Miltenyi Biotec

CD235a (Glycophorin A) MicroBeads
human

Order No. 130-050-501

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1. Description

Components	2 mL CD235a (Glycophorin A) MicroBeads, human: MicroBeads conjugated to monoclonal mouse anti-human CD235a (Glycophorin A) antibodies (isotype: mouse IgG1).
Size	For 10^9 total cells, up to 100 separations.
Product format	Glycopohorin A 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 Glycophorin A$^+$ cells are magnetically labeled with Glycophorin A 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 Glycophorin A$^+$ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of Glycophorin A$^+$ cells. After removal of the column from the magnetic field, the magnetically retained Glycophorin A$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

CD235a (Glycophorin A) MicroBeads are used for the positive selection or depletion of human erythroid cells. The CD235a antigen (Glycophorin A), a single-pass transmembrane glycoprotein, is expressed on mature erythrocytes and erythroid precursor cells.

Examples of applications

- Positive selection or depletion of CD235a (Glycophorin A) expressing cells.
- Positive selection or depletion of CD235a (Glycophorin A$^+$) mature erythrocytes and erythroid precursor cells from peripheral blood, buffy coat, leukapheresis material, cord blood, bone marrow, spleen etc.
- Enrichment of fetal erythroblasts from maternal peripheral blood mononuclear cells (PBMCs).

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 Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: CD235a (Glycophorin A)$^+$ cells can be enriched by using MS, LS or XS Columns (positive selection). CD235a (Glycophorin A) MicroBeads can be used for depletion of Glycophorin A$^+$ cells on LD, CS or D Columns. Cells which strongly express the CD235a (Glycophorin A) 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 CD235a (Glycophorin A) antibodies for flow-cytometric analysis, or CD71-PE (# 130-091-728), CD71-APC # (130-091-727).

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- (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

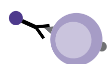
2.1 Sample preparation

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 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).

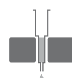
▲ For optimal performance it is important to obtain a single-cell suspension before magnetic separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters # 130-041-407) to remove cell clumps which may clog the column.

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 CD235a (Glycophorin A) 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 CD71-PE (# 130-091-728), 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.

   ▲ **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 CD235a (Glycophorin A)$^+$ 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.

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This MACS® product is for *in vitro* research use only and not for diagnostic or therapeutic procedures.

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 CD235a (Glycophorin A)$^+$ cell fraction.

   When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the CD235a (Glycophorin A)$^-$ cell fraction.

3. Example of a separation using CD235a (Glycophorin A) MicroBeads

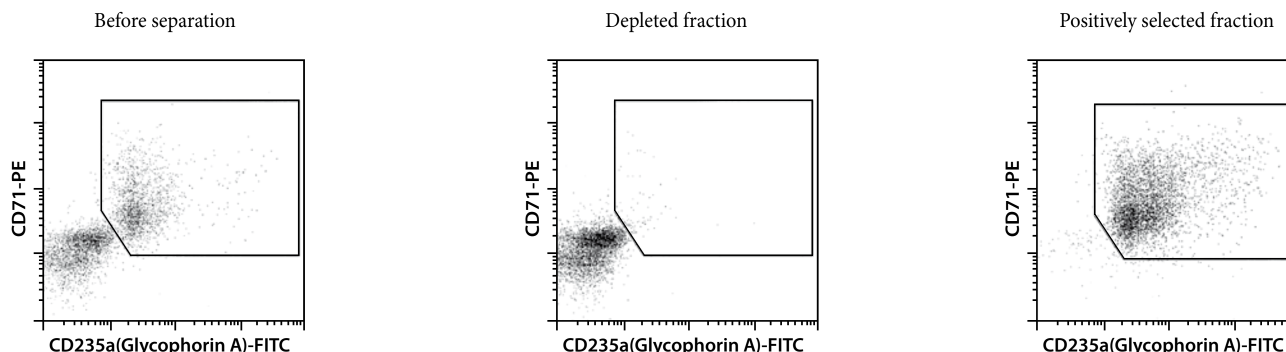
Separation of mononuclear cells from cord blood using CD235a (Glycophorin A) MicroBeads, a MiniMACS™ Separator and an MS Column. Cells are stained with FITC-conjugated anti-Glycophorin A antibody and CD71-PE.

4. Appendix

Isolation of mononuclear cells from peripheral blood, cord blood or bone marrow

1. Start with fresh human blood, leukocyte-rich buffy coat, cord blood or bone marrow aspirate. Cell samples should be treated with anticoagulants, e.g. heparin, citrate, acid citrate dextrose (ACD) or citrate phosphate dextrose (CPD).
2. Dilute cells with 2–4 volumes of PBS containing 2 mM EDTA or 0.6% ACD-A.

   ▲ **Note:** The more diluted the blood sample, the better the purity of the mononuclear cells.
3. Carefully layer 35 mL of diluted cell suspension over 15 mL Ficoll-Paque (1.077 density) in a 50 mL conical tube and centrifuge at 400×g for 35 minutes at 20 °C in a swinging bucket rotor (without brake).
4. Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the interphase.
5. Carefully transfer the interphase cells to a new 50 mL conical tube.
6. Fill the conical tube with PBS containing 2 mM EDTA or 0.6% ACD-A, mix and centrifuge at 300×g for 10 minutes at 20 °C. Carefully remove supernatant completely.
7. For removal of platelets resuspend the cell pellet in 50 mL of PBS containing 2 mM EDTA or 0.6% ACD-A and wash twice: centrifuge for 10–15 minutes at 200×g at 20 °C. Carefully remove supernatant completely.



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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