PNGase F (Peptide-N-Glycosidase F)
Peptide-N4-(acetyl-ß-glucosaminyl)-asparagine amidase
N-Glycosidase F

Source
recombinant from *Elizabethkingia meningosepticum*
was *(Chyrseobacterium/Flavobacterium men.)*

Catalog Number E-RPNG01

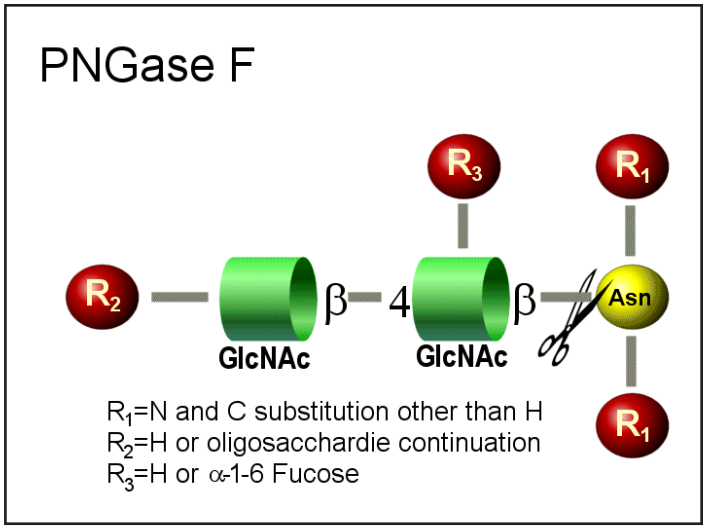
EC 3.5.1.52

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 RNase B in 1 minute at 37°C, pH 7.5. Cleavage is monitored by SDS-PAGE (cleaved RNase B migrates faster).



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 m*M* 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-RPNG01 PNGase F
Specifications - Protocol

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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 production host strain has been extensively tested and does not produce any detectable glycosidases.

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:

Bayer, E.A., F. De Meester, T. Kulik and M. Wilchek. Preparation of deglycosylated egg white avidin. Appl Biochem Biotech 53: 1-9 (1995)

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 an d 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, Inc 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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