

Reagents Provided

Biotinylated rhIL-2 (100 reactions): Lyophilized protein.

2 mL Avidin-Fluorescein: Avidin conjugated with fluorescein (10 µg/mL) at an f:p ratio of 5:1.

600 µL Negative Control Reagent (60 reactions): A protein (soybean trypsin inhibitor) biotinylated to the same degree as the cytokine (5 µg/mL).

300 µL Blocking Antibody (15 reactions): Polyclonal goat IgG anti-human IL-2 antibody.

60 mL RDF1 10X Cell Wash Buffer: A buffered saline-protein solution specifically designed to minimize background staining and stabilize specific binding.

Reagents Not Provided

- PBS (Dulbecco's PBS)
- human or mouse IgG

Storage

All Reagents: 2° - 8° C

Intended Use

Designed to quantitatively determine the percentage of cells bearing cytokine receptors within a population and to estimate the receptor density for the human cytokine IL-2 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the biotinylated cytokine that in turn binds to the cells via specific cell surface receptors. The cells are then directly incubated with avidin-fluorescein, which attaches to the receptor-bound biotinylated cytokine. Unbound biotinylated cytokine participates in an amplification reaction that results in an enhanced signal without compromising specificity. Cells expressing the specific cytokine receptors are fluorescently stained, with the intensity of staining directly proportional to the density of the receptors. Receptor density is then determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Biotinylated rhIL-2: Add 1 mL of sterile distilled water to the lyophilized product. Mix gently until the product is completely dissolved. **Reagent yields optimal activity when used within 60 days after reconstitution.**

Blocking Antibody: If lyophilized, reconstitute by adding 300 µL of sterile distilled water.

1X RDF1 Wash Buffer: Add 1 mL of 10X concentrate to 9 mL of sterile distilled water. Reagent should be maintained on ice during use and stored at 2° - 8° C for no more than 2 months.

Sample Preparation

Peripheral Blood Cells: Whole blood collected in heparinized tubes should be processed by standard Ficoll-Hypaque gradient separation techniques to isolate mononuclear cells. Ficoll and contaminating serum components should be removed by washing the cells twice with 10 mM PBS and then resuspending the cells in 10 mM PBS. Cells should be resuspended to a final concentration of 4×10^6 cells/mL in 10 mM PBS.

Cultured Cells: Cells from continuous or activated cultures should be centrifuged at 500 x g for 5 minutes and then washed twice with 10 mM PBS to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in 10 mM PBS to a final concentration of 4×10^6 cells/mL.

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) Add 10 µL of biotinylated cytokine reagent to 25 µL of the washed cell suspension in a 12 x 75 mm tube for a total reaction volume of 35 µL. As a negative staining control, an identical sample of cells should be stained with 10 µL of biotinylated negative control reagent.
- 2) Incubate the cells for 30 - 60 minutes at 2° - 8° C.
- 3) Add 10 µL of avidin-FITC reagent to each tube. **(DO NOT WASH CELLS PRIOR TO ADDING AVIDIN-FITC).**
- 4) Incubate the reaction mixture for a further 30 minutes at 2° - 8° C **in the dark.**
- 5) Wash the cells twice with 2 mL of 1X RDF1 buffer to remove unreacted avidin-fluorescein and resuspend the cells in approximately 0.2 mL of 1X RDF1 for final flow cytometric analysis.

Specificity Testing

- 1) (Optional) Cells that are to be stained could be pretreated with purified mouse or human IgG (10 µL of 1 mg/mL/ 10^6 cells) for 15 minutes at room temperature in order to block Fc-mediated interactions. *(Cells should not be washed of excess IgG for this assay).*
- 2) **In a separate tube**, anti-human IL-2 blocking antibody is mixed with 10 µL of IL-2-biotin and allowed to incubate for 15 minutes at room temperature.
- 3) To the tube containing the anti-IL-2 blocking antibody and Fluorokine mixture, add 1×10^5 Fc-blocked cells in a volume of 25 µL.
- 4) The reaction is then allowed to proceed as described in steps 2 - 5 above.

Background Information

IL-2 is a 133 amino acid glycoprotein produced by activated T helper cells (1). Approximately 17 kDa in size, IL-2 exhibits a number of growth and maturational effects including T and B cell proliferation (2, 3), stimulation of lactoferrin secretion in neutrophils (4) and the induction of LAK cells from activated NK cells (5). The receptor for IL-2 (IL-2 R) is a three-subunit ($\alpha\beta\gamma$) complex with the signal-transducing γ chain also shared by the receptors for IL-4, 7, 9 and 15 (6 - 11). In general, cells do not express all of the IL-2 R subunits necessary to elicit a physiological response. Nevertheless, cells can be induced to express all three receptor chains upon activation. Fully functional receptors have been reported on T cells, B cells, monocytes and large granular lymphocytes (NK cells) (12). IL-2 has been shown to bind at varying affinities to IL-2 R α , IL-2 R $\alpha\beta$, IL-2 R $\beta\gamma$ and IL-2 R $\alpha\beta\gamma$ (6). The presence of various combinations of receptor subunits coupled with information on the relative number of IL-2 receptors on the surface of cells, can provide information on the general activation state of individual cells, as well as their subsequent biological activity.

References

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Technical Notes

The concentration of Fluorokine in the kit has been optimized for staining peripheral blood lymphocytes. We have observed that some cell lines and transfected cells appear to have unusual background reactivity with some Fluorokines. This is thought to be a consequence of changes in cell surface structures as a result of long term culture or gene transfection. In many cases, simply limiting the amount of Fluorokine in the reaction mixture can circumvent this obstacle. Dilution of the IL-2 Fluorokine to doses ranging from 1 to 0.3 μ g/mL can completely reduce any background staining of IL-2 R transfected NS0 cells while still detecting over 90% of the transfected cells. Additionally, the blocking antibody inhibits > 99% of the Fluorokine signal and 100 molar excess of IL-2 inhibits > 95% of the same signal. We recommend that each user titrate the Fluorokine for the intended test system such that the intensity of the staining reaction on known receptor positive cells gives negligible staining of known receptor negative cells. Suitable cells for testing for the presence of IL-2 receptors are: PHA-activated peripheral blood lymphocytes, the murine cell line HT-2 and IL-2 R transfected cell lines.

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.