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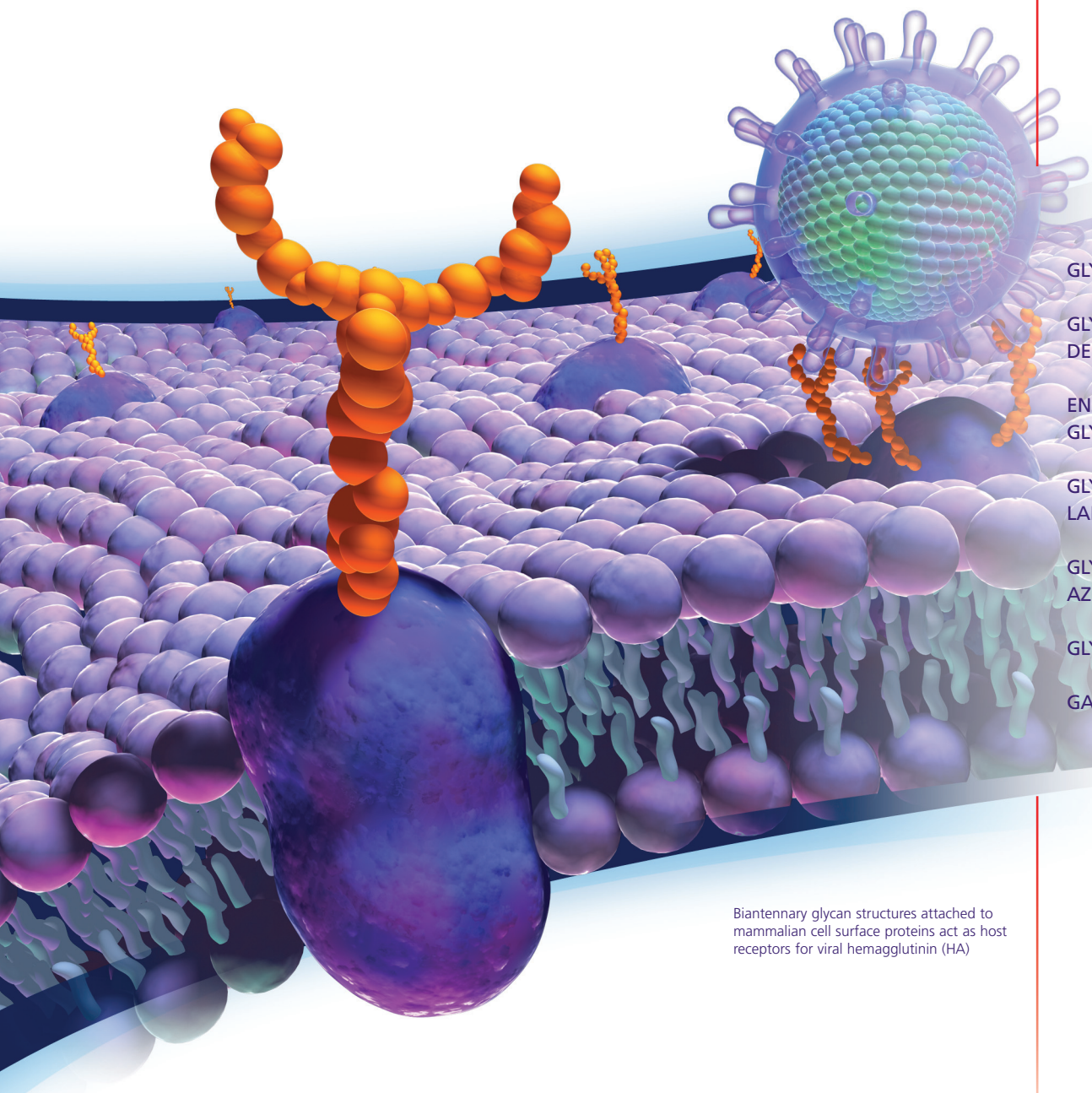

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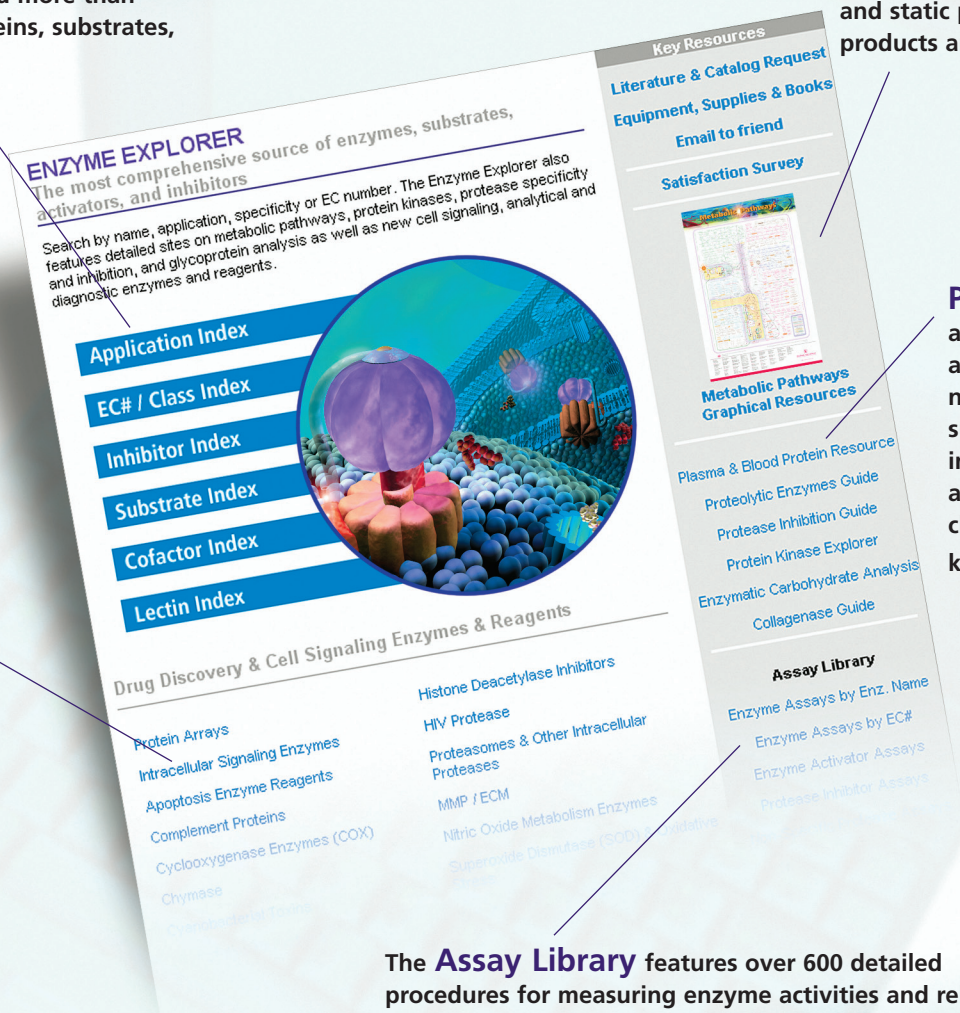

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January 2007

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Glycobiology

Introduction

Glycans are ubiquitous in nature, and their representation on cell surfaces is commonly called the **glycome**. Oligosaccharides and polysaccharides are responsible for much of the structural variation in biological systems and are generated by cells to serve as structural scaffolds, to regulate viscosity, and for energy storage. The carbohydrate moieties of cell surface glycoproteins and glycolipids function in cellular communication processes and physiological responses.[1-4] Cell-surface glycoproteins and glycolipids provide anchors for intercellular adhesion, provide points of attachment for antibodies and other proteins, and function as receptor sites for bacteria and viral particles.[5,6]

Many intracellular processing events are disrupted environmentally or are the result of genomic abnormalities (congenital disorders of glycosylation; CDG) and result in disease states. Altered cell surface glycosylation patterns are associated with cellular differentiation, development, and viral infection, and are diagnostic in certain cancers,[7] correlating to changes in the expression or localization of relevant glycosyltransferases. Multiple studies have evaluated the roles of glycoproteins and proteoglycans in tumor metastasis, angiogenesis, inflammatory cell migration, lymphocyte homeostasis, and congenital disorders of glycosylation. Oligosaccharides and competitive glycoconjugates are potential drug targets in infectious diseases, inflammation and cancer. Glycosylation of proteins and other bioactive molecules has been shown to increase solubility of hydrophobic molecules,[8,9] alter uptake and residency time *in vivo*,[10,11] and decrease antigenicity.[12]

The progress of glycomics in the biopharmaceutical industry is demonstrated by the development of drugs that manipulate carbohydrates and glycoproteins for therapeutic benefit. Research on glycosyltransferases to understand the role of carbohydrate interactions in cancerous cells is also likely to provide further opportunities for application of glycomics. Scientists observing cultured cells that correspond with solid tumors have found expressed glycoprotein antigens that may provide the basis for the development of serum-based biomarker diagnostics for cancer. However, the investigation of the roles of carbohydrates in fundamental biological processes and their potential as novel therapeutic agents has been limited by the low abundance of many glycan structures from natural sources.[3] Cellular systems that overexpress glycoproteins have been found to generate heterogeneous glycan pools.[13,14] Genetic research has tried to identify the genes responsible for glycosylation in specific types of cells. Glycomics is poised to become a dynamic research area as more robust laboratory techniques and targeted reagents become available.

This issue of BioFiles highlights Sigma's key products for glycomics and glycoproteomics research techniques, including enzymatic glycan synthesis, glycoprotein deglycosylation strategies, and glycan detection methods. Glycolytic enzymes and lectins, proteins in which Sigma has historic and core capabilities, are included as fundamental reagents for carbohydrate studies.

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Glycosyltransferases

Tools for Synthesis and Modification of Glycans

The presence of multiple functional groups and stereocenters in complex carbohydrates makes them challenging targets for the organic chemist. Chemical synthesis research has not yielded robust, automated protocols comparable to those developed for the preparation of peptides and oligonucleotides. There are two major obstacles to the large-scale, chemical synthesis of carbohydrates and glycoconjugates:[1-5]

- Multiple hydroxyl groups with similar reactivities must be differentiated in order to create the desired regioselective and stereospecific glycosidic bonds. Laborious manipulation of protecting groups and complex synthetic schemes are required to prevent reactions with undesired hydroxyl sites. The large number of potential linkages between specific monosaccharide units requires effective regioselective and stereospecific activation of either glycosyl donors or acceptors.
- As many carbohydrates are only soluble in water, synthetic manipulation requires either an adaptation of organic reactions to aqueous media or a reversible modification of the carbohydrates to achieve solubility in non-aqueous solvents.

Glycosyltransferases from the Leloir pathway[6-8] have been proven to be viable alternatives to chemical synthesis in the preparation of oligosaccharides.[1,2,9-13] As more of these transferases are isolated from natural sources or produced by recombinant technology, chemists have recognized enzymatic glycosylation as the preferred method to complement classical synthetic techniques. Leloir glycosyltransferases are highly regioselective and stereospecific with respect to the glycosidic linkages formed. They incorporate unprotected sugar precursors, avoid tedious chemical modifications, and provide oligosaccharides in high yields.

The biosynthesis of oligosaccharides, catalyzed by glycosyltransferases from the Leloir pathway, resembles the corresponding chemical procedure (see **Figure 1**). A donor sugar is activated in the first step, followed by the transfer of the activated sugar to an appropriate acceptor sugar. Leloir glycosyltransferases primarily utilize one of eight different nucleotide mono- or diphosphates (UDP-Glc, UDP-GlcNAc, UDP-Gal, UDP-GalNAc, GDP-Man, GDP-Fuc, UDP-GlcA, and CMP-NeuAc) as monosaccharide donors to build a new glycosidic bond. [7]

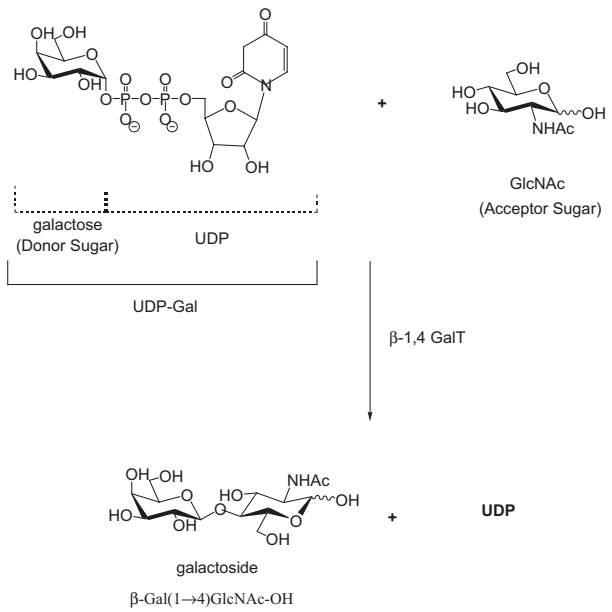

Figure 1. Enzymatic-catalyzed glycosylation using β(1→4)Galactosyltransferase [β(1→4) GalT].

Glycosyltransferases are specific for the type of linkage (α or β), and the linkage position of the glycoside bond formed [e.g. α(1→3) or β(1→4)]. Glycosyltransferases were initially considered to be specific for a single glycosyl donor and acceptor, which led to the "one enzyme–one linkage" concept.[28,29] Subsequent observations have refuted the theory of absolute enzymatic specificity by describing the transfer of analogs of some nucleoside mono- or diphosphate sugar donors.[30-36] Glycosyltransferases can tolerate modifications to the acceptor sugar, as long as the acceptor meets specific structural requirements (e.g. appropriate stereochemistry and availability of the reactive hydroxyl group involved in the glycosidic bond).

A major limitation to enzyme-catalyzed glycosylation reactions is the glycosyltransferase inhibition caused by nucleoside diphosphates generated during the reaction. Two strategies have been identified to prevent enzymatic inhibition (see **Figure 2**):

1. Phosphatase is added to the reaction to degrade the nucleoside diphosphates by removal of the phosphate group (see **Figure 2A**).[23]
2. Nucleoside diphosphates are recycled to the appropriate nucleotide diphosphates by employing multi-enzyme regeneration schemes. Although several different enzymes and cofactors are involved in these *in situ* regeneration schemes, the method avoids the use of stoichiometric amounts of sugar nucleotides (see **Figure 2B**).[24-26]

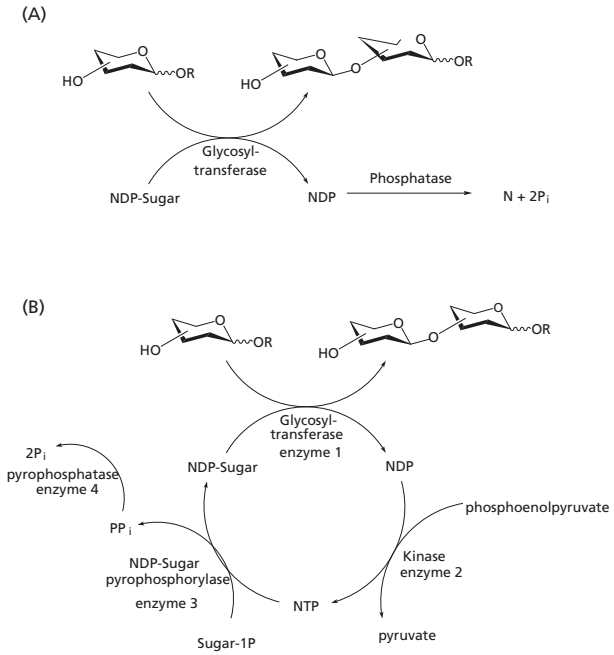

Figure 2. Methods for avoiding enzyme inhibition in glycosyltransferase-catalyzed synthesis: (**A**) Addition of phosphatase. (**B**) Recycling of sugar nucleotides (*NDP = nucleoside diphosphates, NTP = nucleoside triphosphates, N = nucleoside, P_i = phosphate*).

In contrast to organic chemical synthesis, enzymatic glycosylation has potential for application use within biological systems, where the modification of glycosylation sites may be used to investigate the regulation of cell signalling processes. Various application strategies for glycosyltransferases have employed an assortment of glycosyl donors and reaction conditions for the synthesis of carbohydrates and the glycosylation of natural products.[27,28]

α(1→3)Galactosyltransferase

α(1→3)Galactosyltransferase (EC 2.4.1.151; α(1→3)GalT) is responsible for the formation of α-galactosyl epitopes bearing α-Gal(1→3)-β-Gal-OR termini. The interaction of α-Gal epitopes (Galili antigens) on the surface of animal cells (e.g. porcine endothelial cells) with anti-galactosyl antibodies present in human serum is believed to be the main cause in antibody-mediated hyperacute rejection following xenotransplantation.[43-51] Experimental attempts to overcome hyperacute rejection revealed the need for α-Gal oligosaccharides, synthetic α-Gal analogs, and mimetics with high affinity to anti-Gal antibodies. Earlier methods to chemically synthesize α-Gal trisaccharides were tedious,[49-51] while glycosidase-catalyzed transglycosylation reactions to form the desired α-Gal(1→3)-β-Gal-OR linkage resulted in poor yields and regioselectivities.[38-40] Using recombinant α(1→3) galactosyl-transferase, α-Gal epitopes and several derivatives have been synthesized on a preparative scale.[41]

α(1→3)Galactosyltransferase transfers a galactose unit from the activated donor UDP-galactose (UDP-Gal) to the 3-hydroxy site of a terminal β-linked galactose, resulting in an α-linkage. Several studies of α(1→3)galactosyltransferase substrate specificity have been carried out which show a high acceptor promiscuity of the enzyme *in vitro*.[38-40] Acceptors that have been successfully used include lactose, β-lactosyl azide, β-thiophenyl lactoside, N-acetyl-lactosamine derivatives, lactosamine,[41] and a wide range of N-acyl-derivatives of type II disaccharides. Carbamate groups, protected amino acid residues, lipophilic, and hydrophilic aromatic residues can replace the natural occurring N-acetyl group.[6] α(1→3)Galactosyltransferase can transfer galactose to an unnatural hindered tertiary hydroxyl group of the acceptor sugar, yielding an acetal formation reaction with a highly deactivated hydroxyl group that is extremely difficult to synthesize by chemical methods.[42]

β(1→4)Galactosyltransferase

The synthesis and substrate specificity of **β(1→4)Galactosyl-transferase** (EC 2.4.1.22; β(1→4)GalT) from bovine milk has been extensively investigated.[2,9-12,43-49] β(1→4)GalT catalyzes the transfer of galactose from UDP-galactose (UDP-Gal) to the 4-hydroxy site of N-acetyl-D-glucosamine (GlcNAc) and β-linked GlcNAc subunits to yield β-lactosamine (β-LacNAc) and β-Gal(1→4)-β-GlcNAc structures respectively.[50] Both α- and β-glycosides of glucose have been used as acceptors by β(1→4)GalT, with α-glucosides requiring the presence of α-lactalbumin.[26] The enzyme forms a heterodimeric complex with α-lactalbumin, altering the specificity so that D-glucose becomes the preferred acceptor. Thus, addition of α-lactalbumin promotes the formation of lactose (β-Gal(1→4)-Glc-OH). Numerous other acceptor substrates for the β(1→4)GalT-catalyzed transfer of galactose have been described, including 2-deoxyglucose, D-xylose, 5-thioglucose, N-acetylmuramic acid, and myo-inositol. 6-O-Fucosylated and sialyated modifications may also serve as acceptors,[51] as well as 3-O-methyl-GlcNAc,[24] 3-deoxy-GlcNAc, 3-O-allyl-GlcNAc-β-OBu and 3-oxo-GlcNAc.[66] Several modifications of GlcNAc that have been employed as acceptor substrates are illustrated (see **Figure 3**).[9]

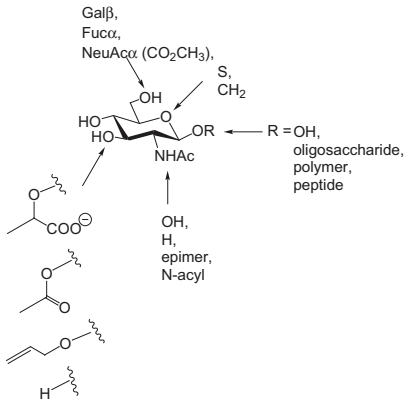

Figure 3. Modifications of GlcNAc employed as acceptors in β(1→4)GalT catalyzed transfer of galactose.

β(1→4)GalT cannot utilize D-mannose, D-allose, D-galactose, or D-ribose as substrates.[11-12] Monosaccharides displaying a negative charge, such as glucuronic acid and α-glucose 1-phosphate, are also not tolerated as substrates. Azasugars and glucals have been shown to be very weak acceptors.[24] Modified nucleotide sugar donor substrates have a slower rate of enzyme-catalyzed transfer.[11,12]

N-Acetylglucosaminyl amino acids and peptides have been successfully galactosylated to produce glycopeptides with a disaccharide moiety. Subsequent extension of the carbohydrate chain was accomplished by employing α(2→6)sialyltransferase.[53-54]

An asparagine-bound trisaccharide was prepared using combined chemo-enzymatic synthesis.[53] Attachment of galactose to a N-acetylglucosaminyl oligopeptide was followed by sialylation with α(2→3)sialyltransferase and fucosylation with α(2→3)-fucosyltransferase, which yielded a glycopeptide containing a tetrasaccharide moiety.[55]

Since different glycosides of N-acetylglucosamine and glucose can be used as acceptors in β(1→4)GalT-catalyzed galactose transfer, the enzymatic method has been used to modify pharmacologically interesting glycosides.[56-59] β(1→4)GalT has been used to attach galactose to the bioactive glycosides elymoclavine-17-O-β-D-glucopyranoside,[56] stevioside and steviolbioside,[60] colchicoside and fraxin,[61] and different ginsenosides.[62] Conjugation of galactose with glycosides demonstrates the potential application in drug delivery by increasing the solubility and bioavailability of large hydrophobic molecules under mild conditions. C-Glycoside analogs of the naturally occurring glycopeptide linkages (N-acetyl-glucosamine β-linked to either asparagine or serine) generated high yields of the corresponding C-lactosides.[63]

β(1→4)GalT has been employed in solid-phase oligosaccharide synthesis on polymer supports such as polyacrylamide or water-soluble poly(vinyl alcohol). The resulting galactosylated oligosaccharides are cleaved from the polymers photochemically or with chymotrypsin.[64]

Glycosyltransferases

Tools for Synthesis and Modification of Glycans

α-1,3-Fucosyltransferase VI

α(1→3)Fucosyltransferase (α(1→3)FucT) catalyzes the transfer of L-fucose from the donor guanosine diphosphate-β-L-fucose (GDP-Fuc) to the free 3-hydroxy position as an α-orientation[65,66] and tolerates a wide range of acceptors (see **Figure 4**).[67]

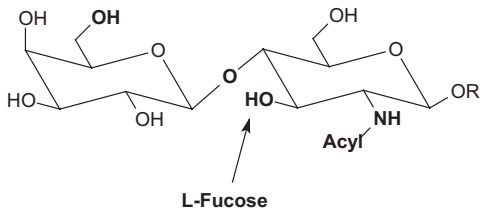

Figure 4. Minimum structural requirements for an acceptor employed in α(1→3)FucT catalyzed transfer of L-fucose include: 6′-OH, free 3-OH, β(1→4)-linkage and 2-NH-acylation. An acyl lactosamine is shown as an example acceptor.

The number and linkage type of fucose residues in N-glycans and the fucosylation pattern varies with the organism, the tissue, and the developmental and physiological status of the cell.[68] Fucose is normally attached:

- To a N-glycan by α(1→2)-linkage to galactose (Gal)
- To a N-glycan by α(1→3), α(1→4), or α(1→6)-linkage to an N-acetylglucosamine (GlcNAc) residue
- To a peptide by direct O-linkage to serine/threonine

The terminal step in the biosynthetic pathway of fucose-containing saccharides is the transfer of L-fucose from GDP-Fuc to the corresponding glycoconjugate acceptor catalyzed by fucosyltransferase.[67-71] Fucosylated glycan structures within glycopeptides, glycoproteins and glycolipids play a central role in cell-cell interactions and cell migration, increasing the significance of the study of fucosyltransferase expression, inhibition and regulation. More than 150 complete or partial sequences of fucosyltransferases can be found through protein sequence databases such as Swiss Institute of Bioinformatics Swiss-Prot system **www.expasy.ch.**

Glycosyltransferase Kits from Sigma

As part of our commitment to biotransformation technologies, Sigma has developed recombinant glycosyltransferases and kits for preparative carbohydrate synthesis and directed modification of carbohydrate moieties. The enzymatic synthesis reactions go to completion rapidly and specifically, eliminating the need to isolate the desired glycan from closely related by-products.

Sigma's glycosyltransferase kits contain the enzyme, the appropriate nucleotide sugar donor, and all other components required for the transfer of a specific monosaccharide moiety to an acceptor substrate on a small preparative scale. Our glycosylation kits include alkaline phosphatase to degrade nucleotide diphosphate and prevent the inhibition of glycosyltransferase activity.

- Unique glycosyltransferases – *deliver regiospecific and stereospecific glycosylation*
- Individual enzyme aliquots for each glycosylation reaction – *prevent enzyme activity loss and cross-contamination*

Glycosyltransferases and nucleotide sugar donors are available separately*.

Glycosyltransferase Kits

Each kit is sufficient for 5 glycosylation reactions.

Cat. No.	Name	Kit Components		Pack Size
74188	α(1→3)Galactosyltransferase Kit	77038	α(1→3)Galactosyltransferase, mouse, recombinant expressed in *Escherichia coli*	1 kit
		40396	Uridine 5′-diphospho-α-D-galactose (UDP-Gal) disodium salt	
		63536	Manganese (II) chloride tetrahydrate	
		93368	Trizma® hydrochloride, BioChemika, pH 7.0	
		05470	Albumin from bovine serum	
		79385	Phosphatase, alkaline from bovine intestinal mucosa	
59505	β(1→4)Galactosyltransferase Kit	48279	β(1→4)Galactosyltransferase from bovine milk	1 kit
		40396	Uridine 5′-diphospho-α-D-galactose (UDP-Gal) disodium salt	
		63536	Manganese (II) chloride tetrahydrate	
		93371	Trizma® hydrochloride, BioChemika, pH 7.4	
		61289	α-Lactalbumin from bovine milk	
		79385	Phosphatase, alkaline from bovine intestinal mucosa	
61843	α(1→3)Fucosyltransferase VI Kit	81106	α(1→3)-Fucosyltransferase VI, human, recombinant expressed in *Pichia pastoris*	1 kit
		55394	Guanosine 5′-diphospho-β-L-fucose disodium salt (GDP-Fuc)	
		63536	Manganese (II) chloride tetrahydrate	
		93368	Trizma® hydrochloride, BioChemika, pH 7.0	
		05470	Albumin from bovine serum	
		79385	Phosphatase, alkaline from bovine intestinal mucosa	

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Glycosyltransferase Enzymes

Cat. No.	Name	Description	Pack Size
77038	α(1→3)Galactosyltransferase, mouse, recombinant, expressed in *Escherichia coli*	*BioChemika*, ~0.5 units/mL. One unit corresponds to the amount of enzyme which catalyzes the transfer of 1 µmol galactose from UDP-galactose to N-acetyllactosamine per minute at pH 7.0 and 37 °C. Solution in 50% glycerol, 25 mM Tris pH 8.0, 0.5 mM DTT.	1 mL
90261	β(1→4)Galactosyltransferase I, human, recombinant, expressed in *Saccharomyces cerevisiae*	*BioChemika*, ≥5 units/g. One unit corresponds to the amount of enzyme which transfers 1 µmol galactose from UDP-galactose to D-glucose per minute at pH 8.4 and 30 °C in the presence of α-lactalbumin. Lyophilized powder containing Tris buffer salts and BSA.	100 mg 500 mg
44498	β(1→4)Galactosyltransferase I, human, recombinant, expressed in *Saccharomyces cerevisiae*	*BioChemika*, ≥0.2 unit/mL. One unit corresponds to the amount of enzyme which transfers 1 µmol galactose from UDP-galactose to D-glucose per minute at pH 8.4 and 30 °C in the presence of α-lactalbumin. Solution in 50% glycerol, 50 mM Tris-HCl, pH 7.5, 2 mM 2-mercaptoethanol.	1 mL
48279	β(1→4)Galactosyltransferase from bovine milk	*BioChemika*, ~1 unit/mg, One unit corresponds to the amount of enzyme which transfers 1 µmol galactose from UDP-galactose to D-glucose per minute at pH 8.4 and 30 °C in the presence of α-lactalbumin.	1 mg 5 mg 25 mg
48281	β(1→4)Galactosyltransferase from bovine milk	*BioChemika*, ~8 unit/g. One unit corresponds to the amount of enzyme which transfers 1 µmol galactose from UDP-galactose to D-glucose per minute at pH 8.4 and 30 °C in the presence of α-lactalbumin.	100 mg 500 mg
81106	α(1→3)Fucosyltransferase VI, human, recombinant, expressed in *Pichia pastoris*	*BioChemika*, ≥1.0 unit/mL. One unit corresponds to the amount of enzyme that transfers 1 µmol L-fucose from GDP-L-fucose to N-acetyl-D-lactosamine per minute at pH 6.2 and 37 °C.	1mL

Nucleoside Phosphate Glycosyl Donor Substrates

Synonyms in Bold are used in the text

Cat. No.	Name	Purity	Pack Size
C8271	Cytidine-5′-monophospho-N-acetylneuraminic acid sodium salt (**CMP-NeuAc**; CMP-sialic acid; CMP-NAN; CMP-NANA)	≥90% (HPLC)	1 mg 5 mg 25 mg
G4401	Guanosine 5′-diphospho-β-L-fucose sodium salt (**GDP-Fuc**; GDP-fucose)	~90%	1 mg 2 mg 5 mg
G5131	Guanosine 5′-diphospho-D-mannose sodium salt from *Saccharomyces cerevisiae* (**GDP-Man**; GDP-mannose)	Type I, ~98%	10 mg 50 mg 100 mg
U5252	Uridine 5′-diphospho-N-acetylgalactosamine disodium salt (**UDP-GalNAc**; UDP-N-acetylgalactosamine)	~98%	5 mg 25 mg 100 mg
U4375	Uridine 5′-diphospho-N-acetylglucosamine sodium salt (**UDP-GlcNAc**; UDP-N-acetylglucosamine; UDPAG)	~98%	25 mg 100 mg 500 mg 1 g
U4500	Uridine 5′-diphosphogalactose disodium salt (**UDP-Gal**; UDP-galactose)	~95%	5 mg 10 mg 25 mg 100 mg
94333	Uridine 5′-diphosphogalactose disodium salt (**UDP-Gal**; UDP-galactose)	≥99%	10 mg 50 mg 250 mg
U4625	Uridine 5′-diphosphoglucose disodium salt from *Saccharomyces cerevisiae* (**UDP-Glc**; UDPG)	≥98%	10 mg 25 mg 100 mg 500 mg 1 g 5 g
U5625	Uridine 5′-diphosphoglucuronic acid triammonium salt (**UDP-GlcA**; UDP-glucuronic acid; UDPGA)	≥98%	100 mg 250 mg 500 mg 1 g

Glycosyltransferases

Tools for Synthesis and Modification of Glycans

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Glycoprotein Deglycosylation

Aparagine-linked (N-linked) and serine/threonine-linked (O-linked) oligosaccharides are major structural components of many eukaryotic proteins. They perform critical biological functions in protein sorting, immune recognition, receptor binding, inflammation, pathogenicity, and many other processes. The diversity of oligosaccharide structures, both O-linked and N-linked, often results in heterogeneity in the mass and charge of glycoproteins. Variations in the structure and different degrees of glycosylation site saturation in a glycoprotein contribute to mass heterogeneity. The presence of sialic acid (N-acetylneuraminic acid) also affects both the mass and charge of a glycoprotein. N-linked oligosaccharides may contribute 3.5 kDa or more per structure to the mass of a glycoprotein (see **Figure 1**). O-linked sugars, although usually less massive than N-linked structures, may be more numerous (see **Figures 2 and 3**).

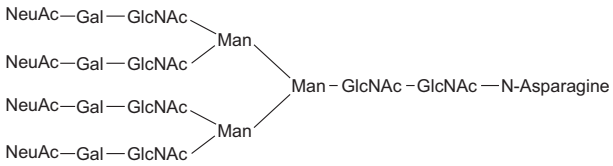


Figure 1. Tetraantennary N-linked Sugar.

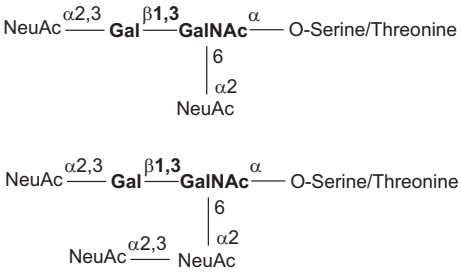


Figure 2. Di- and Trisialyated O-linked Core-1 Saccharides (core shown in bold).

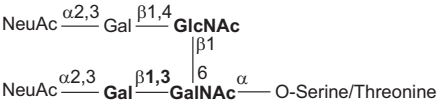


Figure 3. O-linked Core-2 Hexasaccharide.

Abbreviations:

Gal – Galactose, Man – Mannose, GalNAc – N-acetylgalactosamine, GlcNAc – N-acetylglucosamine, NeuAc – N-acetylneuraminic acid (Sialic acid)

To study the structure and function of a glycoprotein, it is often desirable to remove all or a select class of oligosaccharides. This allows assigning specific biological functions to particular components of the glycoprotein. For example, the loss of ligand binding to a glycoprotein after removal of sialic acid may implicate that sugar in the binding process.

Removing carbohydrate groups from glycoproteins is highly recommended for protein identification. Glycoproteins and glycopeptides ionize poorly during mass spectrometry (MS) analysis, leading to inadequate spectral data. Glycopeptides have lower detection sensitivity due to microheterogeneity of the attached glycans, resulting in signal suppression. Proteolytic (tryptic) digestion of native glycoproteins is often incomplete due to steric hindrance from the presence of bulky oligosaccharides. However, proteolytic cleavage is a prerequisite when eluting peptide fragments from gels for identification by MS. Deglycosylation of the glycopeptides before tryptic digestion increases protein sequence coverage and improves protein identification, as well as aids in identifying glycosylation sites on the protein core.

Kits for Chemical and Enzymatic Deglycosylation of Glycopeptides

- Chemical deglycosylation using trifluoromethanesulfonic acid (TFMS) hydrolysis leaves an intact protein component, but destroys the glycans. Glycoproteins from animals, plants, fungi, and bacteria have been deglycosylated by this procedure. It has been reported that the biological, immunological, and receptor binding properties of some glycoproteins are retained after deglycosylation by this procedure, although this may not be true for all glycoproteins. The reaction is non-specific, removing all types of glycans regardless of structure, although prolonged incubation is required for complete removal of O-linked glycans. Also, the innermost Asn-linked GlcNAc residue of N-linked glycans remains attached to the protein. This method removes the N-glycans of plant glycoproteins that are usually resistant to enzymatic hydrolysis.
- Enzymatic deglycosylation is recommended for use with N-linked glycans and can be combined with tryptic digestion. Use of the glycolytic enzyme PNGase F is the most effective method of removing virtually all N-linked oligosaccharides from glycoproteins. Peptide-N-glycosidase F (PNGase F) releases asparagine-linked oligosaccharides from glycoproteins and glycopeptides by hydrolyzing the amide group of the asparagine (Asn) side chain. A tripeptide with the oligosaccharide-linked asparagine as the central residue is the minimum substrate for PNGase F. The oligosaccharides can be high mannose, hybrid, or complex type. However, N-glycans with fucose linked α(1→3) to the asparagine-bound N-acetylglucosamine are resistant to the action of PNGase F (see **Figure 4**).

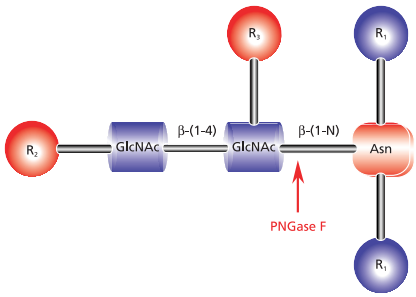


Figure 4. Cleavage site requirements for PNGase F.
R_1 = N- and C- substitution by groups other than H
R_2 = H or the rest of an oligosaccharide
R_3 = H or α(1→6) fucose

Glycoprotein Deglycosylation

GlycoProfile™ IV Chemical Deglycosylation Kit

The optimized GlycoProfile IV Chemical Deglycosylation Kit removes glycans from glycoproteins using trifluoromethanesulfonic acid (TFMS). The deglycosylated protein can then be recovered using a suitable downstream processing method such as gel filtration or dialysis. Unlike other chemical deglycosylation methods, hydrolysis with anhydrous TFMS is very effective at removing O- and N-linked glycans (except the innermost Asn-linked GlcNAc of N-linked glycans) with minimal protein degradation. The extent of deglycosylation may be assessed by mobility shift of the deglycosylated protein versus the intact glycoprotein on SDS-PAGE gels (see **Figure 5**).

- Each reaction processes 1-2 mg of glycoprotein – *Enough output for downstream analysis*
- Minimal degradation of protein core – *For more reliable MS data*
- Complete deglycosylation in as short as 30 minutes – *For increased throughput*

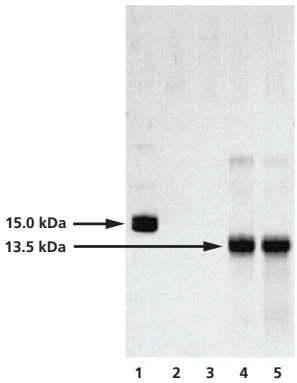


Figure 5. Analysis of the chemical deglycosylation of RNase B on 12% homogeneous SDS=PAGE gel.
Lane 1 is the RNase B control (Cat. No. R1153), while lanes 2 to 5 represent fractions collected from the gel filteration column. Lanes 2 and 3 are pre-void volume fractions and lanes 4 and 5 show bands at 13.5 kDa, corresponding to deglycosylated RNAse B.

GlycoProfile IV Chemical Deglycosylation Kit

Cat. No. PP0510

Contains reagents sufficient to deglycosylate up to 10 samples (1-2 mg each) of a typical glycoprotein or glycoprotein standard.

Kit Components		Pack Size
347817	Trifluoromethanesulfonic acid, anhydrous	5×1.0 g
R1153	Ribonuclease B Glycoprotein Standard	3×1.0 mg
P5496	Pyridine Solution, 60%	10 mL
B1560	Bromophenol Blue Solution, 0.2%	0.5 mL
296295	Anisole, anhydrous	5×1 mL
27265	Reaction Vials	10 each
27273	Caps for Reaction Vials	10 each

Enzymatic Protein Deglycosylation Kit

The Enzymatic Protein Deglycosylation Kit contains all the enzymes and reagents needed to completely remove all N-linked and simple O-linked carbohydrates from glycoproteins, and effect cleavage of complex core-2 O-linked carbohydrates, including those containing polylactosamine.

PNGase F (Peptide-N-glycosidase F) is included for N-linked deglycosylation of glycoproteins and glycopeptides in solution, in-gel digests, or on blot membranes. The enzyme releases asparagine-linked oligosaccharides from glycoproteins and glycopeptides by hydrolyzing the amide group of the asparagine (Asn) side chain.

For degradation of O-linked glycans, monosaccharides must be removed by a series of exoglycosidases until only the Gal-β(1→3)-GalNAc core remains attached to the serine/threonine. The EDEGLY kit contains α(2→3,6,8,9)-Neuraminidase (Sialidase A) for cleavage of terminal sialic acid residues, and O-Glycosidase to remove the core Gal-β(1→3)-GalNAc. β(1→4)-Galactosidase and β-N-Acetylglucosaminidase are also provided to remove sugars associated with specific O-linked glycan structures.

- Deglycosylates up to two mg of glycoprotein – *Sufficient for downstream processing*
- Single reaction at neutral pH – *Retain original peptide structure*
- No protein degradation – *Perform interrogation on peptide structure*
- Removes O-linked sugars containing polysialic acid – *Get more accurate peptide analysis*
- Control glycoprotein provided – *Verification improves confidence and consistency*

Enzymatic Protein Deglycosylation Kit

Cat. No. E-DEGLY

Contains reagents sufficient to deglycosylate and digest minimum of ten samples (each sample 200 µg of average glycoprotein).

Kit Components		Pack Size
P2619	PNGase F	1 vial (20 µL)
G1163	O-Glycosidase (Endo-O-glycosidase)	20 µL
N8271	α(2→3,6,8,9)-Neuraminidase (Sialidase A)	20 µL
G0413	β(1→4)-Galactosidase	20 µL
A6805	β-N-Acetylglucosaminidase	20 µL
F4301	Fetuin Control Glycoprotein Standard	0.5 mg
R2651	5× Reaction Buffer	0.2 mL
D6439	Denaturation Solution	0.1 mL
T3319	TRITON® X-100 (15% Solution)	0.1 mL

GlycoProfile™ I Enzymatic In-Gel N-Deglycosylation Kit

The GlycoProfile I Enzymatic In-Gel N-Deglycosylation Kit robustly removes N-linked glycans and digests protein samples from 1D- or 2D-polyacrylamide gels for MS or HPLC analysis. The kit works well for Coomassie Brilliant Blue, colloidal Coomassie and silver stained gels when properly destained. The glycolytic enzyme PNGase F (Peptide-N-glycosidase F) performs superbly when used for in-gel N-linked deglycosylation of glycoproteins and glycopeptides. Proteomics Grade Trypsin effectively digests the remaining protein. Desalted samples are then concentrated for analysis by MALDI-TOF-MS or ES-MS (see **Figure 6**).

- In-gel deglycosylation and digestion – *Minimizes sample manipulation*
- Highly purified enzymes – *Prevents unwanted activities and by-products*
- Low buffer salt content – *Eliminates interference with MS analysis*
- Destaining reagent included – *Saves time by reducing additional reagent preparation*

GlycoProfile I Enzymatic In-Gel N-Deglycosylation Kit

Cat. No. PP0200

Contains reagents sufficient to deglycosylate and digest up to 10 samples.

Kit Components		Pack Size
P7367	PNGase F from *Elizabethkingia* (*Chryseobacterium/Flavobacterium*) *meningsepticum*, Proteomics Grade, ≥95% (SDS-PAGE)	50 units
T6567	Trypsin from porcine pancreas, Proteomics Grade, Dimethylated	20 µg
D0316	Destaining Solution	1 bottle (10 mL)
T2073	Trypsin Solubilization Reagent	1 mL
R3527	Trypsin Reaction Buffer	1 bottle (11 mL)
I0408	Invertase Glycoprotein Standard, Proteomics Grade	0.5 mg
P0743	Peptide Extraction Solution	10 mL
494445	Acetonitrile, Biotech Grade	50 mL

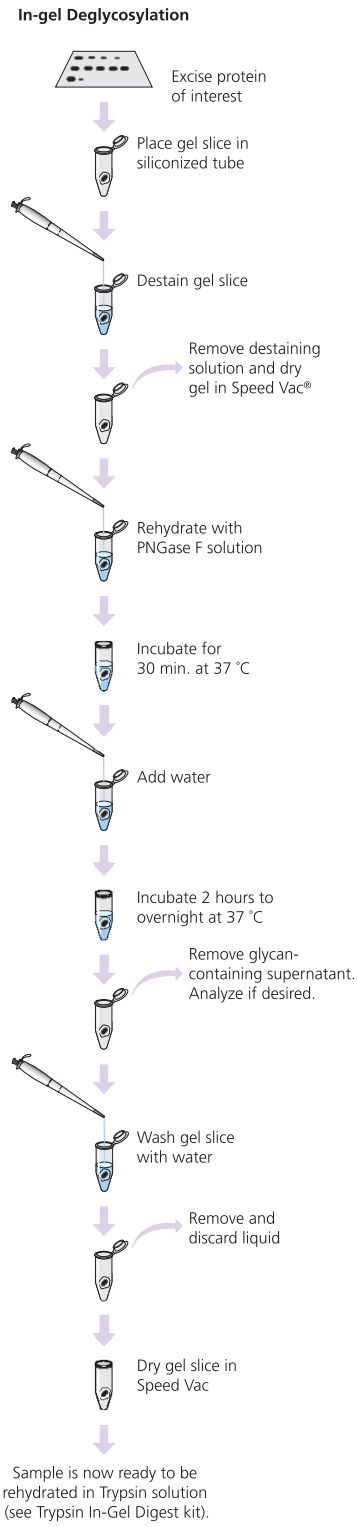


Figure 6. Process for the GlycoProfile I Enzymatic In-Gel N-Deglycosylation Kit.

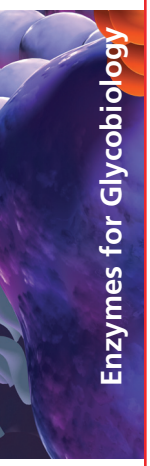
Enzymes for Glycobiology

Complementing our deglycosylation kits, Sigma offers glycolytic enzymes for the removal or partial degradation of glycans.

The use of additional enzymes may be useful for certain glycan structures that resist universal deglycosylation strategies, such as structures that are not cleaved by PNGase F. In addition, sequential hydrolysis of individual monosaccharides from glycans can be used in the analysis of the structure and function of the glycan component.

For additional exoglycosic enzymes, glycosaminoglycan degrading enzymes, and lysing enzymes, as well as suitable substrates and inhibitors please visit the Enzyme Explorer at **sigma-aldrich.com/enzymeexplorer** and discover a new dimension in online resources.

- Indices to more than 3,000 enzymes, proteins, substrates and inhibitors.
- Product highlights address specific new tools for your research.
- Assay Library with over 600 detailed procedures for measuring enzyme activities and related metabolites.



Endoglycosidases

Enzyme	Function	Cat. No.	Name	Unit definition	Physical form	Pack Size
Endoglycosidase F1	Cleaves asparagine-linked or free oligomannose and hybrid, but not complex, oligosaccharides.	**E9762**	Endoglycosidase F1 from *Elizabethkingia (Chryseobacterium/ Flavobacterium) meningosepticum,* ≥16 units/mg	One unit will release N-linked oligosaccharides from 1 µmole of denatured ribonuclease B in 1 minute at 37 °C, pH 5.5.	Aseptically filled solution in 120 mM Tris-HCl, pH 7.5. Supplied with 5× Reaction Buffer.	1 unit
Endoglycosidase F2	Cleaves asparagine-linked or free oligomannose and biantennary complex oligosaccharides.	**E0639**	Endoglycosidase F2 from *Chryseobacterium (Flavobacterium) meningosepticum,* ≥20 units/mg	One unit will release N-linked oligosaccharides from 1 µmole of denatured porcine fibrinogen in 1 minute at 37 °C, pH 4.5.	Aseptically filled solution in 10 mM sodium acetate and 25 mM sodium chloride, pH 4.5. Supplied with 5× Reaction Buffer.	2 units
Endoglycosidase F3	Cleaves asparagine-linked biantennary and triantennary complex oligosaccharides, depending on the state of core fucosylation and peptide linkage.	**E2264**	Endoglycosidase F3 from *Chryseobacterium (Flavobacterium) meningosepticum*, ≥30 units/mg	One unit will release N-linked oligosaccharides from 1 µmole of denatured porcine fibrinogen in 1 minute at 37 °C, pH 4.5.	Aseptically filled solution in 20 mM Tris-HCl, pH 7.5. Supplied with 5× Reaction Buffer.	0.2 unit
Endoglycosidase H	Cleaves between the N-acetylglucosamine residues of the chitobiose core of N-linked glycans, leaving one N-acetylglucosamine residue attached to the asparagine.	**E2406**	Endoglycosidase H from *Streptomyces griseus*	One unit will hydrolyze 1.0 µmole of N-acetyl-(^{14}C)Asn(GlcNAc)$_2$(Man)$_5$ per min at pH 5.0 at 37 °C.	Lyophilized from a solution containing 10 mM Tris HCl, pH 7.2.	0.1 unit
		A0810	Endoglycosidase H from *Streptomyces plicatus*, recombinant, expressed in *Escherichia coli*	One unit will release N-linked oligosaccharides from 60 µmoles of ribonuclease B per hr at 37 °C at pH 5.5.	Solution in 20 mM Tris HCl, pH 7.5, 25 mM NaCl. Supplied with 5× reaction buffer	1 unit
		E7642	Endoglycosidase H from *Streptomyces plicatus,* recombinant, expressed in *Escherichia coli*	One unit will hydrolyze 1.0 µmole of dansyl-Asn-(GlcNAc)$_2$(Man)$_5$ per min at pH 5.5 at 37 °C.	Solution in 0.05 M sodium phosphate, pH 7, containing 25 mM EDTA and preservative	1 unit
Endo-β-galactosidase	Cleaves internal β(1→4)galactose linkages in unbranched, repeating poly-N-acetyllactosamine structures [GlcNAc-β(1→3)Gal-β(1→4)].	**G6920**	Endo-β-galactosidase from *Bacteroides fragilis,* recombinant, expressed in *Escherichia coli*	One unit will release 1.0 µmole of reducing sugar per minute at 37 °C and pH 5.8 from bovine corneal keratan sulfate.	Solution in 20 mm Tris-HCl, pH 7.5	0.5 unit
Glycopeptidase A	Hydrolyzes an N^4-(acetyl-β-D-glycosaminyl)asparagine in which the N-acetyl-D-glucosamine residue may be further glycosylated, yielding a (substituted) N-acetyl-β-D-glucoaminylamine and the peptide containing an aspartic residue.	**G0535**	Glycopeptidase A from almonds, ≥0.05 unit/ml	One unit will hydrolyze 1.0 µmole of ovalbumin glycopeptide per min at pH 5.0 at 37 °C.	Solution in 50% glycerol containing 50 mM citrate-phosphate buffer, pH 5.0	0.005 unit
O-Glycosidase	Releases unsubstituted Ser- and Thr-linked β-Gal-(1→3)-α-GalNAc from glycoproteins.	**G1163**	O-Glycosidase from *Streptococcus pneumoniae*, recombinant, expressed in *Escherichia coli*	One unit will hydrolyze 1 µmole of β-Gal-(1→3)-α-GalNAc-1-O→C_6H_5β-Gal-(1→3)-α-GalNAc-1-O→C_6H_5-p-N per min at 37 °C at pH 6.5.	Solution in 50 mM sodium phosphate, pH 7.5. Supplied with 5× reaction buffer	0.04 unit

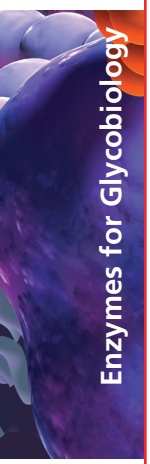
Endoglycosidases cont.

Enzyme	Function	Cat. No.	Name	Unit definition	Physical form	Pack Size
PNGase F (Peptide N-Glycosidase F)	Cleaves an entire glycan from a glycoprotein provided the glycosylated asparagine moiety is substituted on its amino and carboxyl terminus with a polypeptide chain.	**P7367**	PNGase F, Proteomics Grade ≥95% (SDS-PAGE), from *Elizabethkingia (Chryseobacterium/ Flavobacterium) meningsepticum*	One unit will catalyze the release of N-linked oligosaccharides from 1 nmol of denatured ribonuclease B in one min at 37°C at pH 7.5 monitored by SDS-PAGE. One Sigma unit of PNGase F activity is equal to 1 IUB milliunit.	Lyophilized from a solution containing 5 mM sodium phosphate, pH 7.5	50 units 300 units
		G5166	PNGase F from *Elizabethkingia (Chryseobacterium/ Flavobacterium) meningsepticum*	See P7367	Solution in 20 mM Tris HCl, pH 7.5, 50 mM NaCl and 5 mM EDTA	50 units 100 units
		P9120	PNGase F from *Elizabethkingia (Chryseobacterium/ Flavobacterium) meningsepticum* recombinant, expressed in *Escherichia coli*, ≥10 units/mg protein	See P7367	Each set includes enzyme, two formulations of 5x reaction buffer (for routine and Mass Spec downstream analysis), detergent and denaturation solutions	1 set

Exoglycosidases

Enzyme	Function	Cat No.	Name	Unit definition	Physical form	Pack Size
β-N-Acetyl-glucosaminidase (β-N-Acetyl-hexosaminidase)	Reported to liberate terminal β-linked N-acetylglucosamine and N-acetylgalactosamine from a variety of substrates.	**A6805**	β-N-Acetylglucos-aminidase from *Streptococcus pneumoniae*, recombinant, expressed in *Escherichia coli*	One unit will hydrolyze 1.0 µmole of p-nitrophenyl N-acetyl-β-D-glucosaminide to p-nitrophenol and N-acetyl-D-glucosamine per min at pH 5.0 at 37 °C.	Solution in 20 mM Tris-HCl, pH 7.5, 25 mM NaCl. Supplied with 5× reaction buffer.	1 vial
α-L-Fucosidase	Cleaves α(1→2,3,4,6) linked fucose from N- and O-linked glycans; cleaves α(1→6) linked fucose on the trimannosyl core of N-linked glycans more efficiently than other α-fucose linkages.	**F5884**	α-L-Fucosidase from bovine kidney, ≥2 units/mg protein	One unit will hydrolyze 1.0 µmole of p-nitrophenyl α-L-fucoside to p-nitro-phenol and L-fucose per min at pH 5.5 at 25°C.	Suspension in 3.2 M $(NH_4)_2SO_4$, 10 mM NaH_2PO_4 10 mM citrate, pH 6.0	1 unit 2 units 0.5 unit
α(1→2)-Fucosidase	Releases α(1→2)-fucose from the non-reducing end of complex carbohydrates.	**F9272**	α(1→2)-Fucosidase	One unit will release 1.0 µmole of fucose from 2′-fucosyllactose per min at pH 5.0 at 37 °C	Solution in 20 mM Tris-HCl, pH 7.5, 25 mM NaCl. Supplied with 5× reaction buffer.	1 vial
α(1→6)-Fucosidase	Removes branched α(1→6) terminal fucose linked to core N-acetylglucosamine of non-reducing N-linked oligosaccharides. The reducing terminus of the oligosaccharide must be labeled with a reporter molecule, e.g. aminonaphthalenetrisulfonic acid (ANTS).	**F6272**	α(1→6)-Fucosidase recombinant, expressed in *Escherichia coli*	One unit will release 1.0 µmole of methylumbelliferone from 4-methylumbelliferyl α-L-fucoside per min at pH 5.0 at 37 °C	Buffered aqueous solution. Supplied with 5× reaction buffer.	0.04 unit
α(1→2,3,4)-Fucosidase	Cleaves non-reducing terminal fucose when linked α(1→2), α(1→3), or α(1→4) to complex carbohydrates.	**F1924**	α(1→2,3,4)-Fucosidase from *Xanthomonas* sp.	One unit will hydrolyze 1 µmole fucose from 3-fucosyllactose per min at pH 5.0 at 37 °C.	Solution in 20 mM Tris-HCl, pH 7.5, 25 mM NaCl. Supplied with 5× reaction buffer.	1 vial
α(1→3,4)-Fucosidase	Releases non-reducing, terminal α(1→3)-fucose and α(1→4)-fucose from complex carbohydrates.	**F3023**	α(1→3,4)-Fucosidase from *Xanthomonas manihotis*, ≥0.5 unit/mL	One unit will release 1.0 µmole of fucose from Lewis X trisaccharide, 4-methylumbelliferyl glycoside per min at pH 5.0 at 37 °C.	Solution in 20 mM Tris-HCl, pH 7.5, 25 mM NaCl. Supplied with 5× reaction buffer.	1 vial
α-Galactosidase	Cleaves α-linked, non-reducing terminal galactose from complex carbohydrates.	**G8507**	α-Galactosidase from green coffee beans, ~10 units/mg protein	One unit will hydrolyze 1.0 µmole of p-nitrophenyl α-D-galactoside to p-nitrophenol and D-galactose per min at pH 6.5 at 25 °C.	Suspension in 3.2 M $(NH_4)_2SO_4$ solution, pH 6.0, containing BSA. Protein by biuret.	5 units 50 units 25 units
α-Galactosidase, positionally specific	Cleaves α(1→3)- and α(1→6)-linked, non-reducing terminal galactose from complex carbohydrates and glycoproteins.	**G7163**	α-Galactosidase, positionally specific from *Escherichia coli*	One unit will hydrolyze 1 µmole of p-nitrophenyl α-D-galactopyranoside per min at pH 6.5 at 25 °C.	Solution in 20 mM Tris, pH 7.5, 25 mM NaCl. Supplied with 5× reaction buffer.	60 units

Enzymes for Glycobiology



Exoglycosidases cont.

Enzyme	Function	Cat No.	Name	Unit definition	Physical form	Pack Size
β-Galactosidase	Cleaves terminal galactose residues, which are β(1→4)-linked to a monosaccharide, glycopeptide, or oligosaccharide.	**G3153**	β-Galactosidase from *Escherichia coli*, ≥500 units/mg protein	One unit will hydrolyze 1.0 µmole of o-nitrophenyl β-D-galactoside to o-nitrophenol and D-galactose per min at pH 7.3 at 37 °C.	Lyophilized powder, stabilized with phosphate buffer and sucrose. Protein by biuret.	5 mg
β(1→3,6)-Galactosidase	Releases β(1→3)- and β(1→6)-linked galactose from the non-reducing end of complex oligosaccharides.	**G0288**	β-(1→3,6)-Galactosidase, positionally specific recombinant, expressed in *Escherichia coli*	One unit will hydrolyze 1 µmole of p-nitrophenyl β-D-galactopyranoside per min at pH 5.0 at 37 °C.	Solution in 20 mM Tris-HCl, pH 7.5, 25 mM NaCl. Vial of 1.9 units	1 vial
β(1→3,4,6)-Galactosidase	Releases β(1→3)-, β(1→4)-, and β(1→6)-linked galactose from the non-reducing end of complex oligosaccharides	**G1288**	β-(1→3,4,6)-Galactosidase, positionally specific, from *Streptococcus pneumonia* and *Xanthamonas* sp., recombinant, expressed in *Escherichia coli*	See G0288	Solution in 20 mM Tris-HCl, pH 7.5, 25 mM NaCl. Vial of 0.24 unit.	1 vial
β(1→4)-Galactosidase	Releases β(1→4)-linked galactose from the non-reducing end of complex oligosaccharides	**G0413**	β-(1→4)-Galactosidase, positionally specific, from *Streptococcus pneumaniae*, recombinant, expressed in *Escherichia coli*	See G0288	Solution in 20 mM Tris-HCl, pH 7.5, 25 mM NaCl. Vial of 0.06 unit.	1 vial
β(1→6)-Galactosidase	Cleaves β(1→6)-linked, non-reducing terminal galactose from complex carbohydrates and glycoproteins	**G0914**	β-(1→6)-Galactosidase, positionally specific, recombinant, expressed in *Escherichia coli*	See G0288	Solution in 20 mM Tris-HCl, pH 7.5, 25 mM NaCl. Supplied with 5× reaction buffer. Vial of 0.06 unit.	1 vial
α-Glucosidase	Hydrolyzes terminal, non-reducing α(1→4)-, α(1→3)-, and α(1→2)-linked D-glucose residues from oligosaccharides, with preference for the α(1→4) linkage. Cleavage of α(1→6)-linked glucose also takes place, but at a much slower rate.	**G0660**	α-Glucosidase from *Saccharomyces cerevisiae*, recombinant, expressed in unspecified host, ≥125 units/mg protein	One unit will liberate 1.0 µmole of D-glucose from p-nitrophenyl α-D-glucoside per min at pH 6.8 at 37 °C.	Lyophilized powder containing potassium phosphate buffer salt pH 7.15 and approx. 70% lactose. Protein by biuret.	750 units
β-Glucosidase	Cleaves terminal, non-reducing β-D-glucose residues to release D-glucose.	**G4511**	β-Glucosidase from almonds, 20-40 units/mg solid	One unit will liberate 1.0 µmole of glucose from salicin per min at pH 5.0 at 37 °C.	Lyophilized powder	100 units 250 units 1000 units (1 KU)
β-Glucuronidase	Hydrolyzes the O-glycosyl bond of β-D-glucuronosides, resulting in D-glucuronate and an alcohol. Effective in the hydrolysis of steroid glucuronides. Used for the hydrolysis of glucuronide conjugates in urinary metabolite analysis	**G8295** NEW	β-Glucuronidase from *Escherichia coli*, recombinant, expressed in *E. coli* overproducing strain 1,000,000-5,000,000 units/g protein	One Sigma or modified Fishman unit will liberate 1.0 µg of phenolphthalein from phenolphthalein glucuronide per hr at 37 °C at the pH 6.8 (30 min assay).	Lyophilized powder. Contains buffer salts and stabilizer. Approx. 50% protein (biuret)	2 MU 25 KU 500 KU
		G7896	β-Glucuronidase from *Escherichia coli* 20,000,000-60,000,000 units/g protein	See G8295	Highly purified lyophilized powder containing buffer salts and stabilizer. Approx. 30% protein (biuret)	25 KU 100 KU 200 KU
		G4259	β-Glucuronidase from *Helix aspersa* (garden snail) 250,000-500,000 units/g solid	See G8295	Partially purified powder	1000 units (1 KU)
		G8885	β-Glucuronidase from *Helix pomatia*, ≥100,000 units/mL	See G8295	Aqueous solution	1 mL 10 mL 25 mL
		G8132	β-Glucuronidase from *Patella vulgata* (keyhole limpet) 1,000,000-3,000,000 units/g solid	See G8295	Lyophilized powder	100 KU 250 KU 500 KU 1 MU 2 MU
Neuraminidase	Hydrolyzes α(2→3), α(2→6), and α(2→8)-glycosidic linkages of terminal sialic residues of various glycomolecules	**N2133**	Neuraminidase from *Clostridium perfringens* (*C. welchii*), ≥50 units/mg protein (Bradford)	One unit will liberate 1.0 µmole of N-acetylneuraminic acid per min at pH 5.0 at 37 °C using NAN-lactose,	Lyophilized powder, purified by affinity chromatography	1 unit 5 units 10 units 50 units

KU = 1,000 Units MU = 1,000,000 Units

Exoglycosidases cont.

Enzyme	Function	Cat No.	Name	Unit definition	Physical form	Pack Size
α(2→3,6)-Neuraminidase	Releases α(2→3)- and α(2→6)-linked N-acetylneuraminic acid from complex oligosaccharides.	**N5521**	α(2→3,6) Neuraminidase from *Clostridium perfingens*, expressed in *Escherichia coli*	One unit will hydrolyze 1 µmole of 4-methyl-umbelliferyl α-D-N-acetylneuraminide per minute at pH 5.0 at 37 °C.	Solution in 20 mM Tris-HCl, pH 7.5, and 25 mM NaCl. Supplied with 5× reaction buffer.	0.4 unit
α(2→3)-Neuraminidase	Releases α(2→3)-linked N-acetylneuraminic acid from complex oligosaccharides.	**N7271**	α(2→3) Neuraminidase from *Streptococcus pneumoniae*	One unit will hydrolyze 1 µmole of 4-methyl-umbelliferyl α-D-N-acetylneuraminide per minute at pH 5.0 at 37 °C.	Solution in 20 mM Tris-HCl, pH 7.5, 25 mM NaCl. Supplied with 5× reaction buffer.	0.2 unit
α(2→3,6,8,9)-Neuraminidase	Capable of cleaving all non-reducing unbranched N-acetylneuraminic and N-glycolylneuraminic acid residues by hydrolysis of α(2→6), α(2→3), α(2→8), and α(2→9) linkages (affinity in the order given). Branched sialic acids may also be cleaved with the use of high concentrations of enzyme and prolonged incubations.	**N3786**	α(2→3,6,8,9) Neuraminidase Proteomics Grade from *Arthrobacter ureafaciens*	One Sigma unit will release 1 nmole of 4-methylumbelliferone from 2-(4-methylumbelliferyl) α-D-N-acetylneuraminic acid per minute at pH 5.5 at 37 °C. One Sigma unit is equivalent to a standard International milliunit (mIU).	Lyophilized enzyme. Set includes one vial of 25 Sigma units and 5× reaction buffer.	1 set
		N8271	α(2→3,6,8,9) Neuraminidase from *Arthrobacter ureafaciens*, recombinant, expressed in *Escherichia coli*	One unit will hydrolyze 1 µmole of 4-methyl-umbelliferyl α-D-N-acetylneuraminide per min at pH 5.0 at 37 °C.	Solution in 20 mM Tris-HCl, pH 7.5, and 25 mM NaCl. Supplied with 5× reaction buffer.	0.2 unit
α-Mannosidase	Cleaves terminal α-D-mannosyl residues which are α(1→2)-, α(1→3)-, or α(1→6)-linked to the non-reducing end of oligosaccharides. α(1→3)-Linked mannose residues are reported to be hydrolyzed at a lower rate than α(1→2)- and α(1→6)-linked residues.	**M7944**	α-Mannosidase Proteomics Grade, from *Canavalia ensiformis* (Jack bean), 15-20 units/mg protein	One unit will hydrolyze 1.0 µmole of p-nitrophenyl α-D-mannopyranoside to p-nitrophenol and D-mannose per min at pH 4.5 at 37 °C.	Solution in 20 mM Tris HCl, pH 7.5, containing 25 mM NaCl. Supplied with 5× reaction buffer.	10 units
β-Mannosidase	Cleaves single terminal D-mannosyl residues, which are β(1→4)-linked to the non-reducing end of oligosaccharides (glycans) with relative specificity. Other mannose residues linked β(1→3)- and β(1→6)- are reported to be hydrolyzed at much lower rates.	**M7819**	β-Mannosidase Proteomics Grade, from *Helix pomatia*	One unit will hydrolyze 1 µmole of p-nitrophenyl β-D-mannopyranoside to p-nitrophenol (measured at 400 nm) and D-mannose per minute at pH 4.0 at 37 °C.	Lyophilized from 10 mM sodium acetate buffer, pH 4.0, containing BSA and sodium chloride. Supplied with 5× reaction buffer	1 unit
β-Xylosidase	Hydrolyzes 1,4-β-D-xylans to remove successive D-xylose residues from the non-reducing termini.	**X3501**	β-Xylosidase from *Aspergillus niger*, 5-10 units/mg protein	One unit will hydrolyze 1.0 µmole of o-nitrophenyl β-D-xyloside to o-nitrophenol and D-xylose per min at pH 5.0 at 25 °C.	Suspension in 3.5 M $(NH_4)_2SO_4$, 50 mM sodium acetate, pH 5.2. Protein by biuret.	5 units
Xylanase	Hydrolyzes 1,4-β-D-xylosidic linkages in xylans, releasing D-xylose.	**X3876**	Xylanase from *Trichoderma viride*, 100-300 units/mg protein	One unit will liberate 1 µmole of reducing sugar measured as xylose equivalents from xylan (X0627) per min at pH 4.5 at 30 °C.	Lyophilized powder, ~50% protein. Contains sorbitol and sodium acetate buffer salts.	250 units 1000 units (1 KU)

GPI enzymes

Phospholipase C, Phosphatidylino-sitol-specific	Used for the release of GPI anchored proteins from the membrane	**P8804**	Phospholipase C, Phosphatidylinositol-specific from *Bacillus cereus*, ≥1,000 units/mg protein	One unit will liberate one unit of acetylcholinesterase per minute from a membrane-bound crude preparation at pH 7.4 at 30 °C (10 minute incubation).	Solution in 60% (v/v) glycerol containing 10 mM Tris-HCl, pH 8.0 and 10 mM EDTA. Protein by Lowry.	5 units 25 units
		P5542	Phospholipase C, Phosphatidylinositol-specific from *Bacillus cereus*, ≥1,000 units/mg protein	See P8804	Lyophilized powder. Contains phosphate buffer salts, EDTA and stabilizer. Protein by Lowry.	5 units 25 units

GlycoProfile™ Labeling Kits

Useful Fluorescent Dyes for Enhanced Glycan Analysis

Glycan analysis has become an increasingly critical aspect of glycomics and proteomics, as the role of glycoproteins in cell signaling, cell adhesion, immune response, and disease states is emerging through ongoing research. In contrast to proteins and peptides, glycans do not absorb ultraviolet (UV) light strongly, thereby giving a weak detector signal, even at 214 nm. Furthermore, as glycans with various structures may be present in minute amounts in glycoprotein hydrolysates, their detection by UV absorbance may not be practical.

Most glycoproteins exist as a heterogeneous population of glycoforms or glycosylated variants with a single protein backbone and a heterogeneous population of glycans at each glycosylation site. It has been reported that for some glycoproteins, 100 or more glycoforms exist at each glycosylation site. In view of this heterogeneity and the presence of branched structures, the detailed analysis of glycans can be very complex.

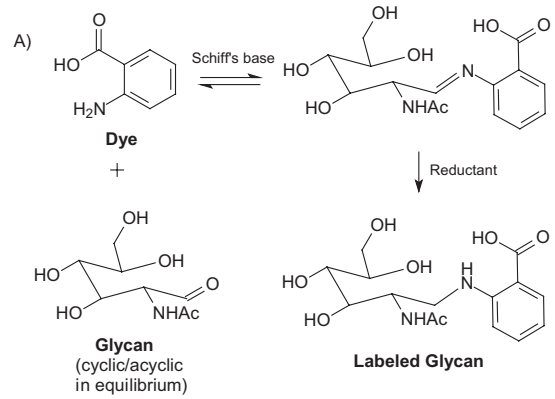
2-AA and 2-AB Labeling of Glycans by Reductive Amination

Once glycans have been cleaved from the glycoprotein, the glycan pool can be labeled with a fluorescent dye and analyzed by HPLC or MS, or both. This strategy can provide a "glycan profile" or a "glycosylation pattern" that is highly characteristic of the glycoprotein. The methodology can be used to compare glycan profiles of glycoproteins found in normal and diseased states, or to compare different batches of recombinant protein products.

Both the GlycoProfile 2-AA and GlycoProfile 2-AB Labeling Kits contain reagents for labeling glycans at their reducing ends by reductive amination. The fluorophores 2-AA (anthranilic acid) and 2-AB (2-aminobenzamide) provide valuable tools for glycan analysis due to their sensitivity and stability when coupled to glycans. Other commonly used methods, such as radioisotopic labels, antibody labels, and various probes do not display the stability, flexibility, and ease of use observed with 2-AA and 2-AB.

Labeling using 2-AA / 2-AB can be performed on either purified or pooled samples, including a variety of sources, such as N-linked, O-linked, and GPI anchored glycans. For samples containing sialated oligosaccharides, sialic acid loss is negligible.

The coupling reaction proceeds through Schiff's base formation of an acyclic reducing sugar with the amine moiety of the dye. The bond is subsequently reduced and stabilized during the coupling reaction (see **Figure 1**).



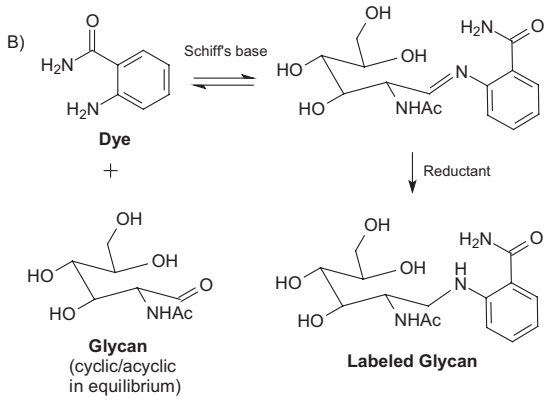


Figure 1. Acyclic glycan and dye form a Schiff's base. Subsequent reduction of the imine with sodium cyanoborohydride results in a stable labeled glycan. (A) 2-AA fluorophore (B) 2-AB fluorophore.

Analysis of 2-AA and 2-AB Labeled Glycans

Once the glycans have been labeled, a variety of methods exist to analyze them. The most common techniques employ fluorescent detection after separation by HPLC or CE. These include separation by ion exchange, normal phase/RP HPLC, and size exclusion chromatography.

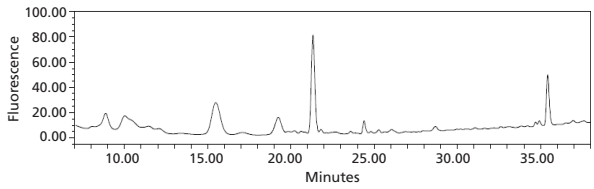


Figure 2. HPLC profile of the 2-AA labeled N-linked glycan library obtained from bovine fetuin. The glycans were cleaved from the glycoprotein using the Enzymatic Protein Deglycosylation Kit (Cat. No. E-DEGLY).

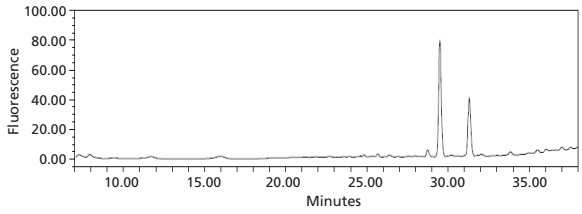


Figure 3. HPLC profile of the 2-AB labeled N-linked glycan library obtained from bovine fetuin. The glycans were cleaved from the glycoprotein using the Enzymatic Protein Deglycosylation Kit (Cat. No. E-DEGLY).

The labeled glycans are undetected by UV detection, but significant peaks are seen by fluorescence (see **Figures 2 and 3**). The different chromatograms are due to the labeling efficiency, sensitivity, and other dye properties. Neither UV nor fluorescent detection was able to detect unlabeled fetuin glycans (data not shown). Labeled glycans can also be detected using mass spectrometry. Mass spectrometry can be performed with either an electrospray ionization (ESI) or matrix assisted laser desorption ionization (MALDI) ion source. Samples containing mixed pools of glycans can often be detected at picomolar concentrations.

GlycoProfile™ 2-AA and 2-AB Labeling Kits

Each GlycoProfile™ Labeling Kit contains sufficient reagents for labeling up to 36 samples. Two sets of components have been provided; each set is sufficient for labeling up to 18 samples based on a reaction volume of 5 µL. Mixed glycan samples should contain between 100 picomoles to 50 nanomoles of purified glycans. With a single pure glycan, as little as 5 picomoles may be labeled and detected in subsequent HPLC analysis.

GlycoProfile™ 2-AA Labeling Kit

Cat. No. PP0530

Kit Components		Pack Size
A6729	2-AA (Anthranilic Acid)	2×6 mg
D4942	DMSO (Dimethyl sulfoxide), 350 µL per vial	2×1 vial
A9353	Acetic acid, glacial	2×200 µL
R5153	Reductant (Sodium cyanoborohydride)	2×6 mg

GlycoProfile™ 2-AB Labeling Kit

Cat. No. PP0520

Kit Components		Pack Size
A9478	2-AB (2-Aminobenzamide)	2×5 mg
D4942	DMSO (Dimethyl sulfoxide), 350 µL per vial	2×1 vial
A9353	Acetic acid, glacial	2×200 µL
R5153	Reductant (Sodium cyanoborohydride)	2×6 mg

Labeling of glycans with 2-AB is covered under US Patent No. 5,747,347 and its foreign equivalents.

GlycoProfile™ Glycan Clean-Up Cartridges

Cat. No. G8169

	Pack Size
For clean up of glycan samples after reductive amination labeling or enzymatic digestion. Recommended for use with the GlycoProfile 2-AA and 2-AB Labeling Kits.	3 each 6 each 12 each

Dextran Ladder

Along with fluorescent labeling of glycans and analysis by normal phase HPLC, an external standard is often used to calibrate the HPLC system. Partially hydrolyzed dextran, consisting of a variable number of monomeric glucose units, may be used as an external standard after fluorescent labeling. This dextran standard has a characteristic ladder profile from monomeric glucose to approximately a 20-mer of glucose oligosaccharide, depending on the chromatographic conditions employed. The elution position of each peak in this ladder is expressed as a glucose unit (gu) and is used to assign gu values to peaks in the released glycan pool.

Dextran Ladder is prepared by partial acid hydrolysis of dextran from *Leuconostoc mesenteroides* with an average molecular weight of 100-200 kDa. A mixture of α-(1→6) linked glucose oligosaccharides of various lengths is produced. The Dextran Ladder may be fluorescently labeled with Sigma's GlycoProfile 2-AB or 2-AA Labeling Kits.

The purity and structural integrity of the ladder is assessed by fluorescently labeling an aliquot and subsequent analysis by normal phase HPLC. The separation of the different glucose oligomers on an amide HPLC column is shown (see **Figure 4**).

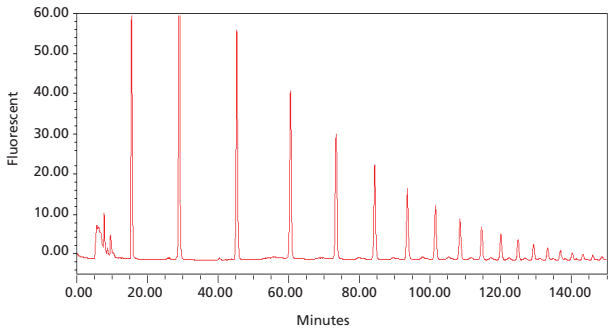


Figure 4.
Normal phase HPLC chromatograph of Dextran Ladder after fluorescent labeling with 2-AB.[1]

Reference

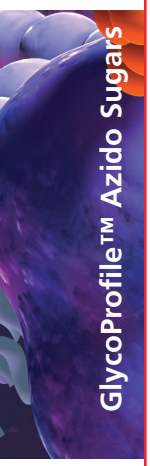
1. Guile, G.R., et al., A rapid high-resolution high-performance liquid chromatographic method for separating glycan mixtures and analyzing oligosaccharide profiles. *Anal. Biochem.*, **240**, 210-226 (1996).
2. Yamashita, K., et al., Analysis of Oligosaccharides by Gel Filtration, *Meth. Enzymol.*, **83**, 105-126 (1983).

Dextran Ladder

Cat. No. D3818

	Pack Size
Glycan standard for HPLC	200 µg

GlycoProfile™ Azido Sugars
Flag Phosphine Technology



Advancing Analysis of Glycoprotein Processing for both Intra and Extra-cellular Evaluation

Many intracellular processing events are disrupted environmentally or are the result of genomic abnormalities (congenital disorders of glycosylation; CDG) and result in disease states. Multiple studies have evaluated the roles of glycoproteins and proteoglycans in tumor metastasis, angiogenesis, inflammatory cell migration, lymphocyte homeostasis, and congenital disorders of glycosylation. Stepwise analysis of the intracellular and surface-displayed sugars provides researchers a more complete picture of the process.

Bioorthogonal Chemical Reporters

While changes in N and O-linked protein glycosylation are known to correlate with disease states, those changes are difficult to monitor in a physiological setting because of a lack of experimental tools. Sigma, in collaboration with the research community, has developed tools for profiling N- and O-linked glycoproteins by labeling cellular glycans using an alternative metabolic-system approach that works both *in vitro* and *in vivo*.[1-5] Non-natural azido-containing monosaccharides (see **Figure 1**) that are bioorthogonal chemical reporters are introduced into a cell and incorporated into glycan structures through endogenous glycosylation processes (see **Figure 2**).

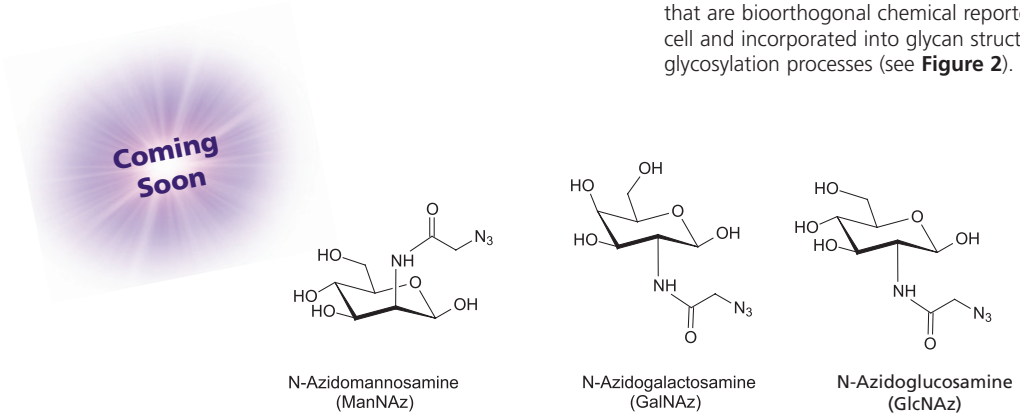


Figure 1. Azido sugars for incorporation into glycan structures.

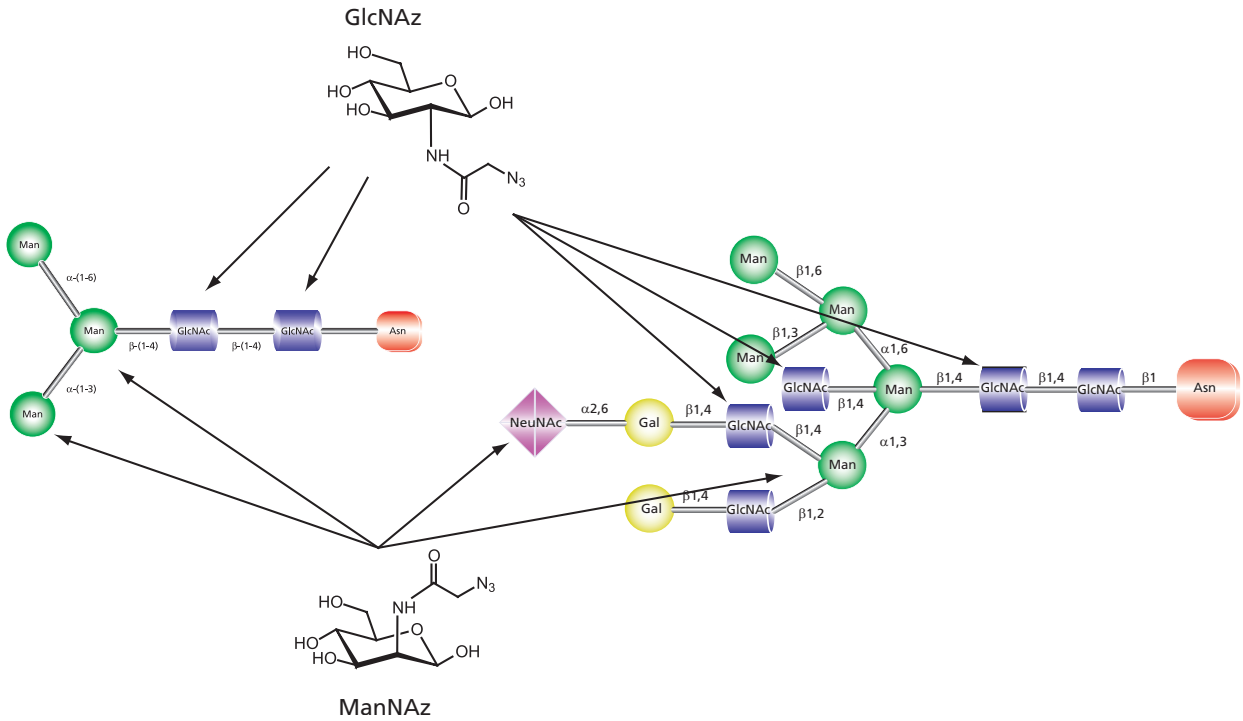


Figure 2. Possible sites of azido-sugar incorporation in simple and complex N-linked glycan structures.

Incorporation of Azido Sugars in Carbohydrate Structures

Cells metabolize the azidosugars using glycosyltransferases and express the sugars on the terminus of a glycan chain both intracellularly and on the cell surface, leaving the azido group unreacted. The azidosugars can also be incorporated into glycans via the sialic acid metabolic pathway. A selective phosphine probe containing a detection epitope such as FLAG® is applied to the cellular extracts containing the azidoglycans. The phosphine group selectively reacts via Staudinger ligation with the displayed azido group, resulting in an epitope tag covalently attached to the glycan (see **Figure 3**).

Although non-natural molecules, both the azido and phosphine moieties are tolerated during cell proliferation. The bound epitope peptide is then detected by using FLAG-specific antibody. This approach permits the analysis of pathways that are regulated by particular glycan post-translational modifications as well as the monitoring of the intracellular glycosylation process itself. Metabolic labeling with bioorthogonal chemical reporters such as azidosugars followed by Staudinger ligation provides a unique mechanism for proteomic analysis of this post-translational modification and for identifying glycoprotein fingerprints associated with disease.

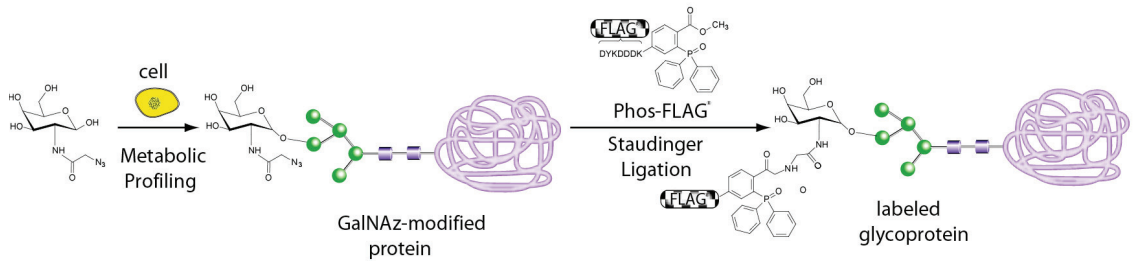


Figure 3. Profiling N-type glycoproteins by metabolic labeling with an azido GalNAc analog (GalNAz) followed by Staudinger ligation with a phosphine probe (phosphine-FLAG). R and R' are oligosaccharide elaborations from the core GalNAc residue.

References:

1. Cell surface engineering by a modified Staudinger reaction. Saxon, E. and Bertozzi, C.R., *Science* , **287**, 2007 (2000).
2. Chemical remodelling of cell surfaces in living animals. Prescher, J.A., Dube, D.H., and Bertozzi, C.R., *Nature*, **430**, 873 (2004).
3. A chemical approach for identifying O-GlcNAc-modified proteins in cells. Vocadlo, D.J., Hang, H.C., Kim, E.J., Hanover, J.A., Bertozzi, C.R., *Proc. Natl. Acad. Sci*. USA, **100**, 9116 (2004).
4. A metabolic labeling approach toward proteomic analysis of mucin-type O-linked glycosylation, Hang H.C., Yu, C., Kato, D.L., Bertozzi, C.R., *Proc. Natl. Acad. Sci.* USA, **100**, 14846 (2003).
5. Probing mucin-type O-linked glycosylation in living animals. Dube, D.H., Prescher J.A., Quang C.N., and Bertozzi, C.R. *Proc. Natl. Acad. Sci*. USA, **103**, 4819 (2006)

Glycan Standards

The analysis of the glycan portion of glycoproteins is necessary for monitoring changes in post-translational modification that occur in disease states, as well as evaluating the consistency of glycoprotein production. In general, the method is to isolate glycan pools from gel electrophoresis bands of a glycoprotein using in-gel digestion techniques.[1] The isolated glycan pools are subsequently separated by a chromatographic method, usually high performance liquid chromatography (HPLC), high-pH anion-exchange chromatography (HPAEC), hydrophilic interaction liquid chromatography (HILC), or high-pH anion-exchange chromatography (HPAE).[2] Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry analysis of the glycan constituents is used to identify the individual glycans after separation.[3,4,5]

Glycan standards function as markers during the separation and purification of glycans isolated from glycoproteins. These compounds are used as internal reference compounds for the peak assignment of glycan constituents separated by chromatography. Additionally, they may be used for calibration in MALDI-TOF mass spectrometry analysis. The following are the most common N-linked and O-linked glycans for research applications.

References

1. Rudd, P. and Dwek, R., Rapid, sensitive sequencing of oligosaccharides from glycoproteins, *Curr. Opin. Biotechnol.*, **8**, 488 (1997).
2. Charlwood, J. et al., Characterization of the glycosylation profiles of Alzheimer's β-secretase protein Asp-2 expressed in a variety of cell lines, *J. Biol. Chem.*, **276**, 16739 (2001).
3. Kremmer, T., et al., Liquid chromatographic and mass spectrometric analysis of human serum acid α-1-glycoprotein, *Biomed. Chromatogr.*, **18**, 323 (2004).
4. Yu, X., et al., Identification of N-linked glycosylation sites in human testis angiotensin-converting enzyme and expression of an active deglycosylated form, *J. Biol. Chem.*, **272**, 3511 (1997).
5. Leibiger, H. et al., Structural characterization of the oligosaccharides of a human monoclonal anti-lipopolysaccharide immunoglobulin M, *Glycobiology*, **8**, 497 (1998).

Key to Monosaccharide Symbols

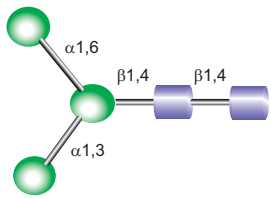
Name and Abbreviation	Symbol
β-D-Glucose (Glc)	Glc
β-D-Mannose (Man)	Man
β-D-Galactose (Gal)	Gal
β-D-N-Acetylglucosamine (GlcNAc)	GlcNAc
α-N-Acetylneuraminic acid; Sialic acid (NeuNAc)	NeuNAc
α-L-Fucose (Fuc)	Fuc

N-Linked High Mannose Glycans

M8418 Man-3 Glycan

$(Man)_3(GlcNAc)_2$; Mannotriose-di-(N-acetyl-D-glucosamine); Oligomannose-3 glycan

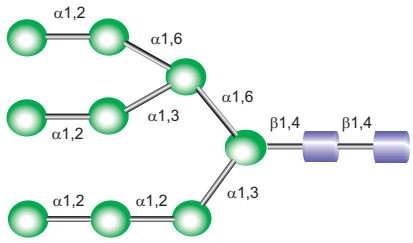
[70858-45-6] $C_{34}H_{58}N_2O_{26}$ **10 µg, 50 µg**



M9037 Man-9 Glycan

$(Man)_9(GlcNAc)_2$; Mannononaose-di-(N-acetyl-D-glucosamine)

[71246-55-4] $C_{70}H_{118}N_2O_{56}$ **10 µg**

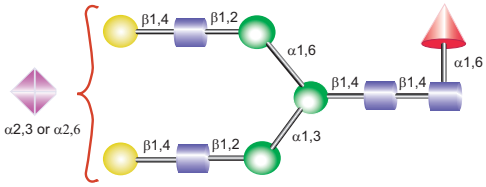


N-Linked Complex Glycans

M3800 A1F Glycan

NeuNAc(Gal-GlcNAc)$_2$Man$_3$(GlcNAc)$_2$Fuc; Mannotriose-(fucosyl-di-[N-acetylglucosamine]), mono-sialyl-bis(galactosyl-N-acetylglucosaminyl); Monosialyl galactosyl biantennary glycan, core fucosylated

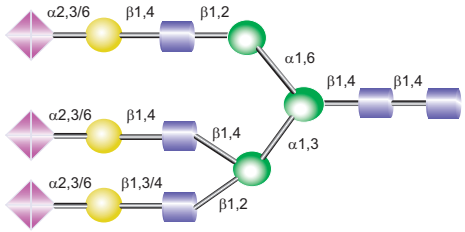
[571188-30-2] $C_{79}H_{131}N_5O_{58}$ **20 µg**



M2925 A3 Glycan

(NeuNAc-Gal-GlcNAc)$_3$Man$_3$(GlcNAc)$_2$; Mannotriose-di-(N-acetyl-D-glucosamine), tris(sialyl-galactosyl-N-acetyl-D-glucosaminyl); Trisialyl galactosyl triantennary glycan

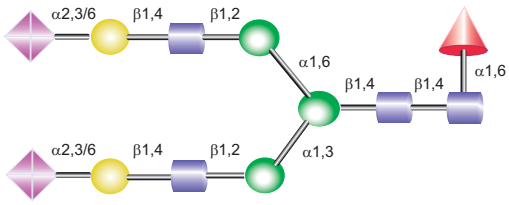
[145164-24-5] $C_{109}H_{178}N_8O_{80}$ **10 µg**



M2800 A2F Glycan

(NeuNAc-Gal-GlcNAc)$_2$Man$_2$(Fuc)(GlcNAc)$_2$; Mannotriose-(fucosyl-di-[N-acetylglucosamine]), bis(sialyl-galactosyl-N-acetylglucosaminyl); Disialylated, galactosylated, fucosylated biantennary glycan

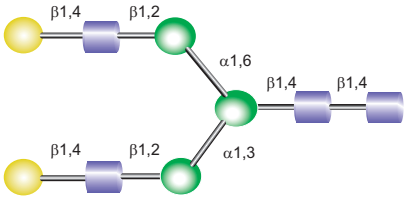
[108341-22-6] $C_{90}H_{148}N_6O_{66}$ **10 µg**



M5925 NA2 Glycan

(Gal-GlcNAc)$_2$Man$_3$(GlcNAc)$_2$; Mannotriose-di-(N-acetyl-D-glucosamine), bis(galactosyl-[N-acetyl-D-glucosaminyl]); Asialo galactosyl biantennary glycan

[71496-53-2] $C_{62}H_{104}N_4O_{46}$ **50 µg**

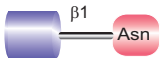


N-Linked Fragments

A6681 N-Asn

2-Acetamido-1-N-(β-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine; β-D-GlcNAc-(1→N)-Asn

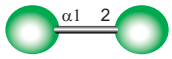
[2776-93-4] $C_{12}H_{21}N_3O_8$ **5 mg, 25 mg, 100 mg**



M1050 2α-Mannobiose

α-D-Man-(1→2)-D-Man; 2-O-α-D-Mannopyranosyl-D-mannopyranose

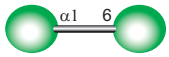
[15548-39-7] $C_{12}H_{22}O_{11}$ **10 mg**



M7788 6α-Mannobiose

α-D-Man-(1→6)-D-Man; 6-O-α-D-Mannopyranosyl-D-mannopyranose

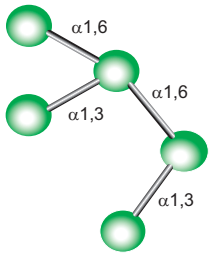
[6614-35-3] $C_{12}H_{22}O_{11}$ **1 mg, 5 mg**



M0925 3α,6α-Mannopentaose

α-Man-(1→3)(α-Man-[1→6])-α-Man-(1→6)(α-Man-[1→3])-Man

[112828-69-0] $C_{30}H_{52}O_{26}$ **5 mg**



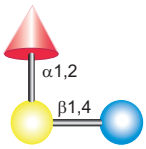
Glycan Standards

O-Linked Neutral Glycans

F0393 2′-Fucosyllactose

α-L-Fuc-(1→2)-β-D-Gal-(1→4)-D-Glc; 2′-FL; 2′-Fucosyl-D-lactose

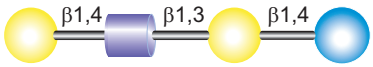
[41263-94-9] $C_{18}H_{32}O_{15}$ **1 mg, 5 mg**



L6543 Lacto-N-Neotetraose

β-D-Gal-(1→4)-β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-D-Glc; LNnT

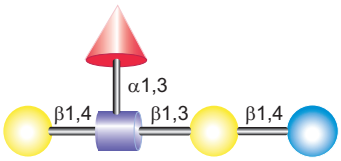
[13007-32-4] $C_{26}H_{45}NO_{21}$ **2 mg**



L 7777 Lacto-N-Fucopentaose III

Le*-lactose, Lewis-X pentasaccharide, β-D-Gal-(1→4)-[α-L-Fuc-(1→3)]-β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-D-Glc; LNFP III

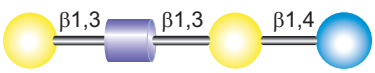
[25541-09-7] $C_{32}H_{55}NO_{25}$ **1 mg**



L6770 Lacto-N-Tetraose

β-D-Gal-(1→3)-β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-D-Glc; LNT

[14116-68-8] $C_{26}H_{45}NO_{21}$ **1 mg, 5 mg**

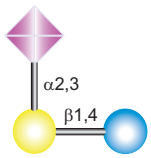


O-Linked Sialylated Glycans

A 8681 3′-Sialyllactose from bovine colostrum

NANA-Lactose; 3′-N-Acetylneuraminyllactose; 3′-SL; α-NeuNAc-(2→3)-β-D-Gal-(1→4)-D-Glc; α-Neu5Ac-(2→3)-β-D-Gal-(1→4)-D-Glc

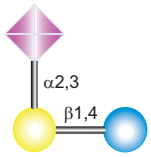
[35890-38-1] $C_{23}H_{39}NO_{19}$ **1 mg**



A 9079 3′-Sialyllactose from human milk

NANA-Lactose; 3′-N-Acetylneuraminyllactose; 3′-SL; α-NeuNAc-(2→3)-β-D-Gal-(1→4)-D-Glc; α-Neu5Ac-(2→3)-β-D-Gal-(1→4)-D-Glc

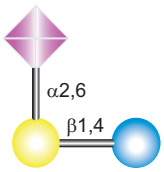
[35890-38-1] $C_{23}H_{39}NO_{19}$ **1 mg**



A 8556 6′-Sialyllactose from bovine colostrum sodium salt

6′-N-Acetylneuraminyllactose; 6′-SL; α-NeuNAc-(2→6)-β-D-Gal-(1→4)-D-Glc; α-Neu5Ac-(2→6)-β-D-Gal-(1→4)-D-Glc

[74609-39-5] $C_{23}H_{38}NNaO_{19}$ **1 mg, 5 mg**



Galectins

Galectins are a family of animal carbohydrate binding proteins; the name is from their description as β-galactoside-specific lectins. They have been strongly implicated in inflammation and cancer and may be useful as targets for the development of new anti-inflammatory and anticancer therapies.

Galectins occur at high concentration in a limited range of cell types, different for each galectin. Galectins bind to sugar molecules on the surface of cells. All galectins bind lactose and other β-galactosides, but they differ in their affinity for more complex saccharides.[1] The galectins are defined by their structural similarities in their carbohydrate recognition domains (CRD) and by their affinity for β-galactosides; fourteen human members have been reported so far.[2] The galectins have been classified into three classes, prototype, chimera, and tandem-repeat galectins. The prototype galectins (-1, -2, -5, -7, -10, -11, -13, -14) all contain one CRD and are either monomers or noncovalent homodimers. The only chimera galectin currently identified (galectin-3) contains one CRD connected to a non-lectin domain. The tandem-repeat galectins (-4, -6, -8, -9, -12) consist of two CRDs joined by a linker peptide.

Extracellular galectins crosslink cell-surface and extracellular glycoproteins and may thereby modulate cell adhesion and induce intracellular signals. Galectins may also bind intracellular non-carbohydrate ligands and have intracellular regulatory roles in processes such as RNA splicing, apoptosis, and, suggested most recently, the cell cycle.[1]

Galectin-1

Galectin-1 has been implicated in metastasis and aggregation of cancer cells based on its association with the glycoprotein 90K.[4,5] It has been shown to induce apoptosis of activated T-cells,[6] T-leukemia cell lines,[7] breast,[8] colon,[9] and prostate[10] cancer cells. Other activities of galectin-1 include cell differentiation and inhibition of CD45 protein phosphatase activity. Galectin-1 binds CD45, CD3, and CD4 in addition to β-galactoside. Galectin-1 bound in the extracellular matrix can induce cell death of adherent T cells at a ten-fold lower concentration than soluble galectin-1.[11] Galectin-1 may play a significant role in cancer through apoptosis, cell adhesion and migration, regulation of the cell cycle, and tumor evasion of immune responses.[12,13]

Galectin-3

Galectin-3, also called Mac-2, L29, CBP35 and εBP, is a chimera galectin that is expressed in tumor cells, macrophages, activated T cells, epithelial cells, and fibroblasts. It binds a variety of matrix glycoproteins including laminin and fibronectin. Intracellularly, galectin-3 acts to prevent apoptosis. Depending on the cell type, galectin-3 can be localized in the extracellular matrix, the cell surface, in the cytoplasm, or in the nucleus. Galectin-3 has been shown to exhibit proinflammatory activities *in vitro* and *in vivo*; it induces pro-inflammatory and inhibits Th2 type cytokine production.[3] High levels of circulating galectin-3 have been shown to correlate with the malignancy potential of several types of cancer. Galectin-3 is known to play a role in tumor growth, metastasis, and cell-to-cell adhesion. It also serves as a preferred substrate for matrix metalloproteinase-9 (MMP-9).[14] Human and mouse Galectin-3 share approximately 80% homology in their amino acid sequence.[15]

Galectin-3C

Galectin-3C is a truncated form of galectin-3 that contains the carboxy-terminus carbohydrate-binding domain. Recombinant galectin-3C competes with endogenous galactin-3 for carbohydrate binding sites and acts as a negative inhibitor of galectin-3[16] in promoting cell adhesion[17] and cell signaling. Galectin-3C has been found to be effective in reducing metastases and tumor volumes and weights in primary tumors in an orthotropic nude mouse model of human breast cancer.[18]

Galectin-8

Galectin-8, also known as prostate carcinoma tumor antigen 1 (PCTA1) in human, is a tandem repeat-type galectin. High levels of circulating galectin-8 have been shown to correlate with lung carcinomas, certain forms of prostate carcinomas, as well as other tumor cells.[19] It binds to a subset of cell surface integrins to modulate ECM-integrin interactions. It acts as a physiological modulator of cell adhesion and cellular growth, and may be involved in neoplastic transformation.[20-22] Human and mouse galectin-8 share approximately 80% homology in their amino acid sequence.[15]

Cat. No.	Name	Description	Pack Size
G7420	Galectin-1	Human, recombinant, expressed in *Escherichia coli*, ≥95% (SDS-PAGE), lyophilized powder	250 µg
G5170	Galectin-3	Human, recombinant, expressed in *Escherichia coli*, lyophilized powder	100 µg
G5295	Galectin-3C	Human, recombinant, expressed in *Escherichia coli*, lyophilized powder A truncated form of galectin-3	100 µg
G3670	Galectin-8	Rat, recombinant, expressed in *Escherichia coli*, ≥90% (SDS-PAGE), buffered aqueous glycerol solution	100 µg

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Lectins

Lectins

Lectins are proteins or glycoproteins from non-immune origins that agglutinate cells and/or precipitate complex carbohydrates. Lectins are isolated from a wide variety of natural sources, both plant and animal. Recombinant human and rat galectins are expessed in *Escherichia coli*. The agglutination activity of these highly specific carbohydrate-binding molecules is usually inhibited by a simple monosaccharide, but for some lectins di-, tri-, and even polysaccharides are required. Sigma offers a wide range of lectins suitable for the following applications:

- Carbohydrate studies
- Mitogenic stimulation
- Fractionation of cells and other particles
- Blood group typing
- Lymphocyte subpopulation studies
- Histochemical studies

Lectin Source	Acronym	Mol. Wt. (kDa)	Subunits	Blood Group Specificity	Carbohydrate Specificity	Mito-genicity	Protein Families[a]	Related Domains[b]	Cat. No.	Conjugates Cat. No.
Agaricus bisporus (Mushroom)	ABA	58.5	—	—	ß-Gal(1→3)GalNac		Fungal fruit body lectin	Fungal fruit body lectin	**L5640**	n/a
Arachis hypogaea (Peanut)	PNA	120	4	T	ß-Gal(1→3)GalNAc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L0881**	Biotin L6135 FITC L7381 Peroxidase L7759 TRITC L3766
Artocarpus integrifolia (Jacalin)		42	4	T	α-Gal-OMe	Mitogenic	Jacalin-like lectin domain	Jacalin-like lectin domain	**L3515**	Agarose L5147
Bandeiraea simplicifolia (Griffonia simplicifolia)	BS-I	114	4	A, B	α-Gal, α-GalNAc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L2380**	Biotin L3759 FITC L9381 TRITC L5264
Isolectin A_4	BS-I-A4	114	4	A	α-GalNAc		Not reported	Not reported	**n/a**	FITC L0890
Isolectin B_4	BS-I-B4	114	4	B	α-Gal		Not reported	Not reported	**L3019**	Biotin L2140 FITC L2895 Peroxidase L5391
Caragana arborescens (Siberian pea tree)		60;120[c]	2;4	—	GalNAc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**n/a**	Biotin L9637
Cicer arietinum (Chick pea)		44	2	—	Fetuin		Not reported	Not reported	**L3141**	n/a
Codium fragile (Green marine algae)		60	4	—	GalNAc		Not reported	Not reported	**L2638**	n/a
Concanavalin A from Canavalia ensiformis (Jack bean) (Con A)		102	4	—	α-Man, α-Glc	Mitogenic	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L7647 C7275 C2010 C5275 (cell culture tested) C0412 (γ-irridated, cell culture tested) C2631**	Biotin C2272 Ferritin C7898 FITC C7642 Gold, 10 nm L5021 Gold, 20 nm L3542 Peroxidase L6397 Agarose C6904 Sepharose® C9017
Succinyl-Concanavalin A		51	2	—	α-Man, α-Glc	Mitogenic[d]	Not reported	Not reported	**L3885**	FITC L9385
Datura stramonium (Jimson weed; Thorn apple)	DSL	86	2(α&ß)[e]	—	$(GlcNAc)_2$		Not reported	Not reported	**L2766**	n/a
Dolichos biflorus (Horse gram)	DBA	140	4	A_1	α-GalNAc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L2785**	Biotin L653 FITC L9142 Peroxidase L1287 TRITC L9658 Agarose L9894

Lectin Source	Acronym	Mol. Wt. (kDa)	Subunits	Blood Group Specificity	Carbohydrate Specificity	Mito-genicity	Protein Families[a]	Related Domains[b]	Cat. No.	Conjugates Cat. No.
Erythrina cristagalli (Coral tree)	ECA	56.8	2(α&ß)[e]	—	ß-Gal(1→4)GlcNAc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L5390**	n/a
Galanthus nivalis (Snowdrop)	GNL	52	4	Rabbit [f]	non-reduc. D-Man		Curculin-like (mannose-binding) lectin	D-mannose binding lectin	**L8275**	Agarose L8775
Glycine max (Soybean)	SBA	110	4	—	GalNAc	Mitogenic [g]	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L1395**	Peroxidase L2650 TRITC L4511
Helix aspersa (Garden snail)	HAA	79	—	A	GalNAc		Not reported	Not reported	**L6635**	Biotin L8764
Helix pomatia (Edible snail)	HPA	79	6	A	GalNAc		Not reported	Not reported	**L3382**	Biotin L6512 FITC L1034 Peroxidase L6387 TRITC L1261
Human Galectin-1	Gal-1	14	2	—	ß-Gal		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Galectin, galactose-binding lectin	Galactoside binding lectin domain	**G7420**	n/a
Human Galectin-3	Gal-3	26		—	ß-Gal		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Galectin, galactose-binding lectin	Galactoside binding lectin domain	**G5170**	n/a
Human Galectin-3C		16		—	ß-Gal		Not reported	Not reported	**G5295**	n/a
Lens culinaris (Lentil)	LcH	49	2	—	α-Man		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L9267**	Biotin L4143 FITC L9262 Sepharose® L8511
Limulus polyphemus (Horseshoe crab)		400	18	—	NeuNAc		Not reported	Lectin-type C domain	**L2263**	n/a
Lycopersicon esculentum (Tomato)	LEA	71	—	—	$(GlcNAc)_3$	Mitogenic [h]	Not reported	Galactose binding lectin domain	**L2886**	Biotin L0651 FITC L0401
Maackia amurensis	MAA	130	2(α&ß)	O	α-Neu NAc (2⟶3)Gal	Mitogenic	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L8025**	n/a
Phaseolus vulgaris Erythroagglutinin (Red kidney bean)	PHA-E	128	4	—	Oligosaccharide	Mitogenic	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L8629**	TRITC L6139
Phaseolus vulgaris Leucoagglutinin (Red kidney bean)	PHA-L	126	4	—	Oligosaccharide	Mitogenic	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L2769**	n/a
Phaseolus vulgaris Phytohemagglutinin (red kidney bean)	PHA-P						Not reported	Not reported	**L8754**	n/a
Phaseolus vulgaris Mucoprotein (red kidney bean)	PHA-M						Not reported	Not reported	**L2646**	n/a

Lectins

Lectin Source	Acronym	Mol. Wt. (kDa)	Subunits	Blood Group Specificity	Carbohydrate Specificity	Mito-genicity	Protein Families[a]	Related Domains[b]	Cat. No.	Conjugates Cat. No.
Phytolacca americana (Pokeweed)	PWM	32[i]	—	—	$(GlcNAc)_3$	Mitogenic	Chitin recognition protein	Chitin-binding, type 1	**L9379**	n/a
Pisum sativum (Garden pea)	PSA	49	4(α&ß)[e]	—	α-Man	Mitogenic	Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L5380**	FITC L0770
Pseudomonas aeruginosa	PA-I	13-13.7	—	—	Gal	Mitogenic [g]	Galactose-binding like	PA-IL-like protein	**L9895**	n/a
Psophocarpus tetragonolobus (Winged bean)		35	1	—	GalNAc, Gal		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**n/a**	Biotin L3014 FITC L3264 TRITC L3389
Rat Galectin-8	Gal-8	34		—	ß-Gal		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Galectin, galactose-binding lectin	Galactoside binding lectin domain	**G3670**	n/a
Ricinus communis Agglutinin (Castor bean)	RCA_{120}	120	4	—	ß-Gal		Ricin B lectin Ricin B-related lectin Ribosome-inactivating protein	Ricin-type β-trefoil lectin domain Ribosome inactivating protein (RIP)	**L7886**	Agarose L2390
Ricinus communis Ricin, A chain (Castor bean)							Ricin B lectin Ricin B-related lectin Ribosome-inactivating protein	Ricin-type β-trefoil lectin domain Ribosome inactivating protein (RIP)	**L9514**	n/a
Ricinus communis Ricin, A chain, deglycosylated (Castor bean)									**L4022**	n/a
Sambucus nigra (Elder)	SNA	140	4(α&ß)[e]	-	α-NeuNAc(2→6) Gal/GalNAc	Mitogenic [g]	Ricin B lectin Ricin B-related lectin	Ricin-type β-trefoil lectin domain	**L6890**	n/a
Solanum tuberosum (Potato)	STA	50; 100 [c]	1;2	-	$(GlcNAc)_3$		Jacalin-related lectin	Jacalin-like lectin domain	**L4266**	n/a
Tetragonolobus purpureas (Lotus tetragonolobus, winged or asparagus pea)		120(A), 58(B), 117(C)	4;2;4	H	α-L-Fuc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L9254**	Biotin L3134
Triticum vulgaris (Wheat germ)	WGA	36	2	—	$(GlcNAc)_2$, NeuNAc		Chitin recognition protein	Chitin-binding, type 1	**L9640**	Biotin L5142 Evans Blue L9884 FITC L4895 Gold, 10 nm L1894 Peroxidase L3892 TRITC L5266 Agarose L1882
Ulex europaeus (Gorse)	UEA I	68	—	H	α-L-Fuc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain	Legume lectin domain	**L5505**	Biotin L8262 FITC L9006 Peroxidase L8146 TRITC L4889
Vicia villosa (Hairy vetch)	VVA	139	4 [e]	A_1+T_n	GalNAc		Not reported	Not reported	**n/a**	Agarose L9388
Vicia villosa Isolectin B4		143	4	T_n	GalNAc		Concanavalin A-like lectin/glucanase Concanavalin A-like lectin/glucanase, subgroup Legume lectin, α Legume lectin, β domain		**L7513**	n/a

Lectin Source	Acronym	Mol. Wt. (kDa)	Subunits	Blood Group Specificity	Carbohydrate Specificity	Mito-genicity	Protein Families[a]	Related Domains[b]	Cat. No.	Conjugates Cat. No.
Viscum album (Mistletoe)		115 [j]	4(αß) [e]	—	ß-Gal		Ricin B lectin Ricin B-related lectin	Ricin-type β-trefoil lectin domain	**L2662**	n/a
Wisteria floribunda	WFA	68	2	—	GalNAc		Not reported	Not reported	**n/a**	Biotin L1516
Wisteria floribunda, Reduced		34	1	—	GalNAc		Not reported	Not reported	**n/a**	Biotin L1766

Notes:

a. Swiss Institute of Bioinformatics Swiss-Prot/European Bioinformatics Institute InterPro protein sequence database
b. Wellcome Trust Sanger Institute Pfam protein sequence database
c. Concentration-dependent mol. wt. change
d. Non-agglutinating and mitogenic
e. Subunits are of different molecular weights
f. Agglutinates rabbit, but not human, erythrocytes
g. Mitogenic for neuraminidase-treated lymphocytes
h. Inhibits mitogenic acitvity of PHA
i. Data given for PWM Pa2
j. Data given for VAA(I)

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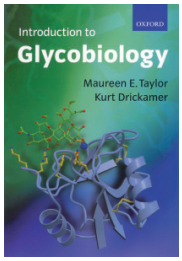
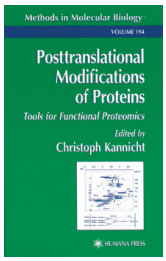


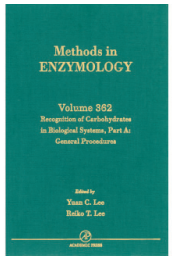
Z700568 Introduction to Glycobiology
M. Taylor and K. Drickamer, Oxford University Press, 2003, 160 pp., softcover

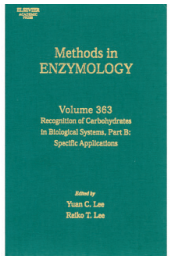
Coherent stories about what sugars do for cells and organisms are the focus--particularly the importance of glycosylation in protein secretion and stability, cell-cell adhesion and signaling, and innate and adaptive immunity. The ways in which glycobiology explains human disease are discussed, giving the book a biomedical context. Illustrated throughout with custom-drawn figures, the book's simple organization, highlighted terms and annotated key reference lists make it readable and accessible.



P8742 Posttranslational Modification of Proteins: Tools for Functional Proteomics
C. Kannicht, *Humana Press*, 2002, 385 pp., hard cover

This volume describes reproducible methods for detecting and analyzing the posttranslational modifications of protein, particularly with regard to protein function, proteome research, and the characterization of pharmaceutical proteins. Methods include those for analyzing the assignment of disulfide bond sites in proteins, protein N-glycosylation and protein O-glycosylation, and oligosaccharides present at specific single glycosylation sites in a protein. Additional techniques facilitate the analysis of glycosylphosphatidylinositols, lipid modifications, protein phosphorylation and sulfation, protein methylation and acetylation, α-amidation and lysine hydroxylation.





Z702110 Recognition of Carbohydrates in Biological Systems, Part A: General Procedures, MIE Vol. 362

Z702129 Recognition of Carbohydrates in Biological Systems, Part B: Specific Applications, MIE Vol, 363
Y. Lee, *Academic Press*, **2003**, 625 pp., hard cover

Recognition of carbohydrates in biological systems has been gaining more and more attention in recent years. Although methodology for studying recognition has been developing, there is no volume that covers the wide area of methodology of carbohydrate recognition. These companion volumes present state-of-the-art methodologies, as well as the most recent biological observations in this area. Volume 362 covers the isolation/synthesis of substances used in studying interactions involving carbohydrates and discusses the methodology for measuring such interactions. Biological roles for such interactions are also covered. Volume 363 covers carbohydrate-binding proteins and discusses glycoproteins and glycolipids. Polysaccharides, enzymes and cells are also covered.

Z513776 Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries
P.H. Seeberger, *John Wiley & Sons*, 2001, 320 pp., hard cover

This book addresses the exciting expectation that solid-phase assembly of oligosaccharides will have a fundamental impact on the field of glycobiology. This publication details the methodologies currently investigated for the attachment of carbohydrates to beads, synthesis including coupling strategies, and removal of the product from beads.

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