



PNGase F (Peptide-N-Glycosidase F)

Peptide-N4-(acetyl-β-glucosaminy)-asparagine amidase
N-Glycosidase F

Source

Elizabethkingia meningosepticum
was (*Chyrseobacterium/Flavobacterium men.*)

Catalog Number E-PNG01

Certification of Analysis Lot Number 604.1A

EC 3.5.1.52

Applications

- Amino acid sequence determination
- X-Ray crystallography
- Removing heterogeneity due to carbohydrates
- Studying carbohydrate ligand binding
- Removing carbohydrate epitopes from antigens
- Studying the role of glycosylation in protein folding and activity.

Contents

1 vial: PNGase F - 60 μl (0.3 U)

1 vial: 5x Reaction Buffer pH 7 - 400 μl

1 vial: Denaturation Solution - 200 μl
2% SDS/ 1 M β-mercaptoethanol

1 vial: 15% Triton X-100 - 200 μl

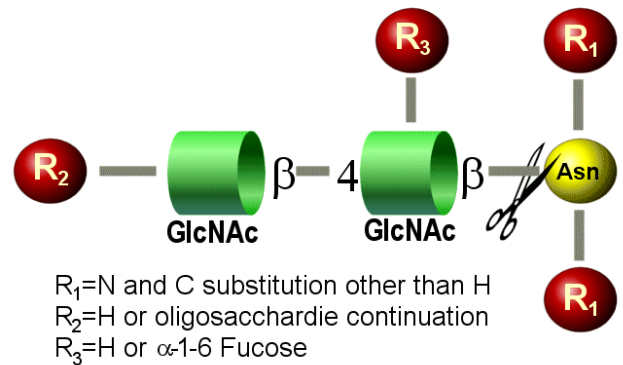
Specific Activity 25 U/mg

Activity 5 U/ml

Specific Activity

One unit of PNGase F activity is defined as the amount of enzyme required to catalyze the release of N-linked oligosaccharides from 1 micromole of denatured BSA and RNase B in 1 minute at 37°C, pH 7.5. Cleavage is monitored by SDS-PAGE (cleaved RNase B migrates faster).

PNGase F



Specificity

QA-Bio™ PNGase F cleaves asparagine-linked (N-linked) oligosaccharides from glycoproteins. PNGase F deaminates asparagine to aspartic acid, leaving the oligosaccharides intact.

Denaturation increases the rate of cleavage up to 100x. 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.

PNGase F will not remove oligosaccharides containing Alpha-(1,3)-linked core fucose commonly found on plant glycoproteins; for this purpose, use peptide N-glycosidase A.

Formulation

The enzyme is provided as a sterile-filtered solution in 20 mM Tris-HCl (pH 7.5).

Molecular Weight approximately 35 kD.

pH optimum: 7.5, active over the range 6-10.

Storage

Store enzyme at 4°C. Do not freeze.

Stability

Several days exposure to ambient temperatures will not reduce activity. Stable at least 12 months when stored properly.

E-PNG01 PNGase F

Specifications - Protocol

Quality & Purity

QA-Bio PNGase F is tested for contaminating protease as follows: 10 µg of denatured BSA is incubated at 37°C for 24 hours with 2 µl of enzyme. SDS-PAGE analysis of the treated BSA shows no evidence of degradation.

The absence of exoglycosidase contaminants is confirmed by extended incubations with the corresponding pNP-glycosides.

PNGase F is isolated from culture supernatants of *Elizabethkingia (Chryseobacterium or Flavobacterium) meningosepticum*. Significant contaminants are the endoglycosidase F enzymes, which cleave within the diacetylchitobiose core of some N-linked oligosaccharides leaving an N-acetylglucosamine residue attached to the asparagine. These contaminants are chromatographically removed from QA-Bio PNGase F preparations.

Directions for use

1. Add up to 200µg of glycoprotein to an Eppendorf tube. Adjust to 35 µl final volume with de-ionized water.
2. Add 10 µl 5x Reaction Buffer 7.5 and 2.5 µl of Denaturation Solution. Heat at 100°C for 5 minutes.
3. Cool. Add 2.5 µl of Triton X-100 and mix.
NOTE: Failure to add Triton X-100 will result in a 3-fold reduction of PNGase F activity.
4. Add 2.0 µl of PNGase F to the reaction. Incubate 3 hours at 37°C.

If SDS or heat denaturation is omitted, increase incubation time to at least 24 hours.

Monitor cleavage by SDS-PAGE.

References:

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- Elder, J.H. and S. Alexander. endo-b-N-Acetylglucosaminidase F: endoglycosidase from *Flavobacterium meningosepticum* that cleaves both high-mannose and complex glycoproteins. *Proc Natl Acad Sci USA* 79: 4540-4544 (1982)
- Tarentino, A. L. , C.M. Gomez and T.H. Plummer, Jr. Deglycosylation of asparagine-linked glycans by peptide :N-glycosidase F . *Biochemistry* 24: 4665-4671 (1985)
- Tarentino A.L. and T.H. Plummer. Enzymatic deglycosylation of asparagine-linked glycans: purification, properties, and specificity of oligosaccharide-cleaving enzymes from *Flavobacterium meningosepticum*. *Meth Enzymol* 230:44-57 (1994)
- Trimble R.B. and A.L. Tarentino. Identification of distinct endoglycosidase (endo) activities in *Flavobacterium meningosepticum*: endo F1 , endo F2 and endo F3. Endo F1 and endo H hydrolyze only high mannose and hybrid glycans. *J Biol Chem* 266:1646-1651 (1991).
- Taga, E. M., A. Waheed and R. L. Van Etten. Structural and chemical characterization of a homogeneous peptide N-glycosidase from almond. *Biochemistry* 23:815-22 (1984).

Warranties and liabilities

QA-Bio warrants that the above product conforms to the specifications described herein. Should the product fail for reasons other than through misuse QA-Bio will, at its option, replace free of charge or refund the purchase price. This warranty is exclusive and QA-Bio makes no other warrants, expressed or implied, including any implied conditions or warranties of merchantability or fitness for any particular purpose. QA-Bio shall not be liable for any incidental, consequential or contingent damages.

This product is intended for *in vitro* research only.

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