

Product Information

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

Catalog Number **A0810**

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

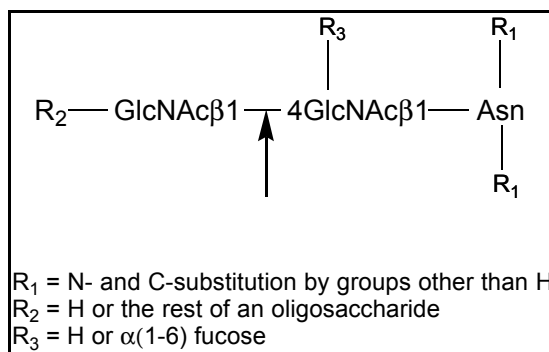
Product Description

One of the distinguishing features of the proteome in eukaryotic 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 composition 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



Endoglycosidase H cleaves between the N-acetylglucosamine 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

Workable pH range: 5.0–6.0 (optimal pH at 5.5)

No loss of activity was observed during incubation at 37 °C for 48 hours over the pH range of 4.5–8.5. However, below pH 4.5, activity is rapidly lost.

The enzyme is supplied as a buffered aqueous solution in 20 mM Tris HCl, pH 7.5, containing 50 mM NaCl and 1 mM EDTA.

Endoglycosidase H is tested and confirmed negative for contaminating activities of other endo- and exo-glycosidases. Protease activity was also not detected.

Unit Definition: One unit will release the N-linked oligosaccharides from 1 μ mole of ribonuclease B per minute at 37 °C at pH 5.5.

Components

- Endoglycosidase H (Catalog number A0810) – The enzyme is supplied in a vial containing 1.0 unit in 20 mM Tris HCl, pH 7.5, with 50 mM NaCl and 1 mM EDTA.
- 5x Reaction Buffer (Catalog number R1271) – 0.2 mL of 250 mM sodium phosphate buffer, pH 5.5, is provided.
- Denaturation Solution (Catalog number S4927) – 0.2 mL of 2% sodium dodecyl sulfate (SDS) with 1 M 2-mercaptoethanol is provided.

Precautions and Disclaimer

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

Storage/Stability

It is recommended to store the product at 2–8 °C. The components are stable for 1 year if stored unopened at 2–8 °C.

Procedure

Denaturation of the glycoprotein prior to digestion with the enzyme may not be necessary. However, longer incubation times as well as more enzyme may be required to deglycosylate a native glycoprotein. SDS does not affect enzyme activity.

1. Add up to 200 µg of glycoprotein to a microcentrifuge tube. Bring the volume to 37.5 µL with deionized water.
2. Add 10 µL of 5x Reaction Buffer (Catalog number R1271) and 2.5 µL of Denaturation Solution (Catalog number S4927, SDS/2-mercaptoethanol).
3. Heat at 100 °C for 5 minutes.
4. Cool on ice and then add 2 µL of enzyme (Catalog number A0810).
5. Incubate at 37 °C for 3 hours.
6. Monitor cleavage by SDS-PAGE.

References

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3. Hsieh, P. *et al.*, J. Biol. Chem., **258**, 2555 (1983).
4. Tarentino, A.L. *et al.*, J. Biol. Chem., **274**, 811 (1974).
5. Trimble, R.E. *et al.*, Anal. Biochem., **141**, 515 (1984).
6. Trimble, R.E. *et al.*, Methods Enzymol., **138**, 763 (1987).

GCY,SG,AE,MAM 06/15-1