Monoclonal
Anti-mouse Complement C5a R1-APC

Catalog Number: FAB6467A
Lot Number: ABVQ01
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated rat monoclonal anti-mouse Complement C5a R1: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 583837

Isotype: rat IgG_{2B}

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

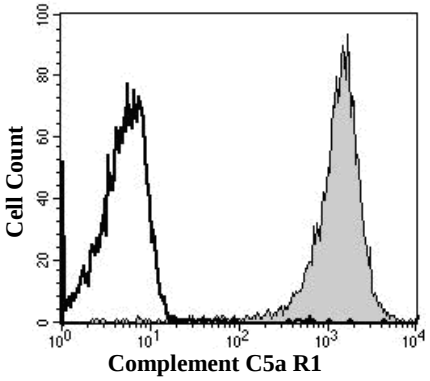
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing Complement C5a R1 within a population and qualitatively determine the density of Complement C5a R1 on cell surfaces by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with NS0 cells transfected with mouse Complement C5a R1 (C5a R1; Accession # P30993). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of Complement C5a R1 is determined by flow cytometry using 620 - 650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.



J774 cells were stained with APC-conjugated anti-mouse Complement C5a R1 (Catalog # FAB6467A, filled histogram) or APC-conjugated isotype control (Catalog # IC013A, open histogram).

Background Information

C5a R1, also known as C5a ligand and CD88, is a 7 transmembrane protein expressed on myeloid, endothelial, epithelial, and smooth muscle cells. C5a R1 binds the activated complement anaphylatoxin C5a. In established allergic environments, this triggers neutrophil and eosinophil chemotaxis and the release of proinflammatory mediators. In contrast, C5a R1/C5a interactions are protective during allergen sensitization. Mouse C5a R1 shares 66% and 77% amino acid sequence identity with human and rat C5a R, respectively.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using J774 cells.

1. Cells may be Fc-blocked with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2. After blocking, 10 μL of conjugated antibody was added to up to 1 x 10^6 cells and incubated for 30 minutes at room temperature.
3. Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Human Lyse Buffer (Catalog # FC002).
4. The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with APC-labeled rat IgG_{2B} antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

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.

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