Monoclonal Anti-human C1qR1/CD93-Fluorescein

Catalog Number: FAB23791F
Lot Number: AANH01
100 Tests

Reagents Provided

Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human C1qR1/CD93: Supplied as 25 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 273107

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

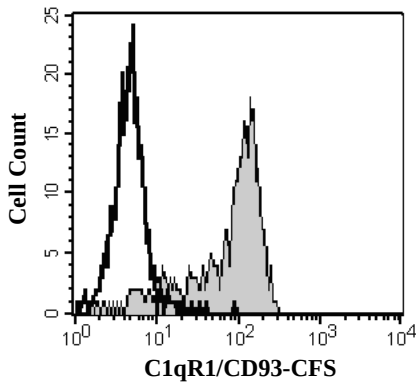
Designed to quantitatively determine the percentage of cells bearing C1qR1/CD93 within a population and qualitatively determine the density of C1qR1/CD93 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody, which binds to cells expressing C1qR1/CD93. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing C1qR1/CD93 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of C1qR1/CD93. Cell surface expression of C1qR1/CD93 is determined by flow cytometry using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515 - 545 nm.

Reagent Preparation

Fluorescein-conjugated mouse anti-human C1qR1/CD93: Use as is; no preparation necessary.



Human monocytes were stained with CFS-conjugated anti-human C1qR1/CD93 (Catalog # FAB23791F, filled histogram) or isotype control (Catalog # IC002F, 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) followed by centrifugation at 500 x g for 5 minutes. 50 μL of packed cells should then be transferred 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) 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 4×10^6 cells/mL and 25 μL of cells (1×10^5) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates 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 (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of CFS-conjugated C1qR1/CD93 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted C1qR1/CD93 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 analysis by flow cytometry.
7) As a control for analysis, cells in a separate tube should be treated with CFS-labeled mouse IgG_1 antibody.

This procedure may need modification, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

C1q R1 is also known as C1q Rp, Collectin Receptor, and AA4 antigen. C1q R1 mediates enhanced phagocytosis by monocytes and macrophages upon interaction with soluble defense collagens including C1q, MBL, and SP-A.[1, 2] It is a type I transmembrane glycoprotein expressed on endothelial cells, hematopoietic progenitor cells, platelets, and cells of myeloid origin. C1q R1 has also been identified as a stem cell marker.[3, 4]

References

1. Kim, T.S., *et al.* (2000) Mol. Immunol. **37**:377.
2. Dean, Y.D., *et al.* (2001) Eur. J. Immunol. **31**:1370.
3. Petrenko, O., *et al.* (1999) Immunity **10**:691.
4. Danet, G.H., *et al.* (2002) Proc. Natl. Acad. Sci. USA **99**:10441.

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.