Miltenyi Biotec

CD4+ Effector Memory T Cell Isolation Kit
human

Order no. 130-094-125

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1. Description

Components	**1 mL CD4+ Effector Memory T Cell Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal antibodies against CD8, CD14, CD15, CD16, CD19, CD34, CD36, CD45RA, CD56, CD123, CD235a (Glycophorin A), TCRγ/δ, and APC-conjugated antibody against CD197 (CCR7). **2 mL CD4+ Effector Memory T Cell MicroBead Cocktail:** MicroBeads conjugated to monoclonal anti-biotin and anti-APC antibodies.
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 CD4+ Effector Memory T Cell Isolation Kit, human CD4+ effector memory T cells are isolated by depletion of non-CD4+ T cells, naive, and central memory CD4+ T cells. Non-CD4+ T cells, naive, and central memory CD4+ T cells are indirectly magnetically labeled with a cocktail of biotin- and APC-conjugated monoclonal antibodies, as primary labeling reagent, and the MicroBead Cocktail, as secondary labeling reagent.

The magnetically labeled non-CD4+ T cells, naive, and central memory CD4+ T cells are depleted by retaining them within a MACS Column in the magnetic field of a MACS Separator, while the unlabeled CD4+ effector memory T cells run through.

1.2 Background information

The CD4+ Effector Memory T Cell Isolation Kit has been developed for the isolation of untouched CD4+ effector memory T cells from human peripheral blood mononuclear cells (PBMCs).

Several developmental stages of T cells can be distinguished on the basis of the expression of CCR7 (chemokine (c-c-motif) receptor 7) and CD45 isoforms. Naive CD4+ T cells are $CD45RA^+CCR7^+$, central memory CD4+ T cells are $CD45RO^+CCR7^+$, and effector memory CD4+ T cells are $CD45RO^+CCR7^-$.[1]

Expression of CCR7 is crucial for homing of T cells to secondary lymphoid organs in the steady state.[2]

To isolate CD4+ effector memory T cells, non-CD4+ T cells , naive CD4+ T cells and central memory CD4+ T cells are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies against CD8, CD14, CD15, CD16, CD19, CD34, CD36, CD45RA, CD123, CD235a (Glycophorin A), and TCRγ/δ as well as APC-conjugated CD197 (CCR7) antibodies. CD45RA and CD197 (CCR7) antibodies are included to deplete naive and central memory CD4+ T cells, respectively, while the other antibodies label the non-CD4+ T cells. In a second step, all antibody-labeled cells are labeled with the MicroBead Cocktail. Isolation of highly pure CD4+ effector memory T cells is achieved by depletion of magnetically labeled cells.

1.3 Applications

- Functional studies on CD4+ effector memory T cells in which effects due to antibody-cross-linking of cell surface proteins should be avoided.
- Studies on signal requirements for induction of CD4+ effector memory T cell activation and proliferation.
- Studies on signal transduction during activation of CD4+ effector memory T cells.
- Studies on cytokine expression of CD4+ effector memory T cells upon restimulation.

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.

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- 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
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
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 antibody for flow cytometric analysis, e.g., CD4-FITC (# 130-080-501), CD45RO-PE, or CD45RA-PE (# 130-092-248). For more information about other fluorochrome conjugates see 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.

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-CD4$^+$ 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.

▲ 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 CD4$^+$ Effector Memory T Cell Antibody Cocktail 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. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
8. Add 20 µL of CD4$^+$ Effector Memory T Cell MicroBead Cocktail per 10^7 total cells.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
10. (Optional) Add staining antibodies, e.g., 10 µL of CD4-FITC (# 130-080-501), CD45RO-PE and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
11. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
12. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
13. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

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 which pass through and wash column with 2×1 mL of buffer. Perform washing steps by adding buffer successively once the column reservoir is empty. Collect total effluent. This contains the unlabeled enriched effector memory CD4$^+$ T cell fraction.

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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.

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: "Deplete025 "
   Collect negative fraction from outlet port neg1.

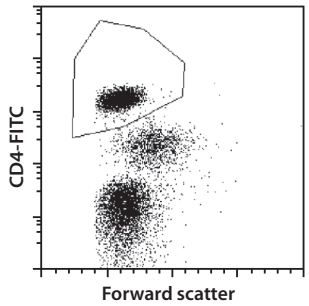
Magnetic separation with the autoMACS™ Pro Separator

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.
3. For a standard separation choose the following program:
   Depletion: "Deplete025"
   Collect negative fraction in row B of the tube rack.

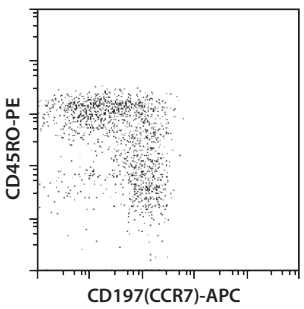
3. Example of a separation using the CD4$^+$ Effector Memory T Cell Isolation Kit

Untouched effector memory CD4$^+$ T cells were isolated from human PBMCs using the CD4$^+$ Effector Memory T Cell Isolation Kit, an LD Column, and a MidiMACS Separator. Cells are fluorescently stained with CD4-FITC (# 130-080-501) and CD45RO-PE. CCR7 is already labeled with CD197 (CCR7)-APC during the separation procedure. Cells were analyzed by flow cytometry using the MACSQuant™ Analyzer.
Cell debris and dead cells are excluded from the analysis based on scatter signals and propidium iodide fluorescence.

Before separation

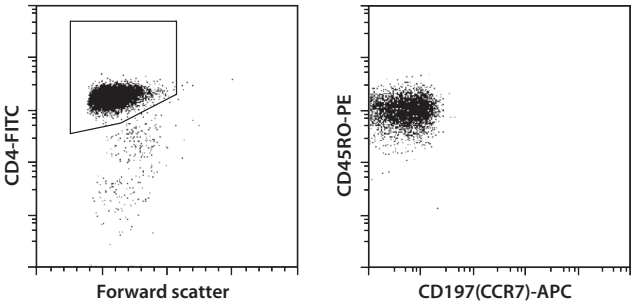


Gated on viable CD4$^+$ T cells



Isolated CD4$^+$ effector memory T cells

Gated on viable CD4$^+$ T cells



4. References

1. Sallusto, D. *et al.* (1999) Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 401: 708–712.
2. Reinhardt, R. L. *et al.* (2001) Visualizing the generation of memory CD4 T cells in the whole body. Nature 410: 101–105.

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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