Fluorokine®
Biotinylated Human CCL2/MCP-1

Catalog Number: NFCP0
100 Tests

Reagents Provided

Biotinylated rhMCP-1 (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 MCP-1 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 MCP-1 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 rhMCP-1: 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 MCP-1 blocking antibody is mixed with 10 µL of MCP-1-biotin and allowed to incubate for 15 minutes at room temperature.
3. To the tube containing the anti-MCP-1 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

CCL2, also called monocyte chemotactic protein-1 (MCP-1) or JE, is a member of the C-C or β chemokine family that is best known as a chemotactic agent for mononuclear cells (1, 2). Human CCL2 cDNA encodes a 99 amino acid (aa) precursor protein with a 23 aa signal peptide and a 76 aa mature protein (2). Removal of the first 5 aa of the mature protein, including the N-terminal pyrrolidone carboxylic acid -modified glutamine, occurs naturally by metalloproteinase cleavage and down-regulates activity but not receptor binding (3). CCL2 may form multiple bands from 8.7 - 13.5 kDa on SDS-PAGE due to non-covalent dimerization and variable carbohydrate content (3). Mature human CCL2 shares 78 - 79% aa identity with canine, porcine and equine CCL2, while mouse and rat express a form of CCL2 that is extended by 49 aa and shares only ~56% aa identity within the common region. Human CCL2 can, however, induce a response in murine cells (4). Fibroblasts, glioma cells, smooth muscle cells, endothelial cells, lymphocytes and mononuclear phagocytes can produce CCL2 either constitutively or upon mitogenic stimulation, but monocytes and macrophages appear to be the major source (1, 2). In addition to its chemotactic activity, CCL2 induces enzyme and cytokine release by monocytes, NK cells and lymphocytes, and histamine release by basophils that express its receptor, CCR2 (2). Additionally, it promotes Th2 polarization in $CD4^+$ T cells (5). CCL2-mediated recruitment of monocytes to sites of inflammation is proposed to play a role in the pathology of atherosclerosis, multiple sclerosis and allergic asthma (6, 7).

References

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3. Proost, P. *et al*. (1998) J. Immunol. **160**:4034.
4. Van Riper, G. *et al*. (1993) J. Exp. Med. **177**:851.
5. Luther, S.A. and J.G. Cyster (2001) Nat. Immunol. **2**:102.
6. Daly, C. *et al*. (2003) Microcirculation **10**:247.
7. Aukrust, P. *et al*. (2008) Arterioscler. Thromb. Vasc. Biol. **28**:1909.

Technical Notes

Human MCP-1 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-MCP-1 blocking antibody. These inhibition reactions were carried out under limiting concentrations of MCP-1-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

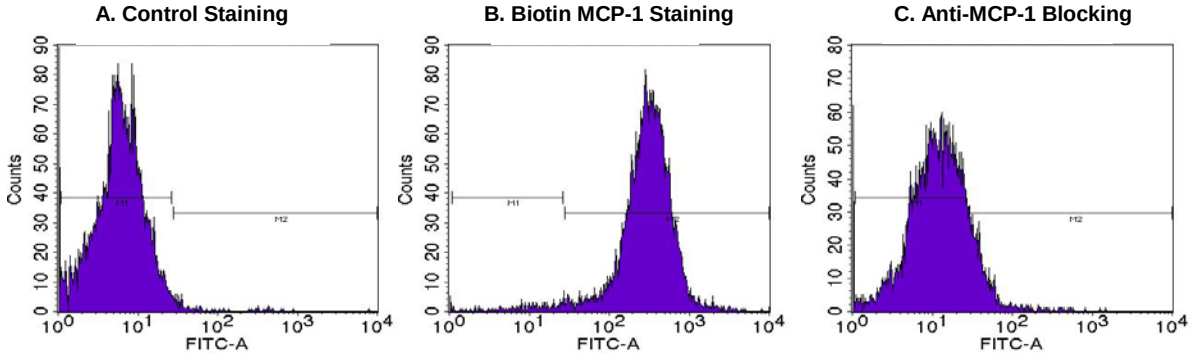

Figure 1. Human Jurkat cells were tested using this MCP-1-biotin kit. Cells stained with **(A)** the Negative Control protein or **(B)** rhMCP-1-biotin are shown. MCP-1 staining can be specifically blocked with **(C)** anti-human MCP-1 antibody. Fluorescein-conjugated Avidin was used as secondary stain.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