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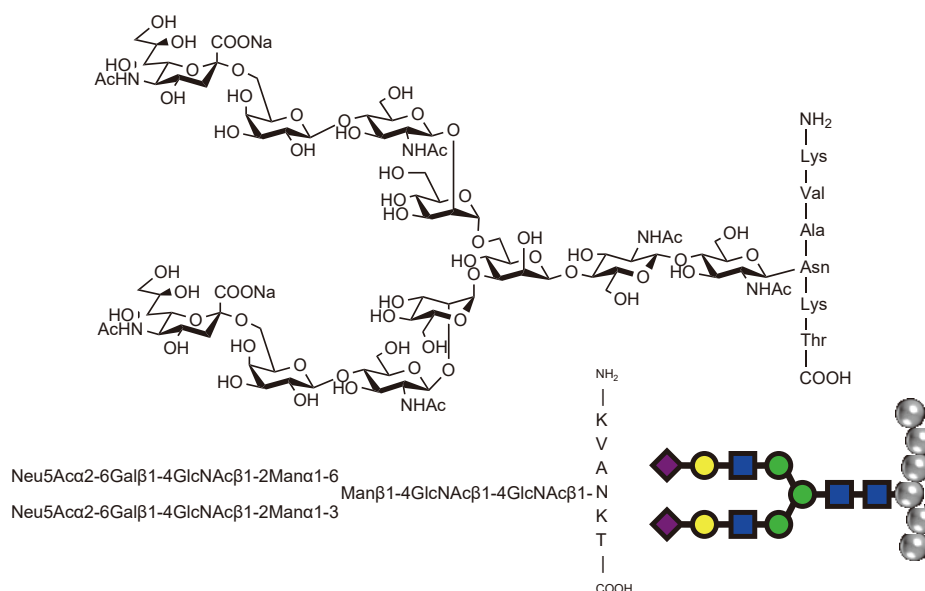
Sialylglycopeptide (SGP) and Disialyloctasaccharide

Structure-defined human-type *N*-glycans indispensable for glycoscience are available at affordable prices.

Sialylglycopeptide (SGP)

Sialylglycopeptide (= SGP)

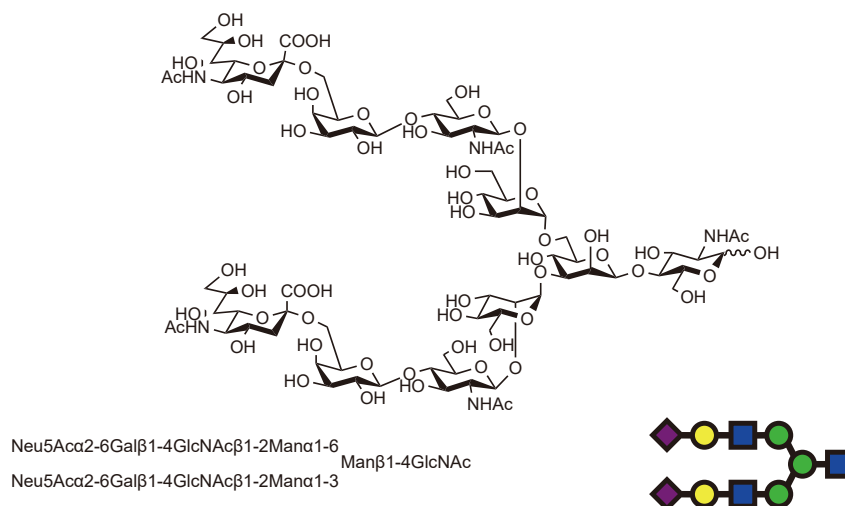
10mg [S0523]



Disialyloctasaccharide

Disialyloctasaccharide

10mg [D4065]



Structure-defined Sialylglycopeptide (SGP) and Disialyloctasaccharide can be offered at high purity (HPLC >95%).

Please contact us about a request of several hundred grams-scale production.

Practical example: Oligosaccharide remodeling of *N*-glycan on antibodies with 2 types of oligosaccharide donors and Endo-M related enzymes

A. Structure-defined oligosaccharide donor

By chemoenzymatic glycoengineering with Endo-M and Glycosynthase, heterogenous *N*-linked oligosaccharides attached to an antibody are replaced by a fine-defined oligosaccharide with focused substrate specificity toward non-corefucosylated biantennary *N*-glycan.^{Ref.7}

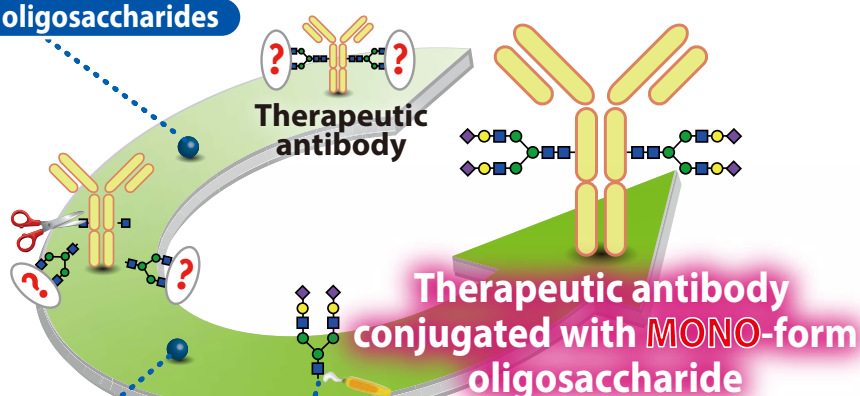
First, Endo-M hydrolyses a glycoside-bond of chitobiose included in *N*-glycan of IgG via an oxazoline intermediate, which subsequently exposes the innermost GlcNAc residue. Next, Glycosynthase bearing a point-mutation on Endo-M performs transglycosylation targeting the GlcNAc residue. The oligosaccharide donors, not only the activated Disialyloctasaccharide with oxazoline formation^{Ref.8} but also the Sialylglycopeptide (SGP), are available to the oligosaccharide remodeling.^{Ref.9}

(The reaction for oligosaccharide remodeling toward IgG with Endo-M and Glycosynthase is conducted under non-reducing conditions, whereas the analysis is performed under reducing conditions with denaturing.)

Endo-M (= *endo*- β -*N*-Acetylglucosaminidase)
[A1651]

? : Multi-form oligosaccharides

Cleavage of multi-form oligosaccharides



Coupling of MONO-form oligosaccharide

Glycosynthase (= Endo-M-N175Q)
[G0365]

Disialyloctasaccharide
[D4065]

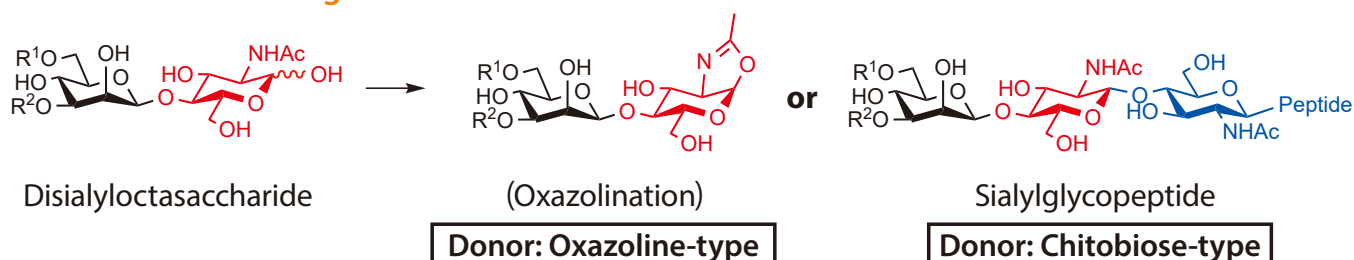
or

Sialylglycopeptide (= SGP)
[S0523]

Activation is required to form the oxazoline intermediate.

This donor is available as is.

Structures of reducing terminals



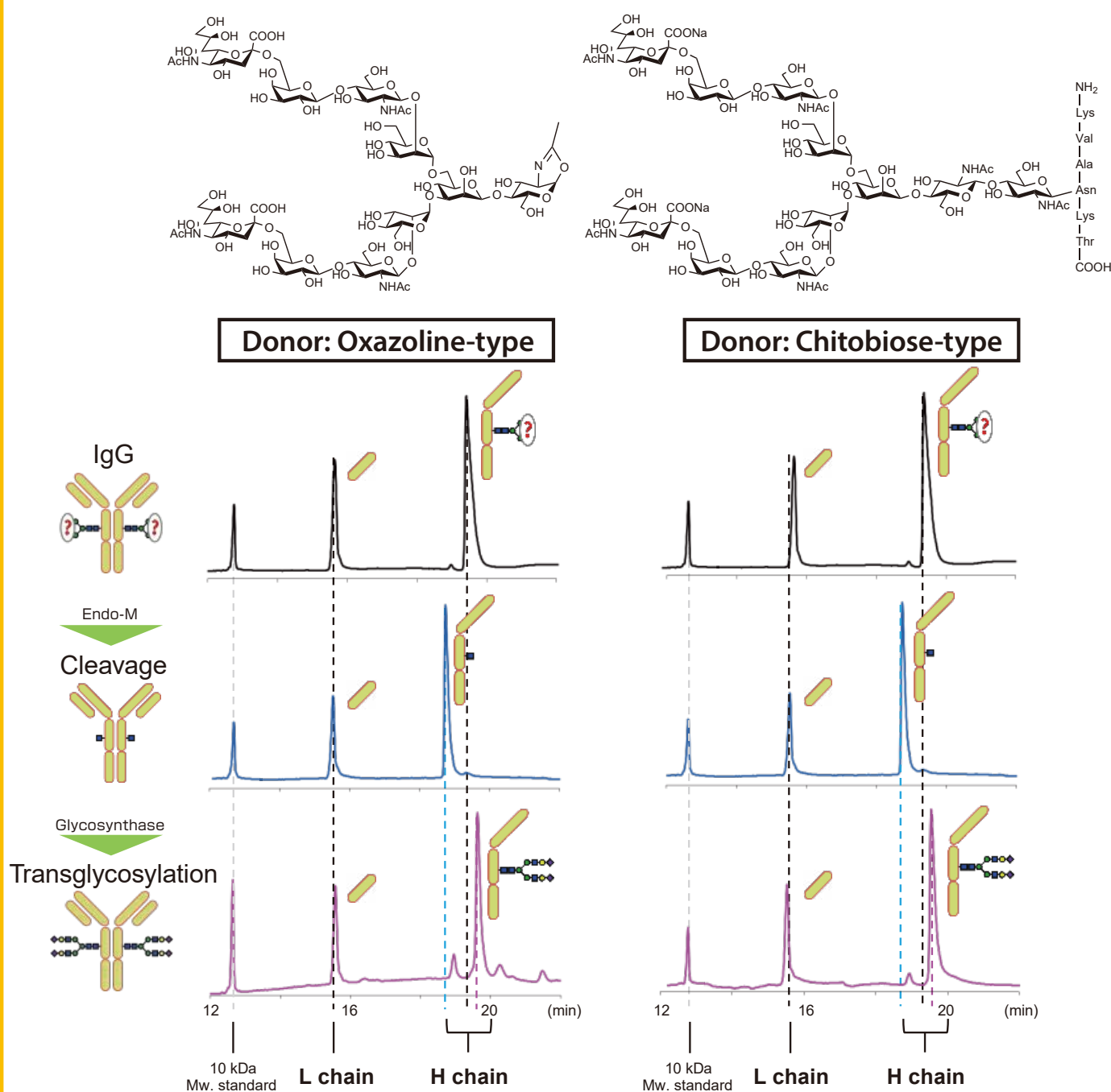
B. Verification of oligosaccharide replacement with a non-fucosylated *N*-glycan attached to a heavy chain of antibody via capillary electrophoresis

Following the hydrolysis reaction for an *N*-glycan attached antibody (IgG) by Endo-M, the size reduction of the heavy chain can be verified via capillary electrophoresis (Blue line).

Next, the coupling between two types of sialylated oligosaccharide donors and a GlcNAc residue remaining on the antibody is conducted by Glycosynthase. Using an activated oxazoline donor derived from an oxazolinated Disialyloctasaccharide, would result in high reactivity. However, non-specific additional incorporation of an oxazoline-activated Disialyloctasaccharide onto any amino acid residue can be observed (Red line left).

Alternatively, the non-specific incorporation of oligosaccharides is not found with SGP donors (Red line right).

Finally, after the coupling of a uniform oligosaccharide to the GlcNAc-exposed antibody, a peak shift in the heavy chain is observed whereas it is not for the light chain.

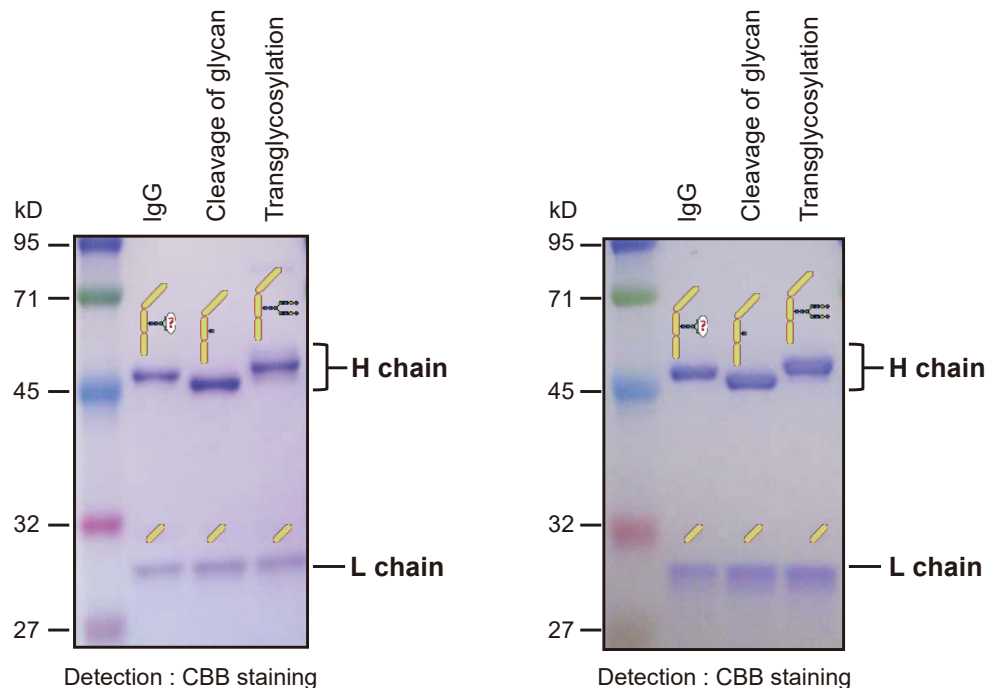


Capillary electrophoresis (under reducing condition)
[BECKMAN COULTER PA 800]
Detection : UV 214 nm

Sialylglycopeptide (SGP) and Disialyloctasaccharide

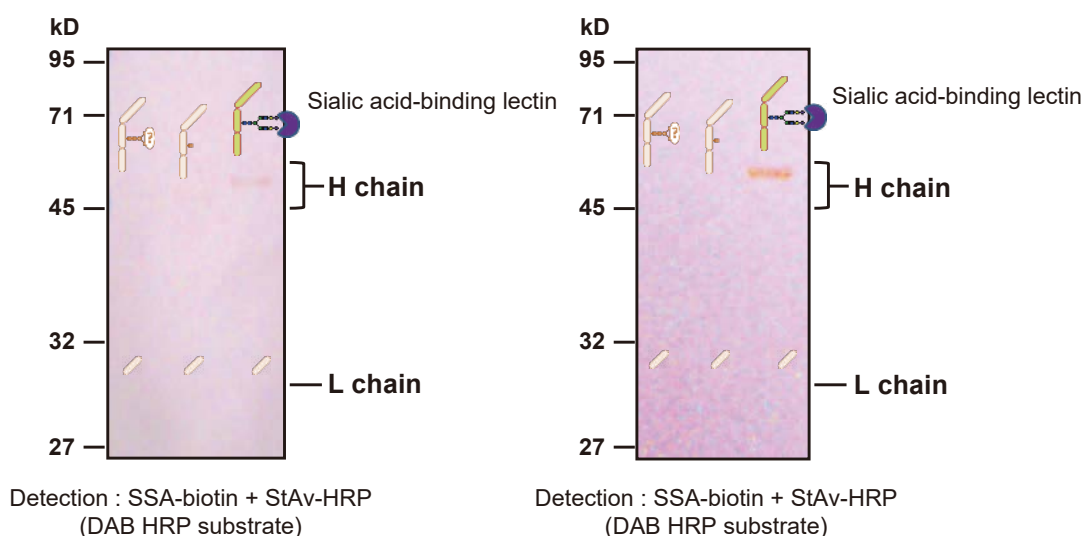
C. Verification of the heavy chain size by SDS-PAGE

Size reduction of the heavy chain is observed when an *N*-glycan attached to the heavy chain is hydrolyzed by Endo-M treatment. With regards to transglycosylation by Glycosynthase, two types of sialylated donors resulted in a larger molecular size compared with original IgG from capillary electrophoresis. A peak shift of the heavy chain is observed but not the light chain.



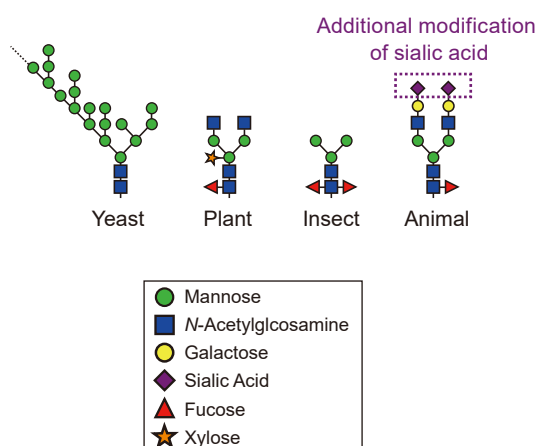
D. Detecting incorporation of sialylated oligosaccharides into Endo-M-treated antibody by lectin-blotting

Incorporation of the sialylated oligosaccharide is validated by lectin-blotting with a sialic acid-binding lectin (SSA: *Sambucus sieboldiana* agglutinin). Only the heavy chain of the transglycosylated antibody appears to exhibit susceptibility to SSA.



Biosynthesis and *N*-glycan

Oligosaccharide attachment during post-translational modification in protein biosynthesis is a ubiquitous biological process. While *N*-linked oligosaccharides are found in various eukaryotes, their oligosaccharide structures are of great diversity and heterogeneity between different organisms.^{Ref.1} Unfortunately, this heterogeneity is difficult to replicate and heterogeneity for (for example) biotechnology-based medical remains a challenging problem to resolve.



Function of human-type *N*-glycan

Sialylated *N*-glycan is a typical human-type glycan that is suggested to be strongly correlated with various physiological phenomena:

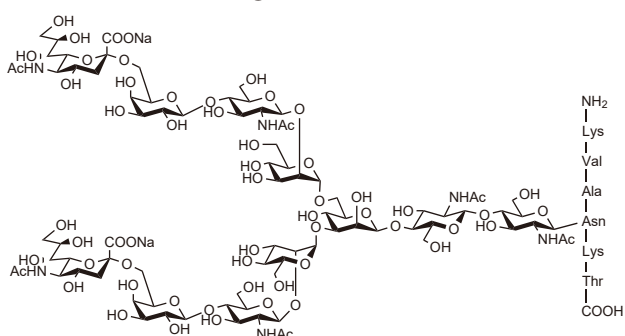
- Viral infection (by human influenzae, etc.)^{Ref.2}
- Delayed blood glycoprotein half-life (owing to hindered clearance by the hepatic asialoglycoprotein receptor)^{Ref.3}
- Anti-inflammatory activity with sialylated *N*-glycan of the IgG Fc region (via endogenous immune-receptors such as DC-SIGN, etc.)^{Ref.4}
- Immunoregulation caused by endogenous lectins (such as Siglecs which preferentially bind to sialylated oligosaccharides)^{Ref.5}
- Relevance to stability of biopharmaceuticals (e.g. erythropoietin: EPO)^{Ref.6}

Sialylated *N*-glycan may contribute to various applications:

- Improvement of biopharmaceutical function (via oligosaccharide remodeling)
- Virus scavenger (based on a matrix of conjugated oligosaccharides)
- Drug delivery systems (as an oligosaccharide-immobilized DDS)
- Oligosaccharide analysis of biological specimens and biopharmaceuticals (internal or external standard)
- Stabilization of proteins (improved solubility)
- General Glycoscience (glycosidase, competitive inhibitors and etc.)

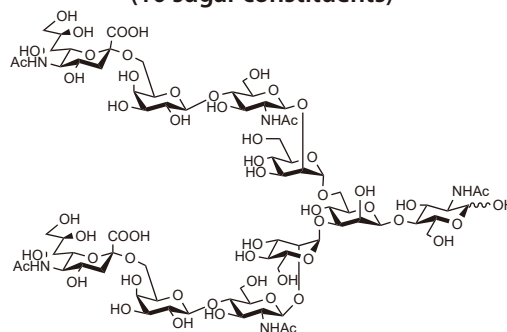
Sialylglycopeptide (SGP)

Glycopeptide containing sialic acids at non-reducing terminal (11 sugar constituents)



Disialyloctasaccharide

Oligosaccharide containing sialic acids at non-reducing terminal (10 sugar constituents)



- References**
- 1) A. Loos, H. Steinkellner, *Arch. Biochem. Biophys.* **2012**, 526, 167.
 - 2) J. E. Stencel-Baerenwald, K. Reiss, D. M. Reiter, T. Stehle, T. S. Dermody, *Nat. Rev. Microbiol.* **2014**, 12, 739.
 - 3) A. G. Morell, G. Gregoriadis, I. H. Scheinberg, J. Hickman, G. Ashwell, *J. Biol. Chem.* **1971**, 246, 461.
 - 4) Y. Kaneko, F. Nimmerjahn, J. V. Ravetch, *Science* **2006**, 313, 670.
 - 5) P. R. Crocker, J. C. Paulson, A. Varki, *Nat. Rev. Immunol.* **2007**, 7, 255.
 - 6) R. J. Darling, U. Kuchibhotla, W. Glaesner, R. Micanovic, D. R. Witcher, J. M. Beals, *Biochemistry* **2002**, 41, 14524.
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 - 8) M. Noguchi, T. Tanaka, H. Gyakushi, A. Kobayashi, S. Shoda, *J. Org. Chem.* **2009**, 74, 2210.
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Related Products

[Reagent for Oxazolation]

2-Chloro-1,3-dimethylimidazolinium Chloride

5g / 25g [C1408]

[Related Oligosaccharides]

DisialylInonasaccharide- β -pNP

1mg [N0913]

DisialylInonasaccharide- β -Ethylazide

1mg [D4217]

DNS-SGN

1mg [D3690]

Neu5Ac α (2-6) N-Glycan

1mg [N1065]

Neu5Ac α (2-6) N-Glycan 2AB

500pmol/vial [N1073]

G2-peptide

5mg [G0466]

G2 Glycan

1mg [G0487]

G2 2AB

500pmol/vial [G0493]

[Endo-M and Related-Reagents]

MANT-M3GN2-DNP (= MM3D)

1mg [M3174]

Endo-M (= endo- β -N-Acetylglucosaminidase)

from *Mucor hiemalis* expressed in *Candida boidinii*

100m units/vial [A1651]

Glycosynthase (= Endo-M-N175Q)

from *Mucor hiemalis* expressed in *Escherichia coli*

100m units/vial [G0365]

Endo-M-W251N

from *Mucor hiemalis* expressed in *Escherichia coli*

100m units/vial [E1339]

Anti-Endo-M Polyclonal Antibody

0.2mg/vial [A2958]

Anti-Endo-M Polyclonal Antibody Biotin Conjugate

0.2mg/vial [A2959]

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