N-GLYCANASE®

(Peptide-N-Glycosidase F)

SPECIFICATIONS

Product Code: GKE-5006
Specific Activity: ≥10 U/mg*
Activity: ≥2.5 U/ml
Shipped on ice pack for next day delivery.
Store enzyme at 2-8°C or -20°C, but avoid repeated freeze-thawing.
Formulation: A sterile-filtered solution in 20 m*M* Tris-HCl, 50 m*M* NaCl, 1 m*M* EDTA (pH 7.5).

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

N-Glycanase [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 *Chryseobacterium [Flavobacterium] meningosepticum*[1,2].

Applications:

- Release of intact N-linked glycans from glycopeptides and glycoproteins
- Structure-function studies of N-glycosylated glycoproteins
- Preparation of deglycosylated proteins for molecular weight estimation or crystallography studies

PRODUCT DESCRIPTION

Supplied Reagents (retail packs only)

WS0010 5x N-Glycanase Reaction Buffer (1 ml; 100 m*M* sodium phosphate, 0.1% sodium azide, pH 7.5)

WS0012 Denaturation Solution (200 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 or 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 according to the method described by Twining [3].

Specificity: N-Glycanase 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.

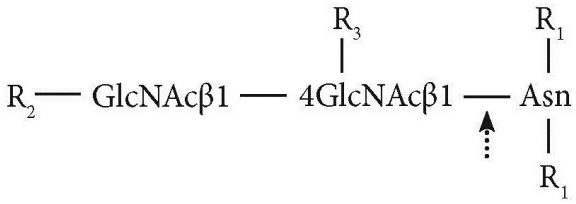

Figure 1 - Specificity
R_1: N & C substitution by other than H
R_2: H or the rest of an oligosaccharide
R_3: H or α(1-6) fucose

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 (Figure 1). The enzyme releases 1-amino oligosaccharide, which is hydrolyzed non-enzymatically 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[4], cleavage does not occur if there is core α(1-3)-linked fucose as commonly encountered in plant glycoproteins[5]. Phosphate, sulfate and sialic acid groups attached to the oligosaccharide do not affect cleavage[6]. As a consequence of hydrolysis the asparagine on the peptide is converted to aspartic acid, but otherwise the polypeptide remains intact[7,8,9,10,11]. 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-acetylglucosamine attached to the polypeptide[7,11,12].

Molecular Weight: ~35,000 daltons[8,11]

pH Range[7,11]:

Optimum: pH 8.6
Range: pH 7.5 - 9.5

Stability: Extended incubations may be performed at 25°C rather than 37°C to promote stability of the N-Glycanase[4]

ASSAY

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

NOTE: One unit of N-Glycanase 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)[13] and Miramutsu (1992)[14] 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[15]. In particular, incubation times may be reduced by using a higher

concentration of N-Glycanase 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[8] Ionic detergents are potent inhibitors of N-Glycanase, however non-ionic detergents (Nonidet P-40, n-octylglucoside or Triton® X-100) are not inhibitory and can be used in approximately 5-fold excess to counteract the inhibitory effects of ionic detergent[16]. Sulfhydryl reagents such as β-mercaptoethanol used for glycoprotein denaturation do not interfere with enzyme activity. N-Glycanase 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 guanidinium thiocyanate[6].

N-Glycanase is compatible with a wide range of buffers[15]. The purified enzyme is free from detectable protease activity. Additional protease inhibitors (*e.g.* 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 m*M* final concentration. Deglycosylation can be conveniently analyzed using SDS-PAGE if the removal of glycans results in a significant reduction of the protein's molecular weight.

Procedure for Deglycosylation (Denaturing Conditions)

1. Prepare 50 to 500 µg glycoprotein solution in 45 µl of 1x Reaction Buffer of choice. Add 2.5 µl of Denaturation Solution (final reaction concentration 0.1% SDS, 50 m*M* β-mercaptoethanol).

2. Denature glycoprotein by heating at 100°C for 5 minutes. Allow mixture to cool.

3. Add 2.5 µl of Detergent Solution (final reaction concentration 0.75% detergent).

4. Add 2 µl of N-Glycanase to the reaction mixture and incubate for 2 hours to overnight at 37°C.

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