Fluorokine®
Biotinylated Human HGF

Catalog Number: NFHGF
100 Tests

Reagents Provided

Biotinylated rhHGF (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).

Blocking Antibody (15 reactions): Polyclonal goat IgG anti-human HGF 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 expressing HGF receptors within a population, and to estimate the receptor density for HGF 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 with the bound cytokine that results in an enhanced signal without compromising specificity. Cells expressing the specific cytokine receptors are fluorescently stained, with the intensity of staining proportional to the density of the receptors. Relative receptor density is then determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Biotinylated rhHGF: 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 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 PBS and then resuspending the cells in PBS or 1X RDF1 buffer. 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. 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 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.

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 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**, 20 μL of anti-human HGF blocking antibody is mixed with 10 μL of HGF-biotin and allowed to incubate for 15 minutes at room temperature.
3. To the tube containing the anti-HGF 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.

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Background Information

Hepatocyte growth factor (HGF) regulates cell growth, cell motility, and morphogenesis by activating the receptor tyrosine kinase, c-Met. HGF is secreted by mesenchymal cells and acts as a multi-functional cytokine primarily on epithelial cells, but also on cells of endothelial origin. Its ability to stimulate mitogenesis, cell motility, and matrix invasion gives HGF a central role in angiogenesis, tumorigenesis, and tissue regeneration. Native human HGF is secreted as a biologically inactive single chain pro-peptide that is cleaved by an extracellular serum serine protease into a 69-kDa α-chain and 34-kDa β-chain. Mature, biologically active HGF consists of a disulfide-linked heterodimer of the two cleavage products, α and β. The α chain NH_2-terminal portion contains the high-affinity c-Met receptor-binding domain, but the β chain is required to interact with the c-Met receptor for receptor activation. The NS0-derived recombinant HGF protein provided in this kit is greater than 95% heterodimeric HGF.

Technical Notes

The human HGF biotin kit has been tested for its ability to stain human adenocarcinoma MDA-MB-231 cells. Staining specificity has been determined by demonstrating a reduction in signal intensity when the staining reaction is carried out in the presence of 1 - 100 molar excess unconjugated HGF or in the presence of the blocking antibody. These inhibition reactions were carried out under limiting concentrations of HGF-biotin.

Some cell lines can exhibit high non-specific staining with labeled cytokines. This effect can be compensated for by reducing the amount of labeled cytokine used in the reaction. We suggest that each user determine the optimal concentration of labeled cytokine 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 cytokine should be made in 1X RDF1 Wash Buffer.

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.