CD5 (Ly-1) MicroBeads
mouse

Order no. 130-049-301

Index

1. Description
   - 1.1 Principle of MACS® Separation
   - 1.2 Background and product applications
   - 1.3 Reagent and instrument requirements
2. Protocol
   - 2.1 Sample preparation
   - 2.2 Magnetic labeling
   - 2.3 Magnetic separation
3. Example of a separation using CD5 (Ly-1) MicroBeads
4. References

1. Description

Components	2 mL CD5 (Ly-1) MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse CD5 (Ly-1; isotype: rat IgG2a; clone: 53-7.3) antibody.
Size	For 2×10^9 total cells; up to 200 separations.
Product format	CD5 (Ly-1) MicroBeads are supplied as a suspension 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 CD5$^+$ cells are magnetically labeled with CD5 (Ly-1) MicroBeads. Then, the cell suspension is loaded onto a column which is placed in the magnetic field of a MACS® Separator. The magnetically labeled CD5$^+$ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of CD5$^+$ cells. After removal of the column from the magnetic field, the magnetically retained CD5$^+$ cells can be eluted from the column as the positively selected cell fraction.

1.2 Background and product applications

CD5 (Ly-1) MicroBeads were developed for positive selection or depletion of CD5$^+$ cells from single cell suspensions of lymphoid organs, non-lymphoid tissue and peripheral blood. CD5 is a T cell differentiation antigen expressed at high levels on most thymocytes and all mature T cells. It is also expressed on a subset of B cells (B-1 cells).[1, 2] Prior to the isolation of highly pure T cells, B-1 cells have to be depleted by using CD45R(B220) MicroBeads (# 130-049-501) or CD19 MicroBeads (#130-052-201). Prior to the isolation of CD5$^+$ B-1 cells by positive selection with CD5 MicroBeads, T cells have to be depleted by using CD90 (Thy1.2) MicroBeads (# 130-049-101).

Examples of applications

- Positive selection or depletion of CD5 expressing cells.
- Positive selection or depletion of T cells from lymphoid organs, non-lymphoid tissue or peripheral blood.
- Positive selection of T cells for analysis of cytokine secretion kinetics during T cell differentiation.[3]
- Positive selection or depletion of the CD5$^+$ B cell subset from previously isolated B cells.

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 mouse serum albumin, mouse serum, or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: CD5$^+$ cells can be enriched by using MS, LS or XS Columns (positive selection). CD5 (Ly-1) MicroBeads can be used for depletion of CD5$^+$ cells on LD, CS or D Columns. Cells which strongly express the CD5 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 VarioMACS or SuperMACS. For details, see MACS Separator data sheets.

- (Optional) Fluorochrome-conjugated CD5 antibody.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells.

140-000-056.04

Miltenyi Biotec GmbH
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach, Germany
Phone +49-2204-8306-0 Fax +49-2204-85197

Miltenyi Biotec Inc.
12740 Earhart Avenue, Auburn CA 95602, USA
Phone 800 FOR MACS, 530 888-8871
Fax 530 888-8925

- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

Prepare a single-cell suspension from lymphoid organs, non-lymphoid tissue or peripheral blood using standard methods. For details see General Protocols in the User Manuals or visit www.miltenyibiotec.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. In case of high numbers of dead cells we recommend to remove dead cells by density gradient centrifugation or using the Dead Cell Removal Kit (# 130-090-101).

2.2 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 (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 90 µL of buffer per 10^7 total cells.
4. Add 10 µL of CD5 (Ly-1) MicroBeads per 10^7 total cells.
5. Mix well and refrigerate for 15 minutes (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.
6. (Optional) Add a fluorochrome-conjugated CD5 antibody according to manufacturer's recommendation, and refrigerate for 5 minutes (4–8 °C).
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 cell pellet in 500 µL of buffer for up to 1.25×10^8 cells.
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 $CD5^+$ 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.

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 the autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose one of the following separation programs:

   Positive selection: "Possel"

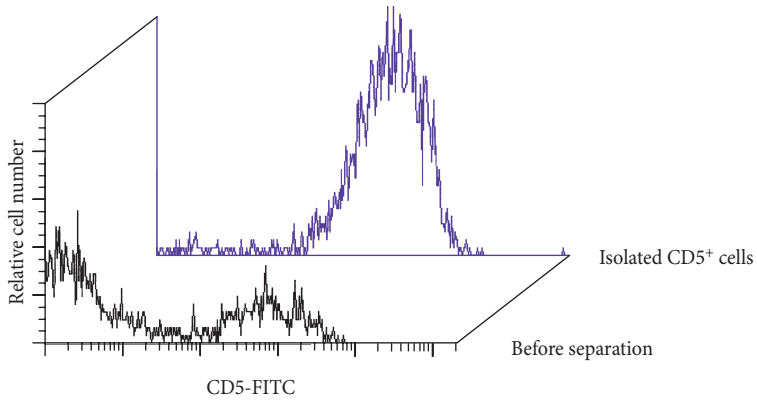
   Depletion: "Depletes"

   ▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see the autoMACS User Manual: "autoMACS Cell Separation Programs".
3. When using the program "Possel", collect positive fraction from outlet port pos1. This is the purified $CD5^+$ cell fraction.

   When using the program "Depletes", collect unlabeled fraction from outlet port neg1. This is the $CD5^-$ cell fraction.

3. Example of a separation using CD5 (Ly-1) MicroBeads

$CD5^+$ cells were isolated from a mouse spleen cell suspension using CD5 (Ly-1) MicroBeads, a MiniMACS™ Separator and an MS Column. The cells are fluorescently stained with CD5-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Ledbetter, JA; Rouse, RV; Micklem, HS; Herzenberg, LA (1980) T Cell Subsets Defined by Expression of Lyt-1,2,3, and Thy-1 Antigens. J. Exp. Med. 152: 280-295
2. Luo, W van de Velde, H; von Hoegen, I; Parnes, JR; Thielemans, K (1992) Ly-1 (CD5), A Membrane Glycoprotein of Mouse T Lymphocytes and a Subset of B Cells, Is a Natural Ligand of the B Cell Surface Protein Lyb-2 (CD72). J. Immunol. 148: 1630-1634
3. Assenmacher, M; Lohning, M; Scheffold, A; Manz, RA; Schmitz, J; Radbruch, A (1998) Sequential production of Il-2, IFN-gamma and Il-10 by individual staphylococcal enterotoxin B-activated T helper lymphocytes. Eur.J. Immunol.28: 1534-1543.

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

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

140-000-056.04