Miltenyi Biotec

CD90.1 MicroBeads
mouse and rat

Order no. 130-094-523

Contents

1. Description

Components	2 mL CD90.1 MicroBeads, mouse and rat: MicroBeads conjugated to monoclonal mouse anti-mouse/rat CD90.1 antibodies (isotype: mouse IgG2a).
Capacity	For 2×10^9 total cells, up to 200 separations.
Product format	CD90.1 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 CD90.1 (Thy1.1)$^+$ cells are magnetically labeled with CD90.1 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 CD90.1$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD90.1$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD90.1$^+$ cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD90.1$^+$ cells is separated over a second column.

1.2 Background information

The mouse monoclonal antibody reacts with rat CD90 (Thy-1) and mouse CD90.1 (Thy1.1), a GPI-anchored conserved membrane glycoprotein. In the rat, the CD90 antigen is expressed on thymocytes[1], recent thymic emigrants[1], hematopoietic stem cells[2], neurons [3,4], and other cell types. In the mouse strains AKR/J, PL, and FVB/N, CD90.1 is a pan T cell marker[5] and can be found on thymocytes, hematopoietic stem cells in the bone marrow, intraepithelial cells (dendritic epidermal cells) in skin[6], and on neurons, such as retinal ganglion cells[7]. The antibody does not cross-react with CD90.2 (Thy1.2).

1.3 Applications

- CD90.1 MicroBeads are suitable for positive selection or depletion of mouse (strains AKR/J, PL, and FVB/N) or rat T lymphocytes from single-cell suspensions of spleen preparations or peripheral blood.
- Isolation of T cells for the adoptive transfer of CD90.1$^+$ T cells into CD90.2$^+$ inbred strains or immunodeficient mice.

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 mouse or rat serum albumin, mouse or rat 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: CD90.1$^+$ cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD, CS, or D Columns. Cells which strongly express the CD90.1 antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion 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
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

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

- (Optional) Fluorochrome-conjugated CD90.1 antibodies for flow cytometric analysis, e.g., CD90.1-PE (# 130-094-528). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.

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- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (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 lymphoid organs, non-lymphoid tissues, or peripheral blood, prepare a single-cell suspension using standard methods.

For details see 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. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 90 µL of buffer per 10^7 total cells.
4. Add 10 µL of CD90.1 MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies, e.g., 10 µL of CD90.1-PE (# 130-094-528), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

   ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 cells in 500 µL of buffer.

9. 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 CD90.1$^+$ cells. For details see 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

▲ To achieve highest purities, perform two consecutive column runs.

1. Place column in the magnetic field of a suitable MACS Separator. For details see 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 effluent 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.

   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a 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. To increase the purity of CD90.1$^+$ cells, enrich the eluted fraction over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details see LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

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

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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. Program recommendations below refer to separation of mouse or rat T cells.

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:

   Positive selection: "Possel"
   Collect positive fraction in row C of the tube rack.

   Depletion: "Deplete"
   Collect negative fraction in row B 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 pos1.
3. For a standard separation choose one of the following programs:

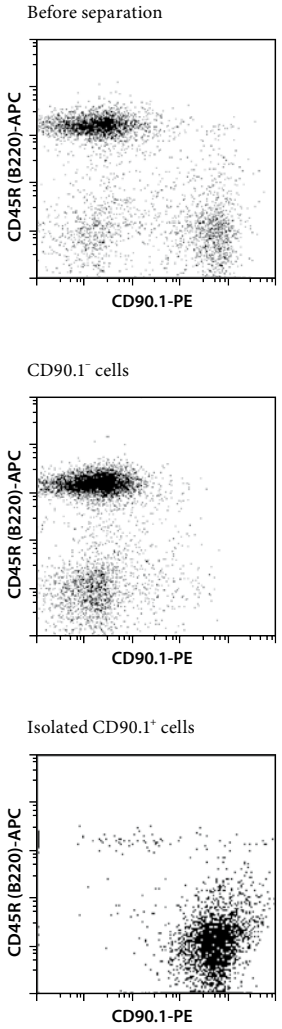
   Positive selection: "Possel"
   Collect positive fraction from outlet port pos1.

   Depletion: "Deplete"
   Collect negative fraction from outlet port neg1.

3. Example of a separation using the CD90.1 MicroBeads

CD90.1$^+$ cells were isolated from a single-cell suspension of mouse spleen (A) or rat spleen (B) using the CD90.1 MicroBeads, an MS Column, and a MiniMACS™ Separator. Cells were fluorescently stained with CD90.1-PE (# 130-094-528) and CD45R (B220)-APC (# 130-091-843) (A) or with CD90.1-PE (B) and analyzed using the MACSQuant™ Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

A)



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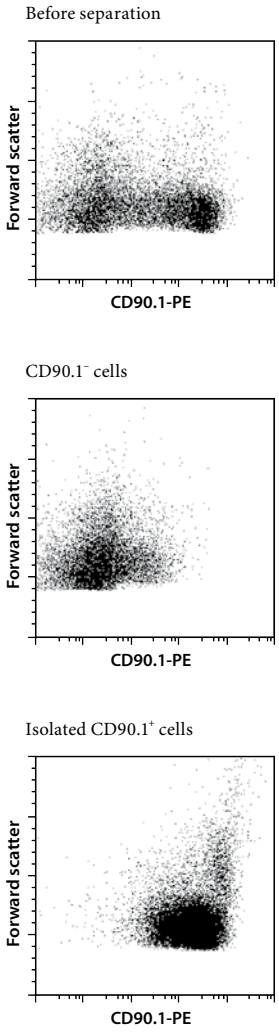
B)



4. References

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All protocols and data sheets are available at **www.miltenyibiotec.com**.

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.

Warranty

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