Miltenyi Biotec

MACSprep™ Chimerism CD66b MicroBeads
human

Order no. 130-111-552

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 Preparation of whole blood
   - 2.2 Magnetic labeling
   - 2.3 Magnetic separation
3. Example of a separation using the MACSprep™ Chimerism CD66b MicroBeads

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 MACSprep™ Chimerism CD66b MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD66b antibodies (isotype: mouse IgG1). **MACSprep Chimerism MicroBeads have been developed for use with the autoMACS® Pro Separator, the MultiMACS™ Cell24 Separator Plus, or the Whole Blood Column Kit.**
Capacity	For 40 mL human whole blood.
Product format	MACSprep Chimerism CD66b MicroBeads 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

First, the CD66b$^+$ cells in a whole blood sample are magnetically labeled with MACSprep Chimerism CD66b MicroBeads. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD66b$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD66b$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD66b$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

MACSprep Chimerism CD66b MicroBeads have been developed for the positive selection of CD66b$^+$ cells directly from anticoagulated whole blood by using the autoMACS Pro Separator, the MultiMACS Cell24 Separator Plus, or the Whole Blood Column Kit. No sample preparation is required, including density gradient centrifugation or erythrocyte lysis, as well as no washing step after labeling. CD66b is a 95–100 kDa glycosylphosphatidylinositol (GPI)-linked protein, also known as CD67, CGM6, and NCA-95. It is expressed on neutrophils and eosinophils but not on basophils and lymphocytes.

1.3 Applications

- Isolation of CD66b$^+$ cells from whole blood. The purified CD66b$^+$ cells are well suited for further flow cytometric, functional, or molecular analysis.

1.4 Reagent and instrument requirements

- Separation 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). Alternatively, use autoMACS Running Buffer. 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.

- Automated separation:
  - autoMACS Pro Starting Kit (# 130-092-545)
  - autoMACS Columns (# 130-021-101)

  or

  - MultiMACS Cell24 Separator Plus (# 130-098-637)
  - Whole Blood Column Kit (# 130-093-545)
- Manual separation:
  - Whole Blood Column Kit (# 130-093-545), including Whole Blood Columns and Whole Blood Column Elution Buffer.
  - MidiMACS™ Separator (# 130-042-302) or QuadroMACS™ Separator (# 130-090-976).
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD15-FITC, CD66b-PE , and CD45-VioBlue®. 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.

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

140-005-146.02

- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)

2. Protocol

▲ MACSprep™ Chimerism MicroBeads have been developed for positive selection of target cells from anticoagulated whole blood samples, ranging in volume from **0.25 mL to 15 mL (autoMACS® Pro Separator or Whole Blood Column Kit)** or **0.25 mL to 10 mL (MultiMACS™ Cell24 Separator Plus)**.

2.1 Preparation of whole blood

▲ Anticoagulants such as EDTA, heparin-EDTA, citrate dextrose formula-A (ACD-A), or citrate phosphate dextrose (CPD) can be used. For subsequent molecular biology applications use EDTA as an anticoagulant. However, replacing EDTA can decrease purity and/or recovery.

▲ 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.

1. Collect up to 15 mL of venous blood in a collection tube containing an appropriate anticoagulant.
2. Proceed to magnetic labeling (2.2).



2.2 Magnetic labeling

▲ Cells can be labeled with MACSprep Chimerism MicroBeads using the autolabeling function of the autoMACS Pro Separator. For more information refer to section 2.3.1.

▲ 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 1 mL of anticoagulated whole blood. When working with larger volumes, scale up all reagent volumes and total volumes accordingly (e.g. for 2 mL, use twice the volume of all indicated reagent volumes and total volumes). When working with volumes below 1 mL (minimal volume 0.25 mL), scale down all reagent and total volumes accordingly.

▲ 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. Add 50 µL MACSprep Chimerism CD66b MicroBeads per 1 mL anticoagulated whole blood.
2. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
3. (Optional) Wash cells by adding 2–5 mL of separation buffer per 1 mL of whole blood and centrifuge at 445×g for 10 minutes at room temperature in a swinging bucket rotor without brake.
   Aspirate supernatant carefully. Do not disturb the cell pellet. Leave a residual volume of supernatant (approximately 1–2 mm in height) to avoid cell loss.
   Resuspend cell pellet by adding separation buffer to a total volume of 1 mL.
4. Proceed to magnetic separation (2.3).



2.3 Magnetic separation

2.3.1 Magnetic separation with the autoMACS® Pro Separator

▲ Refer to the user manual for instructions on how to use the autoMACS® Pro Separator.

▲ All buffer temperatures should be ≥10 °C.

▲ For appropriate resuspension volumes and cell concentrations, please visit www.automacspro.com/autolabeling.

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = negative fraction,

position C = positive fraction.

Fully automated cell labeling and separation

▲ When using the autolabeling feature of the autoMACS® Pro Separator manual magnetic labeling of whole blood samples (section 2.2) is not necessary.

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. Select **Run**.
7. (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×) (# 130-094-183).

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

140-005-146.02

Magnetic separation using manual labeling

▲ When working with the program "Posselwb" on the autoMACS® Pro Separator, samples will be automatically diluted 1:3 with autoMACS Running Buffer before sample uptake. For example, when processing 1 mL, the sample will be diluted with 2 mL of buffer. Make sure that the capacities of the sample tubes and fraction collection tubes are sufficient for the total volumes.

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample and collection tubes into the Chill Rack.
4. Choose program sequence **Posselwb/Rinse**. Collect positive fraction in row C of the tube rack.
5. Choose program **Sleep** after all samples have been processed. The autoMACS Pro Separator can be switched off now.
6. (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×) (# 130-094-183).

2.3.2 Magnetic separation with the MultiMACS™ Cell24 Separator Plus

▲ The MultiMACS™ Cell24 Separator Plus, including the MACS Elution Station, has to be used with the Single-Column Adapter and Whole Blood Columns for magnetic separation with MACSprep Chimerism MicroBeads.

▲ Buffer volumes are as followed:

Equilibration buffer: 3 mL
Wash buffer: 2×2 mL
Elution buffer: 4 mL

▲ If equilibration buffer and negative fraction should be collected in the same Deep Well Plate as waste, select the program **POSSEL_SCA** and follow the on-screen instructions of the MultiMACS Cell24 Separator Plus.

▲ If equilibration buffer and negative fraction should be collected in separate Deep Well Plates, select the program **POSSEL2_SCA** and follow the on-screen instructions of the MultiMACS Cell24 Separator Plus.

▲ (Optional) To increase the purity of the magnetically labeled fraction, the eluted fraction can be enriched over a new, freshly prepared MS Column (# 130-042-201, for up to 10^7 magnetically labeled cells) or LS Column (# 130-042-401, for up to 10^8 magnetically labeled cells).

▲ (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×) (# 130-094-183).

▲ For more detailed instructions on how to use the MultiMACS Cell24 Separator Plus, please refer to the user manual.

2.3.3 Magnetic separation with the Whole Blood Column Kit

1. Place Whole Blood Column in the magnetic field of a suitable MACS® Separator. For details refer to the Whole Blood Column Kit data sheet.
2. Prepare column by rinsing with 3 mL separation buffer.
3. Apply magnetically labeled cell suspension onto the prepared Whole Blood Column. Collect flow-through containing unlabeled cells.

   ▲ **Note:** The reservoir of the Whole Blood Column contains a maximum of 7.5 mL. Samples greater than 7.5 mL should be applied in aliquots to the column.
4. Wash Whole Blood Column with 2×2 mL separation buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

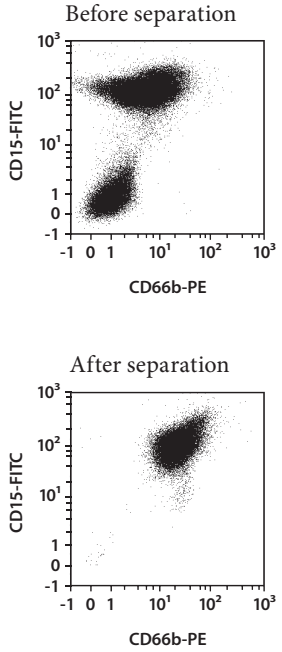
   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove Whole Blood Column from the separator and place it on a new collection tube.
6. Pipette 4 mL **Whole Blood Column Elution Buffer** onto the Whole Blood Column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. (Optional) To increase the purity of the magnetically labeled fraction, the eluted fraction can be enriched over a new, freshly prepared MS Column (# 130-042-201, for up to 10^7 magnetically labeled cells) or LS Column (# 130-042-401, for up to 10^8 magnetically labeled cells).
8. (Optional) Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×) (# 130-094-183).

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

140-005-146.02

3. Example of a separation using the MACSprep™ Chimerism CD66b MicroBeads

Separation of a whole blood sample using the MACSprep™ Chimerism CD66b MicroBeads and the MultiMACS™ Cell24 Separator Plus with the Single-Column Adapter and Whole Blood Columns without washing step after labeling. Cells were fluorescently stained with CD15-FITC, CD66b-PE as well as CD45-VioBlue® and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells were triggered via CD45-VioBlue, 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.

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, MACS, MACSprep, MACSQuant, MidiMACS, the Miltenyi Biotec logo, MultiMACS, QuadroMACS, and VioBlue are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.

Copyright © 2020 Miltenyi Biotec and/or its affiliates. All rights reserved.