Miltenyi Biotec

CD34$^+$CD38$^-$ Cell Isolation Kit
human

Order no. 130-114-822

Contents

Warnings

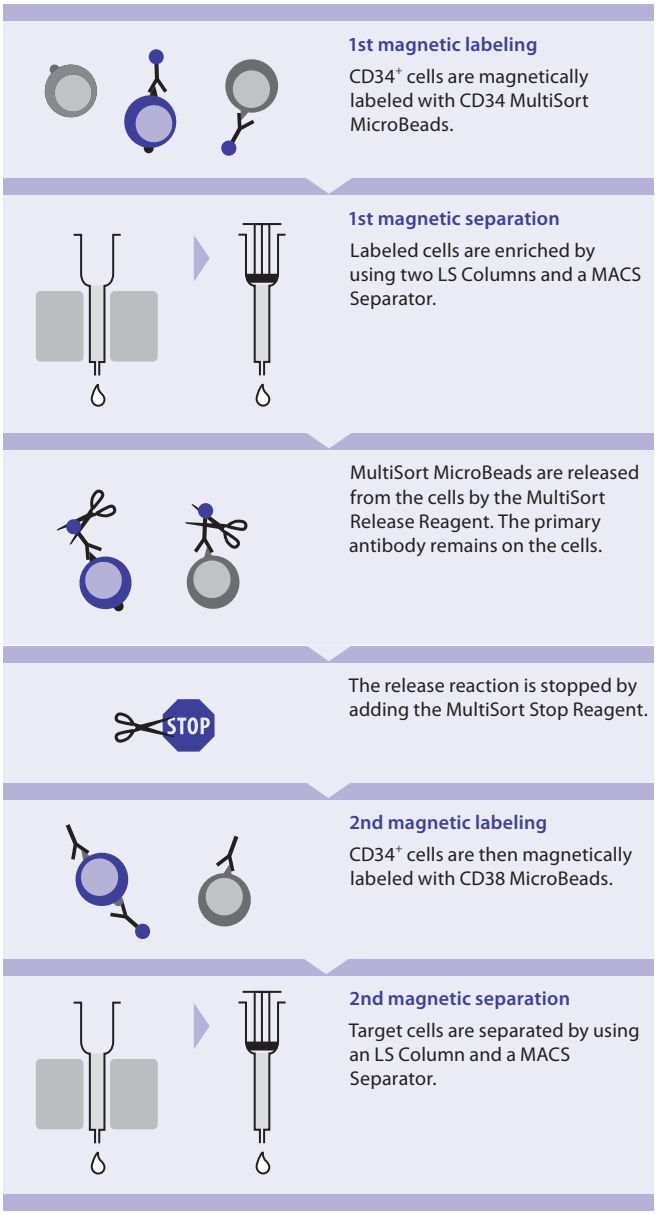
Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components	**2 mL CD34 MultiSort MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD34 antibodies (isotype: mouse IgG1). **1 mL CD38 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD38 antibodies (isotype: mouse IgG2a). **2× 1 mL MultiSort Release Reagent** **2 mL MultiSort Stop Reagent** **2 mL FcR Blocking Reagent, human**
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



1.2 Background information

The CD34$^+$CD38$^-$ Cell Isolation Kit has been developed for the isolation of the more primitive CD38$^-$ subpopulation of CD34$^+$ cells. 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. CD38 is absent on most primitive CD34$^+$ stem cells and upregulated on CD34$^+$ cells upon lymphocyte commitment.

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1.3 Applications

- Isolation of a primitive $CD38^-$ subset of $CD34^+$ cells from cord blood, bone marrow, or apheresis harvest for further phenotypical or functional characterization.

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.
  Always use freshly prepared buffer. Do **not use** autoMACS Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.
  ▲ **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: First magnetic separation can be performed on two LS Columns. The second magnetic separation can be performed on an LS Column.

Column	Max. number of labeled cells	Max. number of total cells	Separator
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II

  ▲ **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) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD45-VioBlue®, CD34-FITC, CD38-APC, Labeling Check Reagent-APC, CD133/2 (293C3)-PE, or CD45RA-APC-Vio® 770. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
  ▲ **Note:** For reliable detection of $CD38^+$ cells after separation use Labeling Check Reagent.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) 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 cord blood or bone marrow, mononuclear cells (MNCs) 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.

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).

When working with leukapheresis material, filter apheresis harvest through a 30 µm nylon mesh (Pre-Separation Filters (30 µm), # 130-041-407) and wash cells once with buffer. Resuspend in a final volume of 300 µL of buffer for up to 10^8 cells.

2.2 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 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 per 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 MultiSort MicroBeads for up to 10^8 total cells.
6. Mix well and incubate for 30 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 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
9. Proceed to first magnetic separation (2.3).

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2.3 First magnetic separation

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

Magnetic separation with LS Columns

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

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. To increase purity of $CD34^+$ cells, the eluted fraction must be enriched over a second 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 (2.4).

2.4 Removal of MultiSort MicroBeads and second magnetic labeling

1. (Optional) 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 at room temperature.
4. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
5. Resuspend cells in 80 µL of buffer per 10^8 initial total cells determined before magnetic labeling with the CD34 MultiSort MicroBeads (2.2).
6. Add 80 µL of MultiSort Stop Reagent per 10^8 initial total cells and mix well.
7. Add 40 µL of the CD38 MicroBeads per 10^8 initial total cells.
8. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
9. Wash cells by adding 5–10 mL of buffer for up to 10^8 initial total cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
10. Resuspend cells in 1 mL of buffer per 10^8 initial total cells.

    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
11. (Optional) Remove a sample for analysis by flow cytometry and proceed with the remaining magnetically labeled fraction.
12. Proceed to second magnetic separation (2.5).

2.5 Second magnetic separation with LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the LS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled $CD34^+CD38^-$ target cells.
4. Wash column with 3×3 mL of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column. The eluted fraction contains $CD34^+CD38^+$ cells.

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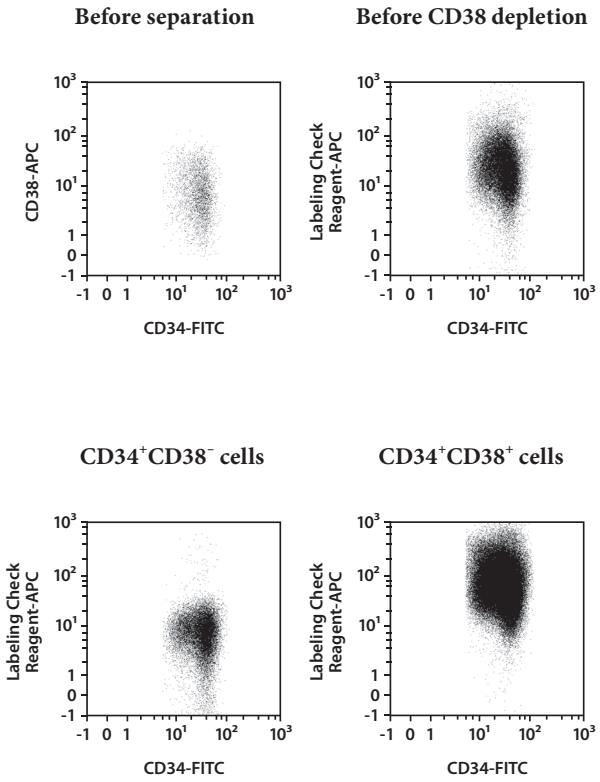
3. Example of a separation using the the CD34$^+$CD38$^-$ Cell Isolation Kit

CD34$^+$CD38$^-$ cells were isolated from human cord blood MNCs by using the CD34 MultiSort MicroBeads, two LS Columns, and a MidiMACS™ Separator for the first separation. In the second separation step, the CD38 MicroBeads, one LS Column, and a MidiMACS Separator have been used. Cells were fluorecently stained with CD34-FITC, CD38-APC, CD45-VioBlue®, Labeling Check Reagent-APC, and Propidium Iodide Solution and analyzed by flow cytometry using the MACSQuant® Analyzer.
Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



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.

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