Human Siglec-5/Siglec-14 Antibody

Antigen Affinity-purified Polyclonal Goat IgG

Catalog Number: AF1072

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Siglec-5/14 in direct ELISAs and Western blots. In direct ELISAs, approximately 5% cross-reactivity with recombinant human (rh) Siglec-7 and rhSiglec-9 is observed and less than 1% cross-reactivity with rhSiglec-2 and rhSiglec-3 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Siglec-5 Glu17-Thr434 Accession # O15389
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

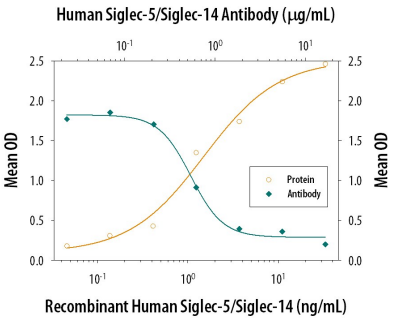
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.1 µg/mL	Recombinant Human Siglec-5 Fc Chimera (Catalog # 1072-SL)
Flow Cytometry	2.5 µg/10^6 cells	See Below
Immunohistochemistry	5-15 µg/mL	Immersion fixed paraffin-embedded sections of human tonsil tissue
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Neutralization	Measured by its ability to neutralize Siglec-5-mediated adhesion of human red blood cells. Kelm, S. *et al.* (1994) Current Biology **4**:965. The Neutralization Dose (ND_{50}) is typically 0.5-2.0 µg/mL in the presence of 5 µg/mL Recombinant Human Siglec-5 Fc Chimera.	

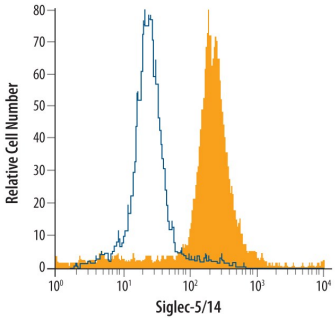
DATA

Neutralization



Cell Adhesion Mediated by Siglec-5 and Neutralization by Human Siglec-5/14 Antibody. Recombinant Human Siglec-5 Fc Chimera (Catalog # Catalog # 1072-SL), immobilized onto a microplate, supports the adhesion of human red blood cells in a dose-dependent manner (orange line). Adhesion elicited by Recombinant Human Siglec-5 Fc Chimera (5 µg/mL) is neutralized (green line) by increasing concentrations of Human Siglec-5/14 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1072). The $ND_{50}$is typically 0.5-2.0 µg/mL.

Flow Cytometry



Detection of Siglec-5/14 in Human Monocytes by Flow Cytometry. Human whole blood monocytes were stained with Human Siglec-5/14 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1072, filled histogram) or control antibody (Catalog # Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # F0107).

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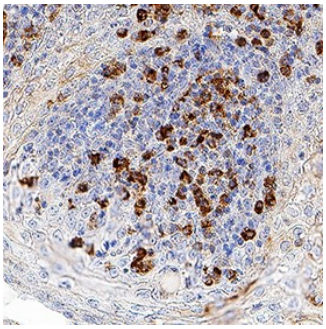
Human Siglec-5/Siglec-14 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1072

Immunohistochemistry



Siglec-5/Siglec-14 in Human Tonsil. Siglec-5/Siglec-14 was detected in immersion fixed paraffin-embedded sections of human tonsil tissue using Goat Anti-Human Siglec-5/Siglec-14 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1072) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes in germinal centers. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

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 Siglec-5 to 11 (4, 5, 6). To date, no Siglec has been shown to recognized 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 cytoplasma tails, suggests that CD33-related Siglecs are involved in the regulation of cellular activation within the immune system.

References:

1. Crocker, P.R. *et al.* (1998) Glycobiology **8**:v.
2. Powell, L.D. *et al.* (1995) J. Biol. Chem. **270**:14243.
3. May, A.R. *et al.* (1998) Mol. Cell 1998. **1**:719.
4. Crocker, P.R. and A. Varki (2001) Trends Immunol. **22**:337.
5. Crocker, P.R. *et al.* (2001) Immunology **103**:137.
6. Angata, T. *et al.* (2002) J. Biol Chem. **277**:24466.

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