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ProductInformation

PNGase F
from *Elizabethkingia meningosepticum*,
recombinant, expressed in *E. coli*

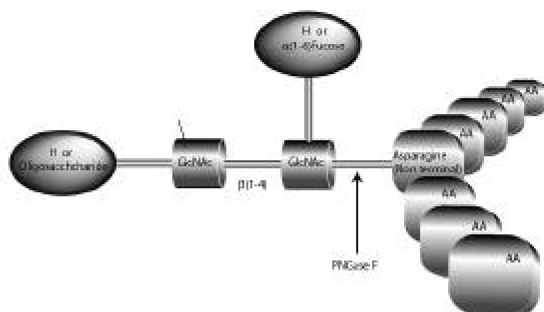
Product Code **P9120**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

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 meningosepticum was formerly known as *Chryseobacterium meningosepticum* or *Flavobacterium meningosepticum*.

Product Description
PNGase F (Glycopeptidase 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.

Figure 1.
Specificity of PNGase F



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.

The product is supplied in a solution of 20 mM Tris-HCl, pH 7.5, containing 1 mM EDTA and 50 mM NaCl.

PNGase F is isolated from *E. coli* expressing the gene for PNGase F from *Elizabethkingia meningosepticum*.

Molecular Weight: 36 kDa.

pH Profile: active in the pH range of 6 to 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.

Components

PNGase F Enzyme (Product Code P3620)	0.1 Unit (100 nanomolar units)
5× Reaction Buffer (Product Code R8277) 100 mM Sodium Phosphate, pH 7.5	1 ml
5× MS Reaction Buffer (Product Code R0154) 50 mM Tris-HCl, pH 8.0	1 ml

Detergent Solution (Product Code D0692) 15% Solution of IGEPAL® CA 630	0.2 ml
Denaturation Solution (Product Code D2317) Solution of 2% SDS and 1 M 2-mercaptoethanol	0.2 ml

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material 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 is stable 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. 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 approximately 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.

1. Prepare the 1× Reaction Buffer by a 5-fold dilution of the appropriate 5× Reaction Buffer with water. The 5× MS Reaction Buffer (50 mM Tris-HCl, pH 8.0, Product Code R0154) should be used with samples for downstream mass spectrometric analysis. The 5× Reaction Buffer (100 mM sodium phosphate, pH 7.5, Product Code R8277) may be used for other downstream procedures. Prepare a solution of 50–500 μg of glycoprotein in 45 μl of 1× Reaction Buffer. Add 2.5 μl of the Denaturation Solution (final reaction concentration 0.1% SDS and 50 mM 2-mercaptoethanol).
2. Denature the glycoprotein by heating at 100 °C for 5 minutes. Allow mixture to cool.
3. Add 2.5 μl of the Detergent Solution (final reaction concentration 0.75% IGEPAL CA 630).
4. Add 2 μl of the PNGase F Enzyme (Product Code P3620) to the reaction mixture and incubate for 2 hours to overnight at 37 °C.

References

1. Bayer, E.A., *et. al.*, Appl. Biochem. and Biotech., **53**, 1-9 (1995).
2. Elder, J.H., and Alexander, S., Proc. Natl. Acad. Sci. USA, **79,** 4540-4544 (1982).
3. Tarentino, A.L., *et. al.*, Biochemistry**, 24**, 4665-4671 (1985).
4. Tarentino, A.L., and Plummer, T.H., Methods in Enzymology, **230**, 44-57 (1994).
5. Trimble, R.B., and Tarentino, A.L., J. Biochem., **266,** 1646-1651 (1991).
6. Taga, E. M., *et al.*, Biochemistry, **23**, 815-22 (1984).

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