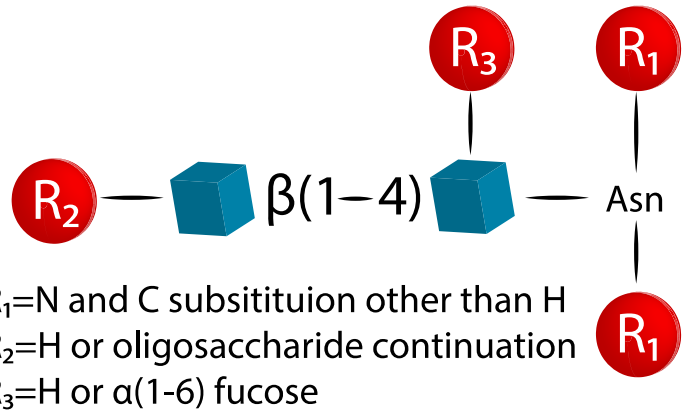

R_1=N and C subsitituion other than H
R_2=H or oligosaccharide continuation
R_3=H or α(1-6) fucose

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

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

Catalog Numbers

E-PNG01	60 µl	0.3 U
E-PNG01-20	20 µl	0.1 U
E-PNG01-200	200 µl	1.0 U

EC 3.5.1.52

Recommended Reagents
included with E-PNG01:

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

Activity 5 U/ml
Specific Activity ≥ 25 U/mg

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).

Formulation
The enzyme is provided as a sterile-filtered solution in 20 m*M* Tris-HCl (pH 7.5).

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 24 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.

continued

PNGase 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

Quality & Purity *continued*
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.

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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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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