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1. Description

Components	2 mL CD56 MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD56 antibodies (isotype: mouse IgG1).
Size	For 10 ⁹ total cells, up to 100 separations.
Product format	CD56 MicroBeads are supplied as a suspension containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of MACS® separation

First the CD56⁺ cells are magnetically labeled with CD56 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 CD56⁺ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of CD56⁺ cells. After removal of the column from the magnetic field, the magnetically retained CD56⁺ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

CD56 MicroBeads are developed for the separation of human cells based on the expression of the CD56 antigen. CD56 is expressed by essentially all human NK cells and its density is increased on the cell membrane after activation.^{1,2} The antigen is also present on a unique subset of CD3⁺ T cells that mediates non-MHC-restricted cytotoxicity and on myoblasts, some neural tissue and tumors.

Examples of applications

- Positive selection or depletion of cells expressing human CD56 antigen.
- Isolation or depletion of NK cells from peripheral blood mononuclear cells (PBMCs) or lymphoid tissue.

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA and 2 mM EDTA by diluting MACS BSA Stock Solution (#130-091-376) 1:20 in autoMACS™ Rinsing Solution (#130-091-222). Keep buffer cold (4–8 °C).
 - ▲ **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 Ca²⁺ or Mg²⁺ are not recommended for use.
- MACS Columns and MACS Separators: CD56⁺ cells can be enriched by using MS, LS or XS Columns (positive selection). CD56 MicroBeads can be used for depletion of CD56⁺ cells on LD, CS or D Columns. Cells which strongly express the CD56 antigen can also be depleted using MS, LS or XS Columns. Positive selection or depletion can also be performed by using the autoMACS 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

▲ **Note:** Column adapters are required to insert certain columns into VarioMACS™ Separator or SuperMACS™ Separator. For details, see MACS Separator data sheets.

- (Optional) Fluorochrome-conjugated antibodies for flow-cytometric analysis, e.g. CD3-FITC (# 130-080-401), CD3-PE (# 130-091-374), CD45-FITC (# 130-080-202), CD45-PE (# 130-080-201), or fluorochrome-conjugated CD56 antibodies.
 - ▲ **Note:** For fluorescent staining against CD56 an antibody clone has to be used which recognizes a different epitope of CD56 than that recognized by clone AF12-7H3, e.g. NCAM16.2.
- (Optional) PI (propidium iodide) 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

When working with anticoagulated peripheral blood or buffy coat, PBMCs should be isolated by density gradient centrifugation (e.g. Ficoll-Paque™, see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** Remove platelets after density gradient separation: resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove supernatant. Repeat washing step and carefully remove supernatant.

When working with tissues, prepare a single-cell suspension by a standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com/protocols).

▲ **Note:** 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 10⁷ total cells. When working with fewer than 10⁷ 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 2×10⁷ 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.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 10⁷ total cells.
4. Add 20 µL of CD56 MicroBeads per 10⁷ total cells.
5. Mix well and incubate for 15 minutes at 4–8 °C.

▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.

6. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.

7. Resuspend up to 10⁸ 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 1.25×10⁸ cells in 500 µL of buffer.

8. 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 CD56⁺ cells (see table in section 1.3).

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
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 which pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times, each time once the column reservoir is empty.
MS: 3×500 µL LS: 3×3 mL.
Collect total effluent. This is the unlabeled cell fraction.
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 fraction with the magnetically labeled cells by firmly applying the plunger supplied with the column.
MS: 1 mL LS: 5 mL.

▲ **Note:** To increase the purity of the magnetically labeled fraction, it can be passed over a new, freshly prepared 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 (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 which pass through and wash column with 2×1 mL of buffer. Collect total effluent. This is the unlabeled cell fraction.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator (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 (see "CS Column data sheet").
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which 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

▲ Refer to the "autoMACS™ User Manual" for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose following separation programs:

Positive selection: "Possel"

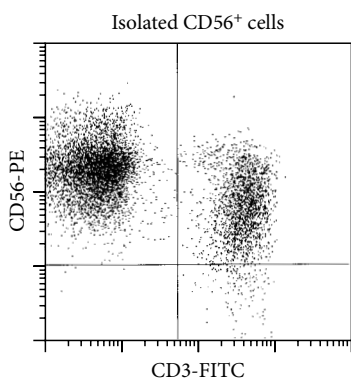
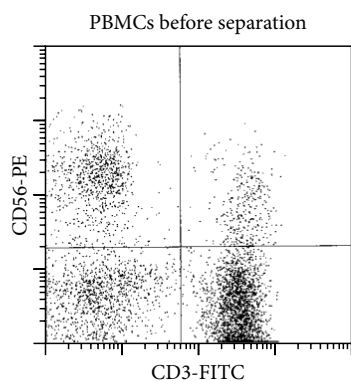
Depletion: "Depletes"

▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see autoMACS User Manual: "autoMACS Cell Separation Programs".

3. When using the program "Possel", collect positive fraction (outlet port "pos1"). This is the purified CD56⁺ cell fraction.
When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the CD56⁻ cell fraction.

3. Example of a separation using CD56 MicroBeads

Separation of PBMCs using CD56 MicroBeads and an MS Column. The cells are fluorescently stained with CD56-PE and CD3-FITC (# 130-080-401). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Robertson, M. J. & Ritz, J. (1990) Biology and clinical relevance of human natural killer cells. *Blood* 76: 2421–2438.
2. Robertson *et al.* (1990) Human natural killer cell adhesion molecules. Differential expression after activation and participation in cytotoxicity. *J. Immunol.* 145: 3194.
3. Kämmerer, U. *et al.* (1999) A subset of CD56⁺ large granular lymphocytes in first-trimester human decidua are proliferating cells. *Fertility and Sterility* 71: 74–79. [589]
4. Pitti, R. M. *et al.* (1998) Genomic Amplification of a Decoy Receptor for Fas Ligand in Lung and Colon Cancer. *Nature* 396: 699–703. [517]

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.

Warranty

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