CD57 MicroBeads
human

Contents

1. Description

Components	2 mL CD57 MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD57 antibodies (isotype: mouse IgM).
Capacity	For 10^9 total cells or up to 100 separations.
Product format	CD57 MicroBeads 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

First, the CD57+ cells are magnetically labeled with CD57 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 CD57+ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD57+ cells. After removing the column from the magnetic field, the magnetically retained CD57+ cells can be eluted as the positively selected cell fraction.

1.2 Background information

CD57 MicroBeads have been developed for the separation of human cells based on the expression of the CD57 antigen. CD57, also known as HNK-1 or Leu-7, is an oligosaccharide antigenic moiety bound to surface proteins of certain cell types. In blood, CD57 is found on 15–20% of mononuclear cells[1], including subsets of NK and T cells, though not on erythrocytes, monocytes, granulocytes, or platelets. Also, CD57 expression can be found on a variety of neural cell types.[1,2]

CD57 has been shown to be expressed on late stage effector CD8+ cells[3]. The frequency of CD3+CD57+ lymphocytes is raised in a variety of diseases, for example, in the blood and synovia of patients with rheumatoid arthritis[4]. Its expression is also increased on proliferation-incompetent or chronically activated CD8+ T cells in persistent viral infections, such as HIV.[5]

1.3 Applications

- Isolation or depletion of CD57+ cells from human peripheral blood mononuclear cells (PBMCs), body fluids or single-cell suspensions from tissue (e.g. lymphoid tissue).

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 bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: CD57+ 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 CD57 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.

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, autoMACS Pro

   ▲ **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 CD57 antibody for flow cytometric analysis, e.g., CD57-FITC (# 130-092-174), CD57-PE (# 130-092-139), or CD57-APC (# 130-092-141).

- (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.

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).

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 CD57 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., 10 μL of CD57-APC, 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 CD57$^+$ 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 the 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 the 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

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 ports neg1 and pos1.

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

    Positive selection: "Possel"
    Collect positive fraction from outlet port pos1.

    Depletion: "Deplete"
   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: "Deplete"
    Collect negative fraction in row B of the tube rack.

3. Example of a separation using the CD57 MicroBeads

CD57+ cells were isolated from human PBMCs using CD57 MicroBeads, an MS Column, and a MiniMACS™ Separator. Cells are fluorescently stained with CD57-APC (# 130-092-141). Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.

Before separation

Cells depleted of CD57+ cells

Isolated CD57+ cells

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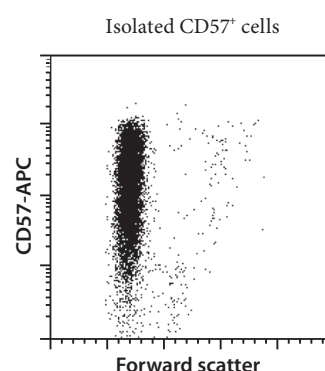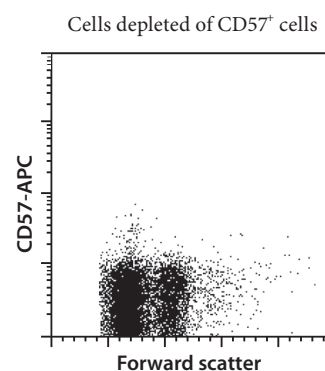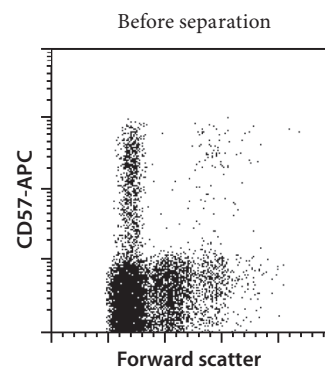
4. References

1. Mechtersheimer, G. *et al.* (1991) Expression of the natural killer cell-associated antigens CD56 and CD57 in human neural and striated muscle cells and in their tumors. Cancer Res. 51: 1300–1307.

2. Ong, E. *et al.* (2002) Biosynthesis of HNK-1 glycans on O-linked oligosaccharides attached to the neural cell adhesion molecule (NCAM): the requirement for core 2 beta 1,6-N-acetylglucosaminyltransferase and the muscle-specific domain in NCAM. J. Biol. Chem. 277: 18182–18190.

3. Brenchley J.M. *et al.* (2003) Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. 101: 2711–2720.

4. Dupuy de'Angeac, A. *et al.* (1993) Increased percentage of $CD3^+$, $CD57^+$ lymphocytes in patients with rheumatoid arthritis. Correlation with duration of disease. Arthritis Rheum. 36: 608–612.

5. Palmer, B.E. *et al.* (2005) Functional and phenotypic characterization of CD57+CD4+ T cells and their association with HIV-1-induced T cell dysfunction. J. Immunol. 175: 8415–8423.

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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