Monoclonal Anti-human Fcγ RI/CD64-Phycoerythrin

Catalog Number: FAB12571P
Lot Number: AAAF01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human Fcγ RI/CD64: Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 276426

Isotype: mouse IgG_1

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

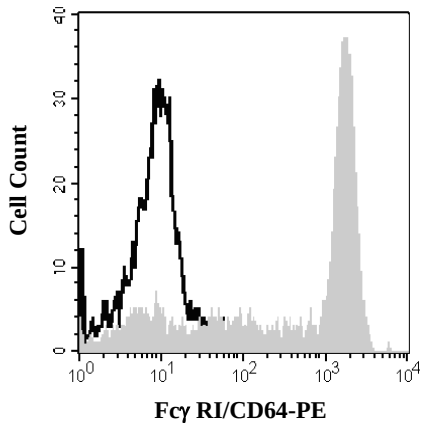
Designed to quantitatively determine the percentage of cells bearing Fcγ RI/CD64 within a population and qualitatively determine the density of Fcγ RI/CD64 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing Fcγ RI/CD64. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing Fcγ RI/CD64 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Fcγ RI/CD64. Cell surface expression of Fcγ RI/CD64 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Phycoerythrin-conjugated mouse anti-human Fcγ RI/CD64: Use as is; no preparation necessary.



Human monocytes were stained with PE-conjugated anti-human Fcγ RI/CD64 (Catalog # FAB12571P, filled histogram) or PE-conjugated isotype control (Catalog # IC002P, 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) Transfer 25 μL of the Fc-blocked cells (up to 1 x 10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
2) Add 10 μL of PE-conjugated Fcγ RI/CD64 reagent.
3) Incubate for 30 - 45 minutes at 2° - 8° C.
4) Following this incubation, remove unreacted Fcγ RI/CD64 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*).
5) Finally, resuspend the cells in 200 - 400 μL of PBS buffer for final flow cytometric analysis.
6) As a control for analysis, cells in a separate tube should be treated with PE-labeled mouse IgG_1 antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

Receptors that recognize the Fc portion of IgG are divided into three groups designated Fcγ RI, RII, and RIII, also known respectively as CD64, CD32 and CD16. In humans, each group of receptors, is encoded by 2-3 closely related genes designated A, B, and C. Fcγ RI binds IgG with high affinity and functions during early immune responses. Fcγ RII and RIII are low affinity receptors that recognize IgG as aggregates surrounding multivalent antigens during late immune responses. Different Fc receptors can function as activators, as inhibitors, or as decoy receptors.

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.