Miltenyi Biotec

CD16 MicroBeads
human

Order no. 130-045-701

Index

1. Description

Components	2 mL CD16 MicroBeads, human: MicroBeads conjugated to monoclonal mouse anti-human CD16 antibodies (isotype: mouse IgM).
Size	For 2×10^9 total cells, up to 200 separations.
Product format	CD16 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 $CD16^+$ cells are magnetically labeled with CD16 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 $CD16^+$ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of $CD16^+$ cells. After removal of the column from the magnetic field, the magnetically retained $CD16^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

CD16 MicroBeads are developed for the isolation of untouched eosinophils from peripheral blood by depletion of neutrophils.[1-11] The CD16 antigen is expressed on virtually all NK cells, neutrophils, activated macrophages and a small subset of T cells. The antigen is the low affinity Fc receptor for aggregated IgG (FcγRIIIA and FcγRIIIB).[12, 13]

Examples of applications

- Isolation of eosinophils with CD16 MicroBeads for the functional analysis of surface molecules[10,11].
- Isolation of eosinophils with CD16 MicroBeads or for studies on signal transduction[15], chemotaxis[10,16], cytokine expression[11,16], degranulation assays[7], and apoptosis[15].

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: CD16 MicroBeads can be used for depletion of $CD16^+$ cells on LD, CS or D Columns. Cells which strongly express the CD16 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 CD16 antibody for flow cytometric analysis, e.g. CD16-FITC (# 130-091-244), CD16-PE (# 130-091-245), CD16-APC (# 130-091-246).
- (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, peripheral blood granulocytes should be isolated by density gradient centrifugation (see Appendix A.)

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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/protocols).

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using 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 5×10^7 total cells. When working with fewer than 5×10^7 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 10^8 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 50 µL of buffer per 5×10^7 total cells.
4. Add 50 µL of CD16 MicroBeads per 5×10^7 total cells.
5. Mix well and incubate for 30 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 CD16-FITC (# 130-091-244), 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 $CD16^+$ 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:
   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. Collect unlabeled $CD16^-$ cell fraction (outlet port "neg1").

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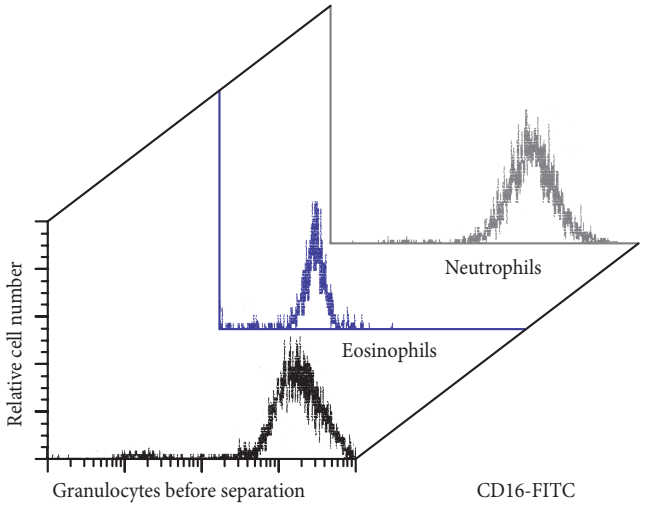
3. Example of a separation using CD16 MicroBeads

Isolation of untouched eosinophils from peripheral blood granulocytes using CD16 MicroBeads. Cells are flourescently stained with CD16-FITC.



4. Appendix

A. Isolation of granulocytes from peripheral blood using Percoll™

1. Start with freshly drawn human blood treated with an anticoagulant (e.g. heparin, EDTA, citrate, ACD-A or CPD). Alternatively, use fresh defibrinated blood or buffy coat treated with an anti-coagulant, not older than 8 hours.
2. Prepare 15 mL Percoll™ gradients with a density of 1.088 (ice-cold) in 50 mL conical tubes by mixing 9.5 mL Percoll with 1.5 mL 10×Hanks balanced salt solution (HBSS) and 4 mL H_2O.
3. Dilute cell suspension 1:5 with buffer (e.g. PBS containing 2–5 mM EDTA).
4. Carefully overlay each 15 mL Percoll gradient with 35 mL of diluted cell suspension without mixing phases.
5. Centrifuge at 400×g for 30 minutes at 20 °C in a swinging-bucket rotor (without brake).
6. Collect white cell layer directly above the red blood cells.Transfer the cells to a new 50 mL conical tube.
7. Dilute with buffer (at least 1:5) and centrifuge at 300×g for 15 minutes at 20 °C. Carefully remove supernatant completely.
8. Resuspend cell pellet in a small volume of buffer and lyse remaining red blood cells by adding 50 mL of lysis buffer (155 mM NH_4Cl; 10 mM $KHCO_3$; 0.1 mM EDTA) and incubate for 5–7 minutes at room temperatur.
9. Centrifuge at 300×g for 10 minutes at 20 °C. Carefully remove supernatant.
10. Wash cells twice by adding buffer. Centrifuge at 300×g for 10 minutes at 20 °C. Carefully remove supernatant.
11. Resuspend cell pellet in an appropriate buffer. Count cells and proceed to magnetic labeling (for details see MACS Reagent data sheets).

▲ Cells may be stored over night in buffer (PBS) supplement with 5% autologous serum or 2% fetal calf serum.

B. Protocol for removal of azide from MicroBead suspension

1. Apply 2 mL MicroBead suspension over a freshly prepared MS Column that is placed in the magnetic field of an appropriate MACS separator. Discard the effluent.
2. Rinse with 3×500 µL of buffer.
3. Remove column from separator and place it on a suitable collection tube. Pipette 2 mL buffer (PBS containing 2 mM EDTA) on top of the column and collect the effluent as azide-free MicroBead suspension.

5. References

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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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