User Guide:
MileCell MagSep™ CD8+ T Cell Isolation Kit, Human

MILECELL

Product Specification

Cat. No.	Product Description	Size	Capacity
AB0052	CD8+ T Cell Isolation Kit, Human	1kit	1×10^9 PBMCs

1. Description

Components:

2mL CD8+ T Cell MicroBead Cocktail, Human. Nanomagnetic Microbeads conjugated to monoclonal antibodies.

2mL CD8+ T Cell Biotin-Antibody Cocktail, Human.

Capacity: For 1×10^9 total cells.

Product Format:

The MicroBead Cocktail is dispersed in phosphate-buffered saline (PBS) containing human serum albumin (HSA) and Poloxamer 188 (P188).

The Biotin-Antibody Cocktail is dispersed in phosphate-buffered saline (PBS).

Storage: Store at 2−8℃. Do not freeze.

Expiration Date: See vial label.

1.1 Principle of Separation

MileCell MagSep™ CD8+ T Cell Isolation Kit comprises two components: Biotin-Antibody Cocktail and Microbead Cocktail. The Biotin-Antibody Cocktail binds to non-target cells in the sample. Subsequently, the Microbead Cocktail is added to magnetically label non-target cells. When the sample passes through a column positioned within the magnetic separator, the magnetically labeled non-target cells are retained in the column, while the unlabeled target CD8+ T cells flow through the column and are collected

1.2 Applications

For in vitro enrichment of CD8+ T cells from fresh or frozen human mononuclear cells (MNCs) derived from peripheral blood, Leukopak, cord blood, bone marrow, or other single-cell suspensions.

1.3 Reagent and Instrument requirements

Isolation Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA) and 2 mM EDTA. Keep the isolation buffer cold (2−8℃).

(Optional) Degas the buffer prior to use to prevent column clogging caused by air bubbles in the solution.

Columns and Separators: For optimal CD8+ T cell enrichment, the use of MileCell or other compatible separators and columns is recommended. Alternative systems demonstrating equivalent performance specifications may be utilized. For optimal $CD8^+$ T cell enrichment, the use of MileCell columns and separators, or other compatible systems, is recommended. Alternative systems demonstrating equivalent performance specifications may also be used.

For detailed operating parameters, refer to the table below:

Column	Maximum Number of Labeled Cells	Maximum Number of Total Cells	Separator
Lc	1×10^8	2×10^9	L1/L4

Note:

When using this kit, non-target cell populations will be magnetically labeled, while target CD8+ T cells remain unlabeled. Depending on the abundance of target cells, the labeled fraction may constitute a significant proportion of the total cell population. To prevent column blocking, strictly adhere to the following protocols:

Estimate labeled cell number: Calculate the total number of labeled cells based on their proportion in the sample.

Split samples when necessary: If labeled cells in a single tube approach the column capacity limit, divide the sample into

multiple aliquots for separate processing.

Use sufficient columns: Select sufficient separation columns to ensure the load per column does not exceed its maximum capacity.

2. Protocol

2.1 Sample Preparation

When processing anticoagulated peripheral blood, Leukopak, or similar samples, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation (e.g. Ficoll) prior to magnetic labeling.

Note:

(Optional) After density gradient separation, if excessive platelets remain, resuspend the cell in isolation buffer and centrifuge at 200×g for 10−15 minutes at 20℃. Aspirate the supernatant.

2.2 Magnetic labeling

Note:

- To prevent nonspecific cell labeling, use pre-cooled magnetic labeling reagents and perform rapid processing.
- The following magnetic labeling reagent volumes are applicable for samples containing up to 10^7 total cells.
  For samples with <1×10^7 total cells, use the reagent volume specified for 1×10^7 cells.
  For samples with >1×10^7 total cells, proportionally scale all reagent volumes and total working volumes (e.g. for processing 2×10^7 total cells, all reagent volumes and total volume should be doubled).
- Recommended incubation temperature: 2-8℃. Higher temperature or prolonged incubation period may lead to nonspecific cell labeling.

a. Determine the total cell number of the sample.
b. Centrifuge the cell suspension at 300×g for 10 minutes. Aspirate the supernatant.
c. Add the CD8+ T Cell Biotin-Antibody Cocktail (20 µL per 10^7 cells) to resuspend the cells.
d. Incubate at 2−8℃ for 10 minutes.
e. Add Isolation buffer (60 µL per 10^7 cells).
f. Add the CD8+ T Cell MicroBead Cocktail (20 µL per 10^7 cells).
g. Mix thoroughly and incubate for 15 minutes at 2−8℃.
h. Resuspend the cells in at least 500 µL of isolation buffer to prepare for magnetic separation.
   Note: For higher cell numbers, increase the buffer volume proportionally (500 µL per 10^7 cells).

2.3 Magnetic separation

Note:

- For all procedures involving liquid/sample addition to the column reservoir, immediately proceed once the residual liquid has completely flowed through.

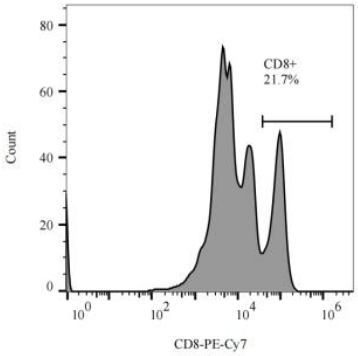
a. Place the column correctly onto the separator.
b. Add isolation buffer to the reservoir of column according to the following volume:
   Lc：3mL
   Allow the buffer to stand until the liquid in the reservoir is nearly drained.
c. Apply the cell suspension to the reservoir of column. Collect the flow-through fraction (containing unlabeled CD8+ T cells), which represents the enriched CD8+ T cell population.
d. Add isolation buffer to the reservoir of column according to the volume and wash cycle specified below to wash the column.
   Lc：3mL×1
   Collect the unlabeled cells that pass through and combine them with the effluent from **Step c**.
   Note: Add new buffer to the reservoir immediately after the liquid in the reservoir has completely drained.
e. (Optional) After the isolation buffer has completely drained, remove the column from the separator and place it

on an appropriate collection tube. Add isolation buffer (as specified below) to the reservoir of column. Immediately assemble the plunger and push it to the bottom rapidly and firmly. Hold for 2 seconds to completely elute the magnetically labeled non-CD8+ T cells.

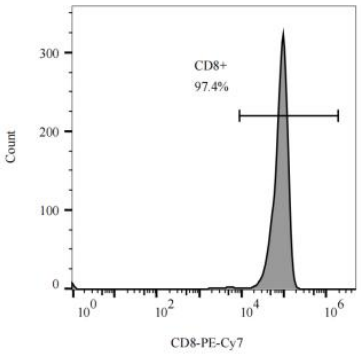
Lc：5ml

3. Example of Cell Separation Using CD8+ T Cell Isolation Kit

CD8+ T cells were isolated from human PBMCs using MileCell MagSep™ CD8+ T Cell Isolation Kit. The obtained cells were fluorescently stained with anti-CD3-APC and analyzed by flow cytometry.



Starting Material



Enriched Population

For inquiries regarding our products, services, or technical assistance, please contact: Info@milecell-bio.com