Miltenyi Biotec

# CD14 MicroBeads UltraPure
## human

Order no. 130-118-906

## 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 CD14 MicroBeads UltraPure, human: MicroBeads conjugated to monoclonal anti-human CD14 antibodies (isotype: mouse IgG2a). |
| **Capacity** | For $10^9$ total cells, up to 100 separations. |
| **Product format** | CD14 MicroBeads UltraPure 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 $CD14^+$ cells are magnetically labeled with CD14 MicroBeads UltraPure. 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 UltraPure are used for the positive selection or depletion of human monocytes and macrophages from cord blood or PBMCs, as well as pleural, peritoneal, or synovial fluids or from various tissues, such as spleen and lymph node. They are especially designed to give better performances working with challenging starting material, i.e., older buffy coats. 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

- Isolation of $CD14^+$ cells from lower than standard quality PBMC preparations.
- Isolation of $CD14^+$ monocytes for *in vitro* generation of dendritic cells[1] or macrophages[2,3].
- Isolation of $CD14^+$ monocytes for studies on cytotoxicity[4] and migration[5].

### 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: $CD14^+$ cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD or D Columns. Cells which strongly express the CD14 antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro Separator or the MultiMACS™ Cell24 Separator Plus.

| Column | Max. number of labeled cells | Max. number of total cells | Separator |
|---|---|---|---|
| **Positive selection** | | | |
| MS | $10^7$ | $2\times10^8$ | MiniMACS, OctoMACS, SuperMACS II |
| LS | $10^8$ | $2\times10^9$ | MidiMACS, QuadroMACS, SuperMACS II, |
| | $10^8$ | $10^9$ | MultiMACS Cell24 Separator Plus |
| XS | $10^9$ | $2\times10^{10}$ | SuperMACS II |
| **Depletion** | | | |
| LD | $10^8$ | $5\times10^8$ | MidiMACS, QuadroMACS, SuperMACS II, MultiMACS Cell24 Separator Plus |
| D | $10^9$ | | SuperMACS II |


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**Positive selection or depletion**

| | | | |
|---|---|---|---|
| autoMACS | $2\times10^8$ | $4\times10^9$ | 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 SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

▲ **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.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis. 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.

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.

▲ 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

▲ Cells can be labeled with MACS MicroBeads using the autolabeling function of the autoMACS Pro Separator. For more information refer to section 2.4.

▲ 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 $2\times10^7$ 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 CD14 MicroBeads per $10^7$ total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies according to manufacturer's recommendations.
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 up to $10^8$ cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

   ▲ **Note:** For depletion with LD Columns, resuspend up to $1.25\times10^8$ cells in 500 µL of buffer.
9. 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 $CD14^+$ 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.





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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) To increase the purity of $CD14^+$ cells, the eluted fraction can 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.

**Magnetic separation with XS Columns**

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

**Depletion with LD Columns**

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total flow-through; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

**Depletion with D Columns**

For instructions on column assembly and separation refer to the D 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.

### 2.4 Cell separation with the autoMACS® Pro Separator

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

▲ All buffer temperatures should be ≥10 °C.

▲ For appropriate resuspension volumes and cell concentrations, please visit www.automacspro.com/autolabeling.

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = negative fraction,

position C = positive fraction.

#### 2.4.1 Fully automated cell labeling and separation

1. Switch on the instrument for automatic initialization.
2. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS Pro Separator. Place the reagent into the appropriate position on the reagent rack.
3. Place sample and collection tubes into the Chill Rack.
4. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu (the correct labeling, separation, and wash protocols will be selected automatically).
5. Enter sample volume into the **Volume** submenu. Press **Enter**.
6. Select **Run**.

#### 2.4.2 Magnetic separation using manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. 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.
4. For a standard separation choose the following program:

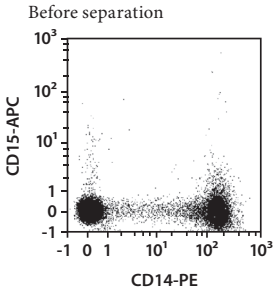
   **Positive selection: Possel**
   Collect positive fraction in row C of the tube rack.

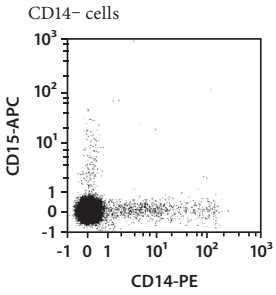
   **Depletion: Depletes**

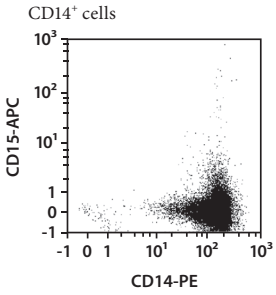
   Collect negative fraction in row B of the tube rack.

## 3. Example of a separation using CD14 MicroBeads UltraPure

$CD14^+$ cells were isolated from human PBMCs using CD14 MicroBeads UltraPure, an MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with CD14-PE and CD15-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.



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