Miltenyi Biotec

Anti-Sca-1 (non-HSC) MicroBeads

mouse

Order no. 130-106-641

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 mL Anti-Sca-1 (non-HSC) MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse Sca-1 antibodies.
Capacity	For 2×10^9 total cells, up to 200 separations.
Product format	Anti-Sca-1 (non-HSC) 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 Sca-1$^+$ cells are magnetically labeled with Anti-Sca-1 (non-HSC) 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 Sca-1$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of Sca-1$^+$ cells. After removing the column from the magnetic field, the magnetically retained Sca-1$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

Sca-1 (stem cell antigen-1) is a 18 kDa GPI-linked surface protein of the Ly-6 family (Ly-6A/E). The anti-Sca-1 antibody specifically recognizes both Ly-6E.1 and Ly-6A.2, which are gene products of the two Ly-6A/E alleles expressed in different mouse strains (Ly-6E.1: e.g. BALB/c, C3H, NZB; Ly6A.2: e.g. C57BL/6, SJL, 129, AKR). Sca-1 is expressed on hematopoietic stem cells (HSCs) and progenitor cells in mouse bone marrow and one of the defining markers for so-called KSL (c-kit$^+$ Sca-1$^+$ Lin$^-$) cells. Sca-1 in combination with CD105 (endoglin) expression has been used to define long-term repopulating hematopoietic stem cells (LTR-HSCs) in mouse bone marrow.[1,2] In addition, Sca-1$^+$ bone marrow cells also contain the mesenchymal stem cell fraction present. Sca-1$^+$ cells have been shown to give rise to hepatocytes *in vivo*[3] and neural cells *in vitro*[4]. Furthermore, Sca-1 is expressed on stem cells in a variety of nonhematopoietic tissues, such as adult liver[5], heart[6], and prostate[7]. Anti-Sca-1 (non-HSC) MicroBeads have been developed for the separation of mouse cells based on the expression of Sca-1 except for HSCs, for which the Anti-Sca-1 MicroBead Kit (VioBright™ FITC), mouse should be used.

1.3 Applications

- Enrichment or depletion of Sca-1 expressing cells, e.g., from solid tissues.

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 serum albumin, mouse 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: Sca-1$^+$ cells can be enriched by using MS or LS or depleted with the use of LD Columns. Cells that strongly express the Sca-1 antigen can also be depleted using MS or LS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro Separator.

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

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LD	1.5×10^7	3×10^7	MidiMACS, QuadroMACS, SuperMACS II
Positive selection or depletion			
autoMACS	5×10^7	10^8	autoMACS Pro

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

- (Optional) Fluorochrome-conjugated Sca-1 antibodies for flow cytometric analysis, e.g., Anti-Sca-1-VioBright FITC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Dissociator with Heaters (# 130-096-427) in combination with the respective dissociation kit for generation of single cell suspensions from solid tissues.
- (Optional) gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) 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 (70 µm) (# 130-095-823) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with solid tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.miltenyibiotec.com/gentlemacs.

▲ 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

▲ Cells can be labeled with MACS MicroBeads using the autolabeling function of the autoMACS Pro Separator. For more information refer to section 2.4.

▲ 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 (70 µm), # 130-095-823) 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 90 µL of buffer per 10^7 total cells.
4. Add 10 µL of Anti-Sca-1 (non-HSC) 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 according to manufacturer's recommendation.
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 5×10^7 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 Sca-1$^+$ 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 as soon as 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 Sca-1$^+$ 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.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the 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 flow-through; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

2.4 Cell 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.

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = negative fraction,

position C = positive fraction.

2.4.1 Fully automated cell labeling and separation

1. Switch on the instrument for automatic initialization.
2. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS Pro Separator. Place the reagent into the appropriate position on the reagent rack.
3. Place sample and collection tubes into the Chill Rack.
4. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu (the correct labeling, separation, and wash protocols will be selected automatically).
5. Enter sample volume into the **Volume** submenu. Press **Enter**.
6. Select **Run**.

2.4.2 Magnetic separation using manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample and collection tubes into the Chill Rack.
4. For a standard separation choose the following program:

   Positive selection: Posseld

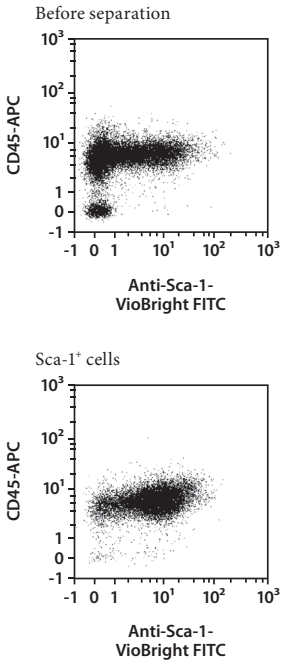
   Collect positive fraction in row C of the tube rack.

   Depletion: Deplete

   Collect negative fraction in row B of the tube rack.

3. Example of a separation using Anti-Sca-1 (non-HSC) MicroBeads

Mouse spleen was dissociated using the Spleen Dissociation Kit, mouse (# 130-095-926) and the gentleMACS Dissociator. Sca-1–expressing cells were isolated using Anti-Sca-1 (non-HSC) MicroBeads, an LS Column, and a QuadroMACS™ Separator. Cells were fluorescently stained with CD45-APC (# 130-110-660) and Anti-Sca-1-VioBright FITC (# 130-106-259) 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/local to find your nearest Miltenyi Biotec contact.

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