PNGase F (Glycerol-free)

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P0705S 041140716072

P0705S

37° 75°

15,000 units **Lot: 0411407** **Exp: 7/16**

500,000 U/ml **Store at 4°C** **Do not freeze**

Description: Peptide: N-Glycosidase F, also known as PNGase F, is an amidase which supplied glycerol free for optimal performance in HPLC intensive methods. PNGase F cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from N-linked glycoproteins (1).

Source: PNGase F is purified from *Flavobacterium meningosepticum* (2).

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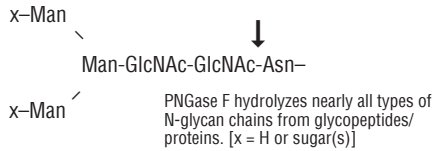
Specificity:



PNGase F hydrolyzes nearly all types of N-glycan chains from glycopeptides/ proteins. [x = H or sugar(s)]

Applications:

- Removal of N-linked glycans from glycoproteins
- Preferred formulation for HPLC intensive methods

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 5 mM Na_2EDTA.

Reagents Supplied with Enzyme:
10X Glycoprotein Denaturing Buffer:
(5% SDS, **0.4 M DTT**)

10X GlycoBuffer 2:
[0.5 M Sodium Phosphate (pH 7.5 @ 25°C)]

10% NP-40

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

Reaction Conditions:
Typical reaction conditions are as follows:

1. Combine 1–20 µg of glycoprotein, 1 µl of 10X Glycoprotein Denaturing Buffer and H_2O (if necessary) to make a 10 µl total reaction volume.
2. Denature glycoprotein by heating reaction at 100°C for 10 minutes.
3. Make a total reaction volume of 20 µl by adding 2 µl 10X GlycoBuffer 2, 2 µl 10% NP-40, H_2O and 1–5 µl PNGaseF.
4. Incubate reaction at 37°C for 1 hour.

Note: Reactions may be scaled-up linearly to accommodate larger reaction volumes.

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl (65 NEB units = 1 IUB milliunit).

Unit Definition Assay: 10 µg of RNase B are denatured with 1X Glycoprotein Denaturing Buffer at 100°C for 10 minutes. After the addition of NP-40 and GlycoBuffer 2, two-fold dilutions of PNGase F are added and the reaction mix is incubated for 1 hour at 37°C. Separation of reaction products are visualized by SDS-PAGE.

Quality Assurance: No contaminating exoglycosidase or Endoglycosidase F_1, F_2 or F_3 activity could be detected. No contaminating proteolytic activity could be detected.

Molecular Weight: 36,000 daltons.

Quality Controls

Glycosidase Assays: 5,000 units of PNGase F were incubated with 0.1 mM of fluorescently-labeled oligosaccharides and glycopeptides, in a 10 µl reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

(See other side)

CERTIFICATE OF ANALYSIS

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No other glycosidase activities were detected (ND) with the following substrates:

β-N-Acetyl-glucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC Galβ1-4
(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4Galα1-3Gal-AMC ND

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3
Galβ1-4Glc-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

β-Mannosidase:
Manβ1-4Manβ1-4Man-AMC ND

Endo F_1, F_2, H:
Dansylated invertase high mannose. ND

Endo F_2, F_3:
Dansylated fibrinogen biantennary. ND

Endoglycosidase F1 Assay: After incubation of 5,000 units of PNGase F with 20 pmol of 2-AA Man-5 fluorescent standard, for 20 hours at 37°C, no endoglycosidase F1 activity could be detected by LC/MS analysis with fluorescence detection.

Protease Assay: After incubation of 10,000 units of PNGase F with 0.2 nmol of a standardized mixture of proteins, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Heat Inactivation: 500 units of enzyme were inactivated by incubation at 75°C for 10 minutes.

Notes: Since PNGase F activity is inhibited by SDS, it is essential to have NP-40 present in the reaction mixture. Why this non-ionic detergent counteracts the SDS inhibition is unknown at present.

To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

PNGase F will not cleave N-linked glycans containing core α1-3 Fucose.

Previously supplied as a recombinant.

Repeated freeze thaw cycles degrade enzyme activity over time.

References:

1. Maley, F. et al. (1989) *Anal. Biochem.* 180, 195–204.
2. Plummer, T.H., Jr. and Tarentino, A.L. (1991) *Glycobiology* 1, 257–263.

Companion Product:
RNase B (NEB #P7817S)

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