Monoclonal
Anti-cotton rat CD8α-APC

Catalog Number: FAB7080A
Lot Number: ACON01
100 Tests

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-cotton rat CD8α: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: JG12

Isotype: mouse IgG_{2A}

Reagents Not Provided

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

Storage

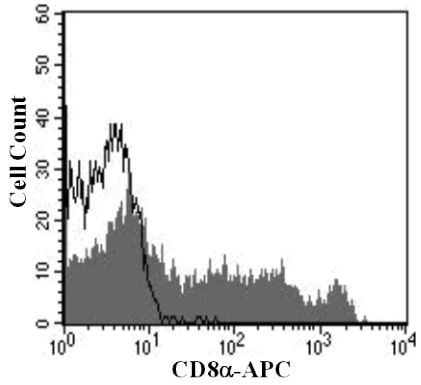
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing CD8α within a population and qualitatively determine the density of CD8α 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 NIH-3T3 cells transfected with cotton rat CD8α and SP2/O cells transfected with cotton rat CD8α (Accession # AAL55392).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 CD8α 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.



Cotton rat splenocytes were stained with APC-conjugated anti-cotton rat CD8α (Catalog # FAB7080A, filled histogram) or APC-conjugated isotype control (Catalog # IC003A, open histogram).

Background Information

CD8 alpha (CD8α; also OX8, Leu-2, and Lyt-2) is a 23 kDa (predicted) member of the Ig superfamily of molecules. It is found on immature thymocytes, cytotoxic and suppressor T cells, select mast cells, and splenic plus bone marrow dendritic cells (in rodent). On T cells, CD8α exists as either a disulfide-linked homodimer, or a heterodimer bound to CD8β. CD8αβ is best known as a co-receptor for the TCR, enhancing TCR signaling. CD8αα serves a different function and acts as a TCR co-repressor that blocks T cell activation. Based on rat, mature *Sigmodon hispidus*/cotton rat CD8α is a 210 amino acid (aa) type I transmembrane protein. It will possess a 161 aa extracellular region (aa 24 - 184) that contains one V-type Ig-like domain (aa 35 - 135), and a 30 aa cytoplasmic tail (aa 206 - 235). Although *Sigmodon hispidus* is called a rat, it is not. It is a rodent, and rat CD8α is the closest ortholog to cotton rat CD8α currently reported. Over the extracellular region, rat and cotton rat CD8α share only 54% aa identity. Over aa 24 - 182, cotton rat CD8α shares 54% and 48% aa identity with rat and mouse CD8α, respectively.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using cotton rat 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 APC-labeled mouse 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.

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