Miltenyi Biotec

Anti-NKp46 MicroBead Kit
mouse

Order no. 130-095-390

Contents

1. Description

Components	**1 mL Anti-NKp46-Biotin, mouse:** monoclonal anti-NKp46 antibody conjugated to biotin (isotype: rat IgG2a). **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells.
Product format	All reagents 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

First, the NKp46$^+$ cells are indirectly magnetically labeled with Anti-NKp46-Biotin antibodies and Anti-Biotin MicroBeads. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled NKp46$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of NKp46$^+$ cells. After removing the column from the magnetic field, the magnetically retained NKp46$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the NKp46$^+$ cells must be separated over a second column.

1.2 Background information

The Anti-NKp46 MicroBead Kit is an indirect magnetic labeling system for the enrichment of mouse NK cells. NKp46, also known as MAR-1 or CD335, is a type I transmembrane protein with two extracellular Ig-like domains. It is a member of the natural cytotoxicity receptor (NCR) family, which triggers cytotoxicity in NK cells. NKp46 is involved in target cell recognition and lysis and seems to be exclusively expressed on NK cells. Staining has been shown on BALB/c, SJL, CBA/CA, C57Bl/6, NOD, DBA/2, and B6.129 mice.[1]

1.3 Applications

- Positive selection of cells expressing mouse NKp46 from single-cell suspensions.

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 Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: For optimal purity of NKp46$^+$ cells the use of two consecutive MS Columns is recommended. Positive selection can also be performed by using the autoMACS Pro or the autoMACS Separator.

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
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.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis of mouse NK cells, e.g., CD3ε-FITC (# 130-092-962), Anti-NKp46-PE (# 130-095-116). For more information about antibodies refer to www.miltenyibiotec.com.
- (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 (# 130-041-407) to remove cell clumps.

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2. Protocol

2.1 Sample preparation

When working with lymphoid organs, non-lymphoid tissues, or peripheral blood, prepare a single-cell suspension using manual methods or the gentleMACS™ Dissociator.

For details refer to 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, 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, # 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 Anti-NKp46-Biotin per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Repeat washing step.
8. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
9. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
10. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
11. (Optional) Add staining antibodies, e.g., 10 µL of Anti-NKp46-PE (# 130-095-116), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
12. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
13. Resuspend up to 10^8 cells in 500 µL of buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
14. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $NKp46^+$ cells. For optimal purity the use of two consecutive MS Columns is recommended. For details refer to the table in section 1.4.

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

Magnetic separation with MS Columns

▲ To achieve highest purities, perform two consecutive column runs.

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details refer to the MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.

   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. To increase purity of $NKp46^+$ cells, enrich the eluted fraction over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

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. Program recommendations below refer to separation of mouse spleen cells.

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:

   Positive selection: Posseld2
   Collect positive fraction in row C of the tube rack.

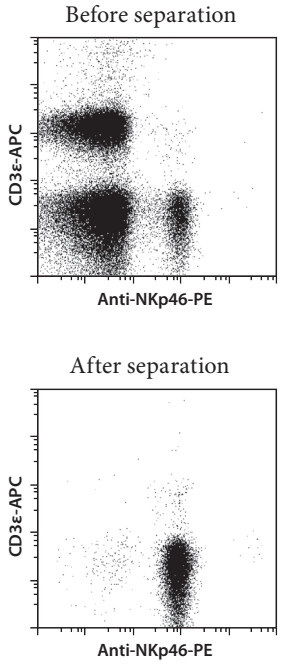
Magnetic separation with the autoMACS® Separator

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 pos2.
3. For a standard separation choose the following program:

   Positive selection: Posseld2
   Collect positive fraction from outlet port pos2.

3. Example of a separation using the Anti-NKp46 MicroBead Kit

NKp46$^+$ cells were isolated from mouse spleen using the Anti-NKp46 MicroBead Kit, two consecutive MS Columns, and a MiniMACS™ Separator. Cells were fluorescently stained with CD3ε-APC (# 130-092-977) and Anti-NKp46-PE (# 130-095-116) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. Reference

1. Walzer, T. *et al.* (2007) Identification, activation, and selective *in vivo* ablation of mouse NK cells via NKp46. Proc. Natl. Acad. Sci. USA 104: 3384–3389.

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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