Miltenyi Biotec

StraightFrom® Whole Blood CD4 MicroBeads
human

Order no. 130-090-877

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1. Description

This product is for research use only.

Components	**2 mL StraightFrom Whole Blood CD4 MicroBeads, human (# 130-090-877):** MicroBeads conjugated to monoclonal anti-human CD4 antibodies (isotype: mouse IgG1). **StraightFrom Whole Blood MicroBeads have been developed for use with the autoMACS® Pro Separator, autoMACS NEO Separator, MultiMACS™ Cell24 Separator Plus, QuadroMACS™ Separator, or QuadroMACS XL Separator.**
Capacity	For 40 mL human whole blood.
Product format	StraightFrom Whole Blood CD4 MicroBeads, human are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store StraightFrom Whole Blood CD4 MicroBeads, human 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 MACS® Separation

First, the $CD4^+$ cells in a whole blood sample are magnetically labeled with StraightFrom Whole Blood CD4 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 $CD4^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD4^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD4^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

The StraightFrom Whole Blood CD4 MicroBeads, human has been developed for the positive selection of $CD4^+$ cells directly from anticoagulated whole blood by using the autoMACS Pro Separator, autoMACS NEO Separator, MultiMACS Cell24 Separator Plus, QuadroMACS Separator, or QuadroMACS XL Separator. No sample preparation is required, including density gradient centrifugation or erythrocyte lysis, as well as no washing step after labeling. StraightFrom Whole Blood CD4 MicroBeads recognize about 65% of all peripheral blood T cells. The CD4 antigen is expressed on T helper cells and at a lower level on monocytes and dendritic cells. The CD4 antigen is an accessory molecule in the recognition of MHC class II/peptide complexes by the TCR heterodimer on $CD4^+$ T helper cells.

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 including lineage-specific chimerism analysis after allogeneic stem cell transplantation[1-3].

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.

- 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)	Max. sample volume	MACS Separator
Columns for manual separation		
XL Whole Blood Column	100 mL (per column)	QuadroMACS XL
Whole Blood Column	0.25–15 mL (per column)	MidiMACS, QuadroMACS
Columns for instruments		
autoMACS Column	28 mL (per sample)	autoMACS NEO

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MACS Column (link to protocol)	Max. sample volume	MACS Separator
autoMACS Column	28 mL (per sample)	autoMACS Pro Separation program: Positive selection: Posselwb/Rinse
Whole Blood Column	0.25–10 mL (per column)	MultiMACS Cell24 Separator Plus
Multi-24 Whole Blood Column Block	0.25–10 mL (per column)	MultiMACS Cell24 Separator Plus

- Automated separation:
  - autoMACS Pro Separator – Starter Kit (# 130-092-545) or autoMACS NEO Separator (# 130-120-327)
  - autoMACS Columns (# 130-021-101)
- Semi-automated separation:
  - MultiMACS Cell24 Separator Plus (# 130-098-637)
  - Whole Blood Column Kit (# 130-093-545), including Whole Blood Columns and Whole Blood Column Elution Buffer, and MultiMACS 12× Single-Column Adapter LS (# 130-108-816)

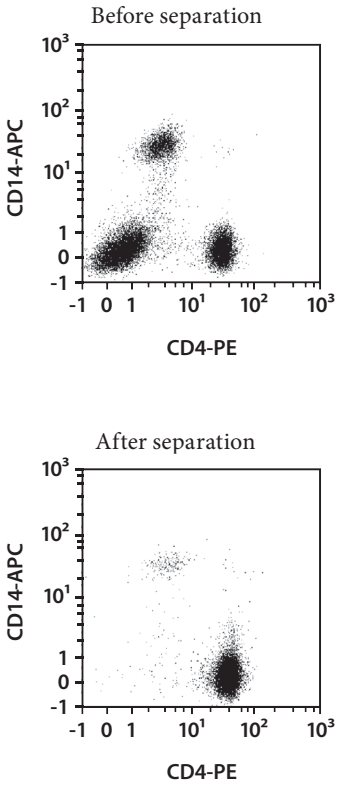
    or
  - Multi-24 Whole Blood Column Block (# 130-130-259) and and Whole Blood Column Elution Buffer (# 130-131-889)
- Manual separation:
  - QuadroMACS XL Separator (# 130-136-311) with XL Whole Blood Columns (# 130-139-767) and optional Whole Blood Column Elution Buffer (# 130-131-889)
    or
    MidiMACS Separator (# 130-042-302) or QuadroMACS Separator (# 130-090-976) with Whole Blood Column Kit (# 130-093-545), including Whole Blood Columns and Whole Blood Column Elution Buffer.
  - MidiMACS Separator (# 130-042-302), QuadroMACS Separator (# 130-090-976), or QuadroMACS XL Separator (# 130-136-311)
  - (Optional) MS Columns (# 130-042-201) or LS Columns (# 130-042-401) as second separation column.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD4-PE, CD14-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)
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.

2. Protocol

▲ 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 StraightFrom Whole Blood CD4 MicroBeads

Separation of a whole blood sample using the StraightFrom Whole Blood CD4 MicroBeads and the MultiMACS Cell24 Separator Plus with the Single-Column Adapter and Whole Blood Columns without washing step after labeling. Cells were fluorescently stained with CD4-PE, CD14-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.



4. References

1. Köhl, U. *et al.* (2003) Quantitative analysis of chimerism after allogeneic stem cell transplantation by PCR amplification of microsatellite markers and capillary electrophoresis with fluorescence detection: the Frankfurt experience. Leukemia 17: 232–236.
2. Adams, S. *et al.* (2004) Cell lineage-specific chimerism in post-hematopoietic stem celltransplant patients. MACS&more 8/2: 16–17.
3. Meyer, R. G. *et al.* (2007) Prophylactic transfer of CD8-depleted donor lymphocytes after T-cell–depleted reduced-intensity transplantation. Blood 109: 374–382.

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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