R&D SYSTEMS
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Fluorokine® Biotinylated Human IL-10

Catalog Number: NF100
100 Tests

REAGENTS PROVIDED

Biotinylated recombinant human (rh)IL-10 (Part 860093;100 reactions): Lyophilized protein.

Avidin-Fluorescein (Part 860069): 2.0 mL of avidin conjugated with fluorescein (10 µg/mL) at an f:p ratio of 5:1.

Negative Control Reagent (Part: 860070; 60 reactions): 600 µL of a protein (soybean trypsin inhibitor) biotinylated to the same degree as the cytokine (5 µg/mL).

Blocking Antibody (Part 860094; 15 reactions): 300 µL of polyclonal goat IgG anti-human IL-10 antibody.

Cell Wash Buffer 10X (Part 860072): 60 mL of a buffered saline protein solution specifically designed to minimize background staining and stabilize specific binding.

OTHER REAGENTS REQUIRED

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

STORAGE AND STABILITY

Store the unopened kit at 2-8 °C. Do not use past kit expiration. Diluted Cell Wash Buffer 1X may be stored at 2-8 °C for up to 2 months. Diluted biotinylated rhIL-10 must be used within 60 days.

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 1.0 mL of sterile distilled water to the lyophilized product. Mix gently until the product is completely dissolved.

1X Cell Wash Buffer: Add 1.0 mL of 10X concentrate to 9.0 mL of sterile distilled water. Reagent should be maintained on ice during use.

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 x 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 Cell 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 x 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 re-attachment to the substrate.*

PRECAUTION

Some components in this kit contain sodium azide which may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.

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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-Fluorescein reagent to each tube. **(DO NOT WASH CELLS PRIOR TO ADDING AVIDIN FLUORESCEIN).**
4) Incubate the reaction mixture for a further 30 minutes at 2-8 °C **in the dark**.
5) Wash the cells twice with 2.0 mL of 1X Cell Wash Buffer to remove unreacted Avidin-Fluorescein and resuspend the cells in approximately 0.2 mL of 1X Cell Wash Buffer 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 1.0 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 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 in Sample Staining.

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 TNFα 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-10 receptors 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).

TECHNICAL NOTES

The IL-10-biotin 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 Fluorescein for 5 minutes at RT, iv) wash cells twice with 1X Cell Wash 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 IL-10-biotin. This can be resolved by limiting the amount of IL-10-biotin in the staining reaction. We recommend that each investigator determine the optimal concentration for their test system. This can be done by diluting the IL-10-biotin up to 1:10 in 1X Cell Wash Buffer and then testing it on known receptor positive and negative cells. The optimal concentration of IL-10-biotin is that which gives optimal signal separation on the two cell types above. Competition experiments that inhibit IL-10-biotin staining and are used to demonstrate the specificity of the reaction are best performed under limiting concentrations of IL-10-biotin.

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