Miltenyi Biotec

CD8 MicroBeads
human

Order no. 130-045-201

Contents

1. Description
   - 1.1 Principle of the MACS® Separation
   - 1.2 Background information
   - 1.3 Applications
   - 1.4 Reagent and instrument requirements
2. Protocol
   - 2.1 Sample preparation
3. Example of a separation using the CD8 MicroBeads

1. Description

This product is for research use only.

Components	2 mL CD8 MicroBeads, human : MicroBeads conjugated to monoclonal anti-human CD8 antibodies (isotype: mouse IgG2a).
Capacity	For 10^9 total cells
Product format	CD8 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 $CD8^+$ cells are magnetically labeled with CD8MicroBeads, 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 $CD8^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD8^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD8^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the $CD8^+$ cells must be separated over a second column.

1.2 Background information

The CD8 antigen forms a complex together with the T cell receptor and acts as an accessory molecule in the recognition of MHC class I/peptide complexes by the TCR heterodimer on $CD8^+$ cytotoxic T cells. The CD8 molecule consists of either an α/β heterodimer or an α/α homodimer. It is strongly expressed on cytotoxic T cells and dimly on a subset of NK cells. CD8 is expressed on most thymocytes and on about one third of all peripheral blood T cells. $CD8^+$ cytotoxic T cells play an important role in the killing of virus-infected cells and tumor cells.
For isolation of highly pure cytotoxic T cells using CD8 MicroBeads, human, depletion of NK cells with CD56 MicroBeads, human (# 130-050-401) prior to positive selection with CD8 MicroBeads, human is recommended.

1.3 Applications

- Positive selection or depletion of cytotoxic T cells from peripheral blood, cord blood, lymph nodes, thymus, skin biopsies, and spleen.
- Isolation of cytotoxic T cells for further functional studies, such as proliferation assays, cytotoxicity assays, and for analysis of *in vitro* cytokine production.
- Depletion of $CD8^+$ T cells from human peripheral blood mononuclear cells (PBMCs) for immune reconstitution experiments in SCID mice.

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
Columns for instruments			
LS Column	10^8	10^9	MultiMACS Cell24 Separator Plus
autoMACS Column	2×10^8	4×10^9	autoMACS NEO

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MACS Column (link to protocol)	Max. number of labeled cells	Max. number of total cells	MACS Separator
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 CD8 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., CD8 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™.

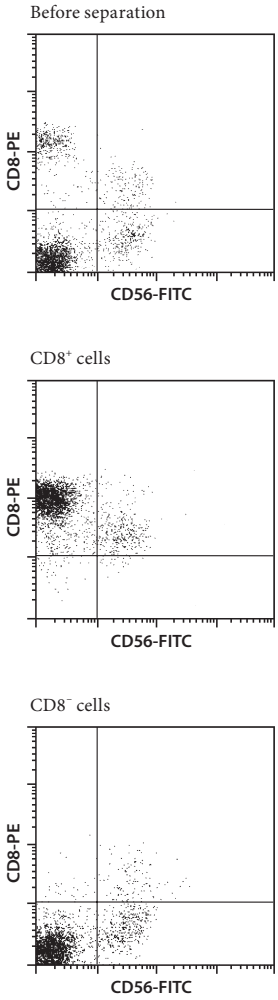
▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200 × g for 10–15 minutes at +20 °C. Carefully aspirate supernatant. Repeat washing step.

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 CD8 MicroBeads, human

$CD8^+$ cells were isolated from human PBMCs using CD8 MicroBeads, human, an LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with CD8-PE and CD56-FITC 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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