Fluorokine®
Biotinylated Mouse TARC/CCL17

Catalog Number: NFMTC0
100 Tests

Reagents Provided

Biotinylated rmTARC (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 TARC 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 chemokine receptors within a population and to estimate the receptor density for TARC on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the biotinylated chemokine that in turn binds to the cells via 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 the specific 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 cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

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

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

TARC/CCL17 is an 8 kDa CC chemokine family member (1, 2) exhibiting 25 - 30% amino acid sequence homology with other known members of the CC or β chemokine family. TARC/CCL17 is most closely related to macrophage-derived chemokine (MDC/CCL22) exhibiting 37% amino acid identity (3). Human and mouse TARC/CCL17 proteins share 64% amino acid identity (1).

TARC/CCL17 mediates its bioactivity through CCR4 (4). CCR4 is a G-protein coupled receptor expressed primarily on CD4+ T cells and cell lines of basophilic and megakaryocytic lineage; including platelets (4, 5). The CCR4 receptor is most often associated with the Th2 subset of $CD4^+$ T cells (6) but has also been described on IL-2 activated NK cells (7) and activated dendritic cells (8). Interactions between CCR4 and TARC/CCL17 are important in allergic responses (9, 10), and may also play a role in Hodgkin's lymphoma (11) and acute lymphoblastic leukemia (12).

References

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Technical Notes

Binding of mouse TARC-biotin has been tested on mouse thymocytes, splenocytes, $CD4^+/CD62^-/CD44^{high}$ memory splenic T cells, and transfectants expressing mCCR4. Additionally, the human T lymphoblastoid cell line CEM that expresses hCCR4 (Cronshaw, D.G. *et al.* 2004, J. Immunol. **172**:7761) also binds mTARC-biotin and can be blocked using the goat anti-mouse TARC polyclonal antibody provided in the kit (in house data).

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.