PNGase F, Recombinant

1-800-632-7799
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www.neb.com

P0708S 002140716071

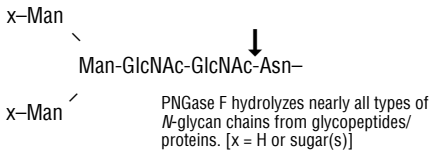
P0708S

15,000 units **Lot: 0021407** **Exp: 7/16**

500,000 U/ml **Store at –20°C**

Description: Peptide: N-Glycosidase F, also known as PNGase F, is a recombinant amidase which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from *N*-linked glycoproteins (1).

Specificity:



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

Source: Cloned from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*) and expressed in *E. coli* (2).

Applications:

- Removal of carbohydrate residues from proteins

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

Reagents Supplied with Enzyme:

10X Glycoprotein Denaturing Buffer:
(5% SDS, 0.4 M DTT)

10X G7 Reaction Buffer:
[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 G7 Reaction Buffer, 2 µl 10% NP40, H_2O and 1–2 µl PNGase F, Recombinant.
4. Incubate reaction at 37°C for 1 hour.

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

MolecularWeight: 36,000 daltons.

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

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 G7 Reaction Buffer, two-fold dilutions of PNGase F, Recombinant 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 activity could be detected. No contaminating proteolytic activity could be detected.

(see other side)

CERTIFICATE OF ANALYSIS

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Specificity:

x–Man
Man-GlcNAc-GlcNAc-Asn–
x–Man

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CERTIFICATE OF ANALYSIS

Quality Controls

Glycosidase Assays: 5,000 units of PNGase F, Recombinant 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.

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

β-N-Acetylglucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

β-*N*-Acetylgalactosaminidase:
GalNAcβ1-4Galβ1-4Glc-AMC ND

α-N-Acetylgalactosaminidase:
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

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

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

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND
Galα1-6Galα1-6Glcα1-2Fru-AMC ND

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

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

α-Glucosidase: Glcα1-6Glcα1-4Glc-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

Protease Assay: After incubation of 5,000 units of PNGase F, Recombinant with 0.2 nmol of a standardized mixture of proteins in a 20 µl reaction, 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.

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

Since PNGase F, Recombinant activity is inhibited by SDS, it is essential to have NP-40 in the reaction mixture. It is not known why this non-ionic detergent counteracts the SDS inhibition at the present time.

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

Recommended storage temperature is 4°C, avoid repeat freeze-thaw cycles

References:

1. Maley, F. et al. (1989) *Anal. Biochem.* 180, 195–204.
2. Chen, M. New England Biolabs, Inc., unpublished results.

Companion Products:

RNase B
#P7817S 250 µg

Endoglycosidase Reaction Buffer Pack
B0701S 4 x 1 ml

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GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

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

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

α-Galactosidase:
Galα1-3Galβ1-4Gal-AMC ND
Galα1-6Galα1-6Glcα1-2Fru-AMC ND

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

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

α-Glucosidase: Glcα1-6Glcα1-4Glc-AMC ND

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

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

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Dansylated invertase high mannose. ND

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