Miltenyi Biotec

StraightFrom™ Buffy Coat CD56 MicroBead Kit
human

Order no. 130-114-963

Contents

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	**4 mL StraightFrom™ Buffy Coat CD56 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD56 antibodies (isotype: mouse IgG1). **50 mL Whole Blood Column Elution Buffer** **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	StraightFrom Buffy Coat CD56 MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide. Whole Blood Column Elution Buffer contains stabilizer and 0.09% sodium azide.
Storage	Store StraightFrom Buffy Coat CD56 MicroBeads and Whole Blood Column Elution Buffer protected from light at 2–8 °C. Do not freeze. Store Whole Blood Columns dry at 10–35 °C and protected from light. The expiration date is indicated on the vial or box label.

1.1 Principle of the MACS® Separation

First, the $CD56^+$ cells in a buffy coat sample are magnetically labeled with StraightFrom Buffy Coat CD56 MicroBeads. Then, the cell suspension is loaded onto a Whole Blood Column, which is placed in the magnetic field of a MACS® Separator. The magnetically labeled $CD56^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD56^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD56^+$ cells can be eluted as the positively selected cell fraction.

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.
- Use Whole Blood Column Elution Buffer to elute positive cell fraction from column.

▲ 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

The StraightFrom Buffy Coat CD56 MicroBead Kit has been developed for the positive selection of $CD56^+$ cells directly from one buffy coat by using the MultiMACS™ Cell24 Separator Plus or the MidiMACS™ Separator or QuadroMACS™ Separator. No sample preparation is required, including density gradient centrifugation or erythrocyte lysis. The CD56 antigen is expressed by most NK cells and a T cell subset. Upon activation of NK cells, the surface expression of CD56 is increased.

1.4 Applications

- Isolation of $CD56^+$ cells from buffy coat. The purified $CD56^+$ cells are well suited for further flow cytometric, functional, or molecular analysis.

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.

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- Semi-automated separation:
  - MultiMACS™ Cell24 Separator Plus (# 130-098-637)
  - MultiMACS 12× Single-Column Adapter LS (# 130-108-816)
  - Single-well Deep Well Plates (# 130-114-966) or 24-well Deep Well Plates (# 130-110-500)
- 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 antibodies for flow cytometric analysis, e.g., CD56-PE (clone REA196), CD3-APC, and CD45-VioBlue®. 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

▲ The StraightFrom™ Buffy Coat 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.

2.1 Preparation of buffy coat

▲ 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.

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 (2.2).

2.2 Magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ 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 2 mL StraightFrom Buffy Coat MicroBeads).

▲ 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.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Add 4 mL StraightFrom Buffy Coat CD56 MicroBeads per buffy coat.
2. Mix well by inverting the tube and incubate for 15 minutes in the refrigerator (2–8 °C).
3. Proceed directly to magnetic separation (2.3).

2.3 Magnetic separation

2.3.1 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 elution plate for magnetic separation with StraightFrom Buffy Coat MicroBeads.

▲ Buffer volumes are as follows:

Equilibration: 3 mL (separation buffer)
Wash: 2×2 mL (separation buffer)
Elution: 4 mL (Whole Blood Column Elution Buffer)

▲ 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.

▲ Divide the sample equally between the 12 Whole Blood Columns, e.g., when starting volume is 84 mL (80 mL buffy coat and 4 mL StraightFrom Buffy Coat MicroBeads), add 7 mL onto each column.

▲ (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×) (# 130-094-183).

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

2.3.2 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 Whole Blood Columns, e.g., when starting volume is 80 mL, add 7 mL onto each column.

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.

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4. Wash Whole Blood Column with 2×2 mL separation buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.

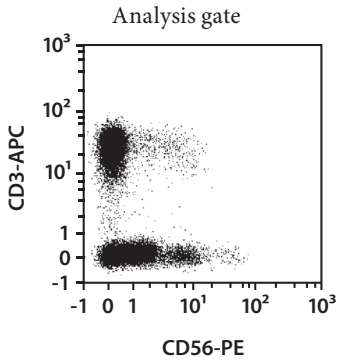
5. Remove Whole Blood Column from the separator and place it on a new collection tube.

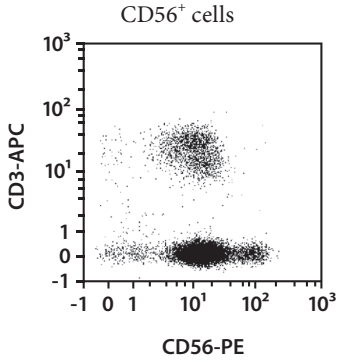
6. Pipette 4 mL **Whole Blood Column Elution Buffer** onto the Whole Blood Column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

7. (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×) (# 130-094-183).

3. Example of a separation using the StraightFrom™ Buffy Coat CD56 MicroBead Kit

Separation of a buffy coat sample using the StraightFrom™ Buffy Coat CD56 MicroBead Kit and the MultiMACS™ Cell24 Separator Plus with the Single-Column Adapter and Whole Blood Columns. Cells were fluorescently stained with CD56-PE, CD3-APC, as well as CD45-VioBlue® and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells were triggered via CD45-VioBlue, cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.





Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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