Miltenyi Biotec

CD4+ Central Memory T Cell Isolation Kit
human

Order no. 130-094-302

Contents

1. Description

Components

1 mL CD4+ Central Memory T Cell Biotin-Antibody Cocktail, human:
Cocktail of biotin-conjugated monoclonal antibodies against CD8, CD14, CD15, CD16, CD19, CD25, CD36, CD45RA, CD56, CD123, TCRγ/δ, and CD235a (Glycophorin A).

2 mL Anti-Biotin MicroBeads:
MicroBeads conjugated to a monoclonal anti-biotin antibody (isotype: mouse IgG1).

100 µL CD197 (CCR7)-PE, human:
Monoclonal anti-human CD197 antibody conjugated to R-phycoerythrin (PE) (isotype: mouse IgG2A).

1 mL Anti-PE MicroBeads:
MicroBeads conjugated to a monoclonal anti-PE antibody.

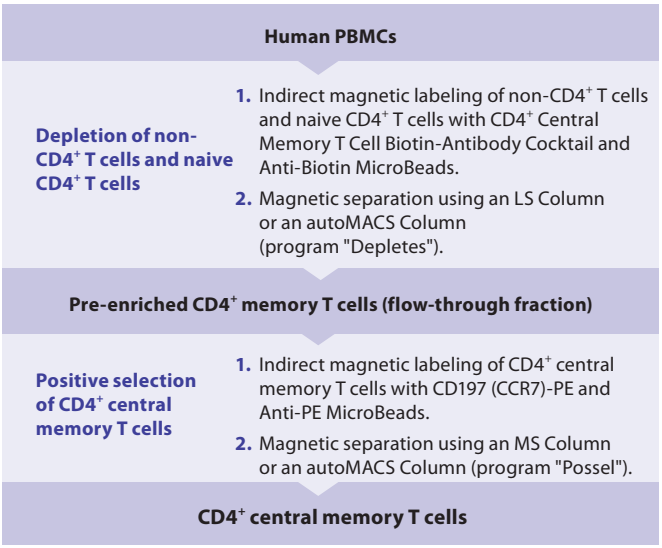
Capacity
For 10^9 total cells.

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 isolation of CD4+ central memory T cells is performed in a two-step procedure. First, non-CD4+ T cells and CD4+ naive T cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies and Anti-Biotin MicroBeads. The labeled cells are subsequently depleted by separation over a MACS® Column.
In the second step, CD4+ central memory T cells are indirectly magnetically labeled with CD197 (CCR7)-PE and Anti-PE MicroBeads and isolated by positive selection from the pre-enriched CD4+ memory T cell fraction. After removing the column from the magnetic field, the magnetically retained CD4+ central memory T cells can be eluted as the positively selected cell fraction.



1.2 Background information

The CD4+ Central Memory T Cell Isolation Kit is an indirect magnetic labeling system for the isolation of CD4+ central 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 pattern of chemokine (C-C motif) receptor 7 (CCR7), designated as CD197, and CD45 isoforms. CD4+ naive T cells are $CD45RA^+CCR7^+$, CD4+ central memory T cells are $CD45RO^+CCR7^+$, and CD4+ effector memory T cells are $CD45RO^+CCR7^-$.[1]
Expression of CCR7 is crucial for homing of T cells to secondary lymphoid organs.[2]
CD4+ central memory T cells are isolated using the CD4+ Central Memory T Cell Isolation Kit based on the expression of CCR7 and absence of CD45RA.
Non-CD4+ T cells and CD4+ naive T cells are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies against CD8, CD14, CD15, CD16, CD19, CD25, CD36, CD45RA, CD56, CD123, TCRγ/δ, and CD235a (Glycophorin A) and Anti-Biotin MicroBeads. CD45RA antibody is included to deplete CD4+ naive T cells, while the other antibodies label the non-CD4+ T cells. After depletion of the non-CD4+ T cells and CD4+ naive T cells, CD4+ central memory T cells are indirectly magnetically labeled with CD197 (CCR7)-PE and Anti-PE MicroBeads for positive selection.

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1.3 Application

- Isolation of $CD4^+$ central memory T cells from human peripheral blood mononuclear cells (PBMCs).

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-$CD4^+$ T cells is performed on an LS Column. The subsequent positive selection of $CD4^+$ central memory T cells is performed on an MS Column. 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
Depletion			
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, 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 antibodies for flow cytometric analysis, e.g., CD4-FITC (# 130-080-501), CD4-APC (# 130-091-232), CD45RA-FITC (# 130-092-247), or CD45RA-APC (# 130-092-249). For more information about 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 protocols section 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.

▲ 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 and $CD4^+$ 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 5×10^7 total cells. When working with fewer than 5×10^7 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 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 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 200 µL of buffer per 5×10^7 total cells.
4. Add 50 µL of $CD4^+$ Central Memory T Cell Biotin-Antibody Cocktail per 5×10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 150 µL of cold buffer and 100 µL of Anti-Biotin MicroBeads per 5×10^7 total cells.
7. Mix well and incubate for an additional 15 minutes in the refrigerator (2–8 °C).
8. Wash cells by adding 5–10 mL of buffer per 5×10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend up to 10^8 cells in 1 mL of buffer.

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

2.3 Magnetic separation: Depletion of non-$CD4^+$ T cells and $CD4^+$ naive T cells

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $CD4^+$ memory T cells. For details see table in section 1.4.

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

Depletion with LS Columns

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details see the LS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×3 mL of buffer. Collect total effluent and combine with the effluent from step 3; this is the unlabeled pre-enriched $CD4^+$ memory T cell fraction.

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. Proceed to 2.4 for further isolation of $CD4^+$ central memory 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

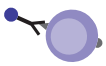
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

   Depletion: "Depletes"
   Collect negative fraction in row B of the tube rack. This fraction represents the unlabeled pre-enriched $CD4^+$ memory T cell fraction.
4. Proceed to 2.4 for further isolation of $CD4^+$ central memory 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. For a standard separation choose the following program:

   Depletion: "Depletes"
   Collect negative fraction from outlet port neg1. This fraction represents the unlabeled pre-enriched $CD4^+$ memory T cell fraction.
4. Proceed to 2.4 for further isolation of $CD4^+$ central memory T cells.



2.4 Magnetic labeling of $CD4^+$ central memory T cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 5×10^7 total cells. When working with fewer than 5×10^7 cells, use the same volumes as indicated. When working with higher initial cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 10^8 total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 50 µL of buffer.
3. Add 5 µL of CD197 (CCR7)-PE.
4. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 500–1000 µL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend cell pellet in 50 µL of Anti-PE MicroBeads.
7. Mix well and incubate for an additional 15 minutes in the refrigerator (2–8 °C).
8. Wash cells by adding 500–1000 µL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend up to 10^8 starting cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
10. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of $CD4^+$ central memory T cells

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $CD4^+$ central memory T cells. For details see table in section 1.4.

Positive selection with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details see the 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. This fraction represents the magnetically labeled $CD4^+$ central memory T cells.

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Positive selection 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.

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. For a standard separation choose the following program:
   Positive selection: "Possel"
   Collect positive fraction in row C of the tube rack. This fraction represents the magnetically labeled $CD4^+$ central memory T cells.

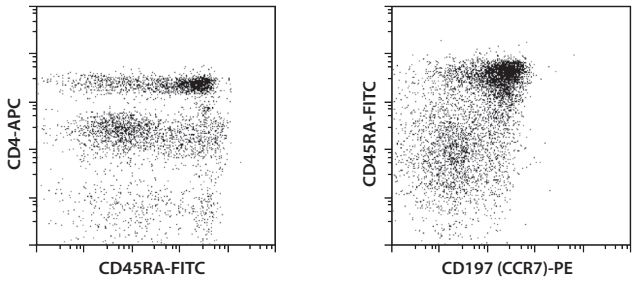
Positive selection with the autoMACS® Separator

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. For a standard separation choose the following program:
   Positive selection: "Possel"
   Collect positive fraction from outlet port pos1. This fraction represents the magnetically labeled $CD4^+$ central memory T cells.

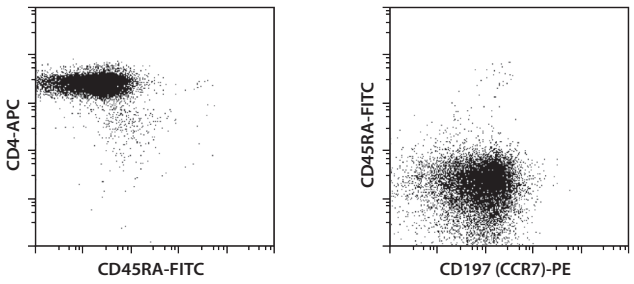
3. Example of a separation using the $CD4^+$ Central Memory T Cell Isolation Kit

$CD4^+$ central memory T cells were isolated from human PBMCs using the $CD4^+$ Central Memory T Cell Isolation Kit, an LS and an MS Column, a MidiMACS™ Separator, and a MiniMACS™ Separator. Cells are fluorescently stained with CD4-APC (# 130-091-232) and CD45RA-FITC (# 130-092-247) and analyzed using the MACSQuant® Analyzer. The cells are already labeled with CD197 (CCR7)-PE during the isolation procedure. Cell debris and dead cells are excluded from the analysis based on scatter signals and propidium iodide fluorescence.

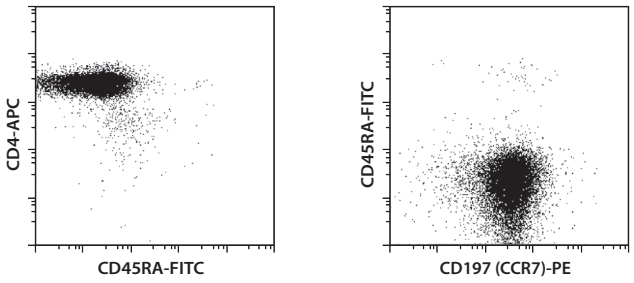
Before separation



Pre-enriched $CD4^+$ memory T cells



$CD4^+$ central memory T 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. 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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