



Endo H (Endoglycosidase H)

endo-beta-N-acetylglucosaminidase H

Source

recombinant gene from *Streptomyces plicatus* in *E. Coli*

Catalog Number

E-EH02 60 µl

E-EH02-20 20 µl

E-EH02-200 200 µl

EC 3.2.1.96

Recommended Reagents

included with E-EH02 and E-EH02-20

1 vial: 5x Reaction Buffer - 400 µl
250 mM sodium phosphate, pH5.5

1 vial: Denaturation Solution - 200 µl 2%
SDS, 1 M Beta-mercaptoethanol

Activity ≥ 5 U/ml

Specific Activity ≥ 40 U/mg

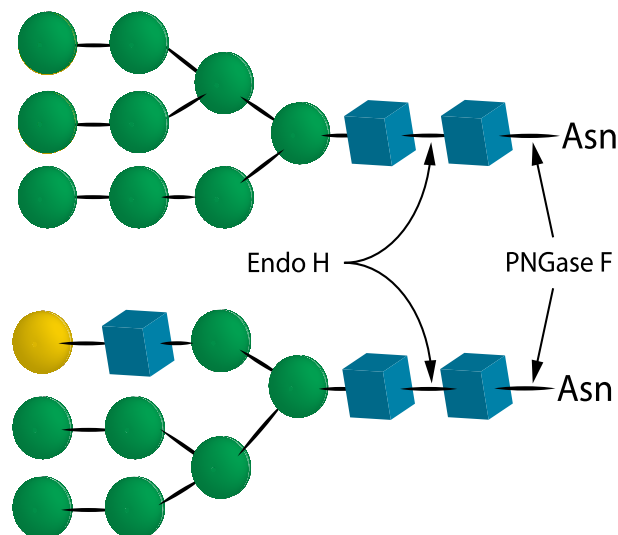
Applications

Releases asparagine-linked hybrid or high mannose oligosaccharides, but not complex oligosaccharides.

Endo H cleaves between the two N-acetylglucosamine residues in the diacetylchitobiose core of the oligosaccharide, generating a truncated sugar molecule with one N-acetylglucosamine residue remaining on the asparagine. In contrast, PNGase F removes the oligosaccharide intact. Detergent and heat denaturation may increase the rate of cleavage for some glycoproteins.

Specific Activity

One unit of Endo H activity is defined as the amount of enzyme required to catalyze the release of N-linked oligosaccharides from 1 µmole of denatured Ribonuclease B. Cleavage is monitored by SDS-PAGE (cleaved Ribonuclease B migrates faster).



Specificity

QA-Bio™ Endo H cleaves Asparagine-linked hybrid or high mannose oligosaccharides, but not complex oligosaccharides. It cleaves between the two N-acetylglucosamine residues in the diacetylchitobiose core of the oligosaccharide, generating a truncated sugar molecule with one N-acetylglucosamine residue remaining on the asparagine. In contrast, PNGase F removes the oligosaccharide intact. Detergent and heat denaturation may increase the rate of cleavage for some glycoproteins.

Formulation

The enzyme is provided as a sterile-filtered solution in 20 mM Tris-HCl, 25mM NaCl, 1 mM EDTA (pH 7.5).

Molecular Weight approximately 29 kD

pH optimum: 5.5, active over the range 5-6.

Storage

Store enzyme at 4°C. Do not freeze.

Stability

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

Endo H
Specifications - Protocol

QA-Bio

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Quality & Purity

QA-Bio Endo H is tested for contaminating protease as follows: 10 µg of denatured BSA is incubated at 37°C for 24 hours with 2 µl of enzyme. SDS-PAGE analysis of the treated BSA shows no evidence of degradation.

The production host strain has been extensively tested and does not produce any detectable glycosidases.

Directions for use

1. Add up to 200 µg of glycoprotein to an Eppendorf tube. Adjust to 37.5 µl final volume with de-ionized water.
2. Add 10 µl 5x Reaction Buffer 5.5 and 2.5 µl of Denaturation Solution. Heat at 100°C for 5 minutes.

NOTE: It is not necessary to add Triton X-100. SDS will not inactivate Endo H.

3. Add 2.0 µl of Endo H 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

Robbins P. W., D. F. Wirth and C. J. Hering. Expression of the *Streptomyces* enzyme endoglycosidase H in *Escherichia coli*. *Biol Chem* 256:10640-10644 (1981).

Robbins P. W., R. B. Trimble, D. F. Wirth, C. Hering, F. Maley, G. F. Maley, R. Das, B. W. Gibson, N. Royal and K. Biemann. Primary structure of the *Streptomyces* enzyme endo-beta-N-acetylglucosaminidase H. *J Biol Chem* 259:7577-7583 (1984).

Trimble R. B., A. L. Tarentino, G. E. Aumick and F. Maley. Endo-beta-N-acetylglucosaminidase L from *Streptomyces plicatus*. *Methods Enzymol* 83:603-610 (1982).

Trimble R. B. and F. Maley. Optimizing hydrolysis of N-linked high-mannose oligosaccharides by endo-beta-N-acetylglucosaminidase H. *Anal Biochem* 141:515-522 (1984).

Trimble R. B., R. J. Trumbly and F. Maley. Endo-beta-N-acetylglucosaminidase H from *Streptomyces plicatus*. *Methods Enzymol* 138:763-770 (1987).

Trumbly R. J., P. W. Robbins, M. Belfort, F. D. Ziegler, F. Maley and R. B. Trimble. Amplified expression of *Streptomyces* endo-beta-N-acetylglucosaminidase H in *Escherichia coli* and characterization of the enzyme product. *J Biol Chem* 260:5683-5690 (1985).

Warranties and liabilities

QA-Bio warrants that the above product conforms to the specifications described herein. Should the product fail for reasons other than through misuse QA-Bio will, at its option, replace free of charge or refund the purchase price. This warranty is exclusive and QA-Bio makes no other warranties, expressed or implied, including any implied conditions or warranties of merchantability or fitness for any particular purpose. QA-Bio shall not be liable for any incidental, consequential or contingent damages.

This product is intended for *in vitro* research only.

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