Monoclonal
Anti-human FCRL1/FcRH1-Allophycocyanin

Catalog Number: FAB2049A
Lot Number: AAEW01
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human FCRL1/FcRH1: Supplied as 25 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 282415

Isotype: mouse IgG_1

Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

Storage

All Reagents: 2° - 8° C in the dark for up to **twelve months** without a significant loss of activity.

Intended Use

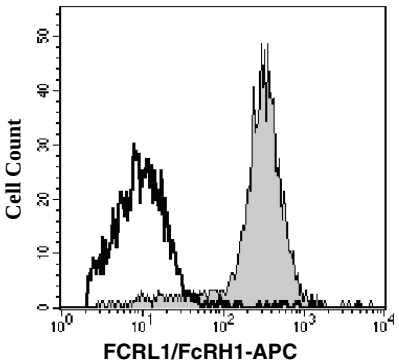
Designed to quantitatively determine the percentage of cells bearing FCRL1/FcRH1 within a population and qualitatively determine the density of FCRL1/FcRH1 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the allophycocyanin-labeled monoclonal antibody, which binds to cells expressing FCRL1/FcRH1. Unbound allophycocyanin-conjugated antibody is then washed from the cells. Cells expressing FCRL1/FcRH1 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of FCRL1/FcRH1. Cell surface expression of FCRL1/FcRH1 is determined by flow cytometric analysis using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.

Reagent Preparation

Allophycocyanin-conjugated mouse anti-human FcRHI/IRTA5: Use as is; no preparation necessary.



Human B-cells were stained with APC-conjugated anti-human FCRL1/FcRH1 (Catalog # FAB2049A, filled histogram) or isotype control (Catalog # IC002A, 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 APC-conjugated FCRL1/FcRH1 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted FCRL1/FcRH1 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 APC-labeled mouse IgG_1 antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

FCRL1, also known as IRTA5 (Immunoglobulin superfamily Receptor Translocation Associated 5), is a type I transmembrane protein having three extracellular Ig-like domains and cytoplasmic ITAM-like motifs. The FcRH subfamily shares sequence homology with the classical receptors for Ig, and the corresponding genes are localized to human chromosome Iq21 - 23, a hotspot for translocation events involved in B-cell malignancy. FCRL1 is expressed primarily in B cells.

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.