Monoclonal Anti-human Siglec-5/Siglec-14-Phycoerythrin

Catalog Number: FAB10721P
Lot Number: AAFE01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human Siglec-5/Siglec-14: Supplied as 25 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 194128

Isotype: mouse IgG_1

Reagents Not Provided

- 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

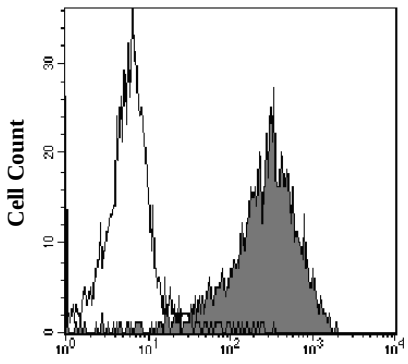
Designed to quantitatively determine the percentage of cells bearing Siglec-5/Siglec-14 within a population and qualitatively determine the density of Siglec-5/Siglec-14 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing Siglec-5/Siglec-14. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing Siglec-5/Siglec-14 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of Siglec-5/Siglec-14. Cell surface expression of Siglec-5/Siglec-14 is determined by flow cytometric analysis using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.

Reagent Preparation

Phycoerythrin-conjugated mouse anti-human Siglec-5/Siglec-14: Use as is; no preparation necessary.



Human monocytes were stained with PE-conjugated anti-human Siglec-5/Siglec-14 (Catalog # FAB10721P, filled histogram) or isotype control (Catalog # IC002P, open histogram).

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. Transfer 50 μL of packed cells to a 5 mL tube for staining with the monoclonal antibody. Whole blood 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 x 10^6 cells/mL and 25 μL of cells (1 x 10^5) 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 should be Fc-blocked by treatment with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (1 x 10^5 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated Siglec-5/Siglec-14 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted Siglec-5/Siglec-14 reagent by washing the cells twice in 4 mL of the same PBS buffer (*note: whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
6) Finally, 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 mouse IgG_1 antibody.

This procedure may need modification, depending upon final utilization.

Specificity

This antibody detects rhSiglec-5 direct ELISAs. In this assay, this antibody shows 100% cross-reactivity with rhSiglec-14 and no cross-reactivity with rhSiglec-3, rhSiglec-7, or rhSiglec-9. Siglec-5/Siglec-14 cross-reactivity by flow has been published. [8]

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Background Information

Siglecs[1] (sialic acid binding Ig-like lectins) are I-type (Ig-type) lectins[2] belonging to the Ig superfamily. They are characterized by an N-terminal Ig-like V-type domain which mediates sialic acid binding,[3] followed by varying numbers of Ig-like C2-type domains.[1, 4] Eleven human Siglecs have been cloned and characterized.[1, 4] They are sialoadhesin/CD169/Siglec-1, CD22/Siglec-2, CD33/Siglec-3, Myelin-Associated Glycoprotein (MAG/Siglec-4a) and the recently identified Siglec 5 to 11.[4, 5, 7] To date, no Siglec has been shown to recognize any cell surface ligand other than sialic acids, suggesting that interactions with glycans containing this carbohydrate are important in mediating the biological functions of Siglecs. Siglec 5 to 11 share a high degree of sequence similarity with CD33/Siglec-3 both in their extracellular and intracellular regions. They are collectively referred to as CD33-related Siglecs. One remarkable feature of the CD33-related Siglecs is their differential expression pattern within the hematopoietic system.[4, 5] This fact, together with the presence of two conserved immunoreceptor tyrosine-based inhibition motifs (ITIMs) in their cytoplasm tails, suggests that CD33-related Siglecs are involved in the regulation of cellular activation within the immune system.

Human Siglec-5 cDNA encodes a 551 amino acid (aa) polypeptide with a hydrophobic signal peptide, an N-terminal Ig-like V-type domain, three Ig-like C2-type domains, a transmembrane region and a cytoplasm tail.[6] Siglec-5 exists as a disulfide-linked homodimer on the cell surface and is expressed on monocytes, neutrophils and B cells.[4, 5, 6] It binds equally well to both α2, 3- and α2, 6-linked sialic acid.[6]

References

1. Crocker, P.R. *et al.*, 1998, Glycobiology **8**:v.
2. Powell, L.D. *et al.*, 1995, J. Biol. Chem. **270**:14243 - 14246.
3. May, A.R. *et al.*, 1998, Mol. Cell 1998. **1**:719 - 728.
4. Crocker, P.R. and A. Varki, 2001, Trends Immunol. **22**:337 - 342.
5. Crocker, P.R. *et al.*, 2001, Immunology **103**:137 - 145.
6. Cornish, A.L. *et al.*, 1998, Blood **92**:2123 - 2132.
7. Angata, T. *et al.*, 2002, J. Biol Chem. **277**:24466.
8. Angata, T. *et al.*, 2006, FASEB J. **20**:1964 - 1973.

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.