Miltenyi Biotec

NK Cell Isolation Kit
human

Order no. 130–092–657

Contents

1. Description

Components	**1 mL NK Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal anti-human antibodies against antigens not expressed by NK cells. **2 mL NK Cell MicroBead Cocktail, human:** Cocktail of MicroBeads conjugated to monoclonal antibodies.
Size	For 10^9 total cells.
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

Using the NK Cell Isolation Kit, human, NK cells are isolated by depletion of non-target cells. Non-target cells are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies against lineage-specific antigens and a cocktail of MicroBeads. In between the two labeling steps no washing steps are required. The magnetically labeled non-target cells are depleted by retaining them within a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled NK cells run through the column.

1.2 Background information

The NK Cell Isolation Kit is an indirect magnetic labeling system for the isolation of untouched NK cells from human peripheral blood mononuclear cells (PBMCs). Non-NK cells, i.e. T cells, B cells, stem cells, dendritic cells, monocytes, granulocytes, and erythroid cells, are indirectly magnetically labeled by using a cocktail of biotin-conjugated antibodies and the NK Cell MicroBead Cocktail. Isolation of highly pure NK cells is achieved by depletion of magnetically labeled cells.

1.3 Applications

- Functional studies on NK cells in which effects due to antibody-cross-linking of cell surface proteins should be avoided.
- Studies on induction of NK cell proliferation or cytotoxic and cytolytic activity.
- Analysis of "self"/"non-self" discrimination by NK cells.
- Studies on NK cell development.
- Analysis of the induction and prevention of cell death in NK cells.
- Studies on perforin, granzyme, and cytokine expression and release in NK cells.
- Analysis of the functional role of NK cell surface receptors.

1.4 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 (2–8 °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 bovine serum (FBS). Buffers or media containing $Ca2^+$ or $Mg2^+$ are not recommended for use.

- MACS Columns and MACS Separators: Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

Column	Max. number of labeled cells	Max. number of total cells	Separator
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	10^9	2×10^{10}	SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

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

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Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD56-PE (# 130-090-755). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.

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, for example, using Ficoll-Paque™.

▲ **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 or lysed blood, prepare a single-cell suspension using standard methods.

For details see the protocols section at www.miltenyibiotec.com/protocols.

▲ 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

▲ Work fast, keep cells cold, 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 10^7 total cells. When working with fewer than 10^7 cells, use same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10^7 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 labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. 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 40 µL of buffer per 10^7 total cells.
4. Add 10 µL of NK Cell Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
6. Add 30 µL of buffer per 10^7 total cells.
7. Add 20 µL of NK Cell MicroBead Cocktail per 10^7 total cells.
8. Mix well and incubate for an additional 10 minutes in the refrigerator (2–8 °C).
9. Resuspend up to 10^8 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
10. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of labeled cells and to the number of total cells. For details see table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched NK cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through, representing the enriched NK cells, and combine with the flow-through from step 3.
   MS: 500 µL LS: 3 mL
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled non-NK cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL

Magnetic separation with XS Columns

For instruction on the column assembly and the separation, refer to the XS Column data sheet.

Magnetic separation with the autoMACS® Pro Separator or the autoMACS® Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator or the autoMACS Separator.

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of ≥10 °C.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS® Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.

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3. For a standard separation choose the following program:

   Depletion: Depletes
   Collect negative fraction in row B of the tube rack.
   This fraction represents the enriched NK cells.

4. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-NK cells.

Magnetic separation with the autoMACS® Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

   Depletion: Depletes
   Collect negative fraction from outlet port neg1.
   This fraction represents the enriched NK cells.

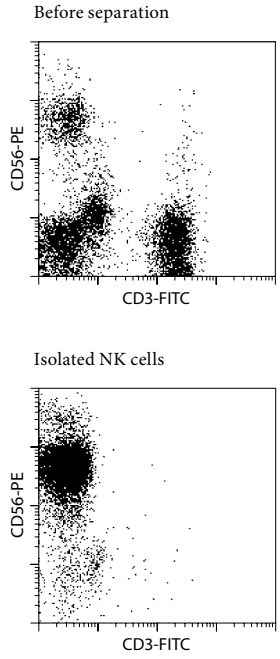
4. (Optional) Collect positive fraction from outlet port pos1. This fraction represents the magnetically labeled non-NK cells.

2.4 (Optional) Evaluation of NK cell purity

The purity of the enriched NK cells can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with a fluorochrome-conjugated antibody against a NK cell marker, e.g., CD56-PE (# 130-090-755) or CD56-APC (# 130-090-843) and optional, against a T cell marker, e.g., CD3-FITC (# 130-080-401) as recommended in the respective data sheets. Analyze cells by flow cytometry or fluorescence microscopy. Labeling of non-NK cells with the Biotin-Antibody Cocktail can be visualized by counterstaining with a fluorochrome-conjugated anti-biotin antibody, e.g. Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856). Staining with fluorochrome-conjugated streptavidin is not recommended.

3. Example of a separation using the NK Cell Isolation Kit

Untouched human NK cells were isolated from PBMCs using the NK Cell Isolation Kit, an LS Column, and a MidiMACS™ Separator. The cells were fluorescently stained with CD56-PE (# 130-090-755) and CD3-FITC (# 130-080-401) and analyzed by flow cytometry. Cell debris and dead cells Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



All protocols and data sheets are available at **www.miltenyibiotec.com**.

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.

Warranty

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