Miltenyi Biotec

TCRγ/δ+ T Cell Isolation Kit
human

Order no. 130-092-892

Index

1. Description

Components	**2 mL TCRγ/δ+ T Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal anti-human antibodies against antigens not expressed by TCRγ/δ+ T cells. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to a monoclonal anti-biotin antibody (isotype: mouse IgG1).
Size	For 10^9 total cells, up to 100 separations.
Product format	The Biotin-Antibody Cocktail is supplied in buffer containing stabilizer and 0.05% sodium azide. Anti-Biotin MicroBeads are supplied in buffer containing 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

Using the TCRγ/δ+ T Cell Isolation Kit, human, untouched TCRγ/δ+ cells are isolated by depletion of non-TCRγ/δ+ cells. Non-TCRγ/δ+ cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. The magnetically labeled non-γ/δ T cells are depleted by retaining them on a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled γ/δ T cells pass through the column.

1.2 Background and product applications

The TCRγ/δ+ T cells represent a subset of T cells expressing a T cell receptor (TCR) variant, composed of gamma and delta chains. The γ/δ TCR is expressed by 2–10% of all T cells in human peripheral blood, whereas the majority of T cells expresses α/β TCRs. γ/δ T cells possess distinct structural and antigen-binding characteristics. They specifically bind small non-peptide antigens, derived from necrotic cells or bacteria. In contrast to α/β T cells, γ/δ T cells directly recognize the antigens—independently of antigen-presenting cells (APCs) and MHC molecules. γ/δ T cells display a range of innate effector functions including rapid secretion of chemokines and cytokines as well as target cell lysis. They also contribute to the adaptive immune system, for example, they exhibit immunoregulatory and memory function. Recently, γ/δ T cells are discussed to have professional antigen-presenting capacity, initiating adaptive immune responses.[1]

The TCRγ/δ+ T Cell Isolation Kit is an indirect magnetic labeling system for the isolation of untouched TCRγ/δ+ T cells from human peripheral blood mononuclear cells (PBMCs). Non-γ/δ T cells, i.e. α/β T cells, NK cells, B cells, dendritic cells, granulocytes, monocytes, stem cells, and erythroid cells are indirectly labeled using a cocktail of biotin-conjugated antibodies and Anti-Biotin MicroBeads. The TCRγ/δ+ T Cell Isolation Kit provides an efficient and fast way to isolate untouched γ/δ T cells by depletion of the magnetically labeled cells.

Example applications

- Functional studies on γ/δ T cells in which effects due to antibody-cross-linking of cell surface proteins should be avoided.
- Isolation of untouched γ/δ T cells from human peripheral blood for phenotypical and functional analysis, e.g. studies on cytokine expression and receptor signaling, antigen recognition, cytotoxicity.

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

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- MACS® Columns and MACS Separators: Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

Column	Max. number of labeled cells	Max. number of total cells	Separator
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
autoMACS	2×10^8	4×10^9	autoMACS

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

- (Optional) Fluorochrome-conjugated antibodies for flow-cytometric analysis, e.g. anti-TCRγ/δ-FITC, anti-TCRγ/δ-PE, anti-TCRγ/δ-APC, Anti-TCRα/β-APC (# 130-091-237), Anti-TCRα/β-PE (# 130-091-236), CD3-PE (# 130-091-374), Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), and Anti-Biotin-APC (# 130-090-856).
- (Optional) PI (propidium iodide) or 7-AAD for the flow-cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

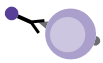
2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, e.g. using Ficoll-Paque™. For details see section General protocols in the user manuals or visit www.miltenyibiotec.com/protocols.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues, prepare a single-cell suspension by a standard preparation method. For details see section General Protocols in the user manuals or visit www.miltenyibiotec.com/protocols.

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).



2.2 Magnetic labeling

▲ Work fast, keep cells cold, 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 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 80 µL of buffer per 10^7 total cells.
4. Add 20 µL of Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well and refrigerate for 10 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. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Add 80 µL of buffer per 10^7 total cells.
8. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
9. Mix well and refrigerate for additional 15 minutes (4–8 °C).
10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
11. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. 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 TCRγ/δ$^+$ cells. For details see table in section 1.3.

Magnetic separation with MS and 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. Allow the cells to pass through and collect effluent as fraction with unlabeled cells, representing the enriched TCRγ/δ$^+$ cell fraction.
5. 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.
6. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled non-TCRγ/δ$^+$ cells.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS 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. Choose program "Depletes".
3. Collect negative fraction from outlet port neg1. This is the TCRγ/δ$^+$ cell fraction.
4. (Optional) Collect positive fraction from outlet port pos1. This is the non-TCRγ/δ$^+$ cell fraction.

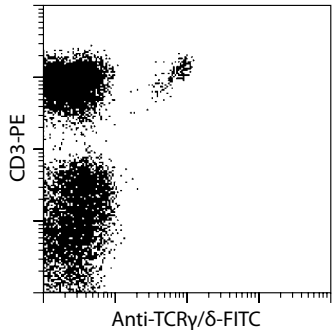
2.4 (Optional) Evaluation of TCRγ/δ$^+$ T cell purity

The purity of the enriched γ/δ T cells can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with fluorochrome-conjugated antibodies against TCRγ/δ. Analyze cells by flow cytometry or fluorescence microscopy. Labeling of non-γ/δ T cells with the Biotin-Antibody Cocktail can be visualized by counterstaining with a fluorochrome-conjugated anti-biotin antibody, e.g. Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856). Staining with fluorochrome-conjugated streptavidin is not recommended.

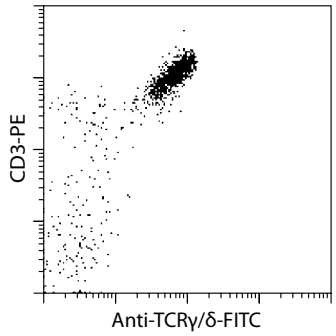
3. Example of a separation using the TCRγ/δ$^+$ T Cell Isolation Kit

Isolation of untouched TCRγ/δ$^+$ cells from PBMCs using the TCRγ/δ$^+$ T Cell Isolation Kit and an LS Column. Cells are fluorescently stained with CD3-PE (# 130-091-374) and anti-TCRγ/δ-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

Before separation



Isolated TCRγ/δ$^+$ T cells



4. Reference

1. Brandes, M. *et al.* (2005) Professional antigen-presentation function by human gammadelta T Cells. Science 309 (5732): 264–268.

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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