

PNGase F (Glycerol Free)



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



P0705S 039120514051

P0705S



15,000 units Lot: 0391205 Exp: 5/14

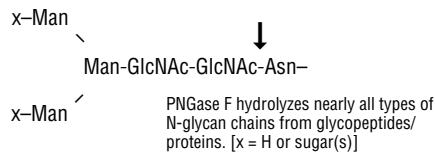
500,000 U/ml Store at 4°C Do not freeze

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).

New Quality Controls

Specificity:



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 Na₂EDTA.

Reagents Supplied with Enzyme:

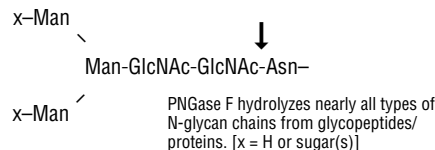
10X Glycoprotein Denaturing Buffer:
(5% SDS, **0.4 M DTT**)

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.

Specificity:



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 Na₂EDTA.

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 1–5 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).

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 1–5 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 G7 Reaction Buffer, 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 F₁, F₂ or F₃ 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

PNGase F (Glycerol Free)



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



P0705S 039120514051

P0705S



15,000 units Lot: 0391205 Exp: 5/14

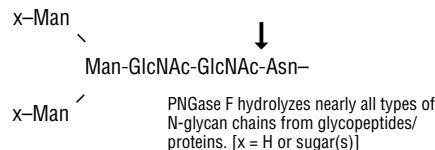
500,000 U/ml Store at 4°C Do not freeze

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).

New Quality Controls

Specificity:



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 Na₂EDTA.

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 1–5 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 G7 Reaction Buffer, 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 F₁, F₂ or F₃ 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

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

β -N-Acetyl-glucosaminidase:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC Gal β 1-4
(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

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

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal α 1-3Gal-AMC ND

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

α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-AMC
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

Page 2 (P0705)

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

β -N-Acetyl-glucosaminidase:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC Gal β 1-4
(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

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

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal α 1-3Gal-AMC ND

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

α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-AMC
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

Page 2 (P0705)

β -Glucosidase:
Glc β 1-4Glc β 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

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.

β -Glucosidase:
Glc β 1-4Glc β 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

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 α 1-3 Fucose.

Previously supplied as a recombinant.

Repeated freeze thaw cycles degrade enzyme activity over time.

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 α 1-3 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)

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)