Miltenyi Biotec

StraightFrom® Buffy Coat REAlease® CD4 MicroBead Kit
human

Order no. 130-127-149

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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.

1. Description

This product is for research use only.

Components	**2 mL StraightFrom Buffy Coat REAlease CD4-Biotin, human** **10 mL StraightFrom Buffy Coat REAlease Anti-Biotin MicroBeads (CD4, human)** **4 mL REAlease Bead Release Reagent (50×)** **4 mL REAlease Release Reagent** **4 mL REAlease Stop Reagent** **12 Whole Blood Columns** columns and plungers, sterile packed.
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 2–8 °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. $CD4^+$ cells are labeled with StraightFrom Buffy Coat REAlease CD4-Biotin (REAlease Biotin Complex). Subsequently, StraightFrom Buffy Coat 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. After removing the column from the magnetic field, the target cells are eluted using the REAlease Bead Release Reagent, which simultaneously removes the MicroBeads from the cell. 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 Technical specifications of Whole Blood Columns

- Columns are “flow stop” and do not run dry.
- Void volume: 600 µL. Reservoir volume: 7.5 mL.
- Whole Blood Columns are for single use only.

▲ To remove clumps and to prevent aggregates in the sample, pass cells through 30 µm nylon mesh (MACS SmartStrainers (30 µm), # 130-098-458) before separation.

1.3 Background information

StraightFrom Buffy Coat REAlease CD4 MicroBead Kit has been developed for the positive selection of cells directly from one buffy coat. 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 Buffy Coat REAlease CD4 MicroBead Kit is an indirect magnetic labeling system that allows to obtain cells free of MicroBeads and REAlease Biotin Complex.

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1.4 Applications

- Isolation of $CD4^+$ cells from buffy coat. 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.

1.5 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 (2–8 °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.

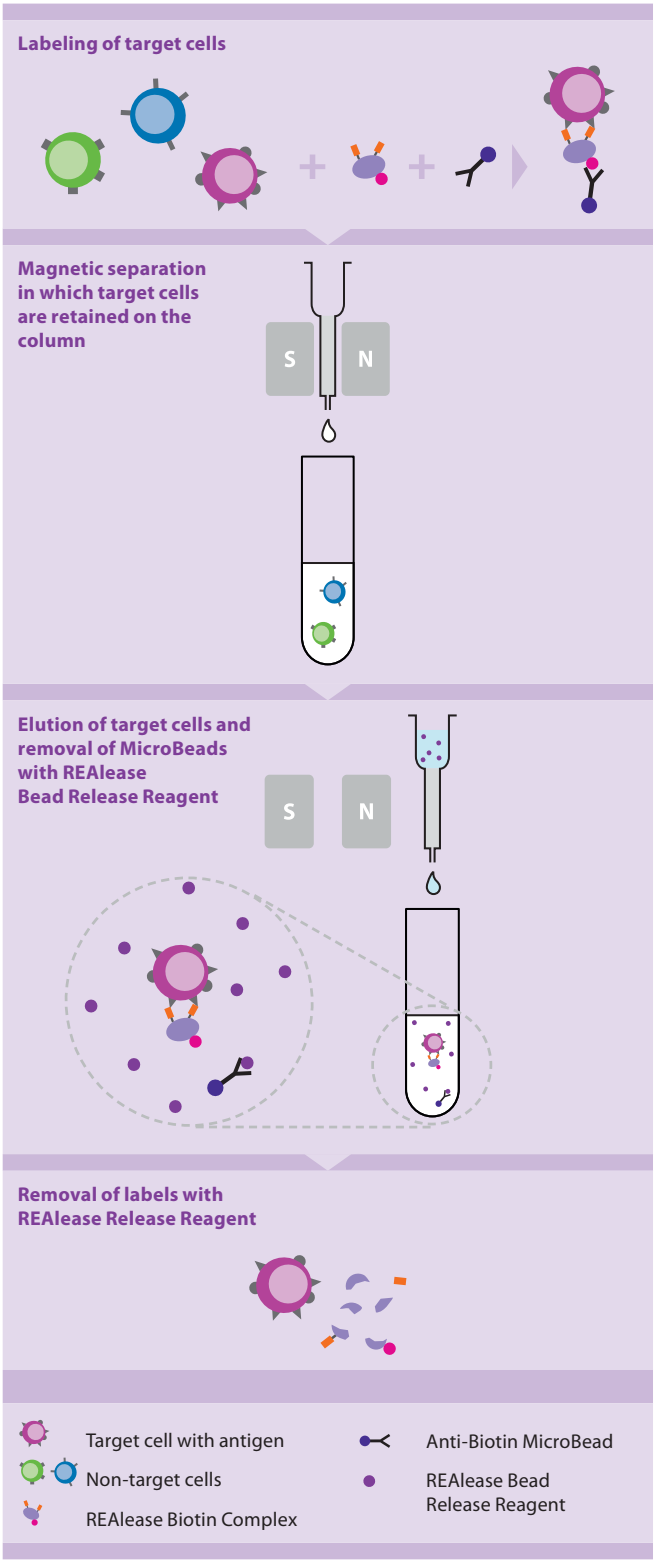
- REAlease Bead Release buffer: Prepare a 1:50 dilution of REAlease Bead Release Reagent (50×), e.g., for 50 mL add 1000 µL of REAlease Bead Release Reagent to 49 mL of separation buffer.

  ▲ **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)
  - 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. 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 (19–25 °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).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (MACS SmartStrainers (30 µm), # 130-098-458) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

1. Add 2 mL of StraightFrom Buffy Coat REAlease CD4-Biotin per buffy coat.
2. Mix well and incubate for 10 minutes at room temperature (19–25 °C).
3. Add 10 mL of StraightFrom Buffy Coat REAlease Anti-Biotin MicroBeads (CD4, human) per buffy coat.
4. Mix well and incubate for 5 minutes at room temperature (19–25 °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.

▲ Buffer volumes per one Whole Blood Column are as follows:

Equilibration: 3 mL (separation buffer)
Wash: 2×2 mL (separation buffer)
Elution: 4 mL (1:50 REAlease Bead Release Reagent)

▲ If equilibration solution and negative fraction should be collected in the same Deep Well Plate as waste, select the program **POSSEL_SCA** and follow the on-screen instructions of the MultiMACS Cell24 Separator Plus.

▲ If equilibration solution and negative fraction should be collected in separate Deep Well Plates, select the program **POSSEL2_SCA** and follow the on-screen instructions of the MultiMACS Cell24 Separator Plus.

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

1. Equilibrate using 3 mL of separation buffer per Whole Blood Column.
2. Divide the sample equally between the 12 Whole Blood Columns, e.g., when starting volume is 92 mL (80 mL buffy coat and 2 mL StraightFrom Buffy Coat REAlease CD4-Biotin plus 10 mL of StraighFrom Buffy Coat REAlease Anti-Biotin MicroBeads (CD4, human)), 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.
3. Wash using 2×2 mL of separation buffer per Whole Blood Column.
4. Elute using 4 mL of REAleaseBead Release Reagent (1:50) per Whole Blood Column.
5. After the separation, mix well and incubate for 10 minutes. Cells are now free from MicroBeads and ready for analysis or downstream applications.
6. (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×) (# 130-094-183).
7. (Optional) Proceed to 2.5 Removal of REAlease Biotin Complex.

2.4.2 Manual magnetic separation with the MidiMACS™ Separator or QuadroMACS™ Separator

▲ The protocol given below is for one Whole Blood Column. Repeat steps for each of the 12 Whole Blood Columns.

▲ Divide the sample equally between the 12 Whole Blood Columns, e.g., when starting 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.

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 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.
5. Remove Whole Blood Column from the separator and place it on a new collection tube.

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6. Pipette 4 mL REAlease Bead Release Reagent (1:50) onto the Whole Blood Column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. Mix well and incubate for 10 minutes.
8. Cells are now free from MicroBeads and ready for analysis or downstream applications.
9. (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×) (# 130-094-183).
10. (Optional) Proceed to 2.5 Removal of REAlease Biotin Complex.

2.5 (Optional) Removal of the REAlease Biotin Complex

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

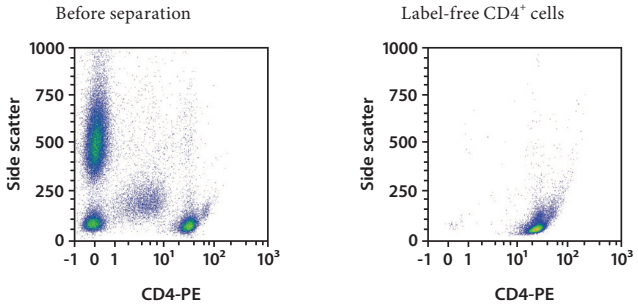
1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in appropriate amount of separation buffer. For a complete buffy coat: 25 mL.
3. Add an appropriate amount of REAlease Release Reagent. For a complete buffy coat: 500 µL.
4. Mix well and incubate 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 4000 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 Buffy Coat REAlease CD4 MicroBead Kit

$CD4^+$ cells were isolated from a complete buffy coat using the StraightFrom Buffy Coat REAlease CD4 MicroBead Kit, Whole Blood Columns, and a MultiMACS™ Cell24 Separator Plus with the Single-Column Adapter. Cells were fluorescently stained with REAfinity™ Antibodies 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 98.6 ± 1.6% (mean ± SD).

A) Cell purity

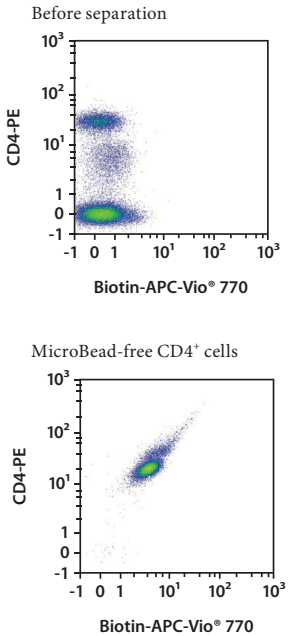


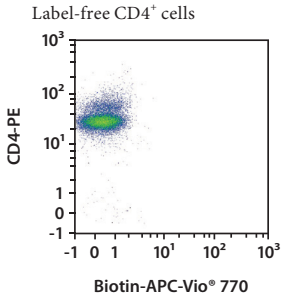
B) Bead-free cells: release efficiency of StraightFrom Buffy Coat REAlease Anti-Biotin MicroBeads

Release efficiency of the REAlease Anti-Biotin MicroBeads was higher than 95% for the StraightFrom Buffy Coat REAlease Anti-Biotin MicroBeads (CD4, human). The efficiency was determined by re-applying the isolated cells to a second MACS Column. The ratio between the numbers of cells in the flow-through and the total number of cells applied to the second column allowed us to calculate the efficiency of magnetic labeling removal.

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

The efficient removal of all labels was shown by using Biotin-APC-Vio® 770 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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