Miltenyi Biotec

CD163 MicroBead Kit
human

Order no. 130-124-420

Contents

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.

1. Description

This product is for research use only.

Components	**2 mL CD163-Biotin, human:** monoclonal CD163 antibody conjugated to Biotin (isotype: recombinant human IgG1). **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	All reagents 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.

1.1 Principle of the MACS® Separation

First, the CD163$^+$ cells are indirectly magnetically labeled with CD163-Biotin antibodies 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 CD163$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD163$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD163$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

The CD163 MicroBead Kit has been developed for the enrichment of human cells based on the expression of the CD163 antigen. CD163 antigen is a single-chain transmembrane protein also known as hemoglobin scavenger receptor or M130. It is most abundantly expressed by mature tissue macrophages and peripheral blood monocytes. The expression of CD163 is up-regulated *in vitro* and *in vivo* by anti-inflammatory mediators, such as interleukin 10 (IL-10) and glucocorticoid and is shed upon activation of macrophages. CD163 functions as a high affinity scavenger receptor for the complex of hemoglobin and haptoglobin. Depending on the ligand, crosslinking of CD163 initiates signal transduction leading to the production of pro-inflammatory cytokines, such as IL-1ß, IL-6, and GM-CSF, or the anti-inflammatory cytokine IL-10.

1.3 Applications

- Positive selection of cells expressing human CD163 antigen.
- Isolation of CD163$^+$ cells from peripheral blood mononuclear cells (PBMCs) or single-cell suspensions from tissue (e.g. tumor tissue).

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 (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: CD163$^+$ cells can be enriched by using MS Columns. Cells which strongly express the CD163 antigen can also be depleted using MS or LS Columns. Positive selection can also be performed by using 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, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, SuperMACS II,
autoMACS	2×10^8	4×10^9	autoMACS Pro

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

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- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD45-VioBlue®, CD11b-PE-Vio® 770, CD206-FITC, CD14-FITC, CD163-VioBlue®, or CD163-PE. 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) Tumor Dissociation Kit, human (# 130-095-929) in combination with the gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427) and gentleMACS C Tubes (# 130-093-237, # 130-096-334) for the dissociation of hman tumor tissue.
- (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 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 protocols section at www.miltenyibiotec.com/protocols.

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.miltenyibiotec.com/gentlemacs.

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 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.

▲ The recommended incubation temperature is 2–8 °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 10^7 total cells.
4. Add 20 µL of CD163-Biotin per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies, e.g., CD14-FITC according to manufacturer's recommendation.
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
9. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
10. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
11. 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 $CD163^+$ 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.
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) If frequency of $CD163^+$ cells is <5% (e.g. in tumor tissues) the eluted fraction should 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.

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Magnetic separation with the autoMACS® Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

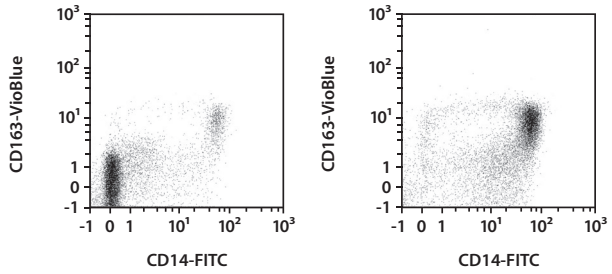
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

   Positive selection:
   Possel if frequency of CD163$^+$ cells is >5%
   or
   Posseld2 if the frequency of CD163$^+$ cells is <5%
   Collect positive fraction in row C of the tube rack.

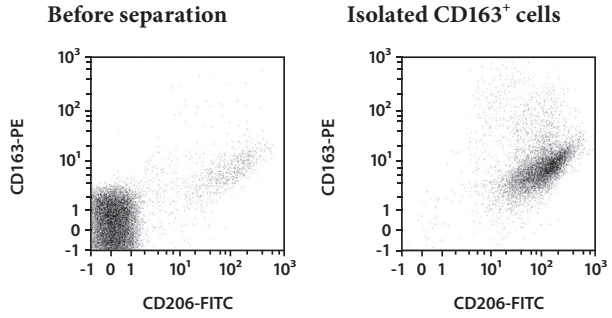
3. Example of a separation using the CD163 MicroBead Kit

CD163$^+$ cells were isolated from human peripheral blood mononuclear cells (PBMCs) (A) or ovarian carcinoma samples (B) using the CD163 MicroBead Kit, an MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with CD45-VioBlue® as well as with CD14-FITC and CD163-VioBlue (A) or CD206-FITC and CD163-PE (B) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

A)



B)



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.

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