

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
3. Example of a separation using the CD66abce MicroBead Kit
4. Reference

1. Description

Components	1 mL CD66abce-Biotin, human: monoclonal anti-human CD66abce antibodies conjugated to biotin (isotype: mouse IgG2b). 2 mL Anti-Biotin MicroBeads: MicroBeads conjugated to monoclonal Anti-Biotin antibodies.
Capacity	For 10 ⁹ total cells, up to 100 separations.
Product format	CD66abce-Biotin and Anti-Biotin 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.

This product is applicable for the separation of cells from rhesus monkey (*Macaca mulatta*). The antibodies have been tested to cross-react with cynomolgus (*Macaca fascicularis*). Cross-reactivity with other non-human primates has not been tested.

1.1 Principle of the MACS® Separation

First, CD66a⁺, CD66b⁺, CD66c⁺, and CD66e⁺ cells are magnetically labeled with the CD66abce-Biotin antibody and Anti-Biotin 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 CD66a⁺, CD66b⁺, CD66c⁺, and CD66e⁺ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD66a⁺, CD66b⁺, CD66c⁺, and CD66e⁺ cells. After removing the column from the magnetic field, the magnetically retained CD66a⁺, CD66b⁺, CD66c⁺, and CD66e⁺ cells can be eluted as the positively selected cell fraction.

1.2 Background information

The human CD66 antigens CD66a, b, c, e belong to the family of carcinoembryonic antigen-related cell adhesion molecules (CEACAM).¹ They either span the plasma membrane (CD66a) or are membrane-associated (CD66b, c, e) and are characterized by extracellular immunoglobulin-like domains. CD66 proteins are involved in a wide variety of cellular processes, including cell adhesion, activation, proliferation, differentiation, survival, and apoptosis.

CD66a (also known as CEACAM1, BGP, NCA-160, and C-CAM) is expressed by certain epithelial, endothelial, and hematopoietic cells. Upon activation, cell surface expression of CD66a is upregulated on various leukocytes, including granulocytes, T cells, and NK cell subsets. CD66b (CEACAM8, NCA-95, CGM6) is expressed on granulocytes. CD66c (CEACAM6, NCA-50/90) is expressed on granulocytes and epithelial cells. CD66e (CEA, CEACAM5) is over-expressed in certain tumors of epithelial origin and is used as a tumor marker.

1.3 Applications

- The CD66abce MicroBead Kit is used for the isolation or depletion of human neutrophils from human peripheral blood.

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 Ca²⁺ or Mg²⁺ are not recommended for use.

- MACS Columns and MACS Separators: CD66a⁺, CD66b⁺, CD66c⁺, and CD66e⁺ 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 CD66a, CD66b, CD66c, or CD66e antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Separator 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 CD15 antibody for flow cytometric analysis, e.g., CD15-FITC (# 130-081-101), CD15-PE (# 130-091-375), CD15-APC (# 130-091-371), CD66abce-PE (# 1303-093-133), or CD66abce-APC (# 130-093-155). Magnetically labeled cells can also be stained with fluorochrome-conjugated Anti-Biotin antibodies for flow cytometric analysis, e.g., Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), and Anti-Biotin-APC (# 130-090-856). For more information about other fluorochrome-conjugates see www.miltenyibiotec.com.
- (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

For the isolation of highly pure rhesus monkey granulocytes, monocytes should be depleted prior to CD66abce enrichment using CD14 MicroBeads, non-human primate (# 130-091-097) and an LS Column. Alternatively, rhesus monkey monocytes can be depleted by using CD56 MicroBeads, non-human primate (# 130-091-094).

When working with human anti-coagulated peripheral blood or buffy coat, leukocytes should be isolated using lysis of erythrocytes.

Lysis of erythrocytes

- Dilute one volume of cell suspension (e.g. freshly drawn blood, buffy coat, leukapheresis material) with 5–10 volumes of lysis buffer (155 mM NH₄Cl; 10 mM KHCO₃ and 0.1 mM EDTA), pH 7.3.
▲ **Note:** Do not use BD FACS™ Lysing Solution.
- Mix gently and incubate for 10 minutes at room temperature. Rotate tube continuously using the MACSmix Tube Rotator (# 130-090-753) or turn tube several times during incubation.
- Centrifuge at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
- Wash cells twice by adding 10 mL of buffer. Centrifuge at 200×g for 10 minutes at room temperature and carefully remove supernatant.
- Resuspend the cell pellet in an appropriate buffer and proceed to magnetic labeling.

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 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. 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.

- Determine cell number.
- Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
- Resuspend cell pellet in 40 µL of buffer per 10⁷ total cells.
- Add 10 µL of CD66abce-Biotin per 10⁷ total cells.
- Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
- Add 30 µL of buffer per 10⁷ total cells.
- Add 20 µL of Anti-Biotin MicroBeads.
- Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).

9. (Optional) Add staining antibodies, e.g., 10 μ L of CD15-FITC (# 130-081-101), CD66abce-PE (# 130-093-133), or Anti-Biotin-PE (# 130-090-756) and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300 \times g for 10 minutes. Aspirate supernatant completely.
11. Resuspend up to 10^8 cells in 500 μ L 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 μ L of buffer.
12. 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 CD66abce⁺ 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 μ L LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 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 \times 500 μ L LS: 3 \times 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
7. (Optional) To increase the purity of CD66abce⁺ 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

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 \times 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 Cell separation programs section 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 below the uptake port and the fraction collection tubes at port neg1 and port pos1/pos2.
3. For a standard separation choose one of the following programs:
 Positive selection: “Possel”
 Collect positive fraction from outlet port pos1.

 Depletion: “Depletes”
 Collect negative fraction from outlet port neg1.

Magnetic separation with the autoMACS™ Pro Separator

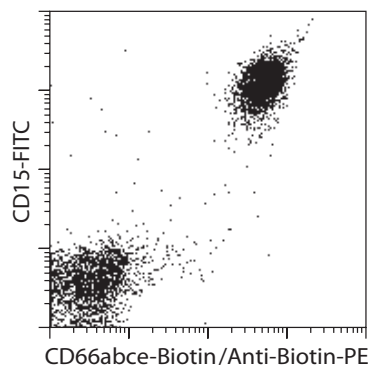
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:
 Positive selection: “Possel”
 Collect positive fraction in row C of the tube rack.

 Depletion: “Depletes”
 Collect negative fraction in row B of the tube rack.

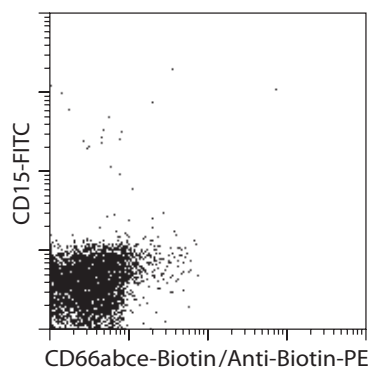
3. Example of a separation using the CD66abce MicroBead Kit

Separation of peripheral blood leukocytes using the CD66abce MicroBead Kit, an MS Column, and an appropriate separator (see table in section 1.4). Cells are fluorescently stained with Anti-Biotin-PE (# 130-090-756) and CD15-FITC (# 130-081-101). Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.

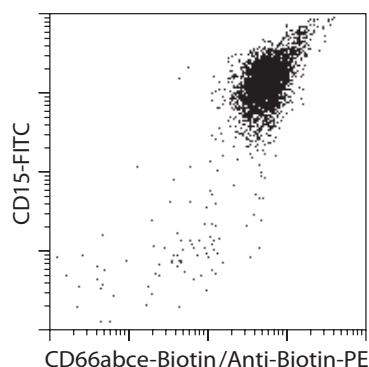
Before separation



Cells depleted of CD66abce⁺ granulocytes



Isolated CD66abce⁺ granulocytes



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.

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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4. Reference

1. Gray-Owen, S. D. and Blumberg R. S. (2006) CEACAM1: contact-dependent control of immunity. *Nat. Rev. Immunol.* 6: 433–446.