Technical Bulletin

PNGase F from Elizabethkingia miricola

Recombinant, expressed in Escherichia coli

P9120

Storage Temperature 2–8 °C

EC 3.5.1.52

CAS RN 83534-39-8

Synonyms: N-Glycanase®, Glycopeptidase F, N-Glycosidase F, Peptide N-Glycosidase F, Peptide-N^4-(acetyl-β-glucosaminyl)-asparagine amidase *Elizabethkingia miricola* was formerly known as *Elizabethkingia, Chryseobacterium,* or *Flavobacterium meningosepticum*.

Product Description

PNGase F (Peptide N-Glycosidase F) cleaves asparagine-linked high mannose as well as hybrid and complex oligosaccharides from glycoproteins. It deaminates the asparagine to aspartic acid, but leaves the oligosaccharide intact (see Figure 1). PNGase F will not remove oligosaccharides containing α(1→3)-linked core fucose, commonly found in plant glycoproteins. A tripeptide with the oligosaccharide-linked asparagine as the central residue is the minimal substrate for PNGase F.

Molecular mass: 36 kDa

pH profile: active in the pH range of 6–10 with an optimal pH of 8.6

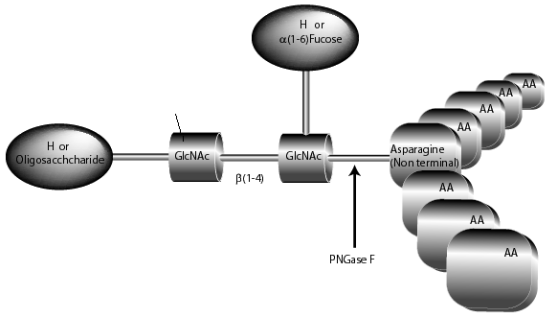
Activity: ≥ 10 units/mg protein and ≥ 2.5 units/mL

Unit definition: One unit will catalyze the release of N-linked oligosaccharides from one µmole of denatured Ribonuclease B in 1 minute at pH 7.5 at 37 °C. One micromolar unit of PNGase F activity is equal to 1,000 nanomolar units (IUB milliunits).

PNGase F is tested for contaminating enzyme activity. No protease or exoglycosidase activity is detected.

Specificity of PNGase F

Figure 1.



Components

- PNGase F Enzyme, Cat. No. P3620, 0.1 unit (0.1 micromolar unit or 100 nanomolar units) Supplied in a solution of 20 mM Tris-HCl, pH 7.5, containing 1 mM EDTA and 50 mM NaCl.
- 5x Reaction Buffer, Cat. No. R8277, 1 mL
- 100 mM sodium phosphate, pH 7.5
- 5x MS Reaction Buffer, Cat. No. R0154, 1 mL
- 50 mM Tris-HCl, pH 8.0
- Detergent Solution, Cat. No. D0692, 0.2 mL
- 15% Solution of IGEPAL® CA 630
- Denaturation Solution, Cat. No. D2317, 1 vial
- 0.2 mL solution of 2% SDS and 1 M 2-mercaptoethanol

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 product ships on wet ice and it is recommended to store the product at 2–8 °C. The product remains active for at least 12 months when stored properly. Exposure for several days to ambient temperatures will not reduce activity.

Procedure

The amount of enzyme required for deglycosylation depends on the substrate, incubation conditions, and the precise application. In the case of glycoprotein substrates, it is recommended to denature the substrate before deglycosylation. Detergent and heat denaturation increases the rate of cleavage 100-fold. Most native proteins can still be completely N-deglycosylated, but incubation time must be increased. PNGase F will remain active under incubation conditions for at least 72 hours.

In general, 0.01 unit of enzyme is sufficient to deglycosylate up to 100 µg of denatured glycoprotein or 20 µg of native glycoprotein in 18 hours at pH 7.5 and 37 °C. In some cases, further optimization may be necessary to achieve complete deglycosylation. In particular, incubation time may be reduced by using a higher concentration of PNGase F in the reaction mixture.

Prior denaturation of the glycoprotein substrate by heating at 100 °C in the presence of up to 1% (w/v) SDS greatly enhances both the rate and extent of deglycosylation. Ionic detergents are potent inhibitors of PNGase F; however, non-ionic detergents (Triton® X-100, IGEPAL CA 630, or octyl β-D-glucopyranoside) are not inhibitory and can be used in ~ 5-fold excess to counteract the inhibitory effects of ionic detergents.

Sulfhydryl reagents such as 2-mercaptoethanol used for glycoprotein denaturation do not interfere with enzyme activity. PNGase F tolerates most chaotrophic agents and is at least 80% active in the presence of < 5 M urea, < 2 M guanidine HCl, and 0.25 M sodium thiocyanate (NaSCN). However, the enzyme is inactivated by the presence of guanidine thiocyanate (SCN). PNGase F is compatible with a wide range of buffers.

Prepare the 1x Reaction Buffer by a 5-fold dilution of the appropriate 5x Reaction Buffer with water.

Notes: The 5x MS Reaction Buffer (50 mM Tris-HCl, pH 8.0, Cat. No. R0154) should be used with samples for downstream mass spectrometric analysis.

1. The 5x Reaction Buffer (100 mM sodium phosphate, pH 7.5, Cat. No. R8277) may be used for other downstream procedures.
2. Prepare a solution of 50–500 µg of glycoprotein in 45 µL of 1x Reaction Buffer. Add 2.5 µL of the Denaturation Solution (final reaction concentration 0.1% SDS and 50 mM 2-mercaptoethanol).
3. Denature the glycoprotein by heating at 100 °C for 5 minutes. Allow mixture to cool.
4. Add 2.5 µL of the Detergent Solution (final reaction concentration 0.75% IGEPAL CA 630).
5. Add 2 µL of the PNGase F Enzyme, Cat. No. P3620, to the reaction mixture and incubate for 2 hours to overnight at 37 °C.

References

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