For Research Use Only.
Not for use in diagnostic procedures.

MONOCLONAL ANTIBODY

Anti-Mouse Sca-1

Code No.	Clone	Subclass	Quantity	Concentration
D052-3	238B	Rat IgG2a	100 µg	1 mg/mL

BACKGROUND: Sca-1 is a member of the Ly-6 antigen family which molecular mass of 8 kDa under nonreducing conditions and of 18 kDa under reducing conditions. Mouse hematopoietic stem cell expresses low levels of Thy-1 antigen (Thy-1^{lo}) and to be lineage-negative (Lin^-); not express markers characteristic of B cells (B220), granulocytes (Gr-1), myelomonocytic cells (Mac-1) and T lymphocytes (CD4 and CD8). Recently, new monoclonal antibody, anti-Sca-1, was used to purify stem cells from the Thy-1^{lo}, Lin^- bone marrow subpopulation. Thy-1^{lo}, Lin^-, Sca-1^+ (but not the Thy-1^{lo}, Lin^-, Sca-1^-) population of bone marrow cells are highly purified pluripotent stem cells. They read out with nearly unit efficiency in assays for primitive myeloerythroid and thymic progenitor, and have a capability to admit lethally irradiated mouse to survive and be restored in all blood-cell lineages. The Thy-1^{lo}, Lin^-, Sca-1^+ subpopulation thought to have all stem cells present in the bone marrow.

SOURCE: This antibody was purified from culture supernatant using protein G agarose. This hybridoma (clone 238B) was established by fusion of mouse myeloma cell P3X with rat splenocyte immunized with mouse Sca-1 transfected LO cells.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with mouse Sca-1 on Flow cytometry.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL

Detailed procedure is provided in the following **PROTOCOL**.

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Not Tested	lymphocyte*, splenocyte*, LO	Not Tested
Reactivity on FCM		+	

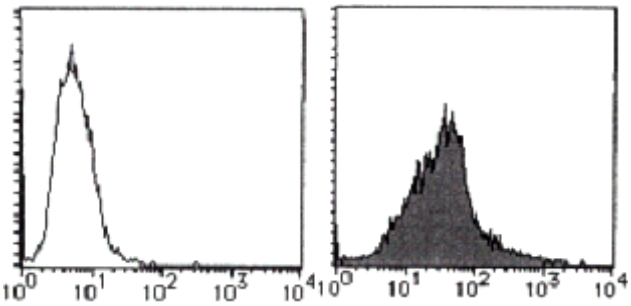
*C57BL/6

REFERENCE:

1) Petersen, B. E., *et al*., *Hepatology* **37**, 632-640 (2003)

RELATED PRODUCTS:

D052-5 PE labeled anti-mouse Sca-1 (238B)
D052-6 Biotin labeled anti-mouse Sca-1 (238B)
D160-3 anti-mouse Ly49Q (2E6)
D160-4 FITC labeled anti-mouse Ly49Q (2E6)
D160-5 PE labeled anti-mouse Ly49Q (2E6)
M081-3 Rat IgG2a isotype control (2H3)



Flow cytometric analysis of mouse Sca-1 expression on LO cells. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D052-3 to the cells.*

PROTOCOL:

Flow cytometric analysis for adherent cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

1) Detach the cells from culture dish by cell dissociation buffer.
2) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
3) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
4) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.

5) Add 10 μL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
6) Add 40 μL of the primary antibody at the concentration as suggest in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
7) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
8) Add 30 μL of 1:40 FITC conjugated anti-rat IgG (MBL; code no. 354) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
9) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; LO, lymphocyte, splenocyte)