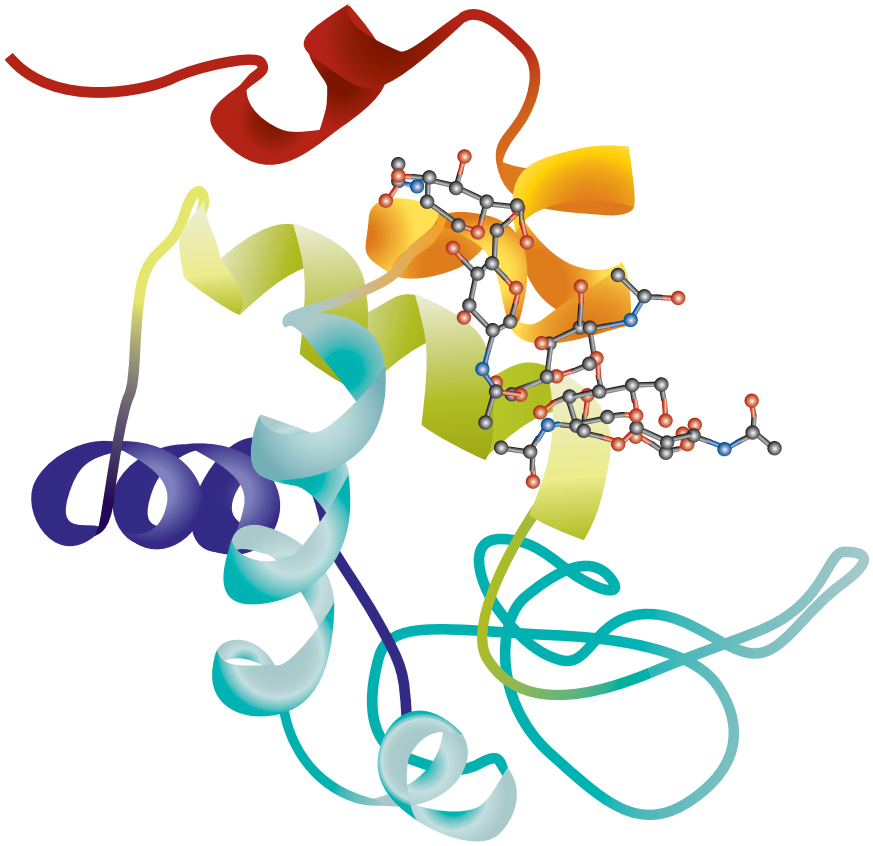
Products for Glycobiology Research



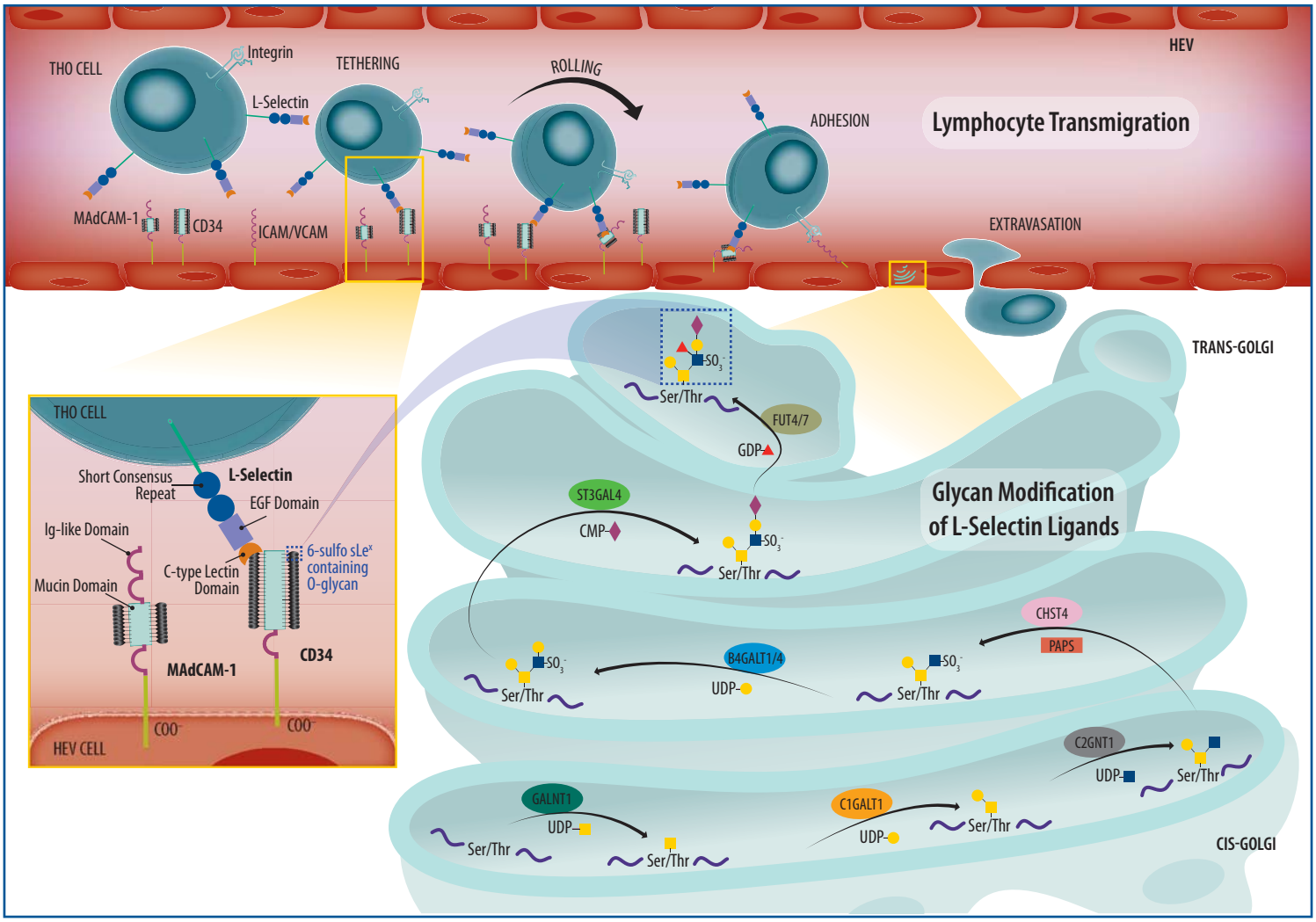
Products for Glycobiology Research

R&D SYSTEMS®

GLYCOBIOLOGY

Glycosylation is an abundant posttranslational modification occurring on a large number of proteins and lipids. It is a dynamic process that produces structural differences that fine tune the functions of glycoconjugates. The modification is known to be important for regulating protein folding, secretion, localization, stability, and activity. In addition, cell surface glycans are crucial for cell recognition and migration in processes such as immune cell trafficking. Glycans are considered to be some of the most structurally varied molecules in nature. This extensive diversity is due both to the large number of existing monosaccharides and the different glycosidic linkages that can form between individual monosaccharide units. Unlike proteins and nucleic acids, glycan synthesis is not template-based. Instead, glycosylation is primarily determined by the availability of specific substrates, and the presence of enzymes that regulate the addition and removal of glycan moieties. The increasing availability of related research reagents, including regulatory enzymes such as glycosyltransferases, glycosidases, sulfotransferases, and sulfatases, offers a unique opportunity to advance the field of glycobiology.

The illustration below highlights the regulatory enzymes involved in the multi-step process of adding the sulfated sialyl Lewis x epitope to L-Selectin ligands in the Golgi. The critical role of this modification in lymphocyte transmigration is an excellent example of the importance of glycosylation in biological processes.



Symbol Key

- Galactose (Gal)
- N-acetylgalactosamine (GalNAc)
- N-acetylglucosamine (GlcNAc)
- N-acetylneuraminic acid (Neu5Ac)
- Fucose (Fuc)

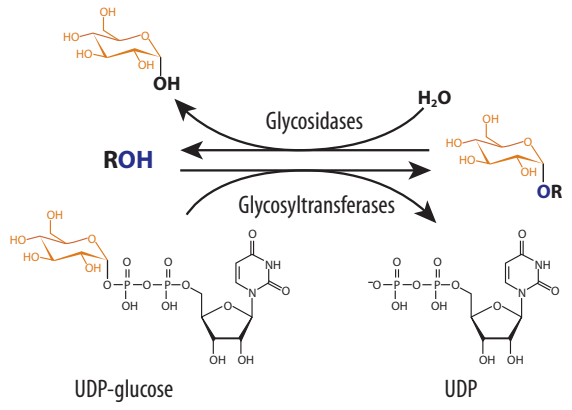
Abbreviation Key

B4GALT1/4	β-1,4-Galactosyltransferase-1/4
C1GALT1	Core 1 Synthase; Glycoprotein-N-Acetylgalactosamine 3-β-Galactosyltransferase 1 (GCNT3-1)
C2GNT1	Core 2 β-1,6-N-Acetylglucosaminyltransferase-1
CHST4	Carbohydrate Sulfotransferase 4; L-Selectin Ligand Sulfotransferase (LSST); N-Acetylglucosamine 6-O-Sulfotransferase (GLCNAC6ST)
FUT4/7	α-1,3-Fucosyltransferase-4/7
GALNT1	Polypeptide N-Acetylgalactosaminyltransferase-1
ICAM	Inter-Cellular Adhesion Molecule
MAdCAM-1	Mucosal Addressin Cell Adhesion Molecule-1
ST3GAL4	α-2,3-Sialyltransferase-4
VCAM	Vascular Cell Adhesion Molecule

Synthesis of the Sulfated Sialyl Lewis x Epitope Present on L-Selectin Ligands. The 6-sulfo sialyl Lewis x (sLe^x) epitope caps the O-glycans that decorate the mucin glycoproteins that function as L-Selectin ligands. These glycoproteins, which include MAdCAM-1 and CD34, are found on the luminal surface of high endothelial venules (HEVs), specialized venules in secondary lymphoid tissue where lymphocytes extravasate into lymph nodes and Peyer's patches. This process is critically dependent on L-Selectin interacting with its glycosylated ligands. Specifically, binding of the C-type lectin domain on L-Selectin to the carbohydrate determinants, such as 6-sulfo sLe^x on sulfated mucin glycoproteins, initiates lymphocyte rolling on the endothelial surface, which in turn leads to integrin-dependent adhesion to HEV cells and lymphocyte extravasation. The posttranslational glycosylation of L-Selectin ligands occurs in the Golgi and requires the coordinated action of fucosyltransferases, sulfotransferases, glycosyltransferases, and sialyltransferases. Biosynthesis of the 6-sulfo sLe^x-containing O-glycan is initiated by GALNT1 catalyzing the addition of GalNAc to serine (Ser) or threonine (Thr) residues of the protein backbone. The branching core enzymes C1GALT1 and C2GNT1 then catalyze the addition of Gal (β1,3 linkage) and GlcNAc (β1,6 linkage), respectively, to GlcNAc . The next step is GlcNAc-6-O-sulfation, which is catalyzed by CHST4, followed by the transfer of Gal to GlcNAc in β1,4 linkage by B4GALT1 and B4GALT4, and the addition of Neu5Ac to Gal by ST3GAL4 in α2,3 linkage. Lastly FUT4 and FUT7 transfer Fuc to GlcNAc in α1,3 linkage.

Glycosyltransferases & Glycosidases

Glycosyltransferases are a family of enzymes that synthesize glycans by transferring a monosaccharide moiety from a glycosyl donor to an acceptor substrate. In contrast, glycan degradation is performed by glycosidases, which hydrolyze glycosidic linkages, thereby releasing monosaccharides or oligosaccharides. R&D Systems provides highly purified, active recombinant glycosyltransferases and glycosidases, and high performance antibodies, which have been generated using the active recombinant proteins as immunogens.



Biosynthesis and Degradation of Glycans. Glycans are synthesized by glycosyltransferases that transfer a monosaccharide moiety from either a nucleotide sugar or lipid phosphosugar to an acceptor substrate. Acceptors are most often glycans, but they can also be proteins and lipids. Glycosidases degrade glycans by hydrolyzing glycosidic linkages to release monosaccharides or oligosaccharides.

For more information please visit our website at www.RnDSystems.com/go/GlycoEnzymes

R&D Systems Products for Glycosyltransferase & Glycosidase Research

Glycosyltransferases

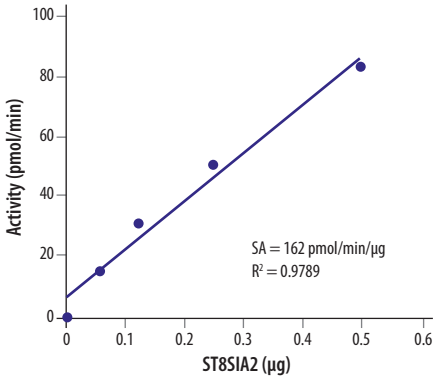
MOLECULE	PROTEINS	ANTIBODIES & APPLICATIONS
β-1,3-Glucuronyltransferase 1/B3GAT1	R	
β-1,3-Glucuronyltransferase 3/B3GAT3	H	
β-1,3-N-acetylglucosaminyltransferase 1/B3GNT1		H M (WB)
β-1,3-N-acetylglucosaminyltransferase 2/B3GNT2	H	H (WB)
β-1,3-N-acetylglucosaminyltransferase 6/B3GNT6	H	
β-1,4-Galactosyltransferase 1/B4GalT1	H	H (IP, WB)
Exostosin-like 1/EXTL1		M (IP, WB)
Exostosin-like 2/EXTL2		M (WB)
Exostosin-like 3/EXTL3		H (IP, WB)
Fucosyltransferase 1/FUT1	H	
Fucosyltransferase 3/FUT3	H	H (IP, WB)
Fucosyltransferase 3/5 (FUT3/5)		H (IP, WB)
Fucosyltransferase 5/FUT5	H	H (IP, WB)
Fucosyltransferase 7/FUT7	H	
Fucosyltransferase 8/FUT8	H	H (IHC, IP, WB)
Fucosyltransferase 11/FUT11	H	H (IHC, IP, WB)
MFNG/O-fucosylpeptide 3-β-N-acetylglucosaminyltransferase		H (IP, WB)
N-Acetylglucosaminyltransferase V/MGAT5	H	
Protein O-Glucosyltransferase 1/POGLUT1	H	
ST6 Gal Sialyltransferase 1/ST6GAL1	H	H (WB)
GalNAc α-2,6-Sialyltransferase V/ST6GALNAC5	H	
ST6 Sialyltransferase 2/ST6GALNAC2	M	
α-2,8-Sialyltransferase 8A/ST8SIA1	H	
α-2,8-Sialyltransferase 8B/ST8SIA2	H	
Toxin B/TcdB	*C. difficile*	
Glucuronosyltransferase 1A1/UGT1A1		H (WB)

Species Key: H Human M Mouse R Rat B Bovine *ELISA Kits are also available for this analyte.
Applications Key: B/N Blocking/Neutralization IHC Immunohistochemistry IP Immunoprecipitation WB Western blot

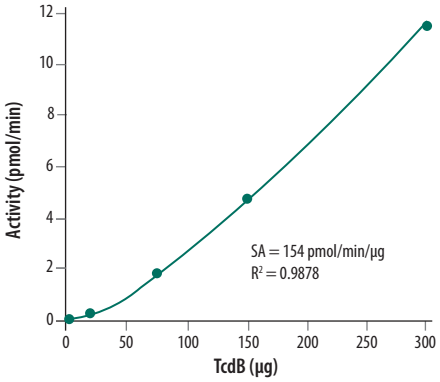
Glycosidases

MOLECULE	PROTEINS	ANTIBODIES & APPLICATIONS
α-Galactosidase/α-Gal	*E. coli*	
α-Galactosidase A/GLA	H	
α-L-Fucosidase	*T. maritima*	
α-N-acetylgalactosaminidase	H *C. perfringens*	
β-Galactosidase-1/GLB1	H	
β-Glucuronidase/GUSB	H	H (WB)
β(1-3)-Galactosidase	*X. campestris*	
β(1-4)-Galactosidase	*S. pneumoniae*	
Chitinase 3-like 1*	H M	H (IHC, WB), M (IP, WB)
Chitinase 3-like 2	H	H (WB)
Chitinase 3-like 3/ECF-L*	M	M (WB)
Chitobiase/CTBS		H (IP, WB)
Chitotriosidase/CHIT1	H M	H (B/N, IP, WB), M (IP, WB)
Cytosolic β-Glucosidase/GBA3	H	H (IP, WB)
Endo-β-N-acetylglucosaminidase F1/Endo F1	*F. meningosepticum*	
Endo-β-N-acetylglucosaminidase F3/Endo F3	*F. meningosepticum*	
Endo-β-N-acetylglucosaminidase H/Endo H	*S. plicatus*	
Heparinase I	*B. thetaiotaomicron*	
Heparinase II	*P. heparinus*	
Heparinase III	*P. heparinus*	
Hexosaminidase A/HEXA	H	
Hyaluronan Lyase	*S. agalactiae*	
α-L-Iduronidase/IDUA	H	H (IP, WB)
Klotho	H M	M (IHC, WB)
Klotho β	H M	M
Lactase-like Protein/LCTL		H
Neuraminidase	*C. perfringens*, *M. viridifaciens*, H1N1 Influenza virus	H1N1 Influenza virus (IP, WB)
O-GlcNAcase/OGA	*B. thetaiotaomicron*	
SPAM1	B	

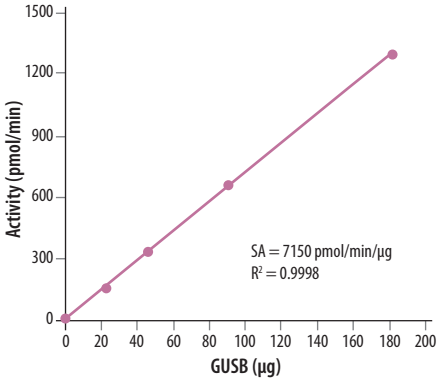
Proteins



Activity of Human ST8SIA2. The ability of Recombinant Human α-2,8-Sialyltransferase 8B/ST8SIA2 (Catalog # 6590-GT) to transfer Neu5Ac (sialic acid) from CMP-Neu5Ac to Recombinant Human NCAM-1/CD56 (Catalog # 2408-NC) was measured. The reaction was carried out in the presence of Recombinant Human 5′-Nucleotidase/CD73 (Catalog # 5795-EN), which cleaves an inorganic phosphate from the CMP leaving group. The released phosphate was detected using the Malachite Green Phosphate Detection Kit (Catalog # DY996). The activity was plotted against enzyme input, and the specific activity of ST8SIA2 was determined to be 162 pmol/min/µg.

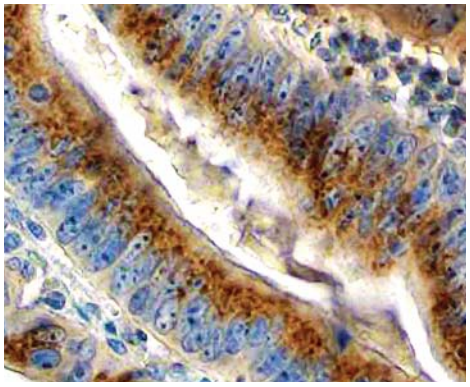


Activity of *C. difficile* TcdB. The ability of Recombinant *Clostridium difficile* Toxin B/TcdB (Catalog # 6246-GT) to hydrolyze UDP-glucose was measured. The reaction was carried out in the presence of Recombinant Human CD39L3/ENTPD3 (Catalog # 4400-EN), which cleaves an inorganic phosphate from the UDP leaving group. The released phosphate was detected using the Malachite Green Phosphate Detection Kit (Catalog # DY996). The activity was plotted against enzyme input, and the specific activity of TcdB was determined to be 154 pmol/min/µg.

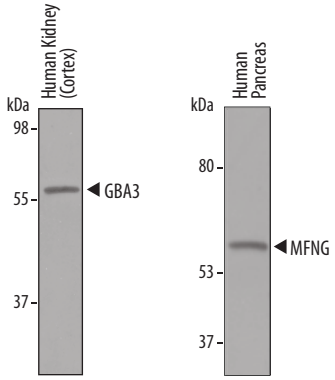


Activity of Human GUSB. The enzymatic activity of Recombinant Human β-Glucuronidase/GUSB (Catalog # 6144-GH) was assayed by hydrolyzing the fluorogenic substrate 4-Methylumbelliferyl (4-MU)-β-D-glucuronide to release the fluorophore 4-MU. Fluorescence from liberated 4-MU was measured, and the specific activity of GUSB, determined by plotting the activity versus enzyme input, was found to be 7150 pmol/min/µg.

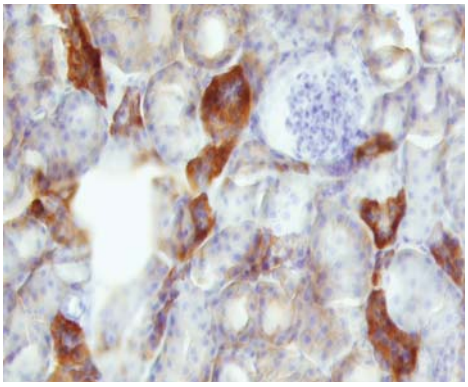
Antibodies



Detection of FUT8 in Human Colon. Fucosyltransferase 8/FUT8 was detected in an immersion-fixed paraffin-embedded section of human colon using a Sheep Anti-Human FUT8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5768). Before incubation with primary antibody, the tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). The tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (Catalog # CTS019; brown) and counterstained with hematoxylin (blue). Please see page 6 for our related Sialyltransferase Activity Kit.

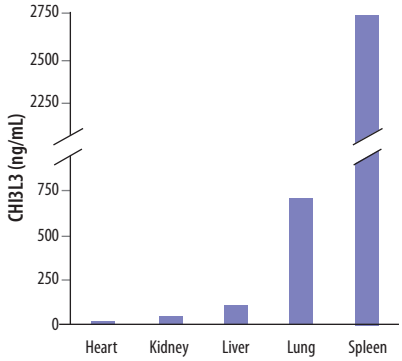


Detection of GBA3 and MFNG by Western Blot. Lysates of human kidney (cortex) and pancreatic tissue were immunoblotted using a Sheep Anti-Human Cytosolic β-Glucosidase/GBA3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5969) or a Sheep Anti-Human MFNG Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6355), followed by a HRP-conjugated Donkey Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band for GBA3 and MFNG was detected at approximately 55 kDa (as indicated). Please see page 6 for our related Glycosyltransferase Activity Kit.

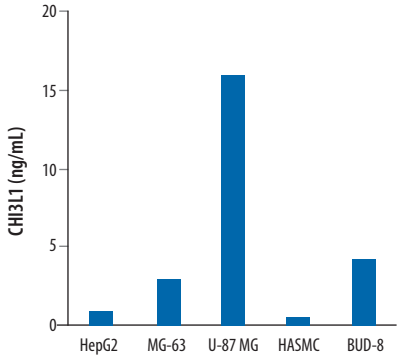


Detection of Klotho in Mouse Kidney. Klotho was detected in perfusion-fixed frozen sections of mouse kidney using a Goat Anti-Mouse Klotho Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1819). The tissue was stained with the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (Catalog # CTS008; brown) and counterstained with hematoxylin (blue).

ELISAs



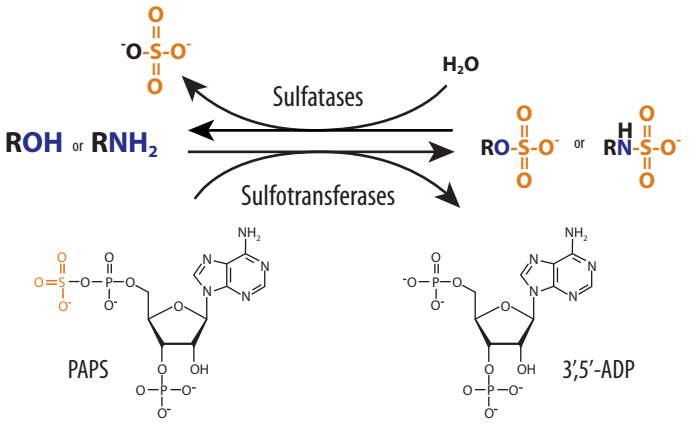
Measurement of the Levels of Mouse CHI3L3 using the CHI3L3 Quantikine® ELISA Kit. Chitinase 3-like 3/ECF-L (CHI3L3) was measured in lysates prepared from mouse heart, kidney, liver, lung, and spleen tissue using the Mouse Chitinase 3-like 3/ECF-L Quantikine ELISA Kit (Catalog # MC3L30).



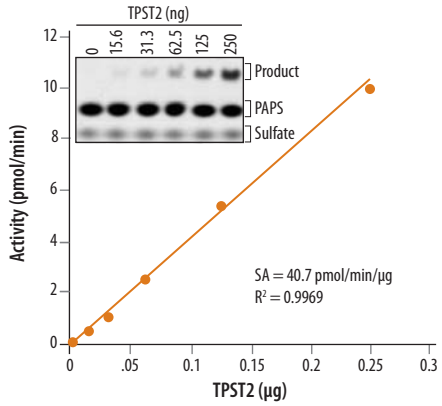
Detection of Human CHI3L1 using the CHI3L1 Quantikine ELISA Kit. Aliquots of cell culture supernates from the HepG2 human hepatocellular carcinoma cell line, MG-63 human osteosarcoma cell line, U-87 MG human glioblastoma/astrocytoma cell line, human aortic smooth muscle cells (HASMC), and BUD-8 human fibroblast cell line were assayed for Chitinase-3-like 1 (CHI3L1) expression using the Human Chitinase-3-like 1 Quantikine ELISA Kit (Catalog # DC3L10).

Sulfotransferases & Sulfatases

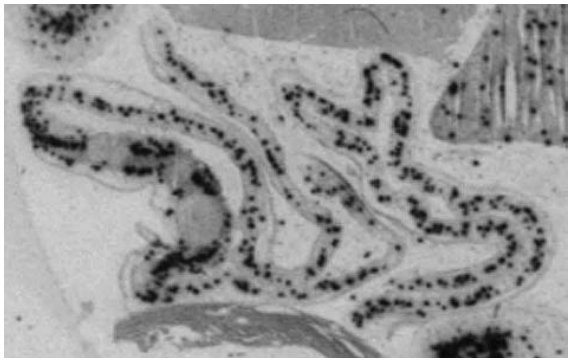
Sulfation is a common modification on glycans. Glycan sulfation is carried out by Golgi-residing carbohydrate-specific sulfotransferases. These enzymes transfer a sulfate group from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to glycans on lipids and proteins as they pass through the secretory pathway. Most sulfatases reside in lysosomes and hydrolyze sulfate esters from sulfated substrates. Improper degradation of sulfated glycans, caused by sulfatase deficiencies, underlies lysosomal storage diseases. R&D Systems provides high performance proteins and antibodies for use in multiple applications, including immunohistochemistry, Western blot, flow cytometry, and more.



Opposing Actions of Sulfotransferases and Sulfatases. Sulfotransferases catalyze the transfer of a sulfate group to a hydroxyl or amine moiety on various molecules, including carbohydrates and proteins. In contrast, sulfatases catalyze the hydrolysis of O- and N-sulfate esters from these molecules.



Activity of Human TPST2. The activity of Recombinant Human Tyrosylprotein Sulfotransferase 2/TPST2 (Catalog # 6236-ST) was analyzed by measuring its ability to catalyze the sulfation of the PSGL-1 peptide using PAP^{35}S as the donor substrate. The enzymatic product and substrates were separated on an 8% SDS polyacrylamide gel (inset). The radioactivity of the hot spots was counted. TPST2 activity was based on ^{35}S incorporation rate. The calculated activity was then plotted against the enzyme inputs and used to determine specific activity, which was measured to be 40.7 pmol/min/µg.



Detection of GlcA in Rat Intestine using PAP^{35}S and CHST10. The high sensitivity of *in vitro* sulfation can be utilized to detect glycans in biological samples. Expression of non-reducing glucuronic acid (GlcA) in intestine was investigated by incubating a perfusion-fixed frozen section of rat intestine with a labeling mixture containing PAP^{35}S and a His-tagged Recombinant Human Carbohydrate Sulfotransferase 10/CHST10 (Catalog # 6140-ST). CHST10 catalyzes the transfer of radioisotope ^{35}S from the sulfur donor (PAPS) to terminal GlcA on both protein- and lipid-linked oligosaccharides. Dried tissue is then exposed to x-ray film. CHST10 labeled discontinuous spots in the mucosal layer of the intestine.

R&D Systems Products for Sulfotransferase & Sulfatase Research

Sulfotransferases		
MOLECULE	**PROTEINS**	**ANTIBODIES & APPLICATIONS**
Carbohydrate Sulfotransferase 1/CHST1	H M	H (B/N, IP, WB)
Carbohydrate Sulfotransferase 2/CHST2	H	H (IP, WB)
Carbohydrate Sulfotransferase 3/CHST3	M	H (B/N, IP, WB)
Carbohydrate Sulfotransferase 4/CHST4	H M	H M (B/N, WB)
Carbohydrate Sulfotransferase 5/CHST5	M	H (IP, WB)
Carbohydrate Sulfotransferase 6/CHST6	H	
Carbohydrate Sulfotransferase 7/CHST7	M	M (IP, WB)
Carbohydrate Sulfotransferase 10/CHST10	H	
Carbohydrate Sulfotransferase 15/CHST15	H	H (FC, WB)
Heparan Sulfate 2-O-Sulfotransferase 1/HS2ST1	H	
Heparan Sulfate 3-O-Sulfotransferase 1/HS3ST1	H	H (IP, WB)
Heparan Sulfate 3-O-Sulfotransferase 4/HS3ST4	H	H M (B/N, IHC, IP, WB)
Heparan Sulfate 6-O-Sulfotransferase 1/HS6ST1	H	H (IHC, WB)
Heparan Sulfate 6-O-Sulfotransferase 2/HS6ST2		H (IP, WB)
Heparan Sulfate 6-O-Sulfotransferase 3/HS6ST3	M	M (IHC)
N-deacetylase/N-sulfotransferase 1/NDST1	H	
Tyrosylprotein Sulfotransferase 1/TPST1	H	
Tyrosylprotein Sulfotransferase 2/TPST2	H	

Sulfatases & Modifying Factors		
MOLECULE	**PROTEINS**	**ANTIBODIES & APPLICATIONS**
Arylsulfatase A/ARSA	H M	H (IHC, IP, WB)
Arylsulfatase B/ARSB	H	H (WB)
Arylsulfatase G/ARSG	M	M (IP, WB)
Glucosamine (N-acetyl)-6-Sulfatase/GNS	H	H (IP, WB)
Iduronate 2-Sulfatase/IDS	H M	H M (IP, WB)
Sulfamidase/SGSH	M	M (IP, WB)
Sulfatase Modifying Factor 1/SUMF1		H M (IP, WB)
Sulfatase Modifying Factor 2/SUMF2		H M (IP, WB)

Species Key: H Human M Mouse

Applications Key: B/N Blocking/Neutralization FC Flow Cytometry IHC Immunohistochemistry IP Immunoprecipitation WB Western blot

For more information, please visit our website at www.RnDSystems.com/go/GlycoEnzymes

ON THE COVER:

Glycosidase in Action: Lysozyme is a major glycosidase that hydrolyzes the bacterial cell wall peptidoglycan by cleaving the 1-4-β-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues. In addition, it also cleaves chitin oligosaccharides. The structure shown here is an active site mutant (E35Q) of hen egg white lysozyme in complex with one of its substrates, chitopentaose (PDB ID 2WAR).

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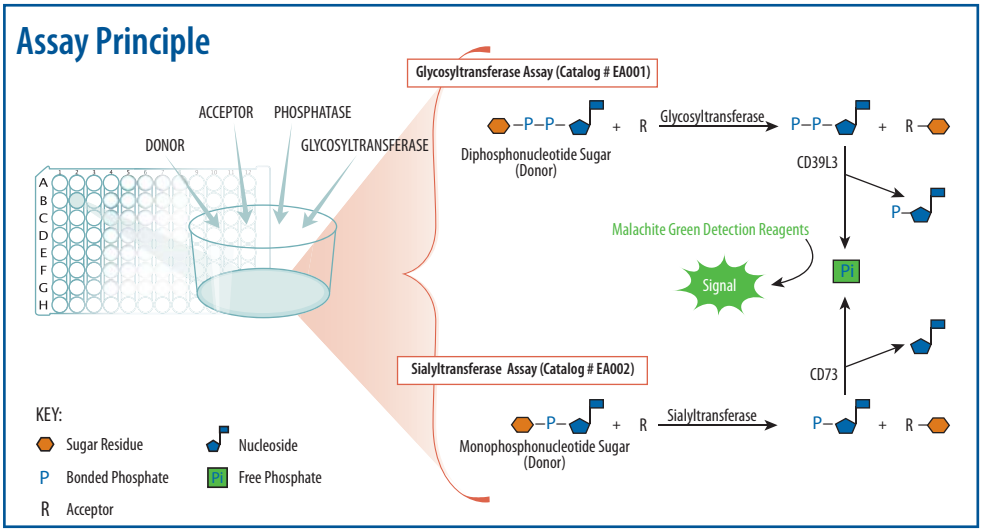
Glycosyltransferase Activity Kits

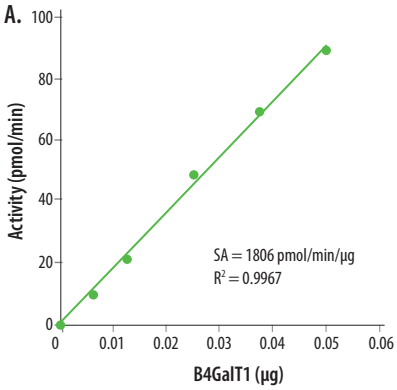
R&D Systems offers two new kits for analyzing the activity of glycosyltransferases that use activated sugar nucleotides as donor substrates. The Glycosyltransferase Activity Kit (Catalog # EA001) and the Sialyltransferase Activity Kit (Catalog # EA002) include specific phosphatases that release inorganic phosphate from nucleotides produced during glycosyltransferase reactions. The released phosphate is then detected using colorimetric reagents. Because the concentration of released phosphate is directly proportional to the number of sugar molecules transferred during the reaction, these kits provide researchers a simple, non-radioactive method for measuring kinetic parameters of glycosyltransferases. Furthermore, the assay is performed in multi-well plates and quantified by a plate reader, making it amenable to high-throughput screening.

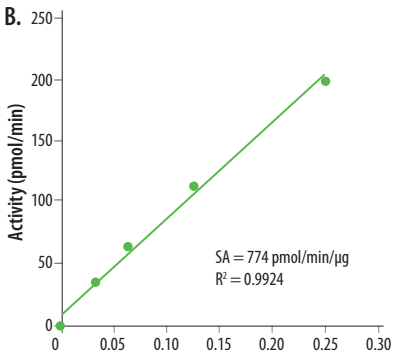
ASSAY KIT	CATALOG #	COMMON KIT CONTENTS	UNIQUE KIT CONTENTS
Glycosyltransferase Activity Kit	EA001	$MnCl_2$, Phosphate Standard, Malachite Green Reagents A & B	ENTPD3/CD39L3, Phosphatase Buffer 1, UDP
Sialyltransferase Activity Kit	EA002	$MnCl_2$, Phosphate Standard, Malachite Green Reagents A & B	5′-Nucleotidase/CD73, Phosphatase Buffer 2, CMP

Assay Principle



The assay procedure for these kits involves preparing a reaction mix containing a donor molecule, acceptor substrate, and coupling phosphatase. The reaction is then initiated by adding the glycosyltransferase or sialyltransferase. Malachite Green Phosphate detection reagents are added and absorbance is read at 620 nm.





Activity of B4GalT1 and ST6GALNAC2. A. The ability of Recombinant Human β-1,4-Galactosyltransferase 1/B4GalT1 (Catalog # 3609-GT) to transfer galactose from UDP-galactose to N-acetyl-α-D-glucosamine was measured using the Glycosyltransferase Activity Kit (Catalog # EA001). **B.** The ability of Recombinant Mouse ST6 Sialyltransferase 2/ST6GALNAC2 (Catalog # 6468-GT) to transfer Neu5Ac from CMP-Neu5Ac to bovine asialofetuin was measured using the Sialyltransferase Activity Kit (Catalog # EA002). For each reaction, activity was plotted against enzyme input, and the specific activities of B4GalT1 and ST6GALNAC2 were determined to be 1806 pmol/min/µg and 774 pmol/min/µg, respectively.

For more information visit our website at
www.RnDSystems.com/go/GlycoActivityKits