Miltenyi Biotec

Classical Monocyte Isolation Kit
human

Order no. 130-117-337

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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	**1 mL Classical Monocyte Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal anti-human antibodies against CD3, CD7, CD16, CD19, CD56, CD123, and CD235a.
	2 mL Anti-Biotin MicroBeads: MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1).
	0.5 mL Thrombocyte Removal Reagent, human: Monoclonal anti-human CD61 antibody conjugated to biotin.
	1 mL FcR Blocking Reagent, human
Capacity	For 10^9 total cells, up to 100 separations.
Product format	All components 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

Using the Classical Monocyte Isolation Kit, human $CD14^+CD16^-$ cells are isolated by depletion of non-monocytes, non-classical monocytes, and, if using the Thrombocyte Removal Reagent, thrombocytes. Non-target cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. In between the two labeling steps and after labeling no washing steps are required. The magnetically labeled non-target cells are depleted by retaining them within a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled $CD14^+CD16^-$ cells run through the column.

1.2 Background information

Monocytes are an extremely plastic population of myeloid cells. In humans three main subpopulations of monocytes are described: classical, intermediate, and non-classical. The classical monocytes represent the largest subpopulation in human blood and are characterized by expression of the monocyte lineage marker CD14 and the lack of CD16, while non-classical monocytes express CD16 and intermediate monocytes co-express both markers. Functional studies suggests that these subpopulations may be distinct and classical monocytes may exert unique functions. Moreover, the number of monocytes and subsets ratio has been correlated to various pathological settings. Monocytes' plasticity has been exploited for the development of culture protocols that allow the generation of so called monocyte-derived dendritic cells and monocyte-derived macrophages. The Classical Monocyte Isolation Kit has been developed for the isolation of classical monocytes for further studies.

1.3 Applications

- Isolation of $CD14^+CD16^-$ cells from human peripheral blood mononuclear cells (PBMCs) for further phenotypical or functional characterization.

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.

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- MACS® Columns and MACS Separators: Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

Column	Max. number of labeled cells	Max. number of total cells	Separator
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	10^9	2×10^{10}	SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro
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 VarioMACS™ or SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD14-FITC. 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 density gradient centrifugation, for example, using Ficoll-Paque™.

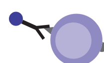
▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

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

▲ To reduce the number of thrombocytes in starting material, it is strongly recommended to perform a thrombocyte removal step. Therefore, resuspend cells in autoMACS® Rinsing Solution (# 130-091-222) and centrifuge at 200×g for 10–15 minutes at room temperature. Thrombocytes will remain in supernatant while the cell pellet will be used for $CD14^+CD16^-$ classical monocyte isolation. If necessary, repeate thrombocyte removal steps.
The Thrombocytes Removal Reagent can be optionally used for further removal of platelets from the sample but may result in lower recovery of monocytes.

▲ 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

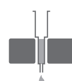
▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 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 room temperature. 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 30 µL of buffer per 10^7 total cells.
4. Add 10 µL FcR Blocking Reagent, human per 10^7 total cells.
5. Add 10 µL of Classical Monocyte Biotin-Antibody Cocktail per 10^7 total cells.
6. (Optional) Add 5 µL of Thrombocyte Removal Reagent per 10^7 total cells.
7. Mix well and incubate for 5 minutes at room temperature.
8. Add 30 µL of buffer per 10^7 cells.
9. Add 20 µL of Anti-Biotin MicroBeads per 10^7 cells.
10. Mix well and incubate for additional 5 minutes at room temperature.
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 $CD14^+CD16^-$ cells. For details refer to 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, representing the enriched CD14$^+$CD16$^-$ monocytes.

4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through, representing the enriched CD14$^+$CD16$^-$ cells, and combine with the flow-through from step 3.

   MS: 500 µL LS: 3 mL

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

5. (Optional) 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 non-monocyte cells by firmly pushing the plunger into the column.

   MS: 1 mL LS: 5 mL

Magnetic separation with XS Columns

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

Magnetic separation with the MultiMACS™ Cell24 Separator

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

Depletion 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 and collection tubes into the Chill Rack.

3. For a standard separation choose the following program:

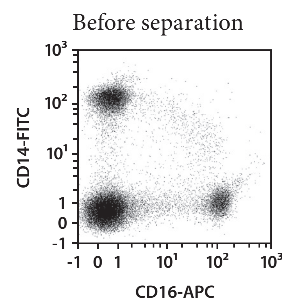
   Depletion: Deplete2
   Collect negative fraction in row B of the tube rack.
   This fraction represents the enriched CD14$^+$CD16$^-$ cells.

4. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-CD14$^+$CD16$^-$ cells.

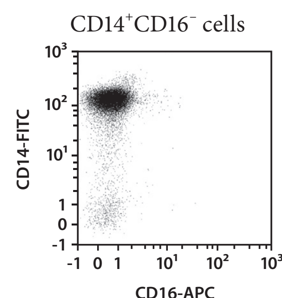
3. Example of a separation using the Classical Monocyte Isolation Kit

CD14$^+$CD16$^-$ cells were isolated from human PBMCs by using the Classical Monocyte Isolation Kit, an LS Columns, and a MidiMACS™ Separator. The cells were fluorescently stained with CD14-FITC and CD16-APC 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.

Before separation



CD14$^+$CD16$^-$ cells



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.

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