Miltenyi Biotec

CD25 MicroBeads II
human – lyophilized

Order no. 130-097-044

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1. Description

Components	**1 vial CD25 MicroBeads II, human – lyophilized**: MicroBeads conjugated to monoclonal anti-human CD25 antibodies (isotype: mouse IgG1).
	2 mL Reconstitution Buffer
Capacity	For 10^9 total cells.
Product format	Lyophilized MicroBeads.
	Reconstitution Buffer contains 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. For information about reconstitution of the lyophilized MicroBeads and storage after reconstitution refer to chapter 2.1.

1.1 Principle of the MACS® Separation

First, the CD25$^+$ cells are magnetically labeled with CD25 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 CD25$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD25$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD25$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

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.

1.3 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.

1.4 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: CD25$^+$ cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD, CS, or D Columns. Cells that 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 Pro or 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 II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	10^9	2×10^{10}	SuperMACS II
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
CS	2×10^8		VarioMACS, SuperMACS II
D	10^9		SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

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

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- (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).
  For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

  ▲ 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.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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) 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 Reconstitution of MicroBeads

Reconstitute the lyophilized MicroBeads by adding all Reconstitution Buffer to the vial. Mix by pipetting up and down until resuspended. After reconstitution the MicroBeads are stable for 6 months at 2–8 °C. Write the new expiration date after reconstitution on the vial label.

2.2 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.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

▲ 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).



2.3 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 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 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 incubate for 15 minutes in the refrigerator (2–8 °C).
5. (Optional) Add staining antibodies, e.g., 10 µL of CD25-PE (# 130-091-024), and incubate for 5 minutes in the dark in the refrigerator (2–8 °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).



2.4 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 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 only when the column reservoir is empty.

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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 $CD25^+$ 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 CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details refer to the 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 refer to the 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 flow-through; 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® Pro Separator or the autoMACS® Separator

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

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of ≥10 °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.

Magnetic separation with the autoMACS® Pro 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 in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

   Positive selection: Posseld2
   Collect positive fraction in row C of the tube rack.

   Depletion: Depl05
   Collect negative fraction in row B of the tube rack.

Magnetic separation with the autoMACS® 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 pos2.
3. For a standard separation choose one of the following programs:

   Positive selection: posseld2
   Collect positive fraction from outlet port pos2.

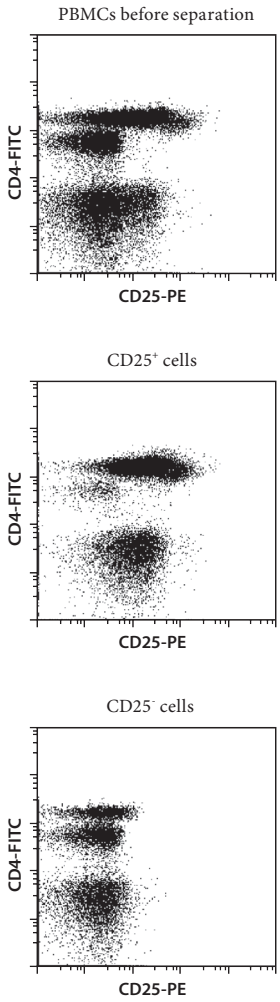
   Depletion: Depl05
   Collect negative fraction from outlet port neg1.

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3. Example of a separation using the CD25 MicroBeads II

CD25 MicroBeads II were reconstituted as described in 2.1. $CD25^+$ cells were enriched from human PBMCs using CD25 MicroBeads, an LD Column, and a MidiMACS™ Separator. Cells were fluorescently stained with CD4-FITC (#130-080-501) and CD25-PE (# 130-091-024) 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.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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.

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