Miltenyi Biotec

CD34 MultiSort Kit
human

Order no. 130-056-701

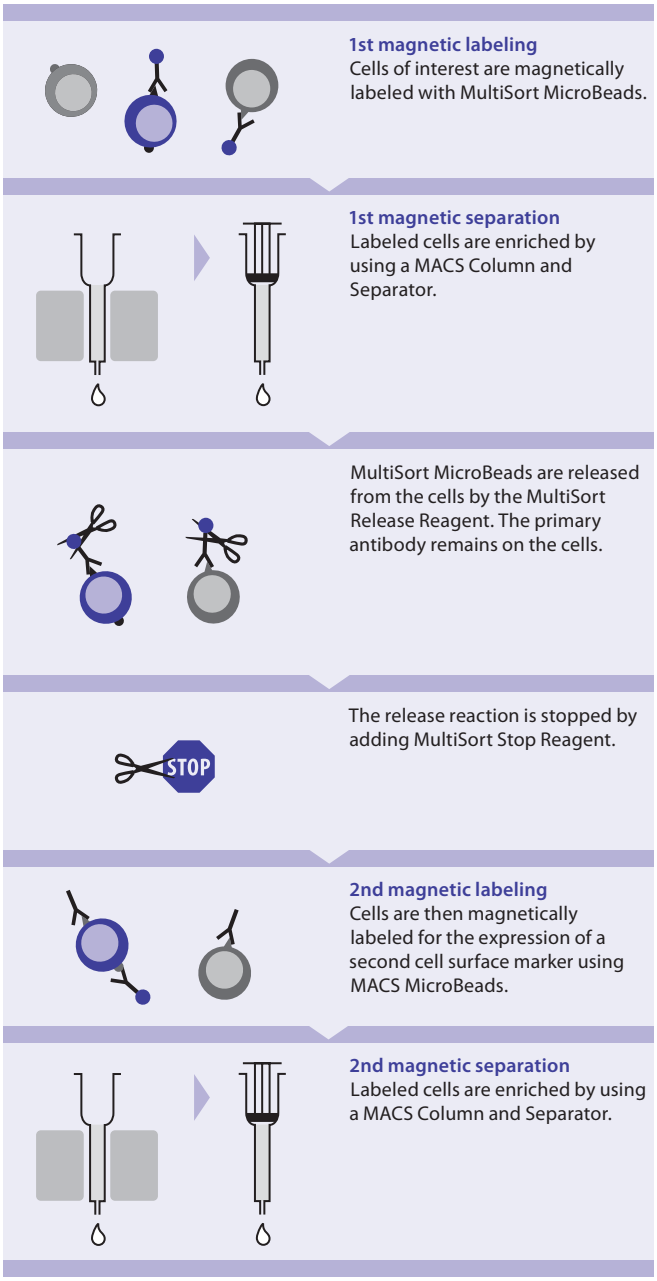
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1. Description

Components	**2 mL CD34 MultiSort MicroBeads, human:** MultiSort MicroBeads conjugated to monoclonal anti-human CD34 antibodies (isotype: mouse IgG1). **1 mL MultiSort Release Reagent** **2 mL MultiSort Stop Reagent** **2 mL FcR Blocking Reagent**
Capacity	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 the MACS® Separation using the CD34 MultiSort Kit



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1.2 Background information

CD34 MultiSort MicroBeads have been developed for the isolation of $CD34^+$ cell subpopulations. CD34 is expressed by hematopoietic progenitor cells and endothelial cells.
The CD34 MultiSort Kit is a direct magnetic labeling system that allows the sorting of cells according to multiple surface markers. Cells are magnetically labeled with CD34 MultiSort MicroBeads. Following the enrichment of the $CD34^+$ cells, using a MACS® Column and Separator, the magnetic particles are removed from the cells by using MultiSort Release Reagent. This allows a second magnetic labeling and separation of the cells for another surface marker of interest. Magnetic labeling for the second marker is achieved by using either direct or indirect magnetic labeling with MACS MicroBeads. For indirect labeling, Anti-FITC MicroBeads, Anti-PE MicroBeads, Anti-APC MicroBeads, or Anti-Biotin MicroBeads are recommended.

1.3 Applications

- The CD34 MultiSort Kit can be used to isolate specific subsets of $CD34^+$ cells. The CD34-selected cells can be further sorted according to their expression of, for example, HLA-DR, CD38, CD117, or CD133.

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 or LS Columns. Positive selection 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
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
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) MC CD34 Stem Cell Cocktail, human (# 130-093-427) for flow cytometric evaluation of separation results.
- (Optional) Fluorochrome-conjugated CD34 antibodies for flow cytometric analysis, e.g., CD34-PE (# 130-081-002). 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 bone marrow or cord blood cells, prepare a single-cell suspension using standard preparation methods.

For details refer to the protocols section 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 Preparation of cells from leukapheresis material

▲ 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, 30 µm, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

1. Determine cell number.
2. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^8 cells in 300 µL of buffer.

   ▲ **Note:** For lower cell numbers, use the same buffer volume.
4. Proceed to first magnetic labeling (2.3).



2.3 First 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, 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 to the cell suspension to inhibit unspecific or Fc-receptor binding of CD34 MultiSort MicroBeads to non-target cells.
5. Add 100 µL of CD34 MultiSort MicroBeads per 10^8 total cells.
6. Mix well and incubate for 30 minutes in the refrigerator (2–8 °C).
7. (Optional) Add staining antibodies recognizing an epitope other than QBEND/10, e.g., 10 µL of CD34-PE (# 130-081-002), and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **Note:** If the second parameter sorting is to be performed using indirect MicroBeads, we recommend to simultaneously label cells with the PE-conjugated primary antibody as well as the primary antibody conjugate to be used in the second parameter. Reduce the volume of the buffer accordingly to accomodate both antibodies in their optimal staining concentration. For the primary antibody conjugate to be used in the second parameter use a staining concentration according to the manufacturer´s recommendations.
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.
10. Proceed to first magnetic separation (2.3).



2.4 First magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of magnetically labeled 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

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.
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. To increase purity of $CD34^+$ 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.
8. Proceed to removal of MultiSort MicroBeads and second magnetic labeling and separation (2.5).

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.
4. Proceed to removal of MultiSort MicroBeads and second magnetic labeling and separation (2.5).

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.
4. Proceed to removal of MultiSort MicroBeads and second magnetic labeling and separation (2.5).

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2.5 Removal of MultiSort MicroBeads and second magnetic labeling and separation

▲ 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.

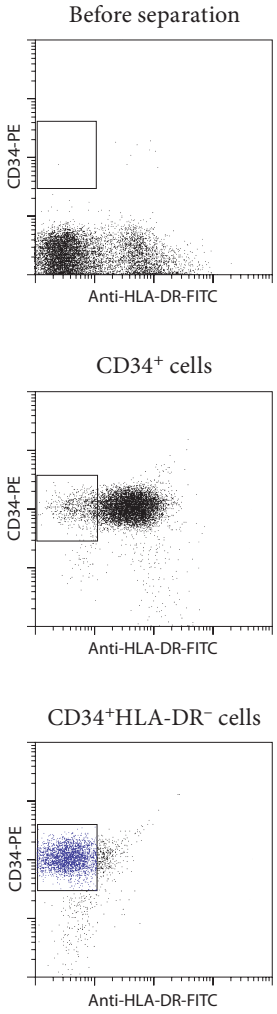
1. Remove a sample for analysis by flow cytometry and proceed with the remaining magnetically labeled fraction.
2. Add 20 µL of MultiSort Release Reagent per 1 mL of cell suspension.
3. Mix well and incubate for 10 minutes in the refrigerator in the dark (2–8 °C).
4. (Optional) To remove any residual magnetically labeled cells, repeat the magnetic separation procedure as described in 2.4. Separate cells over a new column of the same type (MS or LS Column) or use the same autoMACS or autoMACS Pro program to be used in the second parameter separation. Collect magnetic (unreleased) and non-magnetic (released) cell fractions to determine the efficiency of the release reaction.

   ▲ **Note:** This step is extremely important if the target cells of the second parameter separation are present in a low concentration after selection for CD34 expression (<10% target cells in the positive fraction after the first separation).
5. Wash cells from the released fraction carefully by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend cells in buffer at a final concentration of 10^7 total cells per 50 µL of buffer.
7. Add 30 µL of MultiSort Stop Reagent per 10^7 total cells and mix well.
8. Add the recommended amount of direct or indirect MACS MicroBeads to magnetically label the cells for the second marker. For details refer to the respective MACS MicroBead data sheet. Adjust to 100 µL total volume by adding buffer.

   ▲ **Note:** The CD34 antibody is of mouse IgG1 isotype. Thus, Anti-Mouse IgG MicroBeads or Anti-Mouse IgG1 MicroBeads cannot be used for second parameter sorting. When using other Anti-Immunoglobulin MicroBeads for the second parameter sorting, any reactivity with the isotype of the primary antibody of the first parameter sorting must be avoided.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
10. Proceed to magnetic separation. For details refer to the respective MACS MicroBeads data sheet.

3. Example of a separation using the CD34 MultiSort Kit

$CD34^+HLA\text{-}DR^-$ cells were separated from human PBMCs using the CD34 MultiSort Kit, two MS Columns, and a MiniMACS™ Separator for the first positive selection, followed by Anti-HLA-DR-FITC, Anti-FITC MicroBeads, two MS Columns, and a MiniMACS Separator in the second positive selection step. Cells were fluorescently stained with CD34-PE (# 130-081-002) and Anti-HLA-DR-FITC and analyzed by flow cytometry. Cell debris and dead cells are excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. Reference

Vodyanik , M.A. *et al.* (2006) Leukosialin (CD43) defines hematopoietic progenitors in human embryonic stem cell differentiation cultures. Blood 108: 2095–2105.

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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