Human Siglec-2/CD22 Llamabody™ VHH His-tag Antibody

Recombinant Monoclonal Llama V_HH domain Clone # L007.2.5N

Catalog Number: LMAB10732

DESCRIPTION

Species Reactivity	Human
Specificity	
Source	Recombinant Monoclonal Llama V_HH domain Clone # L007.2.5N
Purification	His-tag purified from cell culture supernatant
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

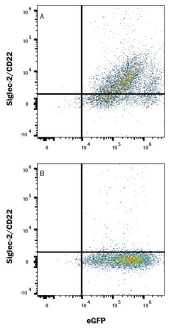
APPLICATIONS

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
Flow Cytometry	0.25 µg/10^6 cells	HEK293 Human Cell Line Transfected with Human Siglec-2/CD22 and eGFP
Immunocytochemistry	5-25 µg/mL	Immersion fixed Transfected HEK293 Human Embryonic Kidney Cell Line (Positive) And Wild Type HEK293 Human Embryonic Kidney Cell Line (Negative) Cells

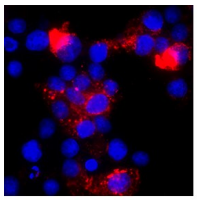
DATA

Flow Cytometry

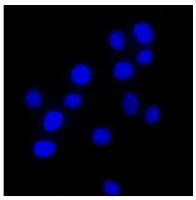


Detection of Siglec-2/CD22 in HEK293 Human Cell Line Transfected with Human Siglec-2/CD22 and eGFP by Flow Cytometry. HEK293 human embryonic kidney cell line transfected with either (A) human Siglec-2/CD22 or (B) irrelevant protein and eGFP was stained with Llama Anti-Human Siglec-2/CD22 Llamabody VHH Monoclonal Antibody (Catalog # LMAB10732) followed by Goat Anti-Llama Secondary Antibody (Catalog # AF011), and then Allophycocyanin-conjugated Anti-Goat IgG Tertiary Antibody (Catalog # F0108). Quadrant markers were set based on secondary plus tertiary antibody staining in the absence of primary antibody. View our protocol for Staining Membrane-associated Proteins.

Immunocytochemistry



Transfected HEK293 (Positive) cells



Wildtype HEK293(Negative) cells

Detection of Siglec-2/CD22 in Transfected HEK293 Cell Line (Positive) And Wild Type HEK293Cell Line (Negative) Cells. Siglec-2/CD22 was detected in immersion fixed Transfected HEK293 Human Embryonic Kidney Cell Line (Positive) and absent in Wild Type HEK293 Human Embryonic Kidney Cell Line (Negative) Cells using Llama Anti-Human Siglec-2/CD22 Llamabody™ VHH His-tag Monoclonal Antibody (Catalog # LMAB10732) at 5 µg/mL for 3 hours at room temperature. Cells were stained using Anti-camelid Vhh conjugated to Rhodamine-Red X (red) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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.
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.

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BACKGROUND

Siglecs are type I transmembrane proteins that belong to the immunoglobulin (Ig) superfamily and function as mammalian lectins (1). They are characterized by an extracellular domain consisting of various numbers of Ig domains with a conserved N-terminal V-set Ig ligand-binding domain. This binds species-specific sialic acid motifs on protein and lipid scaffolds to regulate intracellular signaling pathways (2). The cytoplasmic tail has signaling motifs, in most cases immunoreceptor tyrosine-based inhibitory motif (ITIM) (3). Human Siglec-2, also known as B cell antigen CD22 or B lymphocyte cell adhesion molecule (BL-CAM), is a B cell restricted glycoprotein that is expressed in the cytoplasm of progenitor B and pre B cells and on the surface of mature B cells. Two distinct human Siglec-2/CD22 cDNAs that arise from differential RNA processing of the same gene have been isolated. The predominant Siglec-2/CD22 beta encodes an 847 amino acid (aa) polypeptide with a hydrophobic signal peptide, an N-terminal Ig-like V type domain, six Ig-like C2 type domains, a transmembrane region and a cytoplasmic tail with 4 immunoreceptor tyrosine-based inhibition motifs (ITIMs) (4). The variant Siglec-2/CD22 alpha encodes a 647 aa polypeptide missing two Ig-like C2 type domains and has a truncated (23 aa) cytoplasmic tail (5). Mature human Siglec-2 beta consists of a 668 amino acid (aa) extracellular domain (ECD), a 19 aa transmembrane segment, and a 141 aa cytoplasmic domain. Within the ECD, human Siglec-2 shares 59% and 58% aa sequence identity with the mouse and rat Siglec-2, respectively. Siglec-2/CD22 is an adhesion molecule that preferentially binds alpha 2,6- linked sialic acid on the same (cis) or adjacent (trans) cells. Interaction of CD22 with trans ligands on opposing cells was found to be favored over the binding of ligands in cis (6). Besides its role as an adhesion molecule, Siglec-2/CD22 is a coreceptor that physically interacts with B-cell receptor (BCR) and is rapidly phosphorylated upon BCR ligation. It negatively regulates BCR signals by recruiting tyrosine phosphatase SHP-1 to its ITIMs. Phosphorylated Siglec-2/CD22 can also interact with other intracellular effector proteins such as Syk, PLC gamma, PI3 kinase and Grb-2, suggesting it may play a role in positive signaling (7-9). Another function of CD22 is that it mediates the anti-phagocytic effect of α2,6-linked sialic acid, and inhibition of CD22 promotes the clearance of myelin debris, amyloid-β oligomers and α-synuclein fibrils in vivo(10). CD22 also plays a role in autoimmunity and has great potential for CD22-based immunotherapeutics for the treatment of autoimmune diseases such as systemic lupus erythematosus (SLE) (11).

References:

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