

PNGase F, Recombinant



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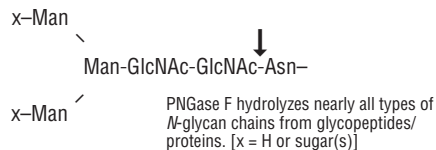


15,000 units Lot: 0021401 Exp: 1/16

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:



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 Na₂EDTA 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 H₂O (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, H₂O 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)

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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 μ l 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:

GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

β -N-Acetylgalactosaminidase:

GalNAc β 1-4Gal β 1-4Glc-AMC ND

α -N-Acetylgalactosaminidase:

GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC ND

α -Fucosidase:

Gal β 1-4 (Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

Fuc α 1-2Gal β 1-4Glc-AMC ND

β -Galactosidase:

Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND

Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC ND

α -Galactosidase:

Gal α 1-3Gal β 1-4Gal-AMC ND

Gal α 1-6Gal α 1-6Glc α 1-2Fru-AMC ND

α -Neuraminidase:

Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC ND

α -Mannosidase:

Man α 1-3Man β 1-4GlcNAc-AMC ND

Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

α -Glucosidase: Glc α 1-6Glc α 1-4Glc-AMC ND

β -Xylosidase:

Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

β -Mannosidase:

Man β 1-4Man β 1-4Man-AMC ND

Endo F₁, F₂, H:

Dansylated invertase high mannose. ND

Endo F₂, F₃:

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 μ l 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 *M*-linked glycans containing core α 1-3 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 μ g

Endoglycosidase Reaction Buffer Pack

B0701S 4 x 1 ml



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