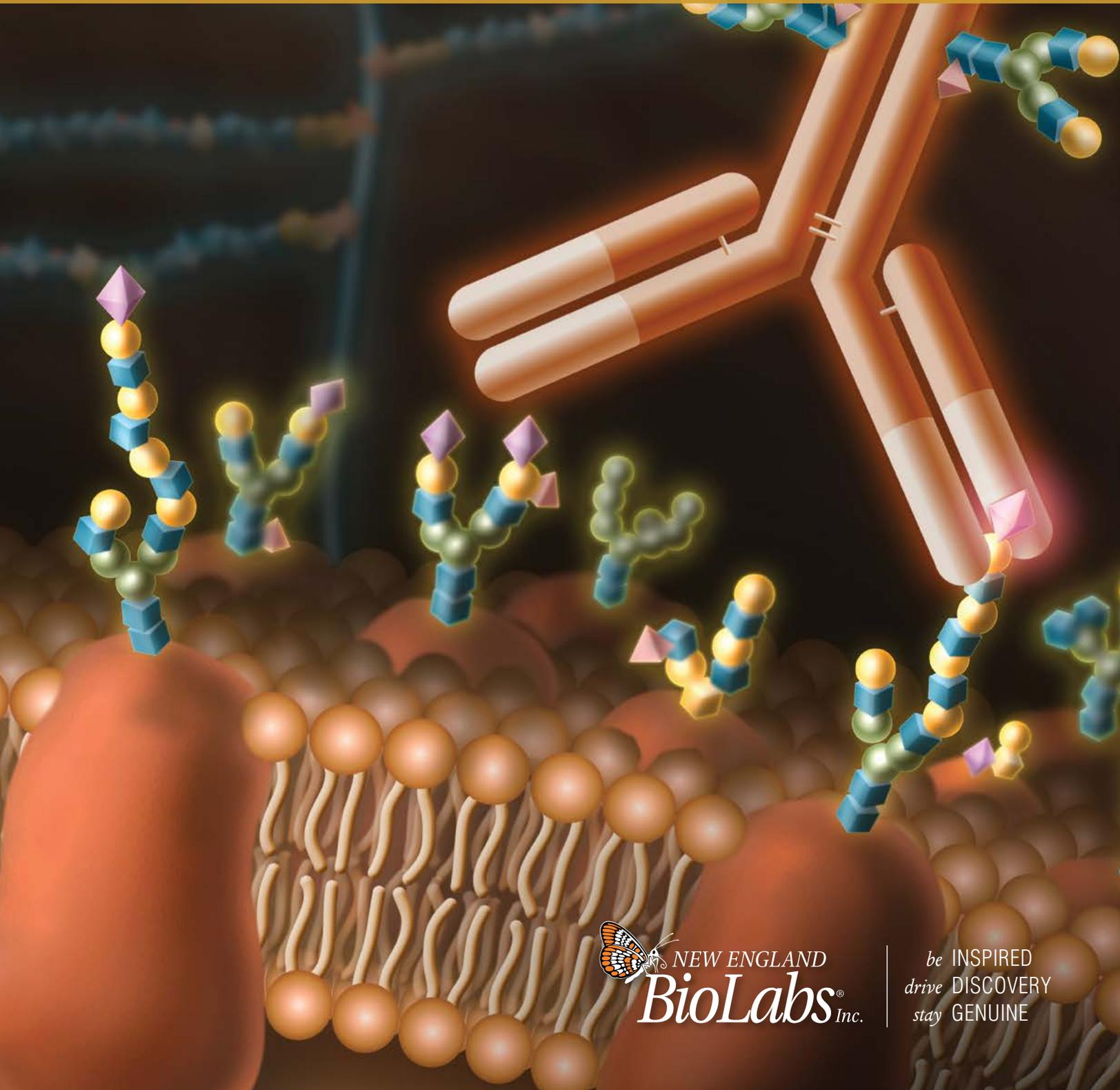


Glycoproteomics

UNDERSTANDING PROTEIN
MODIFICATIONS



 NEW ENGLAND
BioLabs[®] Inc.

be INSPIRED
drive DISCOVERY
stay GENUINE



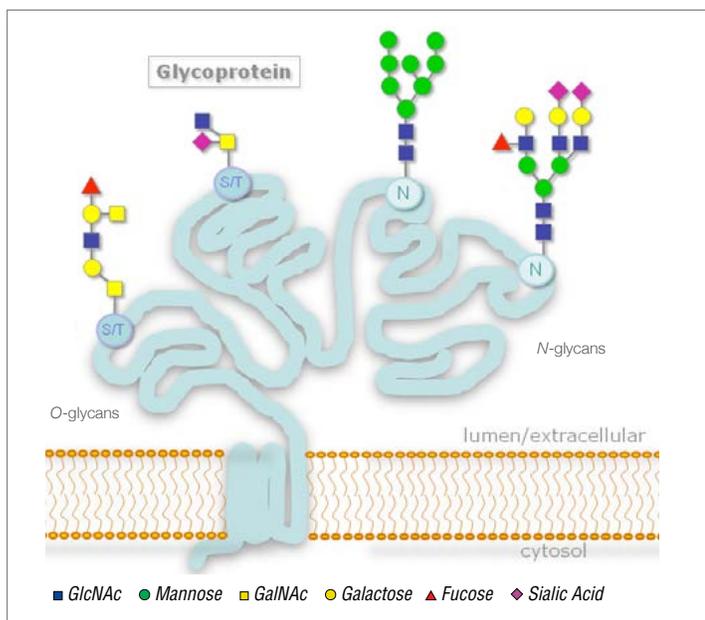
Glycobiology Overview

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.

Reference

1. Spiro, R.G. (2002) *Glycobiology* 12, 43R-56R.

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16 α 1–2,3,4,6 Fucosidase

16 α 1–3,4 Fucosidase

17 β 1–3 Galactosidase

17 β 1–4 Galactosidase

18 β 1–4 Galactosidase S

18 α 1–6 Mannosidase

19 α 1–2,3 Mannosidase

19 α 1–3,6 Galactosidase

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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 DEGLYCOSYLA- TION MIX (NEB #P6039S)	<i>O</i> -GLYCOSIDASE (NEB #P0733S/L & NEB #E0540S)	PNGASE F (NEB #P0704S/L & #P0705S/L)	REMOVE-IT PNGASE F (NEB #P0706S/L)	RECOMBINANT PNGASE F (NEB #P0708S/L & P0709S/L)	RAPID PNGASE F (NEB #P0710S)	RAPID PNGASE F, (NON-REDUCING FORMAT) (NEB #P0711S)	ENDO H (NEB #P0702S/L) & ENDO H ₂ (NEB #P0703S/L)	ENDO S (NEB #P0741S/L)	ENDO D (NEB #P0742S/L)
Deglycosylation of glycoproteins (<i>N</i> - and <i>O</i> -glycans)	•									
Removal of <i>O</i> -glycans	•	•								
Removal of <i>N</i> -glycans from glycoproteins	•		•	•	•	•	•	•	•	•
Removal of high mannose and hybrid <i>N</i> -glycans (leaving a GlcNAc attached to Asn)								•		
Optional removal of the enzyme from the reaction				•					•	•
Removal of paucimannose <i>N</i> -glycans (GlcNAc attached to Asn)					•			•		•
Removal of <i>N</i> -glycans from IgGs (leaving a GlcNAc attached to Asn)								•	•	
Analysis of therapeutic glycoproteins, compliance with regulatory agencies					•	•	•			
High throughput <i>N</i> -glycan analysis of monoclonal anti- bodies, regulatory compliance						•	•			
Glycomics			•	•	•	•	•	•	•	•
Proteomics			• (only GF)	•	• (only GF)	•	•	•	•	•
Determine <i>N</i> -glycan sites								•	•	•

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.



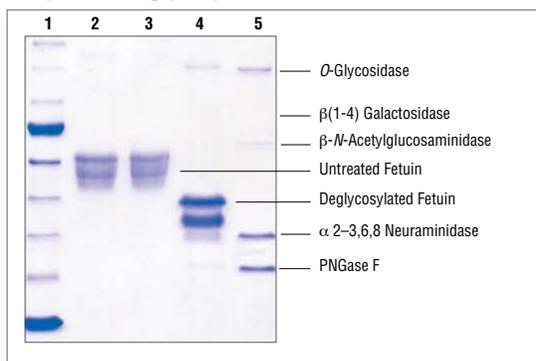
Protein Deglycosylation Mix

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 mono-saccharides on *O*-linked glycans must be removed by a series of exoglycosidases, such as β 1-4 Galactosidase and β -*N*-Acetylglucosaminidase, until only the Gal β 1-3GalNAc (core 1) and/or the GlcNAc β 1-3GalNAc (core 3) cores remain attached to the core protein. *O*-Glycosidase, also called Endo- α -*N*-Acetylglucosaminidase, 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 Neuraminidase.

The Protein Deglycosylation Mix contains all of the enzymes, reagents and controls necessary to remove almost all *N*-linked and simple *O*-linked glycans, as well as some complex *O*-linked glycans. This kit contains enzymes sufficient for 20 reactions or the cleavage of as much as 2 mg of glycoprotein. The Deglycosylation Enzyme Mix (100 μ l) is a single mix made up of equal amounts (20 μ l each) of NEB's PNGase F Glycerol Free, *O*-Glycosidase, α 2-3,6,8 Neuraminidase, β 1-4 Galactosidase, and β -*N*-Acetylglucosaminidase. The mix is supplied with all of the reagents and controls required to complete the experiment, including 10X Glycoprotein Denaturing Buffer, 10% NP-40 Buffer, 10X GlycoBuffer 2, and a Fetuin control containing sialylated *N*-linked and *O*-linked glycans.

Protein Deglycosylation MixP6039S

Enzymatic Deglycosylation of Bovine Fetuin



100 μ g Bovine Fetuin Control was deglycosylated using the denaturing reaction conditions. 25 μ g of the reaction was loaded onto a 10–20% SDS-PAGE gel.

Lane 1: Protein Ladder (NEB #P7703)

Lane 2: 25 μ g untreated Fetuin control

Lane 3: 25 μ g denatured Fetuin control

Lane 4: 25 μ g deglycosylated denatured Fetuin

Lane 5: 5 μ l Protein Deglycosylation Mix

PROTEIN DEGLYCOSYLATION MIX REACTION PROTOCOLS

DENATURING REACTION CONDITIONS

1. Dissolve 100 μ g of glycoprotein into 18 μ l H₂O.
2. Add 2 μ l of 10X Glycoprotein Denaturing Buffer to make a 20 μ l total reaction volume.
3. Denature glycoprotein by heating reaction at 100°C for 10 minutes.
4. Chill denatured glycoprotein on ice and centrifuge 10 seconds.
5. To the denatured glycoprotein reaction add 5 μ l 10X GlycoBuffer 2, 5 μ l 10% NP-40 and 15 μ l H₂O.
6. Add 5 μ l Deglycosylation Enzyme Mix, mix gently.
7. Incubate reaction at 37°C for 4 hours.
8. Analyze by method of choice.

Note: PNGase F and O-Glycosidase are inhibited by SDS, therefore it is essential to have NP-40 in the reaction mixture under denaturing conditions. Failure to include NP-40 in the denaturing reaction may result in loss of activity of this enzyme.

NON-DENATURING REACTION CONDITIONS

When deglycosylating a native glycoprotein it is recommended that an aliquot of the glycoprotein is subjected to the denaturing protocol to provide a positive control for the fully deglycosylated protein. The non-denatured reaction can then be compared to the denatured reaction to determine the extent of reaction completion.

1. Dissolve 100 μ g of glycoprotein into 40 μ l H₂O.
2. To the native glycoprotein add 5 μ l 10X GlycoBuffer 2.
3. Add 5 μ l Deglycosylation Enzyme Mix, mix gently.
4. Incubate reaction at 37°C for 4 hours.

Note: To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

5. Analyze by method of choice.

Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.



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, Endoglycosidase S, Endoglycosidase D and Endoglycosidase H. PNGase F is the most effective enzyme for removing almost all *N*-linked oligosaccharides from glycoproteins. PNGase F cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from *N*-linked glycoproteins (1). PNGase F digestion deaminates the asparagine residue to aspartic acid, and leaves the oligosaccharide intact for further analysis. However, it is critical to note that oligosaccharides containing a fucose $\alpha(1-3)$ -linked to the glycan core, a structure often found in plant and insect glycoproteins, are resistant to PNGase F (2). These substrates would therefore require PNGase A treatment. 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 D cleaves within the chitobiose core of paucimannose *N*-linked glycans, with or without extensions in the antennae; while Endoglycosidase H only deglycosylates glycoproteins containing primarily high mannose *N*-linked structures. All three enzymes will leave one *N*-acetylglucosamine residue attached to the asparagine.

In order to achieve complete removal of *N*-linked glycans from a glycoprotein, it is recommended that the glycoprotein first be denatured by heating with SDS and DTT prior to PNGase F or Endo H treatment. Denaturation of the glycoprotein will decrease the steric hindrances that can inhibit enzyme activity. However, if denaturation of a glycoprotein is not desirable, native conditions may be used. Under native conditions endoglycosidases retain full activity; however more enzyme and longer incubation times may be needed in order to reach complete deglycosylation.

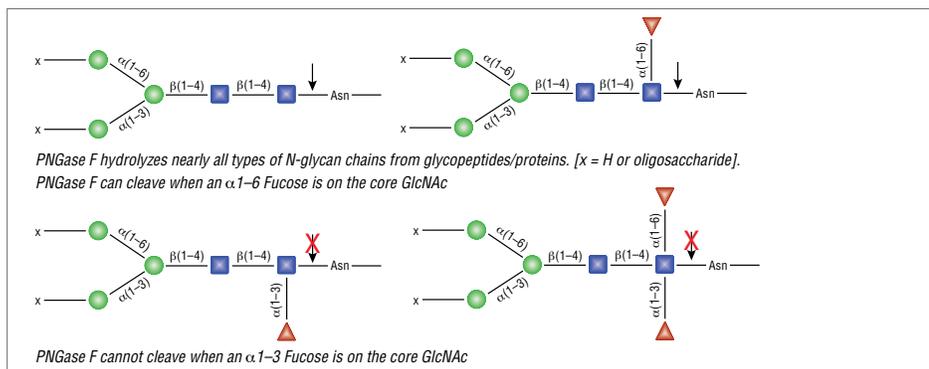
PNGase F (native and recombinant)

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 $\alpha 1-6$ Fucose is on the core GlcNAc. *PNGase F* cannot cleave when an $\alpha 1-3$ 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



Gal ● Glc ● Man ● GalNAc ■ GlcNAc ■ Fuc ▲ NeuAc ◆

PNGASE F & PNGASE F GLYCEROL-FREE REACTION PROTOCOLS

DENATURING REACTION CONDITIONS

1. Combine 1-20 μg of glycoprotein, 1 μl of 10X Glycoprotein Denaturing Buffer and H_2O (if necessary) to make a 10 μl total reaction volume.
2. Denature glycoprotein by heating reaction at 100°C for 10 minutes.
3. Make a total reaction volume of 20 μl by adding 2 μl 10X GlycoBuffer 2, 2 μl 10% NP-40, H_2O and 1-2 μl PNGase F.

Note: PNGase F is inhibited by SDS, therefore it is essential to have NP-40 in the reaction mixture under denaturing conditions. Failure to include NP-40 in the denaturing reaction may result in loss of activity of this enzyme.

4. Incubate reaction at 37°C for 1 hour.
5. Analyze by method of choice.

Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.

NON-DENATURING REACTION CONDITIONS

1. Combine 1-20 μg of glycoprotein, 2 μl 10X GlycoBuffer 2, H_2O and 2-5 μl PNGase F to make a total reaction volume of 20 μl .
2. Incubate reaction at 37°C for 4 hours to overnight.
3. Analyze by method of choice.

Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels. We recommend limiting PNGase F (NEB #P0704) to 1/10 (or less) of the total reaction volume to keep final glycerol concentration equal to (or less than) 5%. Reaction may be scaled-up linearly to accommodate large amounts of PNGase F and larger reaction volumes.

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

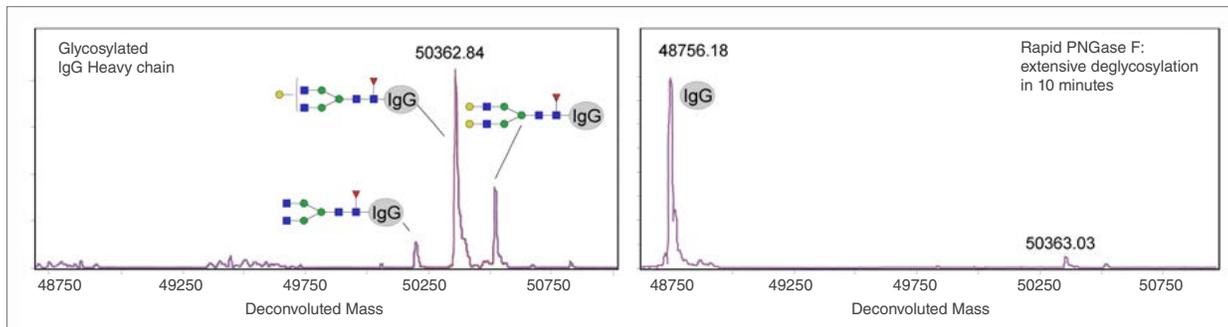
- Complete deglycosylation of antibodies and immunoglobulin fusion proteins in minutes
- Release of all *N*-glycans rapidly and without bias, ready for downstream chromatography or mass spectrometry analysis
- Optimal activity is ensured for 12 months
- Purified to > 99% homogeneity, as determined by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



Remove-iT[®] Endoglycosidases

NEB offers Remove-iT PNGase F, Endo S and Endo D, three tagged endoglycosidases for easy removal from a reaction. PNGase F is the most effective enzymatic method for removing almost all N-linked oligosaccharides from glycoproteins. Endo S is ideal for removing N-linked glycans from the chitobiose core of the heavy chain of native IgG. Endo D cleaves within the chitobiose core of paucimannose N-linked glycans, with or without extensions in the antennae.

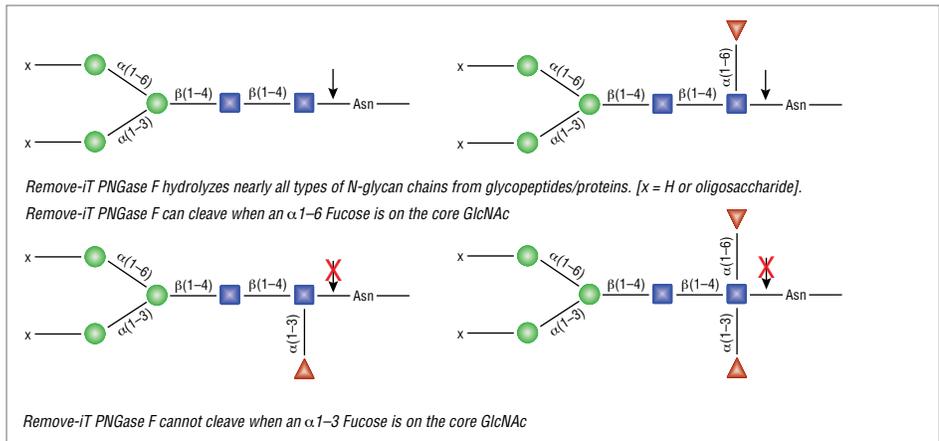
ADVANTAGES

- 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

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



REMOVE-IT PNGASE F REACTION PROTOCOL

1. Combine 10–20 μ g of glycoprotein, 1 μ l of 10X DTT and H₂O (if necessary) to make a 10 μ l total reaction volume.
2. Denature glycoprotein by heating reaction at 55°C for 10 minutes.
3. Make a total reaction volume of 20 μ l by adding 2 μ l 10X GlycoBuffer 2, H₂O and 1–5 μ l Remove-iT PNGase F.
4. Incubate reaction at 37°C for 1 hour.
5. Eliminate Remove-iT PNGase F from the reaction using Chitin Magnetic Beads (NEB #E8036) or analyze deglycosylation reaction by method of choice.

Note: To deglycosylate a native glycoprotein, longer incubation time, as well as more enzyme, may be required. If using Remove-iT PNGase F under typical PNGase F denaturing conditions, it is essential to have NP-40 in the reaction mixture as Remove-iT PNGase F is inhibited by SDS. The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.

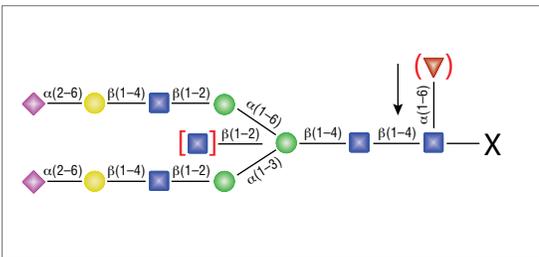


Endo S

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 $\alpha(1-6)$ fucosylation as well as with or without a bisecting *N*-acetylglucosamine. Triantennary and tetrantennary sialylated or asialo glycans are not a substrate for Endo S.

Endo S P0741S/L



ENDO S REACTION PROTOCOL

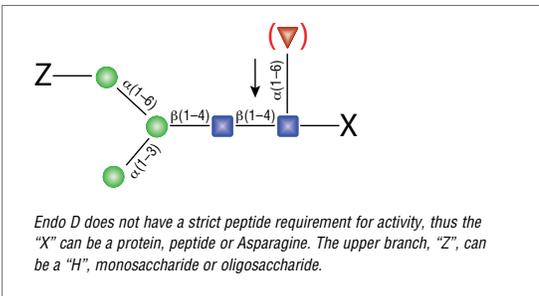
1. Combine 100 μg of native IgG, 1 μl of 10X GlycoBuffer 2 and H_2O (if necessary) to make a 10 μl total reaction volume.
2. Add 1 μl Endo S.
3. Incubate reaction at 37°C for 1 hour.
4. Eliminate Endo S from the reaction using Chitin Magnetic Beads (NEB #E8036) or analyze deglycosylation reaction by method of choice.

Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.

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 REACTION PROTOCOL

1. Combine 10–20 μg of glycoprotein, 1 μl of 10X DTT and H_2O (if necessary) to make a 10 μl total reaction volume.
2. Denature glycoprotein by heating reaction at 55°C for 10 minutes.
3. Make a total reaction volume of 20 μl by adding 2 μl 10X GlycoBuffer 2, H_2O and 1–5 μl Endo D.
4. Incubate reaction at 37°C for 1 hour.
5. Eliminate Endo D from the reaction using Chitin Magnetic Beads (NEB #E8036) or analyze deglycosylation reaction by method of choice.

Notes: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels. To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required. Endo D is not recommended for use with Glycoprotein Denaturing Buffer containing both SDS and DTT, as Endo D is inhibited by SDS and unlike other endoglycosidases, NP-40 does not counteract the SDS inhibition.



Remove-iT PNGase F/Endo S/Endo D Magnetic Chitin Bead Protocol

1. Pipette 50 μ l Chitin Magnetic Beads into an eppendorf tube and place the eppendorf in a Magnetic Separation Rack. Let the magnet attract the chitin beads, then pipette off the liquid supernatant and discard.
2. Wash the magnetic chitin beads with 500 μ l of 50 mM NH_4 Formate pH 4.4 (or buffer of choice) and pull the beads to the side of the tube using the Magnetic Separation Rack. Pipette off the supernatant and discard. Repeat.
3. Add the deglycosylated glycoprotein sample into the eppendorf with magnetic chitin beads.
4. Rock the deglycosylated glycoprotein sample with the magnetic chitin beads for 10 minutes at 4°C.
5. Place the eppendorf back on the magnetic separation rack, and allow the magnet to attract the chitin beads. Pipette off the supernatant and keep.
6. Wash the magnetic chitin beads 3 x 100 μ l with 50 mM NH_4 Formate pH 4.4 (or buffer of choice). Pipette of the supernatant from each wash and keep.
7. Combine all supernatants from steps 5 & 6, as these are the deglycosylated glycoprotein.
8. Analyze sample by method of choice

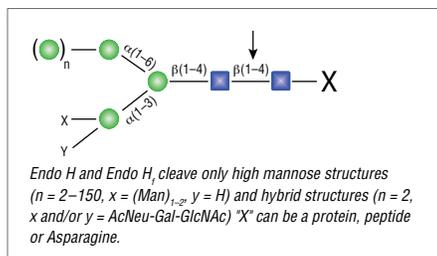
Note - Elimination of Remove-iT enzymes from the deglycosylation reaction can be scaled up linearly with larger magnetic chitin bead volumes. The ideal reaction volume for 50 μ l of chitin beads is in the range of equal volume to no more than 5X bead bed volume. The Magnetic Chitin Beads binding capacity is approximately 0.4 mg/ml of CBD-tagged protein. This binding capacity is calculated in mg of protein per bed volume of resin. The chitin magnetic beads are a 50:50 slurry. Therefore, 50 μ l of slurry will yield 25 μ l bed volume of resin.

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



MATERIALS

- Remove-iT PNGase F (NEB #P0706) or Endo S (NEB #P0741) or Endo D (NEB #P0742)
- Chitin Magnetic Beads (NEB #E8036)
- Magnetic Separation Rack (NEB #S1506, NEB #S1509)

ENDO H AND ENDO H_F REACTION PROTOCOLS

DENATURING REACTION CONDITIONS

1. Combine 1–20 μ g of glycoprotein, 1 μ l of 10X Glycoprotein Denaturing Buffer and H₂O (if necessary) to make a 10 μ l total reaction volume.
2. Denature glycoprotein by heating reaction at 100°C for 10 minutes.
3. Make a total reaction volume of 20 μ l by adding 2 μ l 10X GlycoBuffer 3, H₂O and 1–2 μ l Endo H/H_F.
4. Incubate reaction at 37°C for 1 hour.
5. Analyze by method of choice.

Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.

NON-DENATURING REACTION CONDITIONS

1. Combine 1–20 μ g of glycoprotein, 2 μ l 10X GlycoBuffer 3, H₂O and 2–5 μ l Endo H/H_F to make a total reaction volume of 20 μ l.
2. Incubate reaction at 37°C for 4 hours to overnight.
3. Analyze by method of choice.

Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels. Reaction may be scaled-up linearly to accommodate large amounts of glycoprotein and larger reaction volumes. The pH range of Endo H/H_F is very specific and can effect activity of the enzyme, therefore we recommend using the supplied GlycoBuffer 3 which has a pH of 6.0.



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 Gal β 1-3GalNAc (core 1) and/or the GlcNAc β 1-3GalNAc (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 sialylation, will block the action of the *O*-Glycosidase. Sialic acid residues are easily removed by a general Neuraminidase. In addition, exoglycosidases such as β 1-4 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.

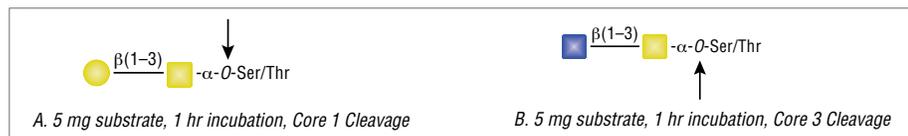
O-Glycosidase

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*.

<i>O</i> -Glycosidase (Endo- α - <i>N</i> -Acetylgalactosaminidase)	P0733S/L
Neuraminidase	P0720S/L
<i>O</i> -Glycosidase & Neuraminidase Bundle	E0540S

Reference

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



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

Gal ● Glc ● Man ● GalNAc ● GlcNAc ● Fuc ▲ NeuAc ◆

O-GLYCOSIDASE REACTION PROTOCOLS

DENATURING REACTION CONDITIONS

1. Combine 1–20 μ g of glycoprotein, 1 μ l of 10X Glycoprotein Denaturing Buffer and H₂O (if necessary) to make a 10 μ l total reaction volume.
2. Denature glycoprotein by heating reaction at 100°C for 10 minutes.
3. Make a total reaction volume of 20 μ l by adding 2 μ l 10X GlycoBuffer 2, 2 μ l 10% NP-40, 2 μ l Neuraminidase, H₂O and 1–5 μ l *O*-Glycosidase.
4. Incubate reaction at 37°C for 1 hour.
5. Analyze by method of choice.

Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.

NON-DENATURING REACTION CONDITIONS

1. Combine 1–20 μ g of glycoprotein, 2 μ l 10X GlycoBuffer 2, 2 μ l Neuraminidase, H₂O and 1–5 μ l *O*-Glycosidase to make a total reaction volume of 20 μ l.
2. Incubate reaction at 37°C for 4 hours to overnight.
3. Analyze by method of choice.

Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels. The enzyme can be used under either denaturing or non-denaturing conditions. However, under denaturing conditions the enzyme activity is increased two-fold. This observation is substrate dependent. The reaction may be scaled-up linearly to accommodate large amounts of glycoprotein and larger reaction volumes.

PNGase F and O-Glycosidase can be used concomitantly in a reaction with 1X GlycoBuffer 2, 10% NP-40, Neuraminidase, H₂O and the glycoprotein of interest.

RNASE B/FETUIN DEGLYCOSYLATION PROTOCOL

1. In a 10 μ l reaction, add 2 μ l of RNase B or Fetuin, 1 μ l of 10X Glycoprotein Denaturing Buffer and 5 μ l of H₂O.
2. Incubate at 100°C for 10 minutes.
3. Add 1 μ l of 10X GlycoBuffer 2 and 1 μ l of 10% NP-40.
4. Add 1 μ l of endoglycosidase.
5. Incubate at 37°C for 1 hour.
6. Visualize reaction products on 10–20% SDS-PAGE gel.



FAQs

Q. What is the difference between PNGase F/Remove-iT PNGase F and Endo H?

A. PNGase F (as well as Remove-iT PNGase F) removes almost all types of *N*-linked (Asn-linked) glycosylation: high mannose, hybrid, bi-, tri- and tetra-antennary. You will choose this enzyme if your goal is to remove all *N*-linked carbohydrates without regard to type. Endo H removes only high mannose and some hybrid types of *N*-linked carbohydrates. You would choose this enzyme to more closely determine the type of *N*-linked glycosylation, or if you know that the protein has a carbohydrate sensitive to Endo H.

Q. What is the difference between Endo H and Endo H_f?

A. Endo H and Endo H_f are the same enzyme, but Endo H_f is the fusion protein of Endo H and MBP. The clone was engineered with the MBP attached to help in the purification of the enzyme. The fusion protein has identical activity to the non-fusion clone. There is no difference in activity between Endo H and Endo H_f on a glycoprotein.

Q. What is a good endoglycosidase substrate?

A. We suggest RNase B (NEB #P7817) for use with PNGase F and Endo H; Galβ1-3GalNAcα1pNP for use with *O*-Glycosidase; and Fetuin (NEB #P6042) for use with the Protein Deglycosylation Mix.

Q. What happens to the asparagine after PNGase F removes the sugar?

A. Since the enzyme is a glycoamidase, the asparagine is converted to aspartic acid.

Q. Is PNGase F compatible with downstream analysis such as HPLC and Mass Spectrometry?

A. NEB sells three versions of the enzyme, PNGase F (NEB #P0704/P0708), PNGase F Glycerol Free (NEB #P0705/P0709) and Remove-iT PNGase F (NEB #P0706). These three versions of the enzyme have identical activity, specificity and concentration. The only difference between the three is that PNGase F is stored in 50% Glycerol, while PNGase F Glycerol Free and Remove-iT PNGase F do not contain glycerol in the storage buffer. PNGase F Glycerol Free or Remove-iT PNGase F are recommended when downstream analysis will include HPLC and/or Mass Spectrometry due to the fact that glycerol is not tolerated in such instruments. Glycoprotein Denaturing Buffer (containing SDS and DTT) is not compatible with Mass Spectrometry applications, and often times hard to remove from the reaction. Therefore, we recommend using PNGase F Glycerol Free or Remove-iT PNGase F under non-denaturing conditions if HPLC or MS will be used for downstream analysis. Non-denaturing conditions often require more enzyme units and a longer incubation time.

Q. Can PNGase F be used for deglycosylation of whole or live cells?

A. All of our enzymes can be used in conditions that are compatible with whole or live cells. Two different protocols, denaturing and non-denaturing, are suggested for deglycosylation with PNGase F. In order to keep cells viable, non-denaturing conditions are typically recommended for use with whole or live cells. The pH of the reaction should be kept between pH 6.0 – 8.0. PNGase F has optimal activity between pH 7.0-7.5.

Typical non-denaturing PNGase F reaction conditions are:

1. Combine 1-20 μg of glycoprotein and H₂O (if necessary) to make a 10 μl total reaction volume.
2. Make a total reaction volume of 20 μl by adding 2 μl 10X GlycoBuffer 2 (0.5M sodium phosphate, pH 7.5), H₂O and 2-5 μl PNGase F.
3. Incubate reaction at 37°C for 4 hours to overnight.

If you incubate the deglycosylation reaction overnight, it is recommended to include a protease inhibitor cocktail to stop protein degradation. Any cocktail may be used, however we recommend avoiding one which includes PMSF, as PMSF can modify basic residues. As the sample becomes more complex (from protein to whole cells) the accessibility to the enzyme is limited because of the complexity of the cell surface. One will have to determine empirically the amount of enzyme needed to remove *N*-linked sugars. As a recommendation, use more enzyme and longer incubation times.

Q. Is it necessary to treat my glycoprotein concomitantly with Neuraminidase and O-Glycosidase?

A. Yes. Neuraminic Acid residues must be removed in order to allow *O*-Glycosidase to cleave the *O*-linked disaccharides. A general Neuraminidase (NEB #P0720) works well.

Q. Are NEB's endoglycosidases compatible with protease inhibitor cocktails?

A. When a protein is denatured it is more susceptible to cleavage by proteases. For this reason a protease cocktail containing the following can be used in a PNGase F, Endo H, Endo S, Endo D, *O*-Glycosidase or Protein Deglycosylation Mix reaction protocol:

Aprotinin (10 μg/ml final concentration), Benzamidine (1 mM final concentration), Pepstatin (10 g/ml final concentration), Leupeptin (1 μM final concentration), EGTA (1 mM final concentration), EDTA (1 mM final concentration), PMSF (1 mM final concentration)

Note: PMSF is not highly recommended as it has the ability to modify basic residues on glycoprotein substrates.



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 fluorescently-labeled oligosaccharides to assay activity and screen for contaminating glycosidases.

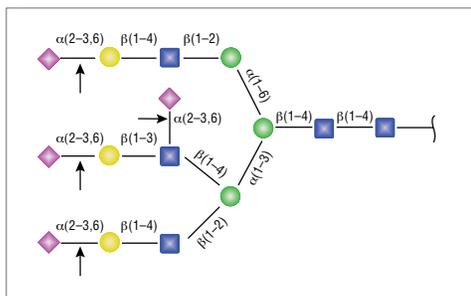
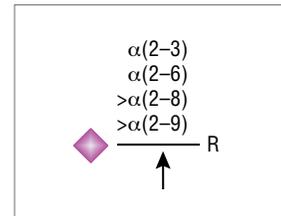
α2-3,6,8,9 Neuraminidase A

Neuraminidase is the common name for Acetyl-neuraminydase (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.

Detailed Specificity:

α2-3,6,8,9 Neuraminidase A will cleave branched sialic acid residues that are linked to an internal residue. This oligosaccharide from fetuin is an example of a side-branch sialic acid residue that can efficiently be cleaved (1).

α2-3,6,8,9 Neuraminidase A P0722S/L



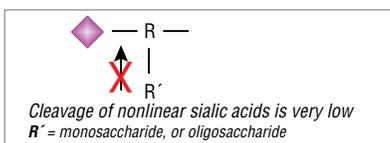
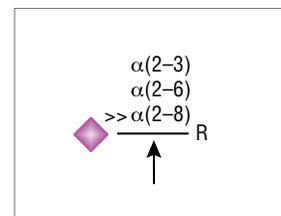
α2-3,6,8 Neuraminidase

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.

Detailed Specificity:

The enzyme is active over a broad range of pH values from 4.5-8.5, and can be used in double digests with endoglycosidases. Neuraminidase has reduced or no activity towards cleavage of side-branch sialic acid residues, depending on the degree of the oligosaccharide and branching structure.

α2-3,6,8 Neuraminidase P0720S/L



Gal ● Glc ● Man ● GalNAc ■ GlcNAc ■ Fuc ▲ NeuAc ◆ R = any sugar

References

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

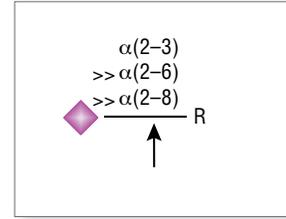


α2-3 Neuraminidase

α2-3 Neuraminidase, cloned from *Salmonella typhimurium* LT2, is a highly specific exoglycosidase that catalyzes the hydrolysis of α2-3 and, at a much lower rate, α2-6 linked *N*-acetylneuraminic acid residues from oligosaccharides.

Detailed Specificity:

This enzyme has a 260-fold preference for α2-3 sialyl linkages over α2-6 sialyl linkages and shows only trace activity against α2-8 sialyl linkages. To hydrolyze α2-3 linkages selectively, an initial 10-fold dilution of the enzyme, using 1X GlycoBuffer 1 supplemented with 100 μg/ml BSA is recommended. α2-3 Neuraminidase does not cleave branched sialic acid residues.

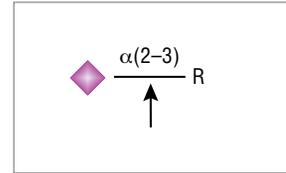


α2-3 Neuraminidase P0728S/L

α2-3 Neuraminidase S

α2-3 Neuraminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of α2-3 linked *N*-acetyl-neuraminic acid residues from glycoproteins and oligosaccharides.

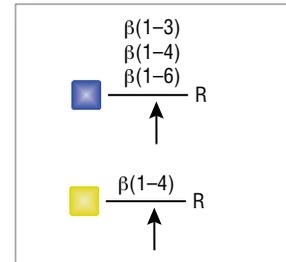
α2-3 Neuraminidase S is cloned from *Streptococcus pneumoniae* and expressed in *E. coli*.



α2-3 Neuraminidase S P0743S/L

β-N-Acetylhexosaminidase_f

β-*N*-Acetylhexosaminidase_p, 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-*D*-*N*-Acetylgalactosamine and glucosamine residues from linear oligosaccharides.



β-*N*-Acetylhexosaminidase_f P0721S/L

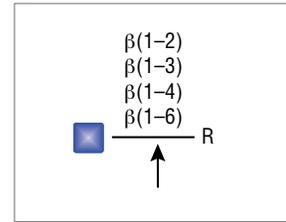


β-N-Acetylglucosaminidase

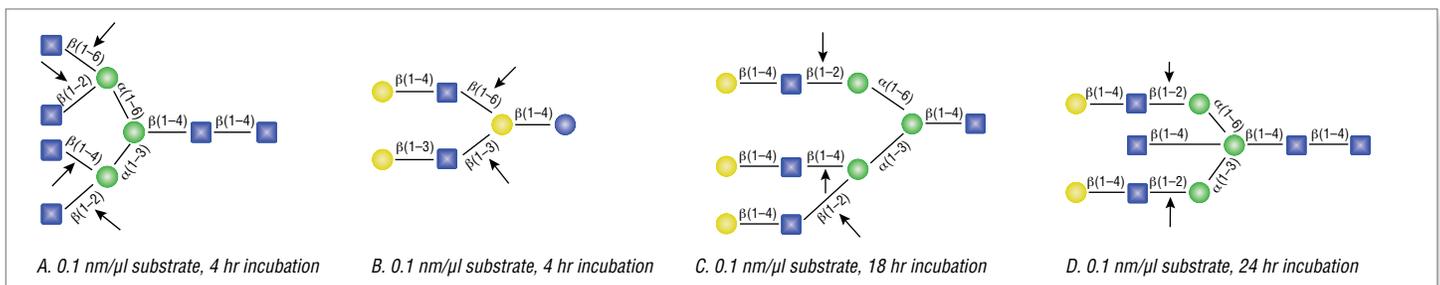
β-N-Acetylglucosaminidase, cloned from *Xanthomonas manihotis*, is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal, non-reducing β-N-Acetylglucosamine residues from oligosaccharides.

Detailed Specificity:

The specificity of the enzyme can vary depending on incubation time and branching structure (see figure below). All reactions contained 4 units of β-N-Acetylglucosaminidase, 1X GlycoBuffer 1 and 1X BSA in a total reaction volume of 10 μl and were incubated at 37°C. Reactions (B), (C) and (D) were treated with 8 units of β1-4 Galactosidase prior to treatment with β-N-Acetylglucosaminidase.



β-N-Acetylglucosaminidase P0732S/L



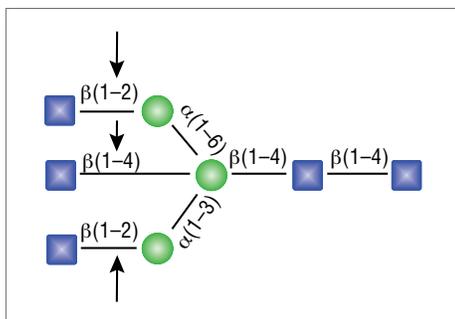
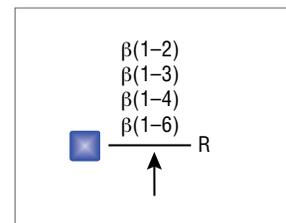
β-N-Acetylglucosaminidase S

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.

Detailed Specificity:

β-N-Acetylglucosaminidase S is able to efficiently cleave bisecting β-N-Acetylglucosaminidase residues.

β-N-Acetylglucosaminidase S P0744S/L





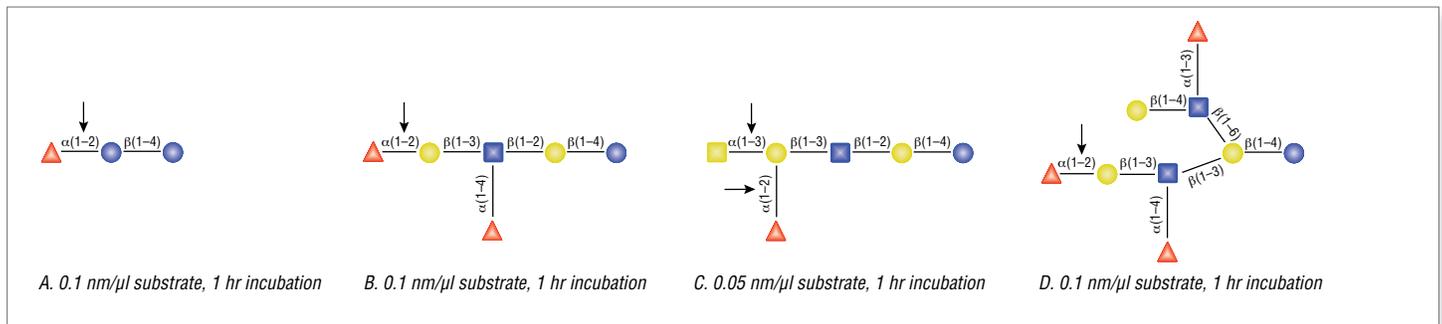
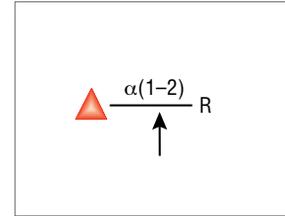
α1-2 Fucosidase

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

Detailed Specificity:

The specificity of the enzyme can vary depending on incubation time and branching structure (see figure below). All reactions contained 1X GlycoBuffer 1 and 1X BSA in a total reaction volume of 10 μl, and were incubated at 37°C. All reactions contained 20 units of α1-2 Fucosidase. Reaction (C) also contained 20 units of α-N-Acetylgalactosaminidase (NEB #P0734). In reaction (C), the branched α1-2 fucose is removed in the presence of both enzymes, but not by α1-2 Fucosidase alone.

α1-2 Fucosidase P0724S/L



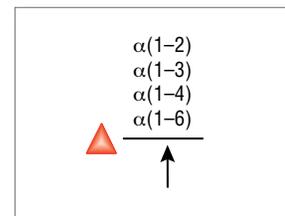
α1-2,3,4,6 Fucosidase

α1-2,3,4,6 Fucosidase, cloned from bovine kidney, is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-2, α1-3, α1-4 and α1-6 linked L-fucopyranosyl residues from oligosaccharides.

Detailed Specificity:

α1-2,3,4,6 Fucosidase cleaves α1-6 fucose residues more efficiently than other linkages, and has slight activity towards α1-3 fucose residues.

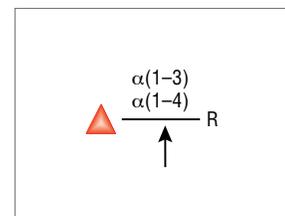
α1-2,3,4,6 Fucosidase P0748S/L



α1-3,4 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



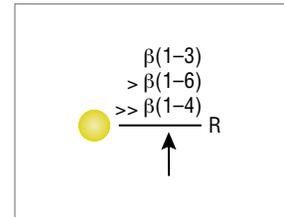


β1-3 Galactosidase

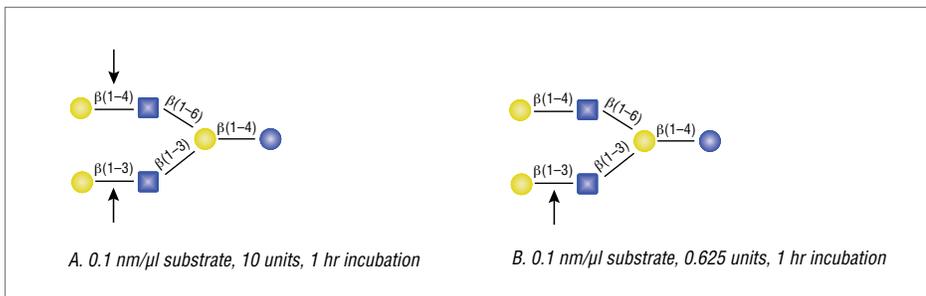
β1-3 Galactosidase, cloned from *Xanthomonas manihotis*, is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-3 and, at a much lower rate, β1-6 linked D-galactopyranosyl residues from oligosaccharides. The approximate kinetic data show >100-fold preference for β1-3 over β1-6 linkages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

Detailed Specificity:

The GlcNAc (β1-6) residue is the only anomeric configuration that can affect the specificity of the enzyme enabling cleavage of the non-reducing β1-4 Galactose. The selling concentration of the enzyme will cut the β1-4 Galactose linkage as shown in A (below), due to the adjacent GlcNAcβ1-6 anomer. This cleavage will not occur if the selling concentration of the enzyme is diluted 16-fold, shown in B (below).



β1-3 Galactosidase P0726S/L

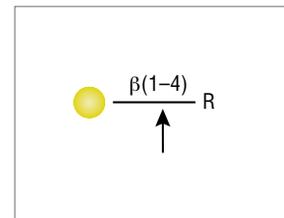


β1-4 Galactosidase

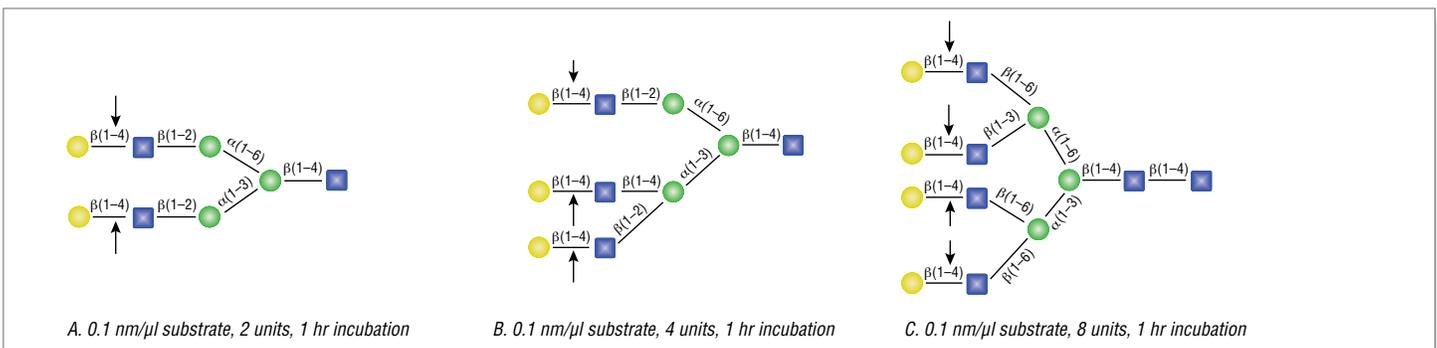
β1-4 Galactosidase, cloned from *Bacteroides fragilis*, is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-4 linked D-galactopyranosyl residues from oligosaccharides.

Detailed Specificity:

Specificity can vary depending on incubation time and branching structure. Reactions (A), (B) and (C) shown below contained 2 units, 4 units and 8 units of β1-4 Galactosidase, respectively and 1X GlycoBuffer 1 in a total reaction volume of 10 μl. Reactions were incubated at 37°C.



β1-4 Galactosidase P0730S/L

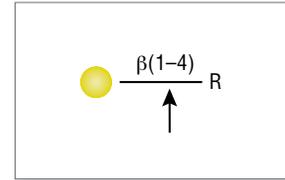




β 1-4 Galactosidase S

β 1-4 Galactosidase S, cloned from *Streptococcus pneumoniae*, is a highly specific exoglycosidase that catalyzes the hydrolysis of β 1-4 linked galactose residues from oligosaccharides.

β 1-4 Galactosidase S P0745S/L



α 1-6 Mannosidase

α 1-6 Mannosidase, cloned from *Xanthomonas manihotis*, is a highly specific exoglycosidase that removes unbranched α 1-6 linked D-mannopyranosyl residues from oligosaccharides. When used in conjunction with α 1-2,3 Mannosidase, the α 1-6 Mannosidase will cleave α 1-6 Mannose residues from branched carbohydrate substrates.

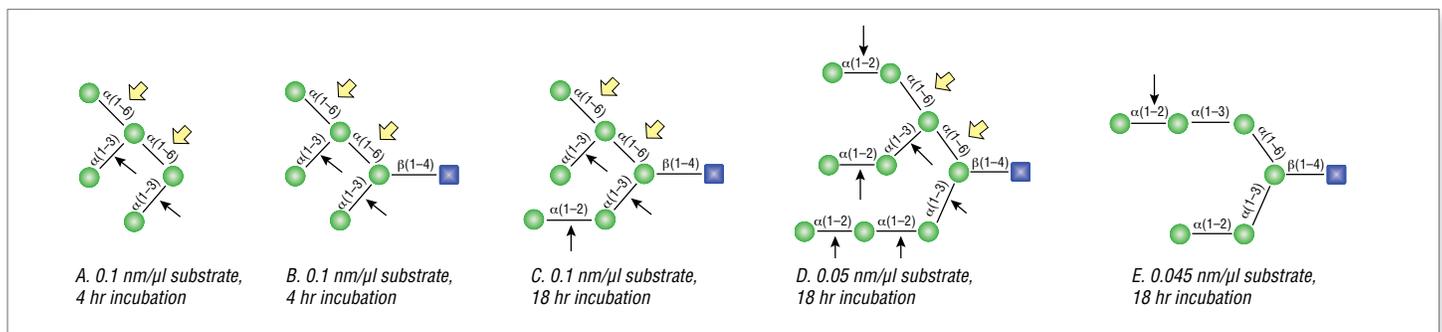
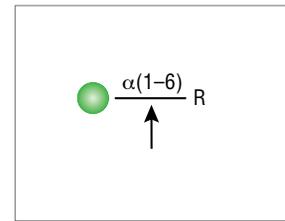
Detailed Specificity:

Specificity can vary depending on incubation time and branching structure (see figure below).

All reactions contained 32 units of α 1-2,3 Mannosidase (NEB #P0729), 40 units of α 1-6 Mannosidase, 1X GlycoBuffer 1 and 1X BSA in a total reaction volume of 10 μ l. Reactions were incubated at 37°C.

The substrate depicted in (E) will not cut to completion. If this structure exists in any substrate it will be impervious to cleavage by α 1-6 Mannosidase. Note: When used alone, α 1-6 Mannosidase will still act only on linear substrates. When used in conjunction with α 1-2,3 Mannosidase, the α 1-6 Mannosidase will cleave α 1-6 Mannose residues from branched carbohydrate substrates.

α 1-6 Mannosidase P0727S/L



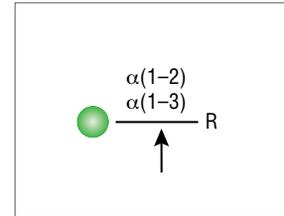


α1-2,3 Mannosidase

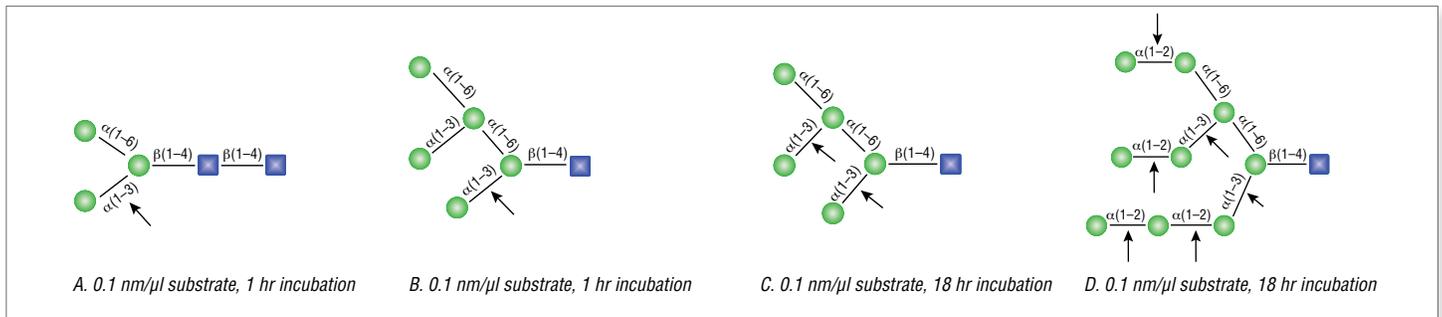
α1-2,3 Mannosidase, cloned from *Xanthomonas manihotis*, is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-2 and α1-3 linked D-mannopyranosyl residues from oligosaccharides.

Detailed Specificity:

Specificity can vary depending on incubation time and concentration of substrate. All reactions contained 32 units of α1-2,3 Mannosidase, 1X GlycoBuffer 1 and 1X BSA in a total reaction volume of 10 μl, and reactions were incubated at 37°C.



α1-2,3 Mannosidase P0729S/L

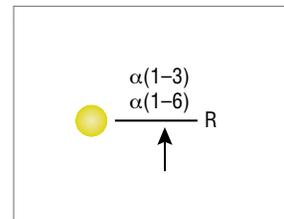


α1-3,6 Galactosidase

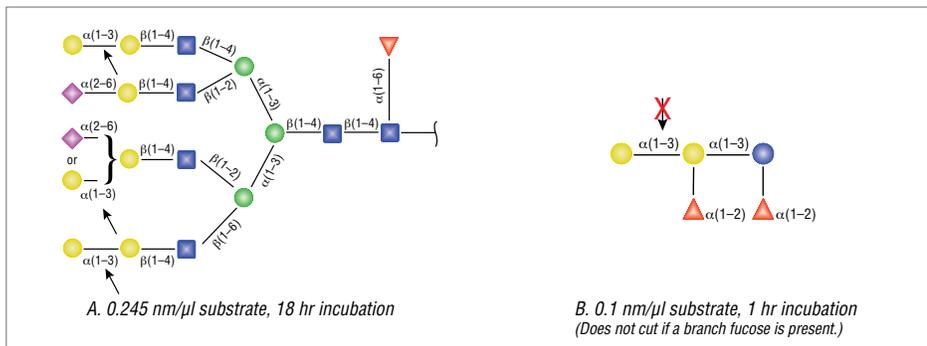
α1-3,6 Galactosidase, cloned from *Xanthomonas manihotis*, is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-3,6 linked D-galactopyranosyl residues from oligosaccharides.

Detailed Specificity:

Specificity can vary depending on incubation time and branching structure (see figure below). Reaction (B) contained 4 units of α1-3,6 Galactosidase, 1X GlycoBuffer 1 and 1X BSA in a total reaction volume of 10 μl. Reaction (A) contained 24 units of α1-3,6 Galactosidase and 100 units of Neuraminidase, followed by a heat kill at 65°C for 10 minutes and a 2 hour digestion with 16 units of β1-4 Galactosidase. The reaction in (A) contained 1X GlycoBuffer 1 and 1X BSA in a total reaction volume of 20 μl. The reactions were incubated at 37°C. Complete digestion by the α1-3,6 Galactosidase was determined by an observation of complete transformation of the substrate in (A) to the non-reducing terminal N-acetylglucosamine tetra-antennary oligosaccharide. Reaction (B) shows that branched fucose inhibits cleavage.



α1-3,6 Galactosidase P0731S/L



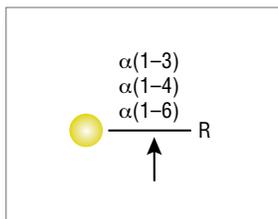
Gal ● Glc ● Man ● GalNAc ● GlcNAc ● Fuc ▲ NeuAc ◆ R = any sugar



α 1-3,4,6 Galactosidase

α 1-3,4,6 Galactosidase, cloned from green coffee bean, is a highly specific exoglycosidase that catalyzes the hydrolysis of α 1-3, α 1-4 and α 1-6 linked D-galactopyranosyl residues from oligosaccharides.

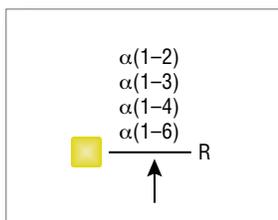
α 1-3,4,6 Galactosidase P0747S/L



α -N-Acetylgalactosaminidase

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

α -N-Acetylgalactosaminidase P0734S/L



EXOGLYCOSIDASE REACTION PROTOCOL

1. Combine 1 nmol of substrate, 1 μ l 10X GlycoBuffer 1, and H₂O to make a total reaction volume of 10 μ l.
2. Add 1 μ l exoglycosidase enzyme, mix gently.
3. Incubate reaction for 1 hour at 37°C.
4. Analyze by method of choice.

Note: The amount of exoglycosidase enzyme required varies when different substrates are used. Start with 1–2 μ l of exoglycosidase for 10–50 μ g of glycoprotein or 1 nmol of oligosaccharide. If after a 1 hour incubation there is still undigested material, let the reaction go overnight.

The protocol can be scaled up linearly to accommodate more substrate and larger reaction volumes. It is imperative to increase the amount of enzyme as you increase the amount of substrate.

Exoglycosidases can be used concomitantly with other endo- and exoglycosidases for double digest reactions. Most exoglycosidase double digests can be done using 1X GlycoBuffer 1 (50 mM NaAcetate, pH 5.5, 5 mM CaCl₂). Whereas, endoglycosidase reactions coupled with exoglycosidases should be done using 1X GlycoBuffer 2 (50 mM sodium phosphate, pH 7.5). First digest your glycoprotein with PNGase F, Endo H or O-Glycosidase, heat-kill the enzyme at 65°C for 10 minutes, and then treat with the exoglycosidase in the same 1X GlycoBuffer 2.

GlycoBuffer Compositions

1X GlycoBuffer 1:

50 mM sodium acetate (pH 5.5 @ 25°C), 5 mM CaCl₂

1X GlycoBuffer 2:

50 mM sodium phosphate (pH 7.5 @ 25°C)

1X GlycoBuffer 3:

50 mM sodium acetate (pH 6.0 @ 25°C)

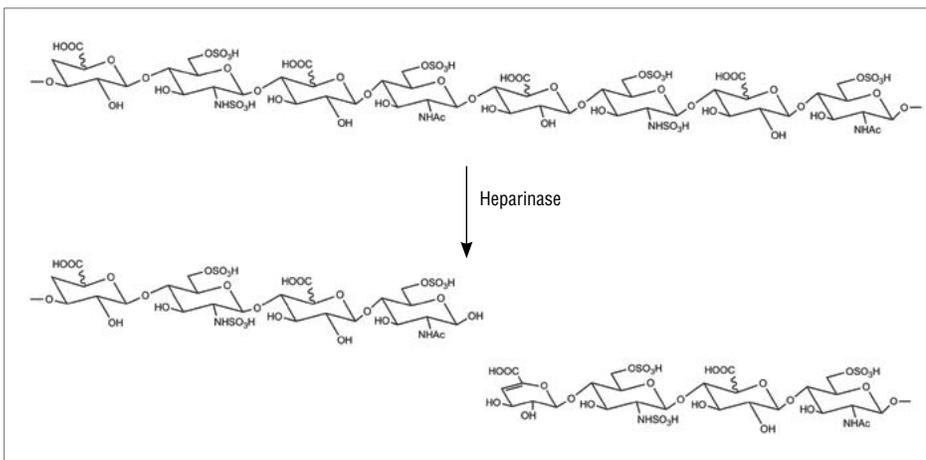


Heparin Lyase Enzymes

Heparin and heparan sulfate (HS) glycosaminoglycans are linear sulfated polysaccharides located on cell-surface membranes and in extracellular matrices in virtually all animal tissues. Heparin and HS have been implicated in cell-biological processes, cell adhesion and regulation of enzymatic catalysis (1). HS chains have been shown to interact with a variety of growth factors, chemokines, ECM proteins, and enzymes, including antithrombin, fibroblast growth factors and vascular endothelial growth factor (2). Heparin has been widely used as an anticoagulant drug (3,4), and it has been shown to regulate cellular process by binding, stabilizing and activating various growth factors (5).

Heparin/HS chains consist of repeating disaccharide units of GlcA β (1-4)GlcNAc α (1-4) with poly-disperse sulfation, N-acetylation and uronosyl epimerization. Repeating disaccharide residues varying between one to three sulfate groups exist in heparin/HS which results in domains of high and low sulfation. During biosynthesis incomplete sulfation by transferases leads to the creation of more structurally complex polysaccharides than that of other glycan classes. The structural elucidation of such complex and diverse polysaccharides is an exceptionally challenging task and cannot be accomplished without enzyme tools.

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 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.



The eliminative mechanism of a Heparinase enzyme degrading a Heparin/HS polysaccharide into oligosaccharides. The double bond on the non-reducing end of the uronic acid absorbs at 232 nm.

References

1. Fritz, T. et al. (1994) *J. Biol. Chem.* 269, 28809–28814.
2. Linhardt, R.J. et al. (1990) *Biochemistry* 29, 2611–2617.
3. Linhardt, R.J., and Gunay, N.S. (1999) *Semin. Thromb. Hemost.* 25 Suppl 3, 5–16.
4. Casu, B. et al. (2002) *Biochemistry* 41, 10519–10528.
5. Knudsen, C.B. and Knudsen, W. (2001) *Semin. Cell Dev. Biol.* 12, 69–78.



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:

In contrast to the *Flavobacterium heparinum* Heparinase I which cleaves the glycosidic bond between *N*-sulfated hexosamines and 2-*O*-sulfated iduronic acid residues, the *Bacteroides* Heparinase I cleaves between these same 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 GlcN-SO₃ does not hinder enzyme activity. While *Bacteroides* Heparinase I cleaves 2-*O* sulfated iduronic acid and 2-*O* sulfated glucuronic acid residues at similar rates, the *Flavobacterium heparinum* Heparinase I has a much higher rate of cleavage for 2-*O* sulfated iduronic acid residues (1). Limited digestion of porcine mucosal heparin with *Flavobacterium heparinum* Heparinase I results in sulfated heparin oligosaccharides structures previously reported (2). Limited digest of porcine mucosal heparin with the *Bacteroides* Heparinase I results in heparin oligosaccharides with a lower extent of sulfation as reported (3).

Bacteroides Heparinase I P0735S/L

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:

Similar to *Flavobacterium heparinum* Heparinase II, *Bacteroides* Heparinase II cleaves the glycosidic bond between *N*-sulfated and 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 III

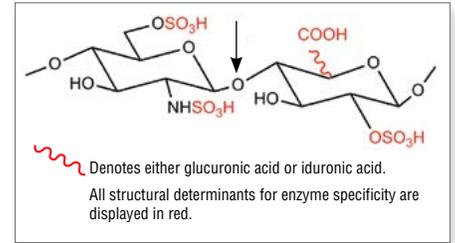
Bacteroides Heparinase III cloned from *Bacteroides eggerthii*, also called Heparin Lyase III, is active on both heparin and heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Detailed Specificity:

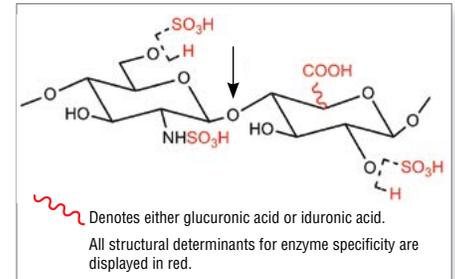
In stark contrast to the *Flavobacterium heparinum* Heparinase III that cleaves the glycosidic bond only between hexosamines and glucuronic acid residues, the *Bacteroides* Heparinase III can cleave the glycosidic bond between hexosamines and either iduronic acid or glucuronic acid residues. *Flavobacterium heparinum* Heparinase III is not active on an *N*-sulfolucosamine with 6-sulfation, whereas the *Bacteroides* Heparinase III is active in the presence of 6-sulfation.

Bacteroides Heparinase III P0737S/L

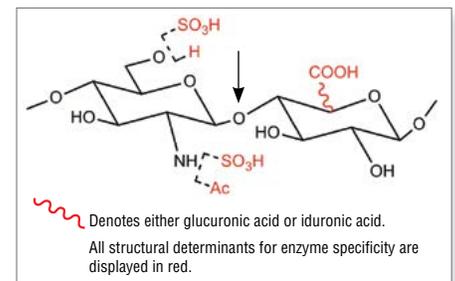
Note: Heparin Hexasaccharide MS Standard 6 (NEB #P0738S) and Heparin Hexasaccharide MS Standard 7 (NEB #P0739S) are available by special order. Contact info@neb.com for details.



Bacteroides Heparinase I specificity.



Bacteroides Heparinase II specificity.



Bacteroides Heparinase III specificity.

References

1. Landry, D. et al. New England Biolabs, Inc., unpublished results.
2. Yamada, S. et al. (1995) *J. Biol. Chem.* 270, 8696–8705.
3. 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.

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: CPAP[▼]ELLG[▼]GPSVF
 human IgG2: CPAPPVA[▼]GPSVF
 murine IgG2a: CPAPNLLG[▼]GPSVF
 murine IgG3: CPPGNILG[▼]GPSVF

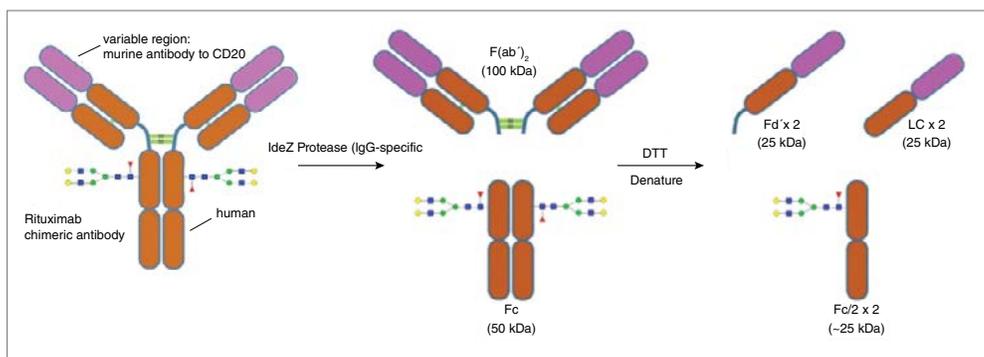
IdeZ PROTEASE (IgG-SPECIFIC) REACTION PROTOCOL

The quantity of enzyme recommended is sufficient for the deglycosylation of 50 µg of IgG under non-denaturing conditions. Reactions may be scaled-up linearly to accommodate larger amounts of IgG. Optimal incubation times may vary for a particular substrate.

1. Combine up to 50 µg of human IgG and H₂O to a volume of 25 µl.
2. Add 2.5 µl of 10X GlycoBuffer 2.
3. Add 1 µl of IdeZ Protease (IgG-specific).
4. Incubate 30 minutes* at 37°C.
5. Prepare IgG sample for SDS-PAGE or mass spectrometry analysis.

**Note: Extend incubation time to 2 hours for murine IgG2a.*

IdeZ Protease (IgG-specific).....P0770S



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

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

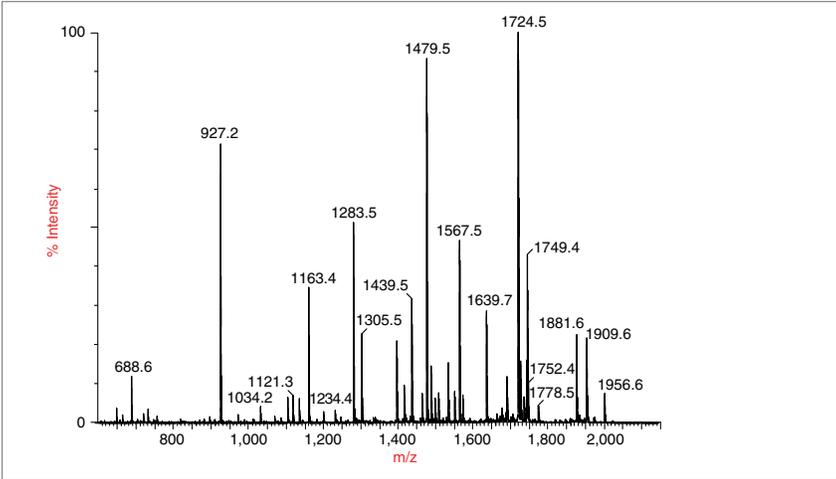
IdeZ Protease does not cleave mouse IgG1 or IgG2b, rat, porcine, bovine or goat IgG. It also does not cleave non-IgG isotypes including IgA, IgM, IgD and IgE.



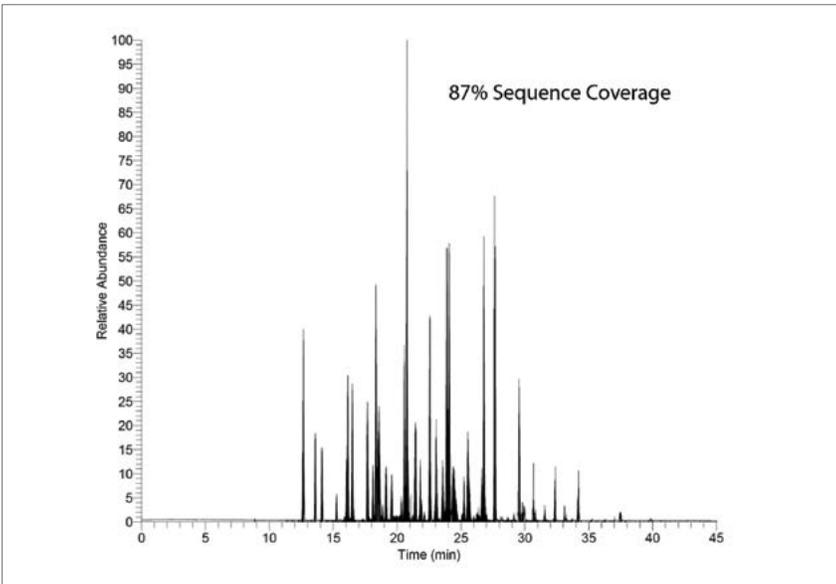
Trypsin-digested BSA MS Standard (CAM-modified)

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



MALDI Analysis of BSA Digest: BSA digest solution diluted to 1 pmol/μl and mixed at a 1:1 ratio with α-cyano-4-hydroxycinnamic acid (10 mg/μl in 50:50 acetonitrile:water with 0.1% trifluoroacetic acid). 2 μl digest/matrix solution was spotted directly onto a MALDI target plate. The sample was analyzed on a MALDI micro MX Mass Spectrometer (Waters Corporation) with a laser energy of 150.



Online analysis of Trypsin digest of BSA: BSA digest solution diluted to 100 fmol/μl with 0.1% formic acid. 1 μl digest solution was injected via a Proxeon Easy n1000 LC system (Thermo Fisher) onto a self-packed C18 column (100 ID x 20 cm, Aqua 3μ C18 packing material). Peptides were separated using a 30 min 5-40% B linear gradient (A = 0.1% formic acid, B = Acetonitrile, 0.1% formic acid) at a flow rate of 400 nl/min and analyzed online by a Q Exactive mass spectrometer (Thermo Fisher) with a nano-electrospray ionization source. Acquisition range was from 400 to 1600 m/z and a source voltage of 2.5 kV was used.



Trypsin-ultra,TM Mass Spectrometry Grade

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

Xxx-Arg▼Xxx

Xxx-Lys▼Xxx

Endoproteinase GluC

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

Xxx-Glu▼Xxx

Xxx-Asp▼Xxx



TRYPSIN DIGESTION PROTOCOL USING NEB TRYPSIN-ULTRA AND THE FASP™ KIT (1)

The starting material can be cells, proteome extracts, protein complexes or pure proteins. The total amount depends on goals and complexity: for whole proteomes use between 5–50 µg, for pure proteins use between 0.5–10 µg. Please note the use of too much material can have a negative effect. Never use more than 50 µg of total protein, as filter clogging will occur.

1. Add 200 µl of 1% SDS to each sample, vortex briefly. The use of SDS can be omitted if sample is soluble proteins or complexes.
2. Heat at 60°C for 5 minutes.
3. Allow samples to cool to room temperature.
4. Add 200 µl 100 mM Tris/8 M Urea/10 mM DTT to each sample. Vortex briefly.
 - a. To prepare urea solution: Add 1 ml 100 mM Tris-HCl pH 8.5 (provided with FASP Kit) to one tube of urea (provided with FASP Kit). Vortex until all powder dissolves and add DTT to a 10 mM concentration.
5. Rock at room temperature for 30 minutes.
6. Transfer protein-urea mixture to spin filter (provided in FASP Kit). Centrifuge at 14,000 x g for 10 minutes.
7. Add 200 µl fresh 100 mM Tris/8 M Urea solution.
 - a. To prepare urea solution: Prepare the same as above, omitting DTT.
8. Centrifuge at 14,000 x g for 10 minutes.
9. Discard flow-through.
10. Add 10 µl prepared iodoacetamide solution and 90 µl 100 mM Tris/8 M urea solution. Incubate without mixing for 20 minutes in the dark
 - a. To prepare iodoacetamide solution: Add 100 µl of 100 mM Tris/8 M urea solution to 1 tube iodoacetamide (provided with FASP Kit). Pipette up and down 10-15 times to mix well.
11. Centrifuge at 14,000 x g for 10 minutes.
12. Add 200 µl 100 mM Tris/8 M urea solution. Centrifuge at 14,000 x g for 10 minutes.
13. Discard flow-through.
14. Add 200 µl 50 mM ammonium bicarbonate solution (provided with FASP Kit). Centrifuge at 14,000 x g for 10 minutes.
15. Transfer filter to new collection tube.
16. Add digestion solution. Pipette up and down to mix on top of filter. Incubate at 37°C for 2 hours (no rocking).
 - a. To prepare digestion solution: Add 1 ml 50 mM ammonium bicarbonate solution (provided with FASP Kit) to 20 µg Trypsin-ultra (NEB #P8101S) to make a 20 ng/µl trypsin solution. Add trypsin solution to top of filter at a 1:50 protein:trypsin ratio, and add 50 mM ammonium bicarbonate solution to bring total digestion solution volume to 100 µl.
17. Add 80 µl 50 mM ammonium bicarbonate solution. Centrifuge at 14,000 x g for 10 minutes.
18. Add 30 µl 50% acetonitrile/0.1% formic acid. Centrifuge at 14,000 x g for 10 minutes.
19. Add 40 µl 0.1% formic acid/water. Centrifuge at 14,000 x g for 10 minutes.
20. Filtrate contains digested peptides.
21. Analyze the peptides by 1D or 2D-LC-MS/MS.

Notes:

1. The FASP™ Protein Digestion Kit is a product of Expedeon, Inc. and is available here: (<http://shop.expedeon.com/products/25-Protein-Digestion-for-MS/>).
2. Trypsin-ultra has been found to have very low levels of autocleavage as compared to other MS grade trypsin. Therefore, using excess Trypsin-ultra will not lower the quality of the data obtained since excess trypsin will remain intact and be retained by the filter.
3. Volumes may be adjusted based on needs and the concentration of the sample. For 1D analysis with an autosampler, where minimal volume is needed, it is possible to cut elution volumes in half without negative effects.



Glossary of Glycobiology Terms

β-elimination- The cleavage of a C-O or C-N bond positioned on the β-carbon with respect to a carbonyl group. The process is used to cleave O-glycans from serine or threonine residues.

Carbohydrate- A generic term used interchangeably with sugar and glycan. This term includes monosaccharides, oligosaccharides and polysaccharides.

Complex N-glycan- In contrast to mannose glycans, complex N-glycans have been processed in the golgi and might contain a variety of monosaccharides.

Endoglycosidase- An enzyme that catalyzes the cleavage of an internal glycosidic linkage in an oligosaccharide or polysaccharide.

Exoglycosidase- An enzyme that cleaves a monosaccharide from the non-reducing end of an oligosaccharide, polysaccharide, or glycoconjugate.

Glycan- A generic term for any sugar, in free form or attached to another molecule, used interchangeably with carbohydrate.

Glycobiology- the study of the structure, function and biology of carbohydrates

Glycomics- the study of glycan diversity in biological systems

Glycoconjugate- A molecule in which one or more glycan units are covalently linked to a non-carbohydrate entity.

Glycoforms- Different molecular forms of a glycoprotein, resulting from variable glycan structure and/or glycan attachment site occupancy.

Glycopeptide- A peptide having one or more covalently attached glycans.

Glycoprotein- A protein with one or more covalently attached glycans.

Glycoproteomics- The systems-level analysis of glycoproteins, including their protein identities, sites of glycosylation and glycan structures.

Glycosaminoglycans- Polysaccharide side chains of proteoglycans or free complex polysaccharides composed of linear disaccharide repeating units each composed of a hexosamine and a hexose or a hexuronic acid.

Glycosylation- The enzyme-catalyzed covalent attachment of a carbohydrate to a polypeptide, lipid, polynucleotide, or another carbohydrate, generally catalyzed by glycosyltransferases.

Hexosamine- Hexose with an amino group in place of the hydroxyl group at the C-2 position. Common examples are the N-acetylated sugars, N-acetylglucosamine and N-acetylgalactosamine.

Hexose- A six-carbon monosaccharide typically with an aldehyde at the C-1 position and hydroxyl groups at all other positions. Common examples are mannose, glucose and galactose.

Hydrazinolysis- A chemical method that uses hydrazine to cleave amide bonds (i.e. the glycosylamine linkage between a glycan residue and asparagine or the acetamide bond in N-acetylhexosamines).

Lectin- A protein that specifically recognizes and binds to glycans without catalyzing a modification of the glycan.

Lyase- a lyase catalyzes the non-hydrolytic removal of a group from a substrate with the resulting formation of a double bond.

Monosaccharide- A carbohydrate that cannot be hydrolyzed into a simpler carbohydrate. It is the building block of oligosaccharides and polysaccharides.

N-linked Glycan- Glycan covalently linked to an asparagine residue of a polypeptide chain in the consensus sequence: -Asn-X-Ser/Thr.

Non-reducing terminus- Outermost end of an oligosaccharide or polysaccharide chain, which is opposite to that of the reducing end.

O-linked Glycan- A glycan glycosidically linked to the hydroxyl group of the amino acids serine or threonine in the consensus GalNAcα1-O-Ser/Thr.

Oligosaccharide- Linear or branched chain of monosaccharides attached to one another via glycosidic linkages. The number of monosaccharide units can vary.

Polysaccharide - Glycan composed of repeating monosaccharides, generally greater than ten monosaccharide units in length.

Proteoglycan- Any protein with one or more covalently attached glycosaminoglycan chains.



Unit Conversion Chart

Reagent companies differ in how a unit of enzyme is defined. This chart can be used to help determine how a unit of enzyme from one company compares to a unit of enzyme from NEB. All enzymes were assayed using NEB's assay protocols as a means of normalization (NEB Assay).

ENZYME	COMPANY	SELLING CONC. (U/ml)	UNITS/VIAL	μl/VIAL	NEB ASSAY (U/ml)	NEB ASSAY UNITS/VIAL	μl CONVERSION (1 NEB μl = x COMPANY μl's)
PNGase F	NEB (P0704/P0705)	500,000	15,000	30	500,000	15,000	1
	Prozyme (GKE-5006A)	2.5	0.1	40	150,000	6,000	3.3
	Prozyme (GKE-5020B, Ultra)	10	0.4	40	500,000	20,000	1
	QA Bio (E-PNG01)	5	0.3	60	200,000	12,000	2.5
	Sigma (P7367)	500	50	50	90,000	4,500	5.5
Endo H	NEB (P0702)	500,000	10,000	20	500,000	10,000	1
	Prozyme (GKE-5002)	0.15	0.03	200	30,000	6,000	16.5
α1-6 Mannosidase	NEB (P0727)	40,000	800	20	40,000	800	1
	Prozyme (GKX-80070)	0.2	.008	40	1,000	40	40
	Prozyme (GKX 5010, α1-2,3,6 Man)	150	10	66	4,000	264	10
	QA Bio (E-AM01, α1-2,3,6 Man)	10	0.6	60	2,000	120	20
α1-2,3 Mannosidase	NEB (P0729)	32,000	640	20	32,000	640	1
	Prozyme (GKX 5010, α1-2,3,6 Man)	150	10	66	4,000	264	8
	QA Bio (E-AM01, α1-2,3,6 Man)	10	0.6	60	700	42	45
β1-4 Galactosidase	NEB (P0730/P0745)	8,000	400	50	8,000	400	1
	Prozyme (GKX-5014)	2	0.2	100	8,000	800	1
	Prozyme (GKX 5013, β1-3,4 Gal)	5	0.5	100	2,000	200	4
	QA Bio (E-BG07)	3	0.18	60	40,000	2,400	0.2
	QA Bio (E-BG02, β1-3,4,6 Gal)	2.5	0.5	200	4,000	800	2
β1-3 Galactosidase	NEB (P0726)	10,000	500	50	10,000	500	1
	Prozyme (GKX 5013, β1-3,4 Gal)	5	0.5	100	10,000	1000	1
	QA Bio (E-BG02, β1-3,4,6 Gal)	2.5	0.5	200	2,000	400	5
β-N-Acetyl glucosaminidase	NEB (P0732)*	4,000	100	25	4,000	100	1
	Prozyme (GK80050)	40	1.6	40	2,000	80	2
	QA Bio (E-GL01)	40	2.4	60	2,000	120	2
α-N-Acetyl galactosaminidase	NEB (P0734)	20,000	3,000	150	20,000	3,000	1
	Prozyme (GKX-5001)	5	0.1	20	600	12	33
α1-3,6 Galactosidase	NEB (P0731)	4,000	100	25	4,000	100	1
	Prozyme (GKX-5007, α1-3,4,6 Gal)	100	5	50	120,000	6,000	0.03
α2-3,6,8 Neuraminidase	NEB (P0720)	50,000	2,000	40	50,000	2,000	1
	Prozyme (GKX-5021)	5	1	200	10,000	2,000	5
	Prozyme (GK80040, α2-3,6,8,9 Neur)	5	1	200	5,000	1,000	10
α2-3 Neuraminidase	NEB (P0728)	50,000	2,500	50	50,000	2,500	1
	Prozyme (GK80020)	20	1	50	80,000	4,000	0.625
β-N-Acetyl hexosaminidase ₁	NEB (P0721)	5,000	500	100	5,000	500	1
	Prozyme (GKX-5003)	50	5	100	500	50	10
α1-2 Fucosidase	NEB (P0724)	20,000	1,000	50	20,000	1,000	1
	Prozyme (GK80170)	0.2	0.004	20	500	10	40

*NEB (P0732) requires a longer incubation time for cleavage of β1-2 linkages.



Citations

NEB Glycosidase Publications:

Magnelli, P., Bielik, A. and Guthrie, E. (2011). Identification and characterization of protein glycosylation using specific endo- and exoglycosidases. pp. 189–211. In J.L. Hartley (Ed.), *Protein Expression in Mammalian Cells, Methods and Protocols*. New York: Humana Press.

Kousioulis, D., Landry, D. and Guthrie, E.P. (2008). Novel endo-beta-N-acetylgalactosaminidases with broader substrate specificity. *Glycobiology* 18, 799–805.

Wong-Madden, S.T., Landry, D., and Guthrie, E.P. (1997). Discovery and Uses of Novel Glycosidases. In Reid Townsend and Arland T. Hotchkiss (Eds.), *Techniques in Glycobiology* (pp. 401–408).

Articles using PNGase F (P0704/P0705) and Endo H (P0702/P0703):

Chen C., et al. (2012). All-trans-retinoic acid modulates ICAM-1 N-glycan composition by influencing GnT-III levels and inhibits cell adhesion and trans-endothelial migration. *PLoS One* 7, e52975.

McCurdy, T.R., et al. (2012). *In vivo* clearance of alpha-1 acid glycoprotein is influenced by the extent of its N-linked glycosylation and by its interaction with the vessel wall. *J. Biomed. Biotechnol.* 2012:292730.

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Ordering Information

Deglycosylation Enzymes

PRODUCT	NEB #	SIZE
Protein Deglycosylation Mix	P6039S	20 reactions
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 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 ₁	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
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
Endoproteinase GluC	P8100S	50 µg

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Exoglycosidase Enzymes

PRODUCT	NEB #	SIZE
α-N-Acetylgalactosaminidase	P0734S/L	3,000/15,000 units (20,000 units/ml)
β-N-Acetylglucosaminidase	P0732S/L	100/500 units (4,000 units/ml)
β-N-Acetylglucosaminidase S	P0744S/L	100/500 units (4,000 units/ml)
β-N-Acetylhexosaminidase ₁	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)
α1-3,4 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)
β1-4 Galactosidase	P0730S/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)
α1-6 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	P0728S/L	2,500/12,500 units (50,000 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
RNase B	P7817S	250 µg
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
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