Miltenyi Biotec

StraightFrom™ Whole Blood Anti-ROR1 MicroBead Kit
human

Order no. 130-105-751

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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	**1 mL StraightFrom™ Whole Blood Anti-ROR1-PE, human:** monoclonal anti-ROR1 antibodies conjugated to PE (isotype: mouse IgG1). **2 mL Anti-PE MicroBeads:** MicroBeads conjugated to monoclonal anti-PE antibodies (isotype: mouse IgG1). **StraightFrom Whole Blood Kits 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	All components 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 ROR1$^+$ cells in a whole blood sample are indirectly magnetically labeled with Anti-ROR1-PE antibodies and Anti-PE 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 ROR1$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of ROR1$^+$ cells. After removing the column from the magnetic field, the magnetically retained ROR1$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

The StraightFrom Whole Blood Anti-ROR1 MicroBead Kit has been developed for the positive selection of ROR1$^+$ 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. ROR1 has characteristics of an oncofetal gene and was recently identified to be overexpressed at the gene and protein levels in chronic lymphocytic leukemia (CLL). ROR1 expression has also been detected in ovarian cancer, renal cancer, melanoma, and lung adenocarcinoma, suggesting a general role of ROR1 in cancer genesis and/or maintenance. Moreover, to a much lower level ROR1 expression has been found in undifferentiated embryonic stem cells, in adipose tissue, in early stage of B cell development, but not in major adult tissues. In blood samples with low frequencies of CLL B cells, e.g., in minimal residual disease, the enrichment of these cells via the StraightFrom Whole Blood Anti-ROR1 MicroBead Kit allows analysis and down stream processing of these cells.

1.3 Applications

- Isolation of ROR1$^+$ cells from whole blood. The purified ROR1$^+$ 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.

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- 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), including Whole Blood Columns and Whole Blood Column Elution Buffer.
- 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., CD45-VioBlue®, CD19-APC, and CD5-FITC. 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.
- (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

▲StraightFrom™ Whole Blood 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 whole blood in a collection tube containing an appropriate anticoagulant.
2. Proceed to magnetic labeling (2.2).



2.2 Magnetic labeling

▲ 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 containing a maximum of 10^7 of white blood cells (WBCs). When working with higher cell numbers, adjust with separation buffer to 10^7 WBCs/mL (e.g. for 5×10^7 WBCs add 4 mL of buffer). When working with fewer than 10^7 cells, use the same volumes as indicated.
When working with larger volumes than 1 mL, 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 0.5 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. Determine WBC count.
2. Adjust WBC count to max. 10^7 cells/mL.
3. Add 25 µL StraightFrom Whole Blood Anti-ROR1-PE per 1 mL anticoagulated whole blood.
4. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
5. Add 50 µL Anti PE MicroBeads.
6. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
7. Wash cells by adding 10 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 one volume of 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.

▲ 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. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. Choose program sequence **Posselwb/Rinse**. Collect positive fraction in row C of the tube rack.
4. Choose program **Sleep** after all samples have been processed. The autoMACS Pro Separator can be switched off now.

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 StraightFrom Whole Blood MicroBeads.

▲ Buffer volumes are as followed:

Equilibration buffer: 3 mL
Wash buffer: 2×2 mL
Whole Blood Column 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.

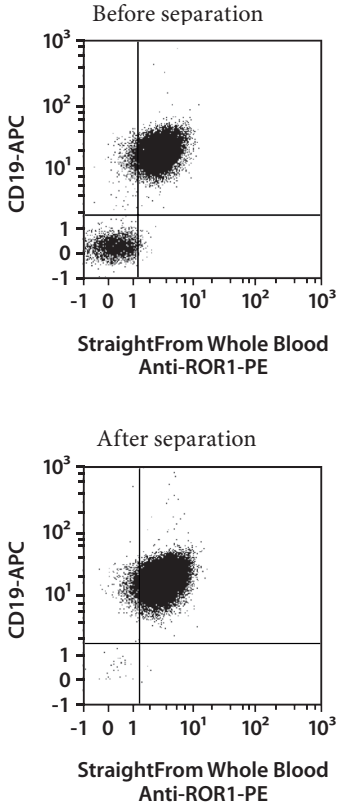
   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove Whole Blood Column from the separator and place it on a new collection tube.
6. Pipette 4 mL **Whole Blood Column Elution Buffer** onto the Whole Blood Column. Immediately flush out the magnetically 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 StraightFrom™ Whole Blood Anti-ROR1 MicroBead Kit

Separation of a B-CLL whole blood sample using the StraightFrom™ Whole Blood Anti-ROR1 MicroBead Kit and the autoMACS® Pro Separator. Cells were fluorescently stained with CD19-APC and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

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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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