

Product Information

Invertase Glycoprotein Standard, Proteomics Grade from *Saccharomyces cerevisiae*

Catalog Number **I0408**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

EC 3.2.1.26

Synonym: β -D-fructofuranoside fructohydrolase

Product Description

Glycosylation is one of the most common post-translational modifications of proteins in eukaryotic cells. Glycoproteins are involved in a wide range of biological functions such as receptor binding, cell signaling, immune recognition, inflammation, and pathogenicity. Mammalian glycoproteins contain three major types of oligosaccharides (glycans): N-linked, O-linked, and glycosylphosphatidylinositol (GPI) lipid anchors. N-Linked glycans are linked to the protein backbone via an amide bond to asparagine residues in an Asn-Xaa-Ser/Thr motif, where Xaa can be any amino acid, except proline.

Invertase is an enzyme that catalyses the hydrolysis of sucrose to fructose and glucose. The Invertase Glycoprotein Standard is the periplasmic or external (glycosylated) invertase with 50% of its mass as polymannan.^{1,2} A non-glycosylated form (cytoplasmic or internal invertase) also exists.³ The periplasmic invertase molecule can exist in a number of association states, each a multiple of the core glycosylated monomer, a 60 kDa peptide with oligosaccharide chains. Depending on extraction, purification, and storage conditions, it will exist as a dimer, tetramer, hexamer, or octamer.⁴ The basic enzyme subunit contains 14 potential N-glycosylation site sequons, 13 of which are glycosylated with high mannose structures. The remaining site is not occupied.⁵ Since yeast provide a system for protein glycosylation similar to mammalian systems,⁶ periplasmic invertase is often used as a model for the study of oligosaccharide function in glycoproteins^{7,8} and for studies of glycoprotein biosynthesis.⁹

Component

The Invertase Glycoprotein Standard is provided as a lyophilized powder containing ≥ 0.5 mg of protein in a screw cap vial. The user may reconstitute the contents in the vial to the required protein concentration.

Reagents Required But Not Provided

(Catalog Numbers have been supplied)
Proteomics Grade PNGase F (50 units),
Catalog Number P7367
2-Mercaptoethanol, Catalog Number M6250
Octyl β -D-glucopyranoside, Catalog Number O9882
Ammonium bicarbonate, Catalog Number A6141

Precautions and Disclaimer

This product is for laboratory use only, not for drug, household, or other uses. Consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

It is recommended to read the entire technical bulletin prior to starting the procedure.

Preparation Instructions

It is recommended to use ultrapure (18 M Ω -cm or equivalent) water when preparing the reagents.

- **Reaction Buffer** – Weigh out 160 mg of ammonium bicarbonate and dissolve in 100 ml of ultrapure water. Store at 2–8 °C and use within two weeks of preparation.
Note: 50 mM sodium phosphate, pH 7.5, may be used as an alternative reaction buffer, but the sample may require desalting prior to mass spectrometric analysis.
- **Invertase Standard Solution** – Centrifuge vial briefly to collect solid at the bottom. Add 0.45 ml of the Reaction Buffer, agitate gently to dissolve the solid, and centrifuge briefly to obtain an ~ 1.1 mg/ml solution. Store at 2–8 °C and use within four weeks of preparation.

- PNGase F Solution – Centrifuge the vial (50 units) briefly to collect solid at the bottom. Add 0.1 ml of ultrapure water, agitate gently, centrifuge briefly, and store on ice for 5 minutes. Mix the contents gently once more and centrifuge briefly. The concentration is 500 units/ml. Store at 2–8 °C and use within one week of preparation.
- Denaturant Solution – Weigh out 100 mg of octyl β -D-glucopyranoside and add to 4 ml of water. Mix to dissolve and then add 35 μ l of 2-mercaptoethanol followed by 0.965 ml of water to obtain a Denaturant Solution containing 2% octyl β -D-glucopyranoside and 0.1 M 2-mercaptoethanol. Store at 2–8 °C and use within four weeks of preparation.

Storage/Stability

This product is stable for at least one year if stored unopened at 2–8 °C. A reconstituted solution can be stored at 2–8 °C for up to 4 weeks.

Procedure

The invertase standard can be used to demonstrate N-deglycosylation by PNGase F with both in-solution or in-gel procedures.

A. In-Solution Deglycosylation

This method has been optimized to provide a convenient and reproducible method to remove N-linked glycans from invertase. It is compatible with subsequent MALDI-TOF mass spectrometric analysis without interference from the reaction components.

The quantity of PNGase F enzyme recommended in the following procedure is sufficient to deglycosylate 50 μ g of invertase in one hour with incubation at 37 °C.

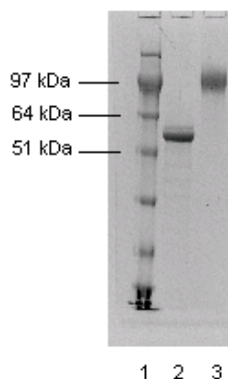
1. Add 90 μ l of the Invertase Standard Solution to a small Eppendorf® tube.
2. Add 5 μ l of Denaturant Solution containing 2% octyl β -D-glucopyranoside and 0.1 M 2-mercaptoethanol. Mix and centrifuge briefly.
3. Incubate at 100 °C for 10 minutes, making sure the cap is firmly closed to prevent evaporation.
4. Allow the solution to cool to room temperature and centrifuge briefly.
5. Add a further 5 μ l of Reaction Buffer, mix, and centrifuge. This makes an ~1 mg/ml solution of invertase.
6. Split the solution by transferring 50 μ l to a separate tube. Label one tube as the control and the other as the test.
7. To the tube labeled test, add 4 μ l of the PNGase F Solution (equivalent to 2.0 units of enzyme). To the control sample, add 4 μ l of water.
Note: The amount of enzyme may be varied for other glycoproteins, depending upon the nature of the glycosylation.
8. Mix and centrifuge briefly.
9. Incubate at 37 °C for 1 hour.
Note: The incubation time may also be varied for partial or complete deglycosylation.
10. Stop the reaction by heating at 100 °C for 10 minutes.
11. Allow the solution to cool and centrifuge briefly.
12. Analyze an aliquot of the reaction mixture by SDS-PAGE to assess deglycosylation.
Note: The reaction mixture may also be lyophilized for subsequent MS analysis of the deglycosylated protein or of the released oligosaccharides (after appropriate treatment).

The provided procedure has been optimized for use with the Invertase Glycoprotein Standard. When using other substrates the reaction conditions may require optimization of incubation time and PNGase F concentration for complete deglycosylation.

Results

One of the simplest methods to assess the extent of deglycosylation is by mobility shift on SDS-PAGE gels. Figure 1 shows the deglycosylation of invertase using 2.0 units of PNGase F Solution. The control invertase sample (lane 3) has an apparent M_r of 97 kDa compared to an apparent M_r of ~60 kDa for the test invertase sample digested with PNGase F (lane 2). The control band is completely absent from lane 2 indicating complete deglycosylation. The ability to detect mobility shifts when the N-linked oligo-saccharides are removed with PNGase F will depend on the molecular mass of the protein and the relative mass contribution by the oligosaccharide.

Figure 1.
In-Solution Deglycosylation of Invertase by
PNGase F Enzyme



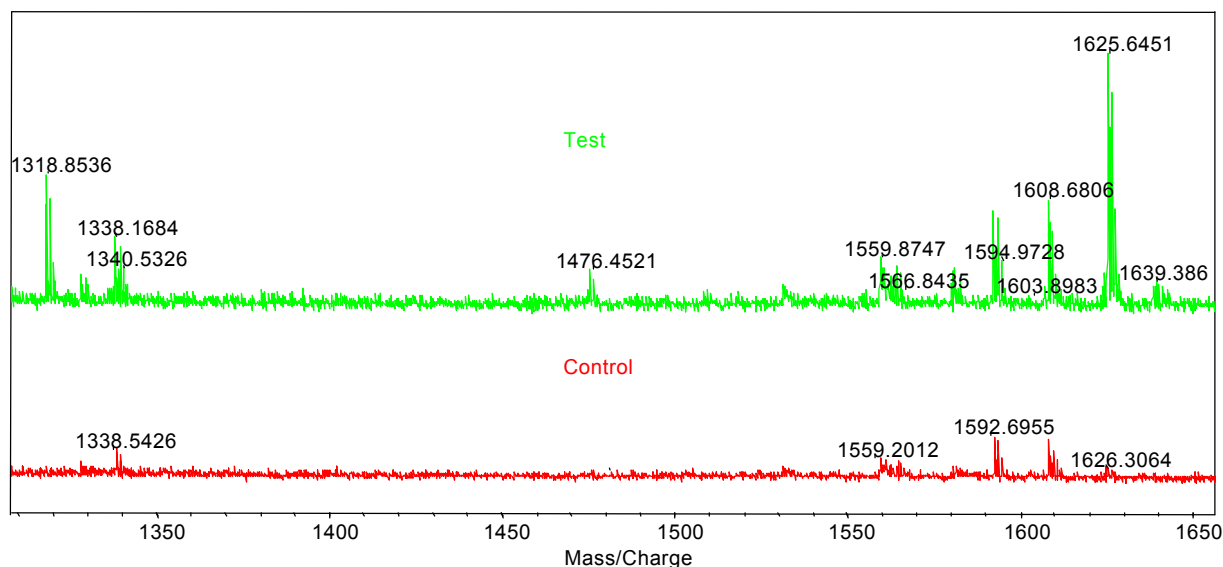
Analysis of the deglycosylation of invertase on 10% SDS-PAGE gel. Lane 1 is a set of molecular weight standards. Lane 2 is the test sample treated with 2.0 units of PNGase F, while Lane 3 is the control sample of denatured invertase.

B. In-Gel Deglycosylation

Full details of this procedure are provided with the GlycoProfile™ I Enzymatic In-Gel N-Deglycosylation Kit, Catalog Number PP0200 or see <http://www.sigma-aldrich.com>

In summary two bands corresponding to invertase are excised from an SDS gel and each slice is destained and dried separately. One sample (test) is rehydrated in a small volume of PNGase F solution and incubated overnight. This gel piece is then washed to remove the released glycans (which can be retained for analysis if desired) and dried. This and the untreated (control) gel slice are both rehydrated in trypsin solution and again incubated overnight. After this step the solution surrounding each gel piece is collected and the slice is washed to ensure maximum recovery of peptides. Each collected sample solution is combined with its respective washes and the volume is reduced. The tryptic peptides in each sample are then analyzed by MALDI-TOF mass spectrometry. Typical results obtained with invertase are shown in Figure 2.

Figure 2.
Comparison (by MALDI-MS) of the invertase peptide profiles with and without in-gel deglycosylation by PNGase F



MALDI-TOF mass spectra of the tryptic peptides of glycosylated (lower spectrum) and deglycosylated (upper spectrum) invertase from a 1D gel (Axima CFR spectrometer in positive reflectron mode using α -cyano-4-hydroxycinnamic acid as matrix). The test piece was deglycosylated in-gel with PNGase F and both the control and test gel pieces were further digested with trypsin, before MS analysis. These results show that two N-deglycosylated peptide fragments (m/z 1318 and m/z 1625) are observed in the MALDI-MS of invertase after N-linked deglycosylation with PNGase F. These fragments are not present in the MS of the untreated glycosylated control sample. Upon deglycosylation the asparagine residue at the site of glycan attachment is converted to an aspartic acid residue and hence the m/z of the corresponding peptide fragment is increased by one unit.

Related Products	Catalog Number
GlycoProfile I Enzymatic In-Gel N-Deglycosylation Kit	PP0200
GlycoProfile II Enzymatic In-Solution N-Deglycosylation Kit	PP0201
Trypsin Profile IGD Kit	PP0100
ProteoMass™ MALDI-MS Calibration Kits Peptide and Protein Peptide Protein	MSCAL1 MSCAL2 MSCAL3
Trypsin, Proteomics Grade	T6567
PNGase F, Proteomics Grade	P7367
RNase B, Glycoprotein Standard, Proteomics Grade	R1153
α-Cyano-4-hydroxycinnamic acid	C8982

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