Fluorokine®
Biotinylated Human TNF-α

Catalog Number: NFTA0
100 Tests

Reagents Provided

Biotinylated rhTNF-α (100 reactions): Lyophilized protein.

2 mL Avidin-Fluorescein: Avidin conjugated with fluorescein (10ug/mL) at an f:p ratio of 5:1.

600uL Negative Control Reagent (60 reactions): A protein (soybean trypsin inhibitor) biotinylated to the same degree as the cytokine (5ug/mL).

300 uL Blocking Antibody (15 reactions): Polyclonal goat IgG anti-human TNF-α 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 TNF-α on cell surfaces by flow cytometer.

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 rhTNF-α: 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 uL of biotinylated cytokine reagent to 25 uL of the washed cell suspension in a 12 x 75 mm tube for a total reaction volume of 35 uL. As a negative staining control, an identical sample of cells should be stained with 10uL of biotinylated negative control reagent.
2) Incubate the cells for 30-60 minutes at 2 - 8° C.
3) Add 10uL 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 uL 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 uL of anti-human TNF-α blocking antibody is mixed with 10 uL of TNF-α-biotin and allowed to incubate for 15 minutes at room temperature.
3) To the tube containing the anti-TNF-α blocking antibody and Fluorokine mixture, add 1×10^5 Fc-blocked cells in a volume of 25uL.
4) The reaction is then allowed to proceed as described in steps 2 - 5 above.

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Background Information

Tumor necrosis factor alpha (TNF-α), also known as cachectin and TNFSF1A, is the prototypic ligand of the TNF superfamily. It is a pleiotropic molecule that plays a central role in inflammation, apoptosis, and immune system development. TNF-α is produced by a wide variety of immune and epithelial cell types (1, 2). Human TNF-α consists of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 177 aa extracellular domain (ECD) (3). Within the ECD, human TNF-α shares 97% aa sequence identity with rhesus and 71% 92% with bovine, canine, cotton rat, equine, feline, mouse, porcine, and rat TNF-α. The 26 kDa type 2 transmembrane protein is assembled intracellularly to form a noncovalently linked homotrimer (4). Ligation of this complex induces reverse signaling that promotes lymphocyte co-stimulation but diminishes monocyte responsiveness (5). Cleavage of membrane bound TNF-α by TACE/ADAM17 releases a 55 kDa soluble trimeric form of TNF-α (6, 7). TNF-α trimers bind the ubiquitous TNF RI and the hematopoietic cell restricted TNF RII, both of which are also expressed as homotrimers (1, 8). TNF-α regulates lymphoid tissue development through control of apoptosis (2). It also promotes inflammatory responses by inducing the activation of vascular endothelial cells and macrophages (2). TNF-α is a key cytokine in the development of several inflammatory disorders (9). It contributes to the development of type 2 diabetes through its effects on insulin resistance and fatty acid metabolism (10, 11).

References

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Technical Notes

The human TNF-α biotin kit has been tested for its ability to stain human monocyte-like histiocytic lymphoma U-937 cells (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-TNF-α blocking antibody. These inhibition reactions were carried out under limiting concentrations of TNF-α 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 labeled protein should be made in 1X Cell Wash Buffer.

Typical Data

A. Control staining **B.** Biotin TNF-α staining **C.** Anti-TNF-α blocking

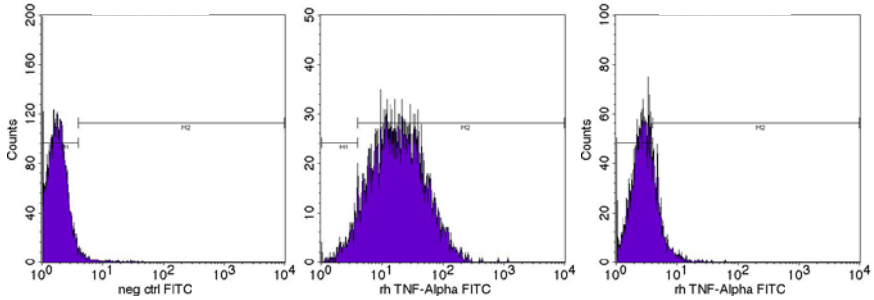

Figure 1. Human U-937 cells were tested using the TNF-α biotin kit (NFTA0). Cells stained with Negative Control protein **(A)** or with rhTNF-α biotin **(B)** are shown. TNF-α staining can be specifically blocked with the anti-human TNF-α antibody **(C).** Fluorescein-conjugated Avidin was used as secondary 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.*