Monoclonal
Anti-mouse Sca-1/Ly6-Phycoerythrin

Catalog Number: FAB1226P
Lot Numbers: LMN03
LMN04
100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated monoclonal anti-mouse Sca-1: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 177228

Ig class: rat IgG_{2A}

Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

Storage

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

Intended Use

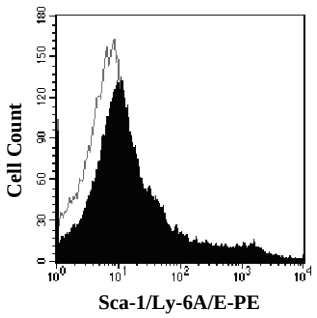
Designed to quantitatively determine the percentage of cells bearing the mouse Sca-1 cell surface antigen within a population and qualitatively determine its density on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody that binds to cells expressing the mouse Sca-1/Ly6 antigen. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing the Sca-1/Ly6 antigen are fluorescently stained, with the intensity of staining directly proportional to the density of Sca-1 expression. Cell surface expression of the Sca-1 antigen is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

PE-conjugated rat anti-mouse Sca-1: Use as is; no preparation is necessary. The investigator should determine the optimal antibody staining concentration by first performing a dilution analysis where decreasing amounts of antibody are used to stain a known tissue sample. Use of excessive amounts of antibody in cell staining reactions can lead to high background signals.



C57BL bone marrow cells, gated on low side scatter, stained with PE-conjugated anti-mouse Sca-1 (Catalog # FAB1226P, filled histogram) or isotype control (Catalog # IC006P, open histogram).

Sample Preparation

Tissues: Whole blood should be collected in tubes containing EDTA or heparin as the anticoagulant. Spleen cells should be first mechanically disaggregated into a single cell suspension. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Blood cells will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4 x 10^6 cells/mL and 25 μL of cells (1 x 10^5) are transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

1) Cells to be used for staining with the antibody may be first Fc-blocked by treatment 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) Transfer 25 μL of the Fc-blocked cells (1 x 10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated anti-mouse Sca-1 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-Sca-1 reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Mouse Erythrocyte Lysing Kit, Catalog # WL2000*).
6) Finally, resuspend the cells in 200 - 400 μL of PBS buffer for final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with PE-labeled rat IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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1-800-343-7475

2/09

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-1antibodies 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 (1) and can be used to enrich progenitor cell populations other than HSC (8). It is suggested that Sca-1 could be involved in regulating both B and T cell activation (9 - 12).

References

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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.