CD4+ CD62L+ T Cell Isolation Kit II
mouse

Order no. 130-093-227

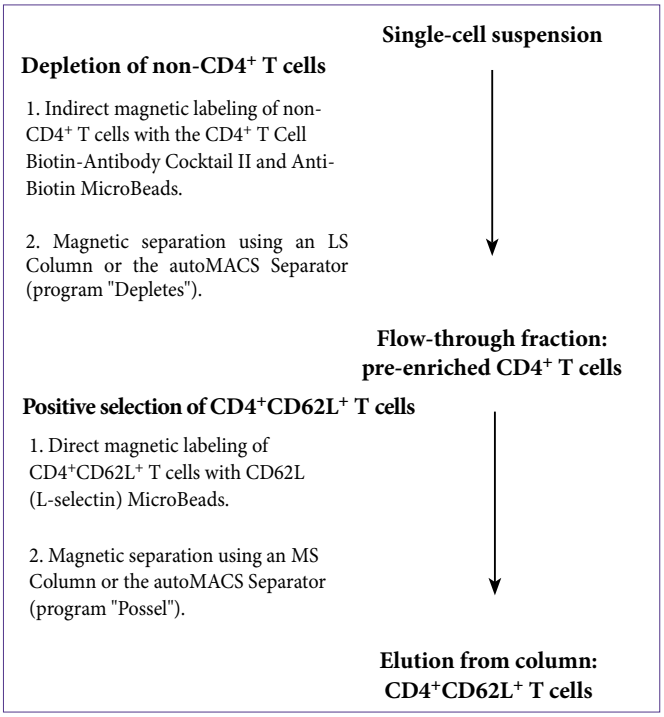
Index

1. Description

Components	**1 mL CD4+ T Cell Biotin-Antibody Cocktail II, mouse:** Cocktail of biotin-conjugated monoclonal anti-mouse antibodies against CD8a, CD45R, CD11b, CD25, CD49b, TCRγ/δ, and Ter-119.
	2 mL Anti-Biotin MicroBeads: MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1).
	2 mL CD62L (L-selectin) MicroBeads: MicroBeads conjugated to monoclonal anti-mouse CD62L (L-selectin; isotype: rat IgG2a) antibody.
Size	For 1×10^9 total cells.
Product format	All components 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

The isolation of mouse CD4+CD62L+ T cells is performed in a two-step procedure. First, non-CD4+ T cells are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies and Anti-Biotin MicroBeads. The labeled cells are subsequently depleted by separation over a MACS® Column.
In the second step, CD4+CD62L+ T cells are directly labeled with CD62L (L-selectin) MicroBeads and isolated by positive selection from the pre-enriched CD4+ T cell fraction.
The magnetically labeled CD4+CD62L+ T cells are retained on the column and eluted after removal of the column from the magnetic field.



1.2 Background and product applications

The CD4+CD62L+ T Cell Isolation Kit II has been developed for the isolation of CD4+CD62L+ T helper cells from spleen and lymph nodes.
CD62L (L-selectin) is highly expressed on naive T cells and down-regulated upon activation. It is also expressed on a small subset of memory T helper cells, the central memory T cells, which can be distinguished from naive T helper cells by their high expression of CD44. Furthermore, CD62L is expressed on most thymocytes, naive CD8+ T cells, B cells, dendritic cells, macrophages, NK cells, neutrophils, eosinophils, regulatory T cells, and TCRγ/δ+ T cells. For isolation of CD4+CD62L+ T helper cells, the non-T helper cells as well as regulatory T cells and TCRγ/δ+ T cells are depleted by indirect magnetic labeling using a cocktail of lineage-specific biotin-conjugated antibodies against CD8a (Ly-2), CD45R (B220), CD49b (DX5), CD11b (Mac-1), and Ter-119, as well as antibodies against

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CD25 and TCRγ/δ in combination with Anti-Biotin MicroBeads. Subsequently, $CD4^+CD62L^+$ T cells are positively selected from the enriched $CD4^+$ T helper cell fraction with CD62L (L-selectin) MicroBeads.

Example applications

Isolation of $CD4^+CD62L^+$ T cells from single cell suspensions of spleen and lymph nodes for:

- analysis of T cell activation by antigen-presenting cells;
- studies on cytokine expression and receptor signaling;
- adoptive transfer experiments.

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 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: Depletion of non-$CD4^+$ T cells is performed on an LS Column. The subsequent positive selection of $CD4^+CD62L^+$ T cells is performed on an MS Column. Depletion and positive selection can also be performed by using the autoMACS Separator.

Column	Max. number of labeled leukocytes	Max. number of total leukocytes	Separator
Depletion			
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
Depletion and positive selection			
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 e.g. CD4-FITC (# 130-091-608), CD4-PE (#130-091-607), CD4-APC (#130-091-611), CD62L-PE (# 130-091-794), CD62L-APC (# 130-091-805), CD25-PE (# 130-091-013), and CD44 antibodies.
- (Optional) Propidium iodide (PI) or 7-AAD for flow-cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

Prepare a single-cell suspension from spleen and lymph nodes using standard methods.

▲ **Note:** Red blood cell lysis or density gradient centrifugation is not necessary, since red blood cells are depleted in the first magnetic separation step on the basis of expression of Ter-119, a red blood cell restricted surface antigen of the mouse.

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. In case of high numbers of dead cells, removal of dead cells by density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101) is recommended.

2.2 Magnetic labeling of non-$CD4^+$ T cells

▲ 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 10^8 leukocytes. When working with fewer than 10^8 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 2×10^8 leukocytes 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 the number of leukocytes.
2. Centrifuge cells at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 400 µL of buffer per 10^8 total cells.
4. Add 100 µL of **$CD4^+$ T Cell Biotin-Antibody Cocktail II** per 10^8 total cells.
5. Mix well and refrigerate for 10 minutes (4–8 °C).
6. Add 300 µL of buffer and 200 µL of **Anti-Biotin MicroBeads** per 10^8 total cells.
7. Mix well and refrigerate for 15 minutes (4–8 °C).
8. Wash cells by adding 10 mL of buffer and centrifuge at 300×g for 10 minutes at 4–8 °C. Aspirate supernatant completely.
9. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
10. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of non-$CD4^+$ T cells

Depletion with LS Column

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details see LS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with 3×3 mL of buffer. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty. Collect total effluent. This contains the unlabeled pre-enriched $CD4^+$ T cell fraction.
5. Proceed to 2.4 for the isolation of $CD4^+CD62L^+$ T cells.

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Depletion 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 the tube containing the magnetically labeled cells in the autoMACS Separator. Choose the separation program "Depletes".
3. Collect the unlabeled fraction from outlet port neg1. This is the pre-enriched $CD4^+$ T cell fraction.
4. Proceed to 2.4 for the isolation of $CD4^+CD62L^+$ T cells.

2.4 Magnetic labeling of naive $CD4^+$ T cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^8 leukocytes. For larger initial cell numbers, scale up volumes accordingly.

1. Centrifuge the cells at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 800 µL of buffer.
3. Add 200 µL of **CD62L (L-selectin) MicroBeads**.
4. Mix well and refrigerate for 15 minutes (4–8 °C).
5. Wash cells by adding 10 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend up to 10^8 cells in 500 µL of buffer.
7. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of $CD4^+CD62L^+$ T cells

Positive selection with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details see MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column.
4. Wash column with 3×500 µL of buffer. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled $CD4^+CD62L^+$ T cells by firmly pushing the plunger into the column.

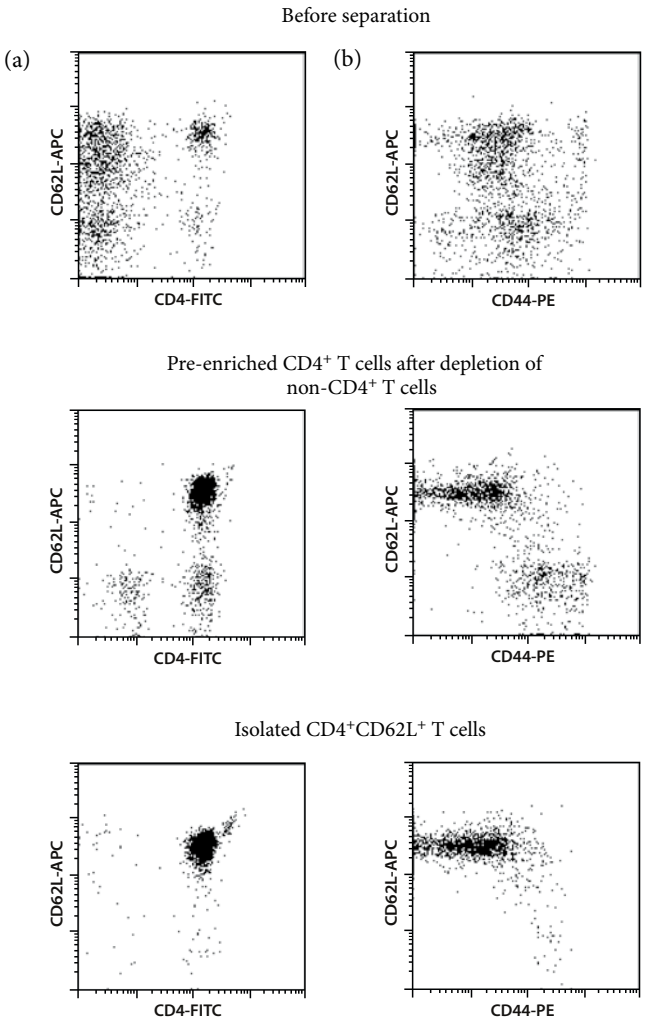
Positive selection with the autoMACS™ Separator

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

1. Prepare and prime the autoMACS Separator.
2. Place the tube containing the magnetically labeled cells in the autoMACS Separator. Choose the separation program "Possel".
3. Collect the positive fraction from outlet port pos1. This is the enriched $CD4^+CD62L^+$ T cell fraction.

3. Example of a separation using the $CD4^+CD62L^+$ T Cell Isolation Kit II

$CD4^+CD62L^+$ T cells were isolated from a mouse spleen cell suspension using the $CD4^+CD62L^+$ T Cell Isolation Kit II, an LS and an MS Column, a MidiMACS™ and a MiniMACS™ Separator. The cells were fluorescently stained with CD4-FITC (# 130-091-608) and CD62L-APC (# 130-091-805) for detection of naive T cells (a) and with CD62L-APC and CD44-PE for detection of central memory T cells (b). Additionally, cells were stained with CD25-PE (# 130-091-013) to illustrate the removal of $CD25^+CD62L^+$ regulatory T cells (c). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



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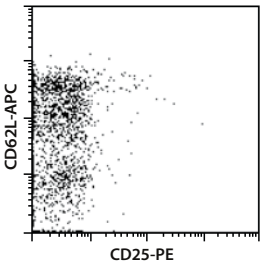
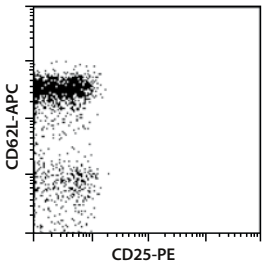
(c)

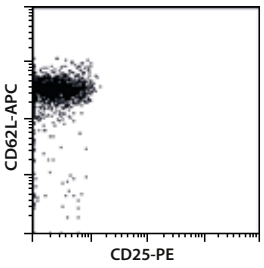
Before separation



Pre-enriched $CD4^+$ T cells after depletion of non-$CD4^+$ T cells



Isolated $CD4^+CD62L^+$ T cells



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.

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