Monoclonal Anti-human Leukotriene B_4 Receptor 1 (BLT1/LTB4R1)-Fluorescein

Catalog Number: FAB099F
Lot Number: LAG02
100 Tests

Reagent Information

Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human BLT1 Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 203/14F11

Ig class: mouse IgG_1

Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing BLT1 on their cell surface within a population and qualitatively determine the density of this receptor on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the fluorescein-labeled monoclonal antibody that binds to the cells expressing BLT1. Unbound fluorescein-conjugated antibody is then washed from the cells. Cells expressing BLT1 become fluorescently stained, with the intensity of staining directly proportional to the density of the BLT1 expressed. Cell surface expression of BLT1 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Fluorescein-conjugated mouse anti-human BLT1: Use as is; no preparation is necessary.

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Blood cells will require lysis of RBC following the staining procedure.

This antibody has been selected for its ability to recognize human BLT1 on human peripheral blood monocytes and granulocytes.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4×10^6 cells/mL and 25 μL of cells (1×10^5) are transferred to a 5 mL tube for staining.

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) Cells to be used for staining with the antibody may be first Fc-blocked by treatment with 1 μg of mouse or human $IgG/10^5$ cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1×10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of fluorescein-conjugated anti-BLT1 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-BLT1 reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Human Erythrocyte Lysing Kit, Catalog # WL1000*).
6) Resuspend the cell pellet in 200 - 400 μL of PBS buffer for final flow cytometric analysis.
7) As a control for analysis, cells in a separate tube should be treated with fluorescein-labeled murine IgG_1 antibody.

This procedure may need to be modified, depending upon final utilization.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Background Information

Polymorphonuclear granulocytes secrete the lipid chemotactic mediator leukotriene B_4 (LTB_4) in response to inflammatory stimuli (1). Neutrophils, monocytes and lymphocytes respond to LTB_4 via specific receptors localized on the cell surface (2 - 4). The high affinity LTB_4 receptor known as BLT1 is only expressed on leukocytes (5 - 7) while a second low affinity receptor BLT2 is expressed more ubiquitously (8, 9). The BLT1 and BLTR2 are G-protein linked seven-transmembrane spanning receptors that share about 37 - 45% amino acid identity (8, 9).

Enhanced LTB_4 production and engagement of the BLT receptors can be important in allergic and inflammatory diseases such as asthma (10), allergic encephalomyelitis (11), endotoxic shock (12), ischemia (12), psoriasis (13), rheumatoid arthritis (14) and inflammatory bowel disease (15). In addition, it has been reported that BLT1 can function as an additional co-receptor for HIV infection of $CD4^+$ T cells (16, 17). Investigations into the mechanisms and potential inhibitors of LTB_4 binding to its' receptors may provide insight into possible treatment modalities for a number of inflammatory disorders.

References

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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.