



# Anti-Sca-1 antibodies mouse

Anti-Sca-1-FITC	130-093-222
Anti-Sca-1-PE	130-093-224
Anti-Sca-1-APC	130-093-223
Anti-Sca-1-Biotin	130-093-421

## Contents

1. Description
  - 1.1 Background information
  - 1.2 Applications
  - 1.3 Recommended antibody dilution
  - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with Anti-Sca-1 antibodies
4. References

## 1. Description

<b>Components</b>	1 mL Anti-Sca-1 antibodies, mouse: monoclonal Anti-Sca-1 antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
<b>Clone</b>	D7 (isotype: rat IgG2a).
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.
<b>Product format</b>	Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

### 1.1 Background information

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). Sca-1 is expressed on stem cells in bone marrow with hematopoietic differentiation potential. Sca-1<sup>+</sup> cells from bone marrow also possess nonhematopoietic differentiation potential as they are able to give rise to hepatocytes *in vivo*<sup>1</sup> and neural cells *in vitro*<sup>2</sup>. Sca-1 expression is also found on distinct subpopulations of bone marrow and peripheral B cells, myeloid cells, thymic and peripheral lymphocytes as well as early intrathymic T-cell precursors. Furthermore, Sca-1 is expressed on stem cells in a variety of nonhematopoietic tissues, such as adult liver<sup>3</sup>, heart<sup>4</sup>, and prostate<sup>5</sup>.

### 1.2 Applications

- Identification and enumeration of Sca-1<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Identification of mouse hematopoietic stem cells in combination with the Lineage Cell Detection Cocktail-Biotin, mouse (# 130-092-613).

- Evaluation of MACS Separations by flow cytometry or fluorescence microscopy. Sca-1<sup>+</sup> cells can be enriched by using the Lineage Cell Depletion Kit, mouse (# 130-090-858) or CD117 MicroBeads, mouse (# 130-091-224). Furthermore, Sca-1<sup>+</sup> cells can be isolated by using the Anti-Sca-1 MicroBead Kit (FITC), mouse (# 130-092-529).

### 1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

Anti-Sca-1 conjugate	FITC	PE	APC
<b>Flow cytometry<sup>a</sup></b>			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11
- Anti-Sca-1 MicroBead-labeled cells	1:11	1:11	1:11
<b>Immunohistochemistry<sup>b</sup></b>			
a) Given antibody dilutions are for a cell concentration of up to 10 <sup>7</sup> cells/100 µL of buffer.			
b) The optimal antibody dilution should be determined.			

### 1.4 Reagent 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).  
▲ **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. Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) CD117-APC (# 130-091-729) or CD117-PE (# 130-091-730).
- (Optional) Lineage Cell Detection Cocktail-Biotin, mouse (# 130-090-857) and Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent.
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

## 2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> 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<sup>7</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.

140-002-0055-01

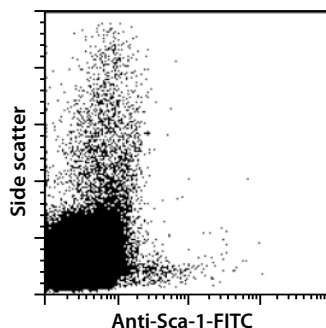


2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10<sup>7</sup> nucleated cells per 100 μL of buffer.
4. Add 10 μL of the Anti-Sca-1 antibody.
  - ▲ **Note:** If Anti-Sca-1-PE is used resuspend 10<sup>7</sup> nucleated cells in 90 μL of buffer and add 10 μL of FcR Blocking Reagent, mouse, directly before addition of Anti-Sca-1-PE.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
  - ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer per 10<sup>7</sup> cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-Sca-1-Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy

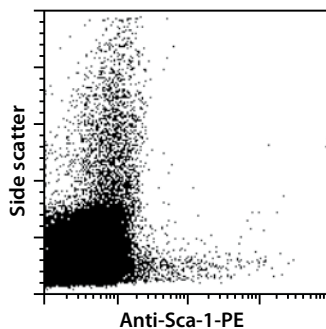
### 3. Examples of immunofluorescent staining with Anti-Sca-1 antibodies

Mouse bone marrow cells were stained with Anti-Sca-1 antibodies conjugated to FITC (a), PE (b), or APC (c), and analyzed by flow cytometry. Cells stained with Anti-Sca-1-PE were incubated with FcR Blocking Reagent in addition. Cells stained with Anti-Sca-1-Biotin (d) were stained with Anti-Biotin-APC (# 130-090-856). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

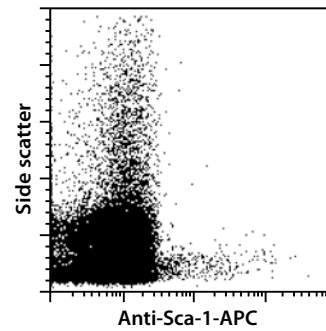
(a) Mouse cells stained with Anti-Sca-1-FITC.



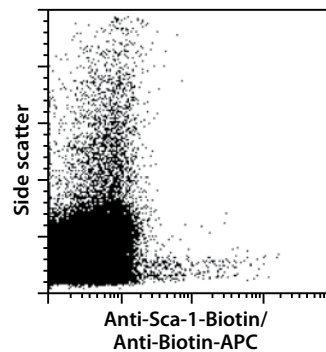
(b) Mouse cells stained with Anti-Sca-1-PE.



(c) Mouse cells stained with Anti-Sca-1-APC.



(d) Mouse cells stained with Anti-Sca-1-Biotin and Anti-Biotin-APC.



### 4. References

1. Lagasse, E. *et al.* (2000) Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. *Nat. Med.* 6(11): 1229–1234.
2. Sanchez-Ramos, J. *et al.* (2000) Adult bone marrow stromal cells differentiate into neural cells *in vitro*. *Exp. Neurol.* 164(2): 247–256.
3. Petersen, B. E. *et al.* (2003) Mouse A6-positive hepatic oval cells also express several hematopoietic stem cell markers. *Hepatology* 37: 632–640 [11259].
4. Matsuura, K. *et al.* (2004) Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. *J. Biol. Chem.* 279(12): 11384–11391 [4144].
5. 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(20): 7180–7185 [7082].

All protocols and data sheets are available at [www.miltenyibiotec.com](http://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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