PNGase F

**Specificity:**

\[ \text{x-Man} \xrightarrow{-} \text{Man-GlcNAc-GlcNAc-Asn} \]

PNGase F hydrolyzes nearly all types of N-glycan chains from glycoproteins and glycoproteins. \( x = \text{H or sugar(s)} \)

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

**Applications:**

- Removal of carbohydrate residues from proteins

Note: Previously supplied as a recombinant.

**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

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**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 glycoprot, 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.
4. Incubate reaction at 37°C for 1 hour.

**Molecular Weight:** 36,000 daltons.

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**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 were 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.

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

- **β-N-Acetyl-glucosaminidase**: GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND
- **α-Fucosidase**: FucC1-2Galβ1-4Glc-AMC Gαlβ1-4 (FucC1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND
- **β-Galactosidase**: Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND
- **α-Galactosidase**: Galα1-3Galβ1-4Galα1-3Gal-AMC ND
- **α-Neuraminidase**: CNeu5Acα2-3Galβ1-4GlcNAcβ1-4Glc-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 Fβ , Fβ , H: Dansylated invertebrate high mannose. ND

Endo Fβ , Fβ : Dansylated fibroin 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.

### References


### Companion Product

RNase B (NEB #P7817S)