Monoclonal Anti-mouse Sca-1-Alexa Fluor® 488

Catalog Number: FAB1226G
Lot Number: ADIQ01
100 Tests

Reagents Provided

Alexa Fluor® 488-conjugated rat monoclonal anti-mouse Sca-1: Supplied as 10 µg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 177228

Isotype: rat IgG_{2A}

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

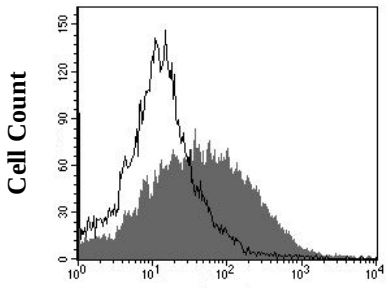
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing Sca-1 within a population and qualitatively determine the density of Sca-1 on cell surfaces by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with purified NS0-derived recombinant mouse Sca-1/Ly-6 C-terminally truncated Ly6E allele (aa 27-119; Accession # CAA28351). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to Alexa Fluor® 488 fluorochrome. Cell surface expression of Sca-1 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515-545 nm.



Sca-1-Alexa Fluor® 488

Mouse splenocytes were stained with Alexa Fluor® 488-conjugated anti-mouse Sca-1 (Catalog # FAB1226G; filled histogram) or Alexa Fluor® 488-conjugated isotype control (Catalog # IC006G; open histogram).

Background Information

Sca-1 is an 18 kDa phosphatidylinositol-anchored protein that is a member of the lymphocyte antigen 6 (Ly-6) family.[1] Sca-1 is encoded by the strain-specific *Ly-6 A/E* allelic gene. Its expression on multipotent hematopoietic stem cells (HSC) has been used as a marker of HSC in mice of both Ly-6 haplotypes.[2-3] Anti-mouse Sca-1 antibodies are frequently used in combination with lineage depletion antibodies to identify and isolate HSC. Sca-1-positive HSC can be found in the fetal liver, adult bone marrow, spleen, and in mobilized peripheral blood in the adult animal.[2-7] However, Sca-1 has also been discovered in several non-hematopoietic tissues, and can be used to enrich progenitor cell populations other than HSC.[1, 8] It is suggested that Sca-1 could be involved in regulating both B cell and T cell activation.[9-12]

References

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2. Spangrude, G.I. *et al*. (1988) Science **241**:58.
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7. Morrison, S.J. *et al*. (1997) Proc. Natl. Acad. Sci. USA **94**:1908.
8. Welm, B.E. *et al*. (2002) Dev. Biol. **245**:42.
9. Codias, E.K. *et al*. (1990) J. Immunol. **144**:2197.
10. Malek, T.R. *et al*. (1986) J. Exp. Med. **164**:709.
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12. Flood, P.M. *et al*. (1990) J. Exp. Med. **172**:115.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse splenocytes.

1. Cells may be Fc-blocked with 1 µg of mouse IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2. After blocking, 5 µL of conjugated antibody was added to up to 1 x 10^6 cells and incubated for 30 minutes at room temperature.
3. Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Mouse Lyse Buffer (Catalog # FC003).
4. The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with Alexa Fluor® 488-labeled rat IgG_{2A} antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.

Legal

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