Miltenyi Biotec

MACSprep™ Chimerism CD34 MicroBead Kit
human

Order no. 130-120-673

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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	**2×25 mL MACSprep™ Sedimentation Buffer** **1 mL MACSprep RBC Removal Cocktail, human** **2 mL MACSprep Chimerism CD34 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD34 antibodies (isotype: mouse IgG1). **4 mL FcR Blocking Reagent, human:** human IgG.
Capacity	For 10×10 mL whole blood.
Product format	MACSprep RBC Removal Cocktail, MACSprep Chimerism CD34 MicroBeads, and FcR Blocking Reagent are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store reagents protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial labels. Do not use after this date.

1.1 Principle of the MACSprep™ Chimerism CD34 MicroBead Kit

The MACSprep™ Chimerism CD34 MicroBead Kit, human has been developed for the fast isolation of $CD34^+$ cells from freshly drawn anticoagulated whole blood without density gradient centrifugation nor red blood cell (RBC) lysis. The isolation of $CD34^+$ cells is performed with only one labeling step without washing afterwards and in a two-step separation procedure. During the first isolation step RBCs are aggregated and sedimented. In the second step, $CD34^+$ cells are magnetically labeled with MACSprep Chimerism CD34 MicroBeads. This can be done either with the autolabeling program of the autoMACS Pro Separator or manually. Then, the cell suspension is separated either by the autolabeling program or manually by loading the suspension 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 MACSprep Chimerism CD34 MicroBead Kit, human contains a cocktail and a buffer to remove most of RBCs. In this RBC-reduced sample CD34-expressing cells are magnetically labeled with MicroBeads.

1.3 Applications

- Isolation of $CD34^+$ cells from whole blood. The purified $CD34^+$ cells are well suited for further flow cytometric, functional, or molecular analysis including PCR, FISH, and lineage-specific chimerism analysis after allogeneic stem cell transplantation.

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). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** EDTA as anticoagulant is recommended. Use of other anticoagulants, e.g., heparin or sodium citrate may decrease the yield and purity of target cells. 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.

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

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- MACSprep™ Chimerism MicroBeads have been developed for positive selection of target cells from anticoagulated whole blood samples, ranging in volume from 1 mL to 6 mL (autoMACS® Pro Separator) or 1 mL to 10 mL (MultiMACS™ Cell24 Separator Plus or LS Column).

  Automated separation:
  - autoMACS Pro Starter Kit (# 130-092-545)
  - autoMACS Columns (# 130-021-101)
- Semi-automated separation
  - MultiMACS Cell24 Separator Plus (# 130-098-637)
  - LS Columns (# 130-042-401)
- Manual separation:
  - LS Columns (# 130-042-401)
  - MidiMACS™ Separator (# 130-042-302) or QuadroMACS™ Separator (# 130-090-976)
- 5 mL polystyrene round-bottom test tube or 15 mL or 50 mL tubes
- MACSmix™ Tube Rotator (# 130-090-753)
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., MC CD34 Stem Cell Cocktail (# 130-093-427), CD34-FITC (clone AC136), CD133/2-Vio® Bright FITC (clone 293C3), CD45-VioGreen™ (clone REA747), or CD45-VioBlue®. 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) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

▲ The use of MACSmix™ Tube Rotator is recommended for all starting volumes to ensure a proper and consistent mixture.

▲ Adjust all reagents and materials to room temperature (19–25 °C) before use.

▲ Pipette gently to avoid foam formation.

▲ (Optional) For the evaluation of purity and recovery of the target cell fraction, take aliquots where indicated in the protocol.

2.2 Protocol overview

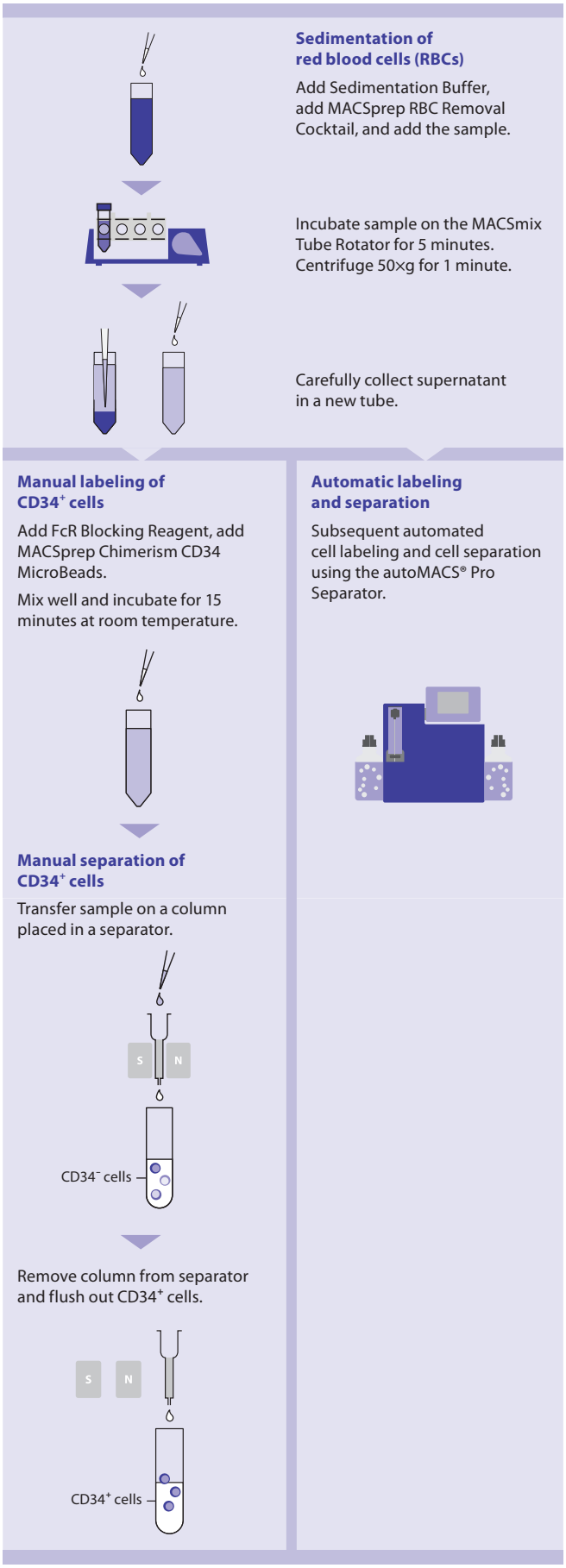


Figure 1: Isolation of CD34⁺ cells from whole blood.

2.3 Removal of red blood cells and magnetic labeling

▲ Cells can be labeled with MACS MicroBeads using the autolabeling function of the autoMACS Pro Separator after step 7. For more information refer to section 2.4.1.

▲ Reagent volumes for magnetic labeling given below are for 10 mL of whole blood. When working with smaller volumes, scale down the reagent volumes accordingly. Minimal sample volume of whole blood is 1 mL. For 1 mL sample volume use reagents as indicated in table 1 below. For appropriate tube size refer to table 2 below.

▲ When using samples from vacutainers with numeric scales: the sample volume can be determined by the numeric scale of the vacutainer.

Component	1 mL whole blood sample
MACSprep Sedimentation Buffer	400 µL
MACSprep RBC Removal Cocktail	10 µL
FcR Blocking Reagent	40 µL
MACSprep Chimerism CD34 MicroBeads	20 µL

Table 1: Component volumes.

Sample volume	Tube size
1–3 mL	5 mL
>3–10 mL	15 mL
>10–35 mL	50 mL

Table 2: Overview of using appropriate tube size.

1. (Optional) Take an aliquot of sample for cell counting and staining to determine target cell frequency in the starting material.
2. Pipette 4 mL of MACSprep Sedimentation Buffer into a 15 mL tube.
3. Add 100 µL of MACSprep RBC Removal Cocktail. Mix well.
4. Add 10 mL of anticoagulated blood to the suspension.
5. Close tube tightly and invert gently three times. Incubate sample for 5 minutes at room temperature using the MACSmix™ Tube Rotator on permanent run at the lowest speed setting.
6. Centrifuge at 50×g for 1 minute.

   ▲ **Note:** Different centrifugation time or speed may yield in decrease of $CD34^+$ cells.
7. Carefully collect the supernatant in a new 15 mL tube.

   ▲ **Note:** Alternatively to steps 8–10 the cells can be labeled using the autolabeling function of the autoMACS Pro Separator. For more information refer to section 2.4.1.
8. Add 400 µL FcR Blocking Reagent to the supernatant.
9. Add 200 µL of MACSprep Chimerism CD34 MicroBeads to the supernatant.
10. Mix well and incubate for 15 minutes at room temperature.
11. Proceed to magnetic separation (2.4).

2.4 Magnetic separation

2.4.1 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.

▲ Pre-cooling of Chill Racks is not required. Operating at room temperature is recommended for optimal results.

▲ 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 with max. sample volume of 6 mL (ChillRack 15) or max. 2 mL (ChillRack 5) manual magnetic labeling of samples (section 2.3, steps 8–10) is not necessary. For process volume and tube limitation, please refer to autoMACS manual.

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 reagents 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**.

   ▲ **Note:** Enter original sample volume as the volume varies after sedimentation of red blood cells The max. sample volume/tube is 6 mL.
6. Select **Run**.

Magnetic separation using manual labeling

1. Label the sample as described in section 2.3.
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 **PosselD2/Clean**. 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.

2.4.2 Semi-automated separation with the MultiMACS™ Cell24 Separator

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

2.4.3 Manual magnetic separation using LS Columns

1. Place LS 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 2 mL of buffer. Discard effluent and change collection tube.
3. Apply sample onto the column. Collect flow-through containing unlabeled cells.

   ▲ **Note:** The reservoir volume of one LS Column is 8 mL. If the sample volume is higher than 8 mL, apply sample in aliquots to the column. Alternatively,

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centrifuge sample at 300×g for 10 minutes and resuspend sample in 2 mL before applying to the column.

4. Wash column with 2×2 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.

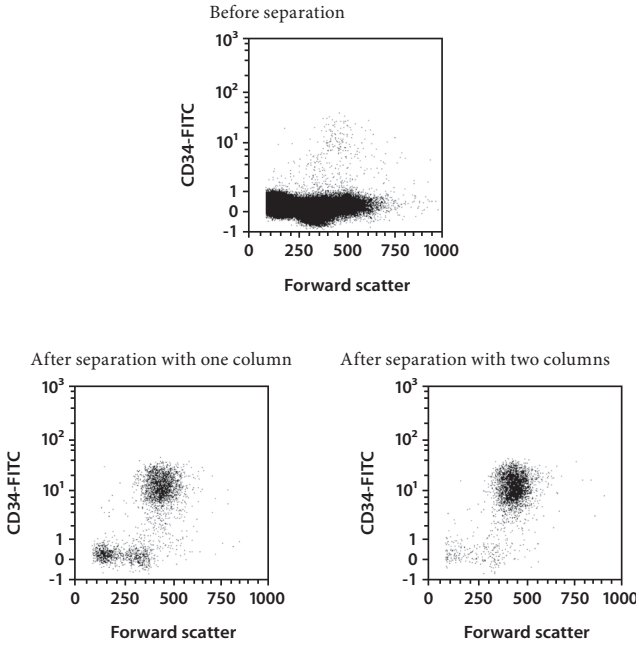
5. Remove column from the separator and place it on a suitable collection tube.

6. Pipette 2 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

7. (Optional) To increase the purity of $CD34^+$ cells, the eluted fraction can be enriched over a second LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

3. Example of a separation using the MACSprep™ Chimerism CD34 MicroBead Kit

Isolation of $CD34^+$ cells from a whole blood sample from a healthy donor using the MACSprep™ Chimerism CD34 MicroBead Kit, two LS Columns, a MACSmix™ Tube Rotator and a MidiMACS™ Separator. Cells were fluorescently stained with CD34-FITC and CD45-VioBlue® and analyzed by flow cytometry using the MACSQuant® Analyzer X. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. The purity of $CD34^+$ cells after separation is defined by percentage of $CD34^+$ cells (viable $CD45^{dim/+}CD34^+$ cells) amongst leukocytes (viable $CD45^{dim/+}$ cells). The $CD34^+$ cells content of the isolated fraction with one column is typically around 69.1 ± 5.8% (mean ± SD); 90.8 ± 3.4% (mean ± SD) with two columns.
The purity of $CD34^+$ cells varies due to the variation of human samples and experimental set-up.



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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