Miltenyi Biotec

CD34 MicroBead Kit
human

2 mL	130-046-702
10 mL	130-046-703

Contents

1. Description

This product is for research use only.

Components	**2 mL CD34 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD34 antibodies (isotype: mouse IgG1). **2 mL FcR Blocking Reagent, human** or **10 mL CD34 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD34 antibodies (isotype: mouse IgG1). **10 mL FcR Blocking Reagent, human**
Capacity	**2 mL:** For 2×10^9 total cells, up to 20 separations. **10 mL:** For 10^{10} total cells, up to 100 separations.
Product format	CD34 MicroBeads, human are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at +2 to +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 CD34MicroBeads, human. 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. To increase the purity, the positively selected cell fraction containing the $CD34^+$ cells must be separated over a second column.

1.2 Background information

The CD34 antigen is a single chain transmembrane glycoprotein expressed on human hematopoietic progenitor cells, endothelial progenitor cells, vascular endothelial cells, embryonic fibroblasts, and some cells in fetal and adult nervous tissue.
The CD34 MicroBead Kit, human contains MicroBeads directly conjugated to CD34 antibodies for magnetic labeling of CD34-expressing cells from peripheral blood, cord blood, bone marrow, apheresis harvest, or differentiated ES and iPS cells. 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

- Positive selection or depletion of cells expressing human CD34 antigen
- Isolation of hematopoietic progenitor cells
- Isolation of endothelial progenitor cells (EPCs)
- Isolation of $CD34^+$ progenitor cells from differentiated ES and iPS cell cultures
- *In vitro* differentiation studies
- Studies on hematologic malignancies

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 to +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. Cells which 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 MultiMACS™ Cell24 Separator Plus or autoMACS Columns on the autoMACS NEO or autoMACS Pro Separators.

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Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, SuperMACS II
	10^8	10^9	MultiMACS Cell24 Separator Plus
XS	10^9	2×10^{10}	SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS NEO Separator, autoMACS Pro Separator
Multi-24 Column Block (per column)	10^8	10^9	MultiMACS Cell24 Separator Plus

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

▲ **Note:** If separating with LS or LD Columns and the MultiMACS Cell24 Separator Plus use the Single-Column Adapter. Refer to the user manual for details.

- (Optional) CD34 Stem Cell Analysis Cocktail, anti-human (# 130-093-427) for flow cytometric analysis of separated cells.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD34 Antibody, anti-human, PE and CD45, anti-human, FITC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (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 anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by using a MACS PBMC Isolation Kit or 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 data sheet or the protocols section at www.miltenyibiotec.com/protocols.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, it is recommended to use density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

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. *

▲ 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.

▲ 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).

▲ The recommended incubation temperature is +2 to +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 MicroBeads, human per 10^8 total cells.
6. Mix well and incubate for 30 minutes in the refrigerator (+2 to +8 °C).
7. (Optional) Add staining antibodies, e.g., CD34 Antibody, anti-human, PE (recognizing another epitope than QBEND/10) and CD45, anti-human, FITC, according to manufacturer's recommendation.
8. Wash cells by adding 5–10 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.

   ▲ **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 as soon as 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. (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 MultiMACS Cell24 Separator Plus

Refer to the the MultiMACS Cell Separator Plus user manual for instructions on how to use the MultiMACS Cell24 Separator Plus.

2.4 Magnetic labeling and separation using autoMACS Separators

▲ Refer to the user manual and the short instructions for instructions on how to use the autoMACS Separators.

▲ Buffers used for operating the autoMACS Separators should have a temperature of ≥ +10 °C.

▲ Place tubes in the following Chill Rack positions:
position A = sample, position B = unlabeled (negative) fraction, position C = labeled (positive) fraction.

2.4.1 Magnetic labeling and separation using the autoMACS NEO Separator

▲ The autoMACS NEO Separator enables stage loading to extend column capacity for selected reagents, minimizing the need to divide larger samples.

▲ For more information on selecting alternative separation programs, stage loading–compatible reagents, autolabeling-compatible reagents, and the minimal and maximal volumes for each reagent and Chill Rack, refer to www.miltenyibiotec.com/automacs-neo-sample-processing.

Magnetic separation after manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Place the Chill Rack on the MACS MiniSampler S.
4. Select the same Chill Rack in the **Experiment** tab. An experiment is created automatically. Tap to select sample positions.
5. Assign a reagent to each sample.
6. Manual labeling is set automatically if autolabeling is not supported or no reagent rack is selected. Alternatively, tap **Labeling** in the reagent placement dialog and select **Manual**.
7. Tap **Sample volume** in the **Sample process** pane and enter the sample volume. Tap the return key.
8 The separation program for highest target cell purity is selected by default. Refer to the **Sample process** pane for all available programs.
9. Place the sample(s) and empty tubes to the Chill Rack.
10. Tap **Run** to start the separation process.

Fully automated magnetic labeling and separation

1. Prepare and prime the instrument.
2. Place the Chill Rack and MACS Reagent Rack 8 on the MACS MiniSampler S.
3. Select the same Chill Rack and MACS Reagent Rack 8 in the **Experiment** tab. An experiment is created automatically.
4. Tap to select sample position(s).
5. To assign a reagent to each sample, tap **Scan reagent** and scan the reagent barcode. Alternatively, tap on a free position of the MACS Reagent Rack 8 for selection out of the reagent list.
6. Unscrew the lids from the reagent vials and place the vials onto the designated positions on the MACS Reagent Rack 8.
7. Tap **Place reagent(s) on reagent rack** button in the dialog box.
8. Automated labeling is set automatically if autolabeling is supported and a reagent rack is selected. Alternatively, tap **Labeling** in the reagent placement dialog and select **Auto**.
9. Tap **Sample volume** in the **Sample process** pane and enter the sample volume. Tap the return key.
10. Tap **Run** to start the separation process.

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2.4.2 Magnetic labeling and separation using the autoMACS Pro Separator

Magnetic separation after manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Apply tube containing the sample.
4. 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.
5. Tap **Run** to start the separation process.

Fully automated magnetic labeling and separation

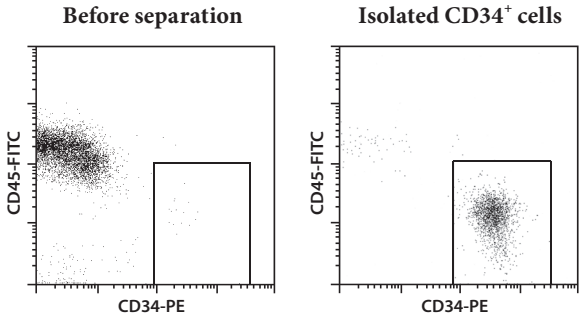
1. Switch on the instrument for automatic initialization.
2. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS Pro Separator. Place the reagent into the appropriate position on the reagent rack.
3. Place sample and collection tubes into the Chill Rack.
4. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu. The correct labeling, separation, and wash protocols will be selected automatically.
5. Enter sample volume into the **Volume** submenu. Press **Enter**.
6. Tap **Run** to start the separation process.

2.5 (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 Antibody, anti-human, PE (clone: AC136)).
For optimal discrimination of $CD34^+$ cells from other leukocytes, counterstain cells with an antibody against CD45 (e.g. CD45 Antibody, anti-human, FITC). $CD34^+$ 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 to +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, human

$CD34^+$ cells were isolated from PBMCs using the CD34 MicroBead Kit, human, two MS Columns, and a MiniMACS™ Separator. Cells were fluorescently stained with CD34-PE and CD45-FITC. 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 for local Miltenyi Biotec Technical Support contact information.

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