Miltenyi Biotec

CD16+ Monocyte Isolation Kit
human

Order No. 130-091-765

Index

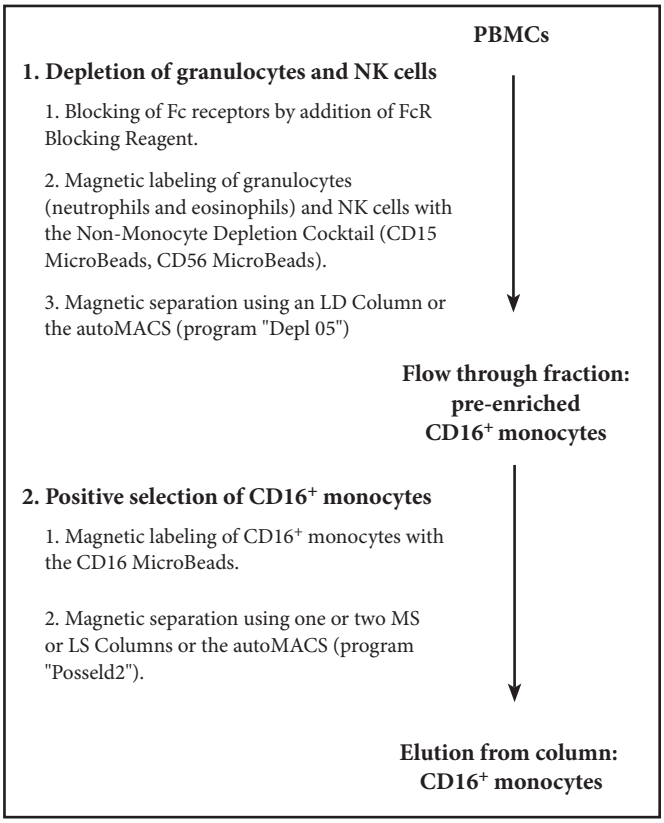
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1. Description

Components	**2 mL FcR Blocking Reagent**: human IgG. **2 mL Non-Monocyte Depletion Cocktail, human:** Cocktail of MicroBeads conjugated to monoclonal anti-human CD15 (isotype: mouse IgM), and anti-human CD56 (isotype: mouse IgG1) antibodies. **2 mL CD16 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD16 (isotype: mouse IgM) antibodies.
Size	For 2×10^9 total cells; up to 20 separations.
Product format	FcR Blocking Reagent is supplied as a solution containing 0.1% gelatine and 0.05% sodium azide. The Non-Monocyte Depletion Cocktail and the 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 dates are indicated on the vial labels.

1.1 Principle of MACS® separation

The CD16+ Monocyte Isolation Kit is developed for the isolation of CD16+ monocytes from peripheral blood mononuclear cells (PBMCs). Isolation of CD16+ monocytes is performed in a two-step procedure. First, PBMCs are labeled with the Non-Monocyte Depletion Cocktail. This cocktail comprises CD15 and CD56 MicroBeads for magnetic labeling of CD15+ granulocytes (neutrophils and eosinophils) and CD56+ NK cells. Upon subsequent magnetic separation of the cells over a MACS® Column, which is placed in a magnetic field of the MACS® Separator, the magnetically labeled granulocytes and NK cells are retained on the column. The flow through fraction with the pre-enriched CD16+ monocytes is then incubated with the CD16 MicroBeads. Upon subsequent magnetic separation, the magnetically labeled CD16+ monocytes are retained on the column and are eluted after removal of the column from the magnetic field.
CD15+ granulocytes and CD56+ NK cells are depleted prior to positive selection of CD16+ monocytes using CD16 MicroBeads, because both cell types express CD16.



1.2 Background and product applications

Blood monocytes can be subdivided into two distinct subpopulations: $CD14^{high}$ $CD16^{-}$ monocytes, which account for about 90–95% of all blood monocytes, and $CD14^{low}$ CD16+ monocytes, which account for about 5–10% of all blood monocytes.[1-3] The CD16+ monocytes can be further subdivided according to expression of 6-sulfo LacNAc (M-DC8), a distinct carbohydrate modification of the P selectin glycoprotein ligand 1 (PSGL-1).[4,5] About 15–25% of all CD16+ monocytes express M-DC8.
CD16+ monocytes share with myeloid blood dendritic cells a potent capacity to prime T cells *in vitro*. However, unlike type-1 myeloid dendritic cells (MDCs1) in blood, CD16+ monocytes do not express CD1c (BDCA-1), and unlike type-2 myeloid dendritic cells (MDCs2) in blood, CD16+ monocytes express only low levels of BDCA-3.[6] Furthermore, CD16+ monocytes produce far more TNF-α in response to LPS than myeloid dendritic cells.[3]

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Examples of applications

- analysis of gene expression in blood CD16$^+$ monocytes
- elucidation of specific signal transduction pathways
- analysis of antigen uptake, processing and presentation
- investigation of blood CD16$^+$ monocyte maturation, migration and differentiation
- studies on the dysfunction and role of blood CD16$^+$ monocytes in disease, e.g. virus infections
- stimulation of T cell responses by antigen-pulsed blood CD16$^+$ monocytes

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:** Depletion of granulocytes and NK cells is performed on an LD Column. The subsequent positive selection of CD16$^+$ monocytes is performed on one or two MS or LS Columns. Depletion and positive selection can also be performed by using the autoMACS™ Separator.

Column	max. number of labeled cells	max. number of total cells	Separator
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Depletion and positive selection			
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 antibodies for flow cytometric evaluation of CD16$^+$ monocyte isolation, for example CD15-APC (# 130-091-371), CD56-APC, CD14-FITC (# 130-080-701), and CD16-PE (# 130-091-245).

  ▲ **Note:** For fluorescence staining against CD56 an antibody clone has to be used which recognizes a different epitope of CD56 than that recognized by clone AF12-7H3, e.g. NCAM16.2.

- (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.



2.2 Magnetic labeling of granulocytes and NK cells

▲ 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 10^8 total cells. When working with fewer than 10^8 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 2×10^8 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 300 µL of buffer per 10^8 total cells.
4. Add 100 µL of **FcR Blocking Reagent** and 100 µL of **Non-Monocyte Depletion Cocktail** per 10^8 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. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
7. Resuspend cell pellet in buffer:
   Depletion with LD Column: 500 µL for up to 1.25×10^8 cells.
   Depletion with autoMACS Separator: 500 µL for up to 1×10^8 cells.

   ▲ **Note:** For larger cell numbers, scale up buffer volume accordingly.
8. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of granulocytes and NK cells

Depletion with LD Column

1. Place LD Column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
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. Perform washing steps by adding buffer successively once the column reservoir is empty. Collect total effluent. This fraction contains the unlabeled cells.
5. Proceed to 2.4 for the isolation of CD16$^+$ monocytes.

Depletion with 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. Choose separation program "Depl 05".
3. Collect the unlabeled fraction (outlet port "neg1").
4. Proceed to 2.4 for the isolation of CD16$^+$ monocytes.



2.4 Magnetic labeling of pre-enriched CD16$^+$ monocytes

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^8 cells (step 2.2). For larger starting cell numbers, scale up volumes accordingly.

1. Centrifuge the cells at 300×g for 10 minutes. Pipette off supernatant completely.
2. Resuspend cell pellet in 400 µL of buffer.
3. Add 100 µL of **CD16 MicroBeads**.
4. 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.
5. Wash cells by adding 5–10 mL of buffer and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
6. Resuspend up to 10^8 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
7. Proceed to magnetic separation (2.5).



2.5 Magnetic separation: Positive selection of CD16$^+$ monocytes

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.
   MS: 1 mL LS: 5 mL.
   Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger supplied with the column.
   ▲ **Note:** To increase the purity of the magnetically labeled fraction, it can be passed over a new, freshly prepared column a second time.

Positive selection with autoMACS™ Separator

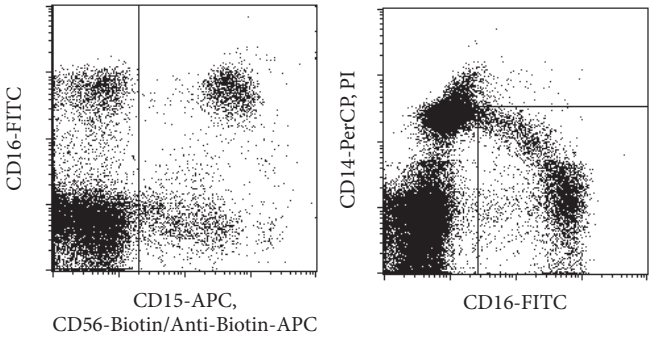
▲ Refer to the "autoMACS™ User Manual" for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose separation program "Posseld2".
3. Collect the positive fraction (outlet port "pos2"). This is the enriched dendritic cell fraction.

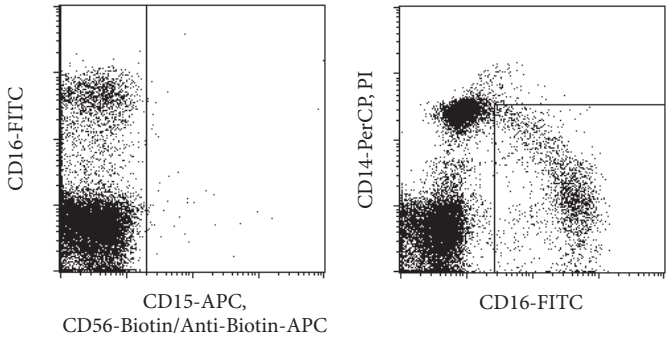
3. Example of a separation using the CD16$^+$ Monocyte Isolation Kit

CD16$^+$ monocytes were isolated from PBMCs using the CD16$^+$ Monocyte Isolation Kit, an LD Column with a MidiMACS™ Separator, and an MS Column with a MiniMACS™ Separator.
Aliquots of the different cell fractions were stained with CD15-APC and CD56-Biotin/Anti-Biotin-APC for identification of granulocytes (neutrophils and eosinophils) and NK cells, and CD16-FITC and CD14-PerCP for identification of CD16$^+$ monocytes (see next page). Additionally cells were stained with Anti-M-DC8 (DD-2)-PE to discriminate between M-DC8$^+$ and M-DC8$^-$ CD16$^+$ monocytes. Cell debris and dead cells were excluded from analysis based on scatter signals and PI fluorescence.

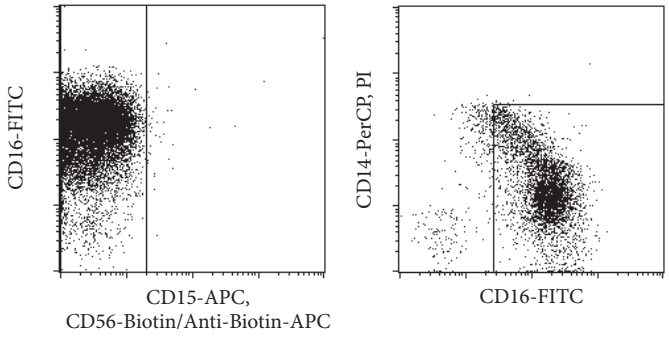
PBMCs before separation

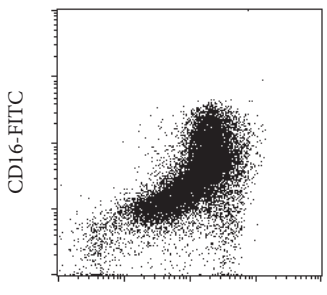


Pre-enriched CD16$^+$ monocytes after depletion of granulocytes and NK cells



Enriched CD16$^+$ monocytes





4. 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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