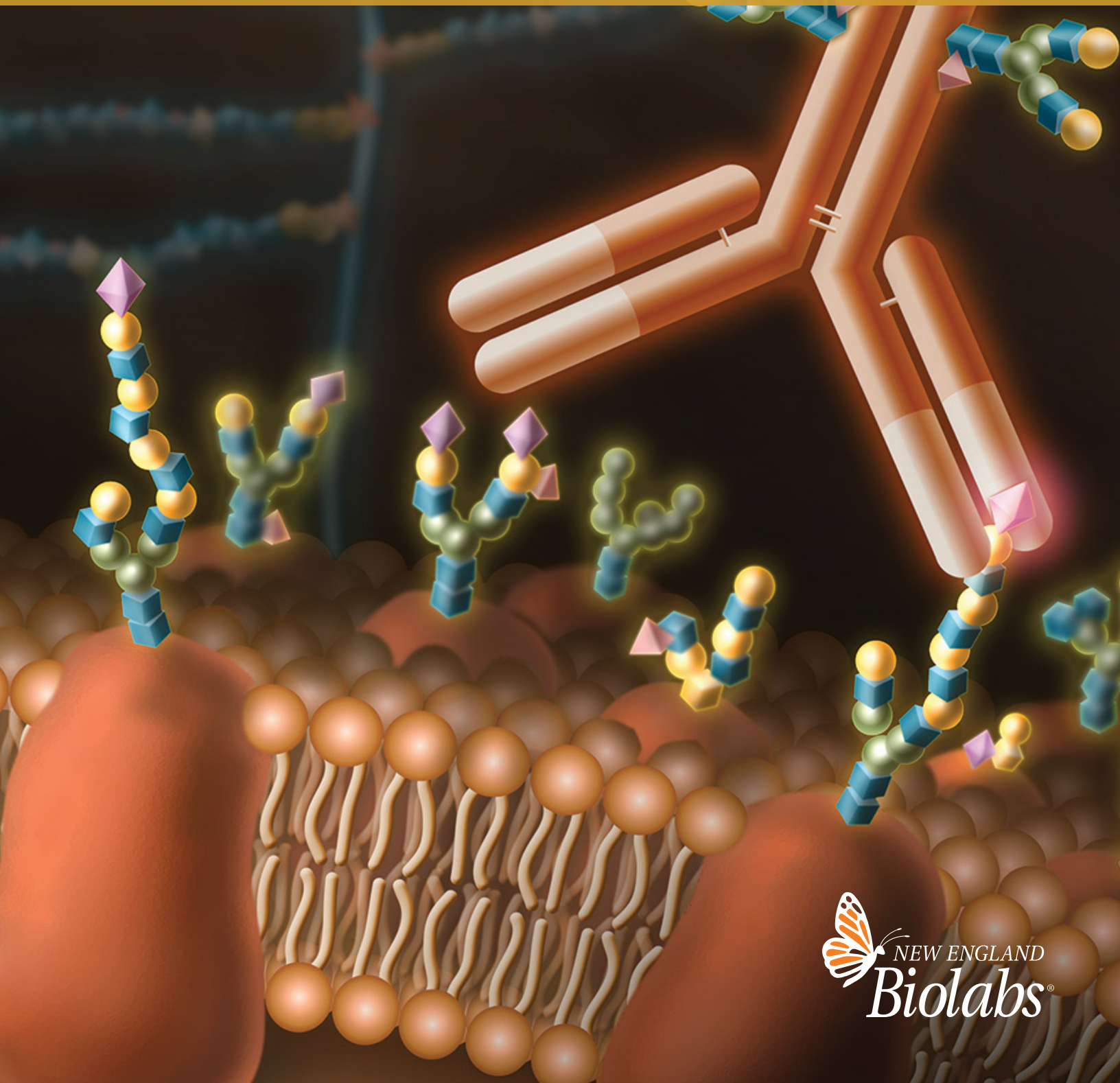
Now includes O-Glycoprotease (IMPa)

Glycoproteomics

UNDERSTANDING PROTEIN MODIFICATIONS



NEW ENGLAND Biolabs®

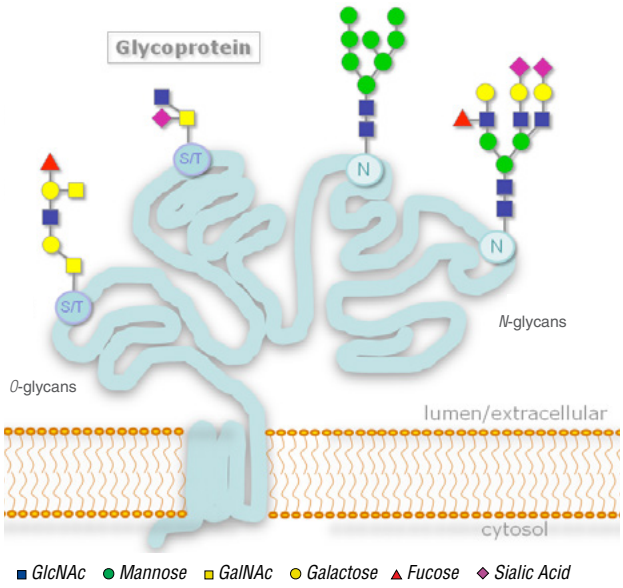
Glycoproteomics Products

New England Biolabs (NEB) offers a selection of endoglycosidases and exoglycosidases for glycobiology research. Many of these reagents are recombinant, and all undergo several quality control assays, enabling us to provide products with lower unit cost, high purity and reduced lot-to-lot variation. All of our glycosidases are tested for contaminants. Since p-nitrophenyl-glycosides are not hydrolyzed by some exoglycosidases, we use only fluorescently-labeled oligosaccharides to screen for contaminating glycosidases.

Glycobiology is the study of the structure, function and biology of carbohydrates, also called glycans, which are widely distributed in nature. It is a small but rapidly growing field in biology, with relevance to biomedicine, biotechnology and basic research. Proteomics, the systematic study of proteins in biological systems, has expanded the knowledge of protein expression, modification, interaction and function. However, in eukaryotic cells the majority of proteins are post-translationally modified (1). A common post-translational modification, essential for cell viability, is the attachment of glycans, shown in Figure 1. Glycosylation defines the adhesive properties of glycoconjugates and it is largely through glycan–protein interactions that cell–cell and cell–pathogen contacts occur, a fact that accentuates the importance of glycobiology. Glycomics, the study of glycan expression in biological systems, relies on effective enzymatic and analytical techniques for correlation of glycan structure with function.

NEB offers a suite of endoglycosidases and exoglycosidases to study glycosylation modifications. Visit **www.NEBglycosidase.com** for the latest list of enzymes and reagents available from NEB.

Figure 1: *N*- and *O*-Glycosylation



N-linked glycosylation occurs through the asparagine residues of the protein, while O-linked glycosylation occurs through serine or threonine.

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14 β1–3,4 Galactosidase
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18 Endoproteinase LysC
18 Endoproteinase AspN
18 Endoproteinase GluC

19 Ordering Information

Reference: 1. Spiro, R.G. (2002) *Glycobiology* 12, 43R–56R.

Deglycosylation Enzymes

Several classes of glycans exist, including *N*-linked glycans, *O*-linked glycans, glycolipids, *O*-GlcNAc, and glycosaminoglycans. *N*-linked glycosylation occurs when glycans are attached to asparagine residues on the protein. *O*-linked glycans are most commonly attached to serine or threonine residues through the *N*-Acetylgalactosamine residue. Removal of oligosaccharides from glycoproteins, termed deglycosylation, is often used to simplify analysis of the peptide and/or glycan portion of a glycoprotein. Detailed knowledge of the glycan structures helps to correlate them to their respective function. To do this, tools are required for highly sensitive analysis of glycan chains. Both chemical and enzymatic methods exist for removing oligosaccharides from glycoproteins. However, chemical methods such as β-elimination with mild alkali (1) or mild hydrazinolysis (2) can be harsh and results in the degradation of the protein; whereas enzymatic methods are much gentler and can provide complete sugar removal with no protein degradation.

ADVANTAGES

- Low cost
- Easy Reaction Setup
- Digestion with a combination of enzymes can yield structural information
- Can be used under native and denaturing conditions

Endoglycosidase Selection Chart

	PROTEIN DEGLYCOSYLATION MIX II (#P6044)	*O*-GLYCOSIDASE (#P0733 & #E0540)	PNGASE A (#P0707)	PNGASE F (#P0704 & #P0705)	REMOVE-IT PNGASE F (#P0706)	RECOMBINANT PNGASE F (#P0708 & #P0709)	RAPID PNGASE F (#P0710)	RAPID PNGASE F, (NON-REDUCING FORMAT) (#P0711)	ENDO H (#P0702) & ENDO H_F (#P0703)	ENDO S (#P0741)	ENDO D (#P0742)	ENDO F2 (#P0772)	ENDO F3 (#P0771)
Deglycosylation of glycoproteins (*N*- and *O*-glycans)	●												
Removal of *O*-glycans	●	●											
Removal of *N*-glycans from glycoproteins	●		●	●	●	●	●	●	●	●	●	●	●
Removal of high mannose and hybrid *N*-glycans (leaving a GlcNAc attached to Asn)									●				
Optional removal of the enzyme from the reaction					●					●	●	●	●
Removal of paucimannose *N*-glycans (GlcNAc attached to Asn)						●			●		●		
Removal of *N*-glycans from IgGs (leaving a GlcNAc attached to Asn)									●	●			
Analysis of therapeutic glycoproteins, compliance with regulatory agencies						●	●	●					
High throughput *N*-glycan analysis of monoclonal antibodies, regulatory compliance							●	●					
Glycomics				●	●	●	●	●	●	●	●	●	●
Proteomics				● (only GF)	●	● (only GF)	●	●	●	●	●	●	●
Determine *N*-glycan sites									●	●	●	●	●
Removal of *N*-glycans from plant and insect glycoproteins			●										

GF = Glycerol Free

References
1. Kakehi, K. et al. (1994) *J. Chromatogr. A.* 680, 209–215.
2. Royle, L. et al. (2002) *Anal. Biochem.* 304, 70–90.

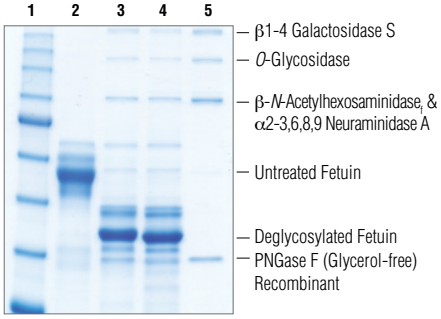
Protein Deglycosylation Mix II

Deglycosylation of a glycoprotein often requires more than one enzyme to completely remove all carbohydrate residues. PNGase F removes almost all *N*-linked oligosaccharides, while monosaccharides on *O*-linked glycans must be removed by a series of exoglycosidases, such as β1-4 Galactosidase S and β-*N*-Acetylhexosaminidase$_F$, until only the Galβ1-3GalNAc (core 1) and/or the GlcNAcβ1-3GalNAc (core 3) cores remain attached to the core protein. *O*-Glycosidase can then remove these core structures leaving serine or threonine residues intact. Sialic acid residues, which will block the action of the *O*-Glycosidase, are easily removed by NEB's general α2-3,6,8,9 Neuraminidase A.

The Protein Deglycosylation Mix II contains all of the enzymes, reagents, and controls needed to remove all *N*-linked and simple *O*-linked glycans as well as some complex *O*-linked glycans. This mix contains enzyme sufficient for 20 reactions or the cleavage of as much as 2 mg of glycoprotein. The Protein Deglycosylation Enzyme Mix II (100 µl) is a single mix made up of five recombinant enzymes: PNGase F Glycerol Free Recombinant, *O*-Glycosidase, α2–3,6,8,9 Neuraminidase A, β1-4 Galactosidase S, and β-*N*-Acetylhexosaminidase$_F$. The mix is supplied with all of the reagents and controls required to complete the experiment under either non-denaturing (native) or reducing conditions. Components include 10X Deglycosylation Mix Buffer 1, 10X Deglycosylation Mix Buffer 2, and a Fetuin control containing sialylated *N*-linked and *O*-linked glycans. All of the enzymes and reagents included in the Protein Deglycosyation Mix II are Mass Spectrometry compatible. Following the deglycosylation reaction, samples are ready to be prepared for mass spectrometry analysis.

Protein Deglycosylation Mix II P6044S

Enzymatic Deglycosylation of Bovine Fetuin



Enzymatic Deglycosylation of Bovine Fetuin under both native (10X Deglycosylation Mix Buffer 1) and reducing (10X Deglycosylation Mix Buffer 2) conditions. 20 µg reactions were loaded onto a 10-20% Tris-glycine SDS-PAGE gel.

Lane 1: Color Prestained Protein Standard, Broad Range (11-245 kDa) (NEB #P7712)

Lane 2: 20 µg untreated Fetuin control

Lane 3: 20 µg Fetuin deglycosylated under native conditions with Deglycosylation Mix Buffer 1

Lane 4: 20 µg Fetuin deglycosylated under reducing conditions with Deglycosylation Mix Buffer 2

Lane 5: 5 µl Protein Deglycosylation Mix II

N-Linked Deglycosylation Enzymes

For structural analysis of asparagine-linked carbohydrates (*N*-linked glycans), sugars are released from the protein backbone by enzymes such as PNGase F, PNGase A, Endoglycosidase S, Endoglycosidase D and Endoglycosidase H.

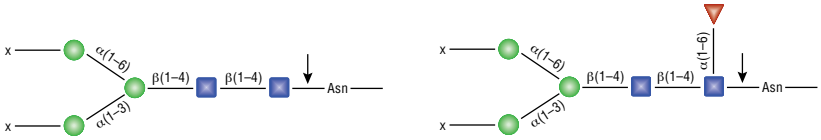
PNGase F (native and recombinant)

Peptide-*N*-Glycosidase F, also known as PNGase F, is an amidase which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid and complex oligosaccharides from *N*-linked glycoproteins. PNGase F from NEB is purified from Flavobacterium meningosepticum. A glycerol-free version of PNGase F is also offered for HPLC methods.

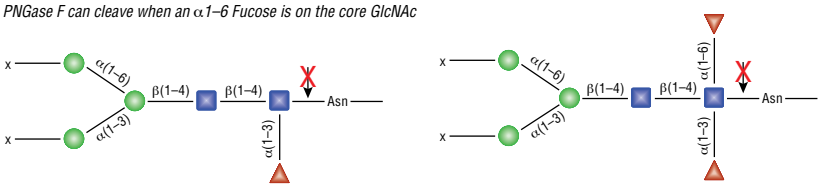
Detailed Specificity:

PNGase F hydrolyzes nearly all types of *N*-glycan chains from glycopeptides/proteins. PNGase F can cleave when an α1–6 Fucose is on the core GlcNAc. PNGase F cannot cleave when an α1–3 Fucose is on the core GlcNAc.

PNGase F P0704S/L
PNGase F, Recombinant P0708S/L
PNGase F (Glycerol-free) P0705S/L
PNGase F (Glycerol-free), Recombinant P0709S/L



PNGase F hydrolyzes nearly all types of N-glycan chains from glycopeptides/proteins. [x = H or oligosaccharide].
PNGase F can cleave when an α1–6 Fucose is on the core GlcNAc



PNGase F cannot cleave when an α1–3 Fucose is on the core GlcNAc

Gal Glc Man GalNAc GlcNAc Fuc NeuAC

References
1. Maley, F. et al. (1989) *Anal. Biochem.,* 180, 195–204.
2. Tretter, V. et al. (1991) *Eur. J. Biochem.* 199, 647–652.

Rapid PNGase F

A growing number of antibodies and antibody fusions are currently used as therapeutic agents. A conserved *N*-glycan at Asn297 of the Fc region of IgG is critical for functional activity. Moreover, some antibodies have additional *N*-glycans that, together with the conserved site, affect recognition, half-life, and immune reactions. Antibody glycosylation is heterogeneous, and variables in cell culture can increase glycan diversity. Monitoring glycosylation during production is essential to obtain the correct glycoprotein forms.

Obtaining an accurate *N*-glycan profile in the shortest time possible is essential for effective process control. Typically, enzymatic release of antibody *N*-glycans using PNGase F requires an incubation time of several hours, followed by glycan derivatization and analysis by liquid chromatography and/or mass spectrometry. In addition, incomplete deglycosylation can lead to biased results. Some glycans are easier to remove than others and unless deglycosylation is extensive, the profile obtained will not represent the correct composition of the therapeutic antibody.

ADVANTAGES

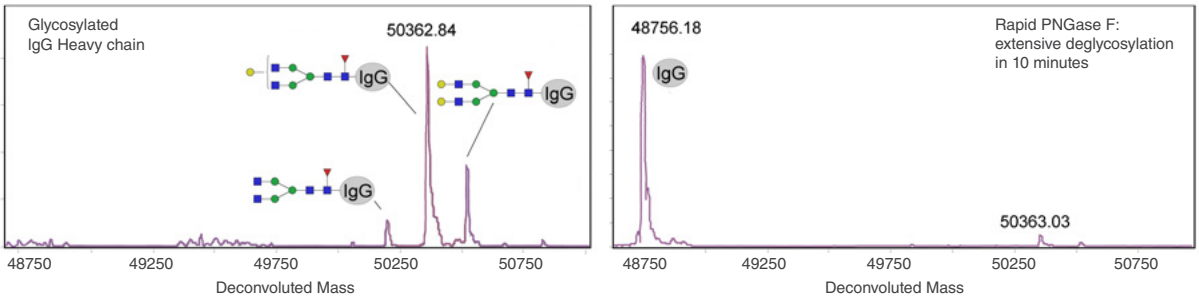
- Complete deglycosylation of antibodies and immunoglobulin fusion proteins in minutes
- Release of all *N*-glycans rapidly and without bias, ready for downstream chromatography or mass spectrometry analysis
- Optimal activity is ensured for 12 months
- Purified to > 99% homogeneity, as determined by SDS-PAGE
- Recombinant source

Rapid PNGase F

Deglycosylation in minutes for *N*-glycan analysis

Rapid PNGase F is an improved reagent that allows the complete and rapid deglycosylation of antibodies and immunoglobulin-fusion proteins in minutes. All *N*-glycans are released rapidly and without bias, and are ready to be prepared for downstream chromatography or mass spectrometry analysis. Rapid PNGase F creates an optimized workflow which reduces processing time without compromising sensitivity or reproducibility.

ESI-TOF analysis of an antibody before and after treatment with Rapid PNGase F

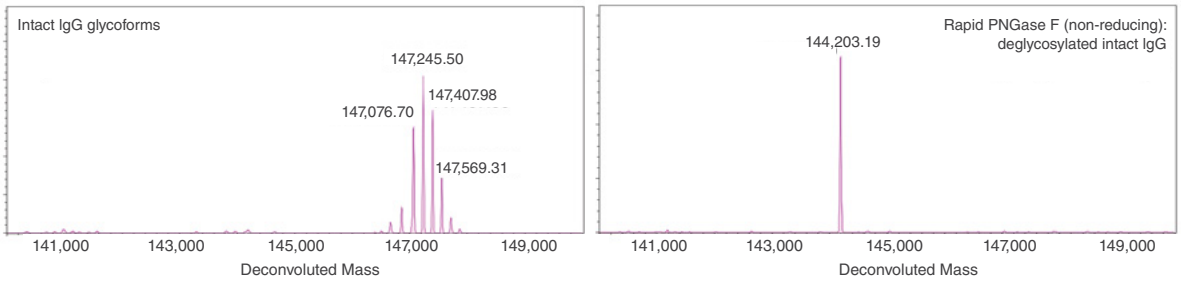


Rapid PNGase F (non-reducing format)

Deglycosylation in minutes for intact antibody analysis

Developed for proteomic applications, Rapid PNGase F (non-reducing format) is a reformulated version of Rapid PNGase F that allows the complete and rapid deglycosylation of antibodies and fusion proteins in minutes, while preserving disulfide bonds. All *N*-glycans are released rapidly and without bias, facilitating high throughput proteomics applications and methods for antibody characterization by mass spectrometry such as intact mass analysis. Rapid PNGase F (non-reducing format) combines the advantages of Rapid PNGase F (fast processing time), with the non-reducing conditions preserving quaternary structure.

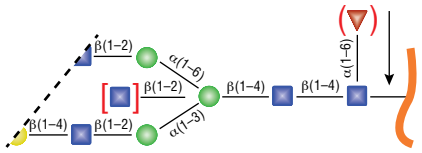
ESI-TOF analysis of an antibody before and after treatment with Rapid PNGase F (non-reducing format)



Detailed Specificity:

Rapid PNGase F & Rapid PNGase F (non-reducing format) cleave all complex, hybrid and high-mannose type glycans from antibodies and related proteins. Core α1-3 fucosylation (found in immunoglobulins expressed in plant or insect cells) is resistant to all forms of PNGase F.

Rapid PNGase F P0710S
Rapid PNGase F (non-reducing format) P0711S



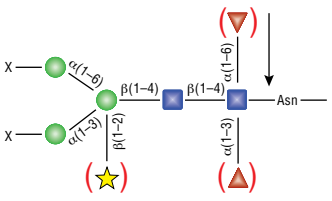
PNGase A

PNGase A is a recombinant amidase, which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and short complex oligosaccharides such as those found in plant and insect cells from *N*-linked glycoproteins and glycopeptides. PNGase A differs from PNGase F in that it cleaves *N*-linked glycans with or without α(1,3)-linked core fucose residues.

Detailed Specificity:

PNGase A hydrolyzes *N*-glycan chains from glycoproteins/peptides regardless of the presence of xylose or fucose. [x=H or Man or GlcNAc]

PNGase A P0707S/L



PNGase A hydrolyzes N-glycan chains from glycoproteins/ peptides regardless of the presence of xylose or fucose. [x = H or Man or GlcNAc].

Gal Glc Man GalNAc GlcNAc Fuc NeuAC

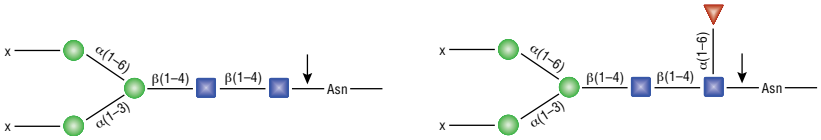
Remove-iT PNGase F

Remove-iT PNGase F is an amidase which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from *N*-linked glycoproteins. Remove-iT PNGase F is purified from *Flavobacterium meningosepticum* and is tagged with a chitin binding domain (CBD) for easy removal from a reaction. It is supplied glycerol free for optimal performance in HPLC and MS intensive methods..

Remove-iT PNGase F P0706S/L

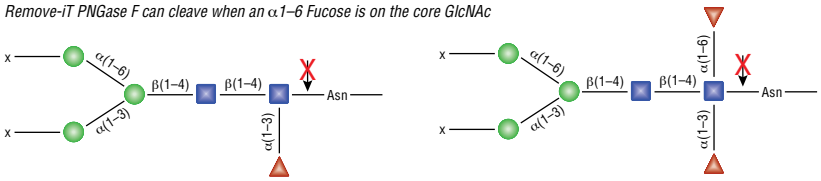
ADVANTAGES

- Chitin Binding Domain (CBD) tag ensures easy removal from a reaction
- Fast reaction setup
- Compatible with protease inhibitor cocktails
- Glycerol-free formulation for optimal performance in HPLC and mass spec analysis



Remove-iT PNGase F hydrolyzes nearly all types of N-glycan chains from glycopeptides/proteins. [x = H or oligosaccharide].

Remove-iT PNGase F can cleave when an α1–6 Fucose is on the core GlcNAc



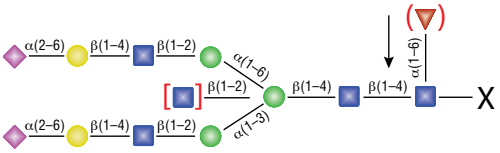
Remove-iT PNGase F cannot cleave when an α1–3 Fucose is on the core GlcNAc

Endo S

Endo S is an endoglycosidase with a uniquely high specificity for removing *N*-linked glycans from the chitobiose core of the heavy chain of native IgG. Endo S is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol free for optimal performance in HPLC and MS intensive methods.

Endo S does not have a strict peptide requirement for activity, thus the "X" can be a protein, peptide, Asparagine, or free glycan. Endo S is active on a substrate with or without core α(1-6) fucosylation as well as with or without a bisecting *N*-acetylglucosamine. Triantennary and tetrantennary sialyted or asialo glycans are not a substrate for Endo S.

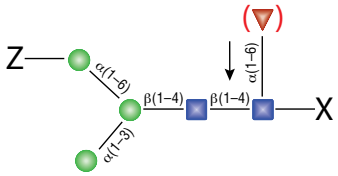
Endo S P0741S/L



Endo D

Endo D also known as Endoglycosidase D is a recombinant glycosidase, which cleaves within the chitobiose core of paucimannose *N*-linked glycans, with or without extensions in the antennae. Endo D is active on both linear and branched upper arm extensions, and is useful for determining *N*-glycosylation sites. Endo D is tagged with a chitin binding domain (CBD) for easy removal from a reaction, and is supplied glycerol-free for optimal performance in HPLC and MS intensive methods.

Endo D..... P0742S/L

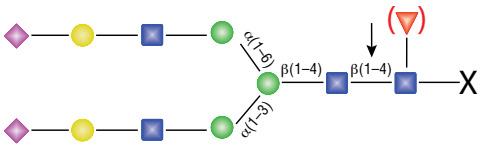


Endo D does not have a strict peptide requirement for activity, thus the "X" can be a protein, peptide or Asparagine. The upper branch, "Z", can be a "H", monosaccharide or oligosaccharide.

Endo F2

Endo F2 is a highly specific recombinant endoglycosidase which cleaves within the chitobiose core of asparagine-linked complex biantennary and high mannose oligosaccharides from glycoproteins and glycopeptides. Endo F2 cleaves biantennary glycans at a rate approximately 20 times greater than high mannose glycans. The activity of Endo F2 is identical on biantennary structures with and without core fucosylation. However, Endo F2 is not active on hybrid or tri- and tetra-antennary oligosaccharides. Endo F2 is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol free for optimal performance in HPLC and MS intensive methods.

Endo F2..... P0772S/L

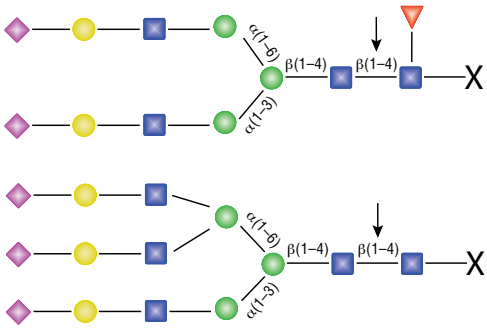


Gal Glc Man GalNAc GlcNAc Fuc NeuAC

Endo F3

Endo F3 is a highly specific recombinant endoglycosidase which cleaves within the chitobiose core of asparagine-linked fucosylated-biantennary and triantennary complex oligosaccharides from glycoproteins. Endo F3 is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol free for optimal performance in HPLC and MS intensive methods.

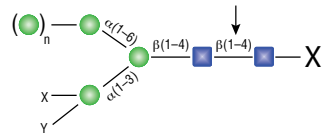
Endo F3 ..... P0771S/L



Endoglycosidase H

Endoglycosidase H (Endo H) is a recombinant glycosidase which cleaves within the chitobiose core of high mannose and some hybrid oligosaccharides from *N*-linked glycoproteins. Endo H_f is a recombinant protein fusion of Endoglycosidase H and maltose binding protein. It has identical activity to Endo H. Endo H and Endo H_f from NEB are cloned from *Streptomyces plicatus* and overexpressed in *E. coli*.

Endoglycosidase H P0702S/L
Endoglycosidase H_f P0703S/L



Endo H and Endo H_f cleave only high mannose structures (n = 2–150, x = $(Man)_{1–2}$, y = H) and hybrid structures (n = 2, x and/or y = AcNeu-Gal-GlcNAc) "X" can be a protein, peptide or Asparagine.

O-Linked Deglycosylation Enzymes

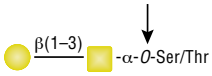
For structural analysis of serine or threonine-linked carbohydrates (*O*-linked glycans), sugars are released from the protein backbone by either chemical or enzymatic methods. Removing *O*-linked glycan chains while rendering a protein intact for further examination can be a difficult task. Chemical methods, such as β-elimination, may result in incomplete sugar removal and degradation of the protein. On the other hand, enzymatic removal of *O*-linked glycans must be performed as a series of exoglycosidase digestions until only the Galβ1-3GalNAc (core 1) and/or the GlcNAc β1-3GalNAc (core 3) cores remains attached to the serine or threonine residue. NEB's *Enterococcus faecalis* *O*-Glycosidase, also known as Endo-α-*N*-Acetylgalactosaminidase, catalyzes the removal of core 1 and core 3 disaccharide structures with no modification of the serine or threonine residues (1). Any modification of the core structures, including sialyation, will block the action of the *O*-Glycosidase. Sialic acid residues are easily removed by a general Neuraminidase. In addition, exoglycosidases such as β1-4 Galactosidase and β-*N*-Acetylglucosaminidase can be included in deglycosylation reactions to remove other complex modifications often known to be present on the core structures.

O-Glycosidase

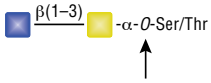
O-Glycosidase, also known as Endo-α-*N*-Acetylgalactosaminidase, catalyzes the removal of Core 1 and Core 3 *O*-linked disaccharides from glycoproteins. *O*-Glycosidase from NEB is cloned from *Enterococcus faecalis* and expressed in *E. coli.*

O-Glycosidase (Endo-α-*N*-Acetylgalactosaminidase) P0733S/L
α2-3,6,8 Neuraminidase P0720S/L
O-Glycosidase & Neuraminidase Bundle E0540S

Reference
1. Koutsioulis, D., Landry, D. and Guthrie, E.P. (2008) *Glycobiology* 18, 799–805.



A. 5 mg substrate, 1 hr incubation, Core 1 Cleavage



B. 5 mg substrate, 1 hr incubation, Core 3 Cleavage

Companion Products

Endoglycosidase Reaction Buffer Pack

The Endoglycosidase Reaction Buffer Pack contains 1 ml of every buffer necessary for optimal activity of a deglycosylation reaction including 10X GlycoBuffer 2, 10X GlycoBuffer 3, 10X Glycoprotein Denaturing Buffer and 10% NP-40.

Endoglycosidase Buffer Pack B0701S

RNase B

RNase B is a high mannose glycoprotein that can be used as a positive control for endoglycosidases that cleave *N*-linked carbohydrates. RNase B has a single *N*-linked glycosylation site which makes it ideal for SDS-PAGE gel shift assays. It has an intact molecular weight of 17,000 daltons, and a molecular weight of 13,683 daltons after deglycosylation.

RNase B P7817S

Fetuin

Fetuin is a glycoprotein containing sialylated *N*-linked and *O*-linked glycans that can be used as a positive control for endoglycosidase enzymes.

Fetuin P6042S

Rapid PNGase F Antibody Standard

Rapid PNGase F Antibody Standard is a murine anti-MBP monoclonal antibody, isotype IgG2a. It is comprised of two heavy chains which are each approximately 49 kDa, as well as two light chains which are each approximately 24.4 kDa. This antibody standard can be used as a positive control for Rapid PNGase F.

Rapid PNGase F Antibody Standard P6043S

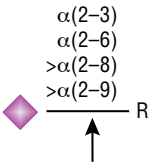
Gal Glc Man GalNAc GlcNAc Fuc NeuAC

Exoglycosidase Enzymes

NEB offers a wide selection of exoglycosidases for glycobiology research. Exoglycosidases cleave a monosaccharide from the non-reducing end of an internal glycosidic linkage in an oligosaccharide or polysaccharide. Many of these reagents are recombinant, and all undergo several quality control assays, enabling us to provide products with lower unit cost, high purity, and reduced lot-to-lot variation. All of our glycosidases are tested for contaminants. Since *p*-nitrophenyl-glycosides are not hydrolyzed by some exoglycosidases, we use only flourescently-labeled oligosaccharides to assay activity and screen for contaminating glycosidases.

α2-3,6,8,9 Neuraminidase A

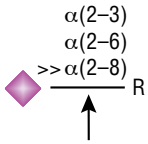
Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). α2-3,6,8,9 Neuraminidase A, cloned from *Arthrobacter ureafaciens*, catalyzes the hydrolysis of all linear and branched non-reducing terminal sialic acid residues from glycoproteins and oligosaccharides. The enzyme releases α2-3 and α2-6 linkages at a slightly higher rate than α2-8 and α2-9 linkages.



α2-3,6,8,9 Neuraminidase A P0722S/L

α2-3,6,8 Neuraminidase

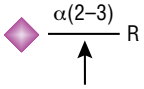
α2-3,6,8 Neuraminidase, cloned from *Clostridium perfringens,* catalyzes the hydrolysis of α2-3, α2-6 and α2-8 linked *N*-acetylneuraminic acid residues from glycoproteins and oligosaccharides.



α2-3,6,8 Neuraminidase P0720S/L

α2-3 Neuraminidase S

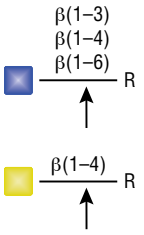
α2-3 Neuraminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of α2-3 linked *N*-acetylneuraminic acid residues from glycoproteins and oligosaccharides. α2-3 Neuraminidase S is cloned from *Streptococcus pneumoniae* and expressed in *E. coli.*



α2-3 Neuraminidase S P0743S/L

β-*N*-Acetylhexosaminidase$_f$

β-*N*-Acetylhexosaminidase$_f$, cloned from *Streptomyces plicatus,* is a recombinant protein fusion of β-*N*-Acetylhexosaminidase and maltose binding protein with identical activity to β-*N*-Acetylhexosaminidase. It catalyzes the hydrolysis of terminal β-*N*-Acetylgalactosamine and glucosamine residues from linear oligosaccharides.



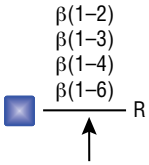
β-*N*-Acetylhexosaminidase$_f$ P0721S/L

References

1. Iwamori, M., Ohta, Y., Uchida, Y. and Tsukada, Y. (1997) *Glycocon. J.*, 1, 67–73.

β-*N*-Acetylglucosaminidase S

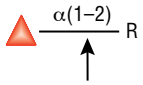
β-*N*-Acetylglucosaminidase S, cloned from *Streptococcus pneumoniae*, is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal, non-reducing β-*N*-Acetylglucosamine residues from oligosaccharides. β-*N*-Acetylglucosaminidase S is able to efficiently cleave bisecting β-*N*-Acetylglucosamine residues.



β-*N*-Acetylglucosaminidase S P0744S/L

α1-2 Fucosidase

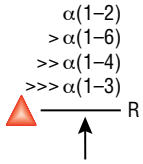
α1-2 Fucosidase, cloned from *Xanthomonas manihotis,* is a highly specific exoglycosidase that catalyzes the hydrolysis of linear α1-2 linked fucose residues from oligosaccharides. A linear substrate is defined as having no branching on the adjacent residue.



α1-2 Fucosidase P0724S/L

α1-2,3,4,6 Fucosidase

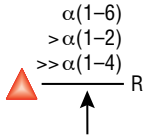
α1-2,3,4,6 Fucosidase, cloned from bovine kidney, is a broad specificity exoglycosidase that catalyzes the hydrolysis of α1-2, α1-3, α1-4 and α1-6 linked fucose residues from oligosaccharides. α1-2,3,4,6 Fucosidase cleaves α1-2 and α1-6 fucose residues more efficiently than other linkages, and has slight activity towards α1-3 fucose residues.



α1-2,3,4,6 Fucosidase P0748S/L

α1-2,4,6 Fucosidase O

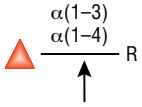
α1-2,4,6 Fucosidase O, cloned from *Omnitrophica*, is a broad specificity exoglycosidase that catalyzes the hydrolysis of terminal α1-2, α1-4 and α1-6 linked fucose residues from oligosaccharides and glycoproteins. α1-2,4,6 Fucosidase O cleaves α1-6 fucose residue more efficiently than other linkages.



α1-2,4,6 Fucosidase O P0749S/L

α1-3,4 Fucosidase

α1-3,4 Fucosidase, (also known as AMF) is a broad specificity exoglycosidase that catalyzes the hydrolysis of α1-3 and α1-4 linked fucose residues from oligosaccharides and glycoproteins.

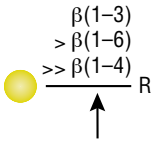


α1-3,4 Fucosidase P0769S/L

Gal Glc Man GalNAc GlcNAc Fuc NeuAC

β1-3 Galactosidase

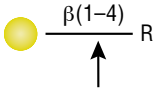
β1-3 Galactosidase, cloned from *Xanthomonas manihotis,* is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-3 and, at a much lower rate, β1-6 linked galactose residues from oligosaccharides. The approximate kinetic data show >100-fold preference for β1-3 over β1-6 linkages and >500-fold preference for β1-3 over β1-4 linkages.



β1-3 Galactosidase P0726S/L

β1-4 Galactosidase S

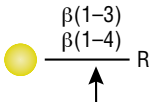
β1-4 Galactosidase S, cloned from *Streptococcus pneumoniae*, is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-4 linked galactose residues from oligosaccharides.



β1-4 Galactosidase S P0745S/L

β1-3,4 Galactosidase

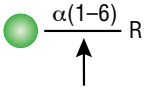
β1-3,4 Galactosidase, cloned from bovine testis and also known as BTG, is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal β1-3 and β1-4 linked galactose residues from oligosaccharides.



β1-3,4 Galactosidase P0746S/L

α1-6 Mannosidase

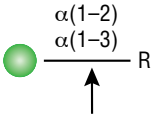
α1-6 Mannosidase, cloned from *Xanthomonas manihotis,* is a highly specific exoglycosidase that removes unbranched α1-6 linked mannose residues from oligosaccharides. When used in conjunction with α1-2,3 Mannosidase, the α1-6 Mannosidase will cleave α1-6 mannose residues from branched carbohydrate substrates.



α1-6 Mannosidase P0727S/L

α1-2,3 Mannosidase

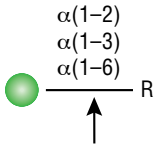
α1-2,3 Mannosidase, cloned from *Xanthomonas manihotis,* is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-2 and α1-3 linked mannose residues from oligosaccharides.



α1-2,3 Mannosidase P0729S/L

α1-2,3,6 Mannosidase

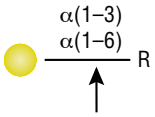
α1-2,3,6 Mannosidase, cloned from Jack Bean, and also known as JBM, is a broad specificity exoglycosidase that catalyzes the hydrolysis of terminal α1-2, α1-3 and α1-6 linked mannose residues from oligosaccharides. α1-2,3,6 Mannosidase has a slight preference for α1-2 mannose residues over α1-3 and α1-6 mannose residues.



α1-2,3,6 Mannosidase P0768S/L

α1-3,6 Galactosidase

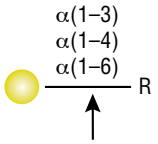
α1-3,6 Galactosidase, cloned from *Xanthomonas manihotis,* is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-3 and α1-6 linked galactose residues from oligosaccharides.



α1-3,6 Galactosidase P0731S/L

α1-3,4,6 Galactosidase

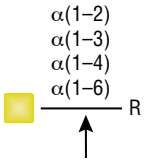
α1-3,4,6 Galactosidase, cloned from green coffee bean, is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-3, α1-4 and α1-6 linked galactose residues from oligosaccharides.



α1-3,4,6 Galactosidase P0747S/L

α-*N*-Acetylgalactosaminidase

α-*N*-Acetylgalactosaminidase, cloned from *Chryseobacterium meningosepticum,* is a highly specific exoglycosidase that catalyzes the hydrolysis of α-*N*-acetylgalactosamine residues from oligosaccharides and *N*-linked glycans attached to proteins.



α-*N*-Acetylgalactosaminidase P0734S/L

GlycoBuffer Compositions

1X GlycoBuffer 1:
50 mM sodium acetate (pH 5.5 @ 25°C), 5 mM $CaCl_2$

1X GlycoBuffer 2:
50 mM sodium phosphate (pH 7.5 @ 25°C)

1X GlycoBuffer 3:
50 mM sodium acetate (pH 6.0 @ 25°C)

1X GlycoBuffer 4:
50 mM sodium acetate (pH 4.5 @ 25°C)

Gal Glc Man GalNAc GlcNAc Fuc NeuAC **R** = any sugar

Heparin Lyase Enzymes

Heparin Lyase enzymes, also called Heparinases, are enzymes that cleave the glycosidic linkage between hexosamines and uronic acids and are known to cleave heparin and heparin sulfate (HS) chains selectively, via an elimination mechanism. Heparinase enzymes create a double bond on the non-reducing end of the uronic acid that absorbs at 232 nm and can be used for the detection of oligosaccharide and disaccharide products. Three Heparinase enzymes are available: *Bacteroides* Heparinase I, Heparinase II and Heparinase III. Heparinase I cleaves highly sulfated heparin/HS chains, Heparinase III cleaves less sulfated HS chains, while Heparinase II cleaves domains of both high and low sulfation on both heparin and HS. Heparinase I, II and III used in combination can produce a near-complete depolymerization of heparin/HS polysaccharide chains to disaccharides.

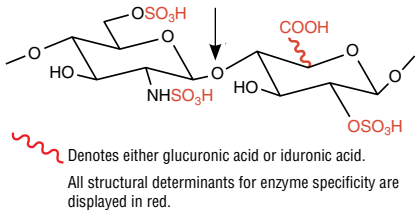
Bacteroides Heparinase I

Bacteroides Heparinase I cloned from *Bacteroides eggerthii*, also called Heparin Lyase I, is active on heparin and the highly sulfated domains of heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Detailed Specificity:

Bacteroides Heparinase I cleaves between *N*-sulfated hexosamines and 2-*O*-sulfated iduronic acid residues as well as the 2-*O*-sulfated glucuronic acid residue. The 2-*O*-sulfated uronic acid residue is essential for the activity of *Bacteroides* Heparinase I and 6-*O*-sulfation of $GlcNSO_3$ does not hinder enzyme activity. Limited digest of porcine mucosal heparin with the *Bacteroides* Heparinase I results in heparin oligosaccharides with a lower extent of sulfation as reported (1).

Bacteroides Heparinase I P0735S/L



Bacteroides Heparinase I specificity.

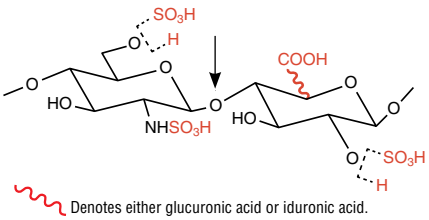
Bacteroides Heparinase II

Bacteroides Heparinase II cloned from *Bacteroides eggerthii*, also called Heparin Lyase II, is a low specificity enzyme that is active on both heparin and heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Detailed Specificity:

Bacteroides Heparinase II cleaves the glycosidic bond between *N*-sulfated glucuronic or iduronic acid residues. When used alone this enzyme rarely yields complete depolymerization of a polysaccharide chain, however disaccharide analysis is enhanced when used in combination with Heparinase I and III.

Bacteroides Heparinase II P0736S/L



Bacteroides Heparinase II specificity.

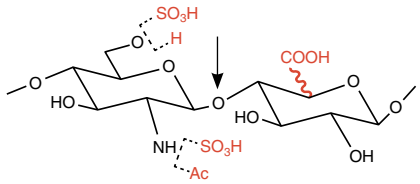
Bacteroides Heparinase III

Bacteroides Heparinase III cloned from *Bacteroides eggerthii*, also called Heparin Lyase III, is active on both heparin and heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Detailed Specificity:

Bacteroides Heparinase III can cleave the glycosidic bond between hexosamines and either iduronic acid or glucuronic acid residues, and is active in the presence of 6-sulfation.

Bacteroides Heparinase III P0737S/L



Bacteroides Heparinase III specificity.

Note: Heparin Hexasaccharide MS Standard 6 (NEB #P0738S) and Heparin Hexasaccharide MS Standard 7 (NEB #P0739S) are available by special order. Contact **info@neb.com** for details.

References
1. Merchant, Z.M., Kim, Y.S., Rice, K.G., Linhardt, R. (1985) *J. Biol. Chem.* 229, 369–377.

Glycoproteomics

NEB offers a selection of high purity proteases and standards for use in concert with our glycosidases for proteomic and glycoproteomic applications. Our proteases are of the highest quality, show no lot-to-lot variation and are offered at exceptional value. They are tested to assure a lack of contaminating proteins as well as high activity. They are used daily at NEB for internal research projects with a wide range of state-of-the-art mass spectrometers.

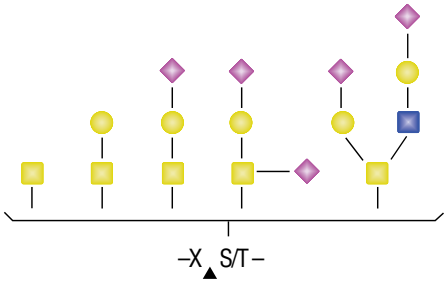
O-Glycoprotease (IMPa)

O-Glycoprotease (IMPa) is a highly specific protease that cleaves the peptide bonds of a glycoprotein or glycopeptide immediately N-terminal to a serine or threonine residue containing a mucin-type O-linked glycan with or without sialylation. O-Glycoprotease (IMPa) has a 6xHis-tag for easy removal from a reaction using nickel affinity resins.

O-Glycoprotease (IMPa) P0761S

ADVANTAGES

- Enables O-glycosylation site mapping and O-glycan structural determination
- Efficiently cleaves glycoproteins with or without sialic acid, no neuraminidase treatment necessary
- Recombinant enzyme with no detectable non-specific protease contaminating activities
- Can be used under native or denaturing conditions
- Glycerol-free for optimal performance in HPLC and mass spectrometry analysis



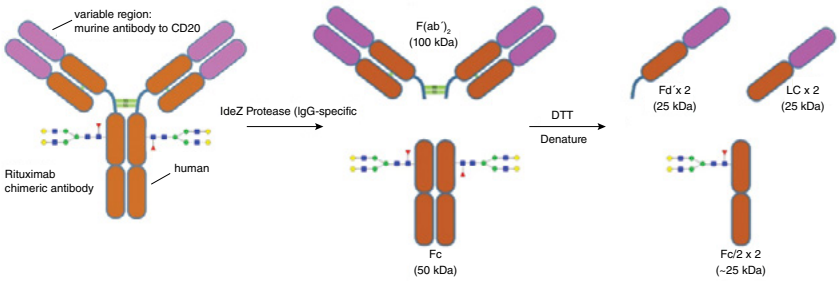
IdeZ Protease (IgG-specific)

IdeZ Protease (IgG-specific) is a recombinant antibody specific protease cloned from *Streptococcus equi* subspecies *zooepidemicus* that recognizes all human, sheep, monkey, and rabbit IgG subclasses, specifically cleaving at a single recognition site below the hinge region, yielding a homogenous pool of $F(ab')_2$ and Fc fragments. IdeZ Protease has significantly improved activity against murine IgG2a and IgG3 subclasses compared to IdeS Protease.

Detailed Specificity:

human IgG1, IgG3, IgG4: CPAPELLG▼GPSVF
human IgG2: CPAPPVA▼GPSVF
murine IgG2a: CPAPNLLG▼GPSVF
murine IgG3: CPPGNILG▼GPSVF

IdeZ Protease (IgG-specific) P0770S



Digestion of IgG with IdeZ Protease (IgG-specific), followed by denaturation.

Notes: IdeZ Protease efficiently cleaves human, humanized, chimeric, sheet, rabbit and monkey IgG as well as mouse IgG2a and IgG3. IdeZ Protease will also cleave Fc-fusion proteins, such as Enbrel.

IdeZ Protease does not cleave mouse IgG1 or IgG2b, rat, porcine, bovine or goat IgG. It also does not cleave non-IgG isotypes including IgA, IgM, IgD and IgE.

Gal Glc Man GalNAc GlcNAc Fuc NeuAC **R** = any sugar

Trypsin-digested BSA MS Standard (CAM-modified)

A complex mixture of peptides produced by the tryptic digest of Bovine Serum Albumin (BSA) that has been reduced and alkylated with iodacetamide (CAM modified). This peptide mixture is free of salts, glycerol and detergents and can therefore be used to standardize Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) or Electrospray Ionization (ESI) mass spectrometers (TOF, Q-TOF, Ion Trap, or Orbitrap) using a standardization range of 500-2400 Da.

Trypsin-digested BSA MS Standard (CAM-modified) P8108S

Trypsin-ultra,™ Mass Spectrometry Grade

Xxx-Arg▼Xxx
Xxx-Lys▼Xxx

Trypsin-ultra, Mass Spectrometry Grade is a serine endopeptidase isolated from Bovine pancreas. Trypsin is the most widely used enzyme in proteomics. It selectively cleaves peptide bonds C-terminal to lysine and arginine residues. Trypsin-ultra is treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inactivate any remaining chymotryptic activity. It is modified by acetylation of the ε-amino groups of lysine residues to prevent autolysis. Trypsin-ultra cleaves at Lys-Pro and Arg-Pro bonds at a much slower rate than when Lys and Arg are N-terminal to other residues.

Trypsin-ultra, Mass Spectrometry Grade P8101S

α-Lytic Protease

XX-T/A/S/V▼XX

α-Lytic Protease (aLP) cleaves after Threonine (T), Alanine (A), Serine (S) and Valine (V) residues. Its specificity makes it an orthogonal and alternative protease to others commonly used in proteomics applications, including trypsin and chymotrypsin. Peptides generated by aLP are of similar average length to those of Trypsin.

α-Lytic Protease P8113S/L

Endoproteinase LysC

XX-Lys▼XXX

LysC is a serine endoproteinase, isolated from *Lysobacter enzymogenes*, that cleaves peptide bonds at the carboxyl side of lysine. LysC is a sequencing grade enzyme and is suitable for proteomics and glycobiology applications.

Endoproteinase LysC P8109S

Endoproteinase AspN

XX▼Asp-XXX

Endoproteinase AspN (flavastacin) is a zinc metalloendopeptidase which selectively cleaves peptide bonds N-terminal to aspartic acid residues.

Endoproteinase AspN P8104S

Endoproteinase GluC

Xxx-Glu▼Xxx
Xxx-Asp▼Xxx

Endoproteinase GluC (*Staphylococcus aureus* Protease V8) is a serine proteinase which preferentially cleaves peptide bonds C-terminal to glutamic acid residues. Endoproteinase GluC also cleaves at aspartic acid residues at a rate 100-300 times slower than at glutamic acid residues. *Staphylococcus aureus* Protease V8 gene was cloned and expressed in *Bacillus subtilis*. Endoproteinase GluC is observed as a single band on SDS-PAGE and TOF-MS and is intended for use in mass spectrometry and protein sequencing.

Endoproteinase GluC P8100S

Ordering Information

Deglycosylation Enzymes

PRODUCT	NEB #	SIZE
Protein Deglycosylation Mix II	P6044S	20 reactions
PNGase A	P0707S/L	150/750 units (5,000 units/ml)
Rapid PNGase F	P0710S	50 reactions
Rapid PNGase F (non-reducing format)	P0711S	50 reactions
PNGase F	P0704S/L	15,000/75,000 units (500,000 units/ml)
PNGase F, Recombinant	P0708S/L	15,000/75,000 units (500,000 units/ml)
PNGase F (Glycerol-free)	P0705S/L	15,000/75,000 units (500,000 units/ml)
PNGase F (Glycerol-free), Recombinant	P0709S/L	15,000/75,000 units (500,000 units/ml)
Remove-iT PNGase F	P0706S/L	6,750/33,750 units (225,000 units/ml)
Endo F2	P0772S	480 units (8,000 units/ml)
Endo F3	P0771S	240 units (8,000 units/ml)
Endo S	P0741S/L	6,000/30,000 units (200,000 units/ml)
Endo D	P0742S/L	1,500/7,500 units (50,000 units/ml)
Endo H	P0702S/L	10,000/50,000 units (500,000 units/ml)
Endo H_f	P0703S/L	100,000/500,000 units (1,000,000 units/ml)
O-Glycosidase	P0733S/L	2,000,000/10,000,000 units (40,000,000 units/ml)
O-Glycosidase & Neuraminidase Bundle	E0540S	2,000,000 units *O*-Glycosidase & 2,000 units Neuraminidase

Heparin Lyases

PRODUCT	NEB #	SIZE
Bacteroides Heparinase I	P0735S/L	240 units/600 units (12,000 units/ml)
Bacteroides Heparinase II	P0736S/L	80 units/200 units (4,000 units/ml)
Bacteroides Heparinase III	P0737S/L	14 units/35 units (700 units/ml)

Glycoproteomics

PRODUCT	NEB #	SIZE
O-Glycoprotease (IMPa)	P0761S	200 reactions
IdeZ Protease (IgG-specific)	P0770S	4,000 units
Trypsin-digested BSA MS Standards (CAM Modified)	P8108S	500 pmol
Trypsin-ultra, Mass Spectrometry Grade	P8101S	100 µg
α-Lytic Protease	P8113S/L	20/100 µg
Endoproteinase LysC	P8109S	20 µg
Endoproteinase AspN	P8104S	50 µg
Endoproteinase GluC	P8100S	50 µg

Exoglycosidase Enzymes

PRODUCT	NEB #	SIZE
α-*N*-Acetylgalactosaminidase	P0734S/L	3,000/15,000 units (20,000 units/ml)
β-*N*-Acetylglucosaminidase S	P0744S/L	100/500 units (4,000 units/ml)
β-*N*-Acetylhexosaminidase$_f$	P0721S/L	500/2,500 units (5,000 units/ml)
α1-2 Fucosidase	P0724S/L	1,000/5,000 units (20,000 units/ml)
α1-2,3,4,6 Fucosidase	P0748S/L	400/2,000 units (8,000 units/ml)
α1-2,4,6 Fucosidase O	P0749S/L	80/400 units (2,000 units/ml)
α1-3,4 Fucosidase	P0769S/L	200/1,000 units (4,000 units/ml)
α1-3,6 Galactosidase	P0731S/L	100/500 units (4,000 units/ml)
α1-3,4,6 Galactosidase	P0747S/L	200/1,000 units (8,000 units/ml)
β1-3 Galactosidase	P0726S/L	500/2,500 units (10,000 units/ml)
β1-3,4 Galactosidase	P0746S/L	400/2,000 units (8,000 units/ml)
β1-4 Galactosidase S	P0745S/L	400/2,000 units (8,000 units/ml)
α1-2,3 Mannosidase	P0729S/L	640/3,200 units (32,000 units/ml)
α1-2,3,6 Mannosidase	P0768S/L	80/400 units (2,000 units/ml)
α1-6 Mannosidase	P0727S/L	800/4,000 units (40,000 units/ml)
α2-3,6,8 Neuraminidase	P0720S/L	2,000/10,000 units (50,000 units/ml)
α2-3,6,8,9 Neuraminidase A	P0722S/L	800/4,000 units (20,000 units/ml)
α2-3 Neuraminidase S	P0743S/L	400/2,000 units (8,000 units/ml)

Companion Products

PRODUCT	NEB #	SIZE
Endoglycosidase Reaction Buffer Pack	B0701S	4 x 1 ml
Fetuin	P6042S	500 µg (10 mg/ml)
RNase B	P7817S	250 µg (5 mg/ml)
Rapid PNGase F Antibody Standard	P6043S	250 µg (5 mg/ml)
Chitin Magnetic Beads	E8036S/L	5/25 ml
6-Tube Magnetic Separation Rack	S1506S	6 tubes
50 ml Magnetic Separation Rack	S1507S	4 tubes
12-Tube Magnetic Separation Rack	S1509S	12 tubes
2-Tube Magnetic Separation Rack	S1510S	2 tubes
96-Well Microtiter Plate Magnetic Separation Rack	S1511S	96-well

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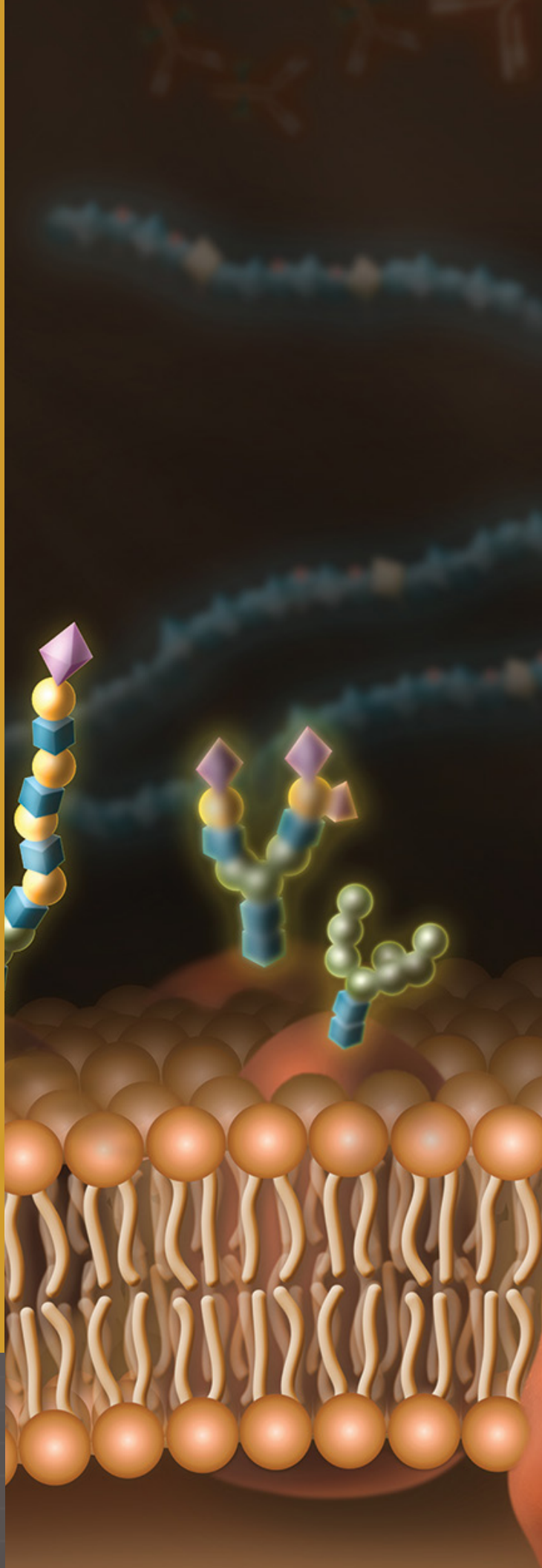
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