Fluorokine®
Biotinylated Human TGF-β1

Catalog Number: NFTG0
100 Tests

Reagents Provided

Biotinylated rhTGF-β1 (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 chicken IgY anti-human TGF-β1 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 TGF-β1 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 rhTGF-β1: 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 ≈ 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**, 20 μL of anti-human TGF-β1 blocking antibody is mixed with 10 μL of TGF-β1-biotin and allowed to incubate for 15 minutes at room temperature.
3) To the tube containing the anti-TGF-β1 blocking antibody and Fluorokine mixture, add 1×10^5 Fc-blocked cells in a volume of not more than 50 μL.
4) The reaction is then allowed to proceed as described in steps 2 - 5 above.

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Background Information

Transforming growth factor-beta (TGF-β) is a 25 kDa disulfide-linked dimer of usually two identical protein chains of 112 amino acids (1). Generally, TGF-β is found to have stimulatory activity on cells of mesenchymal origin and inhibitory action on cells of epithelial and neuroectodermal origin. Five isoforms of TGF-β (TGF-β1-5) have been described (2, 3), all being synthesized from precursor molecules that are biologically inactive when complexed with the latency associated protein (LAP). Dissociation from LAP renders TGF-β biologically active. Although numerous cell surface structures have been described to bind TGF-β (4), three receptor subunits are considered true TGF-β receptors: type I (53 kDa), type II (70 - 85 kDa) and type III (250 - 350 kDa) (5 - 8). Type I and II receptors are transmembrane serine/threonine kinases, while type III receptor is a transmembrane proteoglycan (betaglycan) (9). Intracellular signaling as a result of TGF-β binding to cell surface receptors occurs primarily through the type I and II receptors (10). Functional TGF-β receptors are thought to be homodimers and heteromeric complexes of the type I and II chains (11, 12). One model of TGF-β binding suggests that the role of the type III receptor (betaglycan) is to initially bind TGF-β and present it to the type II receptor unit. The binding of the type I chain in turn releases the type III receptor and produces a high affinity receptor (composed of type I and II chains). Under these conditions, TGF-β1 & 2 bind equally well to these structures. Cells that lack the type III receptor appear to express a high affinity type I and type II receptor and bind TGF-β1 better than TGF-β2 (12, 13).

References

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Technical Notes

Human-TGF-β1-biotin has been tested for its ability to stain cell lines such as TF-1 and prostatic cell tumors. Additionally, peripheral blood monocytes and a TGF-β-receptor type II transfected cell line have been tested for TGF-β1-biotin binding. Specificity of the staining reaction was tested by demonstrating > 95% inhibition of staining of the TGF-β R type II transfectant with the TGF-β1-biotin reagent in the presence of 10 molar excess of unconjugated TGF-β1. Furthermore, the staining was also completely inhibited in the presence of the anti-TGF-β1 blocking antibody. The provided concentration of Fluorokine has been optimized for staining of selected tissues. In view of the varying types and levels of TGF-β-receptors on different tissues, we highly recommend that each investigator determine the optimal concentration of Fluorokine to be used in the staining reaction. We suggest that this be determined, where possible, by testing the staining pattern on known receptor positive (*e.g.* TF-1) and known receptor negative cells (*e.g.* NS0). In the event of unusually high staining in a known receptor negative cell, we suggest diluting the Fluorokine reagent by doubling dilutions in 1X RDF1 until there is a distinguishable difference in the staining pattern between the two types of cells.

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.