Fluorokine®
Biotinylated Human IL-4

Catalog Number: NF400
100 Tests

Reagents Provided

Biotinylated recombinant human IL-4 (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-4 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

- 10 mM 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 human IL-4 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 with the bound cytokine that results in an enhanced signal without compromising specificity. Cells expressing the specific cytokine receptors are fluorescently stained, with the intensity of staining proportional to the density of the receptors. Relative receptor density is then determined by flow cytometry using 488 nm wavelength laser excitation.

Reagent Preparation

Biotinylated IL-4: 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 of reconstitution.**

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. Resuspend the cells in 10 mM PBS to a final concentration of 4 x 10^6 cells/mL.

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 x 10^6 cells/mL.

Note: *Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates 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

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.

1. Incubate the cells for 20-60 minutes at 2-8 °C.
2. Add 10 μL of avidin-FITC reagent to each tube. **(Do not wash cells prior to adding avidin-FITC)**.
3. Incubate the reaction mixture for a further 30 minutes at 2-8 °C **in the dark**.
4. 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-4 blocking antibody is mixed with 10 μL of IL-4-biotin and allowed to incubate for 15 minutes at room temperature.
3. To the tube containing the anti-human IL-4 blocking antibody and labeled cytokine mixture, add 1 x 10^5 Fc-blocked cells in a volume of not more than 25 μL.
4. The reaction is then allowed to proceed as described in steps 1-4 above.

Background Information

R&D Systems, Inc.
1-800-343-7475

Human interleukin 4 (IL-4) is a small, glycosylated, hematopoietically-derived cytokine that exhibits a broad spectrum of activities. Approximately 20 kDa in size, the mature molecule is 129 amino acids long and contains at least one intrachain disulfide bond that is essential for activity. Species-specificity has been demonstrated between human and mouse (1, 2). Production of IL-4 is highly regulated and cells known to produce IL-4 include mast cells, basophils, $CD8^+$ memory T cells, $CD4^+$ ($CD7^-$) Th0 and Th2 cells, and $\gamma\delta$ T cells (1, 3-7). IL-4 is a key regulator in the development of Th2 type responses. The effects of IL-4 are thought to be mediated through a high-affinity receptor (IL-4 R) with a K_D = 20-300 pM. A current model suggests that this IL-4 receptor structure is composed of a 140 kDa, low-affinity (K_D = 30 nM) IL-4 binding α-subunit that is complexed to a 64 kDa, signal-transducing subunit that is also known as the IL-2 receptor gamma chain (common γ-chain; γc) (8-10). Although the 140 kDa component appears to be the cytokine-binding subunit, recent evidence raises questions regarding the necessity for a functional γc (11). The γc is thought to stabilize and regulate the affinity of the IL-4 R. Receptors for IL-4 and IL-13 appear to share some components (11, 12). Normal cells known to express IL-4 R include T cells, thymocytes, B cells, monocytes, fibroblasts and endothelial cells. Cell lines derived from mast cells, lymphocytes, macrophages, keratinocytes, muscle, erythrocytes and melanocytes have been demonstrated to also express IL-4 R (2, 10, 13-15).

References

1. Callard, R.E. (1991) Br. J. Hematol. **78**:293.
2. Paul, W.E. and J. Ohara (1987) Annu. Rev. Immunol. **5**:429.
3. Mueller, R. *et al*. (1994) Eur. J. Immunol. **24**:2935.
4. Ferrick, D.A. *et al*. (1995) Nature **373**:255.
5. Lagoo, A.S. *et al*. (1994) Eur. J. Immunol. **24**:3087.
6. Del Prete, G. *et al*. (1994) Lab Invest. **70**:299.
7. Autran, B. *et al*. (1995) J. Immunol. **154**:1408.
8. Keegan, A.D. *et al*. (1994) Immunol. Today **15**:423.
9. Russell, S.M. *et al*. (1993) Science **262**:1880.
10. Foxwell, B.M.J. *et al*. (1989) Eur. J. Immunol. **19**:1637.
11. Matthews, D.J. *et al*. (1995) Blood **85**:38.
12. Smerz-Bertling, C. and A. Duschl (1995) J. Biol. Chem. **270**:966.
13. De Wit, H. *et al*. (1994) Blood **84**:608.
14. Obiri, N.I. *et al*. (1993) J. Clin. Invest. **91**:88.
15. Lowenthal, J.W. *et al*. (1988) J. Immunol. **140**:456.

Technical Notes

The human IL-4 biotin kit has been tested for its ability to stain the human Burkitt lymphoma Raji cell line (Figure 1). Staining specificity has been determined by demonstrating a reduction in signal intensity when the staining reaction is carried out in the presence of a specific anti-IL-4 blocking antibody. These inhibition reactions were carried out under limiting concentrations of IL-4-biotin. Some cell lines can exhibit high non-specific staining with labeled proteins. This effect can be compensated for by reducing the amount of labeled protein used in the reaction. We suggest that each user determine the optimal concentration of labeled protein by performing a dilution curve staining analysis on known receptor positive and negative cells. Normally, dilutions ranging from 1:2 to 1:10 are sufficient. Dilution of the labeled protein should be made in 1X Cell Wash Buffer.

Typical Data

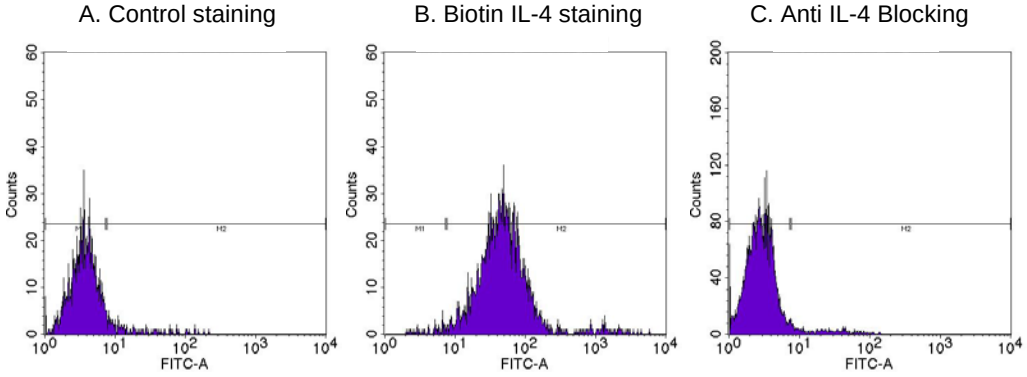

Figure 1. Human Raji Burkitt lymphoma cells were tested using the Human IL-4 Biotinylated Fluorokine Kit (Catalog # NF400). Cells stained with the negative control protein **(A)** or with recombinant human IL-4 biotin **(B)** are shown. IL-4 staining can be specifically blocked with the anti-IL-4 antibody **(C)**. Fluorescein-conjugated Avidin was used as secondary stain. All reagents shown are provided with the kit.

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.