Miltenyi Biotec

CD8+ Dendritic Cell Isolation Kit
mouse

Order no. 130-091-169

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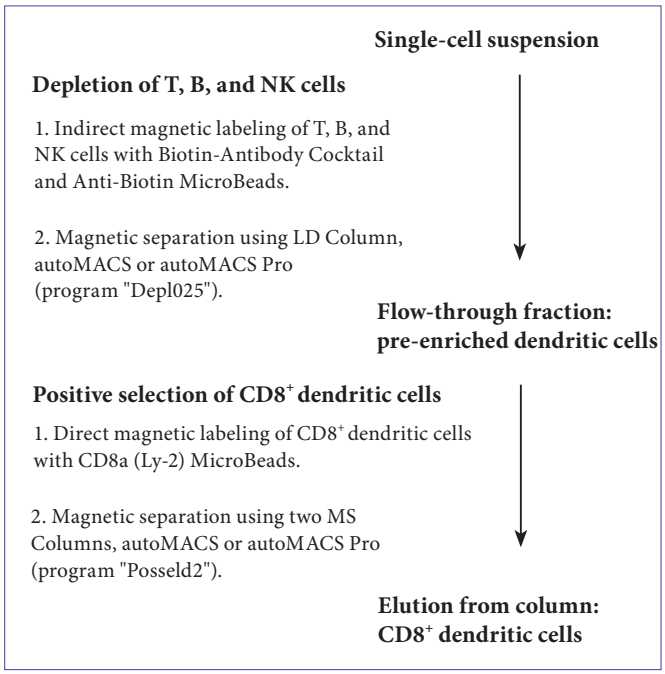
1. Description

Components	**1 mL CD8+ Dendritic Cell Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal anti-mouse antibodies against: CD90.2 (Thy1.2; isotype: rat IgG2b), CD45R (B220; isotype: rat IgG2a), and CD49b (DX5; isotype: rat IgM). **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (clone: Bio3-18E7.2; isotype: mouse IgG1) **2 mL CD8a (Ly-2) MicroBeads:** MicroBeads conjugated to monoclonal anti-mouse CD8a antibody (Ly-2; isotype: rat IgG2a)
Capacity	For 2×10^9 total cells, up to 100 separations.
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 the MACS® Separation

The isolation of CD8+ mouse dendritic cells is performed in a two-step procedure. First, T, B and NK 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, CD8+ dendritic cells are directly labeled with CD8a (Ly-2) MicroBeads and isolated by positive selection from the pre-enriched dendritic cell fraction. The unlabeled cells run through; this cell fraction is thus depleted of CD8+ cells. After removing the column from the magnetic field, the magnetically retained CD8+ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD8+ cells is separated over a second column.



1.2 Background information

Dendritic cells are a rare, heterogeneous population of hematopoietic cells. This kit was designed for the easy isolation of CD8+ dendritic cells, which constitute one of the three major dendritic cell subpopulations in mouse spleen.[1] CD8+ dendritic cells express CD11c, CD205, MHC class II, CD40, CD80 and CD86 and are negative for CD4 and CD11b. In spleen and lymph node, CD8+ dendritic cells are found at moderate levels (23% and 17% of all CD11c+ dendritic cells, respectively) and are located in the T cell areas.[2,3] Skin-draining lymph nodes harbor an additional CD8+ dendritic cell subset (CD4-CD8+CD11b+CD205+) which accounts for 33% of all CD11c+ dendritic cells in this organ and is thought to represent the mature form of Langerhans cells.[1,4]

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1.3 Applications

- antigen-uptake and antigen-processing
- T cell activation or T cell tolerance induction
- cross-priming of cytotoxic T cells
- T helper cell polarization by $CD8^+$ dendritic cells.

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

- MACS Columns and MACS Separators: Depletion of T, B, and NK cells is performed on an LD Column. The subsequent positive selection of $CD8^+$ dendritic cells is performed on two MS Columns. Depletion and positive selection can also be performed by using the autoMACS or the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
Depletion or positive selection			
autoMACS	2×10^8	4×10^9	autoMACS, autoMACS Pro

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

- Collagenase D: 1 mg/mL (Collagenase D >0.15 U/mg, e.g. from Roche Diagnostics, Germany) in 10 mM Hepes-NaOH pH 7.4, 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$.
- (Optional) Fluorochrome-conjugated CD8a and CD11c antibody for flow cytometric analysis, e.g. CD8a-FITC (# 130-091-605), CD8a-PE (# 130-091-603), CD8a-APC (# 1330-091-606), CD11c-FITC (# 130-091-842), CD11c-PE (# 130-091-830), CD11c-APC (# 130-091-844). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

To obtain high numbers of $CD8^+$ dendritic cells with high purities from murine spleen or lymph nodes, single cell suspensions need to be prepared by enzymatic disaggregation with Collagenase D. Protocols which entirely rely on mechanical disruption are not recommended.

1. Place isolated spleen in a 6 cm petri-dish with sufficient Collagenase D solution to completely cover the bottom of the dish (5 mL/spleen).
2. Inject mouse spleen with 500 µL of Collagenase D solution per spleen using a 1 mL syringe and a 25G needle, then cut the tissue in smaller pieces by using a pair of scissors.
3. Incubate the spleen pieces in Collagenase D solution for 30 minutes at 37 °C.
4. Pass the whole material, i.e. remaining fragments and Collagenase D-released cells, through a 70 µm cell strainer using a plunger.
5. Collect all cells in a 50 mL tube and wash the cells by adding buffer to obtain a final volume of 15 mL.

   ▲ **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.
6. Proceed to magnetic labeling (2.2).

2.2 Magnetic labeling of T, B, and NK 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 total cells. 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 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. Wet filter with buffer before use.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 200 µL of buffer per 10^8 total cells.
4. Add 50 µL of Biotin Antibody Cocktail per 10^8 total cells.
5. Mix well and incubate for 10 minutes on ice.
6. Add 150 µL of buffer and 100 µL of Anti-Biotin MicroBeads per 10^8 total cells.
7. Mix well and incubate for 15 minutes on ice.
8. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes at 4 °C. Aspirate supernatant completely.

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9. Resuspend cell pellet in buffer:
   Depletion with LD Column: 500 µL for up to 1.25×10^8 cells
   Depletion with autoMACS: 500 µL for up to 1×10^8 cells
   ▲ **Note:** For larger cell numbers, scale up buffer volume accordingly.
10. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of T, B, and NK cells

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. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
5. Proceed to 2.4 for the isolation of $CD8^+$ dendritic cells.

Depletion with the autoMACS™ Separator or the autoMACS™ Pro Separator

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

▲ Buffers used for operating the autoMACS Separator or the autoMACS Pro 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™ 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.
3. For a standard separation choose the following program:
   Depletion: "Depl025"
   Collect negative fraction from outlet port neg1.

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 the following program:
   Depletion: "Depl025"
   Collect negative fraction in row B of the tube rack.

2.4 Magnetic labeling of $CD8^+$ dendritic cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^8 total cells. 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.

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 400 µL of buffer per 10^8 total cells.
3. Add 100 µL of CD8a (Ly-2) MicroBeads per 10^8 total cells.
4. Mix well and incubate for 30 minutes on ice.
   ▲ **Note:** Mix cells once during the incubation time.
5. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes at 2–8 °C. 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 $CD8^+$ dendritic cells

Positive selection with MS Columns

▲ To achieve highest purities, always perform two cosecutive column runs.

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer:
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 3×500 µL of buffer. Collect total effluent; this is the unlabeled cell fraction. 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.
   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. To increase the purity of $CD8^+$ cells, the eluted fraction must be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Positive selection with the autoMACS™ Separator or the autoMACS™ Pro Separator

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

▲ Buffers used for operating the autoMACS Separator or the autoMACS Pro 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™ Separator

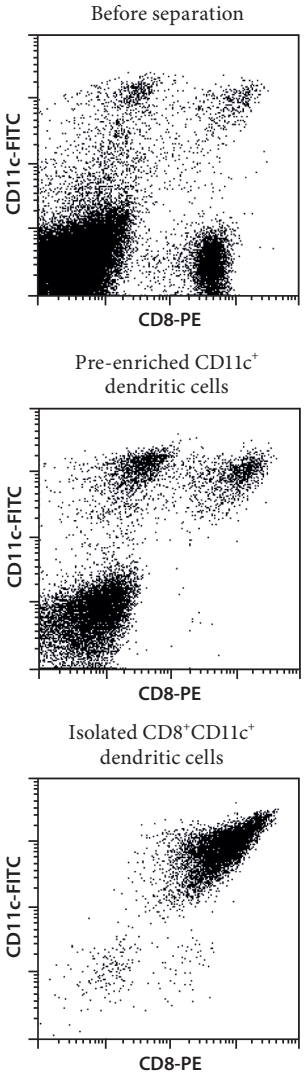
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. For a standard separation choose the following program:
   Positive selection: "Posseld2"
   Collect positive fraction from outlet port pos2.

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

3. Example of a separation using the CD8$^+$ Dendritic Cell Isolation Kit

CD8$^+$ dendritic cells were isolated from a mouse spleen using the CD8$^+$ Dendritic Cell Isolation Kit, an LD and two MS Columns, a MidiMACS™ and a MiniMACS™ Separator. Cells are fluorescently stained with CD11c-FITC (# 130-091-842) and CD8a-PE (# 130-091-603). Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Shortman, K; and Liu, YJ. (2002) Mouse and human dendritic cell subtypes. Nat. Rev. Immunol. 2(3): 151-161.
2. Anjuere, F. *et al.* (1999) Definition of dendritic cell subpopulations present in the spleen, Peyer's patches, lymph nodes, and skin of the mouse. Blood 93(2): 590-598.
3. Vremec, D. *et al.* (2000) CD4 and CD8 expression by dendritic cell subtypes in mouse thymus and spleen. J. Immunol. 164: 2978-2986.
4. Henri, S. *et al.* (2001) The dendritic cell populations of mouse lymph nodes. J.Immunol. 167: 741-748.

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.

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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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