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AM31883RP-L Monoclonal Antibody to Ly6A/E / SCA1 - PE

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:	0.3 mg
Concentration:	0,1 mg/ml
Background:	Ly6A/E is a member of the Ly-6 antigen family. The Thy-1lo, Lin- (lineage-negative, not expressing B220, Gr-1, Mac-1, CD4 or CD8), Sca-1+ population of bone marrow cells are highly purified, perhaps homogenous, pluripotent stem cells. This antigen is also present on various other tissues. Specific staining of the parenchymal cells can be demonstrated in thymus, spleen and kidney where as only vasculature reacts with anti-Sca-1 in brain, heart and liver (and possibly in lung). Also, Sca-1 is a T cell activation antigen, as surface expression of the antigen increases upon Con A activation of T lymphocytes. Sca-1 appears to have a molecular mass of 8 kDa under non-reducing conditions and 18 kDa under reducing conditions, indicating the presence of intra-chain disulfide bonds.
Uniprot ID:	P05533
NCBI:	NP_034868
GeneID:	110454
Host / Isotype:	Rat / IgG2b
Recommended Isotype Controls:	SM19R
Clone:	CT-6A/6E
Format:	**State:** Liquid purified Ig fraction **Purification:** Affinity chromatography on Protein G **Buffer System:** PBS containing 0.02% sodium azide (NaN3) as preservative A highly purified grade of BSA has been added as a stablizing protein to bring the final protein concentration to 4-5 mg/ml after conjugation. **Label:** PE – R - Phycoerythrin
Applications:	Flow Cytometry. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This monoclonal antibody recognizes Sca-1 (Ly-6A.2/6E.1), a cell surface antigen used in the identification of hematopoietic stem cells. **Species:** Mouse. Other species not tested.
Storage:	Store the antibody undiluted at 2-8°C. **DO NOT FREEZE!** Shelf life: one year from despatch.

General Readings:

1. Spangrude, G.J. et al. 1991 Purification and characterization of mouse hematopoletic stem cells, Science 241:58-62.
2. Alhara, Y., Buhring, H.J., Alhara, M, et al. 1986 An attempt to produce “pre-T” cell hybridomas and to identify their antigens, Eur.J.Immunol, 16:1391-1399.
3. van de Rijn M, Heimfeld S, Spangrude GJ, Weissman IL. Mouse hematopoietic stem-cell antigen Sca-1 is a member of the Ly-6 antigen family. Proc Natl Acad Sci U S A. 1989 Jun;86(12):4634-8. PubMed PMID: 2660142.
4. Codias EK, Cray C, Baler RD, Levy RB, Malek TR. Expression of Ly-6A/E alloantigens in thymocyte and T-lymphocyte subsets: variability related to the Ly-6a and Ly-6b haplotypes. Immunogenetics. 1989;29(2):98-107. PubMed PMID: 2492483.
5. Tomonari K. A rat antibody against a structure functionally related to the mouse T-cell receptor/T3 complex. Immunogenetics. 1988;28(6):455-8. PubMed PMID: 2972613.

Protocols:

FLOW CYTOMETRY ANALYSIS:

Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M cell separation medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10e6 cells, representing 1 test).
4. To each tube, add 2.0 µg of this antibody per 10e6 cells.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C. (It is recommended that the tubes are protected from light, since most flurochromes are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 µl ice cold media B.
9. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA.

Media:

A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 ml

Results:

Tissue Distribution by Flow Cytometry Analysis:
Mouse Strain: C57BL/6
Cell Concentration: 1 x 10e6 cells per test
Antibody Concentration Used: 2.0 µg/10e6 cells
Isotypic Control: PE Rat IgG2b