Miltenyi Biotec

Naive CD8$^+$ T Cell Isolation Kit
human

Order no. 130-093-244

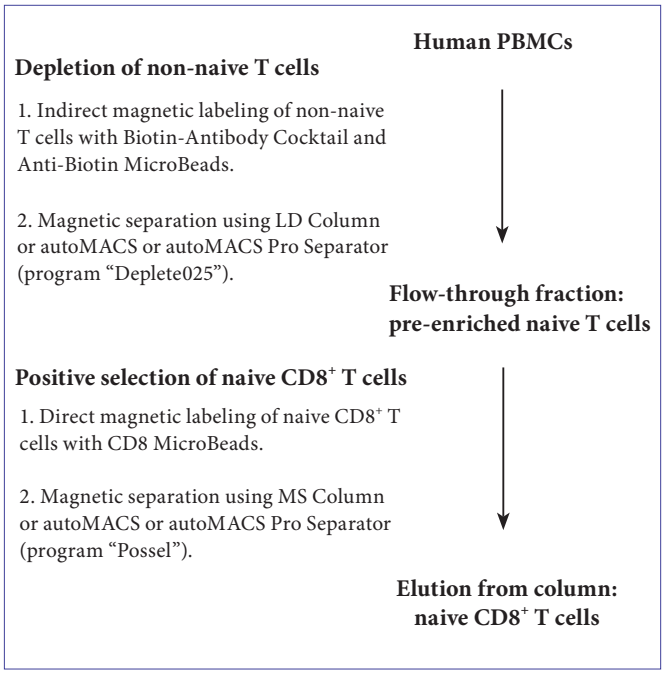
Contents

1. Description

Components	**1 mL Naive CD8$^+$ T Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal anti-human antibodies against CD45RO, CD56, CD57, and CD244. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1). **2 mL CD8 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-CD8 antibodies (isotype: mouse IgG2a).
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

The Naive CD8$^+$ T Cell Isolation Kit is developed for the isolation of human naive CD8$^+$ T cells from peripheral blood mononuclear cells (PBMCs). Isolation of naive CD8$^+$ T cells is performed in a two-step procedure. First, non-naive T cells and NK cells are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies and Anti-Biotin MicroBeads. Upon subsequent magnetic separation of the cells over a MACS® Column that is placed in a magnetic field of a MACS Separator, the magnetically labeled non-naive T cells and NK cells are retained within the column while unlabeled enriched naive T cells pass through. In the second step, the enriched naive T cells are directly labeled with CD8 MicroBeads. After removing the column from the magnetic field, the magnetically retained naive CD8$^+$ T cells can be eluted as the positively selected cell fraction.



1.2 Background information

The Naive CD8$^+$ T Cell Isolation Kit is based on a two-step procedure, the depletion of non-naive T cells and NK cells, followed by positive selection using CD8 MicroBeads. In the first step, non-naive T cells, including memory/effector T cells, activated T cells, and NK cells are depleted using biotin-conjugated antibodies against CD45RO, CD56, CD57, and CD244. CD244, also called 2B4, has been described to be predominantly expressed by effector and effector/memory T cells.[1] CD45RO, the low molecular weight isoform of the leukocyte common antigen, is used to identify Ag-experienced memory T cells.
CD57 is expressed on CD8, CD4, and NK cells and seems to be predominantly expressed by effector CD8 T cells.[2]
CD56 is a marker of human NK cells that is also present on NKT cells.
Thus, using the Antibody-Biotin Cocktail, non-naive T cells and NK cells are depleted. In the second step naive CD8$^+$ T cells are isolated by positive selection using CD8 MicroBeads.

1.3 Applications

- Phenotypic and functional studies on naive CD8$^+$ T cells.
- Studies on signal transduction or cytokine expression after activation of naive CD8$^+$ T cells.
- Analysis of interactions with other cell types.

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

- MACS Columns and MACS Separators: Depletion of non-naive T cells is performed on an LD Column. The subsequent positive selection of naive $CD8^+$ T cells is performed on an MS Column. Positive selection or depletion can also be performed by using the autoMACS or the autoMACS Pro 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, autoMACS Pro

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

- (Optional) Fluorochrome-conjugated CD8 antibody for flow cytometric analysis, e.g., CD8-FITC (# 130-080-601), CD8-PE (# 130-091-084), or CD8-APC (# 130-091-076). For more information about other fluorochrome-conjugates see www.miltenyibiotec.com.
- (Optional) Propidium iodide (PI) 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.

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™. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at 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 or lysed blood, prepare a single-cell suspension using standard methods. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available 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 of non-naive T 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 10^7 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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column. Wet filter with buffer before use.

▲ 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 Naive $CD8^+$ T Cell Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 30 µL of buffer per 10^7 total cells.
7. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
8. Mix well and incubate for an additional 15 minutes in the refrigerator (2–8 °C).
9. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
10. Resuspend up to 10^8 cells in 500 µL of buffer.

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

2.3 Magnetic separation: Depletion of non-naive T cells

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of naive $CD8^+$ T cells. For details see table in section 1.4.

Depletion with LD Columns

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 that pass through and wash column with 2×1 mL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
5. Proceed to 2.4 for further isolation of naive $CD8^+$ T cells.

Depletion with D Columns

For instructions on column assembly and separation refer to the D Column data sheet.

Depletion with the autoMACS™ Separator or the autoMACS™ Pro Separator

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

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

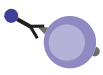
▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual. Program recommendations below refer to separation of human PBMCs.

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.
3. For a standard separation choose the following program:
   Depletion: "Depl025"
   Collect negative fraction from outlet port neg1.
4. Proceed to 2.4 for the isolation of naive $CD8^+$ T 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 fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:
   Depletion: "Depl025"
   Collect negative fraction in row B of the tube rack.
4. Proceed to 2.4 for the isolation of naive $CD8^+$ T cells.



2.4 Magnetic labeling of naive $CD8^+$ T cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^7 total cells. For higher initial cell numbers, scale up all volumes accordingly.

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 80 µL of buffer.
3. Add 20 µL of CD8 MicroBeads.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. (Optional) Add staining antibodies, e.g. CD8-PE-Cy5, CD56-PE (# 130-090-755), CD57-PE, CD45RO-PE, CD45RA-APC, and Anti-CCR7-FITC or add CD56-PE-Cy7, CD8-APC (# 130-091-076), CD45RA-FITC, and CD244-PE and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend up to 10^8 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
8. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of naive $CD8^+$ T 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 that pass through and wash column with 3×500 µL of buffer. Collect total effluent; this is the unlabeled cell fraction. 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 magnetically labeled cells by firmly pushing the plunger into the column.
7. (Optional) To increase the purity of naive $CD8^+$ T cells, the eluted fraction can be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Positive selection with the autoMACS™ Separator or the autoMACS™ Pro Separator

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:
   Positive selection: "Possel"
   Collect positive fraction from outlet port pos1. This is the enriched naive $CD8^+$ T cell fraction.

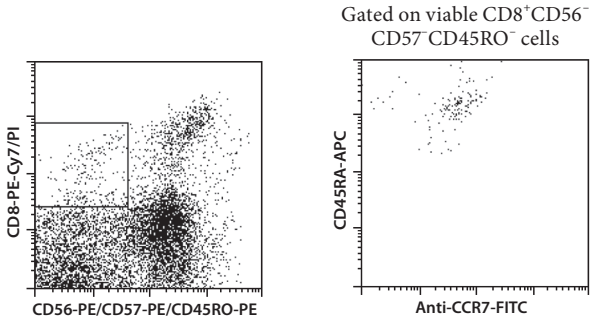
Magnetic separation with the autoMACS™ Pro Separator

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 fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:
   Positive selection: "Possel"
   Collect positive fraction in row C of the tube rack. This is the isolated naive CD8$^+$ T cell fraction.

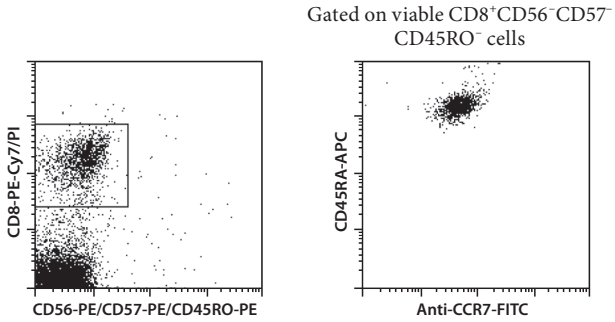
3. Example of a separation using the Naive CD8$^+$ T Cell Isolation Kit

Naive CD8$^+$ T cells were isolated from human PBMCs by using the Naive CD8$^+$ T Cell Isolation Kit, an LD and two MS Columns, a MidiMACS™ Separator and a MiniMACS™ Separator. To evaluate the separation of naive CD8 T cells, PBMCs before separation (a), the pre-enriched naive CD8$^+$ T cells (b) and the isolated naive CD8$^+$ T cells (c) are fluorescently stained and analyzed by flow cytometry using the following antibodies:
CD8-PE-Cy7, CD56-PE, CD57-PE, CD45RO-PE, CD45RA-APC, and Anti-CCR7-FITC.
The expected target cell phenotype is CD8$^+$CD56$^-$CD57$^-$CD45RO$^-$ CD45RA$^+$CCR7$^+$.
The enriched target cells (c) have the phenotype of naive CD8$^+$ T cells.
Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

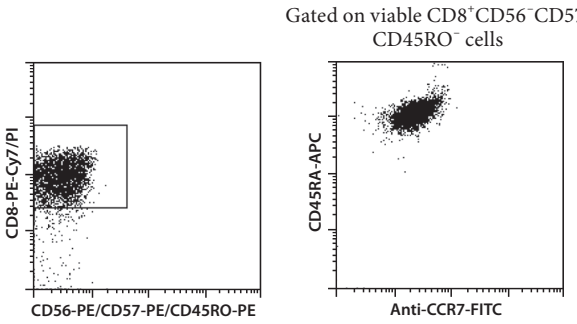
(a) Human PBMCs before separation.



(b) Pre-enriched naive CD8$^+$ T cells.



(c) Isolated naive CD8$^+$ T cells.



4. References

1. Speiser, D. E. *et al.* (2001) The activatory receptor 2B4 is expressed in vivo by human CD8$^+$ effector alpha beta T cells. J. Immunol. 167: 6165–6170.
2. Hamann, D. *et al.* (1997) Phenotypic and functional separation of memory and effector human CD8$^+$ T cells. J. Exp. Med. 186: 1407–1418.

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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