Miltenyi Biotec

CD43 MicroBeads
human

Order No. 130-091-333

Index

1. Description

Components	2 mL CD43 MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD43 antibodies (isotype: mouse IgG1).
Size	For 10^9 total cells, up to 100 separations.
Product format	CD43 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 $CD43^+$ cells are magnetically labeled with CD43 MicroBeads. Then, the cell suspension is loaded onto a column which is placed in the magnetic field of a MACS® Separator. The magnetically labeled $CD43^+$ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of $CD43^+$ cells. After removal of the column from the magnetic field, the magnetically retained $CD43^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

CD43 MicroBeads are developed for the isolation of untouched human B cells based on the magnetic depletion of cells that express the CD43 antigen. CD43 (leukosalin, sialophorin) is a cell surface glycoprotein that is suggested to be involved in adhesion, anti-adhesion, and signal transduction processes.[1] The CD43 antigen is expressed on most leukocytes, i.e. T cells, NK cells, granulocytes, monocyte and macrophages, hematopoietic stem cells and platelets but not on erythrocytes.[2-4] Among B cells, CD43 is expressed on activated B cells and plasma cells but not on resting B cells, e.g. naive B cells.[2] In bone marrow, CD43 is found on pro-B cells but is down regulated during transition to the pre-B cell stage.

Examples of applications

- Positive selection or depletion of cells expressing the human CD43 antigen.
- Isolation of untouched resting B cells from peripheral blood mononuclear cells (PBMC), body fluids (e.g. bronchial lavage) or single-cell suspensions from tissue (e.g. 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 with 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 Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators:
  Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

Column	max. number of labeled cells	max. number of total cells	Separator
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
autoMACS	2×10^8	4×10^9	autoMACS

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

- (Optional) Fluorochrome-conjugated antibodies against a B cell lineage marker (e.g. CD19-APC # 130-091-248, CD19-PE # 130-091-247, CD20-FITC # 130-091-108, or CD20-PE # 130-091-109), CD43 and CD235a (Glycophorin A).
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filter (# 130-041-407).

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, PBMC should be isolated by density gradient centrifugation (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com).

▲ **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.

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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).

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation (e.g. Ficoll-Paque™) 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 Filter # 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 10^7 total cells.
4. Add 20 µL of CD43 MicroBeads per 10^7 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. (Optional) Add staining antibodies, e.g. add 10 µL of CD19-PE (# 130-091-247), and incubate for 5 minutes at 4–8 °C.
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
8. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
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 $CD43^+$ 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 second, 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: "Deplete"

   ▲ **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".

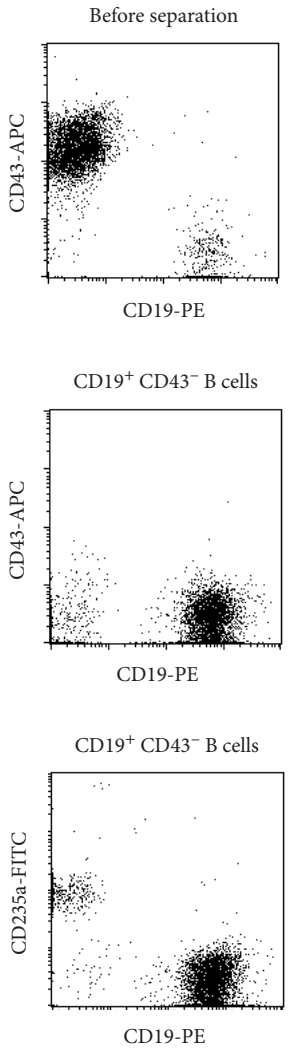
140-001-138.04

3. When using the program "Possel", collect positive fraction (outlet port "pos1"). This is the purified CD43$^+$ cell fraction.

   When using the program "Deplete", collect unlabeled fraction (outlet port "neg1"). This is the CD43$^-$ cell fraction containing the untouched resting B cells.

3. Example of a separation using CD43 MicroBeads

Separation of PBMC using CD43 MicroBeads and a MidiMACS™ Separator with an LS Column. The cells are fluorescently stained with CD19-PE (# 130-091-247), CD43-APC and CD235a (Glycophorin A)-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Woodman *et al.* (1998) J Exp Med. 188: 2181-2186
2. Barclay *et al.* eds. 1997. The Leucocyte Antigen FactsBook. Academic press. London
3. Moore *et al.* (1994) J. Immunol 153: 4978-4987
4. Remold-O'Donnell (1987) Biochemistry. 26: 3908-39134.

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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