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Product Information

Endoglycosidase H, from *Streptococcus plicatus* recombinant, expressed in *E. coli*

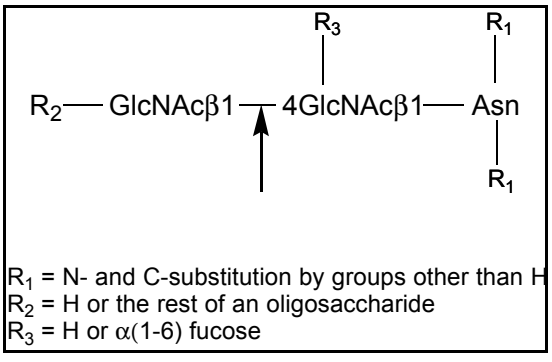
Catalog Number **E7642**
Storage Temperature 2–8 °C

CAS RN 37278-88-9
EC 3.2.1.96
Synonyms: β-N-Acetylglucosaminidase H, Endo H, Endo-β-N-acetylglucosaminidase H

Product Description
One of the distinguishing features of the proteome in eurkaryotic cells is that most proteins are subject to post-translational modification, of which glycosylation is the most common form. It is estimated that more than half of all proteins are glycoproteins. Two major classes of oligosaccharides (glycans) may be attached to proteins. N-linked glycans are attached to the amide side chain of Asn residues, which form part of the consensus sequence AsnXaaSer/Thr, while O-linked glycans may be added to the hydroxyl side chain of Ser or Thr residues.

The core structure and compositon of N-linked glycans are different from those of O-linked glycans. The core structure of N-linked glycans is shown in Figure 1.

Figure 1.
Core Structure of N-linked Glycans



R_1 = N- and C-substitution by groups other than H
R_2 = H or the rest of an oligosaccharide
R_3 = H or α(1-6) fucose

Endoglycosidase H cleaves between the N-acetyl-glucosamine residues of the chitobiose core of N-linked glycans, leaving one N-acetylglucosamine residue attached to the asparagine.

The specificity of this enzyme is such that oligomannose and most hybrid types of glycans, including those that have a fucose residue attached to the core structure, are cleaved; whereas, complex type glycans are not released. Thus, this enzyme is extremely useful for selective release of oligomannose or hybrid type glycans from glycoproteins. The enzyme is also active against dolichol-linked glycans containing these structures. The enzyme has found extensive use in the characterization of glycoproteins,[1] dolichol-linked glycans,[2] and the biosynthetic pathway for N-glycosylation.[3] The action of this enzyme against native N-linked glycans on a glycoprotein can be assessed by a reduction in molecular mass leading to a change in the migration of the protein during SDS-PAGE.

Molecular mass: ~27 kDa

Optimal pH: 5.5
(workable pH range is between 5.0 to 6.0)

This enzyme is isolated from a recombinant glycosidase-free *E. coli* strain, which carries the *Streptomyces plicatus* gene on a plasmid.[4] The enzyme is supplied in 50 mM sodium phosphate, pH 7, containing 25 mM EDTA and preservative.

Unit Definition: One unit will hydrolyze 1.0 μmole of dabsyl-Asn-$(GlcNAc)_2(Man)_5$ per minute at pH 5.5 at 37 °C.

Each lot of enzyme is tested and confirmed negative for the following contaminating activities: α/β-glucosidase, β-galactosidase, α-mannosidase, α-fucosidase, and β-N-acetylhexosaminidase. Protease activity was also not detected.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
It is recommended to store the product at 2–8 °C.
Do Not Freeze. Freezing the solution may lead to the inactivation of the enzyme activity.

Procedure
The extent and rate of the deglycosylation of glycoproteins depend to a high degree on the nature of the glycoprotein. Therefore, no specific instructions with regard to the incubation conditions can be given. The deglycosylation rate can be increased by denaturation of the glycoprotein. In general, 50–250 milliunits of endoglycosidase H should be sufficient to deglycosylate up to 1 mg of high-mannose glycoprotein/ml when incubated overnight at 37 °C.

Note: The enzyme activity against glycoproteins is increased when inter- and intra-molecular disulfide bridges are reduced by addition of 0.1 M 2-mercaptoethanol. In addition, detergents such as Triton™ X-100, n-octylglucoside, or zwitterionic detergents have no influence on the enzyme activity.

References

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6. Trimble, R.E., *et al*., Anal. Biochem., **141**, 515 (1984).

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