Fluorokine®
Biotinylated Human Eotaxin

Catalog Number: NFEO0
100 Tests

Reagents Provided

Biotinylated rhEotaxin (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-Eotaxin 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 chemokine Eotaxin 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 rhEotaxin: 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.**

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 pre-treated 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 Eotaxin blocking antibody is mixed with 10 µL of Eotaxin-biotin and allowed to incubate for 15 minutes at room temperature.
3) To the tube containing the anti-Eotaxin blocking antibody and Fluorokine mixture, add 1 x 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.

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Background Information

Human eotaxin is an 8.3 kDa protein that is a member of the CC or β chemokine family (1, 2). Eotaxin is a potent chemoattractant for eosinophils. Eotaxin exhibits species cross reactivity (3, 4) reflected by the 60% amino acid homology observed between human, murine, and guinea pig eotaxin (1, 5, 6).

Eotaxin bioactivity is mediated through the CCR3 receptor, a G protein-coupled receptor expressed on eosinophils, basophils and on a minor population of Th2 type T cells (1, 7, 8). The Kd for eotaxin binding to the CCR3 receptor is estimated to be 0.5 nM (1, 9). Eotaxin may work in concert with IL-5 to rapidly mobilize eosinophils from the bone marrow following exposure to an allergen (10).

References

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Technical Note

The specificity of the Eotaxin Fluorokine has been established by testing for positive staining on cells transfected with the human CCR3 receptor and on peripheral blood eosinophils. No staining is observed on non-transfected cells, as well as other peripheral blood cell populations. Furthermore, it is possible to inhibit Eotaxin Fluorokine staining in the presence of 50 - 100 fold molar excess of unlabeled eotaxin.

Some cell lines can exhibit unusually high non-specific staining with Fluorokines. Limiting the amount of Fluorokine used in the staining reaction can reduce this effect. We recommend that each user determine the optimal concentration of Fluorokine for staining different cells. This involves diluting the Fluorokine from the provided concentration until a distinguishable positive and negative signal is observed when the Fluorokine is used to stain known receptor-positive and receptor-negative cells. Normally, doubling dilutions up to 1:10 of the provided concentration are adequate. Fluorokine dilutions should be made using 1X RDF1 buffer as the diluent.

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.