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Product datasheet for AM31883PU-N

Ly6a Rat Monoclonal Antibody [Clone ID: CT-6A/6E]

Product data:

Product Type:	Primary Antibodies
Clone Name:	CT-6A/6E
Applications:	FC
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2b
Clonality:	Monoclonal
Specificity:	This monoclonal antibody recognizes Sca-1 (Ly-6A.2/6E.1), a cell surface antigen used in the identification of hematopoietic stem cells.
Formulation:	PBS containing 0.02% sodium azide (NaN3) as preservative State: Purified State: Liquid purified Ig fraction
Concentration:	lot specific
Purification:	Affinity chromatography on Protein G
Conjugation:	Unconjugated
Storage:	Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	lymphocyte antigen 6 complex, locus A
Database Link:	Entrez Gene 110454 Mouse P05533

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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.

Synonyms: Lymphocyte antigen 6A-2/6E-1, Ly-6A.2/Ly-6E.1, T-cell-activating protein, TAP, Stem cell antigen 1, SCA-1

Note: Protocol: **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 1.0 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.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody (FITC Goat anti-rat IgG (H+L)) at 1/500 dilution.
9. Incubate the tubes at 4°C for 30-60 minutes. (It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in media B.
11. Resuspend the cell pellet in 50 µl ice cold media B.
12. 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: Balb/C
Cell Concentration: 1 x 10e6 cells per test
Antibody Concentration Used: 1.0 µg/10e6 cells
Isotypic Control: Purified Rat IgG2b