Miltenyi Biotec

StraightFrom® Whole Blood REAlease® CD4 MicroBead Kit
human

Order no. 130-136-717

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1. Description

This product is for research use only.

Components	**2 mL StraightFrom Whole Blood REAlease CD4-Biotin, human** **2×4 mL StraightFrom Whole Blood REAlease Anti-Biotin MicroBeads (CD4, human)** **4 mL REAlease Bead Release Reagent (50×)** **4 mL REAlease Release Reagent** **4 mL REAlease Stop Reagent**
Capacity	For max. 40 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 CD4-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 sample preparation is required, including density gradient centrifugation or erythrocyte lysis. $CD4^+$ T cells are labeled with StraightFrom Whole Blood REAlease CD4-Biotin (REAlease Biotin Complex). Subsequently, StraightFrom Whole Blood REAlease Anti-Biotin MicroBeads (CD4, 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 $CD4^+$ 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

The StraightFrom Whole Blood REAlease CD4 MicroBead Kit, human has been developed for the positive selection of cells directly from whole blood. CD4 is expressed on on T helper cells, at a lower level on monocytes and dendritic cells, and at a very low level on hematopoietic progenitor cells.

The StraightFrom Whole Blood REAlease CD4 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 $CD4^+$ cells from whole blood. The purified $CD4^+$ cells are well suited for further flow cytometric, functional, or molecular analysis.
- Isolation of $CD4^+$ 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.

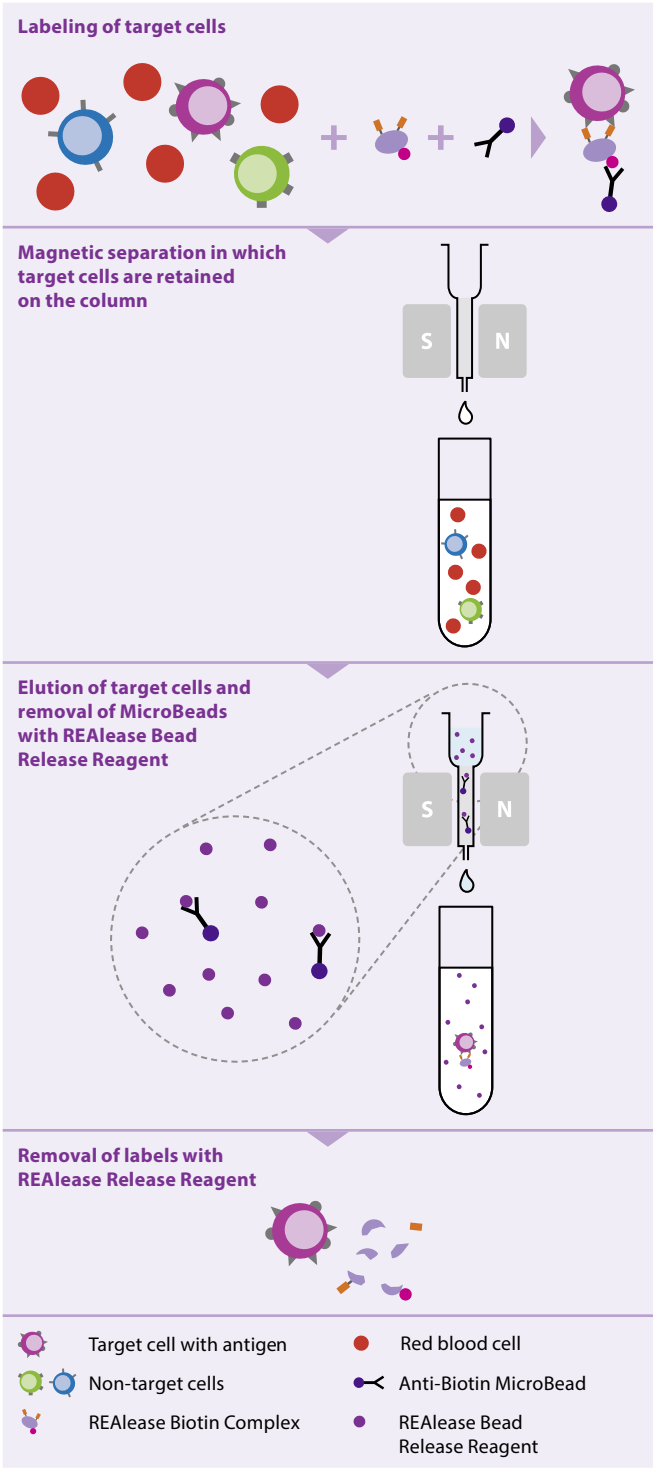
- REAlease Bead Release buffer: Prepare a 1:50 dilution of REAlease Bead Release Reagent (50×), e.g., for 50 mL add 1 mL of REAlease Bead Release Reagent to 49 mL of separation buffer.

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

- Manual separation:
  - MidiMACS Separator (# 130-042-302) or QuadroMACS Separator (# 130-090-976)
  - Whole Blood Column Kit (# 130-093-545)
  - 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. 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) Red Blood Cell Lysis Solution (10×) (# 130-094-183)

2. Protocol

2.1 Protocol overview



2.2 Sample preparation

▲ The StraightFrom Whole Blood REAlease MicroBead Kit has been developed for positive selection of target cells from whole blood samples of up to 40 mL.

▲ 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. Replacing EDTA can decrease purity and/or recovery.

1. Collect up to 15 mL of venous blood in a collection tube containing an appropriate anticoagulant.
2. Proceed to magnetic labeling (2.3).



2.3 Magnetic labeling

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

▲ Volumes for magnetic labeling given below are for 1 mL of anticoagulated whole blood. When working with larger volumes, scale up all reagent volumes and total volumes accordingly (e.g. for 2 mL, use twice the volume of all indicated reagent volumes and total volumes).

1. Add 50 µL of StraightFrom Whole Blood REAlease CD4-Biotin per 1 mL whole blood.
2. Mix well and incubate for 10 minutes at room temperature (+19 to +25 °C).
3. Add 200 µL of StraightFrom Whole Blood REAlease Anti-Biotin MicroBeads (CD4, human) per 1 mL whole blood.
4. Mix well and incubate for 5 minutes at room temperature (+19 to +25 °C).
5. Proceed to magnetic separation (2.4).



2.4 Manual magnetic separation with the MidiMACS Separator or QuadroMACS Separator

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.

   ▲ **Note:** Add max. 12.5 mL labeled cells onto one Whole Blood Column.

   ▲ **Note:** The reservoir of the Whole Blood Column can contain a maximum of 7.5 mL. Samples greater than 7.5 mL should be applied in aliquots to the column.
4. Wash Whole Blood Column with 2×2 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. Apply separation buffer to the edge of the column reservoir to wash out remaining whole blood.
5. Place a new collection tube under the Whole Blood Column.
6. Pipette 4×3.5 mL REAlease Bead Release Reagent (1:50) 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×).
9. (Optional) Proceed to 2.6 Removal of REAlease Biotin Complex.

2.5 Magnetic labeling and separation using the autoMACS NEO Separator

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

▲ Buffers used for operating the autoMACS NEO Separator 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.

▲ 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.
11. (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×).
12. (Optional) After separation, proceed to removal of the Labeling Complex (2.6).

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 1 mL of whole blood: 1 mL.
3. Add an appropriate amount of REAlease Release Reagent. For 1 mL of whole blood: 20 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 100 µL of REAlease Stop Reagent and mix well. Then, proceed with magnetic labeling.

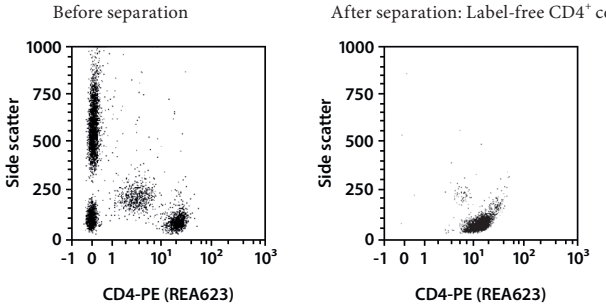
▲ **Note:** For best recovery and purity of cells, the amount of MACS MicroBeads for the second positive labeling may need optimization as the starting frequency of target cells may be different from a PBMC sample.

3. Example of a separation using the StraightFrom Whole Blood REAlease CD4 MicroBead Kit, human

$CD4^+$ cells were isolated from whole blood using the StraightFrom Whole Blood REAlease CD4 MicroBead Kit, human and Whole Blood Columns. Cells were fluorescently stained with the REAfinity Antibody CD4-PE and analyzed by flow cytometry using the MACSQuant® Analyzer X. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

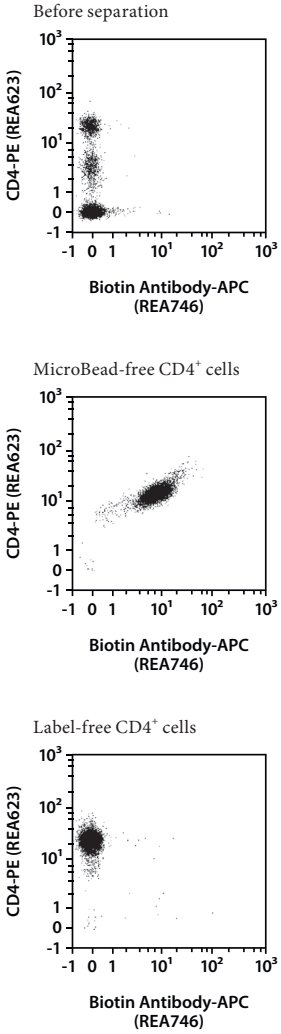
The $CD4^+$ T cell content of the isolated fraction is typically 99% ± 1 (mean ± SD).

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 $CD4^+$ cells"), whereas the label-free $CD4^+$ 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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