Monoclonal
Anti-mouse IL-3 Rβ/CD131-Allophycocyanin

Catalog Number: FAB5492A
Lot Number: LVI02
100 Tests

Reagent Information

Allophycocyanin (APC)-conjugated monoclonal anti-mouse IL-3 Rβ (CD131): Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 130705

Ig class: rat IgG_{2A}

Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

Storage

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

Intended Use

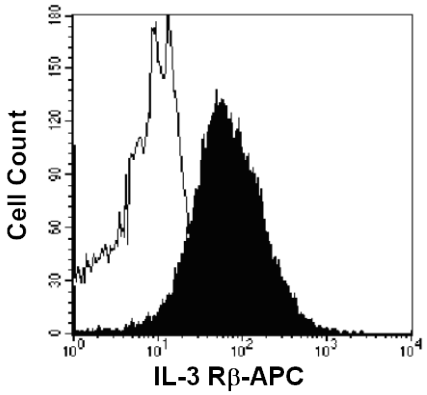
To quantitatively determine the percentage of cells expressing the cell surface receptor IL-3 Rβ (CD131) and qualitatively determine the density of this receptor on cell surfaces within a population by flow cytometry.

Principle of the Test

Cells are incubated with the APC-labeled monoclonal antibody, which binds to cells expressing the mouse IL-3 receptor beta chain. Unbound APC-conjugated antibody is then washed from the cells. Cells expressing the mouse IL-3 Rβ/CD131 are fluorescently stained, with the intensity of staining directly proportional to the density of IL-3 Rβ. Cell surface expression of IL-3 Rβ/CD131 is determined by flow cytometric analysis using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.

Reagent Preparation

APC-conjugated rat anti-mouse IL-3 Rβ/CD131: Use as is; no preparation necessary.



Mouse IL-3 dependent myeloid leukemia DA-3 cells stained with APC-conjugated anti-mouse IL-3 Rβ (Catalog # FAB5492A, filled histogram) and APC-conjugated isotype control (Catalog # IC006A, open histogram).

Sample Preparation

Tissues: Whole blood should be collected in tubes containing EDTA or heparin as the anti-coagulant. Spleen cells should be first mechanically disaggregated into a single cell suspension. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Whole blood cells will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4×10^6 cells/mL and 25 μL of cells (1×10^5) are transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

1) Cells to be used for staining with the antibody may be first Fc-blocked by treatment 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) Transfer 25 μL of the Fc-blocked cells (up to 1×10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of APC-conjugated anti-mouse IL-3 Rβ (CD131) reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove any unreacted anti-IL-3 Rβ (CD131) reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Mouse Erythrocyte Lysing Kit, Catalog # WL2000*).
6) Resuspend the cells in 200 - 400 μL of PBS buffer for final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with APC-labeled rat IgG_{2A} antibody.

This procedure may need to be modified, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

The biological effects of interleukin 3 (IL-3) are mediated by its binding to a heterodimeric receptor complex consisting of an α chain and a β chain subunit (1 - 3). The mouse IL-3 Rα subunit (CD123) is a member of the cytokine receptor superfamily and by itself binds IL-3 with low affinity (2, 4). Two distinct but homologous mouse β subunits, AIC2A (β_{IL-3}) and AIC2B (β_c)/CD131 likely arose from a gene duplication event. Both β subunits are also members of the cytokine receptor superfamily. Either subunit can combine with mouse IL-3 Rα to form a functional high affinity mouse IL-3 receptor. In addition, β_c/CD131 has been shown to be a common β subunit for mouse GM-CSF and IL-5 receptors (2, 5, 6). As a single chain entity, β_{IL-3} binds IL-3 with low affinity, whereas β_c/CD131 does not bind any cytokines, including IL-3, IL-5 and GM-CSF (3, 7). β_{IL-3} and β_c/CD131 have been shown to be co-expressed on IL-3-responsive cells (3, 7). Both the IL-3 Rα and IL-3 Rβ subunits are present on bone marrow progenitor cells, various myeloid lineage cells, as well as a subpopulation of $CD19^+$ B cells (3, 8).

References

1. Schrader, J.W. (1994) in *The Cytokine Handbook*, Thompson, A. ed., Academic Press, New York, p. 81.
2. Hara, T. and A. Miyajima (1992) EMBO J. **11**:1875.
3. Sato, N. *et al*. (1993) Blood **82**:752.
4. Itoh, N. *et al.* (1990) Science **247**:324.
5. Takaki, S. *et al*. (1991) EMBO J. **10**:2833.
6. Kitamura, T. *et al*. (1991) Proc. Natl. Acad. Sci. USA **88**:5082.
7. Gorman, D.M. *et al.* (1990) Proc. Natl. Acad. Sci. USA **87**:5459.
8. Ogorochi, T. and A. Miyajima (1994) in *Guidebook to Cytokines and Their Receptors*, Nicola N.A. ed., Oxford University Press, New York, p. 40.

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.