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1. Description

Components	1 mL Anti-Sca-1-FITC, mouse: monoclonal anti-mouse Sca-1 antibody conjugated to fluorescein isothiocyanate (FITC).
	2 mL Anti-FITC MicroBeads: MicroBeads conjugated to monoclonal anti-FITC Isomer-1 antibody (isotype: mouse IgG1).
Size	For 10 ⁹ total cells, up to 100 separations.
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

Cells are initially immunolabeled with Anti-Sca-1-FITC, after which magnetic labeling of Sca-1⁺ cells can be achieved using Anti-FITC MicroBeads. The cell suspension is then applied to a MACS® Column placed in the magnetic field of a MACS Separator. The magnetically labeled Sca-1⁺ cells are retained within the column, while the unlabeled Sca-1⁻ cells are eluted in the flow through. After removal of the column from the magnetic field the retained Sca-1⁺ cells are eluted.

1.2 Background information

The Anti-Sca-1 MicroBead Kit (FITC) has been developed for the isolation of murine stem cells from bone marrow. Sca-1 (Stem cell antigen-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⁺ ^{1,2} by using the CD105 MultiSort Kit (PE) in combination with the Anti-Sca-1 MicroBead Kit (FITC) for subsequent enrichment of the CD105⁺Sca-1⁺ subpopulation.

Sca-1⁺ cells from mouse bone marrow have further been shown to have nonhematopoietic differentiation potential (e.g. to hepatocytes and neural cells *in vitro*⁴). Additionally, Sca-1⁺ stem cells have been isolated from peripheral blood⁵, fetal⁶, and adult liver⁷ as well as heart⁸ and prostate tissue⁹.

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 (FITC). The 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 murine serum or fetal bovine serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.

- MACS Columns and MACS Separators:

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

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

- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion 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) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g. CD117-PE (# 130-091-730) and CD117-APC (# 130-091-729). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.
- (Optional) Lineage Cell Depletion Cocktail-Biotin, mouse (# 130-090-858) and Anti-Biotin-APC (# 130-090-856) or Anti-Biotin-PE (# 130-090-756) for staining of lineage-committed cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.

2. Protocol

2.1 Sample preparation

When working with tissues, prepare a single-cell suspension by a standard preparation method. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available 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).

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 # 130-041-407) to remove cell clumps which may clog the column. Wet filter with buffer before use.
4. Wash cells by adding buffer and centrifuge at 300 \times g for 10 minutes at 4–8 °C. Aspirate supernatant.
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 separation. Pass cells through 30 μm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column.

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

1. Determine cell number.
2. Centrifuge cell suspension at 300 \times 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-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 \times 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 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 \times g for 10 minutes. Aspirate supernatant completely.
11. Resuspend up to 10^8 cells in 500 μL of buffer.
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 (see table in section 1.4).

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

Positive selection with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
2. Prepare column by rinsing with appropriate amount of buffer:
MS: 500 μL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction (Sca-1⁻ cells). Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
MS: 3 \times 500 μL LS: 3 \times 3 mL
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 appropriate amount of buffer onto the column. Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger supplied with 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™ Separator or the autoMACS™ Pro Separator

- ▲ Refer to the respective user manual for instructions on how to use the autoMACS™ Separator or the autoMACS Pro Separator.
- ▲ Buffers used for operating the autoMACS Separator or the autoMACS Pro 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.

Magnetic separation with the autoMACS™ Separator

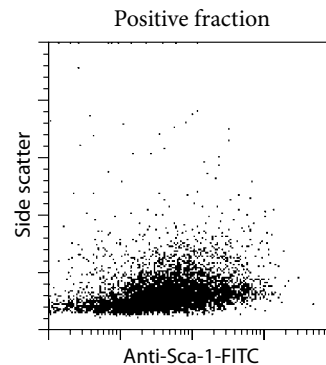
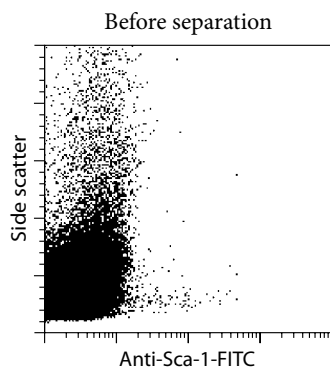
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. Choose the following program:
Positive selection: "Posseld2".
Collect positive fraction from outlet port pos2.

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 fraction collection tubes in rows B and C.
3. 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 (FITC)

Separation of bone marrow cells from BALB/c mice using the Anti-Sca-1 MicroBead Kit (FITC), two MS Columns and then analyzed by flow cytometry. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Chen, C. Z. *et al.* (2002) Identification of endoglin as a functional marker that defines long-term repopulating hematopoietic stem cells. *Proc. Natl. Acad. Sci. U S A* 99: 15468–15473.
2. Chen, C. Z. *et al.* (2003) The endoglin(positive) sca-1(positive) rhodamine(low) phenotype defines a near-homogeneous population of long-term repopulating hematopoietic stem cells. *Immunity* 19: 525–533.
3. Lagasse, E. *et al.* (2000) Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat. Med.* 6: 1229–1234.
4. Sanchez-Ramos, J. *et al.* (2000) Adult Bone Marrow Stromal Cells Differentiate into Neural Cells in Vitro. *Exper. Neurol.* 164: 247–256
5. Yamada, M. *et al.* (2004) Bone Marrow-Derived Progenitor Cells Are Important for Lung Repair after Lipopolysaccharide-Induced Lung Injury. *J. Immunol.* 172: 1266–1272.
6. Cherqui, S. *et al.* (2006) Isolation and Angiogenesis by Endothelial Progenitors in the Fetal Liver. *Stem cells* 24: 44–54.
7. Petersen, B. E. *et al.* (2003) Mouse A 6-positive hepatic oval cells also express several hematopoietic stem cell markers. *Hepatology* 37: 632–640.
8. Matsuura, K. *et al.* (2004) Adult Cardiac Sca-1-positive Cells Differentiate into Beating Cardiomyocytes. *JBC* 279: 11384–11391.
9. Burger, P. E. *et al.* (2005) Sca-1 expression identifies stem cells in the proximal region of prostatic ducts with high capacity to reconstitute prostatic tissue. *Proc. Natl. Acad. Sci. U S A* 102: 7180–7185.

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.

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