Fluorokine®
Biotinylated Human SDF-1α

Catalog Number: NNS00
100 Tests

Reagents Provided

Biotinylated rhSDF-1α (100 reactions): Lyophilized protein.

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

Negative Control Reagent (60 reactions; 600 μL): A protein (soybean trypsin inhibitor) biotinylated to the same degree as the studied protein.

Blocking Antibody (15 reactions): Polyclonal goat IgG anti-human SDF-1α antibody.

10X Cell Wash Buffer (60 mL): 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 SDF-1α 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 cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Biotinylated rhSDF-1α: 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. 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 pre-treatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from the 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

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 an additional 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 can 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 SDF-1α blocking antibody is mixed with 10 μL of SDF-1α-biotin and allowed to incubate for 15 minutes at room temperature.
3. To the tube containing the anti-SDF-1α 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

SDF-1α and SDF-1β are the first cytokines initially identified using the signal sequence trap cloning strategy from a mouse bone marrow stromal cell line. These proteins were subsequently also cloned from a human stromal cell line as cytokines that supported the proliferation of a stromal cell-dependent pre-B cell line. SDF-1α and SDF-1β cDNAs encode precursor proteins of 89 and 93 amino acid residues, respectively. SDF-1α and SDF-1β are encoded by a single gene and arise by alternative splicing. The two proteins are identical except for the four amino acid residues that are present in the carboxy-terminus of SDF-1β and absent from SDF-1α. The amino acid sequence of SDF-1/PBSF identified the protein to be a member of the chemokine α subfamily that lacks the ELR domain. Unlike other known chemokine α and β subfamily members that cluster on chromosomes 4 and 17, respectively, SDF-1/PBSF was mapped to chromosome 10q11.1. SDF-1/PBSF is highly conserved between species, with only one amino acid substitution between the mature human and mouse proteins. SDF-1/PBSF has been found to be a chemoattractant for T lymphocytes and monocytes, but not neutrophils. SDF-1/PBSF was shown to be a ligand for CXCR4 (fusin/LESTR) receptor that functions as a coreceptor for lymphocytetropic HIV-1 strains. SDF-1/PBSF has been found to be a powerful inhibitor of infection by lymphocytetropic HIV-1 strains.

References

1. Tashiro, K. *et al*. (1993) Science **261**:600.

2. Bleul, C. *et al*. (1996) Nature **382**:829.

3. Oberlin, E. *et al*. (1996) Nature **382**:833.

Technical Notes

Human SDF-1α biotin kit has been tested for its ability to stain human acute T cell leukemia Jurkat cell line **(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-SDF-1α blocking antibody. These inhibition reactions were carried out under limiting concentrations of SDF-1α-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

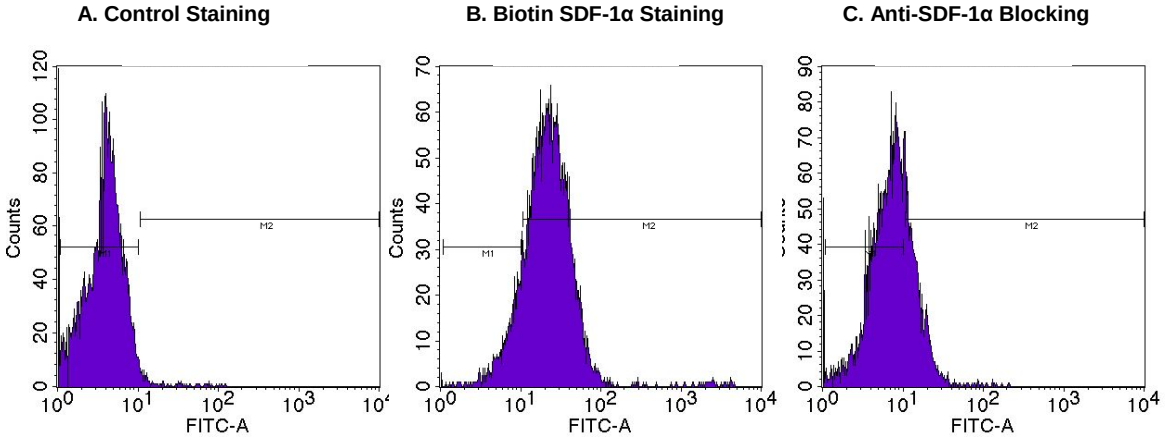

Figure 1. Human Jurkat cells were tested using SDF-1α-biotin kit. Cells stained with **(A)** the Negative Control protein or **(B)** rhSDF-1α-biotin are shown. SDF-1α staining can be specifically blocked with **(C)** anti-human SDF-1α antibody. Fluorescein-conjugated Avidin was used as secondary stain.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