Miltenyi Biotec

StraightFrom® Buffy Coat CD3 MicroBead Kit
human

Order no. 130-121-310

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1. Description

This product is for research use only.

Components	**4 mL StraightFrom Buffy Coat CD3 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD3 antibodies (isotype: mouse IgG2a). **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 CD3 MicroBeads, human 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 CD3 MicroBeads, human and Whole Blood Column Elution Buffer protected from light at +2 to +8 °C. Do not freeze. Store Whole Blood Columns dry at +10 to +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 $CD3^+$ cells in a buffy coat sample are magnetically labeled with StraightFrom Buffy Coat CD3 MicroBeads, human. Then, the cell suspension is loaded onto a MACS Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled $CD3^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD3^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD3^+$ 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 CD3 MicroBead Kit, human has been developed for the positive selection of $CD3^+$ cells directly from one buffy coat by using the MultiMACS™ Cell24 Separator Plus or the QuadroMACS™ Separator. No sample preparation is required, including density gradient centrifugation or erythrocyte lysis. 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.

1.4 Applications

- Isolation of $CD3^+$ cells from buffy coat. The purified $CD3^+$ 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 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.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

- Table 1 provides an overview of MACS Columns and MACS Separators with links to the corresponding complete protocols. Use the link matching the MACS Column and MACS Separator used.

Table 1: MACS Columns and MACS Separators overview.

MACS Column (link to protocol)	Buffy coats	MACS Separator
Columns for manual separation		
Whole Blood Column	1 per 12 columns	QuadroMACS
Columns for instruments		
Whole Blood Column	1 per 12 columns	MultiMACS Cell24 Separator Plus

- 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:
  - 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., CD3 Antibody, anti-human. 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)

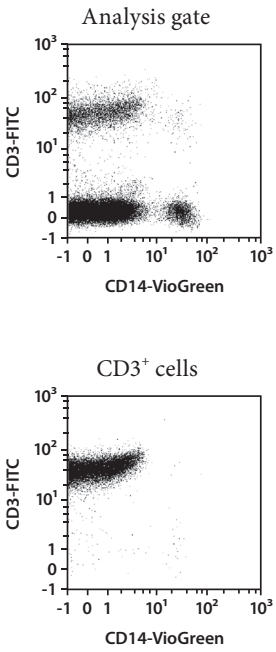
2. Protocol

▲ The StraightFrom Buffy Coat MicroBead Kit, human 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.

▲ Complete labeling and separation protocols are available online. Use the link provided in table 1, based on the used MACS Column and MACS Separator.

3. Example of a separation using the StraightFrom Buffy Coat CD3 MicroBead Kit, human

Separation of a buffy coat sample using the StraightFrom Buffy Coat CD3 MicroBeads, human and the MultiMACS Cell24 Separator Plus with the Single-Column Adapter and Whole Blood Columns. Cells were fluorescently stained withCD3-FITC, CD14-VioGreen™, as well as with CD45-VioBlue® and analyzed by flow cytometry using the MACSQuant® Analyzer. Viable leukocytes were gated for analysis based on scatter signals, propidium iodide fluorescence, and CD45 expression.



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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