Miltenyi Biotec

MACSprep™ Chimerism CD3 MicroBeads
human

Order no. 130-111-551

Contents

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 CD3 MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD3 antibodies (isotype: mouse IgG2a). **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 CD3 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 $CD3^+$ cells in a whole blood sample are magnetically labeled with MACSprep Chimerism CD3 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 $CD3^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of $CD3^+$ cells. After removing the column from the magnetic field, the magnetically retained $CD3^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

MACSprep Chimerism CD3 MicroBeads have been developed for the positive selection of $CD3^+$ 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. MACSprep Chimerism CD3 MicroBeads recognize the CD3 antigen which is associated with the T cell receptor (TCR) heterodimer. CD3 is expressed on all T cells, constituting 15–20% of human peripheral blood leukocytes.

1.3 Applications

- Isolation of $CD3^+$ cells from whole blood. The purified $CD3^+$ cells are well suited for further flow cytometric, functional, or molecular analysis including lineage-specific chimerism analysis after allogeneic stem cell transplantation[1,2].

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., CD3-PE, CD14-APC, and CD45-VioBlue® or the MC CD3 Pan T Cell Cocktail, human. For more information about antibodies refer to

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www.miltenyibiotec.com/antibodies.

- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (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 separation. 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 CD3 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).

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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).

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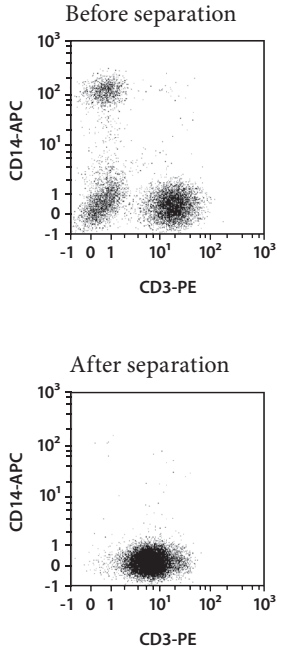
3. Example of a separation using the MACSprep™ Chimerism CD3 MicroBeads

Separation of a whole blood sample using the MACSprep™ Chimerism CD3 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 CD3-PE, CD14-APC, 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.



4. References

1. Köhl, U. *et al.* (2003) Quantitative analysis of chimerism after allogeneic stem cell transplantation by PCR amplification of microsatellite markers and capillary electrophoresis with fluorescence detection: the Frankfurt experience. Leukemia 17: 232–236.
2. Adams, S. *et al.* (2004) Cell lineage-specific chimerism in post-hematopoietic stem celltransplant patients. MACS&more 8/2: 16–17.

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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