Naive CD4⁺ T Cell Isolation Kit
mouse

Contents

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.

1. Description

This product is for research use only.

Components	**1 mL Naive CD4⁺ T Cell Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal antibodies against CD8a, CD11b, CD11c, CD19, CD25, CD45R (B220), CD49b (DX5), CD105, Anti-MHC Class II, Ter-119, and TCRγ/δ.
	2 mL Anti-Biotin MicroBeads: MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1).
	1 mL CD44 MicroBeads: MicroBeads conjugated to monoclonal anti-mouse CD44 antibodies (isotype: rat IgG2b).
Capacity	For 10^9 total cells, up to 10 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 Naive CD4⁺ T Cell Isolation Kit, mouse naive CD4⁺ T cells are isolated by depletion of non-target cells. Non-target cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. Simultaneously, CD44 MicroBeads are added to label memory T cells. No washing steps are required during magnetic labeling. The magnetically labeled non-target cells are depleted by retaining them on a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled target cells pass through the column.

1.2 Background information

The Naive CD4⁺ T Cell Isolation Kit has been developed for the isolation of untouched naive CD4⁺ T helper cells from suspensions of mouse spleen cells or lymph node cells. Non-naive CD4⁺ T cells, i.e., cytotoxic T cells, regulatory T cells, activated T cells, B cells, NK cells, macrophages, granulocytes, endothelial cells, and erythroid cells are indirectly magnetically labeled by using a cocktail of biotin-conjugated antibodies and Anti-Biotin MicroBeads. Memory T cells are directly magnetically labeled with CD44 MicroBeads. Isolation of highly pure naive CD4⁺ T cells is achieved by depletion of magnetically labeled non-target cells.

1.3 Applications

- *In vitro* analysis of T cell differentiation.
- Adoptive transfer experiments.
- Studies on signal requirements for CD4⁺ T cell activation and proliferation.
- Studies on regulation of CD4⁺ T cell cytokine expression.

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 mouse serum albumin, mouse 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: 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
autoMACS	2×10^8	4×10^9	autoMACS Pro

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD45-VioGreen™ (# 130-102-412) CD4-VioBlue® (# 130-102-456), CD62L-PE (# 130-102-543), CD3ε-APC-Vio® 770 (# 130-102-306), and CD44-FITC (# 130-102-511). For more information about fluorochrome-conjugated antibodies refer to www.miltenyibiotec.com/antibodies.

- (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 lymphoid organs, non-lymphoid tissues, or peripheral blood, prepare a single-cell suspension using manual methods or the gentleMACS™ Dissociator.

For details refer to 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

▲ 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, 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. 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 Biotin-Antibody Cocktail per 10^8 total cells.

5. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).

6. Add 200 µL of buffer per 10^8 total cells.

7. Add 200 µL of Anti-Biotin MicroBeads per 10^8 total cells.

8. Add 100 µL of CD44 MicroBeads per 10^8 total cells.

9. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).

10. (Optional) For highest recovery wash cells by adding 1–2 mL of buffer per 10^8 total cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely. Resuspend up to 10^8 cells in 500 µL of buffer.

11. 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 LS Column and a suitable MACS Separator. For details refer to 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 LS 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 effluent 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 the autoMACS® Pro Separator

▲ Refer to the user manual for instructions on how to use the autoMACS® Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

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 enriched naive CD4$^+$ T cells.

4. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-naive CD4$^+$ T cells.
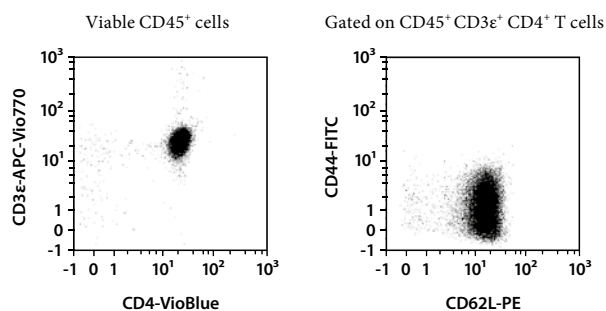
3. Example of a separation using the Naive CD4⁺ T Cell Isolation Kit

Naive CD4⁺ T cells were isolated from a single-cell suspension from mouse spleen using the Naive CD4⁺ T Cell Isolation Kit, an LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with CD45-VioGreen (# 130-102-412) CD4-VioBlue (# 130-102-456), CD62L-PE (# 130-102-543), CD3ε-APC-Vio770 (# 130-102-306), and CD44-FITC (# 130-102-511) and analyzed 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 and a gate was set on CD45⁺ cells.

A) Before separation

Viable CD45⁺ cells   Gated on CD45⁺ CD3ε⁺ CD4⁺ T cells
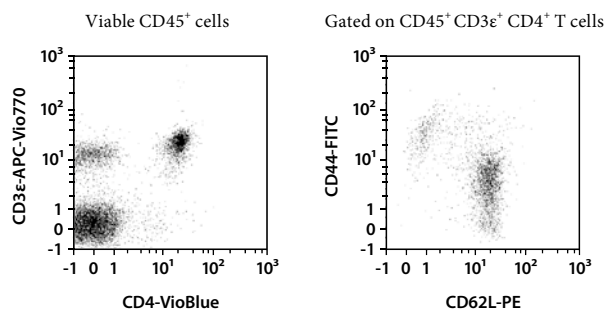
B) Isolated naive CD4⁺ T cells

Viable CD45⁺ cells   Gated on CD45⁺ CD3ε⁺ CD4⁺ T cells

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

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