate for additional 15 minutes (4–8 °C).
9. Wash cells by adding 10–20 mL of buffer and centrifuge at 300 × g for 10 minutes at 4–8 °C. Aspirate supernatant completely.
10. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For larger cell numbers, scale up buffer volume accordingly.
11. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of non-NK 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 $CD56^+CD8^+$ or $CD56^+CD8^-$ 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 $CD56^+CD8^+$ or $CD56^+CD8^-$ NK cells.

2.4 Magnetic labeling of $CD56^+CD8^+$ 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 **CD8 MicroBeads (CM)**.
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, 2.6, or 2.7).

2.5 Magnetic separation: Positive selection of $CD56^+CD8^+$ NK cells

Positive selection with MS Column

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. (Optional) For isolation of $CD56^+CD8^-$ NK cells, keep flow-through and wash fractions (2.7).
6. Remove column from the separator and place it on a suitable collection tube.
7. Pipette 1 mL of buffer onto the column. Immediately flush out the fraction with magnetically labeled cells ($CD56^+CD8^+$ 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 $CD56^+CD8^+$ NK cell fraction.
4. (Optional) Collect negative fraction from outlet port neg 1. This fraction can be used for isolation of $CD56^+CD8^-$ NK cells (2.7).

2.6 Magnetic separation: Enrichment of CD56$^+$CD8$^-$ NK cells

Depletion with an LD Column

1. Place an LD Column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 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 enriched CD56$^+$CD8$^-$ NK cell fraction.

Depletion with the autoMACS™ Separator

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

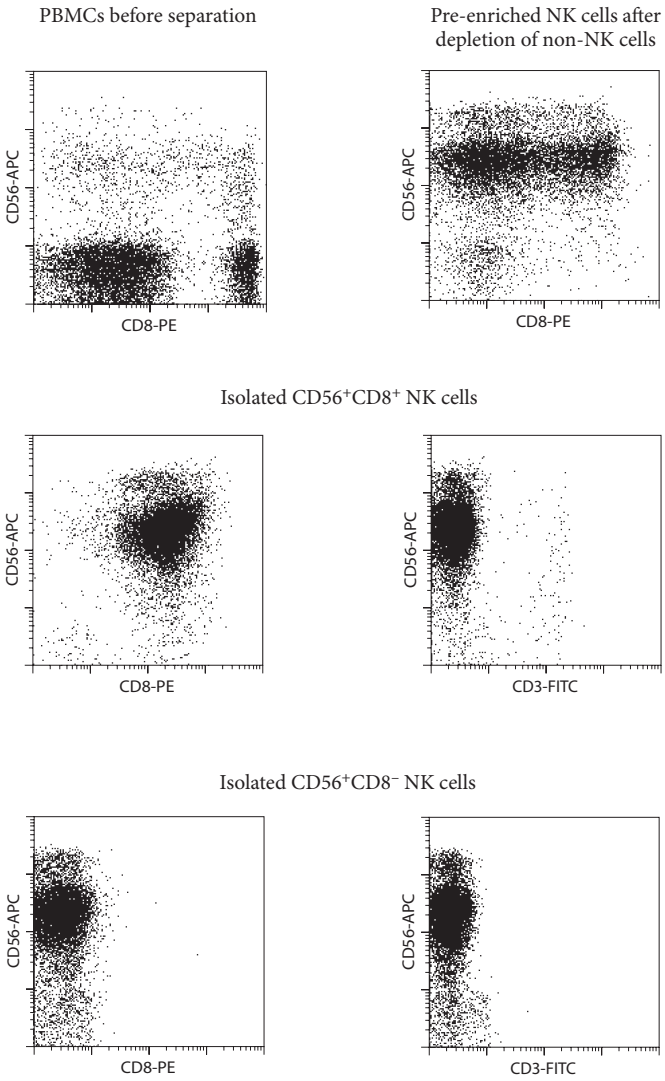
1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose separation program "Depl05".
3. Collect unlabeled fraction from outlet port neg1. This is the enriched CD56$^+$CD8$^-$ NK cell fraction.

2.7 Magnetic separation: Isolation of CD56$^+$CD8$^+$ and CD56$^+$CD8$^-$ NK cells

▲ If both NK cell populations – CD56$^+$CD8$^+$ and CD56$^+$CD8$^-$ – are needed, first isolate the CD56$^+$CD8$^+$ NK cells over an MS Column or using the autoMACS Separator, program "Possel" (see section 2.5). The flow-through fraction of this separation can then be passed over an LD Column or using the autoMACS Separator, program "Depl05" (see section 2.6) to additionally enrich the CD56$^+$CD8$^-$ NK cell population.

3. Examples of a separation using the CD56$^+$CD8$^+$/CD8$^-$ NK Cell Isolation Kit

Both the CD56$^+$CD8$^+$ and CD56$^+$CD8$^-$ NK cell subsets were isolated from human PBMCs using the CD56$^+$CD8$^+$/CD8$^-$ NK Cell Isolation Kit. Non-NK cells were depleted by separation using an LS Column. The enriched NK cells were then magnetically labeled with CD8 MicroBeads (CM), and CD56$^+$CD8$^+$ NK cells were isolated by positive selection using an MS Column. For isolation of the CD56$^+$CD8$^-$ NK cells the wash and flow-through of the MS Column were collected and separated using an LD Column. The CD56$^+$CD8$^-$ NK cells are finally collected in the flow-through of the LD Column. Cells are fluorescently stained with CD8-PE, CD56-APC, and CD3-FITC. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.



4. Reference

1. Addison, E. G. *et al.* (2005) Ligation of CD8α on human natural killer cells prevents activation-induced apoptosis and enhances cytolytic activity. Immunology 116: 254–361.

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.

140-001-859.03

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

autoMACS, MACS, MidiMACS, the Miltenyi Biotec logo, MiniMACS, OctoMACS, QuadroMACS, SuperMACS, and VarioMACS are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Ficoll-Paque is a trademark of GE Healthcare companies.