Miltenyi Biotec

CD3+CD56+ NKT Cell Isolation Kit
human

Order no. 130-093-064

Index

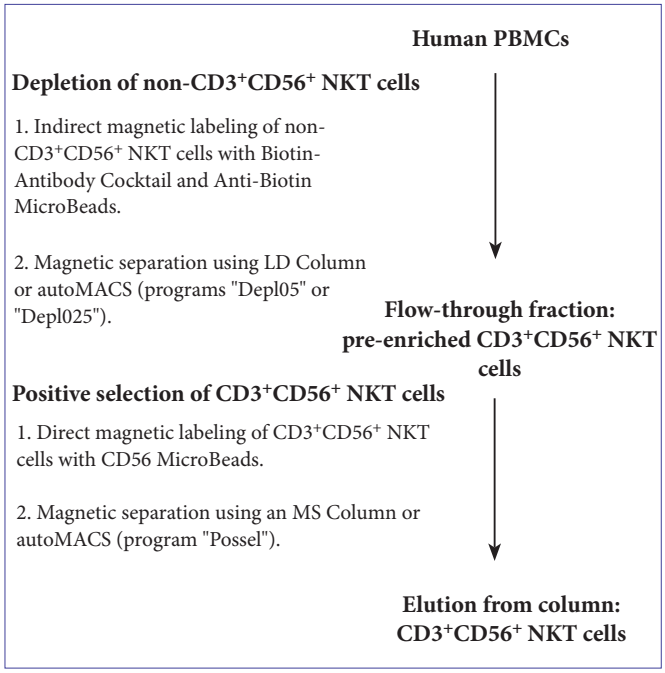
1. Description

Components	**2 mL CD3+CD56+ NKT Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal anti-human antibodies against antigens not expressed by CD3+CD56+ NKT cells. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1). **2 mL CD56 MicroBeads:** MicroBeads conjugated to monoclonal anti-CD56 antibody (clone AF12-7H3; 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 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of MACS® Separation

The CD3+CD56+ NKT Cell Isolation Kit is a two-step magnetic labeling system for the isolation of CD3+CD56+ NKT cells from human peripheral blood mononuclear cells (PBMCs). In the first step, NK cells and monocytes are indirectly magnetically labeled by using a cocktail of biotin-conjugated antibodies and Anti-Biotin MicroBeads. The labeled cells are subsequently depleted by separation over a MACS® Column.

In the second step, CD3+CD56+ NKT cells are directly labeled with CD56 MicroBeads and isolated by positive selection from the pre-enriched NKT cell fraction. The magnetically labeled CD3+CD56+ NKT cells are retained on the column and eluted after removal of the column from the magnetic field.



1.2 Background and product applications

Natural killer (NK) T cells represent a subpopulation of T cells that possess properties of NK cells.[1,2] NKT cells can be stimulated through contact with antigen or by cytokines such as IL-12 to release large amounts of cytokines and to exert cytotoxic effects. NKT cells are a crucial part of the innate immune system[3] and have an influence on the development of autoimmune diseases. They are also involved in tumor immunology as well as immunity against viruses, bacterial[4], fungal, and parasitic pathogens.

Example applications

- Cytokine production analysis after activation/stimulation.
- Gene expression analysis of NKT cell subsets.
- Analysis of the functional role of NKT cell surface receptors.
- Studies on cytotoxic and cytolytic activity.

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- Generation of NKT cell lines.
- Interaction with dendritic cells.

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 human serum albumin, 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-$CD3^+CD56^+$ NKT cells is performed on an LD Column. The subsequent positive selection of $CD3^+CD56^+$ NKT 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			
LD	10^8	5×10^8	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 VarioMACS™ Separator or SuperMACS™ Separator. For details, see MACS Separator data sheets.

- (Optional) Fluorochrome-conjugated antibody for flow cytometric analysis, e.g. CD3-FITC (# 130-080-401) and CD56-PE (BD™ Biosciences, NCAM16.2).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

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

▲ **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.

When working with tissues, prepare a single-cell suspension by a standard preparation method. For details see section General Protocols in the User Manuals or visit www.miltenyibiotec.com/protocols.

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

2.2 Magnetic labeling of non-$CD3^+CD56^+$ NKT 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 total 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 total 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.

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 **$CD3^+CD56^+$ NKT 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. Aspirate supernatant completely.
7. Resuspend cell pellet in 400 µL of buffer per 10^8 cells.
8. Add 100 µL of **Anti-Biotin MicroBeads** per 10^8 cells.
9. Mix well and refrigerate for 15 minutes (4–8 °C).
10. Wash cells by adding 5–10 mL of buffer and centrifuge at 300×g for 10 minutes. 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-CD3$^+$CD56$^+$ NKT cells

Depletion with LD Column

1. Place LD Column in the magnetic field of a suitable MACS® Separator. For details see LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with 2×1 mL of buffer. Perform washing steps by adding buffer successively once the column reservoir is empty. Collect total effluent. This contains the unlabeled pre-enriched CD3$^+$CD56$^+$ NKT cell fraction.
5. Proceed to 2.4 for the isolation of CD3$^+$CD56$^+$ NKT 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 "Depl05". If high purity of the CD3$^+$CD56$^+$ NKT cell population is desired or if the frequency of CD3$^+$CD56$^+$ NKT cells in the sample is below 5%, it is recommended to use "Depl025".
3. Collect unlabeled fraction from outlet port neg1. This is the pre-enriched CD3$^+$CD56$^+$ NKT cell fraction.
4. Proceed to 2.4 for the enrichment of CD3$^+$CD56$^+$ NKT cells.

2.4 Magnetic labeling of CD3$^+$CD56$^+$ NKT 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 per 10^8 initial cells.
3. Add 100 µL of **CD56 MicroBeads** per 10^8 initial cells.
4. Mix well and refrigerate for 15 minutes (4–8 °C).
5. Wash cells by adding 5–10 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend up to 10^8 cells in 500 µL of buffer.
7. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of CD3$^+$CD56$^+$ NKT cells

Positive selection with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details see MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which 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 fraction with magnetically labeled CD3$^+$CD56$^+$ NKT cells by firmly pushing the plunger into the 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 CD3$^+$CD56$^+$ NKT cell fraction.

2.6 (Optional) Evaluation of CD3$^+$CD56$^+$ NKT cell purity

The purity of the enriched CD3$^+$CD56$^+$ NKT cells or any intermediate fraction can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with fluorochrome-conjugated antibodies against CD3, e.g., CD3-FITC (# 130-080-401) and against CD56, e.g., CD56-PE (BD™ Biosciences, NCAM16.2) according to the manufacturer's recommendations.

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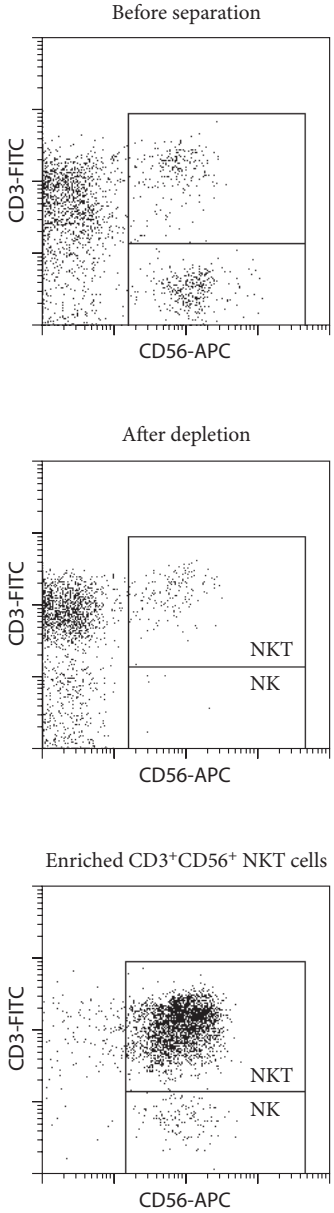
3. Example of a separation using the CD3$^+$CD56$^+$ NKT Cell Isolation Kit

CD3$^+$CD56$^+$ NKT cells were isolated from human PBMCs by using the CD3$^+$CD56$^+$ NKT Cell Isolation Kit, an LD and an MS Column, a MidiMACS™ Separator and a MiniMACS™ Separator. The cells were fluorescently stained with CD3-FITC and CD56-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

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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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