

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Siglec-3/CD33 in direct ELISAs.
Source	Monoclonal Mouse IgG _{2B} Clone # 996810
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Mouse myeloma cell line, NS0-derived human Siglec-3/CD33 Asp18-His259 Accession # P20138
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.

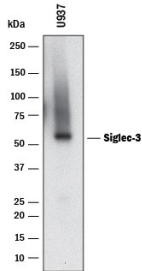
APPLICATIONS

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	2 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Immunohistochemistry	5-25 µg/mL	See Below

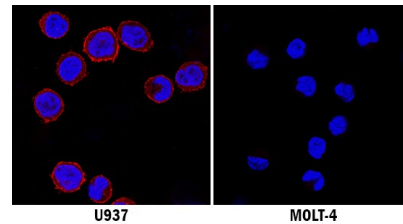
DATA

Western Blot



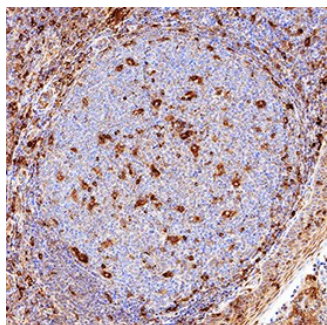
Detection of Human Siglec-3/CD33 by Western Blot. Western blot shows lysates of U937 human histiocytic lymphoma cell line. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human Siglec-3/CD33 Monoclonal Antibody (Catalog # MAB11371) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for Siglec-3/CD33 at approximately 55 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



Siglec-3/CD33 in U937 and MOLT-4 Human Cell Lines. Siglec-3/CD33 was detected in immersion fixed U937 human histiocytic lymphoma cell line (left panel; positive stain) and MOLT-4 human acute lymphoblastic leukemia cell line (right panel; negative stain) using Mouse Anti-Human Siglec-3/CD33 Monoclonal Antibody (Catalog # MAB11371) at 8 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunohistochemistry



Siglec-3/CD33 in Human Tonsil. Siglec-3/CD33 was detected in immersion fixed paraffin-embedded sections of human tonsil using Mouse Anti-Human Siglec-3/CD33 Monoclonal Antibody (Catalog # MAB11371) at 5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Mouse IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC001). 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. View our protocol for [IHC Staining with VisUCyte HRP Polymer Detection Reagents](#).

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	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <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 (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 Siglecs 5 to 11 (1-3). 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 (1, 2). 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.

Human Siglec-3 is alternatively known as myeloid cell surface antigen CD33 and GP67. Human Siglec-3 cDNA encodes a 364 amino acid (aa) polypeptide with a hydrophobic signal peptide, an N-terminal Ig-like V-type domain, one Ig-like C2-type domains, a transmembrane region and a cytoplasmic tail (1, 4). Siglec-3 expression is restricted to cells of myelomonocytic lineage (2). It binds sialic acid preferring α 2,3- linkage over α 2,6- linkage (5). Studies indicated that Siglec-3 recruits SHP-1 and SHP-2 to its ITIMs (6, 7). When co-crosslinking with Fc γ R1, Siglec-3 inhibits tyrosine phosphorylation and calcium mobilization, suggesting Siglec-3 can mediate inhibitory signals (7).

References:

1. Crocker, P.R. and A. Varki (2001) Trends Immunol. **22**:337.
2. Crocker, P.R. and A. Varki (2001) Immunology **103**:137.
3. Angata, T. *et al.* (2002) J. Biol. Chem. **277**:24466.
4. Simmons, D. and B. Seed (1988) J. Immunol. **141**:2797.
5. Freeman, S.D. *et al.* (1995) Blood **85**:2002.
6. Taylor, V.C. *et al.* (1999) J. Biol. Chem. **274**:11505.
7. Ulyanova, T. *et al.* (1999) Eur. J. Immunol. **29**:3440.