Miltenyi Biotec

Memory CD4$^+$ T Cell Isolation Kit
human

Order no. 130-091-893

Contents

1. Description

Components	**1 mL Memory CD4$^+$ T Cell Biotin-Antibody Cocktail,** human: Cocktail of biotin-conjugated monoclonal antibodies against CD8, CD14, CD16, CD19, CD36, CD45RA, CD56, CD123, TCRγ/δ and Glycophorin A. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to a monoclonal anti-biotin antibody.
Capacity	For 10^9 total cells, up to 100 separations.
Product format	The Biotin-Antibody Cocktail and the Anti-Biotin MicroBeads 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 Memory CD4$^+$ T Cell Isolation Kit, human memory CD4$^+$ T cells are isolated by depletion of non–CD4$^+$ T cells and naive CD4$^+$ T cells (negative selection). Non–CD4$^+$ T cells and naive CD4$^+$ T cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and Anti-Biotin MicroBeads, as secondary labeling reagent. No washing steps are required in between the two labeling steps. The magnetically labeled non–CD4$^+$ T cells and naive CD4$^+$ T cells are depleted by retaining them within a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled memory CD4$^+$ T cells run through.

1.2 Background information

The Memory CD4$^+$ T Cell Isolation Kit is an indirect magnetic labeling system for the isolation of untouched memory CD4$^+$ T cells from human peripheral blood mononuclear cells (PBMCs). Naive CD4$^+$ T cells and non-CD4$^+$ T cells, i.e. CD45RA$^+$CD4$^+$ T cells, CD8$^+$ T cells, γ/δ T cells, B cells, NK cells, dendritic cells, monocytes, granulocytes and erythroid cells, are indirectly magnetically labeled using a cocktail of biotin-conjugated antibodies against CD8, CD14, CD16, CD19, CD36, CD45RA, CD56, CD123, TCRγ/δ and Glycophorin A, and Anti-Biotin MicroBeads. Isolation of highly pure memory CD4$^+$ T cells is achieved by depletion of magnetically labeled cells.

1.3 Applications

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

- 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
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	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.

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- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD45RO-FITC or Anti-Biotin-APC (# 130-090-856). 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.

▲ 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

▲ 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 Memory $CD4^+$ 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

▲ Choose an appropriate MACS Column and MACS Separator according to the number of labeled cells and to the number of total cells. For details see table in section 1.4.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the enriched memory $CD4^+$ T cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette the appropriate amount 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 non–$CD4^+$ T cells and naive $CD4^+$ T cells.
   MS: 1 mL LS: 5 mL

Magnetic separation with XS Columns

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

Magnetic separation 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.

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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 optional at port pos1).
3. For a standard separation choose one of the following programs:

   Depletion: "Deplete"
   Collect negative fraction from outlet port neg1. This fraction represents the enriched memory $CD4^+$ T cells.
4. (Optional) Collect positive fraction from outlet port pos1. This fraction represents the magnetically labeled non–$CD4^+$ T cells and naive $CD4^+$ 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 row B (and optional in row C).
3. For a standard separation choose one of the following programs:

   Depletion: "Deplete"
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched memory $CD4^+$ T cells.
4. (Optional) Collect positive fraction from row C. This fraction represents the magnetically labeled non–$CD4^+$ T cells and naive $CD4^+$ T cells.

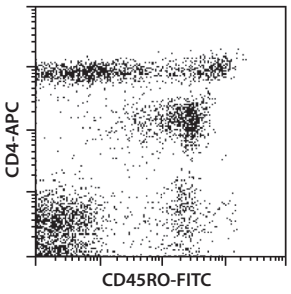
2.4 (Optional) Evaluation of memory $CD4^+$ T cell purity

The purity of the enriched memory $CD4^+$ T cells can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with fluorochrome-conjugated antibodies against CD45RO and CD4, for example, CD4-FITC (# 130-080-501), CD4-PE (# 130-091-231), or CD4-APC (# 130-091-232), as recommended in the respective data sheets. Analyze cells by flow cytometry or fluorescence microscopy. Labeling of non–$CD4^+$ T cells and naive $CD4^+$ T cells with the Memory $CD4^+$ T Cell Biotin-Antibody Cocktail can be visualized by counterstaining with a fluorochrome-conjugated anti-biotin antibody, e.g., Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856). Staining with fluorochrome-conjugated streptavidin is not recommended.

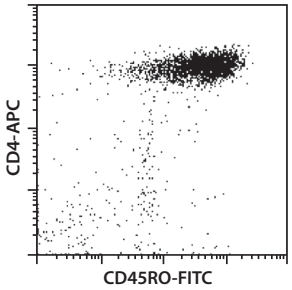
3. Example of a separation using the Memory $CD4^+$ T Cell Isolation Kit

Untouched memory $CD4^+$ T cells were isolated from human PBMCs using the Memory $CD4^+$ T Cell Isolation Kit, an LS Column, and a MidiMACS™ Separator. Cells are fluorescently stained with CD45RO-FITC and CD4-APC (# 130-091-232). Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.

Before separation



Isolated memory $CD4^+$ T cells



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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