Monoclonal Anti-human IL-2 Rβ (CD122)-Phycoerythrin

Catalog Number: FAB224P
Lot Number: LQE02
100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human IL-2 Rβ (CD122): Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 27302

Isotype: mouse IgG_1

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

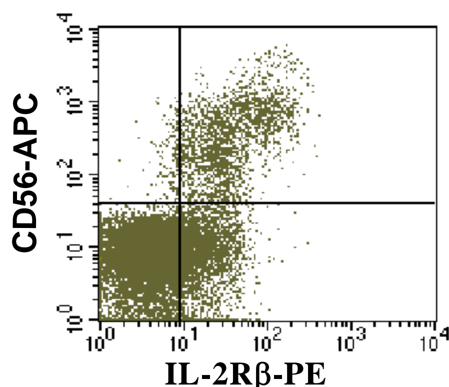
Designed to quantitatively determine the percentage of cells bearing IL-2 Rβ within a population and qualitatively determine the density of IL-2 Rβ on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody that binds to the cells expressing IL-2 Rβ. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing IL-2 Rβ become fluorescently stained, with the intensity of staining directly proportional to the density of IL-2 Rβ. Cell surface expression of IL-2 Rβ is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

PE-conjugated mouse anti-human IL-2 Rβ: Use as is; no preparation is necessary.



Human peripheral blood lymphocytes stained with PE-conjugated anti-human IL-2 Rβ (Catalog # FAB224P) and APC-conjugated anti-human CD56.

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. 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 x 10^6 cells/mL and 25 µL of cells (1 x 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 human 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 (1 x 10^5 cells) or 50 µL of packed whole blood to a 5 mL tube.
3) Add 10 µL of PE-conjugated anti-IL-2 Rβ reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-IL-2 Rβ 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 Whole Blood Lysing Kit, Catalog # WL1000*).
6) The cell pellet is resuspend 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 PE-labeled murine IgG_1 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 IL-2 are mediated by its binding to a multi-chain cellular receptor complex (1 - 4). The IL-2 receptor complex consists of three glycoprotein chains. The alpha chain (IL-2 Rα/CD25) and beta chain (IL-2 Rβ/CD122) act together with the gamma chain (IL-2 Rγ/CD132) to form a high affinity receptor that transduces the signal upon IL-2 binding (1 - 4). Alone, IL-2 Rα binds IL-2 with low affinity, but is unable to transduce a signal. The αβ combination binds IL-2 with intermediate affinity, but is still unable to transmit a signal inside the cell. The βγ complex has intermediate affinity and is capable of signaling if the concentration of IL-2 is relatively high. IL-2Rβ and the common γ chain can also associate with IL-15 Rα (5) to comprise the functional IL-15 receptor.

A soluble form of IL-2 Rα is present in the serum in parallel with its increased cellular expression (3, 6). There are reports of soluble IL-2Rβ as well (6, 8). The biologic function of soluble IL-2 Rβ is unclear because, due to its low affinity for IL-2 (8) and even lower affinity for IL-15 (8), it is expected to be a poor antagonist of cytokine activity. CD122/IL-2 Rβ is expressed by T cells, B cells, NK cells, monocytes/macrophages (1 - 4), keratinocytes (9), synoviocytes (10), fibroblasts (11, 12). Among T cells, expression of CD122 is higher on $CD8^+$ T cells than on $CD4^+$ T cells (13). Finally, IL-2R chain expression on breast cancer tissue epithelium appears to correlate with tumor malignancy (14).

References

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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.