Miltenyi Biotec

Anti-ROR1 MicroBead Kit
human

Order no. 130-103-929

Contents

1. Description
   - 1.1 Principle of the MACS® Separation
   - 1.2 Background information
   - 1.3 Applications
   - 1.4 Reagent and instrument requirements
2. Protocol
   - 2.1 Sample preparation
   - 2.2 Magnetic labeling
   - 2.3 Magnetic separation
3. Example of a separation using the Anti-ROR1 MicroBead Kit
4. References

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 Anti-ROR-1-PE, human:** monoclonal anti-ROR-1 antibody conjugated to PE (clone: 2A2, isotype: mouse IgG1). **2 mL Anti-PE MicroBeads:** MicroBeads conjugated to monoclonal anti-PE antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	All reagents 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 are indirectly magnetically labeled with Anti-ROR-1-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. To increase the purity, the positively selected cell fraction containing the ROR1$^+$ cells can be separated over a second column.

1.2 Background information

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 Anti-ROR1 MicroBead Kit allows analysis and down stream processing of these cells.

1.3 Applications

- Positive selection of cells expressing human ROR1 antigen from PBMCs of CLL patients.

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:** 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.

- MACS Columns and MACS Separators: ROR1$^+$ cells can be enriched by using MS, or LS Columns or depleted with the use of LD, CS, or D Columns. Positive selection can also be performed by using the autoMACS Pro or the autoMACS Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

  ▲ **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.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD19-APC (# 130-091-248) or CD5-FITC (# 130-096-574). For more information about antibodies refer to www.miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

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- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).



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 up to 10^7 total cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ 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, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ 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 cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 100 µL of buffer per 10^7 total cells.
4. Add 10 µL of Anti-ROR-1-PE per 10^7 cells.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
8. Add 20 µL of Anti-PE MicroBeads per 10^7 total cells.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
11. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).



2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of ROR1$^+$ cells. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.
   MS: 3×500 µL LS: 3×3 mL
   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL
7. (Optional) To increase the purity of ROR1$^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with the autoMACS Pro Separator or the autoMACS Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS Pro Separator or the autoMACS Separator.

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of ≥10 °C.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

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Magnetic separation with the autoMACS Pro Separator

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. For a standard separation choose one of the following programs:

   When frequency of ROR1$^+$ cells >5%: **Possels.**

   When frequency of ROR1$^+$ cells <5%: **Posselds.**

   Collect positive fraction in row C of the tube rack.

Magnetic separation with the autoMACS Separator

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 at the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. For a standard separation choose one of the following programs:

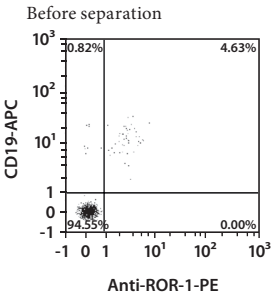
   When frequency of ROR1$^+$ cells >5%: **Possels.**

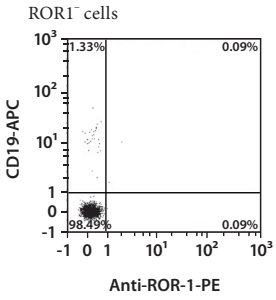
   When frequency of ROR1$^+$ cells <5%: **Posselds.**

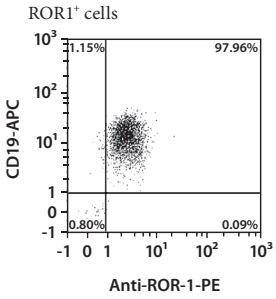
   Collect positive fraction from outlet port pos2.

3. Example of a separation using the Anti-ROR1 MicroBead Kit

ROR1$^+$ cells were isolated from PBMCs (that were previously prepared from B-CLL blood samples) using the Anti-ROR1 MicroBead Kit, and an autoMACS Pro Separator (program "Posselds"). Cells were fluorescently stained with CD19-APC (# 130-091-248) 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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