TCRγ/δ+ T Cell Isolation Kit
mouse

Order No. 130-092-125

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1. Description

Components	**1 mL Non-T Cell Depletion Cocktail, mouse:** Cocktail of MicroBeads conjugated to monoclonal antibodies against mouse CD45R (B220; isotype: rat IgG2a), and CD11b (Mac-1 α-chain; isotype: rat IgG2b) and a biotin-conjugated monoclonal antibody against mouse TCRγ/δ (isotype: hamster IgG2). **1 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1).
Size	For 2×10^9 nucleated cells.
Product format	The Depletion Cocktail is supplied in a solution containing stabilizer and 0.05% sodium azide. The Anti-Biotin MicroBeads are supplied as a suspension containing 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.

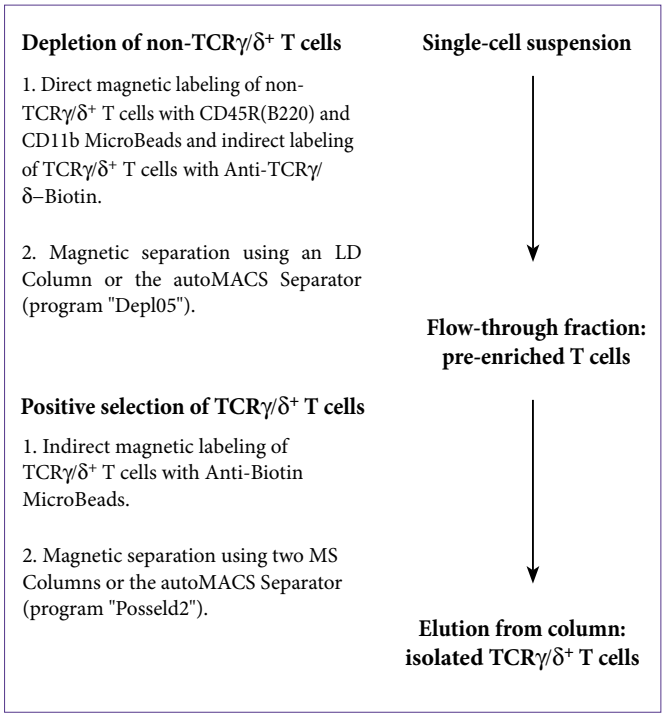
1.1 Principle of MACS® separation

The isolation of mouse TCRγ/δ+ T cells is performed in a two-step procedure. To pre-enrich the target cells, non-T cells are magnetically labeled with a cocktail of CD45R(B220) and CD11b antibodies conjugated to MicroBeads. Concomitantly, TCRγ/δ+ T cells are labeled with Anti-TCR γ/δ-Biotin included in the cocktail. The magnetically labeled, unwanted cells are subsequently depleted by separation over a MACS® Column.

In the second step, TCRγ/δ+ T cells are indirectly magnetically labeled with Anti-Biotin MicroBeads and isolated by positive selection from the pre-enriched T cell fraction. The magnetically labeled TCRγ/δ+ 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 TCRγ/δ+ T Cell Isolation Kit has been developed for the isolation of TCRγ/δ+ T cells from single-cell suspensions of spleen, lymph nodes, or thymus. T cells bearing γ/δ TCRs are a phenotypically and functionally conserved lineage of T cells, which often constitutes the major T cell population in epithelial-rich tissues, e.g. intestine, skin, lung, and reproductive tract. In blood and lymphoid tissues, they are found at low frequency. Depending on their tissue distribution and local microenvironment, TCRγ/δ+ T cells are involved in immune surveillance against tumors, wound healing and pro- and anti-inflammatory responses against pathogens.[1]



Examples of applications

Isolation of TCRγ/δ+ T cells from single-cell suspensions of lymphoid organs for:

- phenotypical and functional analysis;
- studies on cytokine expression and receptor signaling;
- adoptive transfer or co-culture experiments.

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 with autoMACS™ Rinsing Solution (# 130-091-222).
  Keep buffer cold (4–8 °C).

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▲ **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-TCRγ/δ$^+$ T cells is performed on an LD Column. The subsequent positive selection of TCRγ/δ$^+$ T cells is performed on two MS Columns. Depletion and positive selection can also be performed by using the autoMACS 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
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS

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

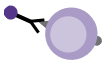
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g. CD3 and Anti-TCRγ/δ.
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

Prepare a single-cell suspension from lymphoid organs using standard methods.

▲ **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-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. Pipette off supernatant completely.
3. Resuspend cell pellet in 450 µL of buffer per 10^8 total cells.
4. Add 50 µL of **Non-T Cell Depletion Cocktail, mouse** per 10^8 total cells.
5. Mix well and incubate for 15 minutes at 4–8°C.
6. Wash cells by adding 10–20× labeling volume of buffer and centrifuge at 300×g for 10 minutes at 4–8 °C. Pipette off supernatant completely.
7. Resuspend cell pellet in buffer:
   Depletion with LD Column: 500 µL for up to 1.25×10^8cells
   Depletion with autoMACS Separator: 500 µL for up to 1×10^8 cells
   ▲ **Note:** For larger cell numbers, scale up buffer volume accordingly.
8. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of non-T cells

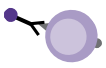
Depletion with LD Column

1. Place LD Column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
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. Perform washing steps by adding buffer successively once the column reservoir is empty. Collect total effluent. This contains the unlabeled pre-enriched T cell fraction.
5. Proceed to 2.4 for the isolation of TCRγ/δ$^+$ T cells.

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 separation program "Depl05".
3. Collect the unlabeled fraction (outlet port "neg1"). This is the pre-enriched T cell fraction.
4. Proceed to 2.4 for the isolation of TCRγ/δ$^+$ T cells.



2.4 Magnetic labeling of TCRγ/δ$^+$ 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. Pipette off supernatant completely.
2. Resuspend cell pellet in 450 µL of buffer.
3. Add 50 µL of **Anti-Biotin MicroBeads**.

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4. Mix well and incubate for 15 minutes at 4–8 °C.
5. Wash cells by adding 10–20× labeling volume of buffer and centrifuge at 300×g for 10 minutes. Pipette off 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 TCRγ/δ+ T cells

Positive selection with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
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 once 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 fraction with magnetically labeled TCRγ/δ+ T cells by firmly applying the plunger supplied with the column.

ĩIJ Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

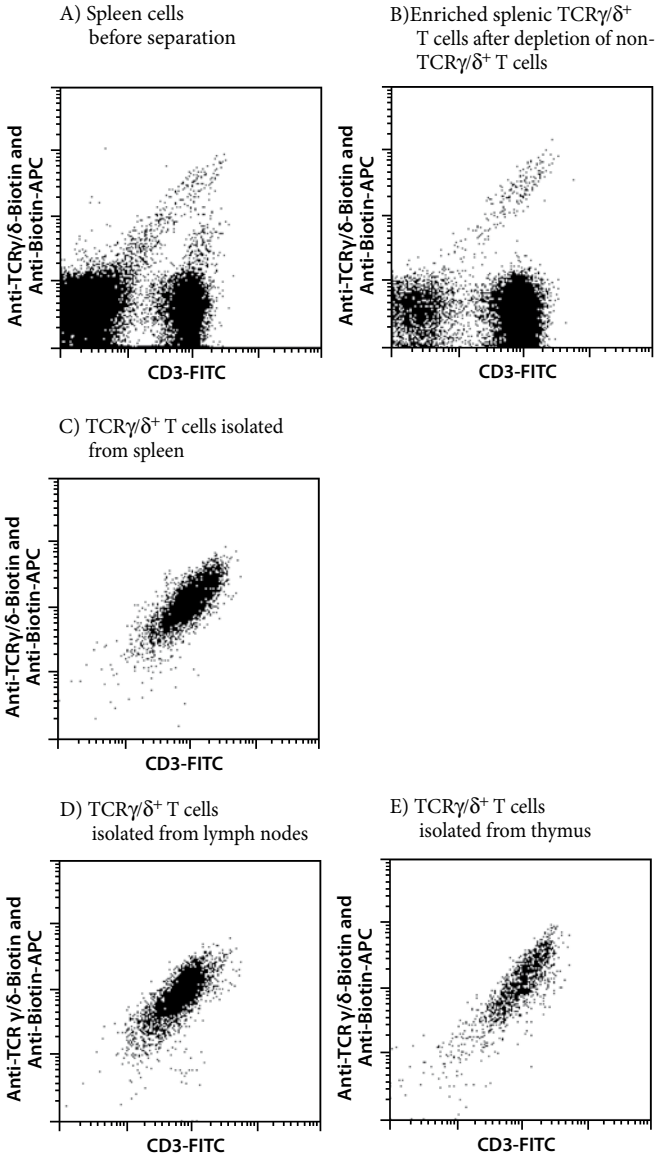
Positive selection with the autoMACS™ Separator

▲ 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 separation program "Posseld2".
3. Collect the positive fraction (outlet port "pos2"). This is the enriched TCRγ/δ+ T cell fraction.

3. Example of a separation using the TCRγ/δ+ T Cell Isolation Kit

TCRγ/δ+ T cells were isolated from single-cell suspensions of mouse spleen (A-C), lymph nodes (D), and thymus (E) using the TCRγ/δ+ T Cell Isolation Kit, an LD and two MS Columns, a MidiMACS™ and a MiniMACS™ Separator. The cells were fluorescently stained with CD3-FITC, Anti-TCRγ/δ-Biotin and Anti-Biotin-APC (# 130-090-856). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. Reference

1. Carding, SR; Egan, PJ (2002) Gammadelta T cells: functional plasticity and heterogeneity. Nat. Rev. Immunol. 5: 336–345.

Warning

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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