Miltenyi Biotec

Indirect CD133 MicroBead Kit
human

Order no. 130-091-895

Index

1. Description

Components	**1 mL CD133/1 (AC133)-Biotin, human:** Biotin-conjugated monoclonal antibody (isotype: mouse IgG1; clone: AC133[1]). **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1; clone: Bio3-18E7.2). **2 mL FcR Blocking Reagent:** Human IgG.
Size	For 2×10^9 total cells, up to 20 separations.
Product format	All components 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 MACS® Separation

First, the $CD133^+$ cells are indirectly magnetically labeled with CD133/1 (AC133)-Biotin antibody and Anti-Biotin 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 $CD133^+$ cells are retained within the column. The unlabeled $CD133^-$ cells pass through; this fraction is thus depleted of $CD133^+$ cells. After removing the column from the magnetic field, the retained $CD133^+$ cells are eluted and once again separated over a new column to achieve highest purities.

1.2 Background and product applications

The CD133 molecule is a 5-transmembrane cell surface antigen with a molecular weight of 117 kD.[2] The CD133/1 (clone AC133) antibody recognizes epitope 1 of the CD133 antigen.[1,3] 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.[4] Additionally, CD133 is expressed by a small portion of $CD34^-$ cells in these tissues.[5] The $CD34^+CD133^+$ cell population, which includes $CD34^+CD38^-$ cells, was shown to be capable of repopulating NOD/SCID mice.[6] Recently, CD133 has also been found to be expressed on circulating endothelial progenitor cells[7,8] and fetal neural stem cells[9,10] as well as on other tissue-specific stem cells, such as renal[11], prostate[12], and corneal[13] stem cells. The putative murine homologue, prominin, is expressed on neuroepithelial and epithelial mouse cells.[14]

Example applications

- $CD133^+$ cells are used in basic stem cell research, stem cell evaluation, and stem cell expansion, as well as in research on hematological malignancies, stem cell plasticity, cellular therapies, and tissue regeneration.

1.3 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 (4–8 °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 calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: $CD133^+$ cells can be enriched by using MS, LS, or XS Columns (positive selection) 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 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
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10^8		VarioMACS, SuperMACS
D	10^9		SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS

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

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- Ficoll-Paque™ and PBS containing 2 mM EDTA or 0.6% anticoagulant citrate dextrose formula-A (6% ACD-A: 22.3 g/L glucose, 22 g/L sodium citrate and 8 g/L citric acid in H2O).
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric or fluorescence microscopic control of $CD133^+$ stem and progenitor cell purification, e.g., CD133/2 (293C3)-PE (# 130-090-853), CD133/2 (293C3)-APC (# 130-090-854), or CD34-FITC (# 130-081-001).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- (Optional) MACS® HSC-CFU media for the evaluation of the hematopoietic engraftment potential. For product details, see our catalog or visit our website www.miltenyibiotec.com.

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, as described below.

When working with tissues, prepare a single-cell suspension by a standard preparation method. For details see section General Protocols in the user manuals or visit www.miltenyibiotec.com/protocols.

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

Preparation of peripheral blood mononuclear cells (PBMCs) using Ficoll-Paque™

▲ The peripheral blood or buffy coat should not be older than 8 hours and supplemented with anticoagulants (e.g. heparin, EDTA, citrate, ACD-A, or citrate phosphate dextrose (CPD)).

▲ PBMCs may be stored in the refrigerator overnight in PBS containing 0.5% BSA supplemented with autologous serum after the last washing step.

1. Dilute cells with 2–4× the volume of PBS, pH 7.2, containing 2 mM EDTA (PBS/EDTA) or 0.6% ACD-A.
   ▲ **Note:** The more diluted the blood sample, the better the purity of the mononuclear cells.
2. Carefully layer 35 mL of diluted cell suspension over 15 mL of Ficoll-Paque™ (ρ = 1.077) in a 50 mL conical tube.
3. Centrifuge at 400×g for 30–40 minutes at 20 °C in a swinging-bucket rotor without brake.
4. Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the interphase.
5. Carefully transfer the interphase cells (lymphocytes, monocytes, and thrombocytes) to a new 50 mL conical tube.
6. Fill the conical tube with PBS containing 2 mM EDTA or 0.6% ACD-A, mix, and centrifuge at 300×g for 10 minutes at 20 °C. Carefully aspirate supernatant completely.
7. For removal of platelets, resuspend the cell pellet in 50 mL of buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully remove the supernatant completely.
   ▲ **Note:** This step increases the purity of the CD133 cell separation.
8. Repeat step 7. Most of the platelets will remain in the supernatant upon centrifugation at 200×g.
9. Resuspend cell pellet in a final volume of 300 µL of buffer for up to 10^8 total cells (PBMCs of about 100 mL of blood). Proceed to magnetic labeling (2.2).

Preparation of cord blood cells

▲ Do not use cord blood older than 4 hours.

▲ The cord blood should be drawn directly into a 50 mL tube containing 5 mL of PBS containing 2 mM EDTA, or 0.6% ACD-A, or 200 U/mL heparin.

▲ The cord blood should be stored at 4 °C prior to separation.

1. Dilute anticoagulated cord blood with 3× the volume of PBS containing 2 mM EDTA or 0.6% ACD-A.
2. Carefully layer 35 mL of diluted cell suspension over 15 mL of Ficoll-Paque in a 50 mL conical tube.
3. Centrifuge at 400×g for 35 minutes at 20 °C in a swinging-bucket rotor without brake.
4. Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the interphase.
5. Carefully transfer the interphase cells (lymphocytes, monocytes, and thrombocytes) to a new 50 mL conical tube.
6. Fill the conical tube with PBS containing 2 mM EDTA or 0.6% ACD-A, mix, and centrifuge at 200×g for 10 minutes at 20 °C. Carefully aspirate supernatant completely.
7. Repeat step 6.
   ▲ **Note:** This step increases the purity of the CD133 cell separation.
8. Resuspend cell pellet in a final volume of 300 µL of buffer for up to 10^8 total cells. Proceed to magnetic labeling (2.2).

Preparation of bone marrow cells

1. Collect bone marrow in 50 mL tubes containing 5 mL PBS containing 2 mM EDTA, or 0.6% ACD-A, or 200 U/mL heparin.
   ▲ **Note:** Store cells at 4 °C if the cells cannot be processed immediately.
2. For preparation of a single-cell suspension of bone marrow cells dilute with 10× the volume of RPMI 1640 containing 0.02% collagenase B and 100 U/mL DNase and shake gently at room temperature for 45 minutes.
3. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407), in order to remove cell clumps. Wet filter with buffer before use.
4. Carefully layer 35 mL of diluted cell suspension over 15 mL of Ficoll-Paque in a 50 mL conical tube.
5. Centrifuge at 400×g for 35 minutes at 20 °C in a swinging-bucket rotor without brake.
6. Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the interphase.
7. Carefully transfer the interphase cells (lymphocytes, monocytes, and thrombocytes) to a new 50 mL conical tube.

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8. Fill the conical tube with PBS containing 2 mM EDTA or 0.6% ACD-A, mix, and centrifuge at 300×g for 10 minutes at 20 °C. Carefully aspirate supernatant completely.
9. Repeat step 8.
   ▲ **Note:** This step increases the purity of the CD133 cell separation.
10. Resuspend cell pellet in a final volume of 300 µL of buffer for up to 10^8 total cells. Proceed to magnetic labeling (2.2).

Preparation of cells from leukapheresis material

1. Filter apheresis harvest through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407), in order to remove cell clumps. Wet filter with buffer before use.
2. Wash cells once with buffer and resuspend in a final volume of 300 µL of buffer for up to 10^8 cells. Proceed to magnetic labeling (2.2).

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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column.

▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 350 µL of buffer per 10^8 total cells.
4. Add 100 µL of FcR Blocking Reagent per 10^8 total cells.
5. Add 50 µL of CD133/1 (AC133)-Biotin per 10^8 total cells .
6. Mix well and refrigerate for 10 minutes(4–8 °C).
7. (Optional) Add staining antibodies, e.g., 50 µL of CD133/2 (293C3)-PE (# 130-090-853), CD34-FITC (# 130-081-001), and CD45-APC (# 130-091-230) and refrigerate for 5 minutes in the dark (4–8 °C).
8. Wash cells by adding 10–20× the labeling volume of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Repeat washing step.
10. Resuspend up to 10^8 total cells in 400 µL of buffer.
11. Add 100 µL of Anti-Biotin MicroBeads per 10^8 total cells.
12. Mix well and refrigerate for 15 minutes (4–8 °C).
13. Wash cells by adding 10–20× the labeling volume of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
14. Resuspend up to 10^8 total 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.
15. 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 see table in section 1.3.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
   Collect total effluent. This is the unlabeled cell fraction.
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 eluting into a collection tube.
6. Pipette an 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 the purity of $CD133^+$ cells, the eluted fraction can be enriched over a second 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 see 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.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS® Separator. For details see 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™ Separator

▲ Refer to the autoMACS™ User Manual for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. For a standard separation, choose one of the following separation programs:

   Positive selection of CD133$^+$ cells from **peripheral blood, bone marrow, or leukapheresis**: "Posseld".

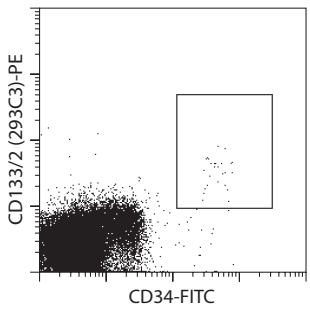
   Positive selection of CD133$^+$ cells from **cord blood:** "Posseld2".

   ▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details see autoMACS User Manual, section autoMACS Cell Separation Programs.
3. When using the programs "Posseld" or "Posseld2", collect positive fraction from outlet port pos2. This is the purified CD133$^+$ cell fraction.

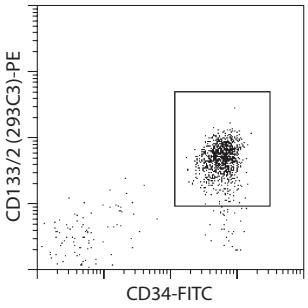
3. Example of a separation using Indirect CD133 MicroBead Kit

Separation of non-mobilized PBMCs using CD133/1 (AC133)-Biotin, Anti-Biotin MicroBeads, MS Columns, and a MiniMACS™ Separator. The cells are fluorescently stained with CD34-FITC and CD133/2 (293C3)-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

Before separation



After separation



4. References

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Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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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