Monoclonal Anti-human CXCL16-Phycoerythrin

Catalog Number: FAB976P
Lot Number: LTZ03
100 Tests

Reagent Information

Phycoerythrin (PE)-conjugated rat monoclonal anti-human CXCL16: Contains 1.0 mL of PE-labeled antibody, at a concentration of 25 µg/mL.

Clone #: 256213

Ig class: rat IgG_{2A}

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

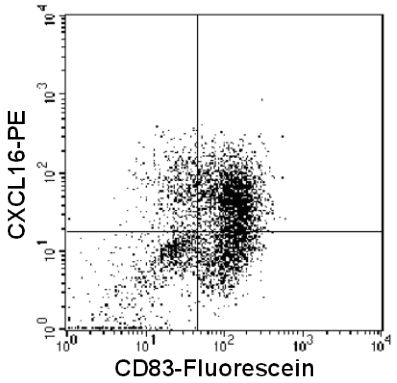
Designed to determine the percentage of cells expressing cell surface CXCL16 and the density of this chemokine on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody, which binds to cells expressing CXCL16. Unbound PE-conjugated antibody is then washed from the cells. Cells expressing CXCL16 are fluorescently stained, with the intensity of staining directly proportional to the density of the CXCL16 protein. Cell surface expression of CXCL16 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

PE-conjugated rat anti-human CXCL16: Use as is; no preparation is necessary.



Human monocyte derived dendritic cells were stained with PE-conjugated anti-CXCL16 antibody (Catalog # FAB976P) and Fluorescein-conjugated anti-CD83 antibody (Catalog # FAB1774F).

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. Whole blood cells will require lysis of RBC following the staining procedure.

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 for 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 a 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 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 (up to 1×10^6 cells) or 50 µL of packed whole blood to a 5 mL tube.
3) Add 10 µL of PE-conjugated anti-CXCL16 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted anti-CXCL16 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 Whole Blood Lysing Kit, Catalog # WL1000).
6) Resuspend the cells 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 PE-labeled rat IgG_{2A} antibody.

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

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Background Information

CXC chemokine ligand 16 (CXCL16) is a 254 amino acid type I transmembrane protein that exhibits a non-ELR motif-containing CXC chemokine domain in its extracellular region (1 - 3). Fractalkine (CX3CL1) and CXCL16 constitute the only two transmembrane chemokines within the chemokine superfamily (1, 2, 4). CXCL16 expression is detected in various lymphoid tissues including; thymus, spleen, Peyer's patchers, and lymph nodes, and in non-lymphoid tissues including; kidney, lung, small intestine, liver, and artherosclerotic lesions (1, 2, 5, 6). CXCL16 is expressed primarily on the surface of antigen presenting cells (APCs) including $CD19^+$ B cells, monocytes, macrophages, and dendritic cells (1, 3), and may be expressed by some T cells, smooth muscle cells, and endothelial cells (6 - 8). Functional CXCL16 can be shed from the cell surface as an approximately 35 kDa soluble protein (1, 2).

The receptor for CXCL16 has been identified as CXCR6 (also known as Bonzo, STRL33, or TYMSTR), a receptor previously shown to be a co-receptor for HIV entry (9 - 12). CXCR6 is expressed on naïve $CD8^+$ T cells, T_H1 polarized $CD8^+$ and $CD4^+$ T cells, NKT cells, plasma cells, and astrocytes (13 - 16). CXCR6 is enriched in cells found at sites of inflammation (13). CXCL16 has the ability to stimulate CXCR6-dependent chemotaxis and is hypothesized to play a role in leukocyte migration (1, 2). In plasma cells, CXCL16 stimulates chemotactic migration and enhances adhesion to fibronectin (15). In addition, CXCL16 was independently cloned as SR-POX (scavenger receptor that binds phosphatidylserine and oxidized lipoprotein) (3). As a scavenger receptor, CXCL16 mediates the uptake of phosphatidylserine and oxidized low density lipoprotein (Ox-LDL), and the phagocytosis by APCs of both gram-positive and gram-negative bacteria (3, 17). The bacterial recognition sequence appears to reside within the chemokine domain of the protein (17).

References

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Technical Notes

Human monocyte-derived dendritic cells used for analysis were generated by culturing $CD14^+$ monocytes (R&D Systems, Catalog # MAGH105) in RPMI complete medium containing 200 U/mL rhIL-4 (R&D Systems, Catalog # 204-IL) plus 50 ng/mL rhGM-CSF (R&D Systems, Catalog # 215-GM) for 7 days. Dendritic cells were then stimulated with 100 ng/mL LPS for an additional 24 hours prior to harvest.

This reagent 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.

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