CD4 (L3T4) MicroBeads
mouse

Order no. 130-049-201

Index

1. Description

Components	2 mL CD4 (L3T4) MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse CD4 antibodies (L3T4; isotype: rat IgG2b;).
Size	For 2×10^9 total cells, up to 200 separations.
Product format	CD4 (L3T4) MicroBeads are supplied as a suspension containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of MACS® separation

First, the $CD4^+$ cells are magnetically labeled with CD4 (L3T4) 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 $CD4^+$ cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of $CD4^+$ cells. After removal of the column from the magnetic field, the magnetically retained $CD4^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

CD4 (L3T4) MicroBeads were developed for the positive selection or depletion of mouse T helper cells from single cell suspensions of lymphoid and non-lymphoid tissue or from peripheral blood. The CD4 antigen is expressed on most thymocytes and mature T helper cells[1,2], on a subset of NK-T cells and at lower levels on a subpopulation of dendritic cells. It is expressed on approximately 90% of thymocytes, 25% of splenocytes and 55% of lymph node cells.

Examples of applications

- Isolation of mouse $CD4^+$ T cells from spleen and lymph nodes,[3-6] brain tissue[7] or bronchoalveolar lavage[8] to study cytokine secretion and surface marker expression.
- Isolation of $CD4^-CD8^+$ thymocytes by depleting $CD4^+$ cells.[9]
- Isolation of $CD4^+$ T cells for adoptive cell transfer experiments to study IgE synthesis,[10] the infectious mechanism of mammary tumor viruses[11] or mouse models for diseases such as diabetes,[12,13] hepatitis,[14] arthritis[15] or leishmaniasis.[16]

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 in autoMACS Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °C).

  ▲ **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 gelatine, 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: $CD4^+$ cells can be enriched by using MS, LS or XS Columns (positive selection). CD4 (L3T4) MicroBeads can be used for depletion of $CD4^+$ cells on LD, CS or D Columns. Cells which strongly express the CD4 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™ Seperator. For details, see MACS Separator data sheets.

- (Optional) Fluorochrome-conjugated CD4 antibodies, e.g. CD4-FITC (# 130-091-608), CD4-PE (# 130-091-607) or CD4-APC (# 130-091-611).
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

140-000-055.05

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2. Protocol

2.1 Sample preparation

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

▲ 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 Filter # 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. Pipette off supernatant completely.
3. Resuspend cell pellet in 90 µL of buffer per 10^7 total cells.
4. Add 10 µL of CD4 (L3T4) MicroBeads per 10^7 total cells.
5. Mix well and incubate 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.
6. (Optional) Add a fluorochrome conjugated CD4 antibody, according to manufacturer's recommendation, and incubate for 5 minutes at 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. Pipette off 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 $CD4^+$ cells (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 (see "Column data sheets").
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 which pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times, each time once 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 appropriate amount of buffer onto the column. Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger supplied with the column.
   MS: 1 mL LS: 5 mL.

   ▲ **Note:** To increase the purity of the magnetically labeled fraction, it can be passed over a second, 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 (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 which 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 (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 (see "CS Column data sheet").
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which 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".

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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: "Possel"

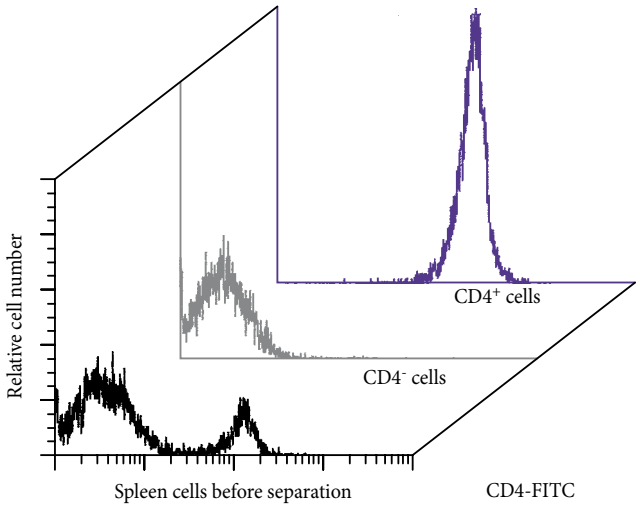
   Depletion: "Depletes"

   ▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see autoMACS User Manual: "autoMACS Cell Separation Programs".
3. When using the program "Possel", collect positive fraction (outlet port "pos1"). This is the purified $CD4^+$ cell fraction.

   When using the program "Depletes", collect unlabeled fraction (outlet port "neg1"). This is the $CD4^-$ cell fraction.

3. Example of a separation using CD4 (L3T4) MicroBeads

$CD4^+$ T cells were isolated from a mouse spleen cell suspension using CD4 (L3T4) MicroBeads, an MS Column and a MiniMACS™ Separator. Cells were stained with CD4-FITC (# 130-091-608). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Dialynas, DP; Quan, ZS; Wall, KA; Pierres, A; Quintáns, J; Loken, MR; Pierres, M; Fitch, FW (1983) Characterization of the Murine T Cell Surface Molecule, Designated L3T4, Identified by Monoclonal Antibody GK1.5: Similiarity of L3T4 To the Human Leu-3/T4 Molecule. J. Immunol. 131: 2445-2451.
2. Dialynas, DP; Wilde, DB; Marrack, P; Pierres, A; Wall, KA; Havran, W; Otten, G; Loken, MR; Pierres, M; Kappler, J; Fitch, FW (1983) Characterization of the Murine Antigenic Determinant, Designated L3T4a, Recognized by Monoclonal Antibody GK1.5: Expression of L3T4a by Functional T Cell Clones Appears to Correlate Primarily with Class II MHC Antigen-Reactivity. Immunological. Rev. 74: 29-56.
3. Gessner, A; Blum, H; Röllinghoff, M (1993) Differential Regulation of IL-9-Expression after Infection with Leishmania major in Susceptible and Resistant Mice. Immunobiol. 189: 419-435. [46]
4. Utermöhlen, O; Tárnok, A; Bönig, L; Lehmann-Grube, F (1994) T Lymphocyte-Mediated Antiviral Immune Responses in Mice are Diminished by Treatment with Monoclonal Antibody Directed against the Interleukin-2 Receptor. Eur. J. Immunol. 24: 3093-3099. [124]
5. Sommer, F; Huber, M; Röllinghoff, M; Lohoff, M (1995) CD44 Plays a Co-Stimulatory Role in Murine T Cell Activation: Ligation of CD44 Selectively Co-Stimulates IL-2 Production, but not Proliferation in TCR-Stimulated Murine Th1 Cells. Int. Immunol. 7: 1779-1786. [167]
6. Martinez, C; Delgado, M; Gomariz, RP; Ganea, D (1996) Vasoactive Intestinal Peptide and Pituitary Adenylate Cyclase-Activating Polypeptide-38 Inhibit IL-10 Production in Murine T Lymphocytes.J. Immunol. 156: 4128-4136. [299]
7. Schlüter, D; Kaefer, N; Hof, H; Wiestler, OD; Deckert-Schlüter, M (1996) Expression Pattern and Cellular Origin of Cytokines in the Normal and Toxoplasma gondii-infected murine brain. submitted. [290]
8. Coulson, PS; Wilson, RA (1993) Pulmonary T Helper Lymphocytes are $CD44^{hi}$, $CD45RB^-$ Effector/Memory Cells in Mice Vaccinated with Attenuated Cercariae of Schistosoma mansoni. J. Immunol. 151: 3663-3671. [28]
9. Lai, ZW; Kremer, J; Gleichmann, E; Esser, C (1994) 3,3',4,4'-Tetrachlorobiphenyl Inhibits Proliferation of Immature Thymocytes in Fetal Thymus Organ Culture. Scand. J. Immunol. 39: 480-488. [82]
10. Schmitz, J; Thiel, A; Kühn, R; Rajewski, K; Müller, W; Assenmacher, M; Radbruch, A (1994) Induction of Interleukin 4 (IL-4) Expression in T Helper (Th) Cells Is not Dependent on IL-4 from Non-Th Cells. J. Exp. Med. 179: 1349-1353. [109]
11. Waanders, GA; Shakhov, AN; Held, W; Karapetian, O; Acha-Orbea, H; Mac Donald, HR (1993) Peripheral T Cell Activation and Deletion Induced by Transfer of Lymphocyte Subsets Expressing Endogenous or Exogenous Mouse Mammary Tumor Virus. J. Exp. Med. 177: 1359-1366. [131]
12. Rohane, PW; Shimada, A; Kim, DT; Edwards, CT; Charlton, B; Shultz, LD; Fathman, CG (1995) Islet-Infiltrating Lymphocytes from Prediabetic NOD Mice Rapidly Transfer Diabetes to NOD-scid/scid Mice. Diabetes 44: 550-554. [269]
13. Ridgway, WM; Fasso, M; Lactot, A; Garvey, C; Fathman, CG (1996) Breaking Self-tolerance in Nonobese Diabtic Mice. J. Exp. Med. 183: 1657-1662. [262]
14. Gossman, J; Löhler, J; Utermöhlen, O; Lehmann-Grube, F (1995) Murine Hepatitis Caused by Lymphocytic Choriomeningitis Virus II. Cells Involved in Pathogenesis. Lab. Invest. 72: 559-570. [166]
15. Petrow, PK; Thoss, K; Katenkamp, D; Bräuer, R (1996) Adoptive Transfer of Susceptibility To Antigen-Induced Arthritis Into Severe Combined Immunodeficient (SCID) Mice: Role of $CD4^+$ and $CD8^+$ T Cells. Immunol. Invest. 25: 341-353.[242]
16. Hoerauf, A; Röllinghoff, M; Solbach, W (1996) Co-transfer of B Cells Converts Resistance into Susceptibility in T Cell-Reconstituted Leishmaia major-Resistant C.B-17 SCID Mice by a Non-Cognate Mechanism. Internat. Immunol. 8: 1569-1575. [329]

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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