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PNGase F (Peptide-N-Glycosidase F)

PNGase F [Peptide-N^4-(acetyl-beta-glucosaminyl)-asparagine amidase, EC 3.5.1.52] cleaves asparagine-linked high mannose as well as hybrid and complex oligosaccharides from glycoproteins. It deaminates the asparagine to aspartic acid but leaves the oligosaccharide intact. PNGase F will not remove oligosaccharides containing α(1,3)-linked core fucose commonly found on plant glycoproteins; use PNGase A for this purpose. In addition, PNGase F will fail to cleave if the asparagine to which the oligosaccharide is linked is not peptide bonded to at least one amino acid residue at both the N and C termini. Detergent and heat denaturation increases the rate of cleavage up to 100X.

Most native proteins can still be completely N-deglycosylated but incubation time must be increased. PNGase F will remain active under incubation conditions for at least 72 hours.

PNGase F is isolated from culture supernatants of Elizabethkingia *miricola*. Significant contaminants are the Endoglycosidase F enzymes, which cleave within the diacetylchitobiose core of some N-linked oligosaccharides leaving an N-acetylglucosamine residue attached to the asparagine. These contaminants are chromatographically removed from the PNGase F preparations.

Proteins deglycosylated with PNGase F have been used for:

- Amino acid sequence determination
- X-Ray crystallography
- Removing heterogeneity due to carbohydrates
- Studying carbohydrate ligand binding
- Removing carbohydrate epitopes from antigens
- Studying the role of glycosylation in protein folding and activity.

Product Code: GE 41

Specifications

Activity: ≥25,000 U/mg, ≥5,000 U/mL

Storage:
Store at 4°C. Do not freeze.

Formulation: The enzyme is provided as a sterile solution in 20 mM Tris HCl pH 7.5.

Stability: Stable at least 12 months when stored properly. Several days exposure to ambient temperatures will not reduce activity.

Product Description

Molecular weight: 34,000 Daltons

Purity: Each lot of PNGase F is tested for contaminating activities by incubating the enzyme for 24 hours at 37°C with the appropriate substrates; the detection limit of this assay is 5 μU/ml (IUB). A passing lot will have no detectable activity.

Contaminant	Substrate
Endo F	Egg white avidin
Beta-Galactosidase	p-nitrophenyl-beta-D-galactopyranoside
N-acetylglucosaminidase	p-nitrophenyl-beta-D-N-acetylglucosaminide
Alpha-Galactosidase	p-nitrophenyl-alpha-D-galactopyranoside
Protease	Denatured BSA

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For the protease assay, 10 µg of denatured BSA is incubated for 24 hours with 2 µL of enzyme. Analysis of the BSA band after SDS-PAGE should show no evidence of degradation. For endoglycosidase assays, 2 mg of native egg white avidin is incubated with 2 µL of enzyme for 1 week. Analysis after gel electrophoresis should show no evidence of cleavage (native avidin is not cleaved by PNGase F).

Specificity: All asparagine-linked complex, hybrid or high mannose oligosaccharides unless α(1-3) core fucosylated. Asparagine must be peptide bonded at both termini.

pH Range: Optimum: pH 7.5
Range: pH 6 – 10

The supplied buffer concentrate provides the optimal pH for enzyme activity with the standard substrate. If glycosidase treatment is performed at suboptimal pH because of glycoprotein solubility or activty requirements, expect some diminution in enzyme activity.

Assay

One unit of PNGase F activity is defined as the amount of enzyme required to catalyze the release of N-linked oligosaccharides from 1 nanomole of denatured Ribonuclease B in 1 min at 37°C, pH 7.5. Cleavage is monitored by SDS-PAGE (cleaved Ribonuclease B migrates faster). One unit of PNGase F is equal to 1 IUB milliunit.

Reagents

- 5X PNGase buffer – 250 mM sodium phosphate pH 7.5
- Denaturation solution- 2% w/v sodium lauryl sulfate, 1 M β-mercaptoethanol
- Triton X-100 solution* - 15% v/v Triton X-100

Suggestions for Use

Procedure for Deglycosylation

1. Add up to 200 µg of glycoprotein to Eppendorf tube. Adjust to 35 µl final volume with deionized water.
2. Add 10 µl 5X PNGase Buffer and 2.5 µl of Denaturation Solution (SDS/β-ME). Heat at 100°C for 5 minutes.
3. Cool, add 2.5 µl of Triton X-100 and mix.
   Note: Failure to add Triton X-100 will result in a 3 fold reduction of PNGase F activity.
4. Add 2.0 µl of PNGase F to the reaction. Incubate 3 hours at 37°C.

If SDS or heat denaturation is omitted, increase incubation time to at least 24 hours. Monitor cleavage by SDS-PAGE.

References

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2. Elder, J.H. and S. Alexander. endo-Beta-N-Acetylglucosaminidase F: Endoglycosidase from Flavobacterium meningosepticum that cleaves both high-mannose and complex glycoproteins. **Proc. Natl. Acad. Sci USA 79:** 4540-4544 (1982)

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6. Taga, E. M., A. Waheed and R. L. Van Etten. Structural and Chemical characterization of a homogeneous peptide N-glycosidase from almond. **Biochemistry 23:** 815-22 (1984).

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This product is intended for in vitro research only.

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