Miltenyi Biotec

MACSprep™ PBMC Isolation Kit
human

Order no. 130-115-169

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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 mL MACSprep™ PBMC Isolation Cocktail, human:** cocktail of biotin-conjugated monoclonal antibodies against CD15, CD61, CD66b, and CD235a (Glycophorin A). **4 mL MACSprep Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1). **2×25 mL PBMC Isolation Buffer** **10 LS Columns:** columns and plungers, sterile packed.
Capacity	For 10×8 mL whole blood.
Product format	MACSprep PBMC Isolation Cocktail and MACSprep 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. Store LS Columns dry at 10–35 °C and protected from light. The expiration date is indicated on the vial or box labels. Do not use after this date.

1.1 Principle of the MACSprep™ PBMC Isolation Kit

The MACSprep™ PBMC Isolation Kit, human has been developed for the fast isolation of human peripheral blood mononuclear cells (PBMCs) from 1–8 mL of freshly drawn anticoagulated whole blood without density gradient centrifugation. The isolation of PBMCs is performed with only one labeling step and in a two-step separation procedure. During the first isolation step erythrocytes are aggregated and sedimented. In a second step, PBMCs are isolated by depletion of non-PBMCs (e.g., neutrophils, eosinophils, platelets, and residual erythrocytes). Non-PBMCs are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies and MACSprep Anti-Biotin MicroBeads. The magnetically labeled non-PBMCs are depleted by retaining them within a MACS Column in the magnetic field of a MACS® Separator while the unlabeled PBMCs run through.

1.2 Background information

The MACSprep PBMC Isolation Kit, human is a magnetic labeling system for the isolation of untouched PBMCs from whole blood. Non-PBMCs cells, such as neutrophils, eosinophils, platelets, and erythrocytes are magnetically labeled by using a cocktail of antibodies against CD15, CD61, CD66b, and CD235a. Isolation of highly pure PBMCs is achieved by depletion of the magnetically labeled cells.

1.3 Applications

- Efficient, fast, and convenient isolation of untouched PBMCs from blood samples of 1–8 mL
- PBMC co-cultures, e.g., functional immune cell assays
- Studies on cytokine expression of immune cells upon restimulation
- Studies on signal transduction during activation of immune cells
- Immune monitoring of antigen-specific T cells

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 (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** In case of subsequent cell culture, replace BSA by 0.5% human AB serum as BSA could lead to non-specific stimulation.

  ▲ **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. This can be attenuated by adding EDTA to a final concentration of 1.25–2.5 mM to the blood sample. 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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- LS Columns: One LS Column can be used for the supernatant from 1–8 mL sedimented whole blood.

  ▲ **Note:** Ten LS Columns are provided with the kit. Further LS Columns can be ordered separately (# 130-042-401).

Column	Max. number of labeled cells	Max. number of total cells	Separator
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II,
	10^8	10^9	MultiMACS Cell24 Separator Plus
Multi-24 Column Block (per column)	10^8	10^9	MultiMACS Cell24 Separator Plus

  ▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

  ▲ **Note:** If separating with LS Columns and the MultiMACS Cell24 Separator Plus use the Single-Column Adapter. Refer to the user manual for details.

- MACS MultiStand (# 130-042-303)
- 15 mL conical centrifuge tubes
- (Optional) MACSQuant® Analyzer 10 (# 130-096-343)
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., 7-Color Immunophenotyping Kit (# 130-098-456). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD (# 130-111-568) for flow cytometric exclusion of dead cells.

2. Protocol

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

▲ Pipette gently to avoid foam formation.

2.1 Magnetic labeling and separation

▲ Reagent volumes for magnetic labeling given below are for 1–8 mL of whole blood. When working with smaller volumes, scale down the reagent volumes accordingly, e.g., per 1 mL whole blood sample use 500 µL PBMC Isolation Buffer, 25 µL MACSprep PBMC Isolation Cocktail, and 50 µL of MACSprep Anti-Biotin MicroBeads.

▲ For the PBMC isolation from 1–8 mL of whole blood use 15 mL conical centrifuge tubes.

1. Pipette 4 mL of PBMC Isolation Buffer into a 15 mL tube.
2. Add 200 µL of MACSprep PBMC Isolation Cocktail.
3. Add 400 µL of MACSprep Anti-Biotin MicroBeads and mix by vortexing.
4. Add 1–8 mL of anticoagulated whole blood to the suspension.
5. Close tube tightly and invert gently three times.
6. Place the tube in an upright position in, e.g., a tube rack and incubate for 3 minutes at room temperature.
7. Place tube in a suited centrifuge and centrifuge for 3 minutes at 50×g at room temperature for erythrocyte sedimentation.
8. Prepare LS Column by rinsing with 2 mL of buffer. Discard effluent and change collection tube. For details refer to the LS Column data sheet at www.miltenyibiotec.com/130-042-401.
9. After erythrocytes have sedimented, carefully collect the supernatant and apply instantly onto the prepared LS Column. Collect flow-through containing unlabeled target cells (PBMCs).

   ▲ **Note:** Leave a residual volume of supernatant (approximately 1–2 mm above erythrocyte pellet) to avoid unintended aspiration of erythrocytes.

10. Wash column with 1×3 mL of buffer. Collect unlabeled cells (PBMCs) that pass through and combine with the effluent from step 9.

    ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.

Magnetic separation with the MultiMACS™ Cell24 Separator

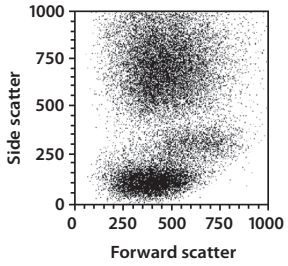
Refer to the the MultiMACS™ Cell Separator user manual for instructions on how to use the MultiMACS Cell24 Separator.

3. Example of a separation using the MACSprep™ PBMC Isolation Kit

Untouched PBMCs were isolated from human EDTA-anticoagulated whole blood using the MACSprep™ PBMC Isolation Kit, a MACSmix™ Tube Rotator, and an LS Column. The isolated cells were fluorescently stained with CD45-VioBlue®, CD3-FITC, CD4-VioGreen™, CD8-APC-Vio® 770, CD14-APC, CD16-PE, CD56-PE, CD20-PE-Vio770, and 7-AAD and analyzed by flow cytometry using the MACSQuant® Analyzer.
Cell debris, non-leukocytes, and dead cells were excluded from the analysis based on CD45 expression, scatter signals, and 7-AAD.

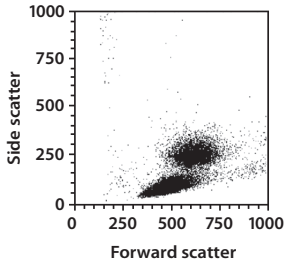
Before separation

Lysed whole blood gated on CD45$^+$ cells

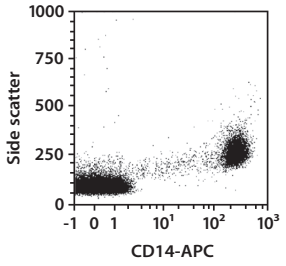


After separation

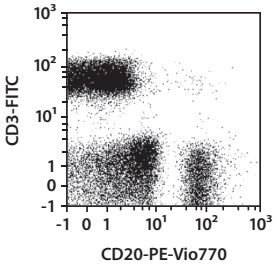
Gated on CD45$^+$ cells



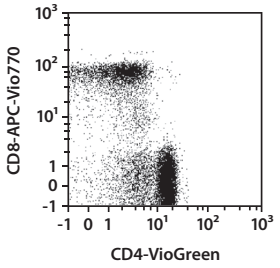
Gated on viable CD45$^+$ cells



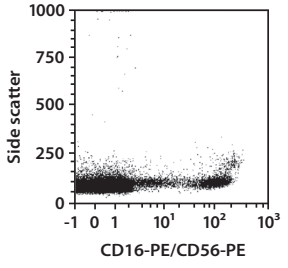
Gated on viable CD45$^+$ cells



Gated on viable CD45$^+$CD3$^+$ cells



Gated on viable CD45$^+$CD14$^-$ cells



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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