Monoclonal Anti-mouse IGSF2/CD101-Phycoerythrin

Catalog Number: FAB3368P
Lot Number: AABY01
100 Tests

Reagents Provided

Phycoerythrin-conjugated rat monoclonal anti-mouse IGSF2/CD101: Contains 1.0 mL of PE-labeled antibody at a concentration of 25 μg/mL.

Clone #: 307707

Isotype: rat IgG_{2A}

Reagents Not Provided

- 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

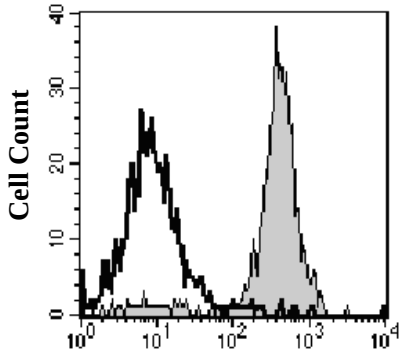
Designed to quantitatively determine the percentage of cells bearing IGSF2/CD101 within a population and qualitatively determine the density of IGSF2/CD101 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the Phycoerythrin-labeled monoclonal antibody, which binds to cells expressing IGSF2/CD101. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing IGSF2/CD101 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of IGSF2/CD101. Cell surface expression of IGSF2/CD101 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Phycoerythrin-conjugated rat anti-mouse IGSF2/CD101: Use as is; no preparation necessary.



Mouse GR-1 positive blood cells were stained with anti-mouse IGSF2/CD101-PE (R&D Systems, Catalog # FAB3368P, filled histogram) or isotype control (R&D Systems, Catalog # IC006P, open histogram).

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. 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. Transfer 50 μL of packed cells to a 5 mL tube for staining with the monoclonal antibody. Whole blood 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) 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 should be Fc-blocked by treatment with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature prior to staining. 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 IGSF2/CD101 reagent.
4) Incubate for 30 - 45 minutes at 2 - 8° C.
5) Following this incubation, remove unreacted IGSF2/CD101 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
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.

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Background Information

IGSF2, also known as CD101, is an Ig-superfamily transmembrane protein. It is expressed on dendritic cells, Langerhans cells, granulocytes, and activated T cells. IGSF2 ligation is involved in T cell activation. Polymorphisms in IGSF2 are associated with susceptibility to type I diabetes. The extracellular domains of mouse and human IGSF2 share 70% amino acid sequence identity.

Note: *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.*