CD133 MicroBead Kit – Hematopoietic Tissue
human

Order no. 130-100-830

Contents

1. Description

Components	**2 mL CD133 MicroBeads – Hematopoietic Tissue, human:** MicroBeads conjugated to monoclonal anti-human CD133 antibodies (isotype: mouse IgG1, clone AC133). **2 mL FcR Blocking Reagent, human:** Human IgG.
Specificity	CD133 antigen, epitope (CD133/1)[1].
Capacity	For 2×10^9 total cells, up to 20 separations.
Product format	CD133 MicroBeads – Hematopoietic Tissue are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS® Separation

First, the $CD133^+$ cells are magnetically labeled with CD133 MicroBeads – Hematopoietic Tissue. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled $CD133^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD133^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD133^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the $CD133^+$ cells is separated over a second column.

1.2 Background information

The CD133 MicroBead Kit – Hematopoietic Tissue is a magnetic labeling system designed for the positive selection of $CD133^+$ cells. It allows the single-step isolation of endothelial and hematopoietic stem and progenitor cells.

The CD133 molecule is a 5-transmembrane cell surface antigen with a molecular weight of 117 kDa.[2] In the hematopoietic system, CD133 expression is restricted to a subset of $CD34^{bright}$ stem and progenitor cells in human fetal liver, bone marrow, cord blood, and peripheral blood.[3] Isolated from hematopoietic sources, $CD133^+$ cells can become adherent and are reported to become $CD133^-$ during culture[4]. The $CD34^+CD133^+$ cell population, which includes $CD34^+CD38^-$ cells, was shown to be capable of repopulating NOD/SCID mice.[5] In addition, the CD133 antigen is also expressed on circulating endothelial progenitor cells[6,7]. The CD133 MicroBead Kit – Hematopoietic Tissue has been optimized for the isolation of $CD133^+$ cells of hematopoietic or endothelial origin. For the isolation of $CD133^+$ cells from solid tissues, e.g., the purification of cancer stem cells from solid tumors, we recommend the CD133 MicroBead Kit – Tumor Tissue (# 130-100-857).

1.3 Applications

- Positive selection or depletion of $CD133^+$ cells of hematopoietic or endothelial origin.
- Isolation or depletion of $CD133^+$ cells from peripheral blood mononuclear cells (PBMCs), cord blood or bone marrow mononuclear cells.
- Isolation of $CD133^+$ cells from debris-rich samples

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 (2–8 °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.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD133/2 (293C3)-PE (# 130-090-853), CD133/2 (293C3)-APC (# 130-090-854), CD133/2 (293C3)-Biotin, CD34-FITC (# 130-081-001), CD34-APC (# 130-090-954), or CD34-PE (# 130-081-002). For more information about fluorochrome-conjugated antibodies refer to www.miltenyibiotec.com/antibodies.

140-004-163.01

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com

- MACS Columns and MACS Separators: CD133$^+$ cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD, CS, or D Columns. Cells which strongly express the CD133 antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro or the autoMACS Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	10^9	2×10^{10}	SuperMACS II
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
CS	2×10^8		VarioMACS, SuperMACS II
D	10^9		SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion 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 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.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

For preparation of cord blood cells, bone marrow cells, or cells from leukapheresis material, please refer to the sample preparation protocols at www.miltenyibiotec.com/protocols.

2.2 Magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^8 total cells. When working with fewer than 10^8 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^8 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 300 µL of buffer per 10^8 total cells.
4. Add 100 µL of FcR Blocking Reagent per 10^8 total cells.
5. Add 100 µL of CD133 MicroBeads – Hematopoietic Tissue per 10^8 total cells.
6. Mix well and incubate for 30 minutes in the refrigerator (2–8 °C).
7. (Optional) Add staining antibody recognizing another epitope than AC133, e.g., CD133/2 (293C3)-PE (# 130-090-853), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
8. Wash cells by adding 1–2 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

   ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 cells in 500 µL of buffer.
10. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of CD133$^+$ cells. For details refer to table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with MS or LS Columns

▲ To achieve highest purities, perform two consecutive column runs.

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1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.
   MS: 3×500 µL LS: 3×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.

   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL
7. To increase purity of $CD133^+$ cells, enrich the eluted fraction over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS Column data sheet.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details refer to the CS Column data sheet.
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way stopcock of the assembled column. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the top. Collect total effluent; this is the unlabeled cell fraction.

Depletion with D Columns

For instructions on column assembly and separation refer to the D Column data sheet.

Magnetic separation with the autoMACS® Pro Separator or the autoMACS® Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator or the autoMACS Separator.

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of ≥ 10 °C.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS® Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

   Positive selection from peripheral blood, bone marrow, or leukapheresis: Posselds
   Collect positive fraction in row C of the tube rack.

   Positive selection from cord blood: Posselds
   Collect positive fraction in row C of the tube rack.

   Depletion: Depletes
   Collect negative fraction in row B of the tube rack.

Magnetic separation with the autoMACS® Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos 2.
3. For a standard separation choose one of the following programs:

   Positive selection from peripheral blood, bone marrow, or leukapheresis: Posselds
   Collect positive fraction from outlet port pos 2.

   Positive selection from cord blood: Posselds
   Collect positive fraction from outlet port pos 2.

   Depletion: Depletes
   Collect negative fraction from outlet port neg1.

140-004-163.01

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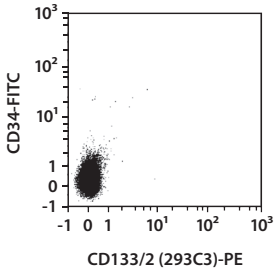
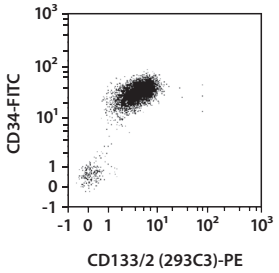
3. Example of a separation using the CD133 MicroBead Kit –Hematopoietic Tissue

$CD133^+$ hematopoietic stem and progenitor cells were isolated from non-mobilized human PBMCs using the CD133 MicroBead Kit – Hematopoietic Tissue, MS Columns, and a MiniMACS™ Separator. Cells were fluorescently stained with CD34-FITC (# 130-081-001) and CD133/2 (293C3)-PE (# 130-090-853) and analyzed by flow cytometry. Cell debris and dead cells werde excluded from the analysis based on scatter signals and propidium iodide fluorescence.

Before separation



$CD133^+$ cell fraction



4. References

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3. Bühring, H. J. *et al.* (1999) Expression of novel surface antigens on early hematopoietic cells. Ann NY Acad Sci: 872: 25–39.
4. Kuci, S. *et al.* (2003) Identification of a novel class of $CD133^+CD34^-$ peripheral blood cells with SCID-repopulating capacity. MACS&more 7/1.
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7. Peichev, M. *et al.* (2000) Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood 95: 952–958.

All protocols and data sheets are available at **www.miltenyibiotec.com**.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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