Fluorokine®
Biotinylated Human CCL5/RANTES

Catalog Number: NFRN0
100 Tests

Reagents Provided

Biotinylated rhRANTES (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 RANTES 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 RANTES 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 rhRANTES: 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 RANTES blocking antibody is mixed with 10 µL of RANTES-biotin and allowed to incubate for 15 minutes at room temperature.
3. To the tube containing the anti-RANTES 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

CCL5, also known as RANTES (Regulated upon Activation, Normal T cell Expressed and presumably Secreted), is an 8 kDa β-chemokine that plays a primary role in the inflammatory immune response by means of its ability to attract and activate leukocytes (1-3). Human and mouse RANTES exhibit cross-species activity on human and mouse cells (4). Mature human CCL5 shares 75% 84% aa seqeuence identity with canine, cotton rat, feline, mouse, and rat CCL5 (5). CCL5 is secreted by many cell types at inflammatory sites, and it exerts a wide range of activities through the receptors CCR1, CCR3, CCR4, and CCR5 (6, 7). Inflammatory responses can be impaired by the sequestration of CCL5 by the cytomegalovirus protein US28 (8). In humans, CCR5 binding to CCL5 inhibits the infectivity of R5 (M-tropic) but not X4 (T-tropic) strains of HIV-1 (9). The two N-terminal residues of CCL5 can be removed by CD26/DPPIV, generating a protein that functions as a chemotaxis inhibitor and more effectively blocks M-tropic HIV-1 infection of monocytes (10). Oligomerization of CCL5 on glycosaminoglycans is required for CCR1mediated leukocyte adhesion and activation as well as CCL5's interaction with the chemokine CXCL4/PF4 (11-13). The deposition of CCL5 on activated vascular endothelial cells is crucial for monocyte adhesion to damaged vasculature, but CCL5 oligomerization is not required for the extravasation of adherent leukocytes (14-15). CCL5 is upregulated in breast cancer and promotes tumor progression through the attraction of proinflammatory macrophages in addition to its actions on tumor cells, stromal cells, and the vasculature.

References

1. Schall, T.J. *et al*. (1990) Nature **347**:669.
2. Bacon, K.B. *et al*. (1995) Science **269**:1727.
3. Fischer, F.R. *et al*. (2001) J. Immunol. **167**:1637.
4. Schall, T.J. *et al*. (1992) Eur. J. Immunol. **22**:1477.
5. Schall, T.J. *et al*. (1988) J. Immunol. **141**:1018.
6. Appay, V. and S.L. Rowland-Jones (2001) Trends Immunol. **22**:83.
7. Levy, J.A. (2009) J. Immunol. **182**:3945.
8. Randolph-Habecker, J.R. *et al*. (2002) Cytokine **19**:37
9. DeVico, A.L. and R.C. Gallo (2004) Nat. Rev. Microbiol. **2**:401.
10. Proost, P. *et al*. (1998) J. Biol. Chem. **273**:7222.
11. Appay, V. *et al*. (1999) J. Biol. Chem. **274**:27505.
12. Proudfoot, A.E.I. *et al*. (2003) Proc. Natl. Acad. Sci. USA **10**:1885.
13. von Hundelshausen, P. *et al*. (2005) Blood **105**:924.
14. von Hundelshausen, P. *et al*. (2001) Circulation **103**:1772.
15. Zernecke, A. *et al*. (2008) Arterioscler. Thromb. Vasc. Biol. **28**:1897.

Technical Notes

Human RANTES biotin kit has been tested for its ability to stain human myeloid erythroleukemia TF-1 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-RANTES blocking antibody. These inhibition reactions were carried out under limiting concentrations of RANTES-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

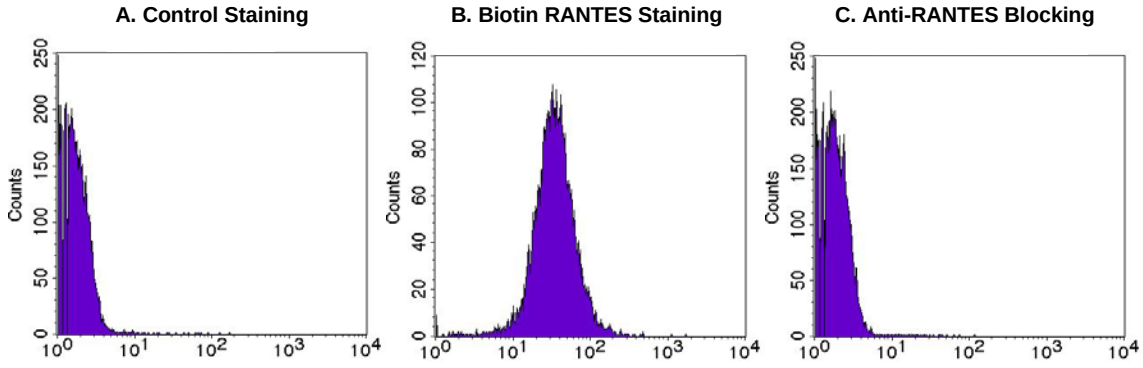

Figure 1. Human TF-1 cells were tested using this RANTES-biotin kit. Cells stained with **(A)** the Negative Control protein or **(B)** rhRANTES-biotin are shown. RANTES staining can be specifically blocked with **(C)** anti-human RANTES antibody. Fluorescein-conjugated Avidin was used as secondary stain.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