Miltenyi Biotec

CD62L MicroBeads
human

Order no. 130-091-758

Contents

1. Description

Components	2 mL CD62L MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD62L antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	CD62L MicroBeads are supplied as a suspension 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 MACS® Separation

First, the CD62L$^+$ cells are magnetically labeled with CD62L MicroBeads. Then, the cell suspension is loaded onto a MACS® Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD62L$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD62L$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD62L$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

The CD62L antigen is a 74 kDa glycoprotein and is a member of the selectin family of cell surface molecules, also referred to as L-selectin, LECAM-1, or LAM-1. CD62L binds 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. The CD62L antigen also contributes to the recruitment of leukocytes from the blood to areas of inflammation. Most hematopoietic cells express CD62L including most peripheral blood B cells, T cells, monocytes, granulocytes and some myeloid cells from bone marrow and thymocytes. CD62L is continuously endoproteolytically cleaved from the cell surface of CD62L-expressing neutrophils and lymphocytes (shedding). Proteolysis is accelerated e.g. after antigen-activation of T cells.

1.3 Applications

- Positive selection or depletion of cells expressing the human CD62L antigen from peripheral blood mononuclear cells (PBMCs), body fluids (e.g. bronchoalveolar lavage) or single-cell suspensions from tissue (e.g. lymphoid and tumor tissue).
- Positive selection of CD62L$^+$ T cells from pre-enriched CD4$^+$ or CD8$^+$ T cells using the CD4$^+$ T Cell Isolation Kit II (# 130-091-155) or the CD8$^+$ T Cell Isolation Kit II (# 130-091-154).
- Isolation of CD4$^+$CD62L$^+$ central memory T cells from pre-enriched CD4$^+$ memory T cells using the Memory CD4$^+$ T Cell Isolation Kit (# 130-091-893).

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 human serum albumin, human serum or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: CD62L$^+$ cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD, CS, or D Columns. Cells which strongly express the CD62L antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS or the 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.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10^8		VarioMACS, SuperMACS
D	10^9		SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-001-184.05

- (Optional) Fluorochrome-conjugated CD62L antibody for flow-cytometric analysis, e.g. CD62L-FITC (# 130-091-757), or CD62L-PE (# 130-091-756), and additional staining antibodies, e.g. CD4-APC (# 130-091-232).
- (Optional) Propidium iodide (PI) or 7-AAD for flow-cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

▲ Always use **fresh material** for positive selection or depletion of $CD62L^+$ cells. For optimal results, the cells should not be older than 8–12 hours. **Keep cells continuously cold**. CD62L-expression may be rapidly lost due to shedding.

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.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available 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).

For the isolation of $CD62L^+$ T cells from pre-enriched $CD4^+$ or $CD8^+$ T cells a special protocol is available from www.miltenyibiotec.com/protocols.

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 10^7 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.

▲ 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 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of CD62L MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies, e.g. add 10 µL of CD62L-FITC (# 130-091-757), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

   ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 cells in 500 µL of buffer.
9. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $CD62L^+$ cells. For details see table in section 1.4.

Magnetic separation with MS or LS Columns

1. Place 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 appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL
7. (Optional) To increase the purity of $CD62L^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation, refer to the XS Column data sheet.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details see LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details see CS Column data sheet.
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way-stopcock of the assembled column. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the top. Collect total effluent; this is the unlabeled cell fraction.

Depletion with D Columns

For instructions on column assembly and separation, refer to the D Column data sheet.

Magnetic separation 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.

▲ 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.

Magnetic separation 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 port pos1.
3. For a standard separation choose one of the following programs:

   Positive selection: "Possel"
   Collect positive fraction from outlet port pos1.
   Depletion: "Depletes"
   Collect negative fraction from outlet port neg1.

Magnetic separation with the autoMACS™ Pro Separator

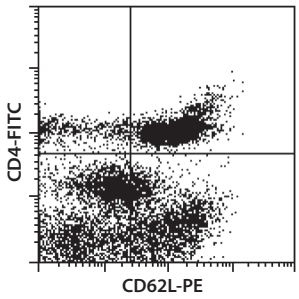
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

   Positive selection: "Possel"
   Collect positive fraction in row C of the tube rack.
   Depletion: "Depletes"
   Collect negative fraction in row B of the tube rack.

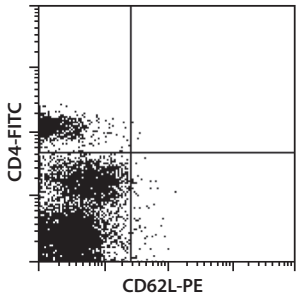
3. Example of a separation using CD62L MicroBeads

CD62L$^+$ cells were separated from PBMCs using CD62L MicroBeads. For positive selection CD62L$^+$ cells were isolated using an MS Column and a MiniMACS™ Separator. For depletion of CD62L$^+$ cells, the sample was separated over an LD Column in a MidiMACS™ Separator. Cells are fluorescently stained with CD62L-PE (# 130-091-756) and CD4-FITC (# 130-080-501). Cell debris and dead cells were excluded from analysis based on scatter signals and PI fluorescence.

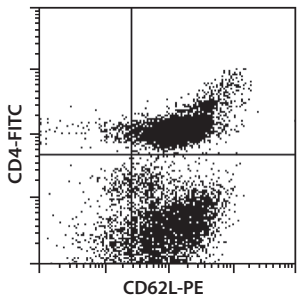
PBMCs before separation



PBMCs depleted of CD62L$^+$ cells



Isolated CD62L$^+$ cells



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.

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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