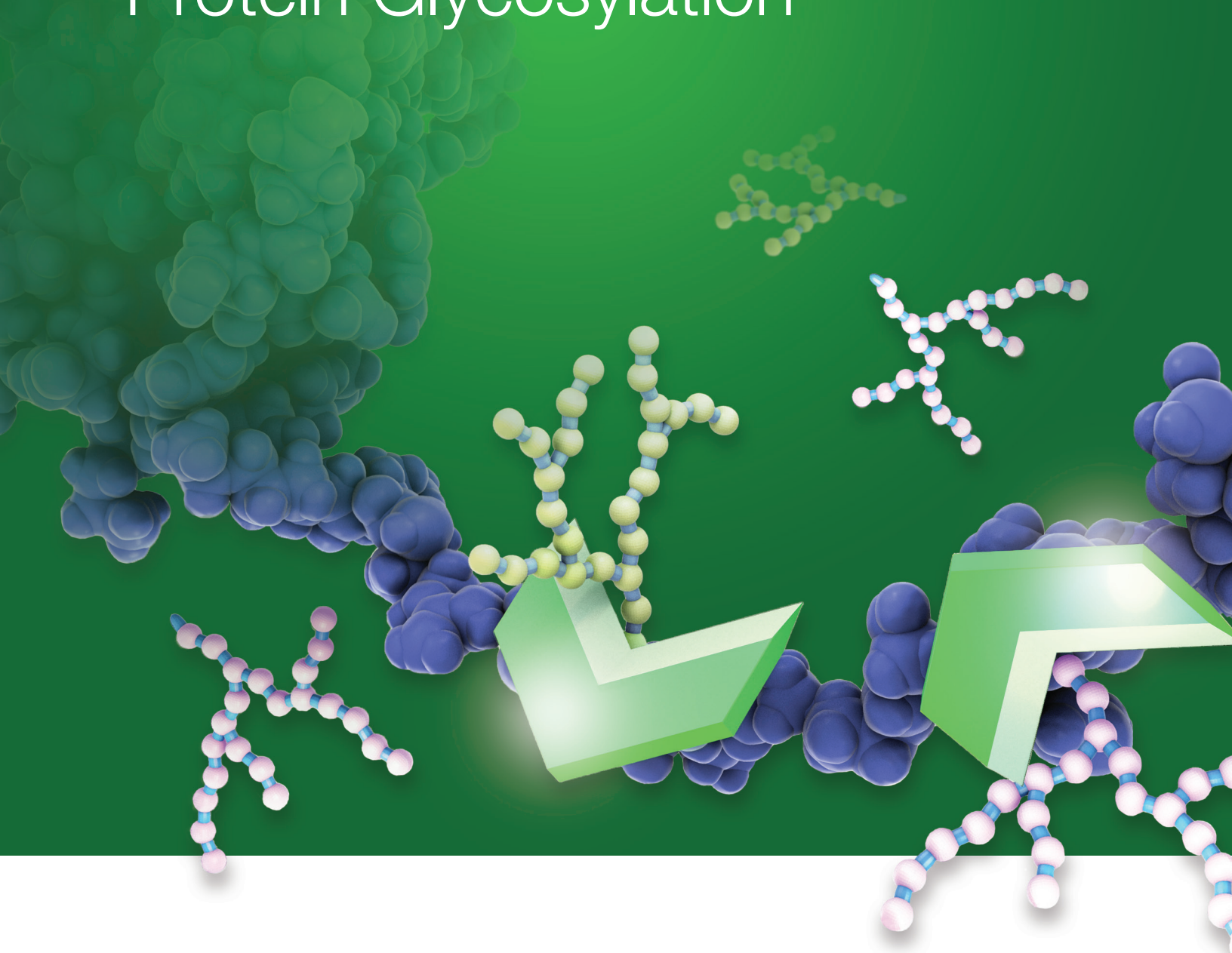


Characterization of Protein Glycosylation



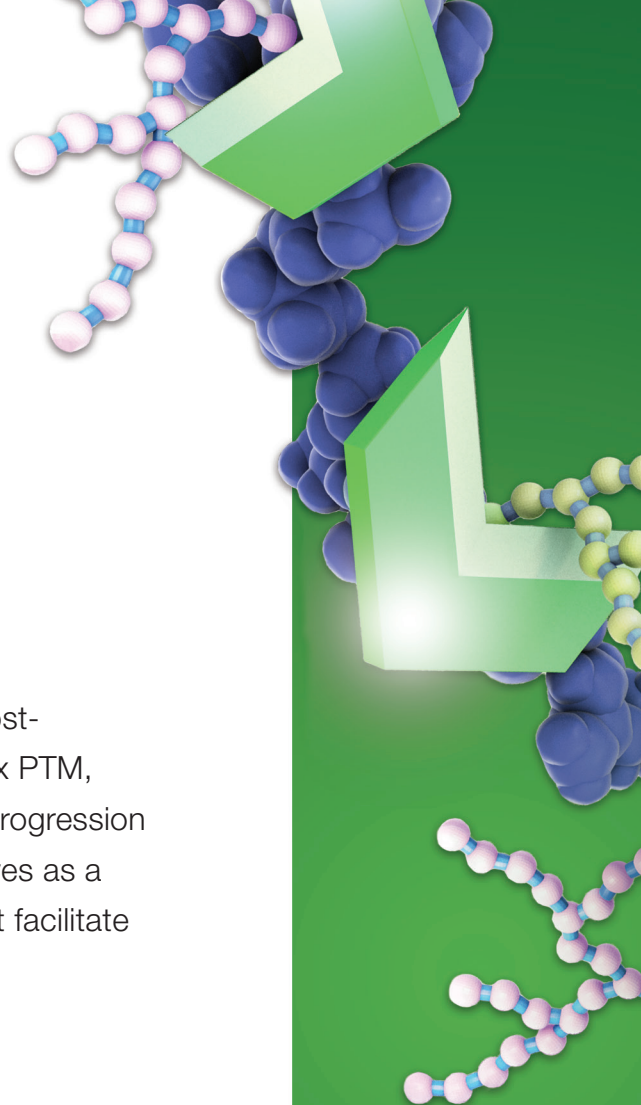
Monitor protein trafficking and determine location
and structure of glycans by mass spectrometry

Characterization of Protein Glycosylation

Glycosylation is among the most abundant of all post-translational modifications (PTMs). A highly complex PTM, glycosylation has diverse roles in biology, disease progression and therapeutic protein function. Glycosylation serves as a biomarker in diseases like cancer, making tools that facilitate glycoprotein characterization extremely important.

 PNGase F

 Endo H



Characterization of Protein Glycosylation

▶ PNGase F

PNGase F, which has a molecular weight of 36kDa, is a recombinant glycosidase cloned from *Elizabethkingia miricola* and overexpressed in *E. coli*. It catalyzes the cleavage of N-linked oligosaccharides between the innermost GlcNAc and asparagine residues of high mannose, hybrid and complex oligosaccharides from N-linked glycoproteins (Figure 1). Despite being active against most N-linked glycans, PNGase F cannot cleave when the innermost GlcNAc is linked to an α -1,3-fucose, most commonly found in plant glycoproteins and in some insect glycoproteins. During glycan removal, the modified asparagine residue is converted to aspartic acid, which imparts a +1Da mass change that can be detected by mass spectrometry workflows utilizing trypsin or alternative proteases.

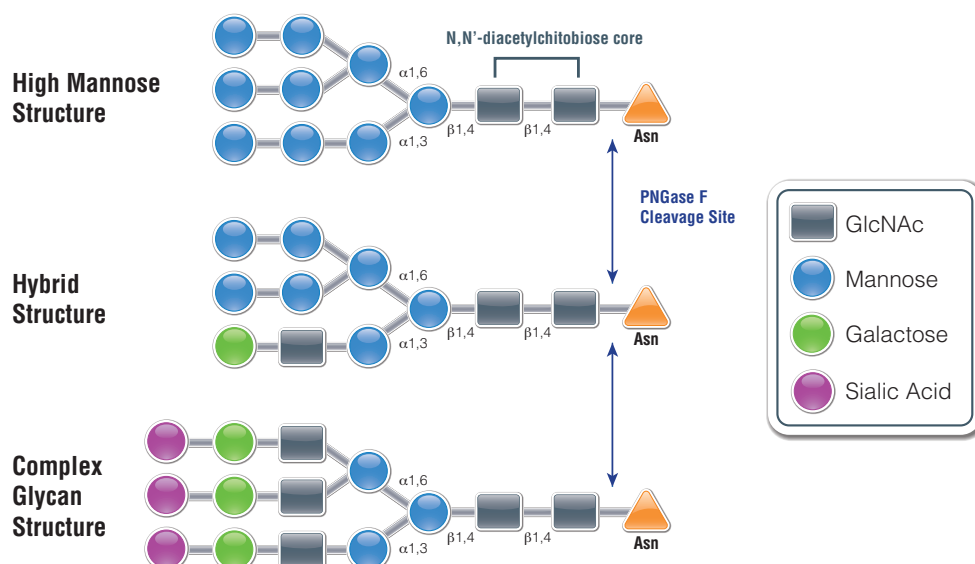


Figure 1. Cleavage specificity of PNGase.

Gel Analysis of Glycoproteins Treated with PNGase F

Determining whether a protein is in fact glycosylated is the initial step in glycoprotein analysis. SDS-PAGE has become a method of choice as the step prior to mass spectrometry analysis. A marked decrease in band width and a change in migration position after treatment with PNGase F is considered evidence of N-linked glycosylation (Figure 2).

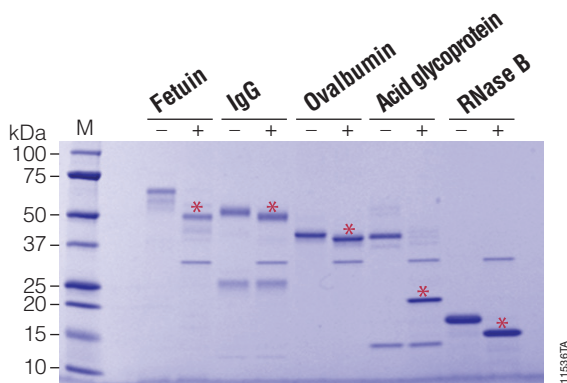


Figure 2. PNGase F treatment of purified glycoproteins. Glycoproteins were incubated in the presence (+) or absence (-) of recombinant PNGase F for two hours. Red asterisks indicate the deglycosylated product.

Gel Analysis of Glycoproteins with LC-MS/MS and PNGase F

Gel-based data is often correlated with information obtained from mass spectrometry analysis. Asn-linked type glycans can be cleaved enzymatically by PNGase F yielding intact oligosaccharides and a slightly modified protein in which Asn residues at the site of de-N-glycosylation are converted to Asp. The deglycosylated peptides are then analyzed by mass spectrometry (Figure 3).

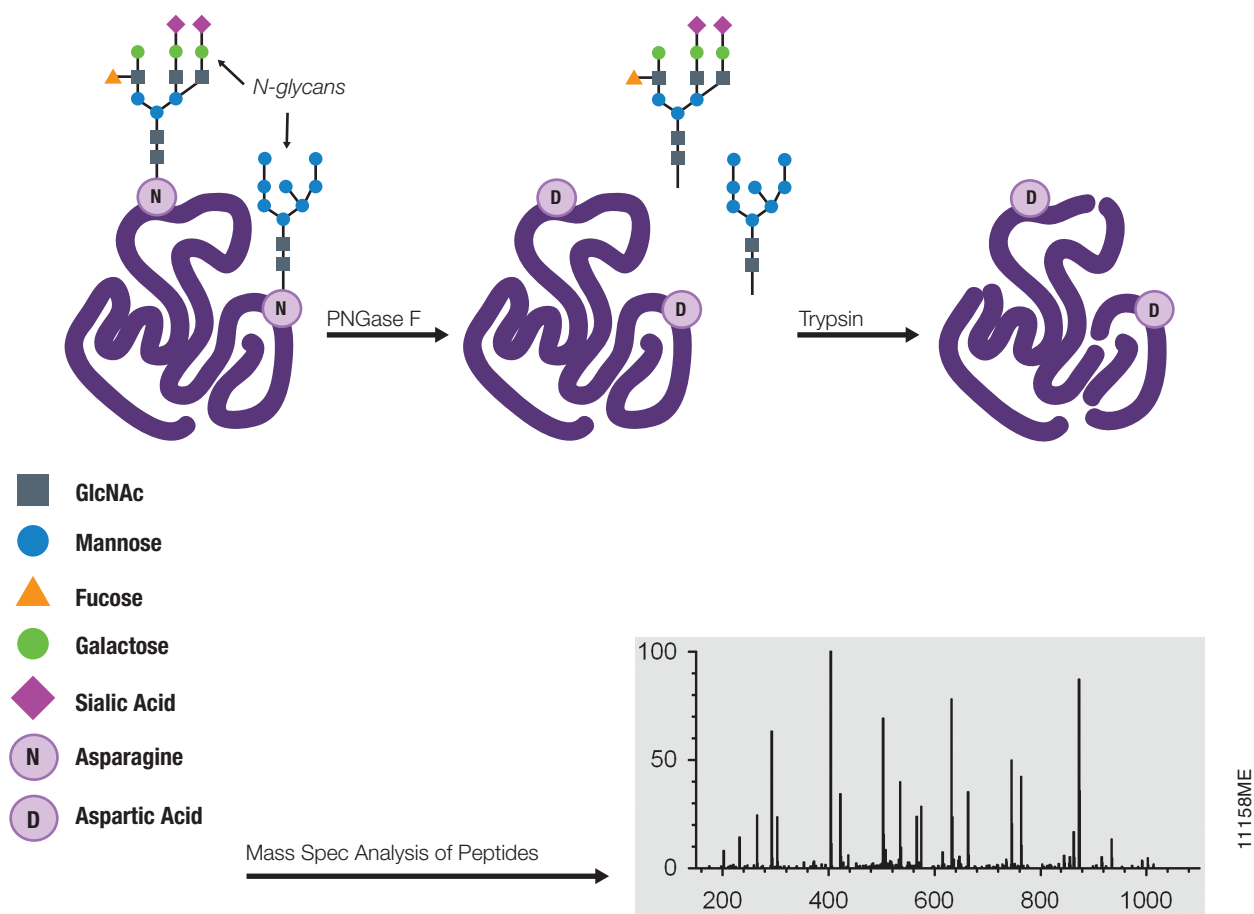


Figure 3. PNGase F used in mass spectrometry analysis of N-linked glycoproteins. The conversion of Asn to Asp results in a monoisotopic mass shift of +1Da that can be measured readily using mass spectrometry.

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Endo H

Endoglycosidase H (Endo H) is a recombinant glycosidase cloned from *Streptomyces plicatus* and over-expressed in *E. coli*. Endo H cleaves the chitobiose core of high mannose and a limited number of hybrid oligosaccharides from N-linked glycoproteins. It does not cleave complex glycans. Enzymatic cleavage is between the two N-acetylglucosamine residues in the diacetylchitobiose core of the oligosaccharide, leaving one N-acetylglucosamine residue on the asparagine. This is in contrast to PNGase F, which cleaves all asparagine-linked oligosaccharides.

Using Endo H and PNGase F to Monitor Protein Trafficking

The unique specificity of Endo H and PNGase F can be used to monitor protein trafficking. Basic N-glycosylation occurs in the endoplasmic reticulum where proteins in this stage are sensitive to Endo H digestion. Proteins that have entered the Golgi body, where additional modifications occur to the glycan, are resistant to Endo H digestion (Figure 4), but are amenable to digestion with PNGase F.

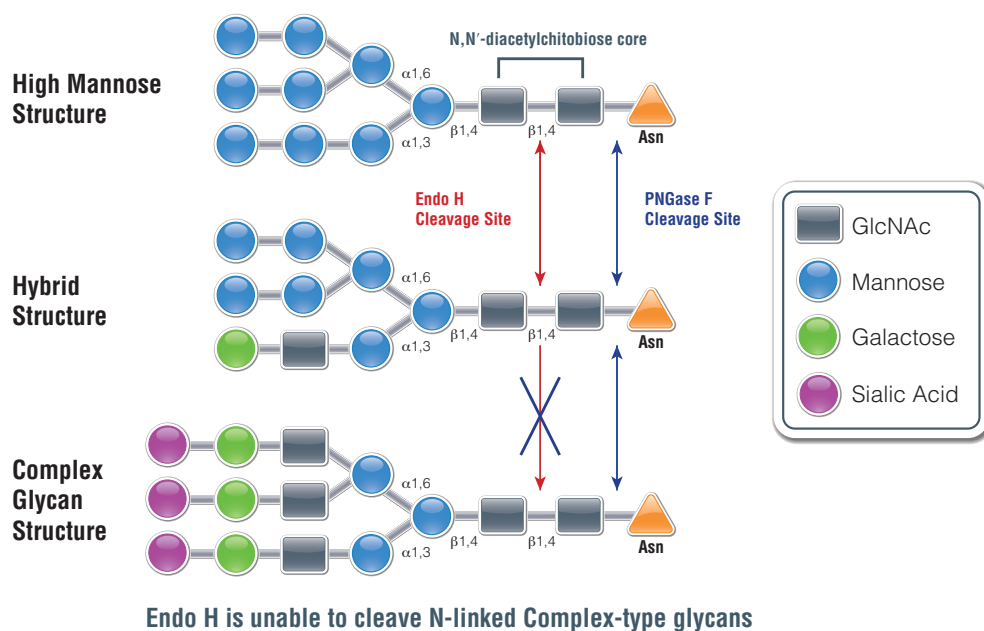
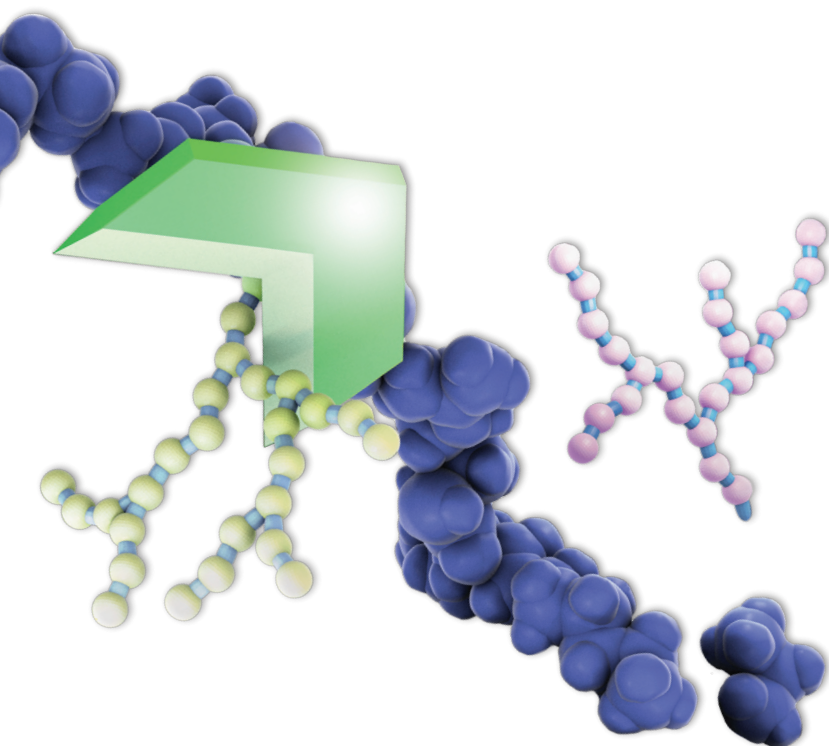


Figure 4. Cleavage specificity of Endo H and PNGase F.

Ordering Information

Related Products	Size	Concentration	Cat. #
PNGase F	500 units	10u/μl	V4831
Endo H	10,000 units	500u/μl	V4871
	50,000 units	500u/μl	V4875
Protein Deglycosylation Mix	20 reactions	–	V4931
Fetuin	500μg	10mg/ml	V4961

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