Miltenyi Biotec

Pan Monocyte Isolation Kit
human

Order no. 130-096-537

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1. Description

This product is for research use only.

Components	**1 mL FcR Blocking Reagent:** human Ig. **1 mL Pan Monocyte Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal antibodies against antigens that are not expressed on human monocytes. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells
Product format	All reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at +2 to +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, human is an indirect magnetic labeling system for the isolation of untouched monocytes from human peripheral blood mononuclear cells (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. 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 to +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.

- Table 1 provides an overview of MACS Columns and MACS Separators with links to the corresponding complete protocols. Use the link matching the MACS Column and MACS Separator used.

Table 1: MACS Columns and MACS Separators overview.

MACS Column (link to protocol)	Max. number of labeled cells	Max. number of total cells	MACS Separator
Columns for manual separation			
MS Column	10^7	2×10^8	MiniMACS, OctoMACS
LS Column	10^8	2×10^9	MidiMACS, QuadroMACS
XLS Column	5×10^8	10^{10}	QuadroMACS XL

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MACS Column (link to protocol)	Max. number of labeled cells	Max. number of total cells	MACS Separator
Columns for instruments			
LS Column	10^8	10^9	MultiMACS Cell24 Separator Plus
autoMACS Column	2×10^8	4×10^9	autoMACS NEO
autoMACS Column	2×10^8	4×10^9	autoMACS Pro Separation program: Depletion: Deplete
Multi-24 Column Block	10^8 per column 2.4×10^9 per block	10^9 per column 2.4×10^{10} per block	MultiMACS Cell24 Separator Plus

▲ **Note:** If separating with LS or LD Columns and the MultiMACS Cell24 Separator Plus, use the Single-Column Adapter. Refer to the user manual for details.

▲ **Note:** For autolabeling compatibilty for the autoMACS NEO Seperator, please see here.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD14 Antibody, anti-human. 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

▲ Complete labeling and separation protocols are available online. Use the link provided in table 1, based on the used MACS Column and MACS Separator.

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by using a MACS PBMC Isolation Kit or by density gradient centrifugation, for example, using Ficoll-Paque™.

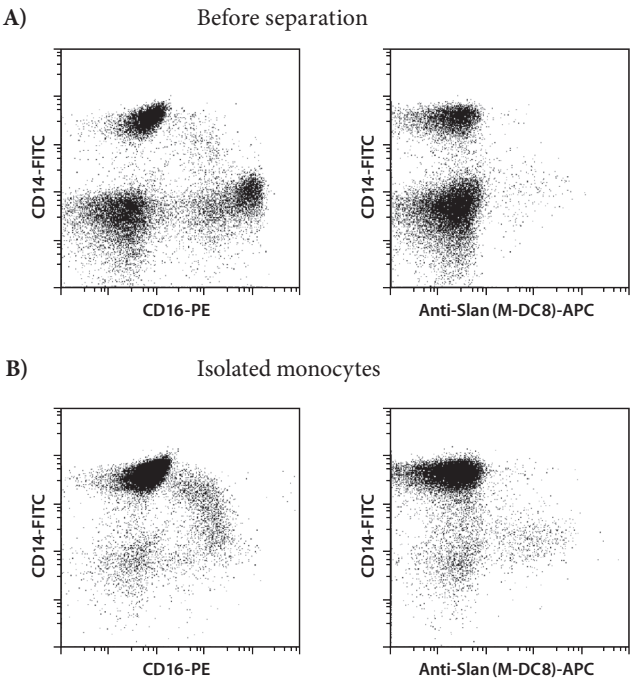
▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200 × g for 10–15 minutes at +20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods. For details refer to the data sheet or the protocols section at www.miltenyibiotec.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, it is recommended to use density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

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

Untouched monocytes were isolated from PBMCs by using the Pan Monocyte Isolation Kit and an LS Column. Cells are fluorescently stained with CD14-FITC, CD16-PE, and Anti-Slan (M-DC8)-APC and analyzed using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



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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