Isolation of CD8$^+$CD62L$^+$ T cells from human PBMCs

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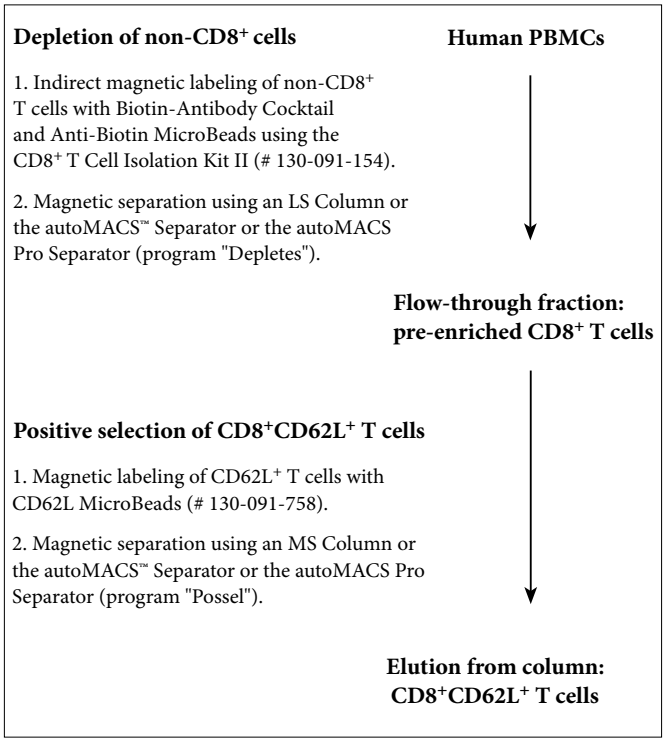

1. Description

1.1 Principle of the MACS® Separation

The isolation of CD8$^+$CD62L$^+$ T cells can be performed in a two-step procedure. After depletion of non-CD8$^+$ T cells using the CD8$^+$ T Cell Isolation Kit II, human (# 130-091-154), the CD8$^+$CD62L$^+$ T cells are positively isolated using CD62L MicroBeads, human (# 130-091-758).

First, non-CD8$^+$ T cells are labeled with a cocktail of biotin-conjugated antibodies and Anti-Biotin MicroBeads. The magnetically labeled cells are subsequently depleted by separation over a MACS® Column. In a second step the CD62L$^+$ T cells are magnetically labeled with CD62L MicroBeads for positive selection. The magnetically labeled CD8$^+$CD62L$^+$ T cells are retained within a MACS Column and eluted after removal of the column from the magnetic field.

1.2 Background information

The CD62L antigen is a 74 kDa glycoprotein and belongs to the selectin family of cell surface molecules, also referred to as L-selectin, LECAM-1, or LAM-1. CD62L binds to a series of glycoproteins, including CD34, GlyCAM-1, and MAdCAM-1 and is important for homing of naive lymphocytes via the high endothelial venules to peripheral lymph nodes and Peyer's patches.[1] The CD62L antigen also contributes to the recruitment of leukocytes from the blood to areas of inflammation.[1] Most hematopoietic cells express CD62L including most peripheral blood B cells, T cells, monocytes, granulocytes, and some myeloid cells from bone marrow and thymocytes. Among T cells CD62L is highly expressed on naive T cells and subsets of memory T cells in particular so called central memory T cells. Thus, the combination of the CD8 T Cell Isolation Kit II with CD62L MicroBeads can be used to enrich naive and central memory CD8$^+$ T cells.

1.3 Application

- Isolation of CD8$^+$CD62L$^+$ T cells from human PBMCs for further phenotypical characterization or functional analysis.

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1.4 Reagent and instrument requirements

- CD8$^+$ T Cell Isolation Kit II (# 130-091-154) containing a Biotin-Antibody Cocktail and Anti-Biotin MicroBeads.
- CD62L MicroBeads (# 130-091-758).
- 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 albumine, human serum, or fetal bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Depletion of non-CD8$^+$ T cells is performed on an LS Column. The subsequent positive selection of CD8$^+$CD62L$^+$ T cells is performed on an MS Column. Depletion and positive selection can also be performed by using the autoMACS™ Separator or the autoMACS Pro Separator.

Column	max. number of labeled cells	max. number of total cells	Separator
Depletion			
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Separator or autoMACS Pro Separator

▲ **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 CD8 antibody for flow cytometric analysis, e.g., CD8-PE (# 130-091-084). For CD62L staining, it is recommended to use CD62L-APC (# 130-091-755). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.
- (Optional) Propidium iodide (PI) 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 (# 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 General Protocols section of the respective separator user manual.

The General Protocols are also available 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).

▲ To achieve a high purity of the CD8$^+$CD62L$^+$ T cell fraction, it is strongly recommended to use fresh samples.

2.2 Magnetic labeling of non-CD8$^+$ cells

▲ 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 10^7 total cells. When working with fewer than 10^7 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 2×10^7 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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column. Wet filter with buffer before use.

1. Determine cell number.
2. Centrifuge cells at 300×g for 5 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 40 µL of buffer per 10^7 cells.
4. Add 10 µL of **Biotin-Antibody Cocktail** per 10^7 cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add an additional 30 µL of buffer and 20 µL of **Anti-Biotin MicroBeads** per 10^7 cells.
7. Mix well and incubate for an additional 15 minutes in the refrigerator (2–8 °C).
8. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
9. Resuspend up to 10^8 cells in 1 mL of buffer.
10. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of non-CD8$^+$ T cells

Depletion with LS Column

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column.

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4. Collect unlabeled cells that pass through and wash column with 3×3 mL of buffer. Perform washing steps by adding buffer successively once the column reservoir is empty. Collect total effluent; this is the unlabeled pre-enriched CD8$^+$ T cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
5. Proceed to 2.4 for the isolation of CD8$^+$CD62L$^+$ T cells.

Depletion with the autoMACS™ Separator or the autoMACS Pro Separator

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

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

Depletion with the autoMACS™ Separator

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 at the uptake port and the fraction collection tubes at port neg1 and pos.
3. Choose program "Depletes".
4. Collect negative fraction from outlet port neg1. This is the pre-enriched CD8$^+$ T cell fraction.
5. Proceed to 2.4 for the isolation of CD8$^+$CD62L$^+$ T cells.

Depletion with the autoMACS™ Pro Separator

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 fraction collection tubes in rows B and C.
3. Choose program "Depletes".
4. Collect negative fraction in row B of the tube rack.
5. Proceed to 2.4 for the isolation of CD8$^+$CD62L$^+$ T cells.

2.4 Magnetic labeling of CD62L$^+$ T cells

▲ Volumes for magnetic labeling of pre-enriched CD8$^+$ T cells given below are for an initial starting cell number of up to 2×10^7 cells.

1. Centrifuge cell suspension at 300×g for 5 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 80 µL of buffer.
3. Add 20 µL of **CD62L MicroBeads**.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
6. Resuspend cell pellet in 500 µL of buffer.
7. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of CD8$^+$CD62L$^+$ T cells

Positive selection with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 3×500 µL of buffer. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells (CD8$^+$CD62L$^+$ T cells) by firmly pushing the plunger into the column.

Positive selection with the autoMACS™ Separator or the autoMACS™ Pro Separator

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

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

Positive selection with the autoMACS Separator

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 at the uptake port and the fraction collection tubes at port neg1 and pos1.
3. Choose program "Possel".
4. Collect positive fraction from outlet port pos1. This is the CD8$^+$CD62L$^+$ T cell fraction.

Positive selection with the autoMACS™ Pro Separator

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 fraction collection tubes in rows B and C.
3. Choose program "Possel".
4. Collect positive fraction in row C of the tube rack.

2.6 (Optional) Evaluation of CD8$^+$CD62L$^+$ T cells

The purity of enriched CD8$^+$CD62L$^+$ T cells or any intermediate fraction can be evaluated by flow cytometry. Stain aliquots of cell fractions with fluorochrome-conjugated antibodies against CD62L, e.g., CD62L-APC (# 130-091-755) and against CD8, e.g., CD8-FITC (# 130-080-601) or CD8-PE (# 130-091-084).

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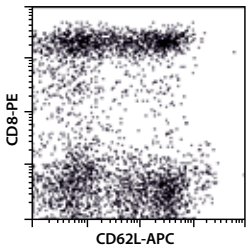
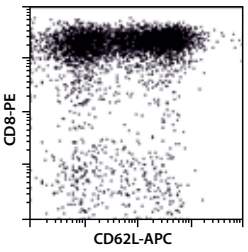
3. Example of a separation using the CD8$^+$ T Cell Isolation Kit II and CD62L MicroBeads

CD8$^+$CD62L$^+$ T cells were isolated from human PBMCs by using the CD8$^+$ T Cell Isolation Kit II and CD62L MicroBeads, an LS Column and an MS Column, and appropriate MACS® Separators. The cells are fluorescently stained with CD62L-APC and CD8-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

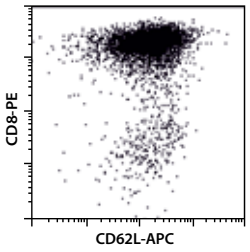
PBMCs before separation



Pre-enriched CD8$^+$ T cells after depletion of non-CD8$^+$ T cells



Isolated CD8$^+$CD62L$^+$ T cells



4. References

1. Khan, A.I. *et al.* (2003) L-selectin ligands in lymphoid tissues and models of inflammation. Inflammation 27: 265–280.
2. Smalley, D.M. and Ley, K. (2005) L-selectin: mechanisms and physiological significance of ectodomain cleavage. J. Cell. Mol. Med. 9: 255–266.

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.

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