

# PNGase F (Glycerol Free)



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www.neb.com



P0705S 036120214021

## P0705S



**15,000 units** Lot: **0361202** Exp: **2/14**

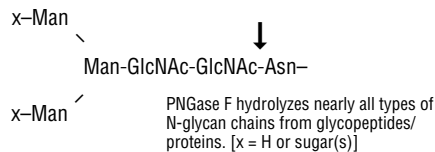
**500,000 U/ml** Store at **4°C** Do not freeze

**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 Na<sub>2</sub>EDTA.

### Reagents Supplied with Enzyme:

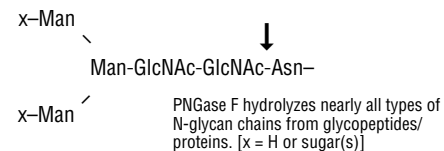
10X Glycoprotein Denaturing Buffer:  
(5% SDS, **0.4 M DTT**)

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.

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### Reaction Conditions:

Typical reaction conditions are as follows:

1. Combine 1–20 µg of glycoprotein, 1 µl of 10X Glycoprotein Denaturing Buffer and H<sub>2</sub>O (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<sub>2</sub>O 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).

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**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 F<sub>1</sub>, F<sub>2</sub> or F<sub>3</sub> 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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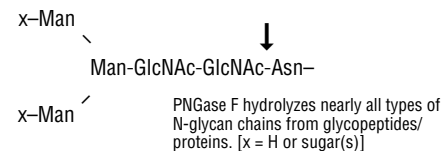
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(See other side)

CERTIFICATE OF ANALYSIS

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

**$\beta$ -N-Acetyl-glucosaminidase:**  
GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-AMC ND

**$\alpha$ -Fucosidase:**  
Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC Gal $\beta$ 1-4  
(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\beta$ -Galactosidase:**  
Gal $\beta$ 1-3GlcNAc $\beta$ 1-4Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Galactosidase:**  
Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal $\alpha$ 1-3Gal-AMC ND

**$\alpha$ -Neuraminidase:**  
Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3  
Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

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**$\alpha$ -Fucosidase:**  
Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc-AMC Gal $\beta$ 1-4  
(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc-AMC ND

**$\beta$ -Galactosidase:**  
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Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal $\alpha$ 1-3Gal-AMC ND

**$\alpha$ -Neuraminidase:**  
Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3  
Gal $\beta$ 1-4Glc-AMC ND

**$\alpha$ -Mannosidase:**  
Man $\alpha$ 1-3Man $\beta$ 1-4GlcNAc-AMC  
Man $\alpha$ 1-6Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man-AMC ND

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**$\beta$ -Glucosidase:**  
Glc $\beta$ 1-4Glc $\beta$ 1-4Glc-AMC ND

**$\beta$ -Xylosidase:**  
Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl-AMC ND

**$\beta$ -Mannosidase:**  
Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC ND

**Endo F<sub>1</sub>, F<sub>2</sub>, H:**  
Dansylated invertase high mannose. ND

**Endo F<sub>2</sub>, F<sub>3</sub>:**  
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.

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Man $\beta$ 1-4Man $\beta$ 1-4Man-AMC ND

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**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  $\alpha$ 1-3 Fucose.

Previously supplied as a recombinant.

Repeated freeze thaw cycles degrade enzyme activity over time.

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**Companion Product:**  
RNase B (NEB #P7817S)

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