Fluorokine®
Biotinylated Mouse IL-9

Catalog Number: NFMIL9
100 Tests

Reagents Provided

Biotinylated rmIL-9 (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 IL-9 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 mouse chemokine IL-9 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, which in turn binds to 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 density of the receptors. Receptor density is then determined by flow cytometry using 488 nm wavelength laser excitation.

Reagent Preparation

Biotinylated rmIL-9: 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 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 them 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 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 their substrates. Cells that require trypsinization to enable removal from their substrates 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 approximately 0.2 mL of 1X RDF1 for analysis by flow cytometry.

Specificity Testing

1) (Optional) Cells that are to be stained could be treated with purified mouse or human IgG (10 µL of 1 mg/mL/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 IL-9 blocking antibody should be mixed with 10 µL of mouse IL-9-biotin and allowed to incubate for 15 minutes at 18° - 24° C.
3) To the tube containing the anti-mouse IL-9 blocking antibody and Fluorokine mixture, 1×10^5 Fc-blocked cells should be added in a volume of 25 µL.
4) The reaction is then allowed to proceed as described in steps 2 - 5 above.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

Mouse IL-9 was originally identified as a T cell-derived T cell growth factor III/P40 which could support the long term growth of certain mouse T helper clones in the absence of antigen or antigen-presenting cells. IL-9 can also prolong the *in vitro* survival of other T cell clones as well as potentiate the IL-2 dependent proliferation of mouse fetal thymocytes. However, this cytokine has no growth-stimulating activity on mouse cytolytic T cell clones or fresh T cells. In addition to its activities on T cells, mouse IL-9 also has mast cell enhancing activity (MEA) and can enhance the mIL-3- or mIL-4-dependent proliferation of mouse bone marrow-derived mast cells. Furthermore, IL-9 will synergize with erythropoietin to support erythroid colony formation *in vitro*.

The gene for mIL-9 has been mapped to mouse chromosome 13. The mouse IL-9 cDNA encodes a 144 amino acid residue precursor protein with an 18 amino acid signal peptide that is cleaved to form the mature cysteine-rich protein with a predicted molecular mass of 14 kDa. Mouse IL-9 contains four potential N-linked glycosylation sites and the native mIL-9 is a highly glycosylated protein.

Human IL-9 was independently cloned as a novel growth factor which is mitogenic for the human megakaryoblastic leukemic cell line, M07e. Human and mouse IL-9 share 56% and 67% homology at the amino acid and nucleotide levels, respectively. Although mouse IL-9 is active on human cells, human IL-9 is not active on mouse cells.[1]

References

1. Renauld, J.E. *et al.* (1995) J. Leukoc. Biol **57**:303.

Technical Notes

The mouse IL-9 biotin kit has been tested for its ability to stain mouse T-cells [isolated with R&D Systems MagCellect (Catalog # MAGM201) or T Cell Enrichment column (Catalog # MTCC-500)]. Staining specificity has been determined by demonstrating a reduction in signal intensity when the staining reaction is carried out in the presence of 1 - 100 molar excess unconjugated mouse IL-9 or in the presence of mouse IL-9 blocking antibody. These inhibition reactions were carried out under limiting concentrations of mouse IL-9-biotin.

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.