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1. Description

Components	<p>1 mL Naive CD4⁺ T Cell Biotin-Antibody Cocktail II, human: Cocktail of biotin-conjugated monoclonal antibodies against CD8, CD14, CD15, CD16, CD19, CD25, CD34, CD36, CD45RO, CD56, CD123, TCRγ/δ, HLA-DR, and CD235a (Glycophorin A).</p> <p>2 mL Naive CD4⁺ T Cell MicroBead Cocktail II, human: MicroBeads conjugated to a monoclonal anti-CD61 antibody (isotype: mouse IgG1) and anti-biotin antibody (isotype: mouse IgG1).</p>
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

Using the Naive CD4⁺ T Cell Isolation Kit II, human naive CD4⁺ T cells are isolated by depletion of non-T helper cells and memory CD4⁺ T cells. Non-T helper cells and memory CD4⁺ T cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies and the Naive CD4⁺ T Cell MicroBead Cocktail II. The magnetically labeled non-T helper cells and memory CD4⁺ T cells are depleted by retaining them on a MACS[®] Column in the magnetic field of a MACS Separator, while the unlabeled naive CD4⁺ T cells pass through the column.

1.2 Background information

The Naive CD4⁺ T Cell Isolation Kit II is an indirect magnetic labeling system for the isolation of naive CD4⁺ T cells from human peripheral blood mononuclear cells (PBMCs).

Naive CD4⁺ T cells can be distinguished from memory CD4⁺ T cells by the different expression pattern of CD45RA and CD45RO, both members of the CD45 family. The CD45RA antigen is expressed on naive T lymphocytes, and is present on effector CD8⁺ T cells, as well as on NK cells and B cells. CD45RO is expressed on primed memory T lymphocytes, as well as monocytes, macrophages, and granulocytes.

CD45RO⁺ memory T cells and non-CD4⁺ T cells are indirectly magnetically labeled using a cocktail of biotin-conjugated antibodies against CD8, CD14, CD15, CD16, CD19, CD25, CD34, CD36, CD45RO, CD56, CD123, TCR γ/δ , HLA-DR and CD235a (Glycophorin A), and Anti-Biotin MicroBeads.

Isolation of highly pure naive CD4⁺ T cells is achieved by depletion of magnetically labeled cells.

1.3 Applications

- Functional studies on naive 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 naive CD4⁺ T cell activation and proliferation.
- Studies on signal transduction during activation of naive CD4⁺ T cells.
- Studies on cytokine expression of naive CD4⁺ T cells upon activation.
- Studies on T helper cell polarization (Th1 versus Th2) and memory versus effector T helper cell generation.

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 Ca²⁺ or Mg²⁺ 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
LS	10 ⁸	2×10 ⁹	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	10 ⁹	2×10 ¹⁰	SuperMACS II
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-APC (# 130-091-232) and CD45RA-FITC (# 130-092-247). 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, 30 μm (# 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

▲ 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 10⁷ total cells. When working with fewer than 10⁷ 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 2×10⁷ 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, 30 μm # 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. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

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 10⁷ total cells.
4. Add 10 μL of Naive CD4⁺ T Cell Biotin-Antibody Cocktail II per 10⁷ total cells.
5. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
6. Add 30 μL of buffer per 10⁷ total cells.
7. Add 20 μL of Naive CD4⁺ T Cell MicroBead Cocktail II per 10⁷ total cells.
8. Mix well and incubate for an additional 10 minutes in the refrigerator (2–8 °C).
9. (Optional) Add staining antibodies, e.g., 10 μL of CD4-APC (# 130-091-232), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
10. Proceed to magnetic separation (2.3 or 2.4).

▲ **Note:** A minimum of 500 μL is required for magnetic separation. If necessary, add buffer to the cell suspension.



2.3 Magnetic separation

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

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

Magnetic separation with LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS 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, representing the enriched naive CD4 T cells.
4. Wash column with 3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched naive CD4⁺ T cells and combine with the flow-through from step 3.
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled non-naive CD4⁺ T cells by firmly pushing the plunger into the column.

Magnetic separation with XS Columns

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

2.4 Cell separation with the autoMACS® Pro Separator and autoMACS Separator

- ▲ Refer to the 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.
- ▲ For appropriate resuspension volumes and cell concentrations, please visit www.automacspro.com/autolabeling.

2.4.1 Cell separation with the autoMACS® Pro Separator using autolabeling for a fully automated procedure

1. Turn on the instrument for automatic initialization (automated preparation and priming procedure).
2. Program autolabeling by selecting Read Reagent in the “reagent menu” tab and scan the 2D barcode on each reagent vial with the barcode scanner on the autoMACS Pro instrument. Place the reagent into the appropriate space on the reagent rack.
3. Place sample and collection tubes into the sample rack. Sample tube should be in row A, and the collection tubes in rows B and C.
4. Select the reagent name for each sample from the labeling submenu (the correct labeling, separation and wash protocols will be selected automatically).
5. Enter sample volume into the Volume submenu.
6. Select run.

For more details on complete walk away automation on the autoMACS Pro Separator, please refer to the autoMACS Pro Separator user manual.

2.4.2 Magnetic separation with the autoMACS® Pro Separator using manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling
2. Prepare and prime the instrument.
3. 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.
4. For a standard separation choose the following program:
Depletion: “Depletes”
Collect negative fraction in row B of the tube rack. This fraction represents the enriched naive CD4⁺ T cells.
5. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-CD4⁺ T cells and memory CD4⁺ T cells.

2.4.3 Magnetic separation with the autoMACS® Separator

1. Label the sample as described in section 2.2 Magnetic labeling
2. Prepare and prime the instrument.
3. 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.

4. For a standard separation choose the following program:

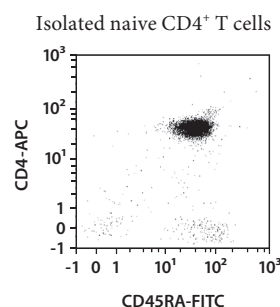
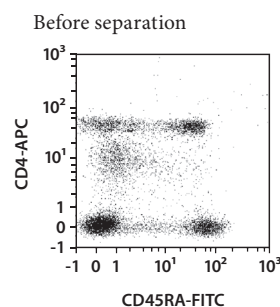
Depletion: “Depletes”

Collect negative fraction from outlet port neg1. This fraction represents the enriched naive CD4⁺ T cells.

5. (Optional) Collect positive fraction from outlet port pos1. This fraction represents the magnetically labeled non-CD4⁺ T cells and memory CD4⁺ T cells.

3. Example of a separation using the Naive CD4⁺ T Cell Isolation Kit II

Naive CD4⁺ T cells were isolated from human PBMCs using the Naive CD4⁺ T Cell Isolation Kit II, an LS Column, and a MidiMACS™ Separator. Cells are fluorescently stained with CD45RA-FITC (# 130-092-247) and CD4-APC (# 130-091-232). Cell debris and dead cells are excluded from the analysis based on scatter signals and propidium iodide fluorescence.



All protocols and data sheets are available at www.miltenyibiotec.com.

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.

Warranty

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