Data Sheet

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Agilent AdvanceBio N-Glycanase-PLUS Peptide-N-Glycosidase F (PNGase F), ≥10 U/mL

(Peptide-N-Glycosidase F) Specifications	
Product Code	GKE-5010
Specific Activity	≥10 U/mg One unit of N-Glycanase-PLUS is defined as the amount of enzyme required to catalyze the release of N-linked oligosaccharides from 1 µmole of denatured ribonuclease β per minute at pH 7.5 and 37 °C.
Activity	≥10 U/mL
Shipping	Shipped on ice pack for next day delivery.
Storage	2 to 8 °C or –20 °C, but avoid repeated freeze-thawing.
Formulation	A sterile-filtered solution in 20 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA (pH 7.5)

Introduction

N-Glycanase-PLUS [PNGase F: Peptide-N^4-(acetyl-β-glucosaminyl)-asparagine amidase, EC 3.5.1.52] is isolated from a strain of *E. coli*, expressing a cloned gene from Chryse*obacterium [Flavobacterium] meningosepticum* (formerly ProZyme).

N-Glycanase is widely used for the removal of N-glycans from glycoproteins and glycopeptides. It is well known that denaturation of glycoprotein substrates before enzyme digestion dramatically increases the efficiency of their deglycosylation, allowing complete removal of most classes of N-glycans from glycoproteins. In contrast, deglycosylation proceeds rather slowly, and in some cases incompletely, with native glycoprotein substrates presumably due to steric constraints.[11] However, it is often desirable to obtain deglycosylation of glycoprotein in the absence of denaturants and detergents to allow structural or functional studies of the folded protein. Studies have shown that to obtain efficient deglycosylation of the native glycoprotein substrates, it is important to use a high starting concentration of the enzyme. Recombinant N-Glycanase-PLUS is recommended for all applications requiring deglycosylation of glycoproteins in the absence of denaturants. The high activity also allows microscale reaction volumes and shorter reaction times to be explored. The highly concentrated enzyme preparation is the reagent of choice for efficient deglycosylation in the absence of detergents, which facilitates subsequent analysis by electrospray or MALDI-TOF mass spectrometry.[19]

Product description

Supplied reagents (retail pack only)

- WS0010 5x N-Glycanase Incubation Buffer (1 mL; 100 m*M* sodium phosphate, 0.1% sodium azide, pH 7.5)
- WS0012 Denaturation Solution (500 µL; 2% SDS, 1 *M* β-mercaptoethanol)
- WS0013 Detergent Solution (200 µL; 15% nonionic detergent solution)
- WS0145 5x N-Glycanase Tris Reaction Buffer (1 mL; 50 m*M* Tris-HCl, pH 8.0)

Note: Tris Reaction Buffer has been included as an alternative reaction buffer because phosphate buffers should be avoided if mass spectrometry is used in downstream analysis.

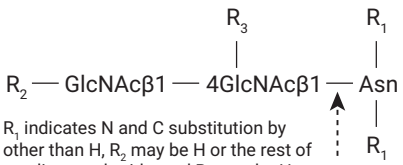
Purity

The absence of exoglycosidase contaminants was confirmed by extended incubations with the corresponding pNP and MU-glycosides.

No protease activity was detectable after incubation of the enzyme with 0.2 mg resorufin-labeled casein for ~18 hours at 37 °C.[16]

Specificity

N-Glycanase-PLUS releases intact N-linked oligosaccharides from glycoproteins and glycopeptides. Prior denaturation of the glycoprotein substrate by treatment with heat/SDS greatly enhances the rate and reliability of N-glycan removal, although at high concentrations the enzyme can remove intact glycans from undenatured glycoproteins.



R_1 indicates N and C substitution by other than H, R_2 may be H or the rest of an oligosaccharide, and R_3 may be H or α(1-6) fucose.

The site of enzyme cleavage is highly specific, with hydrolysis occurring between asparagine and proximal N-acetyl-glucosamine of most oligomannose, hybrid-and complex-type N-glycans. The enzyme releases 1-amino oligosaccharide, which is hydrolyzed nonenzymatically to form ammonia and free oligosaccharides having an intact chitobiose reducing terminus. The peptide backbone is an important structural determinant since glycan cleavage will not occur from an asparagine having unsubstituted α-amino and carboxyl groups. While di-N-acetylchitobiose is the minimum glycan structural determinant,[2] cleavage does not occur if there is core α(1-3)-linked fucose as commonly encountered in plant glycoproteins.[15] Phosphate, sulfate, and sialic acid groups attached to the oligosaccharide do not affect cleavage.[4] As a consequence of hydrolysis, the asparagine on the peptide is converted to aspartic acid, but the polypeptide remains intact.[8,11,12,9,13] True endoglycosidases, such as endo F and endo H, have more restricted specificities, and do not release intact oligosaccharides since they cleave within the chitobiose core, and leave a single N-acetyl-glucosamine attached to the polypeptide.[8,13,3]

Molecular weight

~35,000 daltons[11,13]

pH range[8,13]

Optimum: pH 8.6

Range: 7.5 to 9.5

Stability

To promote stability of the N-Glycanase-PLUS, extended incubations may be performed at 25 °C rather than 37 °C.[2]

Assay

One unit of N-Glycanase-PLUS is defined as the amount of enzyme required to catalyze the release of N-linked oligosaccharides from 1 µmole of denatured ribonuclease β per minute at pH 7.5 and 37 °C.

Note: One unit of N-Glycanase-PLUS is equal to one IUB Unit.

Suggestions for use

Before use, briefly centrifuge the vial to ensure all material is at the base of the vial. Ensure that reagents, substrates, and laboratory-ware are free from contaminants and proteases.

The amount of enzyme required for deglycosylation depends on the substrate, incubation conditions, and the precise application. For a review of methods see Montreuil *et al.* (1994)[17] and Miramutsu (1992)[18] in addition to references cited therein. In the case of glycoprotein substrates, it is recommended to denature the substrate before deglycosylation. In general, 10 mU of enzyme is sufficient to deglycosylate up to 100 µg denatured glycoprotein or 20 µg native glycoprotein in 18 hours at pH 7.5 and 37 °C. In some cases, further optimization of the method may be necessary to achieve complete deglycosylation.[5]

In particular, incubation times may be reduced by using a higher concentration of N-Glycanase-PLUS in reaction mixtures. Prior denaturation of the glycoprotein substrate by heating at 100 °C in the presence of up to 1% (w/v) SDS greatly enhances both the rate and extent of deglycosylation.[11] Ionic detergents are potent inhibitors of N-Glycanase-PLUS; however, nonionic detergents (Triton X-100, Nonidet P-40 or *n*-octylglucoside) are not inhibitory, and can be used in approximately 5-fold excess to counteract the inhibitory effects of ionic detergent.[10]

Sulfhydryl reagents such as β-mercaptoethanol used for glycoprotein denaturation do not interfere with enzyme activity. N-Glycanase-PLUS tolerates most chaotrophic agents, and is at least 80% active in the presence of <5 M urea, <2 M guanidine HCl and 0.25 M NaSCN; however, the enzyme is inactivated by the presence of guanidine thiocyanate.[4]

N-Glycanase-PLUS is compatible with a wide range of buffers.[5] The purified enzyme is free from detectable protease activity. Additional protease inhibitors (for example, PMSF, pepstatin A, benzamidine, aprotinin, leupeptin, and 1,10-phenanthroline) can be included in enzyme digestions to inhibit any other types of proteases present in samples. This is particularly important when deglycosylation under native conditions is performed, and retention of protein conformation is desirable. Deglycosylation efficiency against metalloprotein substrates has been suggested to be enhanced by inclusion of EDTA at between 0.1 and 1 mM final concentration. Deglycosylation can be conveniently analyzed using SDS-PAGE if the loss of glycans results in a significant lowering of the protein's molecular weight.

Procedure for deglycosylation (denaturing conditions)

1. Prepare 50 to 500 µg glycoprotein solution in 45 µL of 1x Incubation Buffer. Add 2.5 µL of SDS/β-mercaptoethanol (final reaction concentration 0.1% SDS, 50 mM β-mercaptoethanol).
2. Denature glycoprotein by heating at 100 °C for five minutes. Allow the mixture to cool.
3. Add 2.5 µL of NP-40 (final reaction concentration 0.75% detergent).
4. Add 1 µL N-Glycanase-PLUS to reaction mixture and incubate for two hours to overnight at 37 °C.

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