Miltenyi Biotec

CD8$^+$CD45RA$^+$ Effector T Cell Isolation Kit
human

Order no. 130-094-485

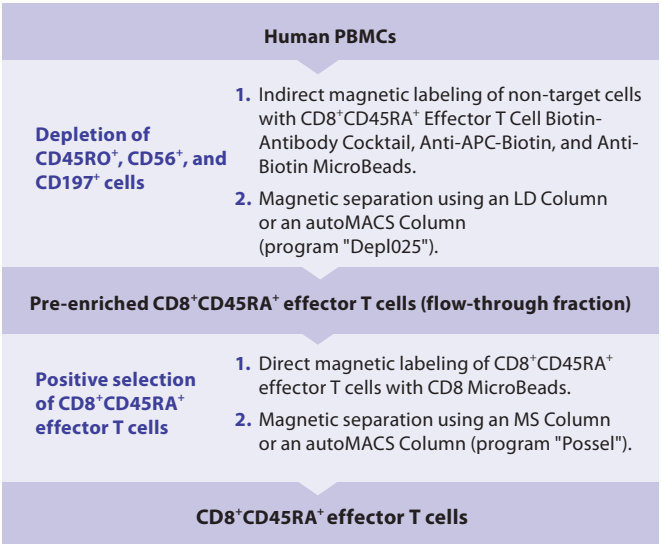
Contents

1. Description

Components	**2 mL CD8$^+$CD45RA$^+$ Effector T Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal antibodies against CD45RO, and CD56 and APC-conjugated antibody against CD197 (CCR7). **2 mL FcR Blocking Reagent, human** **1 mL Anti-APC-Biotin** **2×2 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 antibody (isotype: mouse IgG2a).
Capacity	For 2×10^9 total cells, up to 20 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 CD8$^+$CD45RA$^+$ Effector T Cell Isolation Kit has been developed for the isolation of human CD8$^+$CD45RA$^+$ effector T cells from peripheral blood mononuclear cells (PBMCs). The isolation of CD8$^+$CD45RA$^+$ effector T cells is performed in a two-step procedure. First, the non-target cells are indirectly magnetically labeled with a cocktail of biotin- and APC-conjugated antibodies, Anti-APC-Biotin 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-target cells are retained within the column while unlabeled enriched CD8$^+$CD45RA$^+$ effector T cells pass through.
In the second step, the CD8$^+$CD45RA$^+$ effector T cells are directly labeled with CD8 MicroBeads and isolated by positive selection from the pre-enriched cell fraction. The magnetically labeled CD8$^+$CD45RA$^+$ effector T cells are retained on the column and eluted after removing the column from the magnetic field.



1.2 Background information

Several developmental stages of T cells can be distinguished on the basis of the expression of CD197, also known as CCR7 (chemokine (c-c motif) receptor 7), and CD45 isoforms. Naive T cells are CD45RA$^+$CD197$^+$, central memory (T_{CM}) and effector memory (T_{EM}) T cells are CD45RO$^+$CD197$^+$ and CD45RO$^+$CD197$^-$, respectively.[1,2] Additionally, CD8 T cells are characterized by a CD45RA$^+$CD197$^-$ population that is termed effector memory RA (T_{EMRA}).[3] CD8$^+$ T_{EMRA} cells express high levels of perforin and granzyme B.
CD45RO and CD197 antibodies are included to deplete naive, T_{CM}, and T_{EM} cells, respectively. Natural killer T cells (NKT) are depleted by the addition of CD56. Using the CD8$^+$CD45RA$^+$ Effector T Cell Isolation Kit, a highly pure population of human effector T cells with the phenotype CD8$^+$CD45RA$^+$ and CD197$^-$CD56$^-$ is isolated.

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1.3 Applications

- Phenotypic and functional studies on $CD8^+CD45RA^+$ effector T cells.
- Studies on signal transduction or cytokine expression during activation of $CD8^+CD45RA^+$ effector T cells.
- Analysis of interactions with other cell types and degranulation or secretion of effector molecules of $CD8^+CD45RA^+$ effector T cells.

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

- MACS Columns and MACS Separators: $CD45RO^+$, $CD56^+$, and $CD197^+$ cells can be depleted with the use of LD Columns. $CD8^+CD45RA^+$ effector T cells can be enriched by using MS Columns. Positive selection or depletion 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
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

  ▲ **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 antibodies 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 fluorochrome-conjugated antibodies 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™.

▲ **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.

For details see 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 of $CD45RO^+$, $CD56^+$, and $CD197^+$ 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 10^8 total cells. When working with fewer than 10^8 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 2×10^8 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. 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 400 µL of buffer per 10^8 total cells.
4. Add 100 µL of **$CD8^+CD45RA^+$ Effector T Cell Biotin-Antibody Cocktail** per 10^8 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 10–20 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 350 µL of buffer per 10^8 total cells.
8. Add 100 µL of **FcR Blocking Reagent** per 10^8 total cells.
9. Add 50 µL of **Anti-APC-Biotin** per 10^8 total cells.
10. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
11. Wash cells by adding 10–20 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
12. Resuspend cells in 800 µL of buffer per 10^8 total cells.
13. Add 200 µL of **Anti-Biotin MicroBeads** per 10^8 total cells.
14. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).

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15. Wash cells by adding 10–20 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
16. Resuspend up to 10^8 cells in 1 mL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
17. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of CD45RO$^+$, CD56$^+$, and CD197$^+$ cells

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

Depletion with LD Column

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 pre-enriched CD45RO$^-$CD56$^-$CD197$^-$ cell fraction.
5. Proceed to 2.4 for the isolation of CD8$^+$CD45RA$^+$ effector T cells.

Depletion 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.

Depletion 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. Choose the following program:
   Depletion: "Depl025"
   Collect negative fraction in row B of the tube rack.
   This is the pre-enriched CD45RO$^-$CD56$^-$CD197$^-$ T cell fraction.
4. Proceed to 2.4 for the isolation of CD8$^+$CD45RA$^+$ effector T cells.

Depletion 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. Choose the following program:
   Depletion: "Depl025"
   Collect negative fraction from outlet port neg1.
   This is the pre-enriched CD45RO$^-$CD56$^-$CD197$^-$ T cell fraction.
4. Proceed to 2.4 for the isolation of CD8$^+$CD45RA$^+$ effector T cells.

2.4 Magnetic labeling of CD8$^+$CD45RA$^+$ effector T cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^8 cells. For larger initial cell numbers, scale up volumes accordingly.

1. Centrifuge cells at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 400 µL of buffer per 10^8 total cells.
3. Add 100 µL of **CD8 MicroBeads** per 10^8 total cells.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend up to 10^8 cells in 500 µL of buffer.
7. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of CD8$^+$CD45RA$^+$ effector T cells

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

Positive selection with MS Column

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. Wash column with 3×500 µL of buffer.
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 1mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

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

Positive selection 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. Choose the following program:
   Positive selection: "Possel"
   Collect positive fraction in row C of the tube rack.
   This is the enriched $CD8^+CD45RA^+$ effector T cell fraction.

Positive selection 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. Choose the following program:
   Positive selection: "Possel"
   Collect positive fraction from outlet port pos1.
   This is the enriched $CD8^+CD45RA^+$ effector T cell fraction.

2.6. (Optional) Evaluation of $CD8^+CD45RA^+$ effector T cell purity

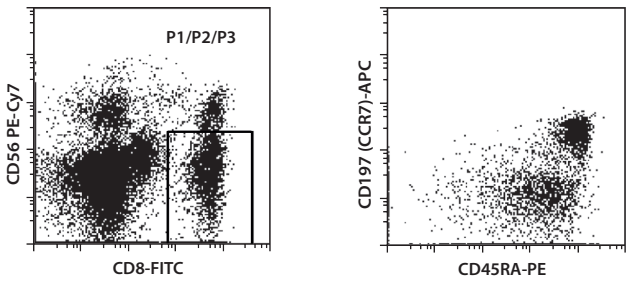
The purity of the enriched $CD8^+CD45RA^+$ effector T cells can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with a fluorochrom-conjugated antibody against, e.g., CD8-FITC (# 130-080-601), and CD45RA, e.g., CD45RA-PE (# 130-092-248). Cells are already stained with CD197-APC (see 2.2 Magnetic labeling of $CD45RO^+$, $CD56^+$, and $CD197^+$ ($CCR7^+$) cells).

3. Example of a separation using the $CD8^+CD45RA^+$ Effector T Cell Isolation Kit

$CD8^+CD45RA^+$ effector T cells were isolated from human PBMCs using the $CD8^+CD45RA^+$ Effector T Cell Isolation Kit, an LD Column, an MS Column, a MidiMACS™ Separator, and a MiniMACS™ Separator. Cells were fluorescently stained with CD8-FITC (# 130-080-601), CD45RA-PE (# 130-092-248), CD56 PE-Cy™7 (NCAM 16.2), and CD197 (CCR7)-APC (included in the kit) and analysed 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.

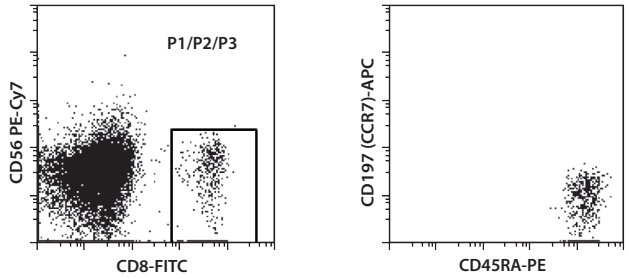
PBMCs before separation

Gated on viable $CD8^+CD56^-$ cells.



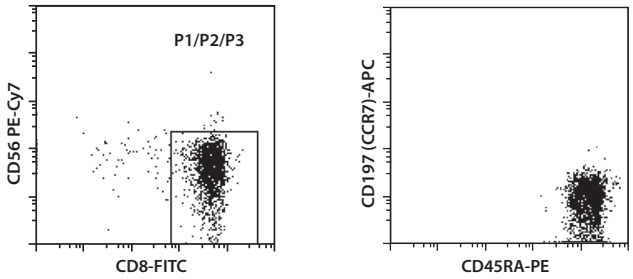
Pre-enriched $CD8^+CD45RA^+$ effector T cells

Gated on viable $CD8^+CD56^-$ cells.



Isolated $CD8^+CD45RA^+$ effector T cells

Gated on viable $CD8^+CD56^-$ cells.



4. References

1. Sallusto, F. *et al.* (1999) Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 401: 708–712.
2. Hamann, D. *et al.* (1997) Phenotypic and Functional Separation of Memory and Effector Human $CD8^+$ T Cells. J. Exp. Med. 186: 1407–1418.
3. Geginat, J. *et al.* (2003) Proliferation and differentiation potential of human $CD8^+$ memory T-cell subsets in reponse to antigen or homeostatic cytokines. Blood 101: 4260–4266.

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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