Miltenyi Biotec

NK Cell Isolation Kit
mouse

Order no. 130-115-818

Contents

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.

1. Description

This product is for research use only.

Components	**1 mL NK Cell Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal anti-mouse antibodies against non–NK cells. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 100 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 the MACS® Separation

Using the NK Cell Isolation Kit, mouse NK cells are isolated by depletion of non-target cells. Non-target cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. 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, mouse is an indirect magnetic labeling system for the isolation of untouched NK cells from suspensions of murine spleen cells. Non-NK cells, i.e. T cells, dendritic cells, B cells, granulocytes, macrophages, and erythroid cells are indirectly magnetically labelled by using a cocktail of biotin-conjugated antibodies and Anti-Biotin MicroBeads.
Isolation of highly pure unlabeled NK cells is achieved by depletion of the magnetically labeled cells.

1.3 Applications

- Isolation of NK cells from suspensions of murine spleen cells for further phenotypical or functional characterization.

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 mouse serum albumin, mouse 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
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro

▲ **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.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD45-VioGreen™, CD3-FITC, Anti-NK1.1-PE, Anti-NKp46-PE, or CD49b-APC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233), 7-AAD Staining Solution (# 130-111-568), or DAPI Staining Solution (# 130-111-570) for flow cytometric exclusion of dead cells.

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- (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.

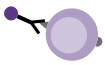
2. Protocol

2.1 Sample preparation

Prepare a single-cell suspension from spleen using manual methods or the gentleMACS™ Dissociators.

For details refer to www.gentleMACS.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, 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 10^7 total cells. When working with fewer than 10^7 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 2×10^7 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. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

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. Wash cells by adding 2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Add 80 µL of buffer per 10^7 cells.
8. Add 20 µL of Anti-Biotin MicroBeads per 10^7 cells.
9. Mix well and incubate for additional 10 minutes in the refrigerator (2–8 °C).
10. (Optional) Add staining antibodies, e.g., 10 µL of CD49b-PE, and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
11. Proceed to magnetic separation (2.3).

▲ **Note:** A minimum of 500 µL is required for magnetic separation. If necessary, add buffer to the cell suspension.

2.3 Magnetic separation

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

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

Magnetic separation with MS or LS Columns

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

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
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 the autoMACS® Pro Separator

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

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

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 and collection tubes into the Chill Rack.
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.

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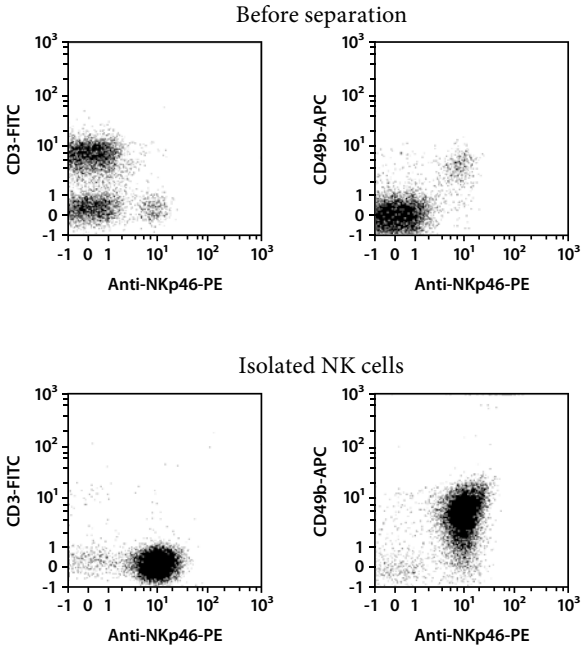
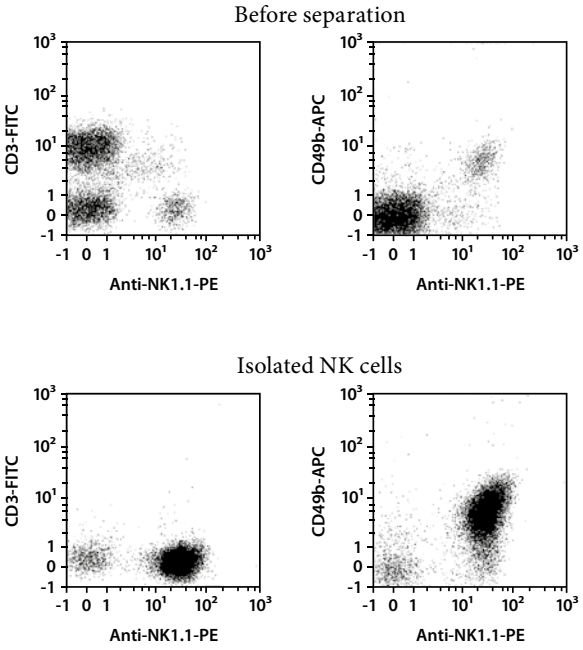
3. Example of a separation using the NK Cell Isolation Kit

NK cells were isolated from a BALB/c (A) or C57BL/6 (B) mouse spleen cell suspension by using the NK Cell Isolation Kit, an LS Column, and a MidiMACS™ Separator. The cells were fluorescently stained with CD3-FITC, CD49b-APC, and Anti-NKp46-PE (A), or CD3-FITC, CD49b-APC, and Anti-NK1.1-PE (B) and analyzed by flow cytometry using the MACSQuant® Analyzer 10. Cell debris and dead cells were excluded from the analysis based on scatter signals, CD45-VioGreen™, and 4',6-diamidino-2-phenylindole (DAPI) fluorescence.

A) BALB/c mouse spleen cell suspension



B) C57BL/6 mouse spleen cell suspension



Refer to **www.miltenyibiotec.com** for all data sheets and protocols.

Warranty

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