Miltenyi Biotec

NK Cell Activation/ Expansion Kit
human

Order no. 130-094-483

Contents

1. Description

This product is for research use only.

Components	**2 mL MACS® Anti-Biotin MACSiBead™ Particles**, cell culture grade, corresponding to 4×10^8 MACSiBead Particles; MACSiBead Particles conjugated to monoclonal anti-biotin antibodies. **0.4 mL CD335 (NKp46)-Biotin, human – functional grade** (100 µg/mL). **0.4 mL CD2-Biotin, human – functional grade** (100 µg/mL).
Product format	All components are supplied in azide-free buffer, Anti-Biotin MACSiBead Particles contain stabilizer.
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 NK Cell Activation/Expansion Kit

TThe NK Cell Activation/Expansion Kit is designed to activate and expand human NK cells. The kit consists of Anti-Biotin MACSiBead Particles and biotinylated antibodies against human CD335 (NKp46) and CD2. Anti-Biotin MACSiBead Particles loaded with biotinylated antibodies are used to activate and expand resting NK cells purified from human blood or PBMCs.

1.2 Background information

In a first step the Anti-Biotin MACSiBead Particles are loaded with biotinylated antibodies. Best activation is achieved by using equal amounts of the provided biotinylated antibodies against CD335 (NKp46) and CD2.

▲ **Note:** Other combinations of biotinylated antibodies may be experimentally tested for their suitability, if required.

Loaded Anti-Biotin MACSiBead Particles are subsequently used for the expansion of NK cells. Best activation and expansion of NK cells is accomplished by using one loaded Anti-Biotin MACSiBead Particle per two cells (bead-to-cell ratio 1:2). The cells are cultured for further expansion.

NK cells, activated by using Anti-Biotin MACSiBead Particles, can be used for any downstream processing such as cytokine analysis, cytolytic activity, gene expression, or functional studies.

MACS Anti-Biotin MACSiBead Particles show no autofluorescence and normally do not need to be removed prior to flow cytometric analysis. However, if desired, removal of Anti-Biotin MACSiBead Particles is easily achieved by using the MACSiMAG™ Separator (see 2.6).

1.3 Applications

- Activation and expansion of resting NK cells from peripheral blood mononuclear cells (PBMCs) or after isolation with the NK Cell Isolation Kit (# 130-092-657) or CD56 MicroBeads (# 130-050-401).

1.4 Reagent and instrument requirements

- Buffer: Phosphate-buffered saline (PBS) pH 7.2, supplemented with 0.5% human serum albumin (HSA) and 2 mM EDTA. Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** HSA can be replaced by other proteins such as bovine serum albumin, fetal calf serum or human AB serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- NK Cell Isolation Kit (# 130-092-657) or CD56 MicroBeads (# 130-050-401).
- 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

▲ **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.

- Medium: NK MACS Medium (# 130-107-879) supplemented with 5% AB serum and 500 IU/mL human Interleukin 2 (IL-2).
- Human IL-2, e.g., Human IL-2 IS, premium grade (# 130-097-748).
- (Optional) MACS GMP Cell Expansion Bags (# 170-076-403) or flat bottom cell culture plates with lids.

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- Humidified incubator.
- MACSmix™ Tube Rotator (# 130-090-753) for loading of MACSiBead Particles.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis. 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.

2. Protocol

▲ All steps in the protocol have to be performed under sterile conditions.

2.1 Loading of Anti-Biotin MACSiBead™ Particles

▲ Resuspend Anti-Biotin MACSiBead™ Particles thoroughly by vortexing **before use**, to obtain a homogenous suspension.

▲ Anti-Biotin MACSiBead Particles are supplied without preservative. Remove aliquots under aseptic conditions.

▲ It is recommended to load Anti-Biotin MACSiBead Particles in batches of 1×10^8 Anti-Biotin MACSiBead Particles. Loaded Anti-Biotin MACSiBead Particles are stable for up to 2 months when stored at 2–8 °C.

1. Pipette 100 µL of CD335 (NKp46)-Biotin and 100 µL CD2-Biotin into sealable 2 mL tube and mix well.

   ▲ **Note:** This antibody combination is optimized for achieving maximum NK cell activation and expansion.

2. **Resuspend** Anti-Biotin MACSiBead Particles **thoroughly** by vortexing.
3. Remove 500 µL Anti-Biotin MACSiBead Particles (1×10^8 Anti-Biotin MACSiBead Particles) and add to antibody mix.
4. Add 300 µL buffer to adjust to a total volume of 1 mL.

   ▲ **Note:** Anti-Biotin MACSiBead Particles can be loaded in a flexible manner with biotinylated antibodies or ligands other than those supplied. If desired, add other biotinylated antibodies or ligands at appropriate concentrations and adjust with buffer to a total volume of 1 mL, accordingly.

5. Incubate for 2 hours at 2–8 °C under constant, gentle rotation by using the MACSmix Tube Rotator at approximately 4 rpm (slowest permanent run program).
6. The **loaded Anti-Biotin MACSiBead Particles** (1×10^8 Anti-Biotin MACSiBead Particles/mL) are now ready to use. **Do not remove the loaded Anti-Biotin MACSiBead Particles from the antibody mix.** Store at 2–8 °C for up to 2 months.

2.2 Magnetic separation of NK cells using the NK Cell Isolation Kit

▲ Isolate the NK cells according to the NK Cell Isolation Kit data sheet.

2.3 NK cell activation and expansion protocol

This NK cell activation and expansion protocol is optimized for NK cells that have been purified using the NK Cell Isolation Kit, using one loaded Anti-Biotin MACSiBead Particle per two NK cells (bead-to-cell ratio 1:2).

▲ **Note:** Other ratios than 1:2 of loaded Anti-Biotin MACSiBead Particles per cell may be required for other applications.

▲ Volumes for activation given below are for 10^6 purified NK cells or 10^6 PBMCs. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10^6 total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend **loaded Anti-Biotin MACSiBead Particles** thoroughly and transfer 5 µL (5×10^5 loaded Anti-Biotin MACSiBead Particles) per 10^6 NK cells to a suitable tube.

   ▲ **Note: :** If unloaded MACSiBead Particles shall be used for negative control experiments, replace loaded Anti-Biotin MACSiBead Particles by adding 5×10^5 of unloaded Anti-Biotin MACSiBead Particles per 10^6 NK cells.

2. Add 100 µL culture medium to the **loaded Anti-Biotin MACSiBead Particles** and centrifuge at 300×g for 5 minutes.
3. Aspirate supernatant and resuspend **loaded Anti-Biotin MACSiBead Particles** in 50 µL of fresh culture medium.
4. Resuspend PBMCs or purified NK cells at a density of 10^6 cells per 950 µL of culture medium (NK MACS Medium supplemented with 5% AB serum and 500 IU/mL IL-2).
5. Add the prepared Anti-Biotin MACSiBead Particles from step 3 to the 950 µL of cell suspension and mix well.
6. Add the mixture to a suitable cell culture vessel at a density of 10^6 cells per mL, for example, in the wells of a 24 well cell culture plate.
7. Incubate at 37 °C and 5% CO_2.

▲ Inspect cultures daily, and add fresh medium if required.

▲ For NK cell expansion the addition of culture medium is required regularly. In the following a guideline for the stimulation and expansion of NK cells is described.

8. At day 6, gently pipette cell suspension up and down to break up clumps.
9. Determine cell number and dilute to $1–1.5\times10^6$ cells per mL by adding fresh culture medium (NK MACS Medium supplemented with 5% AB serum and 500 IU/mL IL-2). Transfer to a fresh culture vessel of appropriate size.

▲ NK cell expansion is donor-dependent. Best results are achieved when NK cells are maintained in a cell density of $1–1.5\times10^6$ NK cells per mL. Depending on the expansion rate it might be necessary to split culture daily.

2.4 Immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for 10^6 total cells. When working with fewer than 10^6 cells and up to 10^7 cells, use the same volumes as indicated.

▲ MACSiBead Particles show no autofluorescence and do not need to be removed prior to flow cytometric analysis.

▲ Scatter properties of cells may be altered due to strong interaction between cells and MACSiBead Particles.

1. Resuspend cells to break up cell clumps.
2. Wash cells by adding 1–2 mL of buffer per 10^6 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Add 10 µL of each staining antibody, e.g., CD3-FITC (# 130-080-401) and CD56-PE (# 130-090-755) to 10^6 cells resuspended with buffer to a total volume of 110 µL.

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4. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **Note:** Working on ice requires increased incubation time. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.

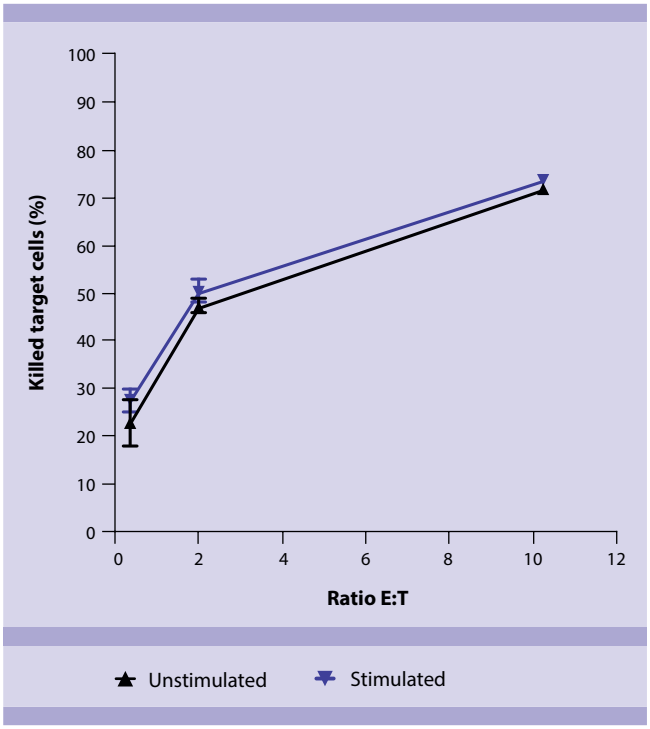
5. Wash cells by adding 1–2 mL of buffer per 10^6 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

6. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Example of NK cell activation and expansion using the NK Cell Activation/Expansion Kit

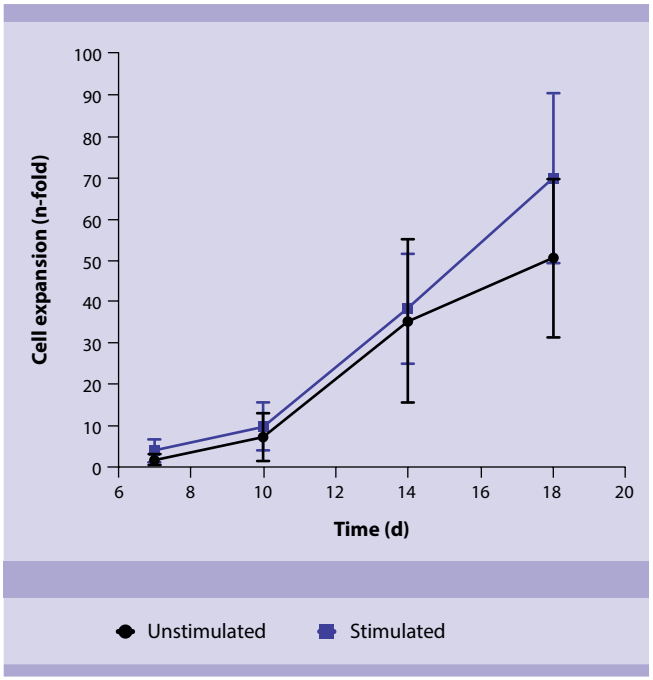
A) Cytotoxicity of expanded NK cells

Anti-Biotin MACSiBead Particles were loaded with CD335 (NKp46) and CD2 antibodies. NK cells were activated and expanded from PBMCs using one loaded MACSiBead particle per viable cell. Cells were cultured in NK MACS Medium supplemented with 5% AB serum and 500 IU/mL IL-2. After 2 weeks, NK cells were incubated with K562 target cells in different ratios. In addition, NK cells incubated in NK MACS Medium supplemented with 5% AB serum and 500 IU/mL IL-2 alone were analyzed. Specific target cell lysis by NK cells was determined after 4 hours.



B) NK cell expansion rates

Anti-Biotin MACSiBead Particles were loaded with CD335 (NKp46) and CD2 antibodies. NK cells were isolated using the NK Cell Isolation Kit and expanded using 1 loaded Anti-Biotin MACSiBead Particle per 2 NK cells. Cells were cultured in NK MACS Medium supplemented with 5% AB serum and 500 IU/mL IL-2 at an initial density of 10^6 NK cells per mL. Cells were expanded for 18 days.
For comparison NK cells were cultured in medium supplemented with 5% AB serum and 500 IU/mL IL-2 alone.



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

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