Miltenyi Biotec

Anti-Sca-1 MicroBead Kit (Vio® Bright FITC)

mouse

Order no. 130-123-124

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-Sca-1 MicroBead Kit (Vio Bright FITC)**
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	**200 µL Anti-Sca-1-Vio Bright FITC, mouse:** monoclonal anti-mouse Sca-1 antibody conjugated to Vio Bright FITC. **2 mL Anti-FITC MicroBeads:** MicroBeads conjugated to monoclonal anti-FITC Isomer-1 antibody (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 Sca-1$^+$ cells are indirectly magnetically labeled with Anti-Sca-1-Vio Bright FITC antibodies and Anti-FITC 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. To increase the purity, the positively selected cell fraction containing the Sca-1$^+$ cells must be separated over a second column.

1.2 Background information

The Anti-Sca-1 MicroBead Kit (Vio Bright FITC) has been developed for the isolation of murine stem cells from bone marrow. Stem cell antigen-1 (Sca-1) is an 18 kDa GPI-linked surface protein of the Ly-6 family (Ly-6A/E). The anti-Sca-1 monoclonal antibody recognizes both Ly-6E.1 and Ly-6A.2, which are gene products of two Ly-6A/E alleles expressed in different mouse strains (e.g. BALB/c, C3H, NZB mice express only Ly-6E.1, while C57BL/6, SJL, 129, AKR express only Ly-6A.2). Hematopoietic stem cells in mouse bone marrow are defined as negative for lineage markers (lin$^-$), and positive for Sca-1 and CD117 (c-kit). Long-term repopulating hematopoietic stem cells (LTR-HSCs) can be enriched by their expression of CD105$^+$Sca-1$^+$ using the CD105 MultiSort Kit (PE) in combination with the Anti-Sca-1 MicroBead Kit (Vio Bright FITC).
Sca-1$^+$ stem cells have also been isolated from peripheral blood, fetal, and adult liver as well as heart and prostate tissue.

1.3 Applications

- Positive selection or depletion of cells expressing the mouse Sca-1 antigen.
- Isolation of Sca-1$^+$ cells from murine bone marrow after depletion of so-called "lineage-positive" cells using the Lineage Cell Depletion Kit, mouse (# 130-090-858).
- Isolation of LTR-HSCs by subsequent separation of CD105$^+$Sca-1$^+$ cells using the CD105 MultiSort Kit (PE) in combination with the Anti-Sca-1 MicroBead Kit (Vio Bright FITC). A special protocol is available at www.miltenyibiotec.com.

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.

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- MACS Columns and MACS Separators: Sca-1$^+$ cells can be enriched by using MS, LS, or XS Columns. Positive selection 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	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	10^9	2×10^{10}	SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro

▲ **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 CD117 antibodies for flow cytometric analysis, e.g., CD117-PE. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (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) CD105 MultiSort Kit (PE), mouse (# 130-092-924) for the enrichment of CD105$^+$Sca-1$^+$ LTR-HSCs.
- (Optional) Lineage Cell Depletion Kit, mouse (# 130-090-858) for the depletion of lineage-committed cells.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with 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)

Preparation of bone marrow cells

▲ All steps should be performed on ice.

1. Collect murine bone marrow cells from femur (and tibias) by flushing the shaft with buffer using a syringe and a 26G needle.
2. Disaggregate cells by gentle pipetting them several times.
3. 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.
4. Wash cells by adding buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
5. Resuspend cell pellet in buffer and take an aliquot for cell counting.

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 (30 µm), # 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 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 98 µL of buffer per 10^7 total cells.
4. Add 2 µL of Anti-Sca-1-Vio Bright FITC per 10^7 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Wash cells by adding 1–2 mL of buffer per 10^7 total 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-FITC 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 total 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 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.

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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. 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.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation, refer to the XS Column data sheet.

Magnetic separation with the autoMACS® Pro Separator

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

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

1. Prepare and prime the instrument.

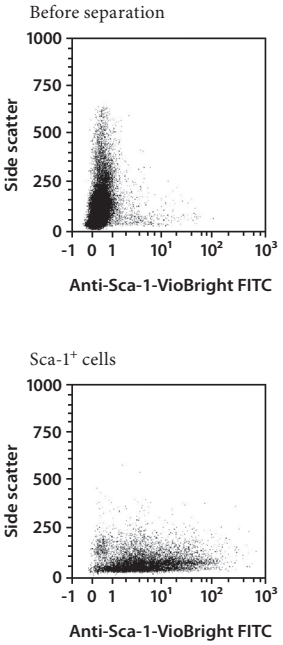
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.

3. For a standard separation choose the following program:

   Positive selection: Posseld2
   Collect positive fraction in row C of the tube rack.

3. Example of a separation using the Anti-Sca-1 MicroBead Kit (Vio Bright FITC)

Sca-1$^+$ cells were isolated from bone marrow cells from BALB/c mice using Anti-Sca-1 MicroBead Kit (Vio Bright FITC), two MS Columns, and a MiniMACS™ Separator. Cells were 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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