Polyclonal
Anti-canine IL-2 Rα-Phycoerythrin

Catalog Number: FAB6227P
Lot Number: ACKS01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated sheep polyclonal anti-canine IL-2Rα: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Isotype: sheep IgG

Reagents Not Provided

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

Storage

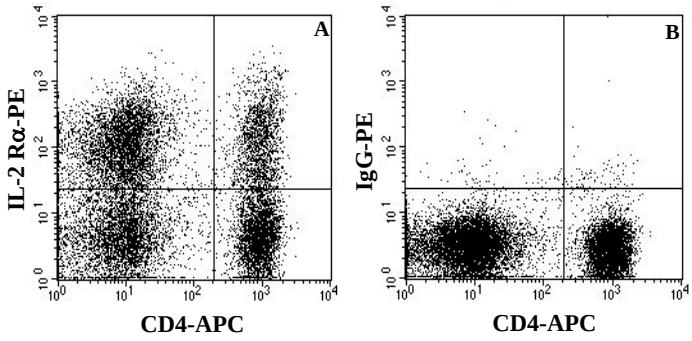
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing IL-2 Rα within a population and qualitatively determine the density of IL-2 Rα on cell surfaces by flow cytometry.

Product Description

This antibody was produced in sheep immunized with purified, NS0-derived, recombinant canine interleukin 2 receptor alpha (rcaIL-2 Rα; aa 1 - 238; Accession # BAI49682). IL-2 Rα specific IgG was purified by canine IL-2 Rα affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of IL-2 Rα 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.



Lymphocytes in canine PBMCs were stained with either A) PE-conjugated anti-canine IL-2 Rα (Catalog # FAB6227P) or B) PE-conjugated isotype control (Catalog # IC016P), followed by staining with APC-conjugated anti-canine CD4 (Catalog # FAB2410A).

Background Information

IL-2 receptor alpha (IL-2 Rα), also known as CD25, is a 55 kDa type I membrane glycoprotein that belongs to a family of cytokine receptors that utilize the common gamma chain subunit (γc). IL-2 Rα is primarily expressed on activated T cells and on regulatory T cells (Treg). IL-2 Rβ (CD122) and γc (IL-2 Rγ/CD132) dimerize to form a constitutively expressed intermediate affinity IL-2 receptor. By itself, IL-2 Rα binds IL-2 with low affinity. It associates with IL-2 Rβ and γc to generate a ternary high affinity IL-2 receptor complex. A soluble form of IL-2 Rα can be generated by proteolytic cleavage of the cell surface receptor, rendering the T cell unresponsive to IL-2. Increased serum levels of soluble IL-2 Rα are found in some cancers and immune disorders. IL-2 Rα is required for activation-induced cell death (AICD) of naive T cells, a mechanism responsible for deleting autoreactive T cell clones. IL-2 Rα is also required for the development of $CD4^+CD25^+$ Treg which suppress autoreactive $CD4^+$ T cells, thereby contributing to peripheral T cell homeostasis. Within the ECD, canine IL-2 Rα shares 49% - 60% amino acid sequence identity with human, mouse, and rat IL-2 Rα.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using lymphocytes in canine PBMCs.

1. Cells may be Fc-blocked with 1 µg of canine 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 sheep IgG 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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