

Contents

1. Description
 - 1.1 Principle of the MACS® Separation
 - 1.2 Background information
 - 1.3 Applications
 - 1.4 Reagent and instrument requirements
2. Protocol
 - 2.1 Sample preparation
 - 2.2 Magnetic labeling
 - 2.3 Magnetic separation
 - 2.4 Magnetic labeling and separation using autoMACS® Separators
3. Example of a separation using the CD235a (Glycophorin A) MicroBeads, human

1. Description

This product is for research use only.

Components	2 mL CD235a (Glycophorin A) MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD235a (Glycophorin A) antibodies (isotype: mouse IgG1).
Capacity	For 10 ⁹ total cells, up to 100 separations.
Product format	CD235a (Glycophorin A) MicroBeads, human are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at +2 to +8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS Separation

First, the CD235a (Glycophorin A)⁺ cells are magnetically labeled with CD235a (Glycophorin A) MicroBeads, human. Then, the cell suspension is loaded onto a MACS Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD235a (Glycophorin A)⁺ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD235a (Glycophorin A)⁺ cells. After removing the column from the magnetic field, the magnetically retained CD235a (Glycophorin A)⁺ cells can be eluted as the positively selected cell fraction.

1.2 Background information

CD235a (Glycophorin A) MicroBeads, human 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.

1.3 Applications

- Positive selection or depletion of cells expressing human CD235a (Glycophorin A) antigen.
- Isolation 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).

1.4 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 to +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 (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.

- MACS Columns and MACS Separators: CD235a (Glycophorin A)⁺ cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD 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 MultiMACS™ Cell24 Separator Plus or autoMACS Columns on the autoMACS NEO or autoMACS Pro Separators.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10 ⁷	2×10 ⁸	MiniMACS, OctoMACS, SuperMACS II
LS	10 ⁸	2×10 ⁹	MidiMACS, QuadroMACS, SuperMACS II
	10 ⁸	10 ⁹	MultiMACS Cell24 Separator Plus
XS	10 ⁹	2×10 ¹⁰	SuperMACS II
Depletion			
LD	10 ⁸	5×10 ⁸	MidiMACS, QuadroMACS, SuperMACS II, MultiMACS Cell24 Separator Plus
D	10 ⁹		SuperMACS II
Positive selection or depletion			
autoMACS	2×10 ⁸	4×10 ⁹	autoMACS NEO Separator, autoMACS Pro Separator

Multi-24 Column Block (per column)	10 ⁸	10 ⁹	MultiMACS Cell24 Separator Plus
---	-----------------	-----------------	------------------------------------

▲ **Note:** Column adapters are required to insert certain columns into the SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

▲ **Note:** If separating with LS or LD Columns and the MultiMACS Cell24 Separator Plus use the Single-Column Adapter. Refer to the user manual for details.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD235a (Glycophorin A) Antibody, anti-human. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) 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 (30 µm) (# 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 using a MACS PBMC Isolation Kit or by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **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.

For details refer to the data sheet or the protocols section at www.miltenyibiotec.com/protocols.

When working with tissues, prepare a single-cell suspension using standard protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, it is recommended to use density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).



2.2 Magnetic labeling

▲ Cells can be labeled with MACS MicroBeads using the autolabeling function of the autoMACS NEO or autoMACS Pro Separators. For more information refer to section 2.4.

▲ 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.

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

▲ 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).

▲ The recommended incubation temperature is +2 to +8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

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 10⁷ total cells.
4. Add 20 µL of CD235a (Glycophorin A) MicroBeads, human per 10⁷ total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (+2 to +8 °C).
6. (Optional) Add staining antibodies, e.g., CD235a (Glycophorin A) Antibody, anti-human, FITC, according to manufacturer's recommendation.
7. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. 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.

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. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to 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. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.
MS: 3×500 µL LS: 3×3 mL

▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.

- Remove column from the separator and place it on a suitable collection tube.
- 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

- (Optional) To increase the purity of CD235a (Glycophorin A)⁺ 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

- Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the LD Column data sheet.
- Prepare column by rinsing with 2 mL of buffer.
- Apply cell suspension onto the column.
- Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total flow-through; 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 D Columns

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

Magnetic separation with the MultiMACS Cell24 Separator Plus

Refer to the the MultiMACS Cell Separator Plus user manual for instructions on how to use the MultiMACS Cell24 Separator Plus.

2.4 Magnetic labeling and separation using autoMACS Separators

▲ Refer to the user manual and the short instructions for instructions on how to use the autoMACS Separators.

▲ Buffers used for operating the autoMACS Separators should have a temperature of $\geq +10^\circ\text{C}$.

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = unlabeled (negative) fraction,
position C = labeled (positive) fraction.

2.4.1 Magnetic labeling and separation using the autoMACS NEO Separator

▲ The autoMACS NEO Separator enables stage loading to extend column capacity for selected reagents, minimizing the need to divide larger samples.

▲ For more information on selecting alternative separation programs, stage loading-compatible reagents, autolabeling-compatible reagents, and the minimal and maximal volumes for each reagent and Chill Rack, refer to www.miltenyibiotec.com/automacs-neo-sample-processing.

Magnetic separation after manual labeling

- Label the sample as described in section 2.2 Magnetic labeling.
- Prepare and prime the instrument.
- Place the Chill Rack on the MACS MiniSampler S.
- Select the same Chill Rack in the **Experiment** tab. An experiment is created automatically. Tap to select sample positions.
- Assign a reagent to each sample.
- Manual labeling is set automatically if autolabeling is not supported or no reagent rack is selected. Alternatively, tap **Labeling** in the reagent placement dialog and select **Manual**.
- Tap **Sample volume** in the **Sample process** pane and enter the sample volume. Tap the return key.
- The separation program for highest target cell purity is selected by default. Refer to the **Sample process** pane for all available programs.
- Place the sample(s) and empty tubes to the Chill Rack.
- Tap **Run** to start the separation process.

Fully automated magnetic labeling and separation

- Prepare and prime the instrument.
- Place the Chill Rack and MACS Reagent Rack 8 on the MACS MiniSampler S.
- Select the same Chill Rack and MACS Reagent Rack 8 in the **Experiment** tab. An experiment is created automatically.
- Tap to select sample position(s).
- To assign a reagent to each sample, tap **Scan reagent** and scan the reagent barcode. Alternatively, tap on a free position of the MACS Reagent Rack 8 for selection out of the reagent list.
- Unscrew the lids from the reagent vials and place the vials onto the designated positions on the MACS Reagent Rack 8.
- Tap **Place reagent(s) on reagent rack** button in the dialog box.
- Automated labeling is set automatically if autolabeling is supported and a reagent rack is selected. Alternatively, tap **Labeling** in the reagent placement dialog and select **Auto**.
- Tap **Sample volume** in the **Sample process** pane and enter the sample volume. Tap the return key.
- Tap **Run** to start the separation process.

2.4.2 Magnetic labeling and separation using the autoMACS Pro Separator

Magnetic separation after manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Apply tube containing the sample.
4. 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.

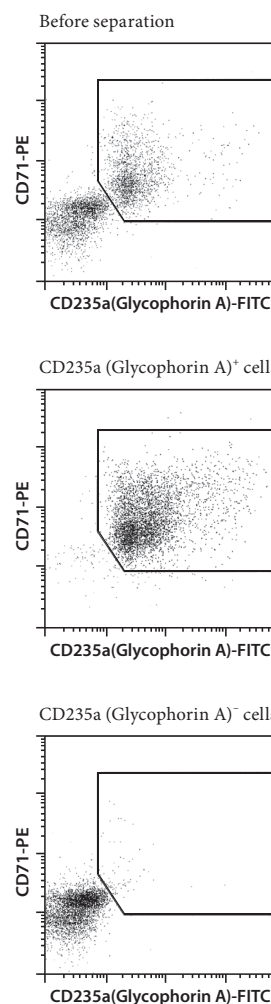
5. Tap **Run** to start the separation process.

Fully automated magnetic labeling and separation

1. Switch on the instrument for automatic initialization.
2. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS Pro Separator. Place the reagent into the appropriate position on the reagent rack.
3. Place sample and collection tubes into the Chill Rack.
4. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu. The correct labeling, separation, and wash protocols will be selected automatically.
5. Enter sample volume into the **Volume** submenu. Press **Enter**.
6. Tap **Run** to start the separation process.

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

CD235a (Glycophorin A)⁺ cells were isolated from human cord blood using CD235a (Glycophorin A) MicroBeads, human, an MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with CD235a (Glycophorin A)-FITC and CD71-PE and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



Refer to www.miltenyibiotec.com for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

autoMACS, gentleMACS, MACS, MidiMACS, the Miltenyi Biotec logo, MiniMACS, MultiMACS, OctoMACS, QuadroMACS, and SuperMACS are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Ficoll-Paque is a trademark of GE Healthcare companies.

Copyright © 2024 Miltenyi Biotec and/or its affiliates. All rights reserved.