Miltenyi Biotec

Naive CD8a$^+$ T Cell Isolation Kit

mouse

Order no. 130-096-543

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 CD8a$^+$ T Cell Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal anti-mouse antibodies against CD4, CD11b, CD11c, CD19, CD45R, CD49b, CD105, Ter-119, MHC II, and TCRγ/δ. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (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 CD8a$^+$ T Cell Isolation Kit, murine naive CD8a$^+$ 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 reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary reagent. Simultaneously, CD44 MicroBeads are added to label memory T cells. In between both labeling steps no washing steps are required. 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 non-target cells pass through the column.

1.2 Background information

The Naive CD8a$^+$ T Cell Isolation Kit has been developed for the isolation of untouched naive CD8a$^+$ cytotoxic T cells from suspensions of mouse spleen cells or lymp node cells. Non-naive CD8a$^+$ T cells, i.e., T helper 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 CD8a$^+$ 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.

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
XS	10^9	2×10^{10}	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) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427)

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- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD3ε-APC, CD8a-VioBlue®, CD44-FITC, CD62L-PE. For more information about 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™ Dissociators.

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. 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 400 µL of buffer per 10^8 total cells.
4. Add 100 µL of Naive CD8a$^+$ T Cell 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 an additional 10 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
11. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. 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 the number of total cells. For details refer to the 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 CD8a$^+$ T cell fraction.
4. Wash column with 3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched naive CD8a$^+$ 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-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.

Depletion with the autoMACS® Pro Separator

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

▲ Buffers used for operating the autoMACS Pro Separator hould 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.

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.

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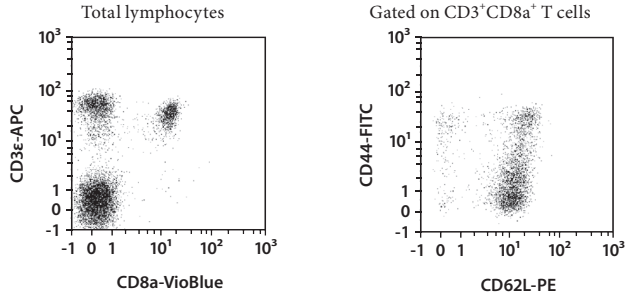
3. For a standard separation choose the following program:
   Depletion: Depletes
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched naive CD8a$^+$ cells.
4. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-naive CD8a$^+$ cells.

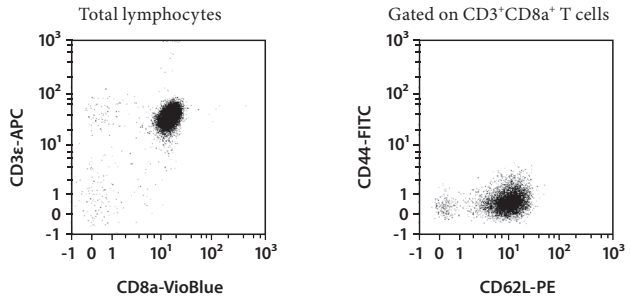
3. Example of a separation using the Naive CD8a$^+$ T Cell Isolation Kit

Naive CD8a$^+$ T cells were isolated from a single-cell suspension from mouse spleen using the Naive CD8a$^+$ T Cell Isolation Kit, an LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with CD3ε-APC, CD8a-VioBlue®, CD44-FITC, and CD62L-PE 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.

A) Before separation



B) Isolated naive CD8a$^+$ T cells



4. Reference

1. Wherry, E. J. and Ahmed R. (2004) Memory CD8 T cell differentiation during viral infection. J. Virol. 78 (11): 5535–5545.

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.

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