Endo H (Endoglycosidase H)
endo-beta-N-acetylglucosaminidase H

Source
recombinant from *Streptomyces plicatus*

Catalog Number
E-EH02

Certification of Analysis Lot Number
1011.1A

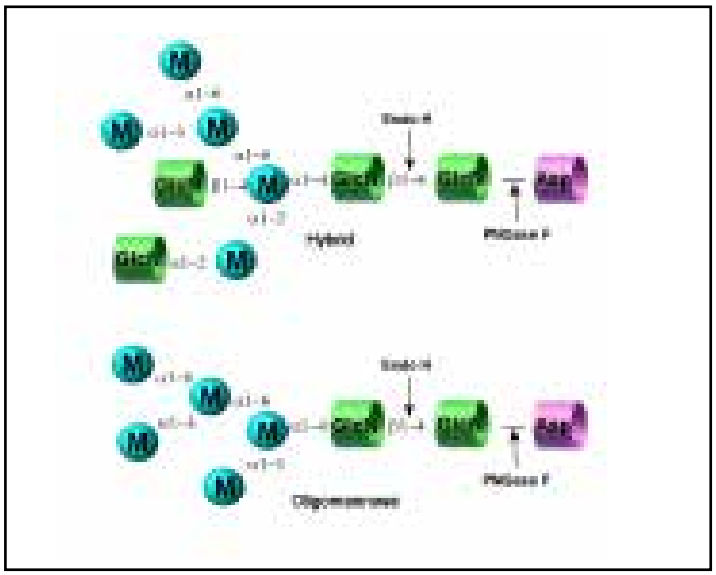
EC 3.2.1.96

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.

Contents
1 vial: Endo H- 60 µl (0.3 U)
Tris-HCl, 25 mM NaCl, 1 mM EDTA (pH 7.5)
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

Specific Activity >30 U/mg
Activity >5 U/ml

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 m*M* 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.

E-EH02 Endo H
Specifications - Protocol

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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 absence of exoglycosidase contaminants is confirmed by extended incubations with the corresponding pNP-glycosides.

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.

4. 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-Nacetylglucosaminidase H. Anal Biochem 141:515-522 (1984).

Trimble R. B., R. J. Trumbly and F. Maley. Endo-beta-Nacetylglucosaminidase 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 warrants, 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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