Fluorokine®
Biotinylated Mouse JE

Catalog Number: NFJE0
100 Tests

Reagents Provided

Biotinylated rmJE (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-mouse JE 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)
- human or mouse IgG

Storage

All Reagents: 2° - 8° C

Intended Use

Designed to quantitatively determine the percentage of cells bearing murine chemokine JE receptors within a population and to estimate the receptor density on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the biotinylated chemokine that in turn binds cells via specific cell surface receptors. The cells are then directly incubated with avidin-fluorescein, which attaches to the receptor-bound biotinylated chemokine. Unbound biotinylated chemokine participates in an amplification reaction that results in an enhanced signal without compromising specificity. Cells expressing chemokine receptors are fluorescently stained, with the intensity of staining directly proportional to the receptor density. Receptor density is then determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Biotinylated rmJE: 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 be collected in heparinized tubes. Tissues should be disaggregated into a single cell suspension. Cells may then be processed by standard gradient separation 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 at 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 chemokine 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 approx. 0.2 mL of 1X RDF1 for final 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 18° - 24° C 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 JE blocking antibody is mixed with 10 μL of mouse JE-biotin and allowed to incubate for 15 minutes at 18° - 24° C.
3) To the tube containing the anti-mouse JE 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 2 - 5 above.

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Background Information

Mouse JE is a 25 - 30 kDa glycosylated protein that is a member of the C-C or β chemokine family (1, 2). Based on function, amino acid structure and receptor usage, JE appears to be the homologue of human monocyte chemotactic protein-1 (MCP-1) (2). However, with the cloning of murine MCP-5 (3), this suggestion is being re-evaluated. Extensive O-linked glycosylation at the C-terminus of JE appears to negatively impact its bioactivity when compared to non-glycosylated JE (4). Cells known to express JE include fibroblasts (1), macrophages, astrocytes (5), mast cells, endothelial cells (3) and osteoblasts (6).

The receptor for JE is murine CCR2 (7, 8). Various Kd's have been reported for binding of JE to CCR2 [average value is 770 pM (7, 8)]. Although JE has also been shown to chemoattract lymphocytes (5), CCR2 is preferentially expressed on monocytes and macrophages. The physiological roles of JE may likely include: monocyte recruitment at sites of inflammation, attraction of lymphocyte progenitors to sites of differentiation within the thymus (4) and perhaps also promotion of the development of a Th2 type T cell response through its ability to down regulate IL-12 production from macrophages (9) as well as inducing IL-4 production from T cells (10).

References

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5. Luo, Y. *et al*. (1994) J. Immunol. **153**:3708.
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Technical Notes

Some cell lines can exhibit unusually high non-specific staining with Fluorokines. Limiting the amount of Fluorokine used in the staining reaction can reduce this effect. We recommend that each user determine the optimal concentration of Fluorokine for staining different cells. This involves diluting the Fluorokine from the provided concentration until a distinguishable positive and negative signal is observed when the Fluorokine is used to stain known receptor-positive and receptor-negative cells. Normally, doubling dilutions up to 1:10 of the provided concentration are adequate. Fluorokine dilutions should be made using 1X RDF1 buffer as the diluent.

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.