Glycobiology and Glycoproteomics

Enrichment

Deglycosylation Enzymes and Kits

Cell Surface Modification

Downstream Analysis

Glycan Synthesis and Modification

Glycobiology and Glycoproteomics

The contribution of glycobiology and glycoproteomics to life science is increasingly being recognized. In eukaryotic cells, most proteins are subject to post-translational modifications, of which glycosylation is the most common form. It is estimated that more than half of all eukaryotic proteins have been characterized as glycoproteins.

The structural diversity of glycans is much greater than that of nucleic acids and proteins. The enzymes involved in glycan biosynthesis are characteristic of the biological species, organ, and tissue, in addition to being contingent on the life cycle, time frame, and environment.

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. 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.

See the intricacies of glycobiology and carbohydrate analysis more clearly with the unique portfolio of carbohydrates, enzymes, and kits from Sigma Life Science.

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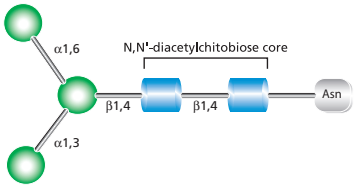
Key Mammalian Glycan Structure Types

N-Glycans

N-linked glycans are linked to the protein backbone via an amide bond to asparagine (Asn) residues in an Asn-X-Ser (serine) or an Asn-X-Thr (threonine) motif, where X represents any amino acid except proline. All *N*-linked glycans are based on the common core pentasaccharide, $Man_3GlcNAc_2$.

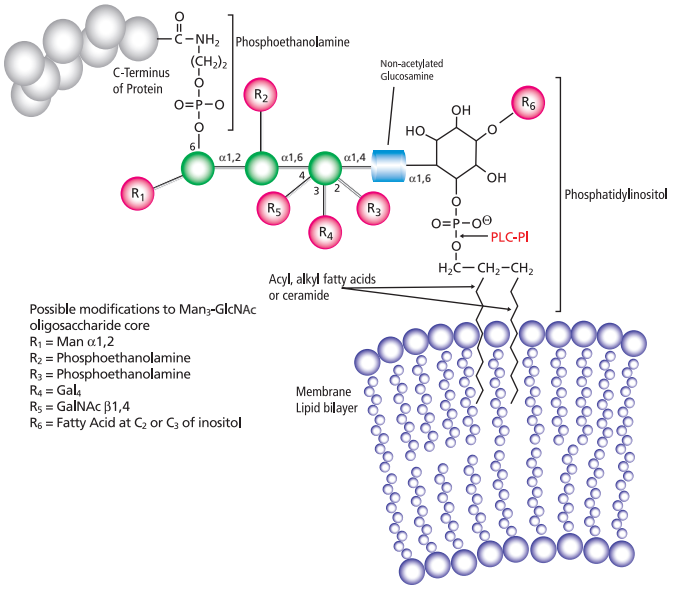


O-Glycans

O-linked glycans are most commonly linked to the hydroxyl group of Ser or Thr, with no consensus sequence required. Other *O*-linkages do occur. The most common *O*-linked glycans are the mucin-type glycans, which contain an initial GalNAc residue. *O*-linked glycans tend to be linear or biantennary.

GPI

GPI (Glycosylphosphatidylinositol)-anchored proteins are attached at their carboxy termini through a phosphodiester linkage of phosphoethanolamine to a trimannosyl glucosamine core structure. The reducing end of the latter moiety is bound to the hydrophobic region of the membrane via a phosphatidylinositol group.



Enrichment

Enrichment is often one of the first steps taken in analyzing glycoproteins. This allows for the selection of glycosylation proteins in general or specific selection of glycans of interest.

Affinity Matrices

Purification of glycoproteins by selectively capturing the glycan component is commonly done utilizing affinity chromatography. The most common affinity matrices are *m*-aminophenylboronic acid-agarose for nonspecific binding of saccharides, and immobilized lectins for binding specific carbohydrates.

m-Aminophenylboronic Acid Matrices

m-Aminophenylboronic acid matrices are capable of forming temporary bonds with any molecule that contains a 1,2-cis-diol group.

Cat. No.	Name	Matrix	Extent of Labeling	Binding Capacity	Size
A8530	*m*-Aminophenylboronic acid–Agarose	Cross-linked 6% beaded agarose	5–20 µmol per 1 mL	-	5 mL
A8312	*m*-Aminophenylboronic acid– Agarose	6% beaded agarose	40–80 µmol per 1 mL	≥8 mg/mL peroxidase	5 mL

Immobilized Lectins

Lectins are glycan-binding proteins (GBPs) or glycoproteins of non-immune origin that specifically and reversibly bind carbohydrates. Through this affinity for specific glycans, lectins are able to agglutinate cells and precipitate complex carbohydrates. Lectins are found in a wide variety of natural sources including plants, animals and humans, bacteria, and viruses (hemagglutinins). The binding of these glycan-binding proteins can usually be inhibited by a simple monosaccharide, but for some lectins, di-, tri-, and even polysaccharides are required. Immobilized lectins are commonly used in affinity chromatography to selectively capture glycoproteins and glycopeptides.

Cat. No.	Name	Matrix	Extent of Labeling	Carbohydrate Specificity	Size
L2507	Lectin from *Arachis hypogaea* (peanut)	Cross-linked 4% beaded agarose	2–4 mg/mL	ß-Gal(1→3)GalNAc	1 mL 2 mL
L5147	Lectin from *Artocarpus integrifolia*	Cross-linked 4% beaded agarose	~ 5 mg/mL	α-Gal-OMe	2 mL 5 mL
C7555	Concanavalin A from *Canavalia ensiformis* (Jack bean)	Cross-linked 4% beaded agarose	Concanavalin A protein 15–30 mg/mL	α-Man, α-Glc	5 mL 25 mL 100 mL
C9017	Concanavalin A from *Canavalia ensiformis* (Jack bean)	Sepharose® 4B	10–15 mg/mL	α-Man, α-Glc	25 mL 100 mL
L8775	Lectin from *Galanthus nivalis* (snowdrop)	Cross-linked 4% beaded agarose	2–4 mg protein/mL	non-reducing D-Man	2 mL
L0511	Lectin from *Lens culinaris* (lentil)	Sepharose 4B	~ 2 mg/mL	α-Man	5 mL 10 mL 25 mL
L1394	Lectin from *Triticum vulgaris* (wheat)	6% agarose macrobeads	~ 6 mg/mL	$(GlcNAc)_2$, NeuNAc	1 mL 5 mL 10 mL
L1882	Lectin from *Triticum vulgaris* (wheat)	Cross-linked 4% beaded agarose	5–10 mg/mL	$(GlcNAc)_2$, NeuNAc	1 mL 5 mL

Deglycosylation Enzymes and Kits

Both selective and nonselective deglycosylation methods may be applied to determine the structure of the protein core and its glycosylation sites. Enzymatic methods are relatively mild and allow removal of a selected class of glycans, although complete removal often requires denaturation of the protein. Advances in chemical methods have greatly improved over the years to reflect many of the benefits seen with enzymatic methods. Depending upon the method used, either the protein core or the glycans can be recovered intact without significant degradation.

Enzymes for Deglycosylation

Endoglycosidases and exoglycosidases are used for deglycosylation procedures. Endoglycosidases are enzymes that catalyze the cleavage of an internal glycoside bond in an oligosaccharide. Exoglycosidases are enzymes that remove terminal carbohydrates from the nonreducing end of a glycan, but do not cleave internal bonds between carbohydrates.

PNGase F

***N*-Glycosidase F, Peptide *N*-glycosidase**
EC 3.5.1.52

PNGase F cleaves all asparagine-linked (*N*-linked) complex, hybrid, or high-mannose oligosaccharides from a glycopeptide or glycoprotein unless the core contains an α(1→3) fucose (see **Figure 1**).

Unit definition: One unit will catalyze the release of *N*-linked oligosaccharides from 1 nanomole of denatured ribonuclease B in one minute at 37 °C at pH 7.5 monitored by SDS-PAGE. One Sigma unit of PNGase F activity is equal to 1 IUB milliunit.

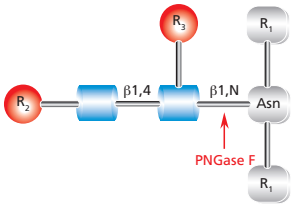


Figure 1. Cleavage site and structural requirements for PNGase F.

R1 = N- and C-substitution by groups other than H
R2 = H or the rest of an oligosaccharide structure
R3 = H or α(1→6)fucose

PNGase F from *Elizabethkingia meningoseptica* BioReagent, ≥95% (SDS-PAGE), for proteomics

- Excellent for applications requiring *N*-linked deglycosylation (see **Figure 2**)
- Superior performance for on-blot, in-gel, and in-solution digestion methods
- High specific activity; ≥25,000 units/mg
- Compatible for use in MALDI-TOF mass spectrometry

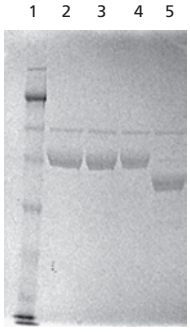


Figure 2. SDS-PAGE analysis of native and PNGase F-treated α_1-antitryspin. The test sample (Lane 5) was deglycosylated in solution with 5 units of PNGase F (**Cat. No. P7367**) for 1 hour at 37 °C prior to separation on SDS-PAGE. Note the shift in mobility of the band upon deglycosylation.

Lanes

1: Molecular weight marker
2, 3, 4: Control, native α_1-antitryspin
5: In-solution deglycosylated α_1-antitryspin

PNGase F from *Elizabethkingia meningoseptica* BioReagent, ≥95% (SDS-PAGE), for proteomics

Cat. No.	Description	Size
P7367	Extensively purified and lyophilized from dilute potassium phosphate buffer to produce a stable product. The product is free from glycerol and other stabilizers, and contains very low levels of buffer salts.	50 units 100 units

PNGase F from *Elizabethkingia miricola*

Cat. No.	Description	Size
G5166	**Buffered aqueous solution** Solution in 20 mM Tris HCl, pH 7.5, 50 mM NaCl and 1 mM EDTA	50 units 100 untis

Enzymatic Deglycosylation Kits

GlycoProfile™ II Enzymatic In-Solution *N*-Deglycosylation Kit
Optimized kit provides a convenient and reproducible method to remove *N*-linked glycans from glycoproteins. Compatible with subsequent MALDI-TOF mass spectrometric analysis without interference from any of the reaction components.

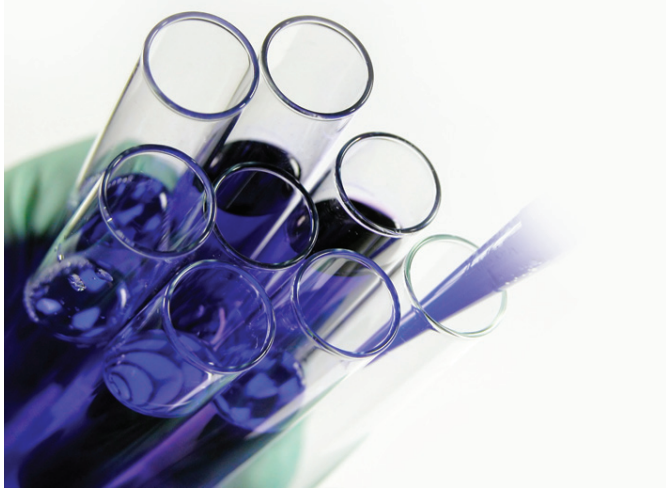
Cat. No.	Description	Size
PP0201-1KT	**Components** PNGase F 50 units Ribonuclease B 0.5 mg 10× Reaction Buffer 1 vial Octyl β-D-glucopyranoside 100 mg 2-Mercaptoethanol 0.90 mL	1 kit

Enzymatic Protein Deglycosylation Kit
Contains all enzymes and reagents needed to completely remove all *N*- and simple *O*-linked glycans including polysialylated carbohydrates from glycoproteins as well as additional enzymes and reagents needed to remove complex *O*-linked carbohydrates.

Cat. No.	Description	Size
EDEGLY-1KT	**Components** PNGase F 1 vial *O*-Glycosidase 20 µl α-(2→3,6,8,9)-Neuraminidase 20 µl Fetuin Control 0.5 mg 5× Reaction Buffer 0.2 mL Denaturation Solution 0.1 mL Triton® X-100 0.1 ml β-(1→4)-Galactosidase, positionally specific 20 µl β-N-Acetylglucosaminidase 20 µl	1 kit

Native Protein Deglycosylation Kit
Designed for the deglycosylation of *N*-linked oligosaccharides from PNGase F-resistant native proteins. Endoglycosidases F1, F2, and F3 are less sensitive to protein conformation than PNGase F and are more suitable for removal of all classes of *N*-linked oligosaccharides without protein denaturation.

Cat. No.	Description	Size
NDEGLY-1KT	**Components** Endoglycosidase F1 0.3 un Endoglycosidase F2 0.1 un Endoglycosidase F3 0.1 un Endoglycosidase F1 reaction buffer 200 µl Endoglycosidase F2 & 3 reaction buffer 200 µl	1 kit



The Enzyme Explorer is your source for endoglycosidases, exoglycosidases, and other carbohydrate-active enzymes.
sigma.com/enzymeexplorer

Chemical Deglycosylation Kits

TFMS Deglycosylation

The GlycoProfile™ IV Kit utilizes trifluoromethanesulfonic acid (TFMS) in a deglycosylation system that completely removes all *N*- and *O*-linked glycans while preserving the protein/polypeptide structure. TFMS hydrolysis of glycoproteins results in minimal protein degradation while the released glycans are destroyed (see **Figure 3**). For high molecular weight or complex, non-mammalian glycoproteins, an optional scavenger species is included.

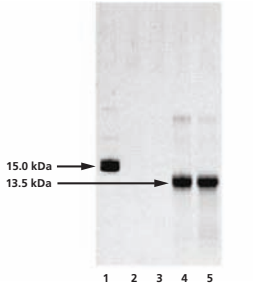


Figure 3. Analysis of the chemical deglycosylation of RNase B on 12% homogeneous SDS-PAGE gel after treatment with GlycoProfile IV kit (**Cat. No. PP0510**).

Lane 1 is the RNase B control (**Cat. No. R1153**), while lanes 2 to 5 represent fractions collected from the gel filtration 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.

- Removes both *N*- and *O*-linked glycans from proteins — *Permits sequence analysis of the protein core by mass spectrometry or DNA-to-protein sequence comparison*
- Eliminates glycans which affect molecular radius — *Allows molecular mass determination by electrophoresis*
- Complete removal in a single process — *Saves time by eliminating need for multiple enzyme reactions, overcoming enzyme resistance*

GlycoProfile™ IV Chemical Deglycosylation Kit
TFMS Deglycosylation System

Cat. No.	Description	Size
PP0510-1KT	Components	1 kit
	Trifluoromethanesulfonic Acid (TFMS) 5 × 1 g	
	RNase B Glycoprotein Standard 3 × 1 mg	
	Pyridine solution, 60% 10 mL	
	Bromophenol blue solution, 0.2% 0.5 mL	
	Anisole, anhydrous 5 × 1 mL	
	Reaction vials 10 each	

β-Elimination

The GlycoProfile β-Elimination Kit contains a novel non-reducing reagent to efficiently and specifically remove *O*-linked carbohydrates from glycoproteins with minimal protein or glycan destruction (see **Figure 4**). Consequently, additional downstream proteomics and glycomic analyses may be employed.

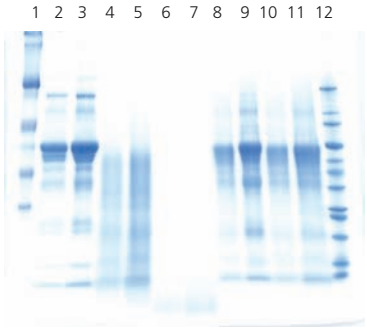


Figure 4. SDS-PAGE gel of fetuin before and after ß-elimination deglycosylation using GlycoProfile ß-Elimination kit (**Cat. No. PP0540**). The GlycoProfile kit does not completely degrade the protein as seen with traditional methods of ß-elimination.

Lane 1: ColorBurst™ Marker, High Range (**Cat. No. C1992**)

Lanes 2 and 3: Fetuin prior to ß-elimination

Lanes 4 and 5: Fetuin incubated in 50 mM NaOH overnight

Lanes 6 and 7: Fetuin incubated in $NaBH_4$ and NaOH overnight (traditional ß-elimination)

Lanes 8 and 9: Fetuin incubated in Sigma's ß-elimination reagent at 4–8 °C overnight.

Lanes 10 and 11: Fetuin incubated in Sigma's ß-elimination reagent at room temperature overnight

Lane 12: SigmaMarker™, Wide Range (**Cat. No. S8445**)

The GlycoProfile β-Elimination Kit allows researchers to:

- Perform complete glycoproteomics research by preserving both the *O*-glycans and protein
- Specifically remove *O*-glycans
- Label *O*-glycans prior to analysis
- Have confidence in uniformity of procedure

GlycoProfile™ β-Elimination Kit

Cat. No.	Description	Size
PP0540-1KT	Components	1 kit
	β-Elimination Reagent 940 µL	
	Sodium hydroxide 60 µL	
	Centrifugal filter unit 24 ea	

Cell Surface Modification

FSL Constructs for Labeling of Cells and Real-Time Imaging

FSL constructs are compounds based on KODE™ technology designed to label hydrophobic surfaces, including living cells with carbohydrates, biotin, or fluorophores (see **Figure 5**). All KODE FSL constructs consist of three essential designable features (see **Figure 6**):

- a functional component (**F**)
- a spacer (**S**)
- and a diacyl lipid (**L**)

All FSL constructs disperse in biocompatible media and spontaneously and stably incorporate into cell membranes. Cells modified with KODE constructs are known as kodecytes[1] and usually maintain their normal vitality and functionality. Constructs with human blood group A and B carbohydrate may be used to create live cells (kodecytes) with surface blood group antigens, but the constructs can also be used to modify other hydrophobic surfaces including fixed cells and solid-phase surfaces. FSL-carbohydrate constructs can also be used to attach reproducible and controlled levels of blood group antigens to group O or B erythrocytes.[2] These kodecytes will react with most antibody-based blood grouping reagents including polyclonal reagents.

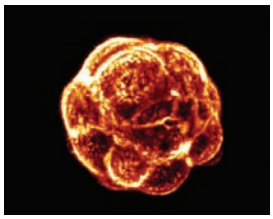


Figure 5. Confocal image of murine embryo blastocyte (external view) labeled with FSL-biotin (**Cat. No. F9182**) and detected using Alexa Fluor® 488 avidin conjugate

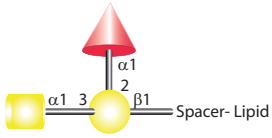
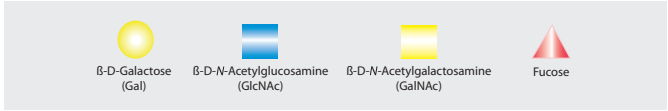


Figure 6. Structure of FSL-A (tri)



Cat No.	Name	Functional Group	Application	Size
F9307	**FSL-A(tri)** (FSL-A(GALNa3[Fa2]GALb)-SA1-L1)	Blood group A trisaccharide GalNAcα3(Fucα2)Galβ	Used to attach reproducible and controlled levels of blood group A antigens (generic A due to its trisaccharide structure) to group O or B erythrocytes.	1 mg
F9557	**FSL-B(tri)** FSL-B(GALa3[Fa2]GALb)-SA1-L1	Blood group B trisaccharide Galα3(Fucα2)Galβ	Used to attach reproducible and controlled levels of blood group B antigens (generic B due to its trisaccharide structure) to group O or A erythrocytes.	1 mg
F9432	**FSL-Galili(tri)** FSL-GALILI(GALa3GALb4GLCNb)-SA1-L1	Galili trisaccharide Galα3Galβ4GlcNAcβ; ubiquitous animal antigen	Used to modify Galili negative red cells to create kodecytes that can quantitate antibodies directed against the Galili antigen. These Galili-kodecytes will react with almost all human serum; the degree of reactivity is determined by the level of antibody present and level of FSL antigen inserted.	1 mg
F9807	**FSL-GB3** FSL-GB3(GALa4GALb4GLCb)-SA1-L1	Blood group GB3 trisaccharide Galα4Galβ4Glcβ (also known as Pk)	Used to create live cells having GB3 antigens but can also be used to modify other hydrophobic surfaces including fixed cells and solid phase surfaces.	1 mg
F9682	**FSL-Le^a(tri)** FSL-LEA(GALb3[Fa4]GLCNb)-SA1-L1	Lewis blood group Le^a trisaccharide Galβ3(Fucα4)GlcNAcβ	Used to attach reproducible and controlled levels of blood group Le^a antigens to erythrocytes. These kodecytes will react with most anti-Le^a blood grouping reagents including polyclonal reagents.	1 mg
F9182	**FSL-biotin** FSL-CONJ(1Biotin)-SC2-L1	Biotin (vitamin B7)	Used to create biotinylated live cells (kodecytes) but can also be used to modify other hydrophobic surfaces including fixed cells and solid phase surfaces.	1 mg

References

(1) Henry, S.M., Modification of red blood cells for laboratory quality control use. *Curr. Opin. Hematol.* **16**, 467–72 (2009).

(2) Frame, T., et al., Synthetic glycolipid modification of red blood cell membranes. *Transfusion* **47**, 876–82 (2007).

KODE Constructs are supplied by KODE Biotech Materials Limited under license from KODE Biotech Limited.

Downstream Analysis

Initial detection of glycoproteins *in vitro* is routinely accomplished on SDS-PAGE gels and Western blots. Cellular localization of glycoproteins is normally accomplished utilizing lectin fluorescent conjugates. Sigma's glycobiology portfolio includes:

- Fluorescent stains
- Colorimetric stains
- Post-translational modified protein markers
- Lectins conjugated with FITC, TRITC, biotin, or Atto fluorescent dyes
- Biotin labeling of glycoproteins

For information on our products and fluorescent applications, visit
sigma.com/fluorescence

Quantification

In contrast to proteins and peptides, glycans do not absorb ultraviolet (UV) light strongly, thereby giving a weak detector signal. Furthermore, as glycans with various different structures may be present in minute amounts, their detection via UV may not be practical. As a result, a wide range of alternative techniques have been developed for detection and subsequent quantification.

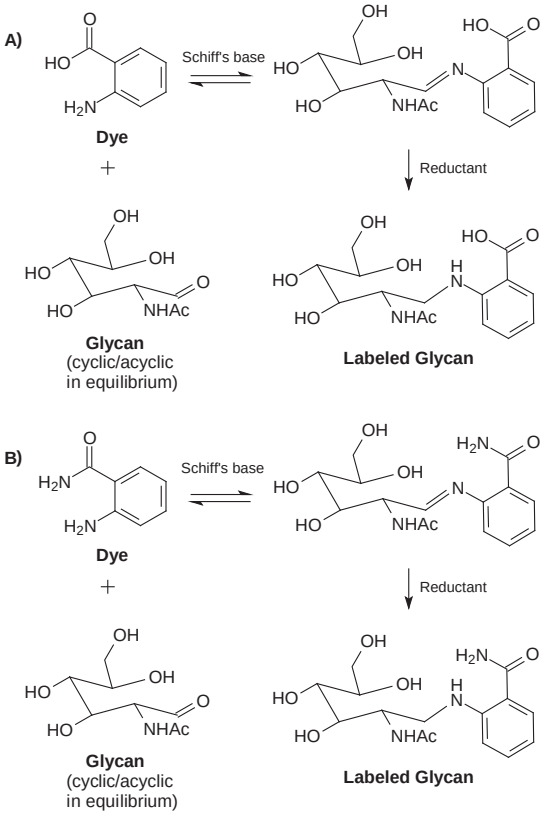


Figure 7. Acyclic glycan and dye form a Schiff's base. Subsequent reduction of the imine with sodium cyanoborohydride results in a stable labeled glycan. (A) 2-AA fluorophore (B) 2-AB fluorophore.

GlycoProfile Labeling Kits

Designed for efficient labeling of *N*-linked, *O*-linked, and glycosylphosphatidylinositol (GPI)-anchored glycans with either 2-aminobenzamide (2-AB) or 2-aminobenzoic acid (anthranilic acid; 2-AA) (see **Figure 7**). These small fluorophores increase the spectral absorption of glycans, improving the detection of labeled glycans by HPAE (high-performance anion exchange chromatography), HPLC, and ESI-MS (electrospray mass spectroscopy) methods. Binding is robust and the resulting dye-glycan conjugates are stable, with no degradation during post-labeling analysis.

While 2-AB is slightly less sensitive than 2-AA, it is suitable for downstream glycan analysis by HPAE, HPLC, and ESI-MS. Although suitable for the same applications, 2-AA is better suited for SDS-PAGE.

GlycoProfile 2-AA Labeling Kit

Cat. No.	Description	Size
PP0530-1KT	Components 2-AA (Anthranilic acid) 2 × 6 mg DMSO 2 × 350 µl Acetic acid, glacial 2 × 200 µl Reductant (sodium cyanoborohydride) 2 × 6 mg	1 kit

GlycoProfile 2-AB Labeling Kit

Cat. No.	Description	Size
PP0520-1KT	Components 2-AB (2-Aminobenzamide) 2 × 5 mg DMSO 2 × 350 µl Acetic acid, glacial 2 × 200 µl Reductant (sodium cyanoborohydride) 2 × 6 mg	1 kit

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

Identification by Mass Spectrometry

Mass spectrometry (MS) is widely used in glycosylated protein analysis due to its high selectivity, sensitivity, and ability to analyze complex mixtures rapidly. The use of matrices is necessary for MS analysis of glycans. Different approaches and matrices are used for the analysis of neutral and acidic glycans by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS.

Matrices for Mass Spectrometry Analysis of Glycans

Cat. No.	Name	Purity	Application
08658	8-Aminonaphthalene-1,3,6-trisulfonic acid disodium salt (ANTS)	≥90%, CE	Fluorescent label for saccharides and glycoproteins used for oligosaccharide sequencing.
07336	3-Aminoquinoline (3-AQ)	≥99.0%, GC	Fluorescently labels glycans containing a free reducing terminus. Forms a liquid composite matrix with 4-HCCA and glycerol for analysis of neutral and acidic glycans.
A89804	Anthranilamide (2-AB)	≥98%	Fluorescently labels glycans containing a free reducing terminus.
10678	Anthranilic acid (2-AA)	≥99.0%, T	Fluorescently labels glycans containing a free reducing terminus.
82393	6-Aza-2-thiothymine	≥99.0%, HPLC	Used in MALDI analysis of acidic glycans in negative ion mode.
C8982	α-Cyano-4-hydroxycinnamic acid (4-HCCA; α -CCA) Suitable for MALDI-TOF MS	—	Specially purified and qualified as a matrix for peptide MALDI-MS calibration standards.
37468	2′,6′-Dihydroxyacetophenone	≥99.5%, HPLC	Used with diammonium hydrogen citrate for MALDI-MS of PMP-labeled acidic and neutral glycans.
85707	2,5-Dihydroxybenzoic acid (DHB)	>99.0%, HPLC	Most commonly used matrix for carbohydrates. For MALDI-MS of free neutral glycans. Produces $[M+Na]^+$ ion; may show a weaker $[M+K]^+$ ion. Other species may be generated by the addition of the appropriate inorganic salt.
49771	Glycerol	≥99.0%, GC	Forms a liquid composite matrix with 4-HCCA and 3-aminoquinoline for analysis of neutral and acidic glycans. Glycerol has also been used as a matrix for fast atom bombardment MS.
55547	2-Hydroxy-5-methoxybenzoic acid	≥98.0%, HPLC	Used in combination with DHB to form a "super DHB" with higher sensitivity. For MALDI-MS of free neutral glycans.
55433	1-Isoquinolinol	≥99.0%, HPLC	Used as co-matrix with DHB for analysis of oligosaccharides. Tolerant of buffers, salts, and sodium dodecyl sulfate (SDS).
N6252	Norharmane	—	Used as matrix for analysis of cyclodextrins and for sulfated oligosaccharides in combination with DHB as co-matrix.
85590	Spermine	≥99.0%, GC	Used as co-matrix with DHB for MALDI-MS of sialylated glycans in negative ion mode.
88639	1-Thioglycerol	≥98.5%, GC	Used as a matrix for fast atom bombardment MS.
91928	2′,4′,6′-Trihydroxyacetophenone monohydrate	≥99.5%, HPLC	Used in MALDI analysis of acidic glycans and glycopeptides in negative ion mode. Lower limit of detection than 6-aza-2-thiothimidine.

Glycan Synthesis and Modification

The use of glycosyltransferases is a viable alternative to chemical synthesis in the preparation of oligosaccharides. Enzymatic glycosylation complements techniques for chemical synthesis of carbohydrates and glycoconjugates (see **Figure 8**). Glycosyltransferases are highly regioselective and stereospecific with respect to the glycosidic linkages formed.

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 signaling processes.

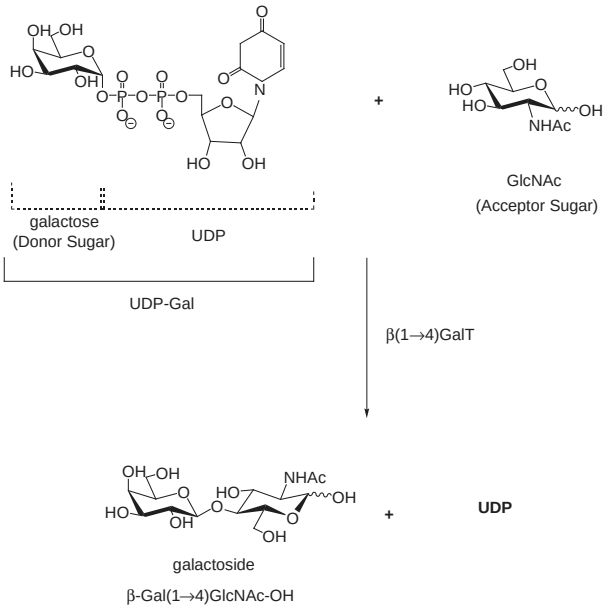


Figure 8. Enzymatic-catalyzed glycosylation using β(1→4)Galactosyltransferase [β(1→4)GalT] and UDP-Gal as donor substrate.

Glycosyltransferase Enzymes

Cat. No.	Name	Unit Definition	Size
C1999	CMP-Sialic Acid Synthetase from *Neisseria meningitidis group B*	One unit will catalyze the formation of 1 µmol CMP-Neu-5-Ac from Neu-5-Ac and CTP per minute at 37 °C at pH 8.5.	10 units
G5507	Galactosyltransferase from bovine milk (UDP-galactose:D-glucose; 4-β-galactosyltransferase; Lactose synthase)	One unit will transfer 1.0 µmole of galactose from UDP-galactose to D-glucose per min at pH 8.4 at 30 °C in the presence of 0.2 mg α-lactalbumin per mL of reaction mixture. (without added α-lactalbumin the activity is zero)	1 unit 5 units 10 units 25 units
48279	β-1,4-Galactosyl Transferase from bovine milk (Lactose synthase)	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
90261	β-1,4-Galactosyl Transferase I human, recombinant from *Saccharomyces cerevisiae*	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
44498	β-1,4-Galactosyl Transferase I human, recombinant from *Saccharomyces cerevisiae*	One unit corresponds to the amount of enzyme which transfers 1 µmol galactose from UDP-galactose to *N*-acetyl-D-glucosamine per minute at pH 7.4 and 37 °C in the presence of α-lactalbumin	1 mL
S1951	α-2,3-Sialyltransferase from *Pasteurella multocida*	One unit will catalyze the formation of 1.0 µmol Neu-5-Ac-α-2,3-LacMU from CMP-Neu-5-Ac and Lac-β–OMU per minute at 37 °C at pH 8.0	1 unit
S2076	α-2,6-Sialyltransferase from *Photobacterium damsela*	One unit will catalyze the formation of 1 µmol Neu-5-Ac-α-2,6-LacMU from CMP-Neu-5-Ac and Lac-β–OMU per minute at 37 °C at pH 8.0	1 unit

Nucleoside Phosphate Glycosyl Donor Substrates

Cat. No.	Name	Assay	Size	
C8271	**Cytidine-5′-monophospho-*N*-acetylneuraminic acid sodium salt** CMP-NANA; CMP-Sialic acid; CMP-NAN; CMP-Neu5Ac	≥85%, HPLC	1 mg 5 mg 25 mg	
G4401	**Guanosine 5′-diphospho-β-L-fucose sodium salt** 6-Deoxy-β-L-galactopyranosylguanosine 5′-diphosphate; GDP-Fuc; GDP-fucose	≥85%	1 mg 2 mg 5 mg	
G7502	**Guanosine 5′-diphosphoglucose sodium salt** GDPG; GDP-Glucose; GDP-Glc	–	10 mg 25 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** UDPAG; UDP-GlcNAc; UDP-*N*-acetylglucosamine	≥98%	25 mg 100 mg 500 mg 1 g	
U4500	**Uridine 5′-diphosphogalactose disodium salt** UDP-Gal; UDP-galactose	≥97.0%	5 mg 10 mg 25 mg 100 mg	
U4625	**Uridine 5′-diphosphoglucose disodium salt from *Saccharomyces cerevisiae*** UDPG; UDP-Glc	≥98%	10 mg 25 mg 100 mg	500 mg 1 g 5 g
U5625-	**Uridine 5′-diphosphoglucuronic acid triammonium salt** UDP-GlcA; UDPGA; Uridine-diphosphate-glucuronic acid	98-100%	100 mg 250 mg	500 mg 1 g
U6751	**Uridine 5′-diphosphoglucuronic acid trisodium salt** UDP-GlcA; UDPGA; Uridine-diphosphate-glucuronic acid	98-100%	25 mg 100 mg 250 mg	500 mg 1 g

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