Fluorokine®
Biotinylated Human IL-10

Catalog Number: NF100
100 Tests

Reagents Provided

Biotinylated rhIL-10 (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-10 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 human IL-10 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-10: 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. Cells should be then 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 1X RDF1 wash buffer 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-10 blocking antibody is mixed with 10 μL of IL-10-biotin and allowed to incubate for 15 minutes at room temperature.
3) To the tube containing the anti-IL-10 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.

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Background Information

Interleukin 10 (IL-10) is a variably glycosylated polypeptide ranging from 32 kDa to 40 kDa in molecular weight (1). Produced by B cells, keratinocytes, macrophages, $CD4^+CD45RA^+$ cells and $CD4^+$ TH2 and $CD8^+$ TH2-like cells, IL-10 exhibits approximately 75% interspecies amino acid similarity (1,3). Differences between murine IL-10 (mIL-10) and human IL-10 (hIL-10) include glycosylation of mIL-10 but not hIL-10, and cross-reactivity of hIL-10 in the murine system but no activity of mIL-10 on the human cells (4). Functionally, IL-10 stimulates macrophages to produce IL-1 receptor antagonist and CD64, the latter leading to an increase in ADCC (5). IL-10 also induces IgA synthesis in CD40-activated B cells, and IgG_1 and IgG_3 synthesis in $CD40^+IgD^+$ (naive) B cells (6, 7). IL-10 indirectly suppresses IFN-γ production by TH1 cells (8, 9), Class II MHC and proinflammatory cytokine production by macrophages (10, 11), and TNF-α plus chemokine secretion by eosinophils and neutrophils (12, 13). A 110 kDa receptor for IL-10 (IL-10 R) has been characterized that most closely resembles the receptor for IFN-γ (14, 15). Results from transfection studies utilizing murine and human IL-10Rs confirm functional studies that show mIL-10 and hIL-10 binding to murine IL-10 R but only hIL-10 binding to human IL-10 R (14). Within the murine system, cell lines known to express IL-10 R include B cells, $CD4^+$ TH1 cells, macrophages, mast cells and LPS-stimulated fibroblasts (16, 17).

References

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Technical Notes

The IL-10-biotin Fluorokine has been tested on peripheral blood lymphocytes, monocytes/macrophages. Specificity of the staining reaction has been established by the ability to inhibit the IL-10-biotin staining in the presence of 1 - 10 molar excess of unconjugated IL-10 or polyclonal anti-IL-10 antibody. Where unconjugated IL-10 is used to compete with IL-10-biotin for receptor binding, we recommend the following test protocol: i) incubate cells with IL-10 for 30 minutes at RT, ii) add 10 molar excess of IL-10-biotin to the tube and incubate for an additional 10 minutes also at RT, iii) develop the reaction with 10 μL of avidin-FITC for 5 minutes at RT, iv) wash cells twice with 1X RDF1 buffer and then read as indicated above. This staining strategy minimizes the modulation of IL-10 receptor density on the surface of cells exposed to IL-10, which in turn can confound data interpretation. Some cell lines exhibit unusual background staining with Fluorokines. This can be resolved by limiting the amount of Fluorokine in the staining reaction. We recommend that each investigator determine the optimal concentration for their test system. This can be done by diluting the Fluorokine up to 1:10 in 1X RDF1 buffer and then testing it on known receptor positive and negative cells. The optimal concentration of Fluorokine is that which gives optimal signal separation on the two cell types above. Competition experiments that inhibit Fluorokine staining and are used to demonstrate the specificity of the reaction are best performed under limiting concentrations of Fluorokine.

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.