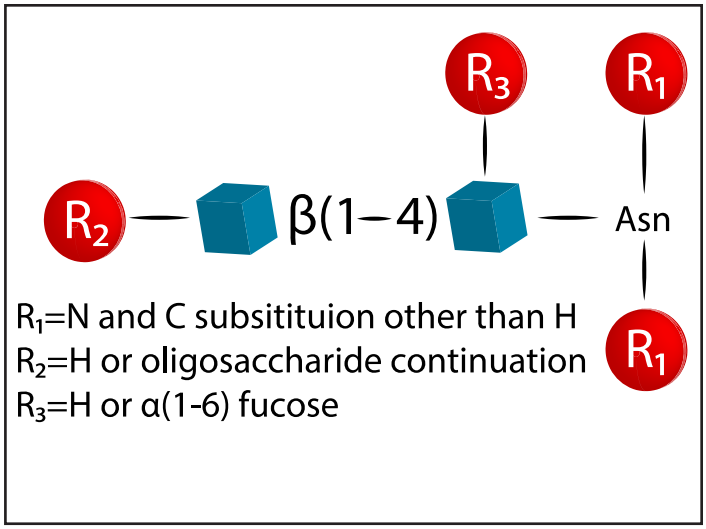

PNGase F (Peptide-N-Glycosidase F)
Peptide-N4-(acetyl-ß-glucosaminyl)-asparagine amidase, N-Glycosidase F

Source
recombinant gene from *Elizabethkingia miricola* in *E. Coli*

Catalog Number

E-RPNG01	60 µl	0.3 U
E-RPNG01-20	20 µl	0.1 U
E-RPNG01-200	200 µl	1.0 U

EC 3.5.1.52

Recommendd Reagents
included with E-RPNG01:
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

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.

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.

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

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.

rPNGase F

Specifications - Protocol

QA-Bio
www.QA-Bio.com
info@QA-Bio.com

phone (Asia) +65 3158 9855
phone (Europe) +48 22 104 0892
phone (USA) +1 760 249 2664

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.

revised on January 16, 2025