Product Information

PNGase F from *Elizabethkingia miricola*

BioReagent, ≥95% (SDS-PAGE), for proteomics

P7367

Product Description

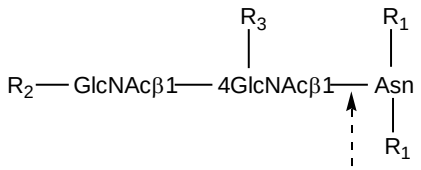
CAS Registry Number: 83534-39-8

Enzyme Commission (EC) Number: 2.5.1.52

Synonym: Peptide-N-Glycosidase F

(Note: *Elizabethkingia miricola* was formerly known as *Elizabethkingia*, *Chryseobacterium*, or *Flavobacterium meningosepticum*.)

PNGase F (Peptide-N-glycosidase F) is one of the most widely used enzymes for the deglycosylation of glycoproteins. It occurs in the culture fluid of the pathogenic microorganism *Elizabethkingia miricola*. PNGase F releases asparagine-linked oligosaccharides from glycoproteins and glycopeptides by hydrolyzing the amide of the asparagine (Asn) side chain. A tripeptide with the oligosaccharide-linked asparagine as the central residue is the minimal substrate for PNGase F. The oligosaccharides can be high mannose, hybrid, or complex type. However, *N*-glycans with fucose linked α(1→3) to the Asn-bound *N*-acetylglucosamine are resistant to the action of PNGase F.



R_1 = N- and C-substitution by groups other than H
R_2 = H or the rest of an oligosaccharide
R_3 = H or α1,6 fucose

The enzyme has a molecular mass of ~36 kDa, with a reported pH optimum of 8.6.[1]

Proteomics Grade PNGase F has been extensively purified and lyophilized from dilute potassium phosphate buffer to produce a stable product. The material is free of glycerol and other stabilizers, and contains very low levels of buffer salts. The manufacturing process yields a highly purified material that is suitable both for proteomics and glycobiology work, and that is compatible with MALDI-TOF MS analysis.

This PNGase F product may be used for *N*-linked deglycosylation of glycoproteins/glycopeptides in solution, in-gel digests, or on blot membranes. Several dissertations[8-16] have cited use of product P7367 in their protocols.

Reagent

Unit Definition: One unit will catalyze the release of *N*-linked oligosaccharides from 1 nanomole of denatured Ribonuclease B in 1 minute at 37 °C at pH 7.5, monitored by SDS-PAGE. One Sigma unit of PNGase F activity is equal to 1 IUB milliunit.

Purity: ≥95% (SDS-PAGE)

Each lot of the enzyme is tested for contaminating exoglycosidase and endoglycosidase activity. The purity of the product is confirmed by the absence of activity during extended incubation of the enzyme with specific *p*-nitrophenyl glycoside substrates. Protease activity is not detectable after incubation of the enzyme with denatured bovine serum albumin (BSA) for 24 hours.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The lyophilized powder has a shelf-life of at least one year if stored unopened at 2-8 °C. A reconstituted solution of ≥500 units/mL can be stored for one week at 2-8 °C or at –20 °C without significant loss of activity. The solution retains activity for at least 3 freeze-thaw cycles.

Preparation Instructions

- Before opening the vial containing PNGase F, centrifuge the vial briefly to ensure the lyophilized material is at the bottom of the tube.
- Prepare a 500 units/mL solution by adding 100 µL of high purity water to a vial of 50 units. This results in an enzyme solution containing ~5 mM potassium phosphate buffer, pH 7.5.
- Depending on the application, solutions containing higher or lower enzyme activity may be prepared with the appropriate volume of water.

Procedure

The following procedure has been developed to achieve optimal deglycosylation with minimal interference for subsequent mass spectrometric analysis of either the intact protein or peptide fragments, or for structural studies on the released glycans. The procedure presented here, which may be used as a general guideline, is for the deglycosylation of RNase B (Cat. No. R7884) by PNGase F. A control substrate, such as RNase B (Glycoprotein Standard, Cat. No. R1153), should be run with all experiments, since the composition of the reaction buffer may influence the deglycosylation of a glycoprotein.

Solution Digestion Procedure

1. Prepare a 1 mg/mL solution of denatured glycoprotein.

   1.1. Add 50 µg of RNase B to 45 µL of 20 mM ammonium bicarbonate (pH 8).

   1.2. Then add 5 µL of denaturation solution (0.2% SDS with 100 mM 2-mercaptoethanol).

   1.3. Heat the solution to 100 °C for 10 minutes to denature the glycoprotein.

   Note: 50 mM sodium phosphate, pH 7.5, may be used as an alternative reaction buffer.

2. Allow the solution to cool.

   Note: For protein analysis by techniques other than MS, add 5 µL of 15% TRITON® X-100, and mix.

3. Add 2-10 µL of the prepared PNGase F enzyme solution (500 units/mL) to the reaction mixture.

   Note: The amount of enzyme added can be varied, depending on the nature of the glycoprotein and the incubation time.

4. Incubate at 37 °C for 1-3 hours.

5. Stop the reaction by heating to 100 °C for 5 minutes.

6. Remove a 5-10 µL aliquot to assess deglycosylation by SDS-PAGE.

7. The remainder of the reaction mix may be lyophilized or frozen for subsequent MS analysis of the deglycosylated protein or, after appropriate treatment, of the released carbohydrate.

In-gel Digestion Procedure

The following procedure starts with a Coomassie®-stained 1D or 2D polyacrylamide gel of a reduced and alkylated protein sample.

For silver-stained gels, a gel destaining step different than that used for dye-stained gels is required. The ProteoSilver™ Plus Silver Staining Kit (Cat. No. PROTSIL2) is recommended for silver staining prior to tryptic digestion and MS analysis. It contains destaining solutions for silver-stained gels and a procedure for preparing gel slices for tryptic digestion.

As a control, load ~5 µg of the glycoprotein standard per gel lane.

1. Carefully cut the band of interest from a 1D gel or the protein spot from a 2D gel, using a scalpel or razor blade, taking care to include only stained gel. Lift out the gel piece using clean flat-nosed tweezers.

2. Place the gel piece in a siliconized Eppendorf® tube or equivalent. A siliconized tube reduces binding of the peptides to the tube surface. If unsure of chemicals leaching from the tube, which could interfere or suppress the MALDI-MS signal, prewash the tube with 100 µL of a 0.1% TFA (trifluoroacetic acid) in 50% acetonitrile solution, and then allow it to dry before use.

   Note: The gel piece may be cut into equal sections of 1-1.5 mm size and the sections may be used in place of the intact piece.

3. Cover the gel piece with 200 µL of 200 mM ammonium bicarbonate with 40% acetonitrile. Incubate at 37 °C for 30 minutes. Remove and discard the solution from the tube.
4. Repeat Step 3 once.
5. Dry the gel piece in a SpeedVac™ for 15-30 minutes.
6. Add 10 µL (5 units) of the prepared PNGase F Solution to the sample tube. Centrifuge briefly.

   Note: The amount of enzyme added can be varied, depending on the nature of the glycoprotein and the incubation time.
7. Allow the gel piece to incubate for 30 minutes at 37 °C.
8. Add 10-25 µL of water to the sample such that the gel piece is just covered by the liquid. Continue incubation for an additional 2 hours.

   Note: The user must determine the actual digestion time, which can vary from 1 hour to overnight. For overnight incubation, ensure that the gel piece remains covered with liquid at all times.
9. Centrifuge briefly. Remove the supernatant from the tube.

   Note: The supernatant from Step 9 and the washings from Steps 10 and 11 can be combined and retained for glycan analysis, if desired.
10. Add 200 µL of water to the tube. Mix at room temperature in a sonic bath for 30 minutes. Remove the supernatant.
11. Repeat Step 10 three times.
12. Dry the gel piece in a SpeedVac™ for 15-30 minutes.
13. The deglycosylated protein present in the gel piece can then be digested with trypsin (such as either Proteomics Grade Trypsin, Cat. No. T6567, or Trypsin Profile IGD Kit, Cat. No. PP0100), and the peptide fragments eluted for subsequent MS analysis.

References

1. Tarentino, A.L., and Plummer, T.H., *Methods Enzymol*., **230**, 44-57 (1994).
2. Fan, J.Q., and Lee, Y.C., *J. Biol. Chem*., **272(43)**, 27058-27064 (1997).
3. Tarentino, A.L. *et al*., *Biochemistry*, **24(17)**, 4665-4671 (1985).
4. Elder, J.H., and Alexander, S., *Proc. Natl. Acad. Sci. USA*, **79(15)**, 4540-4544 (1982).
5. Taga, E.M. *et al*., *Biochemistry*, **23(5)**, 815-822 (1984).
6. Trimble, R.B., and Tarentino, A.L., *J. Biol. Chem*., **266(3)**, 1646-1651 (1991).
7. Bayer, E.A. *et al*., *Appl. Biochem. Biotech*., **53(1)**, 1-9 (1995).
8. Uribesalgo, Miren Zumárraga, "Evolución Molecular Dirigida de Lacasas Fúngicas en *Saccharomyces Cerevisiae*: Tolerancia a Disolventes Orgánicos y Estudios Semi-Racionales" ("Directed Molecular Evolution of Fungal Laccases in *Saccharomyces Cerevisiae*: Tolerance to Organic Solvents and Semi-Rational Studies"). Universidad Autónoma de Madrid, Ph.D. dissertation, p. 44 (2007).
9. Nejad, Daniel Nouri, "Biochemical and Functional Analyses of PANX1 Variants". University of Western Ontario, Ph.D. dissertation, p. 67 (2019).
10. Bohne, Jana, "Etablierung und Anwendung einer analytischen Methode zur Untersuchung der *N*-Glykosylierung des Influenza-Virus Hämagglutinin" ("Establishment and application of an analytical method for investigating the *N*-glycosylation of the influenza virus haemagglutinin"). Otto-von-Guericke-Universität Magdeburg, Dr. ing. dissertation, p. CLXXXIV (2013).
11. Kiss, Katalin, "A humán ABCB6 transzmembrán fehérje sejten belüli lokalizációjának és funkciójának tanulmányozása" ("The human ABCB6 transmembrane protein: intracellular studies of its localization and function"). Eötvös Loránd Tudományegyetem (Loránd Eötvös University), Ph.D. dissertation, p. 30 (2014).

12. Tescari, Simone, "Production and characterization of therapeutic proteins/peptides: Human Recombinant FSH-beta subunit expressed in Plant Cells and Chemical Synthesis of Human Osteocalcin and Neuritogenic Peptides". Università degli Studi di Padova, Ph.D. dissertation, p. 27 (2017).
13. Li, Linhan, "Investigations of Barley Powdery Mildew Effectors (CSEPs) in the Non-host Plant Wheat and Host Plant Barley". Imperial College London, Ph.D. dissertation, p. 88 (2018).
14. Mallikarjun, Venkatesh J., "Using proteomics to analyse how ageing and senescence affect tissue mechanics and mechanotransduction". University of Manchester, Ph.D. dissertation, p. 112 (2016).
15. Schroeder, Holly A., "IgG-mucin crosslinking as a universal mucosal protective mechanism". University of North Carolina at Chapel Hill, Ph.D. dissertation, pp. 68, 94 (2018).
16. Venturini da Silva, Gabriela, "Identificação de marcadores proteicos de alto e baixo *shear stress*" ("Identification of high and low *shear stress* protein markers"). Universidade de São Paulo, Ph.D. dissertation, p. 33 (2018).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

P7367pis Rev 05/22 RBG,ER,VNC,GCY,MAM