Miltenyi Biotec

MACSprep™ CD3 and CD19 Selection Kit
human

Order no. 130-136-718

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1. Description

This product is for research use only.

Components	**4 mL REAlease Start Reagent** **4 mL Anti-CD3 Labeling Complex, human** **4 mL Anti-CD19 Labeling Complex, human** **2 × 8 mL Anti-CD3 Complex MicroBeads, human** **2 × 8 mL Anti-CD19 Complex MicroBeads, human** **4 mL CD3 Bead Release Reagent (50×)** **4 mL CD19 Bead Release Reagent (50×)** **4 mL REAlease Release Reagent** **4 mL REAlease Stop Reagent**
Capacity	For 40 mL human whole blood.
Product format	REAlease Start Reagent, CD3 Bead Release Reagent (50×), and REAlease Stop Reagent are 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 MACSprep Selection Kit

The MACSprep CD3 and CD19 Selection Kit, human in combination with MACS Columns allows to simultaneously isolate two independent cell types, e.g. $CD3^+$ T cells and $CD19^+$ B cells, and elute them in individual target fractions.
Building on the REAlease Technology, cell isolation bases 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 Labeling Complex (i.e. Anti-CD3 Labeling Complex, human) 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. $CD3^+$ T cells and $CD19^+$ B cells are simultanously labeled with Anti-CD3 and Anti-CD19 Labeling Complex, human. Subsequently, Anti-CD3 and Anti-CD19 Complex MicroBeads bind to the corresponding Labeling Complexes on target cells. 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 both $CD3^+$ T cells and $CD19^+$ B cells.
The CD3 and CD19 Bead Release Reagent (50×) usage allows independent sequential release of the MicroBeads from the cells. Target cells are eluted within the magnetic field in a plunger-free workflow.
Optionally, upon the subsequent incubation with the REAlease Release Reagent, the Labeling Complex monomerizes and dissociates from the cell surface leaving the isolated target cells free of all labels.

1.2 Background information

The MACSprep CD3 and CD19 Selection Kit, human has been developed for the simultaneous positive selection of $CD3^+$ T cells and $CD19^+$ B cells directly from whole blood. The indirect magnetic labeling allows to obtain two autologous cell populations using one MACS Column from the same whole blood sample with a plunger-free protocol, where targets cells are obtained with epitopes free from MicroBeads and Labeling Complexes.
The CD3 antigen is associated with the T cell receptor (TCR) heterodimer. CD3 is expressed on all T cells, constituting 15–20% of human peripheral blood leukocytes. The CD19 antigen is expressed from early pro-B cells to the B cell blast stage. It is also present on most malignant B cells. The expression of the CD19 antigen is down-regulated upon terminal differentiation of B cells to plasma cells.

1.3 Applications

- Isolation of autologous $CD3^+$ T cells and $CD19^+$ B cells directly from whole blood. The isolated $CD3^+$ T cells and $CD19^+$

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B cells are well suited for further flow cytometric, cell culture, functional or molecular analysis.

- Isolation of single CD3$^+$ T cell and CD19$^+$ B cell subsets that needs 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). 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.

- CD3 and CD19 Bead Release Reagent (50×) buffers:
  Prepare separate 1:50 dilutions of CD3 and CD19 Bead Release Reagent (50×), e.g., for 50 mL add 1 mL of CD3 Bead Release Reagent (50×) to 49 mL of separation buffer.

  ▲ **Note:** Use freshly prepared buffer the same day. Store at +19 to +25 °C.

- 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

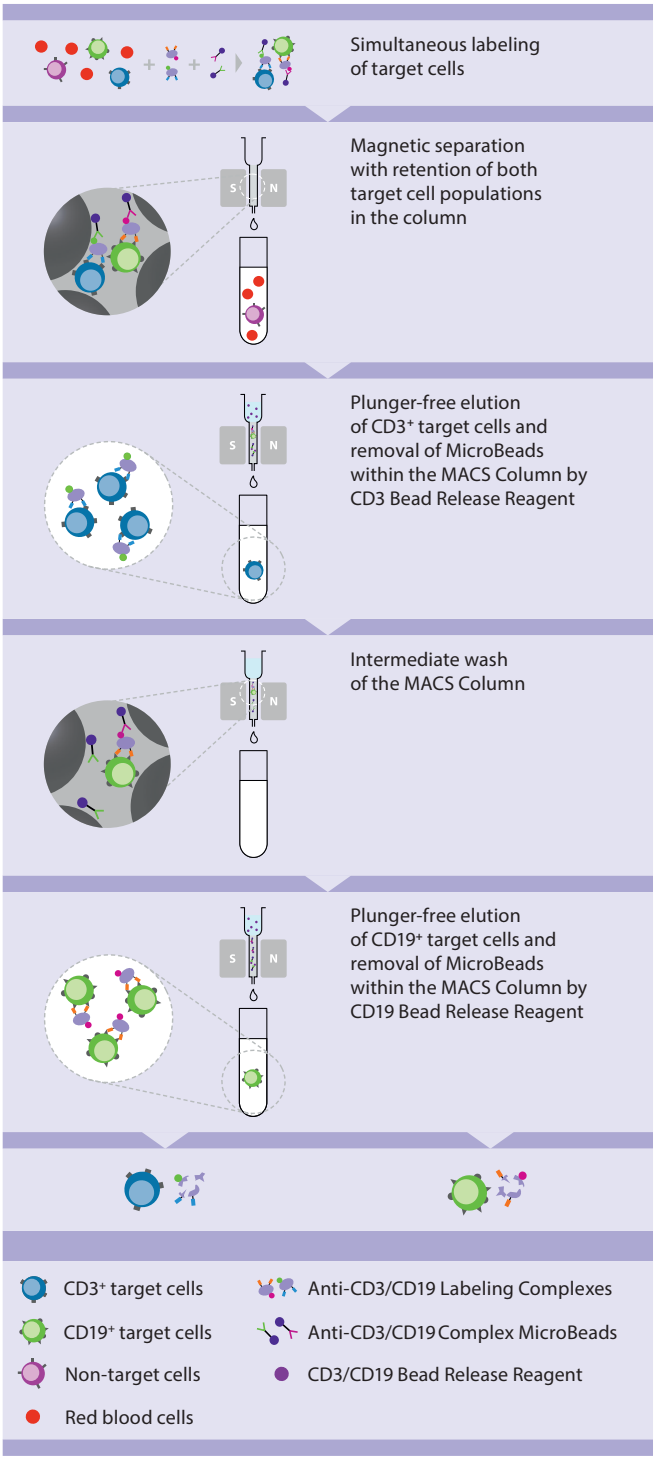
▲ The MACSprep CD3 and CD19 Selection Kit, human has been developed for the simultaneous positive selection of two target cells from 5 mL whole blood samples.

▲ **Note:** A range of 0.5–7.5 mL is possible but can reduce the cell separation performance.

Use reagents in following order:

Order of use	Reagent
1	REAlease Start Reagent
2	Anti-CD3 Labeling Complex, human Anti-CD19 Labeling Complex, human
3	Anti-CD3 Complex MicroBeads, human Anti-CD19 Complex MicroBeads, human
4	CD3 Bead Release Reagent (50×)
5	CD19 Bead Release Reagent (50×)
6	REAlease Release Reagent
7	REAlease Stop Reagent

2.1 Protocol overview



2.2 Sample preparation

Preparation of whole blood

▲ Anticoagulants such as EDTA, heparin-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 5 mL of whole blood in a collection tube containing an appropriate anticoagulant.
2. Proceed to magnetic labeling (2.3).



2.3 Magnetic labeling

▲ Volumes for magnetic labeling given below are for 5 mL of anticoagulated whole blood. When working with smaller or larger volumes, scale up or down all reagent volumes and total volumes accordingly.

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

1. Prepare a 15 mL tube with 250 µL of REAlease Start Reagent.
2. Add 5 mL of whole blood sample. Vortex gently to ensure proper mixing.
3. Add 500 µL Anti-CD3 Labeling Complex, human and 500 µL Anti-CD19 Labeling Complex, human.
4. Mix well and incubate for 10 minutes at +19 to +25 °C.
5. Add 2 mL Anti-CD3 Complex MicroBeads, human and 2 mL Anti-CD19 Complex MicroBeads, human.
6. Mix well and incubate for 5 minutes at +19 to +25 °C.
7. Proceed to magnetic separation (2.4).



2.4 Manual magnetic separation with the MidiMACS Separator or QuadroMACS Separator

▲ Always wait until the column reservoir is empty before proceeding to the next step.

1. Place a Whole Blood Column in the magnetic field of a MidiMACS Separator or QuadroMACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with 3 mL of separation buffer.
3. Apply magnetically labeled whole blood sample in aliquots with a maximum of 7.5 mL onto the column, e.g., add 2 × 5.125 mL when starting volume is 5 mL. Collect flow-through containing unlabeled cells (non-target cells).

   ▲ **Note:** The reservoir of the Whole Blood Column can contain a maximum of 7.5 mL.

4. Wash column with 2 × 2 mL of 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 rinse remaining whole blood off the reservoir.

5. Place a new collection tube under the Whole Blood Column.
6. Pipette 2 × 7 mL CD3 Bead Release Reagent onto the Whole Blood Column and remove collection tube. This fraction contains magnetically released target $CD3^+$ T cells.
7. Place a new collection tube under the Whole Blood Column. Wash column by pipetting 2 × 7 mL separation buffer onto the Whole Blood Column and discard flow-through.
8. Place a new collection tube under the Whole Blood Column.
9. Pipette 2 × 7 mL CD19 Bead Release Reagent onto the Whole Blood Column and remove collection tube. This fraction contains magnetically released target $CD19^+$ B cells.
10. Target cells are now free from MicroBeads and ready for analysis or downstream applications.
11. (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×).
12. (Optional) Proceed to removal of the Labeling Complex (2.6).

2.5 (Optional) Removal of the Labeling Complex and second magnetic labeling with MACS MicroBeads or REAlease MicroBeads

▲ Volumes given below are for target cells from 5 mL of anticoagulated whole blood starting material. When working with smaller or larger volumes, scale up or down all reagent volumes and total volumes accordingly.

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

2.5.1 Removal of the Labeling Complex

1. Centrifuge cell suspension at 300 × g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 5 mL of separation buffer.
3. Add 100 µL of REAlease Release Reagent.
4. Mix well and incubate for 5 minutes at +19 to +25 °C.
5. Cells are now free from Labeling Complexes and MicroBeads and are ready for analysis or downstream applications.
6. (Optional) For second magnetic labeling with MACS MicroBeads or REAlease MicroBeads continue with 2.6.2.

2.5.2 Second magnetic labeling with MACS MicroBeads or REAlease MicroBeads

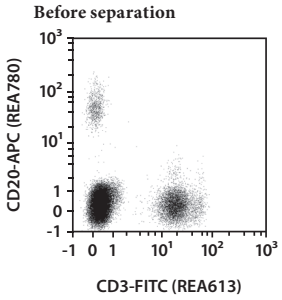
1. Centrifuge cell suspension at 300 × g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 500 µL REAlease Stop Reagent.
3. Mix well.
4. For a second magnetic labeling follow the labeling protocol in the respective MicroBead data sheet.

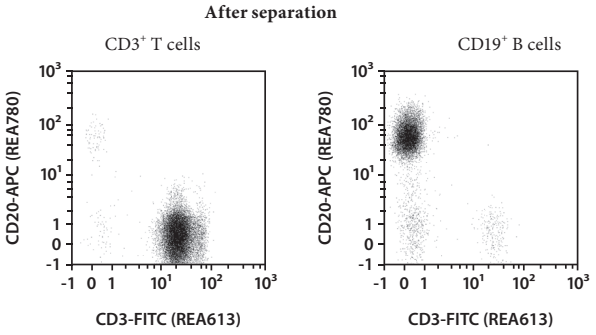
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3. Example of a separation using the MACSprep CD3 and CD19 Selection Kit, human

CD3$^+$ T cells and CD19$^+$ B cells were isolated from whole blood using the MACSprep CD3 and CD19 Selection Kit, human and Whole Blood Columns. Cells were fluorescently stained with the REAfinity Antibody CD45-VioBlue®, CD3-FITC, and CD20-APC and analyzed by flow cytometry using the MACSQuant® Analyzer 16. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

A) Cell purity

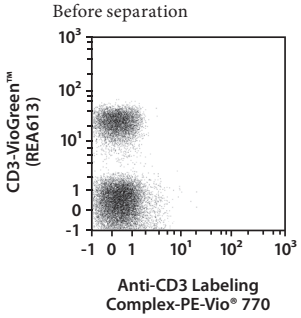


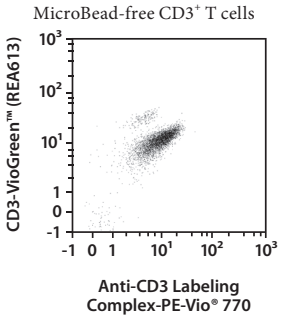


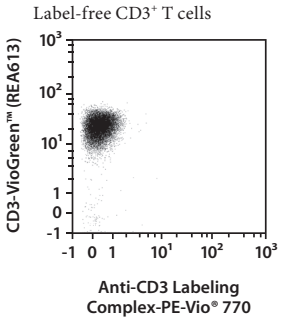
B) Label- and MicroBead-free cells

The efficient removal of all labels was shown by using Anti-CD3 Labeling Complex for isolated T cells and Anti-CD19 Labeling Complex for B cells to analyze the cells by flow cytometry for the presence of Labeling Complexes. Directly after isolation, the target CD3$^+$ T cells and CD19$^+$ B cells showed positive staining of the respective Labeling Complexes ("MicroBead-free target cells"), whereas after the release of the Labeling Complex, label-free target CD3$^+$ T cells and CD19$^+$ B cells showed negative staining signal similar to the non-labeled cells before separation.

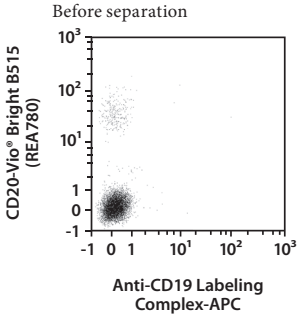
CD3$^+$ T cells

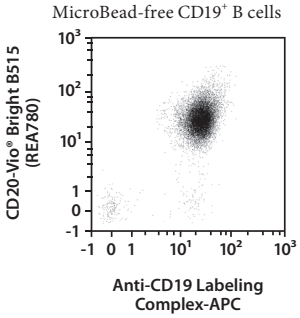


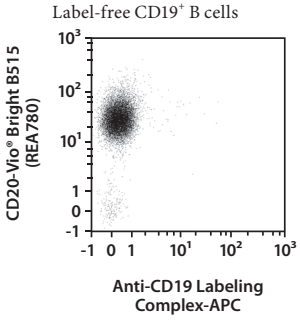




CD19$^+$ B cells







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