Miltenyi Biotec

StraightFrom® Leukopak® REAlease® Anti-TCRγ/δ MicroBead Kit

human

Order no. 130-135-465

Contents

1. Description
   - 1.1 Principle of the REAlease MACS® Separation
   - 1.2 Background information
   - 1.3 Applications
   - 1.4 Reagent and instrument requirements
2. Protocol
   - 2.1 Protocol overview
   - 2.2 Preparation of Leukopak®
   - 2.3 Magnetic labeling
   - 2.4 Magnetic separation and removal of magnetic labeling
     - 2.4.1 Semi-automated magnetic separation with the MultiMACS™ Cell24 Separator Plus
   - 2.5 (Optional) Removal of the REAlease Biotin Complex
3. Example of a separation using the StraightFrom Leukopak® REAlease Anti-TCRγ/δ MicroBead Kit, human

1. Description

This product is for research use only.

Components	**5 mL StraightFrom Leukopak® REAlease Anti-TCRγ/δ-Biotin, human** **2×5 mL StraightFrom Leukopak® REAlease Anti-Biotin MicroBeads (Anti-TCRγ/δ, human)** **4 mL REAlease Bead Release Reagent (50×)** **4 mL REAlease Release Reagent** **4 mL REAlease Stop Reagent**
Capacity	For one ½ or 100 mL Leukopak®.
Product format	REAlease Stop Reagent is supplied in buffer containing 0.05% sodium azide. All other reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at +2 to +8 °C. The expiration date is indicated on the vial or box label.

1.1 Principle of the REAlease MACS Separation

The REAlease Technology relies on recombinantly engineered antibody fragments to label specific cell surface markers. The fragments are developed to have low affinity for epitopes. However, when fragments are multimerized as a REAlease Biotin Complex (i.e. REAlease Anti-TCRγ/δ-Biotin) they bind to epitopes with high avidity. REAlease Technology can control the multimer/monomer state of antibody fragments. With this technology a controlled label release is possible where monomerized antibody fragments dissociate from the cell surface, enabling users to obtain bead- and label-free cells.

No density gradient centrifugation or erythrocyte lysis is needed. Anti-TCRγ/δ^+ T cells are labeled with StraightFrom Leukopak® REAlease Anti-TCRγ/δ-Biotin, human (REAlease Biotin Complex). Subsequently, StraightFrom Leukopak® REAlease Anti-Biotin MicroBeads (Anti-TCRγ/δ, human) bind to the REAlease Biotin Complex. Then, the cell suspension is loaded onto a Multi-24 Whole Blood Column Block, which is placed in the magnetic field of a MultiMACS Cell24 Separator Plus. The magnetically labeled cells are retained within the column. The unlabeled non-target cells flow through; this cell fraction is thus depleted of TCRγ/δ^+ T cells. Using the REAlease Bead Release Reagent, which removes the MicroBeads from the cell, target cells are eluted within the magnetic field. Finally, during the subsequent incubation with the REAlease Release Reagent, the REAlease Biotin Complex monomerizes and dissociates from the cell surface leaving the cells free of all labels.

1.2 Background information

During leukapheresis white blood cells are separated from whole blood and collected as highly concentrated leukocytes in Leukopaks®. Thus, Leukopaks® are ideal for the isolation of large numbers of various leukocyte subsets.

TCRγ/δ^+ T cells express TCRγ/δ instead of TCRα/β and are characterized by their HLA-independent antigen recognition. TCRγ/δ^+ T cells comprise 1–5% of T cells in the blood.

The StraightFrom Leukopak® REAlease Anti-TCRγ/δ MicroBead Kit, human has been developed for the positive selection of TCRγ/δ^+ T cells directly from a Leukopak® by using the MultiMACS Cell24 Separator Plus. The StraightFrom Leukopak® REAlease Anti-TCRγ/δ MicroBead Kit, human is an indirect magnetic labeling system that allows to obtain cells free of MicroBeads and REAlease Biotin Complex.

1.3 Applications

- Isolation of TCRγ/δ^+ T cells from Leukopak®. The purified TCRγ/δ^+ T cells are well suited for further flow cytometric, functional, or molecular analyses.
- Isolation of TCRγ/δ^+ T cells that need to be label-free.

1.4 Reagent and instrument requirements

- Separation 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 to +8 °C). Alternatively, use autoMACS Running Buffer. 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 bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not

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recommended for use.

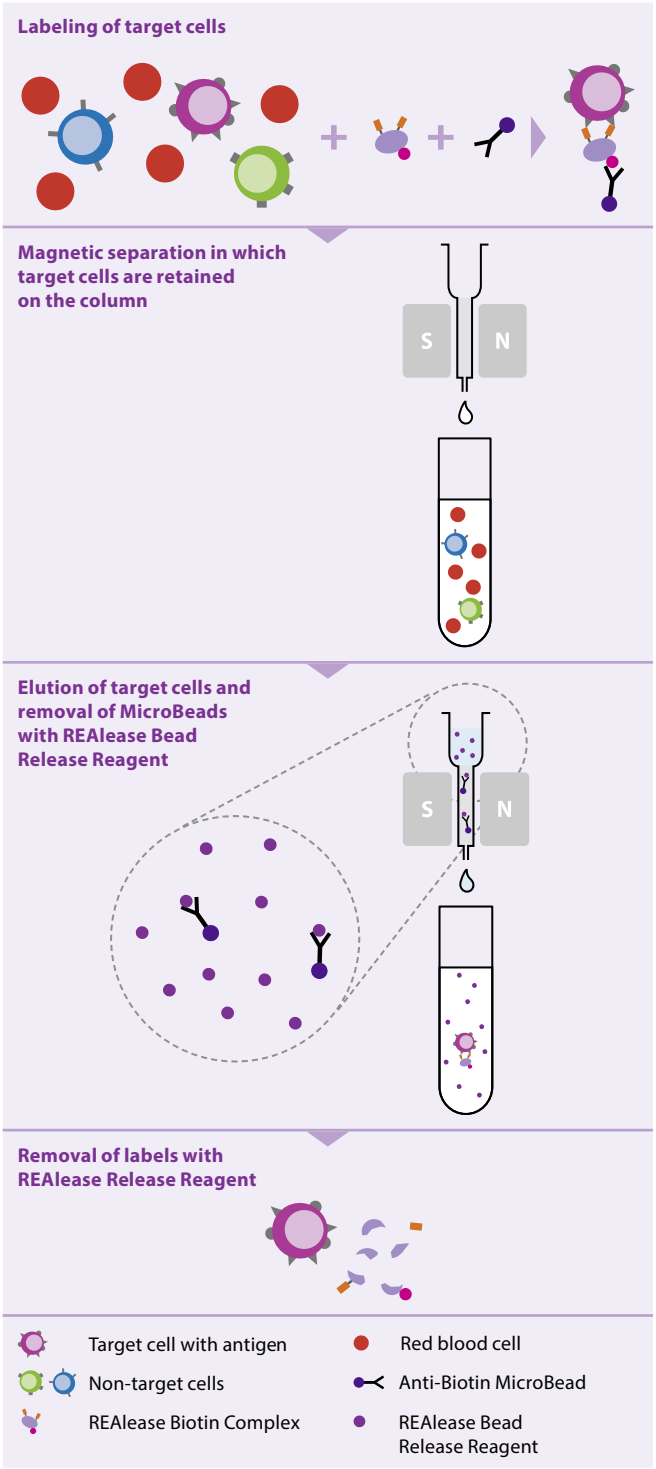
- REAlease Bead Release buffer: Prepare a 1:50 dilution of REAlease Bead Release Reagent (50×), e.g., for 50 mL add 1 mL of REAlease Bead Release Reagent (50×) to 49 mL of separation buffer.

  ▲ **Note:** Use freshly prepared buffer the same day. Store at room temperature.

- Semi-automated separation:
  - MultiMACS Cell24 Separator Plus (# 130-098-637).
  - Multi-24 Whole Blood Column Block (# 130-130-259).
  - Single-well Deep Well Plates (# 130-114-966).
- (Optional) Fluorochrome-conjugated REAfinity™ Antibodies for flow cytometric analysis, e.g., TCRγ/δ Antibody, anti-human, FITC, CD3 Antibody, anti-human, APC, and Biotin Antibody, APC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) MACS SmartStrainers (30 µm) (# 130-098-458) to remove cell clumps.
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)

2. Protocol

2.1 Protocol overview



2.2 Preparation of Leukopak®

1. Divide ½ Leukopak® equally into four collection tubes (max. 25 mL per tube). If the volume per tube is less than 25 mL, fill up to 25 mL with separation buffer.
2. Proceed to magnetic labeling (2.3).



2.3 Magnetic labeling

▲ The StraightFrom Leukopak® Anti-TCRγ/δ MicroBead Kit, human has been developed for positive selection of target cells from one ½ Leukopak® with up to 100 mL (5×10^9 cells). When working with smaller volumes, scale down all reagents and total volumes accordingly (e.g. for ¼ Leukopak®, fill two tubes with 25 mL and use 1/2 vial of each reagent and buffer as well as half of the Multi-24 Whole Blood Column Block).

▲ Perform labeling and separation steps for each tube one after the other.

▲ The recommended incubation temperature is room temperature (+19 to +25 °C).

1. Add 1.25 mL of StraightFrom Leukopak® REAlease Anti-TCRγ/δ-Biotin, human to each of the four tubes containing 25 mL Leukopak®.
2. Mix well by inverting the tube and incubate for 10 minutes at room temperature (+19 to +25 °C).
3. Add 2.5 mL of StraightFrom Leukopak® REAlease Anti-Biotin MicroBeads (Anti-TCRγ/δ, human) to each of the four tubes containing 25 mL Leukopak®.
4. Mix well by inverting the tube and incubate for 5 minutes at room temperature (+19 to +25 °C).
5. Fill up with the equal volume of separation buffer, e.g., add 25 mL separation buffer to 25 mL Leukopak®.
6. Proceed to magnetic separation (2.4).



2.4 Magnetic separation and removal of magnetic labeling

2.4.1 Semi-automated magnetic separation with the MultiMACS Cell24 Separator Plus

▲ The MultiMACS Cell24 Separator Plus, including the MACS Elution Station, has to be used with a Multi-24 Whole Blood Column Block and two Single-well Deep Well Plates for magnetic separation with StraightFrom Leukopak® REAlease MicroBeads.

▲ **Note:** To reach maximum cell recovery, rinse the Deep Well Plate after removal of the positive fraction and combine with the positive fraction. For the collection of the negative fraction and the wash fractions, use one Deep Well Plate.

▲ Buffer volumes per column are as follows:

Equilibration: 2 mL separation buffer
Wash: 2×2 mL separation buffer
Elution: 3×3 mL REAlease Bead Release buffer

▲ Select the program **POSSEL2** and follow the protocol below.

▲ For more detailed instructions on how to use the MultiMACS Cell24 Separator Plus, refer to the user manual.

1. Equilibrate using 2 mL of separation buffer per column.
2. Add 9 mL of magnetically labelled Leukopak® per column of the Multi-24 Whole Blood Column Block in two consecutive steps (i.e. 5 mL and 4 mL).
3. Select **Move back** to move the magnet up.
4. Change the Single-well Deep Well Plate.
5. Press **OK** to move down the magnet again.
6. Wash using 2×2 mL of separation buffer per column.
7. Select **Move back** to move the magnet up.
8. Change the Single-well Deep Well Plate.
9. Elute using 3×3 mL of REAlease Bead Release buffer (see 1.4) per column.
10. Stop the program by pressing **Esc** and follow the screen to remove the column and the Single-well Deep Well Plate.
11. (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×).
12. (Optional) Proceed to 2.5 Removal of REAlease Biotin Complex.

2.5 (Optional) Removal of the REAlease Biotin Complex

▲ The recommended incubation temperature is room temperature (+19 to +25 °C).

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in appropriate amount of separation buffer. For ½ Leukopak®: 25 mL.
3. Add an appropriate amount of REAlease Release Reagent. For ½ Leukopak®: 500 µL.
4. Mix well and incubate for 5 minutes.
5. Cells are now free from REAlease Biotin Complex and MicroBeads and are ready for analysis or downstream applications.
6. (Optional) For second magnetic labeling, centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely. Resuspend cell pellet in 4 mL of REAlease Stop Reagent and mix well. Then, proceed with magnetic labeling.

▲ **Note:** For best recovery and purity of cells, the amount of MACS MicroBeads for the second positive labeling may need optimization as the starting frequency of target cells may be different from a PBMC sample.

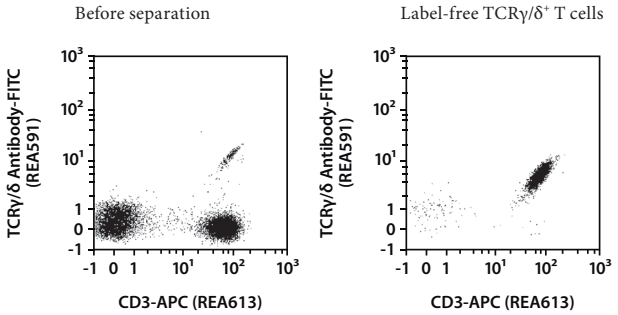
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3. Example of a separation using the StraightFrom Leukopak® REAlease Anti-TCRγ/δ MicroBead Kit, human

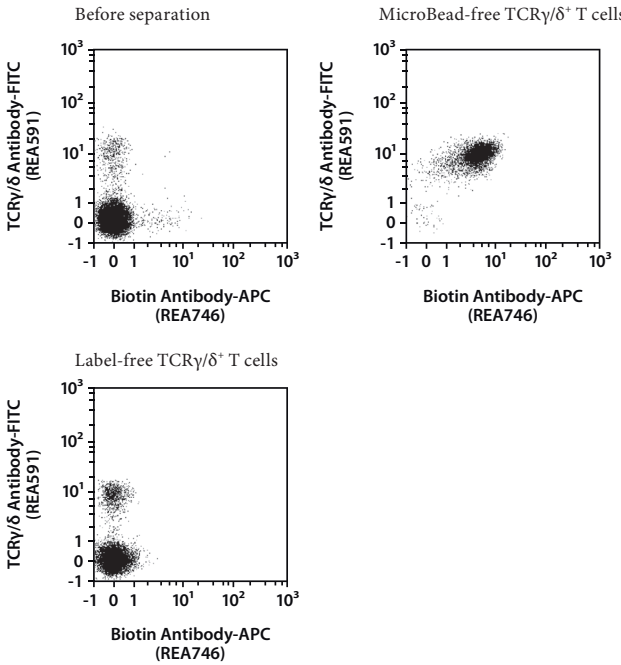
TCRγ/δ$^+$ T cells were isolated from ½ Leukopak® using the StraightFrom Leukopak® REAlease Anti-TCRγ/δ MicroBead Kit, human, the Multi-24 Whole Blood Column Block, and a MultiMACS Cell24 Separator Plus. Cells were fluorescently stained with TCRγ/δ Antibody-FITC, CD3-APC, and Biotin Antibody-APC and analyzed by flow cytometry using the MACSQuant® X. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.
The TCRγ/δ$^+$ cell content of the isolated fraction is typically 97 ± 2% (mean ± SD) for Leukopak®.

A) Cell purity



B) Label-free cells: release of REAlease Biotin Complex

The efficient removal of all labels was shown by using Biotin Antibody-APC to analyze the cells by flow cytometry for the presence of REAlease Biotin Complex. Directly after isolation, the cells showed staining of biotin ("MicroBead-free TCRγ/δ$^+$ T cells"), whereas the label-free TCRγ/δ$^+$ T cells after the REAlease Biotin Complex release were negative for biotin similar to the non-labeled cells before separation.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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