Miltenyi Biotec

Indirect CD34 MicroBead Kit
human

Order no. 130-046-701

Contents

1. Description

Components	**2 mL CD34-Hapten-Antibody**: monoclonal hapten-conjugated CD34 antibody (isotype: mouse IgG1). **2 mL Anti-Hapten MicroBeads:** MicroBeads conjugated to an anti-hapten antibody. **2 mL FcR Blocking Reagent:** Human IgG.
Capacity	For 2×10^9 total cells.
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

First, the $CD34^+$ cells are indirectly magnetically labeled using an hapten-conjugated primary monoclonal CD34 antibody and Anti-Hapten 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 $CD34^+$ 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

The Indirect CD34 MicroBead Kit is an indirect magnetic labeling system for the isolation of hematopoietic progenitor cells from peripheral blood, cord blood, bone marrow or apheresis harvest by positive selection of CD34 expressing cells. With the Indirect CD34 MicroBead Kit, hematopoietic progenitor cells, present at a frequency of about 0.05–0.2% in peripheral blood, 0.1–0.5% in cord blood and 0.5–3% in bone marrow, can be rapidly and efficiently enriched.

1.3 Applications

- Characterization of hematopoietic progenitor cells and their developmental pathways.
- Studies on stimulation of proliferation and maturation by cytokines.

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. 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 can also be performed by using the autoMACS or the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
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
autoMACS	2×10^8	4×10^9	autoMACS, autoMACS Pro

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

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, for example, CD34-FITC (# 130-081-001), CD34-PE (# 130-081-002), CD34-APC (# 130-090-954), or CD133/1 (AC133)-PE (# 130-090-853), CD133/1 (AC133)-APC (# 130-090-854), or CD133/1 (AC133)-Biotin (# 130-090-852) in combination with CD45-FITC (# 130-080-202), CD45-PE (# 130-080-201), or CD45-APC (# 130-091-230). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.
- (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.

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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™. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at www.miltenyibiotec.com/protocols.

▲ **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 General Protocols section of the respective separator user manual. The General Protocols are also available at www.miltenyibiotec.com/protocols.

▲ 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).

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. Wet 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 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 400 µL of buffer per 10^8 total cells.
4. Add 100 µL FcR Blocking Reagent per 10^8 total cells and mix well.
5. Add 100 µL CD34-Hapten-Antibody per 10^8 total cells.
6. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
7. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10^8 cells in 400 µL of buffer.
9. Add 100 µL of Anti-Hapten MicroBeads per 10^8 total cells.
10. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
11. (Optional) Add staining antibodies, e.g. 10 µL of CD34-FITC (# 130-081-001), CD34-PE (# 130-081-002), or CD34-APC (# 130-090-954) and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
12. Wash cells by adding 1–2 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
13. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
14. 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 see table in section 1.4.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see 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.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. 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
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™ Separator or the autoMACS™ Pro Separator

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

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

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▲ 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™ 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 from **peripheral blood, bone marrow, or leukapheresis:** "Posseld".

   Positive selection from **cord blood:** "Posseld2".

   Collect positive fraction from outlet port pos2.

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:** "Posseld".

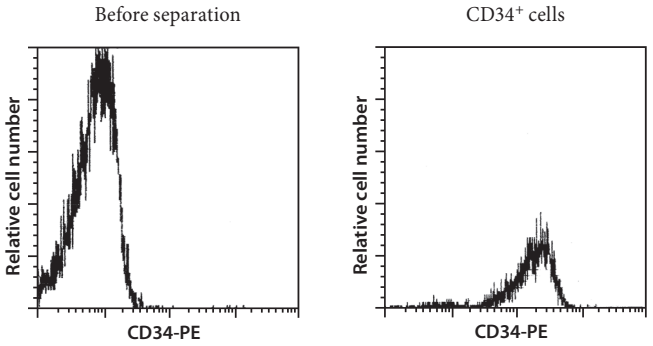
   Positive selection from **cord blood:** "Posseld2".

   Collect positive fraction in row C of the tube rack.

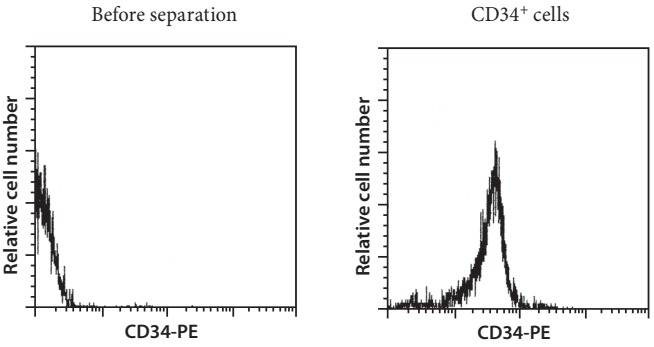
3. Example of a separation using the Indirect CD34 MicroBead Kit

CD34$^+$ cells were isolated from human PBMCs, cord blood, and bone marrow using the Indirect CD34 MicroBead Kit, an MS Column, and a MiniMACS™ Separator. Cells are fluorescently stained with CD34-PE (# 130-081-002). Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.

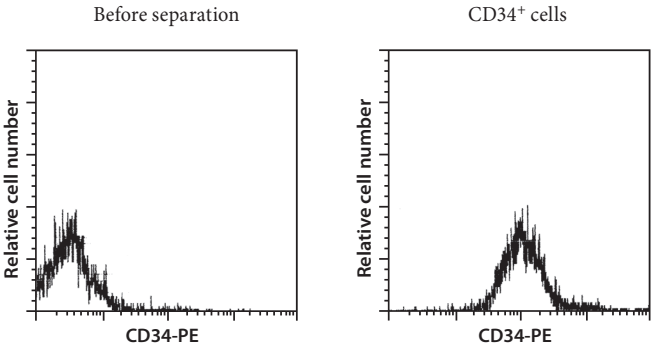
PBMCs



Cord blood



Bone marrow



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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