Miltenyi Biotec

Diamond CD133 Isolation Kit
human

Order no. 130-094-913

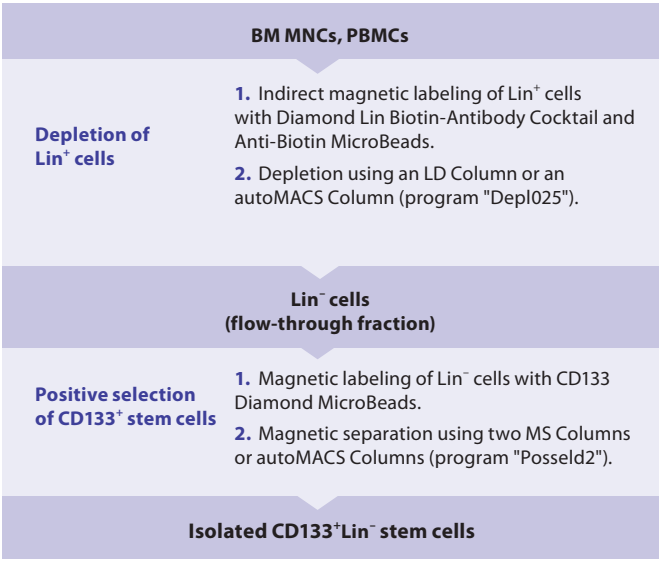
Contents

1. Description

Components	**2 mL Diamond Lin Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal anti-human antibodies against CD2, CD3, CD11b, CD14, CD15, CD16, CD19, CD56, CD61, and CD235a (Glycophorin A). **2×2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1) **2 mL CD133 Diamond MicroBeads, human:** MicroBeads conjugated to monoclonal CD133 antibodies (isotype: mouse IgG1)
Capacity	For 2×10^9 total cells, up to 40 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 the MACS® Separation

The Diamond CD133 Isolation Kit, human has been developed for the isolation of extremely pure CD133$^+$ stem cells (HSCs) from peripheral blood mononuclear cells (PBMCs) and bone marrow mononuclear cells (BM MNCs). The expected purity is almost 100%. Isolation of stem cells is performed in a two-step procedure. First, Lin$^+$ cells are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies against lineage-specific antigens and Anti-Biotin MicroBeads. Upon subsequent magnetic separation of the cells over a MACS® Column that is placed in a magnetic field of a MACS Separator, the magnetically labeled Lin$^+$ cells are retained within the column while the unlabeled Lin$^-$ cells pass through. In the second step, the enriched stem cells are directly labeled with CD133 Diamond MicroBeads. Upon subsequent magnetic separation, the CD133$^+$ stem cells are eluted after removal of the column from the magnetic field. To achieve highest purities, the positively selected cell fraction containing the CD133$^+$ stem cells is separated over a second column.



1.2 Background information

The CD133 molecule is a 5-transmembrane cell surface antigen with a molecular weight of 117 kDa. CD133$^+$ stem cells can be isolated from peripheral blood, cord blood, bone marrow and leukapheresis product. CD133$^+$ cells can become adherent and are reported to become CD133-negative during culture.[1,2]

The Diamond CD133 Isolation Kit, human combines lineage depletion and a CD133 enrichment to obtain pure Lin$^-$CD133$^+$ stem cells, for example, for expression profiling.

Using the Diamond CD133 Isolation Kit, human primitive CD133 cells can be isolated from BM MNCs or PBMCs even if the starting frequency is low.

▲ **Note:** CD133 MicroBeads recognize the epitope CD133/1. For quality control staining of separated cells using the Diamond CD133 Isolation Kit, the application of CD133/2 (293C3)-PE or -APC is recommended.

1.3 Applications

- Isolation of highly pure Lin$^-$CD133$^+$ stem cells, e.g., for expression profiling especially when using the autoMACS Pro Separator.
- Characterization of undifferentiated stem cells and their developmental potential.
- Studies on stimulation of proliferation and maturation by cytokines

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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.

- MACS Columns and MACS Separators: Depletion of Lin^+ cells is performed on an LD Column. The subsequent positive selection of stem cells is performed on two MS 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
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, 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™ Separators. For details see the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD34-FITC, CD34-PE, CD34-APC, CD45-FITC, CD45-PE, CD45-APC, CD133/2 (293C3)-PE, CD133/2 (293C3)-APC, or Biotin Antibody-PE . For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (Optional) MC CD34/CD133 Stem Cell Cocktail, human (# 130-092-882) for flow cytometric evaluation of separation results.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 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 see the protocols section at www.miltenyibiotec.com/protocols.

For preparation of bone marrow from human aspirates, please refer to the sample preparation protocols at www.miltenyibiotec.com/protocols.

2.2 Magnetic labeling of Lin^+ cells

▲ 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 5×10^7 total cells. When working with fewer than 5×10^7 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 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, # 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. 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 200×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 200 µL of buffer per 5×10^7 total cells.
4. Add 50 µL of Diamond Lin Biotin-Antibody Cocktail per 5×10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 2.5–5 mL of buffer per 5×10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 400 µL of buffer per 5×10^7 total cells.
8. Add 100 µL of Anti-Biotin MicroBeads per 5×10^7 total cells.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 5–10 mL of buffer per 5×10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
11. Resuspend up to 10^8 cells in 1000 µL of buffer.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).

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2.3 Magnetic separation: Depletion of Lin$^+$ cells

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

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 Lin$^-$ cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
5. Proceed to 2.4 for further enrichment of CD133$^+$ stem cells.

Depletion 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.

Depletion 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 the following program:

   Depletion: "Depl025"

   Collect negative fraction in row B of the tube rack. This fraction represents the unlabeled Lin$^-$ HSCs.

   (Optional) Collect positive fraction in row C of the tube rack.
4. Proceed to 2.4 for further enrichment of CD133$^+$ stem cells.

Depletion 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 pos1.
3. For a standard separation choose the following program:

   Depletion: "Depl025"

   Collect negative fraction from outlet port neg1. This fraction represents the unlabeled and Lin$^-$ HSCs.

   (Optional) Collect positive fraction from outlet port pos1.
4. Proceed to 2.4 for further enrichment of CD133$^+$ stem cells.

2.4 Magnetic labeling of CD133$^+$ stem cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 5×10^7 cells. For larger initial cell numbers, scale up volumes accordingly.

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 200 µL of buffer per 5×10^7 total cells.
3. Add 50 µL of CD133 Diamond MicroBeads per 5×10^7 total cells.
4. Mix well and incubate for 30 minutes in the refrigerator (2–8 °C).
5. (Optional) Add staining antibodies, e.g., 25 µL of CD133/2 (293C3)-PE and CD34-FITC, and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
6. Wash cells by adding 2.5–5 mL of buffer per 5×10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 500 µL of buffer per 10^8 total cells.

2.5 Magnetic separation: Positive selection of CD133$^+$ stem cells

Positive selection with MS Columns

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

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

   ▲ **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 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. Enrich the eluted fraction over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

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Positive selection 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.

Positive selection 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 the following program:

   Positive selection: "Posseld2"
   Collect positive fraction in row C of the tube rack.

Positive selection with the autoMACS® Separator

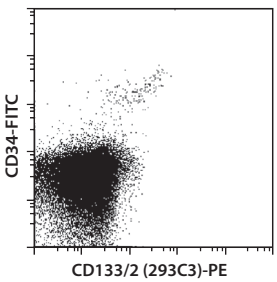
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

   Positive selection: "Posseld2"
   Collect positive fraction from outlet port pos1.

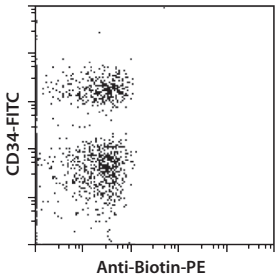
3. Example of a separation using the Diamond CD133 Isolation Kit

Lin^-CD133^+ cells were isolated from human PBMCs using the Diamond CD133 Isolation Kit, an LD Column, two MS Columns, a MidiMACS™ Separator, and a MiniMACS™ Separator. Cells were fluorescently stained with CD34-FITC, CD133/2 (293C3)-PE, and Anti-Biotin-PE and analyzed using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

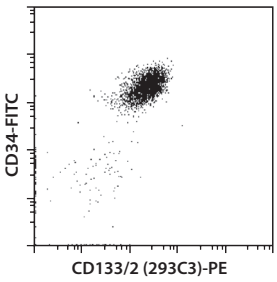
PBMCs before separation



Lin^- cell fraction



Isolated Lin^-CD133^+ cells



4. References

1. Miraglia, S. *et al.* (1997) A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. Blood 90: 5013–5021.
2. Bühring, H. J. *et al.* (1999) Expression of novel surface antigens on early hematopoietic cells. Ann NY Acad Sci: 872: 25–39.

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.

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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