

Description of MACS CD14 MicroBeads

MACS colloidal super-paramagnetic MicroBeads conjugated with monoclonal mouse anti-human CD14 antibodies. Isotype: mouse IgG2a.

The product is supplied as suspension containing 0.1 % BSA and 0.05 % sodium azide.

Applications

CD14 MicroBeads were developed for separation of human cells based on the expression of the CD14 antigen. CD14 is expressed in large quantities on monocytes/macrophages and in low amounts on granulocytes.

- ▲ Positive selection or depletion of cells expressing human CD14 antigen.
- ▲ Positive selection or depletion of monocytes/macrophages from peripheral blood, lymphoid tissue, body fluids (e.g. peritoneal, pleural and synovial fluid) and non-hematopoietic tissue (liver, muscle, skin etc.).
- ▲ Positive selection of CD14⁺ cells for subsequent generation of dendritic cells.

Principle of MACS Separation

For MACS separation, cells are magnetically labeled with CD14 MicroBeads and separated on a column which is placed in the magnetic field of a MACS separator. The magnetically labeled CD14⁺ cells are retained in the column while the unlabeled CD14⁻ cells run through. The unlabeled cells are depleted of CD14⁺ cells. After removal of the column from the magnetic field, the magnetically retained CD14⁺ cells can be eluted as positively selected cell fraction.

How to Use MACS CD14 MicroBeads

CD14 MicroBeads can be used to enrich CD14⁺ monocytes from peripheral blood, lymphoid tissue, body fluids etc. on positive selection columns (MS⁺/RS⁺ or LS⁺/VS⁺). Positive selection of CD14⁺ cells can also be performed using depletion columns type AS and BS. CD14 MicroBeads are suitable for depletion of CD14⁺ cells on depletion columns (AS, BS, CS or D). Cells which strongly express CD14 antigen can also be depleted using positive selection columns (MS⁺/RS⁺ or LS⁺/VS⁺).

Instrument and Reagent Requirement

Magnetic cell separators MiniMACS, MidiMACS, VarioMACS or SuperMACS (plus RS⁺ or VS⁺ column adaptor).

Positive selection column(s) or depletion column(s).

Buffer: phosphate buffered saline pH 7.2, supplemented with 0.5 % bovine serum albumin and 2 mM EDTA (see "Important Notes").

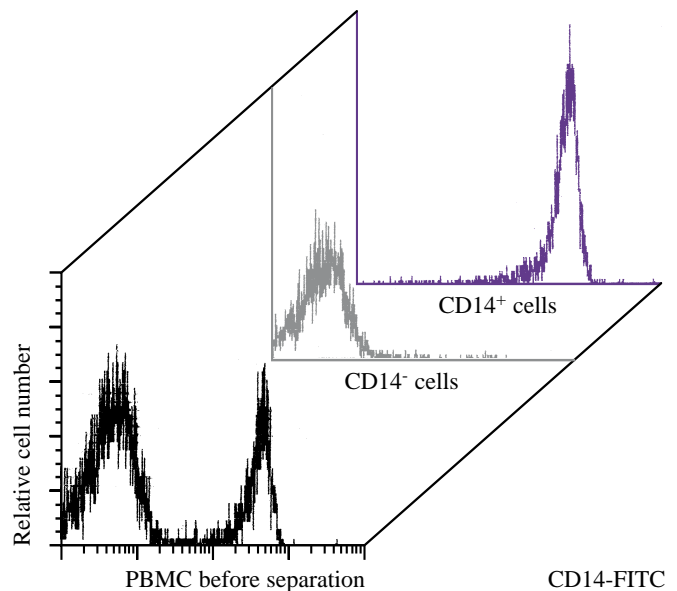
(Optional) Fluorochrome conjugated CD14 antibody, e.g. CD14-FITC (Order No. 130-080-701).

Storage of MACS MicroBeads

Store protected from light at 4°C. Do not freeze.

Example for a Separation Using MACS CD14 MicroBeads

Separation of PBMC using MACS CD14 MicroBeads and MiniMACS with positive selection column type MS⁺. Cells are fluorescently stained with CD14-FITC.



Protocol for Magnetic Labeling of Cells with CD14 MicroBeads

- Isolate PBMC from anti-coagulated human blood or buffy coat or prepare single cell suspension from body fluid or tissue by standard preparation method. For the isolation of PBMC and to remove dead cells, we recommend density gradient centrifugation using Ficoll-Paque[®]. To remove clumps, pass cells through 30 µm nylon mesh or filter (Order No. 130-041-407). Wet filter with buffer before use.
- Wash cells, remove supernatant completely and resuspend cell pellet in 80 µl of buffer per 10⁷ total cells. For fewer cells, use same volume.
- Add 20 µl of MACS CD14 MicroBeads per 10⁷ total cells, mix well and incubate for 15 minutes at 6°–12°C.
- (Optional) Add fluorochrome conjugated CD14 antibody, e.g. 10 µl of CD14-FITC (Order No. 130-080-701), at appropriate titer and incubate for additional 5–10 minutes to evaluate the efficiency of the magnetic separation by flow cytometry or fluorescence microscopy.

- Wash cells by adding 10–20x the labeling volume of buffer, centrifuge at 300xg for 10 minutes, remove supernatant completely and resuspend cell pellet in appropriate amount of buffer (500 µl of buffer per 10⁸ total cells). Proceed to magnetic separation.

Magnetic Separation with Positive Selection Columns (Protocol for 10⁴–10⁸ Positive Cells)

- Choose a positive selection column type MS⁺/RS⁺ (for up to 10⁷ positive cells), or LS⁺/VS⁺ (for up to 10⁸ positive cells) and place the column in the magnetic field of an appropriate MACS separator (see "Column Data Sheets").
- Prepare column by washing with appropriate amount of buffer (MS⁺/RS⁺: 500 µl, LS⁺/VS⁺: 3 ml; for details, see "Column Data Sheets").
- Apply cell suspension in appropriate amount of buffer onto the column (MS⁺/RS⁺: 500–1000 µl, LS⁺/VS⁺: 1–10 ml). Let the negative cells pass through. Rinse with appropriate amount of buffer (MS⁺/RS⁺: 3 x 500 µl, LS⁺/VS⁺: 3 x 3 ml).
- Remove column from separator, place column on a suitable collection tube, pipette appropriate amount of buffer (MS⁺/RS⁺: 1 ml; LS⁺/VS⁺: 5 ml) onto the column and flush out positive cells using the plunger supplied with the column.

Magnetic Separation with Depletion Columns (Protocol for 10⁴–2 x 10⁸ Positive Cells)

- Choose a depletion column type AS (for up to 3 x 10⁷ positive cells), BS (for up to 10⁸ positive cells) or CS (for up to 2 x 10⁸ positive cells), assemble the column and place it in the magnetic field of an appropriate MACS separator (see "Column Data Sheet").
- Prepare column by filling and washing with appropriate amount of buffer. Attach a flow resistor to the 3-way-stopcock of the assembled column (AS: 25G, BS: 23G, CS: 22G; for details, see "Column Data Sheet").
- Apply cell suspension in appropriate amount of buffer on top of the depletion column (AS: 500 µl, BS: 1 ml, CS: 2 ml).
- Let the negative cells pass through. Rinse with 3–5 column volumes of buffer from top (AS: 3 ml; BS: 15 ml; CS: 30 ml). Collect effluent as negative fraction.

Magnetic Separation with Depletion Column Type D (Protocol for up to 10⁹ Positive Cells)

- Assemble the column type D and place it in the magnetic field of SuperMACS (see "Column Data Sheet").
- Prepare column by filling with 70 % ethanol and rinsing with 200 ml of buffer. Attach a flow resistor (21G) to the 3-way-stopcock of the column (for details, see "Column Data Sheet").
- Apply cells resuspended in 10 ml of buffer on top of the depletion column.
- Let the negative cells pass through. Wash with 3–5 column volumes (150–200 ml) of buffer from top. Collect effluent as negative fraction.

Protocol for the Isolation of CD14⁺ and CD14⁻ Cells from One Sample

For some experiments it is desirable to obtain both, the CD14⁺ cells and the CD14⁻ cells, from one cell sample with high purity. If magnetic labeling of CD14⁺ cells is strong due to a high expression level of the CD14 antigen on all CD14⁺ cells, it is possible to obtain pure CD14 positive and negative cells by the use of LS⁺/VS⁺ columns. However, if CD14 is weakly expressed on some cells, another possibility is provided by using the depletion columns type AS and BS as described below.

- Start with depletion of CD14⁺ cells as described above.
- Close stopcock and remove column from magnetic separator.
- Turn 3-way-stopcock to position "filling". Back-flush retained cells to top of column (reservoir) with buffer from the side-syringe.
- Replace separation column in magnetic separator.
- Change flow resistor to higher flow rate (AS: 23G; BS: 22G). Turn 3-way-stopcock to position "run" and allow the cell suspension to run through.
- Wash the column with 3–5 column volumes of buffer (AS: 3 ml; BS: 15 ml). Collect effluent as wash fraction.
- (Optional) Repeat back-flush and rinse.
- Turn 3-way-stopcock to position "closed", remove and discard flow resistor. Refill syringe with buffer, if necessary.
- Remove separation column from magnetic separator.
- Turn 3-way-stopcock to position "filling". Back-flush the retained cells into top-reservoir, change stopcock position to "run" and elute the positive fraction by washing the column from top with 5 column volumes of buffer (AS: 5 ml; BS: 15 ml). Collect effluent.
- Repeat back-flush and rinse. Collect effluent. Pool eluted cells from both steps as positive fraction.

Important Notes

- ▲ Avoid capping of antibodies on the cell surface during staining. Work fast, keep cells cold, use cold solutions only. **Attention:** Working on ice requires increased incubation times for MACS MicroBeads. Incubate in refrigerator at 6°–12°C.
- ▲ EDTA in the buffer can be replaced by other supplements such as acid citrate dextrose (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other protein such as gelatin, HSA or FCS. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- ▲ Higher temperatures and longer incubation times for fluorescent staining and magnetic labeling may lead to unspecific cell labeling
- ▲ Large numbers of cells in the starting sample require a larger buffer volume when applying cells onto separation column. Use a maximum cell concentration of 10⁸ cells per 500 µl of buffer.
- ▲ MACS MicroBeads may bind unspecifically to dead cells. Therefore, dead cells should be removed before staining, e.g. by Ficoll-Paque® density gradient centrifugation.
- ▲ For convenient and efficient evaluation of MACS CD14 separation we recommend using CD14-FITC (Order No. 130-080-701).

- ▲ Increased MACS CD14 MicroBead concentrations may impair antibody fluorochrome staining when using antibody of the same specificity.
- ▲ To increase sensitivity of the magnetic separation, cells can be passed over the column a second time.

Warning

Reagents contain sodium azide. Sodium azide yields hydrazoic acid under acid conditions, which is extremely toxic. Azide compounds should be diluted with running water before discarded. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop..

Warranty

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