Fluorokine®
Biotinylated Mouse CCL3/MIP-1α

Catalog Number: NFM1A0
100 Tests

Reagents Provided

Biotinylated rmMIP-1α (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-MIP-1α 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)
- 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 MIP-1α 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 rmMIP-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 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 collected in heparinized tubes. Tissues should be disaggregated into a single cell suspension. Cells may then be processed by gradient sedimentation 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 resuspending the cells 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 rmMIP-1α 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.

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 MIP-1α blocking antibody is mixed with 10 μL of MIP-1α-biotin and allowed to incubate for 15 minutes at room temperature.
3) To the tube containing the anti-MIP-1α blocking antibody and Fluorokine mixture, add 1×10^5 Fc-blocked cells in a minimal volume, not exceeding 50 μL.
4) The reaction is then allowed to proceed as described in steps 2 - 5 above.

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Background Information

MIP-1 comprises two closely related, but distinct proteins, 69 amino acid MIP-1α and 67 amino acid MIP-1β (1). MIP-1α and MIP-1β are expressed in a variety of stimulated cells including T cells, B cells, monocytes, and mast cells. MIP-1α is preferentially expressed in smooth muscle, endothelium, neutrophils and fibroblasts (2, 3, 4, 5). Murine and human MIP-1α and MIP-1β share approximately 75% similarity at the amino acid level (2). Among the activities attributed to MIP-1α are monocyte, B cell and $CD8^+$ T cell chemotaxis (6, 7) and eosinophil and basophil degranulation (8, 9). Functions for MIP-1β include monocyte and $CD4^+$ chemotaxis (6). At least two receptors for murine MIP-1α have been cloned (10, 11), and two other MIP-1α Receptor-like proteins have been reported (10). Cells known to express mouse MIP-1α receptors include peripheral blood leukocytes (10) and P388D tumor cells (11).

References

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10. Gao, J-L. and Murphy, P.M. (1995) J. Biol. Chem. **270**:17494.
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Technical Notes

When tested on mouse spleen cells, the blocking antibody included in the kit reduced the number of cells that stained with MIP-1α-biotin to < 6.0%. Alternatively, unlabeled MIP-1α can be used to compete with the biotinylated MIP-1α for staining of cells. Mouse MIP-1α-biotin has been tested on mouse splenocytes.

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 chemokine 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.