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

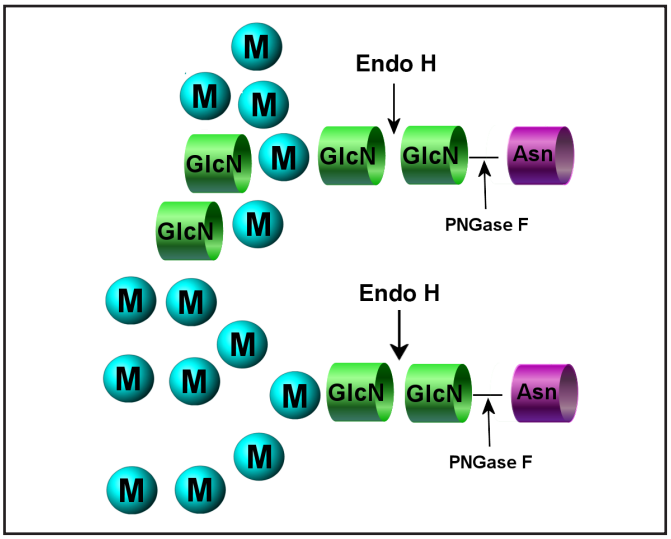
Recommended Reagents
included with E-EH02 and E-EH02-20
1 vial: 5x Reaction Buffer - 400 µl
250 mM sodium phosphate, pH5.5
1 vial: Denaturation Solution - 200 µl 2%
SDS, 1 M Beta-mercaptoethanol

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 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.

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

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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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