Miltenyi Biotec

StraightFrom® Buffy Coat REAlease® Anti-TCRγ/δ MicroBead Kit

human

Order no. 130-135-452

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1. Description

This product is for research use only.

Components	**4 mL StraightFrom Buffy Coat REAlease Anti-TCRγ/δ-Biotin, human** **7.5 mL StraightFrom Buffy Coat 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 buffy coat (max. 80 mL) from max. 500 mL whole blood.
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. Do not freeze. The expiration date is indicated on the vial 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. TCRγ/δ$^+$ T cells are labeled with StraightFrom Buffy Coat REAlease Anti-TCRγ/δ-Biotin, human (REAlease Biotin Complex). Subsequently, StraightFrom Buffy Coat REAlease Anti-Biotin MicroBeads (Anti-TCRγ/δ, human) bind to REAlease Biotin Complex. Then, the cell suspension is loaded onto a MACS Column, which is placed in the magnetic field of a MACS Separator. 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

StraightFrom Buffy Coat REAlease Anti-TCRγ/δ MicroBead Kit, human has been developed for the positive selection of cells directly from one buffy coat.

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 Buffy Coat REAlease Anti-TCRγ/δ MicroBead Kit 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 buffy coat. The purified TCRγ/δ$^+$ T cells are well suited for further flow cytometric, functional, or molecular analysis.
- Isolation of the TCRγ/δ$^+$ T cell subset that needs to be label-free.

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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 recommended for use.

- REAlease Bead Release buffer: Prepare a 1:50 dilution of REAlease Bead Release Reagent (50×), e.g., for 50 mL add 1000 µL 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).
  - MultiMACS 12× Single-Column Adapter LS (# 130-108-816).
  - Multi-24 Whole Blood Column Block (# 130-130-259).
  - Whole Blood Column Kit (# 130-093-545).
  - Deep Well Plates for collection of waste and target cells, e.g. Single-well Deep Well Plates (# 130-114-966), 2-well Deep Well Plates (# 130-120-010), or 3-well Deep Well Plates (# 130-120-009) depending on the number of target cell fractions.

- Manual separation:
  - MidiMACS Separator (# 130-042-302) or QuadroMACS Separator (# 130-090-976)
  - MACS Acrylic Tube Rack (# 130-041-406) or MACS 15 mL Tube Rack (# 130-091-052)

- (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.

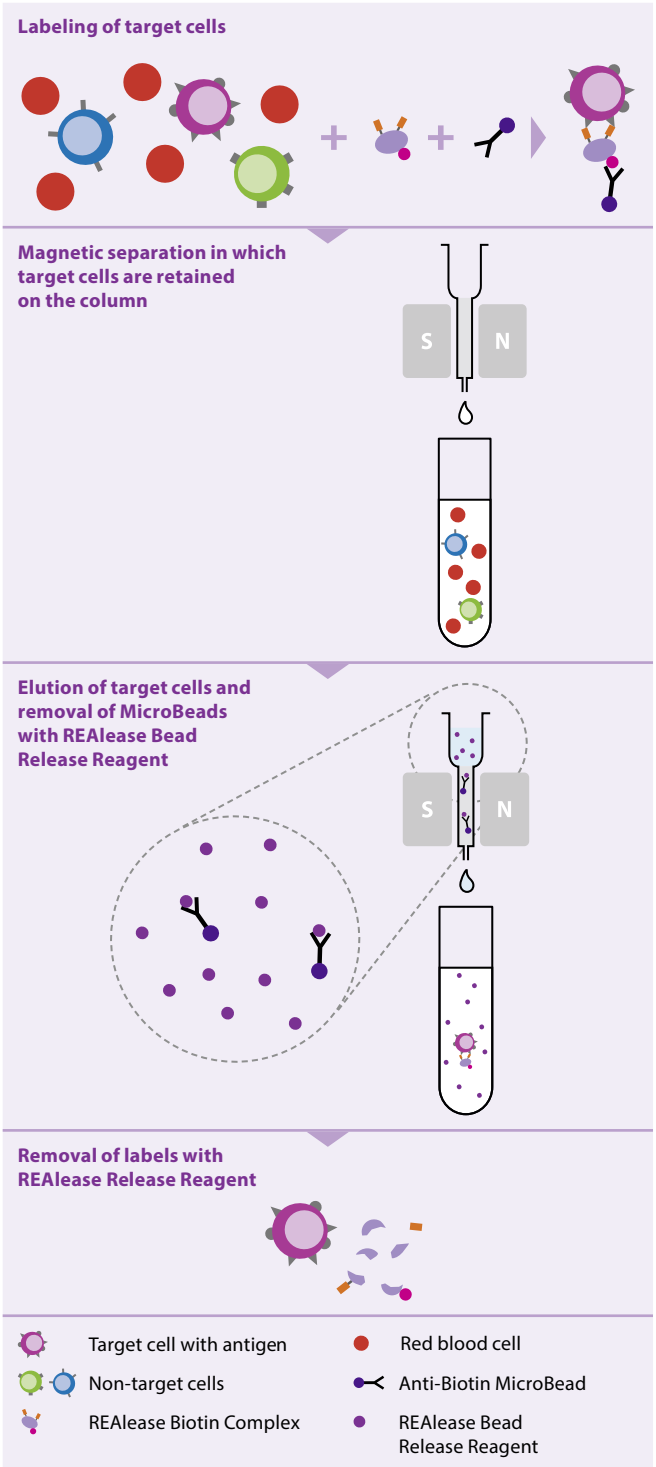
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.

- (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 Sample preparation

▲ The StraightFrom Buffy Coat REAlease MicroBead Kit has been developed for positive selection of target cells from buffy coat samples of up to 80 mL. When working with higher volumes, please contact our Technical Support team.

2.2.1 Preparation of buffy coat

▲ Anticoagulants such as EDTA, citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD) can be used. For subsequent molecular biology applications use EDTA as an anticoagulant.

1. Transfer up to 80 mL of one buffy coat into a collection tube. If the volume is less than 80 mL, fill up to 80 mL with separation buffer.
2. Proceed to magnetic labeling (2.3).



2.3 Magnetic labeling

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

▲ Volumes for magnetic labeling given below are for one entire buffy coat (max. 80 mL). When working with fractions of buffy coat, scale down all reagents and total volumes accordingly (e.g. for half a buffy coat, fill up to 40 mL and use half volume of all indicated reagents).

1. Add 4 mL of StraightFrom Buffy Coat REAlease Anti-TCRγ/δ-Biotin, human per buffy coat.
2. Mix well and incubate for 10 minutes at room temperature (+19 to +25 °C).
3. Add 7.5 mL of StraightFrom Buffy Coat REAlease Anti-Biotin MicroBeads (Anti-TCRγ/δ, human) per buffy coat.
4. Mix well and incubate for 5 minutes at room temperature (+19 to +25 °C).
5. 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 the Single-Column Adapter, Whole Blood Columns, and Deep Well Plate for magnetic separation.

▲ Select the program **POSSEL2_SCA** and follow the protocol below.

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

▲ Cells can be separated using the Whole Blood Column Block or Whole Blood Columns and a Single-Column Adapter.

Semi-automated magnetic separation using the Whole Blood Column Block

1. Equilibrate using 3 mL of separation buffer per Whole Blood Column.
2. Add 7.7 mL (in two steps) of magnetically labeled buffy coat per column to twelve columns of the Whole Blood Column Block.
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.

Semi-automated magnetic separation using Whole Blood Columns and the Single-Column Adapter

1. Equilibrate using 3 mL of separation buffer per Whole Blood Column.
2. Add 7.7 mL (in two steps) of magnetically labeled buffy coat per column to twelve columns of the Whole Blood Column Block.
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 1×4 mL and 1×2 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 4×3.5 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.

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2.4.2 Manual magnetic separation with the MidiMACS Separator or QuadroMACS Separator

▲ Divide the sample equally between the twelve Whole Blood Columns, e.g., when the separation volume is 92 mL, add 7.7 mL onto each column. Since the reservoir of the Whole Blood Column can only be filled with a volume of 7 mL, apply the sample in two steps.

▲ The protocol given below is for one Whole Blood Column. Repeat steps for each of the twelve Whole Blood Columns.

1. Place Whole Blood Column in the magnetic field of a MidiMACS™ Separator or QuadroMACS™ Separator. Insert Whole Blood Column with the column wings to the front into the separator.
2. Prepare column by rinsing with 3 mL separation buffer.
3. Apply magnetically labeled cell suspension onto the prepared Whole Blood Column. Collect flow-through containing unlabeled cells.
4. Wash Whole Blood Column with 1×4 mL and 1×2 mL separation buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.
   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Place a new collection tube under the Whole Blood Column.
6. Pipette 4×3.5 mL REAlease Bead Release buffer onto the Whole Blood Column.
7. Cells are now free from MicroBeads and ready for analysis or downstream applications.
8. (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×) (# 130-094-183).
9. (Optional) Proceed to 2.6 Removal of REAlease Biotin Complex.

2.5 Magnetic labeling and separation using autoMACS Separators

▲ Refer to the user manual and the short instructions for instructions on how to use the autoMACS Separators.

▲ Buffers used for operating the autoMACS Separators should have a temperature of ≥ +10 °C.

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = unlabeled (negative) fraction,

position C = labeled (positive) fraction.

2.5.1 Magnetic labeling and separation using the autoMACS NEO Separator

▲ The autoMACS NEO Separator enables stage loading to extend column capacity for selected reagents, minimizing the need to divide larger samples.

▲ For more information on selecting alternative separation programs, stage loading–compatible reagents, autolabeling-compatible reagents, and the minimal and maximal volumes for each reagent and Chill Rack, refer to www.miltenyibiotec.com/automacs-neo-sample-processing.

Fully automated magnetic labeling and separation

1. Prepare and prime the instrument.
2. Place the Chill Rack and MACS Reagent Rack 8 on the MACS MiniSampler S.
3. Select the same Chill Rack and MACS Reagent Rack 8 in the **Experiment** tab. An experiment is created automatically.
4. Tap to select sample position(s).
5. To assign a reagent to each sample, tap **Scan reagent** and scan the reagent barcode. Alternatively, tap on a free position of the MACS Reagent Rack 8 for selection out of the reagent list.
6. Unscrew the lids from the reagent vials and place the vials onto the designated positions on the MACS Reagent Rack 8.
7. Tap **Place reagent(s) on reagent rack** button in the dialog box.
8. Automated labeling is set automatically if autolabeling is supported and a reagent rack is selected. Alternatively, tap **Labeling** in the reagent placement dialog and select **Auto**.
9. Tap **Sample volume** in the **Sample process** pane and enter the sample volume. Tap the return key.
10. Tap **Run** to start the separation process.

2.6 (Optional) Removal of the REAlease Biotin Complex

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

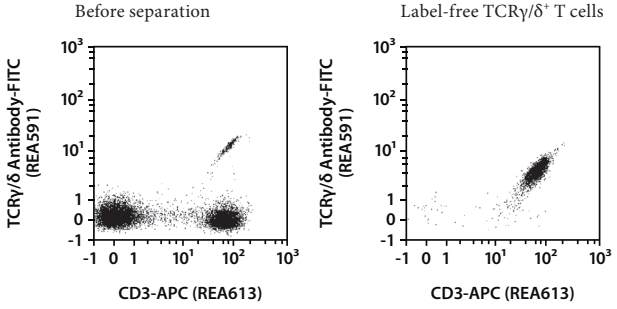
1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in appropriate amount of separation buffer. For a complete buffy coat: 25 mL.
3. Add an appropriate amount of REAlease Release Reagent. For a complete buffy coat: 500 µL.
4. Mix well and incubate 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 with REAlease MicroBeads or MACS Anti-Biotin MicroBeads, 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 Buffy Coat REAlease Anti-TCRγ/δ MicroBead Kit, human

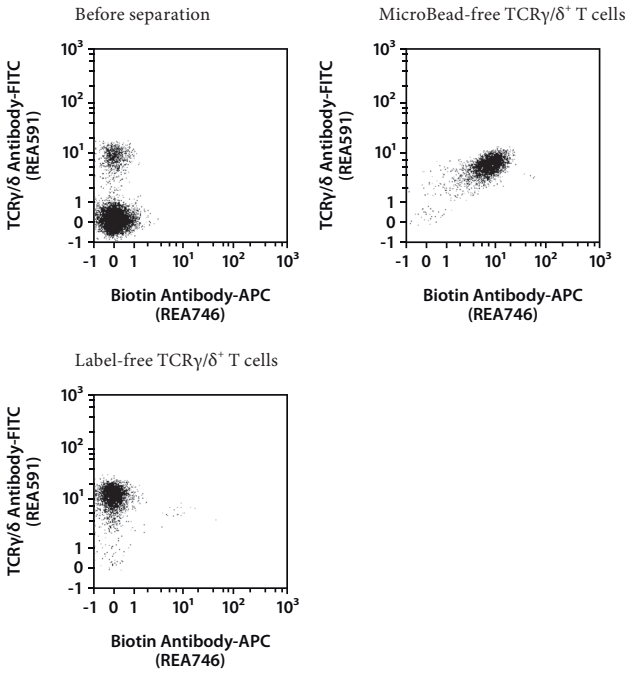
TCRγ/δ$^+$ T cells were isolated from a complete buffy coat using the StraightFrom Buffy Coat REAlease Anti-TCRγ/δ MicroBead Kit, human, Whole Blood Columns, and a MultiMACS Cell24 Separator Plus with the Single-Column Adapter. Cells were fluorescently stained with TCRγ/δ Antibody-FITC, CD3-APC, and 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$^+$ T cell content of the isolated fraction is typically 94 ± 4% (mean ± SD) for buffy coat.

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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