Miltenyi Biotec

MACSprep™ CD19 CAR MicroBead Kit
human

Order no. 130-127-866

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	**2×25 mL MACSprep™ Sedimentation Buffer** **1 mL MACSprep RBC Removal Cocktail, human** **1 mL FcR Blocking Reagent, human:** human IgG. **100 µL CD19 CAR Detection Reagent, human, Biotin** **2 mL Anti-Biotin MicroBeads UltraPure**
Capacity	For 100 mL whole blood.
Product format	MACSprep RBC Removal Cocktail, FcR Blocking Reagent, CD19 CAR Detection Reagent, human, Biotin and Anti-Biotin MicroBeads 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 CD19 CAR MicroBead Kit

The MACSprep CD19 CAR MicroBead Kit, human has been developed for the fast isolation of CD19 CAR$^+$ cells from freshly drawn anticoagulated whole blood without density gradient centrifugation nor red blood cell (RBC) lysis. During the first isolation step RBCs are aggregated and sedimented. In the second step, CD19 CAR$^+$ cells are labeled with CD19 CAR Detection Reagent, human, Biotin.
After one washing step the CD19 CAR Detection Reagent-bound cells are magnetically labeled with Anti-Biotin MicroBeads. Then, the cell suspension is separated by loading it onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD19 CAR$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD19 CAR$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD19 CAR$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the target cells can be separated over a second column.

1.2 Background information

The MACSprep CD19 CAR MicroBead Kit, human has been developed for the separation of cells that are engineered to express CD19-specific chimeric antigen receptor (CAR) on the cell surface. The kit contains a cocktail and a buffer to remove most of RBCs. In this RBC-reduced blood sample the engineered CD19 CAR$^+$ T cells can be detected via the CD19 CAR Detection Reagent, human, Biotin and can be enriched by magnetic labeling using Anti-Biotin MicroBeads.

1.3 Applications

- Isolation of CD19 CAR$^+$ cells from whole blood. The purified CD19 CAR$^+$ cells are well suited for further flow cytometric, functional, or molecular analysis.

1.4 Reagent and instrument requirements

- PEB 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.

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- 15 mL polystyrene round-bottom test tubes
- MidiMACS™ Separator (# 130-042-302) or QuadroMACS™ Separator (# 130-090-976)
- LS Columns (# 130-042-401)
- Tube rotor, e.g. MACSmix™ Tube Rotator (# 130-090-753)
- (Optional) MS Columns (# 130-042-201) and MiniMACS™ Separator (#130-042-102)
- (Optional) CD3 Antibody, anti-human, FITC, REAfinity™ (clone REA613), CD45 Antibody, anti-human, VioBlue®, REAfinity (clone REA747), Biotin Antibody, PE, REAfinity (clone REA746)
- (Optional) 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.

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

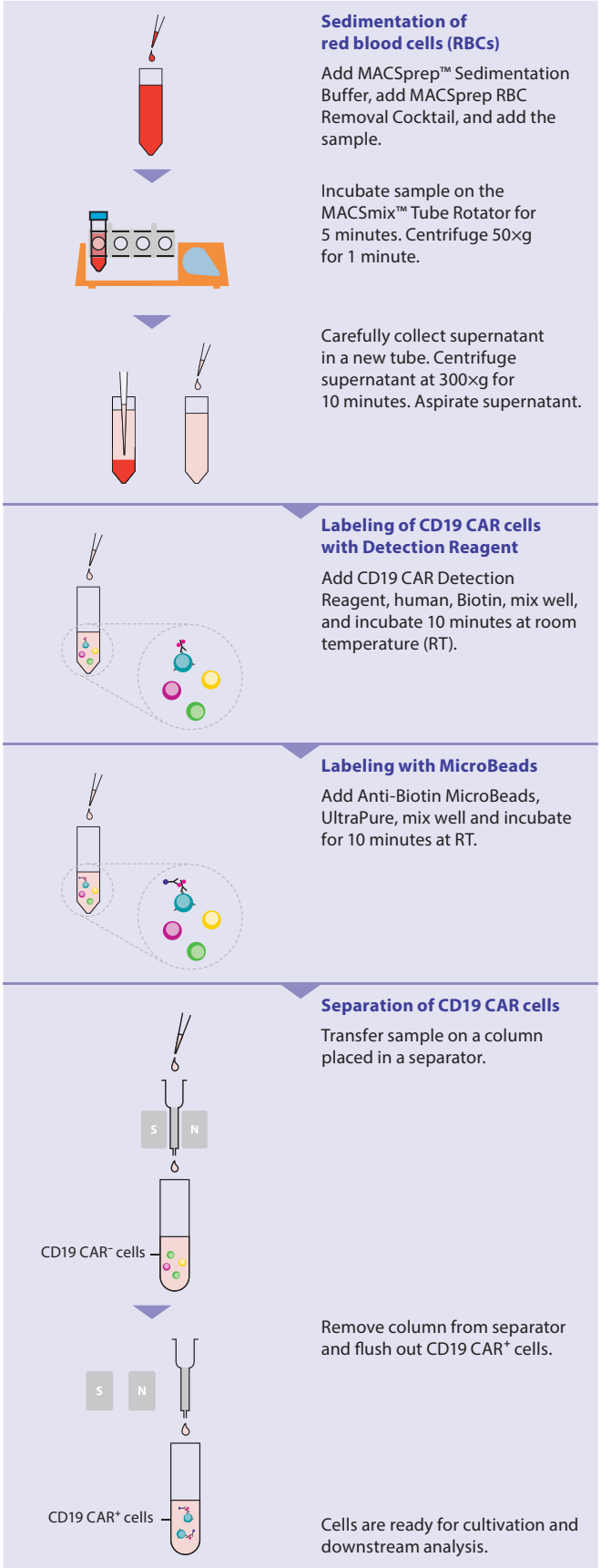

Figure 1: Isolation of CD19 CAR⁺ cells from whole blood.

2.3 Removal of red blood cells and magnetic labeling

▲ Reagent volumes for magnetic labeling given in table 1 are for 10 mL of whole blood. When working with smaller volumes, scale down the reagent volumes accordingly. Recommended minimal sample volume of whole blood is 5 mL.

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

Component	10 mL whole blood sample
MACSprep Sedimentation Buffer	4 mL
MACSprep RBC Removal Cocktail	100 µL
CD19 CAR Detection Reagent, human, Biotin	10 µL
FcR Blocking Reagent	100 µL
Anti-Biotin MicroBeads UltraPure	200 µL

Table 1: Component volumes.

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 target cells.
7. Carefully collect the supernatant in a new 15 mL tube.
8. Centrifuge the supernatant at 300×g for 10 minutes. Aspirate supernatant.
9. Resuspend pellet in 100 µL of PEB buffer.
10. Add 10 µL CD19 CAR Detection Reagent, human, Biotin. Mix well.
11. Incubate for 10 minutes at room temperature (19–25 °C).
12. Add 300 µL of PEB buffer.
13. Add 100 µL of FcR Blocking Reagent. Mix well.
14. Incubate for 10 minutes at room temperature (19–25 °C).
15. Add 7.5 mL of PEB buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant.
16. Resuspend cells in 1.8 mL of PEB buffer.
17. Add 200 µL of Anti-Biotin MicroBeads UltraPure. Mix well.
18. Incubate for 10 minutes at room temperature (19–25 °C).
19. (Optional) Take an aliquot of sample for cell counting and staining to determine target cell frequency in the material before separation.
20. Proceed to magnetic separation (2.3).

2.4 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 3 mL of PEB 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.
4. Wash column with 3×3 mL of PEB 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 PEB 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 the magnetically labeled fraction, the eluted fraction can be enriched over a new, freshly prepared MS Column (for up to 10^7 magnetically labeled cells) or LS Column (for up to 10^8 magnetically labeled cells).
8. Cells can be used for further analysis.

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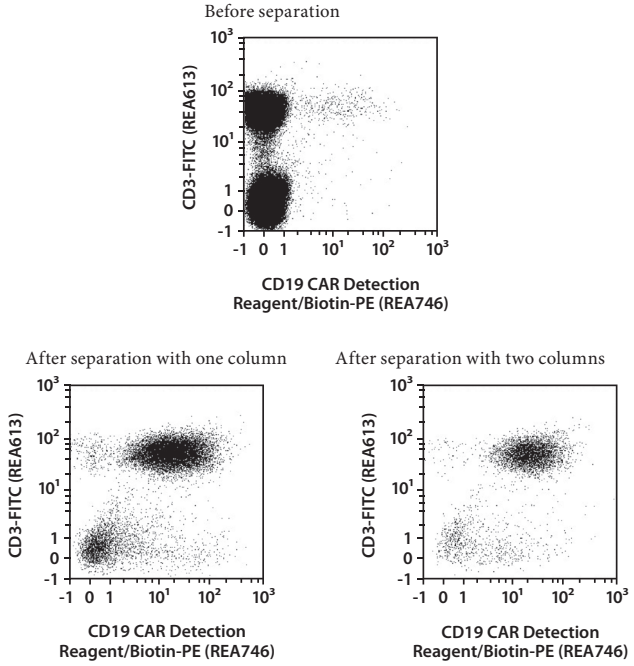
3. Example of a separation using the MACSprep CD19 CAR MicroBead Kit

CD19 CAR$^+$ T cells were spiked into a whole blood sample from a healthy donor and isolated using the MACSprep CD19 CAR MicroBead Kit, one LS Column, one MS Column, a MACSmix Tube Rotator, and a QuadroMACS Separator. Cells were fluorescently stained with CD19 CAR Detection Reagent, human, Biotin, Biotin Antibody-PE, CD45-VioBlue®, 7-AAD Staining Solution, CD3-FITC, and analyzed by flow cytometry using the MACSQuant® Analyzer 10. Cell debris and dead cells were excluded from the analysis based on scatter signals and 7-AAD fluorescence. The purity of CD19 CAR$^+$ T cells after separation is defined by the percentage of CD19 CAR$^+$ T cells (viable CD45$^{dim/+}$, 7-AAD$^-$ cells). The purity of CD19 CAR$^+$ T cells varies due to the variation of human samples, experimental set-up, and starting frequency of CD19 CAR$^+$ T cells.



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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