Miltenyi Biotec

CD34 MicroBead Kit UltraPure
human

Order no. 130-100-453

Contents

1. Description
   - 1.1 Principle of the MACS® Separation
   - 1.2 Background information
   - 1.3 Applications
   - 1.4 Reagent and instrument requirements
2. Protocol
   - 2.1 Sample preparation
   - 2.2 (Optional) Evaluation of hematopoietic progenitor cell purity
3. Example of a separation using the CD34 MicroBead Kit UltraPure, human

1. Description

This product is for research use only.

Components	**2 mL CD34 MicroBeads UltraPure, human:** MicroBeads conjugated to monoclonal anti-human CD34 antibodies (isotype: mouse IgG1). **2 mL FcR Blocking Reagent, human:** Human IgG.
Capacity	For 2×10^9 total cells
Product format	CD34 MicroBeads 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 UltraPure, 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.

1.2 Background information

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.

The CD34 MicroBead Kit UltraPure, human contains MicroBeads directly conjugated to CD34 antibodies for magnetic labeling of CD34-expressing cells from peripheral blood, cord blood, bone marrow, or apheresis harvest. Hematopoietic progenitors present at a frequency of 0.05–0.2% among peripheral blood mononuclear cells (PBMCs), 0.1–0.5% among cord blood mononuclear cells (MNCs) or 0.5–3% among bone marrow MNCs can be rapidly and efficiently enriched.
The CD34 MicroBead Kit UltraPure, human is suited for all routine $CD34^+$ cell isolations. In addition, its unique formulation provides particular advantages with debris-rich sample material.

1.3 Applications

- Positive selection or depletion of cells expressing human CD34 antigen
- Isolation of $CD34^+$ cells from debris-rich samples
- Isolation of hematopoietic progenitor cells, especially from low frequency samples
- Isolation of endothelial progenitor cells (EPCs)
- *In vitro* differentiation studies
- Isolation of $CD34^+$ progenitor cells from differentiated ES and iPS cell cultures

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

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-004-115.05

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, CD133/2 Antibody, anti-human, CD45 Antibody, anti-human. 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

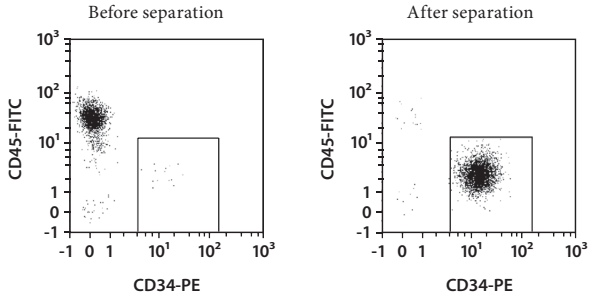
The purity of the isolated hematopoietic progenitor cells can be evaluated by flow cytometry or fluorescence microscopy. Analysis of $CD34^+$ cells can be accomplished by direct immunofluorescent staining using an antibody recognizing an epitope different from that recognized by the CD34 monoclonal antibody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

Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

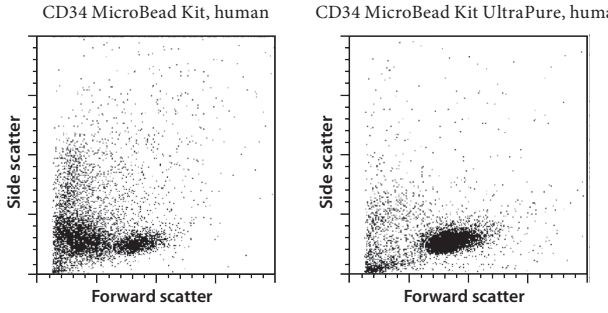
140-004-115.05

3. Example of a separation using CD34 MicroBead Kit UltraPure, human

CD34$^+$ cells were isolated from a debris-rich human PBMC sample using the CD34 MicroBead Kit UltraPure, human, two MS Columns, and a MiniMACS™ Separator. Cells were fluorescently stained with CD34-PE and CD45-FITC and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



A comparison of the ungated forward scatter versus side scatter profile of the same sample after separation with the CD34 MicroBead Kit, human (# 130-046-702) or the CD34 MicroBead Kit UltraPure, human (# 130-100-453) demonstrates reduced debris carry-over with the CD34 MicroBead Kit UltraPure, human.



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.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

autoMACS, gentleMACS, MACS, MACSQuant, MidiMACS, the Miltenyi Biotec logo, MiniMACS, MultiMACS, OctoMACS, and QuadroMACS are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Ficoll-Paque is a trademark of GE Healthcare companies.

Copyright © 2026 Miltenyi Biotec and/or its affiliates. All rights reserved.