

## Reagents Provided

**Peridinin-Chlorophyll-Protein-Complex (PerCP)-conjugated rat monoclonal anti-mouse IL-3 R $\alpha$ :** Supplied as 25  $\mu$ g of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 151231

Isotype: rat IgG<sub>2A</sub>

## Reagents Not Provided

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

## Storage

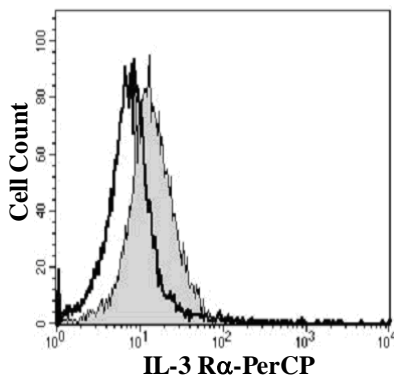
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing IL-3 R $\alpha$  within a population and qualitatively determine the density of IL-3 R $\alpha$  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, Sf21-derived, recombinant mouse Interleukin 3 Receptor alpha (rmIL-3 R $\alpha$ ) extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PerCP fluorochrome. Cell surface expression of IL-3 R $\alpha$  is determined by flow cytometry. PerCP has a maximum absorption of 482 nm and 564 nm and a maximum emission of 675 nm.



DA3 cells were stained with PerCP-conjugated anti-mouse IL-3 R $\alpha$  (Catalog # FAB983C, filled histogram) or isotype control (Catalog # IC006C, open histogram).

## Background Information

Mouse IL-3 R $\alpha$  is the ligand binding subunit in the functional heterodimeric IL-3 receptor complex. IL-3 R $\alpha$  associates either with an IL-3-specific beta subunit (IL-3 R $\beta$ /AIC2A) or with a beta subunit that is shared with receptors for IL-5 and GM-CSF (common  $\beta$  subunit/AIC2B).

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using DA3 cells.

- Cells may be Fc-blocked with 1  $\mu$ g of mouse IgG/ $10^5$  cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10  $\mu$ L of conjugated antibody was added to 1 -  $2.5 \times 10^5$  cells and incubated for 30 minutes at room temperature.
- 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).
- 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 PerCP-labeled rat IgG<sub>2A</sub> antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine 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.