

CD14 MicroBeads, human (51-01-0004)

[Components]

2 mL CD14 MicroBeads, human: MicroBeads conjugated to monoclonal anti- human CD14 antibodies (isotype: mouse IgG2a).

[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 labels.

[Principle of the Separation]

First, the CD14+ cells are magnetically labeled with CD14 MicroBeads. Then, the cell suspension is loaded onto a Column which is placed in the magnetic field of a 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.

[Background information]

CD14 MicroBeads 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. 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.

[Applications]

- ▲ Isolation of CD14+ monocytes for in vitro generation of dendritic cells or macrophages
- ▲ Isolation of CD14+ monocytes for studies on cytotoxicity and migration

[Reagent and instrument requirements]

1. Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA. 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. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
2. Columns and Separators: CD14+ cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD, CS, 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 Separator.

▲ **Note:** Column adapters are required to insert certain columns into the Separators.

[Protocol]

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.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at $200\times g$ 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 General Protocols section of the respective separator user manual.

▲ **Dead cells may bind non-specifically to MicroBeads.** To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit.

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 separation. Pass cells through 30 μm nylon mesh to remove cell clumps which may clog the column. Wet filter with buffer before use.

▲ 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 $300 \times g$ 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, e.g., 10 μL of CD14-FITC, and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at $300 \times g$ 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 1.25×10^8 cells in 500 μ L of buffer.

9. Proceed to magnetic separation .

3. Magnetic separation

▲ Choose an appropriate Column and Separator according to the number of total cells and the number of CD14+ cells.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable Separator. For details see the respective Column data sheet.

2. Prepare column by rinsing with the appropriate amount of buffer:

MS: 500 μ L LS: 3 mL

3. Apply cell suspension onto the column.

4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer.

Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.

MS: $3 \times 500 \mu$ L LS: 3×3 mL

5. Remove column from the separator and place it on a suitable collection tube.

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 Separator..
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 effluent; 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 CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable Separator.

2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way stopcock of the assembled column.

3. Apply cell suspension onto the column.

4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the top. Collect total effluent; this is the unlabeled cell fraction.

Depletion with D Columns

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

▲ Buffers used for operating the Separator should have a temperature of $\geq 10^{\circ}\text{C}$.

▲ 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. Program recommendations below refer to separation of human PBMCs.

Magnetic separation with the 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 one of the following programs:

Positive selection: “Pssel”

Collect positive fraction from outlet port pos1.

Depletion: “Depletes”

Collect negative fraction from outlet port neg1.