PNGase F

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

**Applications:**
- Removal of carbohydrate residues from proteins

**Note:** Previously supplied as a recombinant.

**Reagents Supplied with Enzyme:**
- 10X Glycoprotein Denaturing Buffer: (5% SDS, 0.4 M DTT)
- 10X GlycoBuffer 2: [0.5 M Sodium Phosphate (pH 7.5 @ 25°C)]
- 10% NP-40

**Specificity:**
\[
\text{PNGase F hydrolyzes nearly all types of N-glycan chains from glycopeptides/proteins.} \quad [x = H or sugar(s)]
\]

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

**Reaction Conditions:**
- Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

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

**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 GlycoBuffer 2, 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.

**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 GlycoBuffer 2, 2 µl 10% NP-40, H₂O and 1–2 µl PNGase F.
4. Incubate reaction at 37°C for 1 hour.

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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**: Fuc+Galβ1-4Glc-AMCαGalβ1-4Glc-AMC ND
- **β-Galactosidase**: Galβ1-4GlcNAcβ1-4Galβ1-4Glc-AMC ND
- **α-Galactosidase**: Galα1-3Galβ1-4Glcx1-3Gal-AMC 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.

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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 #P7817)