Monoclonal Anti-mouse CD55/DAF-Phycoerythrin

Catalog Number: FAB5376P
Lot Number: ABOW01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated rat monoclonal anti-mouse CD55/DAF: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 583905

Isotype: rat IgG_{2A}

Reagents Not Provided

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

Storage

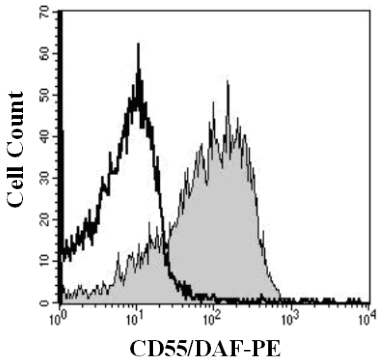
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing CD55/DAF within a population and qualitatively determine the density of CD55/DAF 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 rat immunized with purified, NS0-derived, recombinant mouse CD55 (rmCD55; aa 35-359; Accession # Q61475). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of CD55/DAF is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565-605 nm.



Mouse splenocytes were stained with PE-conjugated anti-mouse CD55/DAF (Catalog # FAB5376P, filled histogram) or PE-conjugated isotype control (Catalog # IC006P, open histogram).

Background Information

CD55 (Decay-accelerating factor/DAF) is a glycoprotein member of the RCA family of molecules. It is found on blood cells, epithelium, and endothelium and serves both as a receptor for CD97 and a negative regulator of the C3 convertases, C4b2a and C3bBb. Mature mouse CD55 is the product of two genes that arose by duplication. There is a 55-60 kDa, 356 amino acid (aa), GPI-linked form that is ubiquitously expressed. This molecule contains four SUSHI domains (aa 35-285), a Ser/Thr-rich region (aa 288-362) and a GPI-anchor at Gly362. There is also a 50 kDa, 379 aa, type I transmembrane form that is testis-associated. It shows the same domain architecture and is 93% aa identical to the GPI-form. At least four GPI gene isoforms exist. They diverge after Ile285 and show deletions and substitutions. Over aa 35-359, mouse CD55 is 66% and 50% aa identical to rat and human CD55, respectively.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse splenocytes.

1. Cells may be Fc-blocked with 1 µg of mouse 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 Mouse Lyse Buffer (Catalog # FC003).
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 PE-labeled rat IgG_{2A} 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.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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