CD25 MicroBeads II
human

Order no. 130-092-983

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1. Description

Components	2 mL CD25 MicroBeads II, human: MicroBeads conjugated to monoclonal anti-human CD25 antibodies (isotype: mouse IgG1).
Size	For 10^9 total cells, up to 100 separations.
Product format	CD25 MicroBeads II 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 MACS® Separation

First, the $CD25^+$ cells are magnetically labeled with CD25 MicroBeads II. Then the cell suspension is loaded onto a MACS® Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled $CD25^+$ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of $CD25^+$ cells. After removal of the column from the magnetic field, the magnetically retained $CD25^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

CD25 MicroBeads II are developed for depleting or isolating $CD25^+$ cells from human peripheral blood mononuclear cells (PBMCs), lymphoid tissue or *in vitro* stimulated lymphocytes. CD25, the low-affinity interleukin-2 receptor alpha chain (IL–2Ra), is expressed on activated T cells, B cells, NK cells and monocytes as well as on $CD4^+$ regulatory T cells. The CD25 antigen shows three epitope regions, called A, B and C. The CD25 MicroBeads II recognize epitope A of the CD25 molecule.

Example applications

- Positive selection or depletion of cells expressing the human CD25 antigen, such as activated T cells, B cells, NK cells or monocytes.
- Depletion of allo-reactive T lymphocytes.
- Isolation of antigen-, mitogen- or allogen- activated T lymphocytes.
- Isolation or depletion of activated T cell subsets, B cells or NK cells in combination with MACS Isolation Kits for separation of untouched cells or with MultiSort MicroBeads.

1.3 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 (4–8 °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 calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: $CD25^+$ cells can be enriched by using MS, LS or XS Columns (positive selection) or depleted with the use of LD, CS or D Columns. Cells which strongly express the CD25 antigen can also be depleted using MS, LS or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10^8		VarioMACS, SuperMACS
D	10^9		SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS

▲ Note: Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibodies for flow-cytometric analysis, for example, CD25-PE (# 130-091-024), CD25-APC (# 130-092-858), CD4-FITC (# 130-080-501), CD4-PE (# 130-091-231), or CD4-APC (# 130-091-232).

  ▲ Note: CD25-PE and CD25-APC recognize epitope B. Their use is recommended for staining of cells isolated with CD25 MicroBeads II, which bind to epitope A.

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- (Optional) Propidium iodide (PI) or 7-AAD for flow-cytometric exclusion of dead cells. For cell fixation and flow-cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.
- (Optional) Pre-Separation Filters (# 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™. For details see section General Protocols in the user manuals or visit www.miltenyibiotec.com/protocols.

▲ **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, prepare a single-cell suspension by a standard preparation method. For details see section General Protocols in the user manuals or visit www.miltenyibiotec.com/protocols.

▲ **Note:** 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

▲ Work fast, keep the 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 (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in buffer and add CD25 MicroBeads II according to the following table per 10^7 total cells:

Application	Buffer	CD25 MicroBeads II
Positive selection of CD25$^+$ cells	90 µL	10 µL
Depletion of CD25$^+$ cells	80 µL	20 µL

4. Mix well and refrigerate for 15 minutes at 4–8 °C.

   ▲ Note: Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.

5. (Optional) Add staining antibodies, for example, 10 µL of CD25-PE (# 130-091-024), and refrigerate for 5 minutes in the dark (4–8 °C).
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. 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 µL of buffer.

8. 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 CD25$^+$ cells. For details see table in section 1.3.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
   Collect total effluent; this is the unlabeled cell fraction.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette an 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

   ▲ **Note:** To increase the purity of the magnetically labeled fraction pass the cells over a new, freshly prepared 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 see 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 effluent. This is the unlabeled cell fraction.

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Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details see CS Column data sheet.
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. For details see CS Column data sheet.
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.

Magnetic separation with the autoMACS™ Separator

▲ Refer to the autoMACS™ User Manual for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose following separation programs:

   Positive selection: "Posseld2"

   Depletion: "Depl05"

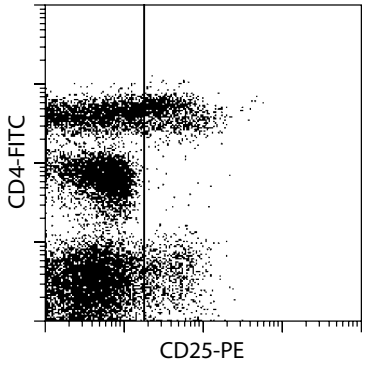
   ▲ Note: Program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see autoMACS user manual, section autoMACS Cell Separation Programs.
3. When using the program "Posseld2", collect positive fraction from outlet port pos2. This is the purified $CD25^+$ cell fraction.

   When using the program "Depl05", collect unlabeled fraction from outlet port neg1. This is the $CD25^-$ cell fraction.

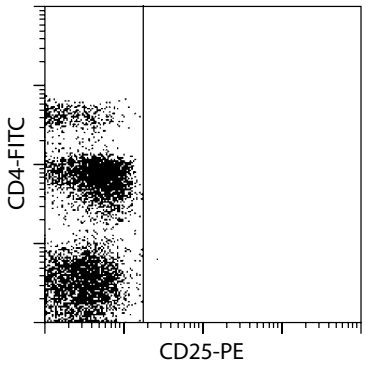
3. Example of a separation using CD25 MicroBeads II

$CD25^+$ cells were depleted from human PBMCs using CD25 MicroBeads II, an LD Column, and a MidiMACS™ Separator. Cells were stained with CD4-FITC (# 130-080-501) and CD25-PE (130-091-024). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

PBMCs before separation



$CD25^-$ cell fraction



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.

Warranty

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