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1. Description

This product is for research use only.

Components	2 mL CD14 MicroBeads, human : MicroBeads conjugated to monoclonal anti-human CD14 antibodies (isotype: mouse IgG2a).
Capacity	For 10 ⁹ total cells
Product format	CD14 MicroBeads 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

First, the CD14⁺ cells are magnetically labeled with CD14 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 CD14⁺ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD14⁺ cells. After removing the column from the magnetic field, the magnetically retained CD14⁺ cells can be eluted as the positively selected cell fraction.

1.2 Background information

CD14 MicroBeads are used for the positive selection or depletion of human monocytes and macrophages from cord blood or peripheral blood mononuclear cells (PBMCs), as well as pleural, peritoneal, or synovial fluids or from various tissues. The CD14 antigen belongs to the LPS receptor complex. Binding of antibody to CD14 does not trigger signal transduction since CD14 lacks a cytoplasmatic domain. CD14 is strongly expressed on most monocytes and macrophages and weakly on neutrophils and some myeloid dendritic cells.

1.3 Applications

- Positive selection or depletion of cells expressing human CD14 antigen.
- Isolation of CD14⁺ monocytes for *in vitro* generation of dendritic cells or macrophages.
- Isolation of CD14⁺ monocytes for studies on cytotoxicity and migration.

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 Ca²⁺ or Mg²⁺ 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
LD Column	10 ⁸	5 × 10 ⁸	MidiMACS, QuadroMACS
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: Positive selection: Possel Depletion: Depletes

MACS Column (link to protocol)	Max. number of labeled cells	Max. number of total cells	MACS Separator
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:** Cells which strongly express the CD14 antigen can also be depleted using MS, LS or XLS Columns.

▲ **Note:** For autolabeling compatibility for the autoMACS NEO Separator, 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

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™.

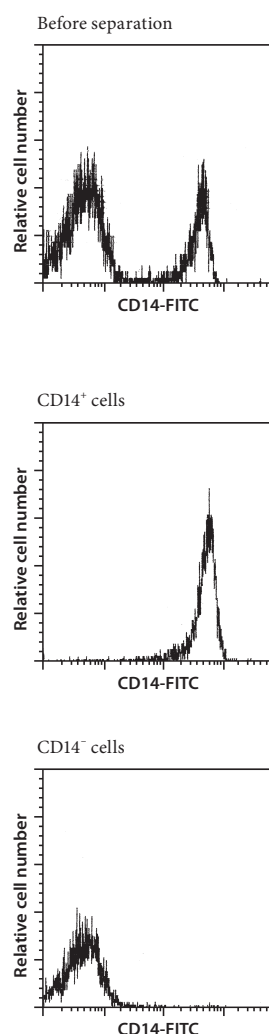
▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200 × g for 10–15 minutes at +20 °C. Carefully aspirate supernatant. Repeat washing step.

For details refer to the data sheet or the protocols section at www.miltenyibiotec.com/protocol.

▲ 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 CD14 MicroBeads

CD14⁺ monocytes were isolated from human PBMCs using CD14 MicroBeads, an MS Column, and a MiniMACS Separator. Cells were fluorescently stained with CD14-FITC and analyzed by flow cytometry. 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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