OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-888-267-4436
Fax: +1-301-340-8606
techsupport@origene.com

OriGene Technologies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info-de@origene.com

AM26430AF-N Monoclonal Antibody to Ly6A/E / SCA1 - Azide Free

Alternate names:	Ly-6A.2/Ly-6E.1, Lymphocyte antigen 6A-2/6E-1, SCA-1, Stem cell antigen 1, T-cell-activating protein, TAP
Quantity:	0.1 mg
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-1lo) 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-1lo, Lin- bone marrow subpopulation. Thy-1lo, Lin-, Sca-1+ (but not the Thy-1lo, 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-1lo, Lin-, Sca-1+ subpopulation thought to have all stem cells present in the bone marrow.
Uniprot ID:	P05533
NCBI:	NP_034868.1
GeneID:	110454
Host / Isotype:	Rat / IgG2a
Recommended Isotype Controls:	SM26A
Clone:	238B
Immunogen:	Mouse Sca-1 transfected LO cells
Format:	**State:** Liquid Ig fraction **Purification:** Protein G agarose **Buffer System:** PBS containing 50% glycerol, pH 7.2. No preservative is contained.
Applications:	Flow cytometry: 10 µg/ml. For details see protocol below. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This antibody reacts with Sca-1.
Species Reactivity:	**Tested:** Mouse
Add. Information:	This product was originally produced by MBL International.
Storage:	Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing. Shelf life: one year from despatch.
General Readings:	1. Petersen, B. E., et al., Hepatology 37, 632-640 (2003).

Protocols:

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% NaN3].
3) Resuspend the cells with washing buffer (5x10e6 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 (20~25oC). Remove supernatant by careful aspiration.
5) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN3 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 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)

Pictures:

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 AM26430AF-N to the cells.