

Reagents Provided

Peridinin-Chlorophyll-Protein-Complex (PerCP)-conjugated mouse monoclonal anti-human IL-3 R α : Supplied as 50 μ g of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 32703

Isotype: mouse IgG₁

Reagents Not Provided

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

Storage

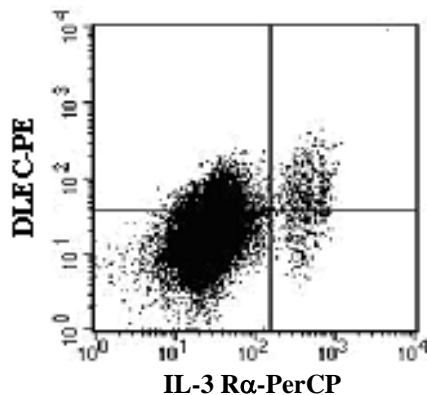
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing IL-3 R α within a population and qualitatively determine the density of IL-3 R α 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 purified, insect cell line *Sf21*-derived, recombinant human interleukin 3 soluble receptor alpha (rhIL-3 sR α). The IgG fraction of ascites fluid was purified by Protein A affinity chromatography. The purified antibody was then conjugated to PerCP fluorochrome. Cell surface expression of IL-3 R α is determined by flow cytometry. PerCP has a maximum absorption of 482 nm and 564 nm and a maximum emission of 675 nm.



PBMC monocytes were stained with PerCP-conjugated anti-human IL-3 R α (Catalog # FAB301C) and PE-conjugated anti-human DLEC (Catalog # FAB1376P). Quadrant markers are set based on isotype control staining (Catalog # IC002C and IC108P).

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FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

Background Information

IL-3 is a pleiotropic cytokine that can stimulate proliferation and differentiation of pluripotent hematopoietic stem cells as well as various lineage committed progenitors.^{1,2} IL-3 exerts its activity through binding to a specific cell surface receptor known as IL-3 R. IL-3 R is a heterodimeric structure composed of a 70 kDa IL-3 R α subunit (CDw123 or beta common) and a 120 - 140 kDa IL-3 R β subunit (CDw131).^{3,4} IL-3 R α binds IL-3 with relatively low affinity. In the presence of IL-3 R β , however, IL-3 R α has a much higher affinity for IL-3. It is not clear how signal transduction occurs following IL-3 binding. The IL-3 R α chain has a very short intracellular domain while the IL-3 R β chain has a very large cytoplasmic domain. Recent studies suggest that formation of β - β homodimers initiates signaling events.⁵ The IL-3 R β chain is also shared by the receptors for IL-5 and GM-CSF. Cells known to express IL-3 receptors include hematopoietic progenitors, epithelial cells, double negative T cells, mast cells, basophils and blood monocytes.⁶

References

- Moore, M.A.S. *et al.* (1991) *Blood* **72**:944.
- Warren, D.J. *et al.* (1988) *J. Immunol.* **140**:94.
- Plant M. *et al.* (1989) *Nature* **339**:150.
- Budel, L.M. *et al.* (1990) *Blood* **75**:1439.
- Orban, P.C. *et al.* (1999) *Blood* **94**:1614.
- Schrader, J.W. *et al.* (1988) In *Interleukin-3: The Panspecific hemopoietin* (ed. J.W. Schrader), Academic Press, San Diego, CA.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using THP-1 cells.

- Cells may be Fc-blocked with 1 μ g of human IgG/ 10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10 μ L of conjugated antibody was added to 1 - 2.5 x 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 Human Lyse Buffer (Catalog # FC002).
- 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 mouse IgG, antibody. This procedure may need to be modified, depending upon 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.