Fluorokine®
Biotinylated Mouse SCF

Catalog Number: NFMKL0
100 Tests

Reagents Provided

Biotinylated rmSCF (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 goat IgG anti-mouse SCF 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 murine cytokine stem cell factor (SCF) 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 rmSCF: 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

Blood, Lymph Node or Spleen Cells: Whole blood should be collected in heparinized tubes. Tissues should be disaggregated into a single cell suspension. Cells may then be processed by standard gradient separation techniques to isolate mouse mononuclear cells. Contaminating RBC should be removed by either gradient sedimentation or by hypotonic lysis methods. Excess gradient media and contaminating serum components should be removed by washing the cells twice with 10 mM PBS and then resuspended in 10 mM PBS 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 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 approximately 0.2 mL of 1X RDF1 for final analysis.

Recommended Specificity Testing

1) (Optional) Cells that are to be stained could be pretreated with purified mouse or human IgG (10 µg /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-mouse SCF blocking antibody is mixed with 10 µL of SCF-biotin and allowed to incubate for 15 minutes at room temperature.
3) To the tube containing the anti-SCF 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 2 - 5 above.

R&D Systems, Inc.
1-800-343-7475

Background Information

SCF (also known as *c-kit* ligand, or KL) exists as either a membrane-bound or soluble molecule (1, 2, 3, 4). Two membrane-bound molecules are currently known (45 kDa and 32 kDa) which are the result of an alternative splicing event (3). Soluble molecules are derived from the membrane forms of SCF by proteolytic cleavage, generating a 31 kDa and a 23 kDa protein, respectively (2, 4). Cells known to produce SCF include endothelial cells (5), macrophages (6), Sertoli cells (7), and Schwann cells (8). Functionally, SCF can induce the proliferation and maturation of mast cells (9). It also acts in synergy with other growth factors to stimulate erythroid and myeloid colony formation from $CD34^{+}/Lin^{-}$ cells (10). In concert with IL-7, SCF is reported to expand the B cells (11). The receptor for SCF (also known as *c-kit*) was discovered prior to the isolation of the growth factor (12). *c-kit* is a transmembrane tyrosine kinase of approximately 145 kDa in size that is expressed on endothelial cells (13), mast cells (14), megakaryocytes (15), stem cells (BFU-E and CFU-Mix) (16) and multiple embryonic cells, such as melanoblasts and primordial germ cells (17). Mouse and rat SCF are highly homologous to human SCF and as a result murine SCF demonstrates bioactivity on human cells while the human protein is minimally active on murine cells (18).

References

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Technical Notes

When tested on MO7e cells, this blocking antibody reduced the number of cells that stained with mSCF-biotin to < 2.0%. Alternatively, unlabeled SCF, used at 100 and 10 molar excess, reduced the number of cells that stained with SCF-biotin to < 90%. Other cell lines useful for positive control staining with mSCF-biotin are the human TF-1 cell line and the murine FDCP-1 cell line.

Some cell lines can exhibit unusual background staining with Fluorokines. This can be resolved by limiting the amount of Fluorokine in the staining reaction. We recommend that each investigator determine the optimal concentration for their test system. This can be done by diluting the Fluorokine up to 1:10 in 1X RDF1 buffer and then testing it on known receptor positive and negative cells. The optimal concentration of Fluorokine is that which gives optimal signal separation on the two cell types above. Additionally, inhibition of staining in the presence of unconjugated cytokines is best demonstrated under limiting concentrations of Fluorokine.

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.