Miltenyi Biotec

CD56 MicroBeads
human

Order no. 130-050-401

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1. Description

This product is for research use only.

Components	2 mL CD56 MicroBeads, human : MicroBeads conjugated to monoclonal anti-human CD56 antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells
Product format	CD56 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 $CD56^+$ cells are magnetically labeled with CD56 MicroBeads. Then, the cell suspension is loaded onto a MACS 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 Background information

CD56 MicroBeads, human have been developed for the separation of human cells based on the expression of the CD56 antigen. CD56 is expressed by essentially all human NK cells and its density is increased on the cell membrane after activation. The antigen is also present on a unique subset of $CD3^+$ T cells that mediates non-MHC–restricted cytotoxicity and on myoblasts, some neural tissue, and tumors.

1.3 Applications

- Positive selection or depletion of cells expressing human CD56 antigen
- Isolation or depletion of $CD56^+$ cells from peripheral blood mononuclear cells (PBMCs) or lympoid tissue

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
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 CD56 antigen can also be depleted using MS, LS, or XLS Columns.

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

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

140-000-069.11

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD56 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™.

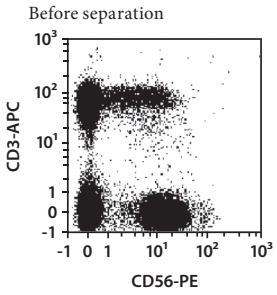
▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200 × g for 10–15 minutes at +20 °C. Carefully aspirate supernatant. Repeat washing step.

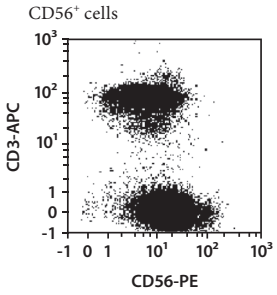
For details refer to the data sheet or the protocols section at www.miltenyibiotec.com/protocol.

▲ 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 CD56 MicroBeads, human

$CD56^+$ cells were isolated from human PBMCs using CD56 MicroBeads, human, an LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with CD56-PE and CD3-APC and analyzed by flow cytometry using the MACSQuant® Analyzer. 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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