Miltenyi Biotec

CD34 MicroBead Kit
human

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

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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 **10 mL:** For 10^{10} total cells
Product format	All reagents 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 CD34 MicroBeads, 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.

- Table 1 provides an overview of MACS Columns and MACS Separators with links to the corresponding complete protocols. Use the link matching the MACS Column and MACS Separator used.

Table 1: MACS Columns and MACS Separators overview.

MACS Column (link to protocol)	Max. number of labeled cells	Max. number of total cells	MACS Separator
Columns for manual separation			
MS Column	10^7	2×10^8	MiniMACS, OctoMACS
LS Column	10^8	2×10^9	MidiMACS, QuadroMACS

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MACS Column (link to protocol)	Max. number of labeled cells	Max. number of total cells	MACS Separator
XLS Column	5×10^8	10^{10}	QuadroMACS XL
Columns for instruments			
LS Column	10^8	10^9	MultiMACS Cell24 Separator Plus
autoMACS Column	2×10^8	4×10^9	autoMACS NEO
autoMACS Column	2×10^8	4×10^9	autoMACS Pro Separation program: Positive selection from peripheral blood, bone marrow, or leukapheresis: Posseld Positive selection from cord blood: Posseld2
Multi-24 Column Block	10^8 per column 2.4×10^9 per block	10^9 per column 2.4×10^{10} per block	MultiMACS Cell24 Separator Plus

▲ **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.

▲ **Note:** Cells which strongly express the CD34 antigen can also be depleted using MS, LS, or XLS Columns.

▲ **Note:** For autolabeling compatibilty for the autoMACS NEO Seperator, please see here.

- (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

▲ Complete labeling and separation protocols are available online. Use the link provided in table 1, based on the used MACS Column and MACS Separator.

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, prepare a single-cell suspension using the gentleMACS® Dissociator.

For details refer to www.miltenyibiotec.com/gentlemacs.

▲ 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 (Optional) Evaluation of hematopoietic progenitor cell purity

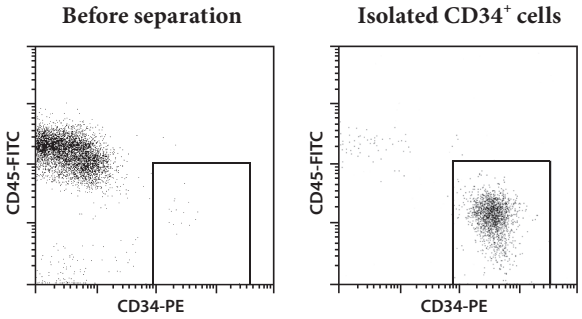
The purity of the isolated hematopoietic progenitor cells can be evaluated by flow cytometry or fluorescence microscopy. Analysis of $CD34^+$ cells can be accomplished by direct immunofluorescent staining using an antibody recognizing an epitope different from that recognized by the CD34 monoclonal antibody 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.

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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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