Miltenyi Biotec

Pan Monocyte Isolation Kit
human

Order no. 130-096-537

Contents

1. Description

Components	**1 mL FcR Blocking Reagent:** human Ig.
	1 mL Pan Monocyte Biotin-Antibody Cocktail, human: Cocktail of biotin-conjugated monoclonal anti-human antibodies against antigens that are not expressed on human monocytes.
	2 mL Anti-Biotin MicroBeads: MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1).
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 Pan Monocyte Isolation Kit, human monocytes are isolated by depletion of non-monocytes (negative selection). Non-monocytes 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 no washing steps are required. The magnetically labeled non-monocytes are depleted by retaining them on a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled monocytes pass through the column.

1.2 Background information

The Pan Monocyte Isolation Kit is an indirect magnetic labeling system for the isolation of untouched monocytes from human PBMCs. Non-monocytes, such as T cells, NK cells, B cells, dendritic cells, and basophils, are indirectly magnetically labeled using a cocktail of biotin-conjugated antibodies and Anti-Biotin MicroBeads. This Kit contains an improved biotin-cocktail for the simultaneous enrichment of classical ($CD14^{++}/CD16^{-}$), non classical ($CD16^{++}/CD14^{+}$) and intermediate ($CD16^{+}/CD14^{++}$) monocytes[1]. Highly pure unlabeled monocytes are obtained by depletion of the magnetically labeled cells.

1.3 Applications

- Functional studies on monocytes in which any possible effect due to antibody binding to target cells should be avoided.
- Studies on monocyte activation, differentiation, cytokine secretion etc.
- Studies on signal transduction in monocytes.
- Studies on antigen uptake (e.g. phagocytosis) and antigen presentation by monocytes.
- *In vitro* differentiation to dendritic cells from blood monocytes.

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: 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
Positive selection			
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, autoMACS

▲ **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.

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- (Optional) Fluorochrome-conjugated antibodies (e.g. Anti-Slan (M-DC8)-FITC (# 130-093-027), CD14-FITC (# 130-080-701), CD14-PE (# 130-091-242), CD14-APC (# 130-091-243), CD16-APC (# 130-091-246), Anti-Biotin-PE (# 130-090-756), Anti-Biotin-APC (# 130-090-856)). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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 (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 see the protocols section 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 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. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

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 per 10^7 total cells.
5. Add 10 µL of Biotin-Antibody Cocktail per 10^7 total cells.
6. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
7. Add 30 µL of buffer per 10^7 total cells.
8. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
9. Mix well and incubate for an additional 10 minutes in the refrigerator (2–8 °C).
10. Proceed to magnetic separation (2.3).

▲ **Note:** A minimum of 500 µL is required for magnetic separation. If necessary, add buffer to the cell suspension.

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of total 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.

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 monocyte fraction.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through, representing the enriched monocyte cells, 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 only when 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.

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Depletion with the autoMACS® Pro Separator or the autoMACS® Separator

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

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS 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 section describing the cell separation programs in the respective user manual.

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 the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

   Depletion: Deplete
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched monocyte cells.
4. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-monocyte cells.

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 at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

   Depletion: Deplete
   Collect negative fraction from outlet port neg1. This fraction represents the enriched monocyte cells.
4. (Optional) Collect positive fraction from outlet port pos1. This fraction represents the magnetically labeled non-monocyte cells.

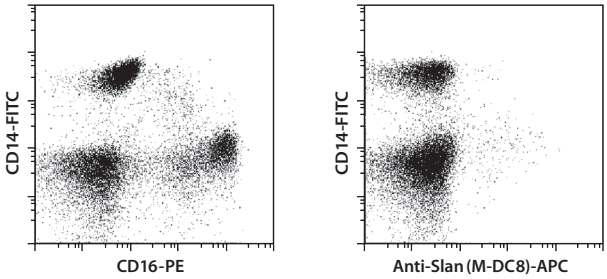
2.4 (Optional) Evaluation of monocyte purity

The purity of the enriched monocytes can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with a fluorochrome-conjugated antibody to discriminate classical ($CD14^{++}CD16^{-}$), non-classical ($CD16^{++}CD14^{+}$) and intermediate ($CD16^{+}CD14^{++}$) monocytes (e.g. CD14-FITC #130-080-701 and CD16-PE #130-091-245) as recommended in the respective data sheets. Analyze cells by flow cytometry or fluorescence microscopy. Labeling of non-monocytes with the Biotin-Antibody Cocktail can be visualized by counterstaining with a fluorochrome-conjugated anti-biotin antibody, e.g. Anti-Biotin-PE (#130-090-756) or Anti-Biotin-APC (#130-090-856). Staining with fluorochrome-conjugated streptavidin is not recommended.

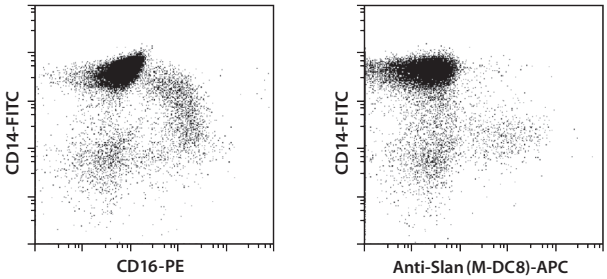
3. Example of a separation using the Pan Monocyte Isolation Kit

Isolation of untouched monocytes from PBMCs by using the Pan Monocyte Isolation Kit and an LS Column. Cells are fluorescently stained with CD14-FITC (#130-080-701), CD16-PE (#130-091-245), and Anti-Slan (M-DC8)-APC (#130-093-031) and analyzed using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

A) Before separation



B) Isolated monocytes



4. References

1. Ziegler-Heitbrock, L. *et al.* (2010) Nomenclature of monocytes and dendritic cells in blood. Blood. 116(16): e74-80.

All protocols and data sheets are available at **www.miltenyibiotec.com**.

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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

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