

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Siglec-9 in direct ELISAs and Western blots. In direct ELISAs, less than 10% cross-reactivity with recombinant mouse Siglec-E is observed and less than 1% cross-reactivity with recombinant human (rh) Siglec-3, rhSiglec-5, rhSiglec-6, rhSiglec-7, rhSiglec-8 or rhSiglec-10 is observed. In Western blots, approximately 100% cross-reactivity with recombinant mouse Siglec-E is observed under non-reduced conditions.
Source	Monoclonal Mouse IgG _{2A} Clone # 191240
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Siglec-9 Gln18-Gly348 (predicted) Accession # Q9Y336
Endotoxin Level	<1.0 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 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	1 µg/mL	Recombinant Human Siglec-9 Fc Chimera (Catalog # 1139-SL) under non-reducing conditions only
Flow Cytometry	0.25 µg/10 ⁶ cells	See Below
Human Siglec-9 Sandwich Immunoassay		Reagent
ELISA Capture	2-8 µg/mL	Human Siglec-9 Antibody (Catalog # MAB1139)
ELISA Detection Standard	0.1-0.4 µg/mL	Human Siglec-9 Biotinylated Antibody (Catalog # BAF1139) Recombinant Human Siglec-9 Fc Chimera (Catalog # 1139-SL)
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-9-mediated adhesion of human red blood cells. Kelm, S. <i>et al.</i> (1994) <i>Current Biology</i> 4:965. The Neutralization Dose (ND ₅₀) is typically 0.015-0.075 µg/mL in the presence of 10 µg/mL Recombinant Human Siglec-9 Fc Chimera.	

DATA

Flow Cytometry

Detection of Siglec-9 in Human Blood Granulocytes by Flow Cytometry. Human peripheral blood granulocytes were stained with (A) Mouse Anti-Human Siglec-3/CD33 PE-conjugated Monoclonal Antibody (Catalog # FAB1137P) and (B) Mouse Anti-Human Siglec-9 Monoclonal Antibody (Catalog # MAB1139, filled histogram) or isotype control antibody (Catalog # MAB003, open histogram), followed by PerCP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0114).

Neutralization

Cell Adhesion Mediated by Siglec-9 and Neutralization by Human Siglec-9 Antibody. Recombinant Human Siglec-9 Fc Chimera (Catalog # 1139-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-9 Fc Chimera (10 µg/mL) is neutralized (green line) by increasing concentrations of Mouse Anti-Human Siglec-9 Monoclonal Antibody (Catalog # MAB1139). The ND₅₀ is typically 0.015-0.075 µg/mL.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 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. <ul style="list-style-type: none"> ● 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 belonging to the Ig superfamily. They are characterized by an N-terminal Ig-like V-type domain which mediates sialic acid binding, followed by varying numbers of Ig-like C2-type domains (1, 2). Eleven human Siglecs have been cloned and characterized. They are sialoadhesin/CD169/Siglec-1, CD22/Siglec-2, CD33/Siglec-3, Myelin-Associated Glycoprotein (MAG/Siglec-4a) and Siglec-5 to -11 (1-4). 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. Siglecs 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 (2, 3). This fact, together with the presence of two conserved immunoreceptor tyrosine-based inhibition motifs (ITIMs) in their cytoplasmic tails, suggests that CD33-related Siglecs are involved in the regulation of cellular activation within the immune system.

The cDNA of human Siglec-9 encodes a 463 amino acid (aa) polypeptide with a hydrophobic signal peptide, an N-terminal Ig-like V-type domain, two Ig-like C2-type domains, a transmembrane region and a cytoplasmic tail (5, 6). In peripheral blood leukocytes, Siglec-9 is expressed on neutrophils, monocytes, a fraction of NK cells, B cells, and a minor subset of CD8+ T cells (5). It binds equally well to both 2,3- and 2,6-linked sialic acid (5, 6). Siglec-9 is closely related to Siglec-7, and they share ~80% amino acid sequence identity. The gene encoding siglec-9 was mapped to chromosome 19q13.4.

References:

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2. Crocker, P.R. and A. Varki (2001) *Trends Immunol.* **22**:337.
3. Crocker, P.R. and A. Varki (2001) *Immunology* **103**:137.
4. Angata, T. *et al.* (2002) *J. Biol. Chem.* **277**:24466.
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6. Angata, T. *et al.* (2000) *J. Biol. Chem.* **275**:22127.