**PNGase F (Peptide-N-Glycosidase F)**
Peptide-N4-(acetyl-β-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

**Recommended 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 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.

**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).

**rPNGase F**
**Specifications - Protocol**

- R₁= N and C substituition other than H
- R₂= H or oligosaccharide continuation
- R₃= H or α(1-6) fucose

**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.
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:


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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