Miltenyi Biotec

CD34 MicroBead Kit UltraPure
human

Order no. 130-100-453

Contents

1. Description

Components	**2 mL CD34 MicroBeads UltraPure, human:** MicroBeads conjugated to monoclonal mouse anti-human CD34 antibodies (isotype: mouse IgG1). **2 mL FcR Blocking Reagent, human:** Human IgG.
Capacity	For 2×10^9 total cells, up to 20 separations.
Product format	CD34 MicroBeads 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 $CD34^+$ cells are magnetically labeled with CD34 MicroBeads UltraPure. Then, the cell suspension is loaded onto a MACS® Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled $CD34^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD34^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD34^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

CD34 is a well-established marker of human hematopoietic stem and progenitor cells and additionally expressed on hemangioblasts, endothelial progenitor cells, and mature endothelial cells.
The CD34 MicroBead Kit UltraPure contains MicroBeads directly conjugated to CD34 antibodies for magnetic labeling of CD34-expressing cells from peripheral blood, cord blood, bone marrow, or apheresis harvest. Hematopoietic progenitors present at a frequency of 0.05–0.2% among peripheral blood mononuclear cells (PBMCs), 0.1–0.5% among cord blood mononuclear cells (MNCs) or 0.5–3% among bone marrow MNCs can be rapidly and efficiently enriched.
The CD34 MicroBead Kit UltraPure is suited for all routine $CD34^+$ cell isolations. In addition, its unique formulation provides particular advantages with debris-rich sample material.

1.3 Applications

- Positive selection or depletion of cells expressing human CD34 antigen.
- Isolation of $CD34^+$ cells from debris-rich samples.
- Isolation of hematopoietic progenitor cells, especially from low frequency samples.
- Isolation of endothelial progenitor cells (EPCs).
- *In vitro* differentiation studies.
- Isolation of $CD34^+$ progenitor cells from differentiated ES and iPS cell cultures.

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: $CD34^+$ cells can be enriched by using MS, LS, or XS Columns (positive selection). Cells that strongly express the CD34 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
Positive selection			
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.

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- (Optional) MC CD34 Stem Cell Cocktail (# 130-093-427) for flow cytometric analysis of separated cells.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD34-FITC (# 130-081-001), CD34-PE (# 130-081-002), CD34-APC (# 130-090-954), CD133 (293C3)-PE (# 130-090-853), CD133 (293C3)-PE (# 130-090-854), CD45-FITC (# 130-080-202), CD45-PE (# 130-080-201), or CD45-APC (# 130-091-230). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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 density gradient centrifugation, for example, using Ficoll-Paque™.

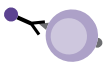
▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

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.

Preparation of cells from leukapheresis material

1. Filter apheresis harvest through 30 µm nylon mesh (Pre-Separation Filters, 30 µm # 130-041-407), in order to remove cell clumps.
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 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 for up to 10^8 total cells.
4. Add 100 µL of FcR Blocking Reagent for up to 10^8 total cells.
5. Add 100 µL of CD34 MicroBeads UltraPure for up to 10^8 total cells.
6. Mix well and incubate for 30 minutes in the refrigerator (2–8 °C).
7. (Optional) Add fluorochrome-conjugated CD34 antibody recognizing another epitope than QBEND/10 (e.g. clone AC136: CD34-PE, # 130-081-002) or fluorochrome-conjugated CD45 antibody (e.g. CD45-FITC, # 130-080-202), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
8. Wash cells by adding 5–10 mL of buffer for up to 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 $CD34^+$ cells. For details refer to the 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

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 flow-through 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.

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5. Remove column from the separator and place it on a suitable 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. (Optional) To increase the purity of CD34$^+$ cells, the eluted fraction can be enriched 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.

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 of CD34$^+$ cells from peripheral blood, bone marrow or leukapheresis: Posseld.

   Positive selection of CD34$^+$ cells from cord blood: Posseld2.
   Collect positive fraction in row C 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 pos2.
3. For a standard separation choose one of the following programs:

   Positive selection of CD34$^+$ cells from peripheral blood, bone marrow or leukapheresis: Posseld.

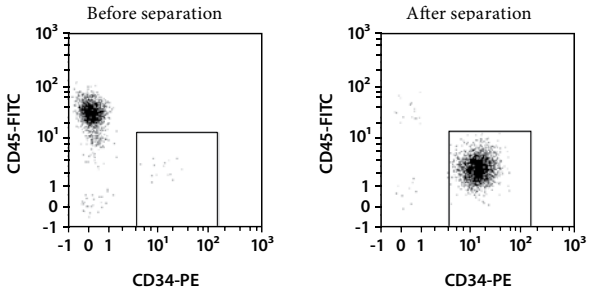
   Positive selection of CD34$^+$ cells from cord blood: Posseld2.
   Collect positive fraction from outlet port pos2.

2.4 (Optional) Evaluation of hematopoietic progenitor cell purity

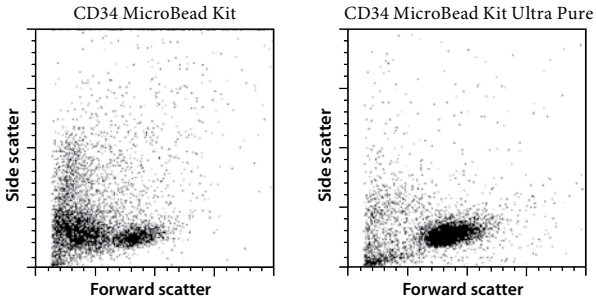
The purity of the isolated hematopoietic progenitor cells can be evaluated by flow cytometry or fluorescence microscopy. Analysis of CD34$^+$ cells can be accomplished by direct immunofluorescent staining using an antibody recognizing an epitope different from that recognized by the CD34 monoclonal antibody QBEND/10 (e.g. CD34-PE, clone: AC136, # 130-081-002).
For optimal discrimination of CD34$^+$ cells from other leukocytes, counterstain cells with an antibody against CD45 (e.g. CD45-FITC, # 130-080-202). CD34$^+$ cells express CD45 at a lower level as compared to lymphocytes.
Use the antibodies in appropriate concentrations as recommended by the manufacturers. Typically, staining for 5 minutes at 2–8 °C should be sufficient. After fluorescent staining, cells should be washed and resuspended in buffer.

3. Example of a separation using the CD34 MicroBead Kit UltraPure

Isolation of CD34$^+$ cells from a debris-rich PBMCs sample using the CD34 MicroBead Kit UltraPure, two MS Columns, and a MiniMACS™ Separator. Cells were stained with CD34-PE (# 130-081-002), CD45-FITC (# 130-090-202) and Propidium Iodide Solution (# 130-093-233). Cells were analyzed after gating on propidium iodide-, lymphoid cells



A comparison of the ungated forward scatter versus side scatter profile of the same sample after separation with the CD34 MicroBead Kit (# 130-046-702) or the CD34 MicroBead Kit UltraPure (# 130-100-453) demonstrates reduced debris carry-over with the CD34 MicroBead Kit UltraPure.



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

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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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

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