Fluorokine®
Biotinylated Human β-NGF

Catalog Number: NFNGF
100 Tests

Reagents Provided

Biotinylated recombinant human (rh) β-NGF (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 studied protein.

Blocking Antibody (15 reactions): Polyclonal goat IgG anti-human β-NGF antibody.

60 mL 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 qualitatively and quantitatively determine the presence of cells expressing NGF receptors or other interacting proteins by flow cytometry.

Principle of the Test

Washed cells are incubated with the biotinylated protein that in turn binds to the cells via specific cell surface receptors or other interacting proteins. The cells are then directly incubated with avidin-fluorescein, which attaches to the bound biotinylated protein. Cells expressing the receptor/interacting protein are fluorescently stained, with the intensity of staining proportional to the density of such protein. Relative density is then determined by flow cytometry using 488 nm wavelength laser excitation.

Reagent Preparation

Biotinylated rhβ-NGF: 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 of reconstitution.**

Blocking Antibody: If lyophilized, reconstitute by adding 300 μL of sterile distilled water.

1X Cell 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 PBS and then resuspending the cells in PBS or 1X Cell Wash Buffer. The cells should be resuspended to a final concentration of 4×10^6 cells/mL.

Cultured Cells: Cells from continuous or activated cultures should be centrifuged at 500 x g for 5 minutes and then washed twice with PBS to remove any residual growth factors that may be present in the culture medium. The cells should then be resuspended in 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 their substrates. Cells that require trypsinization to enable removal from their substrates 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

Add 10 μL of biotinylated protein 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.

1. Incubate the cells for 30-60 minutes at 2°-8° C.
2. Add 10 μL of avidin-FITC reagent to each tube. **(DO NOT WASH CELLS PRIOR TO ADDING AVIDIN-FITC)**.
3. Incubate the reaction mixture for a further 30 minutes at 2-8 °C **in the dark**.
4. Wash the cells twice with 2 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 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**, 20 μL of anti-human NGF blocking antibody is mixed with 10 μL of NGF-biotin and allowed to incubate for 15 minutes at room temperature.
3. To the tube containing the anti-NGF 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 1-4 above.

Background Information

NGF was initially isolated in the mouse sub-mandibular gland as a 7S complex composed of three non-covalently linked subunits, α, β, and γ. Both the α and γ subunits of NGF are members of the kallikrein family of serine proteases. The γ-NGF subunit is capable of processing the precursor form of β-NGF, whereas α-NGF is an inactive serine protease. β-NGF is a 12.5-kDa protein that contains all the survival and neurite-promoting activities associated with NGF. The human protein shares approximately 90% homology at the amino acid level with both mouse and rat β-NGF and exhibits cross species activity.

NGF is a well characterized neurotropic protein that plays a critical role in the development of sympathetic and some sensory neurons in the peripheral nervous system. In addition, NGF can also act in the central nervous system as a trophic factor for basal forebrain cholinergic neurons. NGF has also been shown to have biological effects on non-neuronal tissues. NGF is mitogenic for a factor-dependent human erythroleukemic cell line, TF1. NGF has been found to increase the number of mast cells in neonatal rats and to induce histamine release from peritoneal mast cells. NGF will enhance histamine release and strongly modulate the formation of lipid mediators by basophils in response to various stimuli. NGF will also induce the growth and differentiation of human B lymphocytes as well as suppress apoptosis of murine peritoneal neutrophils. These results, taken together, suggest that NGF is a pleiotropic cytokine which, in addition to its neurotropic activities, may have an important role in the regulation of the immune system.

Technical Notes

The human NGF biotin kit has been tested for its ability to stain human myelogenous leukemia K562 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-β-NGF blocking antibody. These inhibition reactions were carried out under limiting concentrations of β-NGF-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 β-NGF staining **C.** Anti-β-NGF Blocking

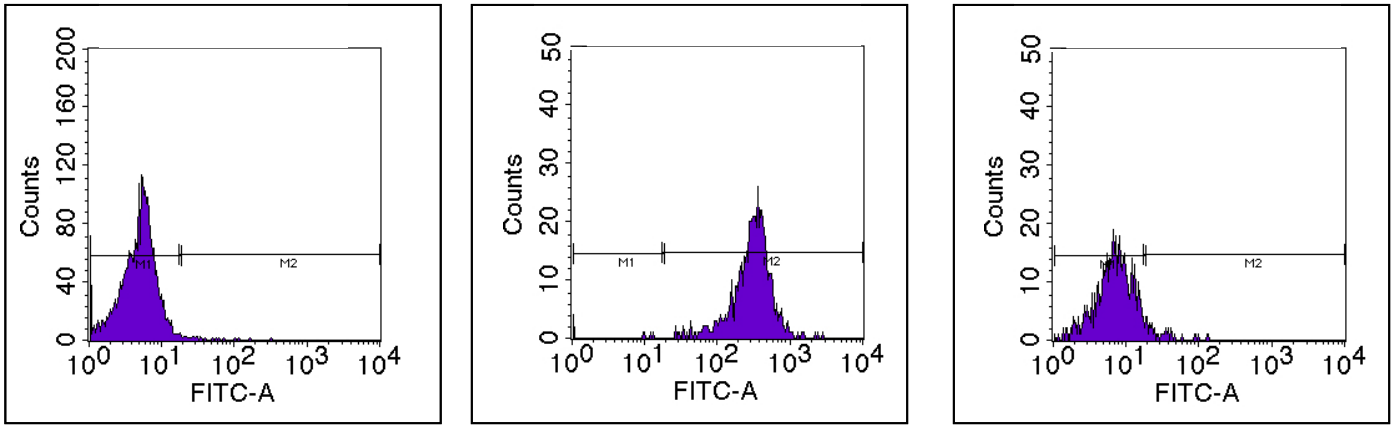

Figure 1. Human K562 myelogenous leukemia cells were tested using the Human β-NGF Biotinylated Fluorokine Kit (Catalog # NFNGF). Cells stained with the Negative Control protein (**A**) or with rhβ-NGF biotin (**B**) are shown. NGF staining can be specifically blocked with the anti-human β-NGF antibody (**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.