Miltenyi Biotec

CD4 MicroBeads
human

Order no. 130-045-101

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1. Description

This product is for research use only.

Components	2 mL CD4 MicroBeads, human : MicroBeads conjugated to monoclonal anti-human CD4 antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells
Product format	CD4 MicroBeads 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 MACS Separation

First, the $CD4^+$ cells are magnetically labeled with CD4MicroBeads, 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. To increase the purity, the positively selected cell fraction containing the $CD4^+$ cells must be separated over a second column.

1.2 Background information

CD4 MicroBeads have been developed for the separation of human cells based on the expression of the CD4 antigen. CD4 is a 55 kDa protein that is highly expressed on T helper cells in the thymus and peripheral blood. In T helper cells, CD4 functions as an accessory molecule for the recognition of MHC class II/peptide complexes by TCR heterodimers. CD4 is also expressed on monocytes and dendritic cells, albeit at lower levels than on $CD4^+$ T helper cells. In conjunction with certain chemokine receptors, CD4 is the primary high-affinity receptor for HIV.

1.3 Applications

- Positive selection or depletion of $CD4^+$ T cells from peripheral blood, leukapheresis, cord blood, lymph nodes, thymus, spleen, skin biopsies, synovial fluids, bronchial lavages, or cell cultures.
- Functional studies on $CD4^+$ T cells, such as proliferation or cytotoxicity assays.
- *In vitro* culture of $CD4^+$ T cells for evaluation of cytokine production.
- Coculture experiments of $CD4^+$ T cells with dendritic cells or B cells for evaluation of the activation of antigen-presenting cells.
- Numerous applications for HIV research, for example, *in vitro* infection of cells with HIV, evaluation of the infectivity of HIV-infected follicular dendritic cells, assessment of immune reconstitution after antiviral therapy, and monitoring of aberrations in the immune system of children from HIV-infected mothers.

1.4 Reagent and instrument requirements

- 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). 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. number of labeled cells	Max. number of total cells	MACS Separator
Columns for manual separation			
MS Column	10^7	2×10^8	MiniMACS, OctoMACS
LS Column	10^8	2×10^9	MidiMACS, QuadroMACS
XLS Column	5×10^8	10^{10}	QuadroMACS XL
LD Column	10^8	5×10^8	MidiMACS, QuadroMACS

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MACS Column (link to protocol)	Max. number of labeled cells	Max. number of total cells	MACS Separator
Columns for instruments			
LS Column	10^8	10^9	MultiMACS Cell24 Separator Plus
autoMACS Column	2×10^8	4×10^9	autoMACS NEO
autoMACS Column	2×10^8	4×10^9	autoMACS Pro Separation program: Positive selection: Possel Depletion: Depletes
Multi-24 Column Block	10^8 per column 2.4×10^9 per block	10^9 per column 2.4×10^{10} per block	MultiMACS Cell24 Separator Plus

▲ **Note:** If separating with LS or LD Columns and the MultiMACS Cell24 Separator Plus, use the Single-Column Adapter. Refer to the user manual for details.

▲ **Note:** Cells which strongly express the CD4 antigen can also be depleted using MS, LS, or XLS Columns.

▲ **Note:** For autolabeling compatibilty for the autoMACS NEO Seperator, please see here.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD4 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) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by using a MACS PBMC Isolation Kit or by density gradient centrifugation, for example, using Ficoll-Paque™.

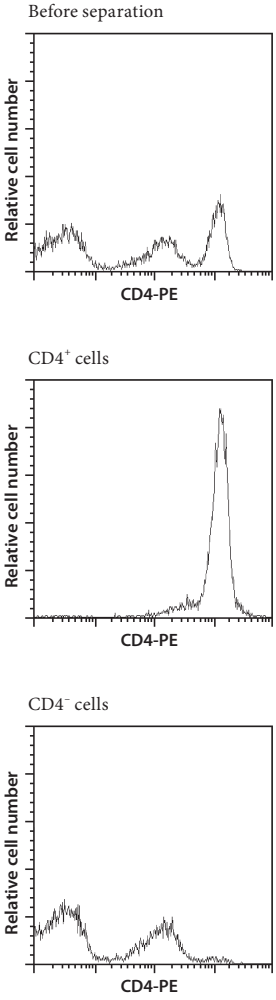
▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200 × g for 10–15 minutes at +20 °C. Carefully aspirate supernatant. Repeat washing step.

For details refer to the data sheet or the protocols section at www.miltenyibiotec.com/protocol.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, it is recommended to use density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

3. Example of a separation using CD4 MicroBeads, human

$CD4^+$ cells were isolated from human PBMCs using CD4MicroBeads, human, an MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with CD4-PE and analyzed by flow cytometry. 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 for local Miltenyi Biotec Technical Support contact information.

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