User Guide:
MileCell MagSep™ CD8 MicroBeads, Human

MILECELL

Product Specification

Cat. No.	Product Description	Size	Capacity
AB0002	CD8 MicroBeads, Human	2mL	1×10^9 PBMCs

1. Description

Components: 2mL MileCell MagSep™ CD8 Microbeads, Human. Superparamagnetic nanoparticles conjugated to monoclonal anti -human CD8 antibodies (isotype: mouse IgG1).

Capacity: For 1×10^9 total cells.

Product Format: Supplied in phosphate-buffered saline(PBS), containing Human Serum Albumin (HSA) and Poloxamer 188.

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

Expiration Date: See vial label.

1.1 Principle of Separation

CD8+ cells are magnetically labeled using MileCell MagSep™ CD8 MicroBeads during the co-incubation process. The labeled cell suspension is then loaded onto a separation column placed in a suitable magnetic separator. Magnetically labeled CD8+ cells are retained within the column, while unlabeled cells flow through. After removing the column from the magnetic field, the magnetically retained CD8+ cells can be eluted from the column.

1.2 Applications

For in vitro positive selection or depletion of CD8+ 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.

Note:

- Buffers or culture media containing Ca^{2+} or Mg^{2+} are not recommended.
- EDTA can be substituted with other additives such as Anticoagulant Citrate Dextrose Solution A (ACD-A) or Citrate Phosphate Dextrose (CPD).
- BSA can be replaced with other proteins including Human Serum Albumin (HSA), human serum, or Fetal Bovine Serum (FBS).

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

For detailed operating procedures, refer to the table below:

Column	Maximum Number of Labeled Cells	Maximum Number of Total Cells	Separator
Mc	1×10^7	2×10^8	M1 M8
Lc	1×10^8	2×10^9	L1 L4

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.
- Dead cells may exhibit nonspecific binding to MileCell MagSep™ magnetic beads. Density gradient centrifugation is recommended for dead cell removal.

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. Resuspend cells in isolation buffer (80μL per 10^7 cells).
d. Add CD8 MicroBeads (20μL per 10^7 cells).
e. Mix thoroughly and incubate at 2−8℃ for 15 minutes.
f. Wash cells by adding isolation buffer (1-2 mL per 10^7 cells), mix by gentle pipetting, and then centrifuged at 300×g for 10 minutes. Aspirate the supernatant.
g. Resuspend cells in isolation buffer (500μL per 10^8 cells) for magnetic separation.
   Note: For higher cell quantities, adjust buffer volumes proportionally.

2.3 Magnetic separation

Note:

- Choose appropriate columns and separators based on both the total cell count and the estimated CD8+ cell count in your sample.(For detailed specifications, refer to the table in Section 1.3)
- 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 volumes:
   Mc：500μL Lc：3mL
   Allow the buffer to stand until the liquid in the reservoir is nearly drained.
c. Apply the entire sample to the reservoir of the column.
   Note: Flow-through liquid can be collected as needed, containing unlabeled cells.
d. Add isolation buffer to the reservoir of column according to the volume and wash cycle specified below to wash the column.
   Mc：500μL×3 Lc：3mL×3
   Collect the unlabeled cells that pass through and combine them with the flow-through from **Step c**.
   Note: Add new buffer to the reservoir immediately when the liquid in the reservoir has completely drained.
e. Remove the column from the separator and place it on an appropriate collection tube when the isolation buffer has

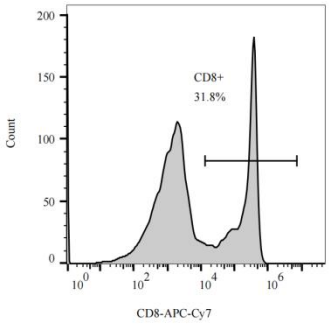
completely drained.

f. 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 labeled cells.

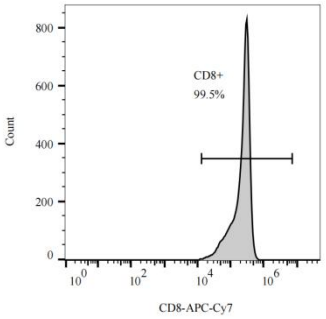
Mc：1mL Lc：5ml

3. Example of Cell Separation Using CD8 MicroBeads

CD8+ cells were isolated from human PBMCs using MileCell MagSep™ CD8 Microbeads. The obtained cells were fluorescently stained with anti-CD8-APC-Cy7 and analyzed by flow cytometry.



Starting Material



Enriched Population

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