Fluorokine®
Biotinylated Human I-309/CCL1

Catalog Number: NFCC10
100 Tests

Reagents Provided

Biotinylated rhI-309/CCL1 (100 reactions): Lyophilized protein.

Avidin-Fluorescein: 2 mL of 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-human I-309 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

- 10 mM 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 receptors for human I-309/CCL1 and to estimate the receptor density for human I-309/CCL1 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 I-309/CCL1: 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

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 10 mM PBS. Cells should be resuspended in 10 mM PBS to a final concentration of 4 x 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 x 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 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**, anti-human I-309/CCL1 blocking antibody is mixed with 10 μL of I-309/CCL1-biotin and allowed to incubate for 15 minutes at room temperature.
3. To the tube containing the anti-human I-309/CCL1 blocking antibody and labeled cytokine mixture, add 1 x 10^5 Fc-blocked cells in a volume of not more than 25 μL.
4. The reaction is then allowed to proceed as described in steps 1 - 4 above.

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

Background Information

Human I-309/CCL1 is a member of the CC (β) family of chemokine molecules and was first identified as a gene expressed from activated T cells (1). I-309/CCL1 has been shown to have chemotactic activity for monocytes and an anti-apoptotic effect on thymocytes (2, 3). The receptor for I-309/CCL1 is CCR8, which was previously known as TER1, ChemR1 and CKR-L1 (4, 5). Expression of CCR8 has been documented on moncytes, thymocytes and Th2 polarized T cells (5 - 9). Additional ligands for CCR8 have been reported, including herpes and pox virus encoded MIP-I, MIP-II and MCC-I (10) as well as TARC/CCL17, MIP-1β/CCL4 and HCC-4/CCL16 (11, 12). The virally encoded molecules do impart cellular signals, however, it is not clear whether the latter three molecules are true receptor ligands since they appear unable to generate a calcium signal.

References

1. Miller, M.D. *et al*. (1990) J. Immunol. **145**:2737.
2. Miller, M.D. and M.S. Krangel (1992) Proc. Natl. Acad. Sci. USA **89**:2950.
3. Van Snick, J. *et al*. (1996) J. Immunol. **157**:2570.
4. Roos, R.S. *et al*. (1997) J. Biol. Chem. **272**:17251.
5. Tiffany, H.L. *et al*. (1997) J. Exp. Med. **186**:165.
6. Napolitano, M. *et al*. (1996) J. Immunol. **157**:2759.
7. Zaballos, A. *et al*. (1996) Biochem. Biophys. Res. Commun. **227**:846.
8. Zingoni, A. *et al*. (1998) J. Immunol. **161**:547.
9. D'Ambrosio, D. *et al*. (1998) J. Immunol. **161**:5111.
10. Dairaghi, D.J. *et al*. (1999) J. Biol. Chem. **274**:21569.
11. Bernardini, G. *et al*. (1998) Eur. J. Immunol. **28**:582.
12. Zack Howard, O.M. *et al*. (2000) Blood **96**:840.

Technical Notes

I-309/CCL1-biotin staining has been tested on a variety of cell types including, peripheral blood monocytes, CCR8 transfected cells and various tumor cell lines. This Fluorokine yields optimal results when used at limited staining concentrations. We recommend that each investigator first determine the optimal staining dose with each cell type. This is most easily done by performing a serial 2-fold dilution of the biotinylated I-309/CCl1 and then determining the lowest concentration of biotinylated Fluorokine which results in the positive staining of a predetermined percentage of cells. Once this optimal staining concentration has been defined, it is then possible to perform a variety of blocking experiments by using either "cold" cytokine or blocking antibodies to demonstrate the staining specificity of the biotinylated I-309/CCL1.

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.