Endo H_f

1-800-632-7799
info@neb.com
www.neb.com

P0703S 018140816081

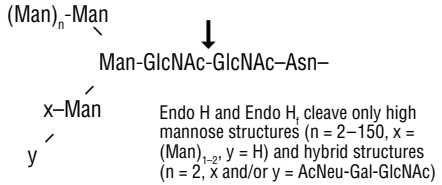
P0703S

100,000 units **1,000,000 U/ml** **Lot: 0181408**

RECOMBINANT **Store at –20°C** **Exp: 8/16**

Description: Endo H_f is a recombinant protein fusion of Endoglycosidase H and maltose binding protein. Endo H_f cleaves the chitobiose core of high mannose and some hybrid oligosaccharides from N-linked glycoproteins (1) equally as well as Endo H.

Specificity:



Endo H and Endo H_f cleave only high mannose structures (n = 2–150, x = $(Man)_{1-2}$, y = H) and hybrid structures (n = 2, x and/or y = AcNeu-Gal-GlcNAc)

Source: Cloned from *Streptomyces plicatus* (2) and overexpressed in *E. coli* (3)

Applications:

- Removal of high mannose N-glycans from glycoproteins

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 5 mM Na_2EDTA.

Reagents Supplied with Enzyme:
10X Glycoprotein Denaturing Buffer:
5% SDS, **0.4 M DTT**

10X G5 Reaction Buffer:
0.5 M Sodium Citrate (pH 5.5 @ 25°C)

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

Reaction Conditions:
Typical reaction conditions are as follows:

1. Combine 1–20 µg of glycoprotein, 1 µl of 10X Glycoprotein Denaturing Buffer and H_2O (if necessary) to make a 10 µl total reaction volume.
2. Denature glycoprotein by heating reaction at 100°C for 10 minutes.
3. Make a total reaction volume of 20 µl by adding 2 µl of 10X G5 Reaction Buffer, H_2O and 1–5 µl Endo H.
4. Incubate reaction at 37°C for 1 hour.

Note: Reactions may be scaled-up linearly to accommodate larger reaction volumes.

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl (10 NEB units = 1 IUB milliunit).

Unit Definition Assay: 10 µg of RNase B are denatured with 1X Glycoprotein Denaturing Buffer at 100°C for 10 minutes. After the addition of 1X G5 Reaction Buffer, two-fold dilutions of Endo H_f are added and the reaction mix is incubated for 1 hour at 37°C. Separation of reaction products are visualized by SDS-PAGE.

Specific Activity: ~232,000 units/mg.

Molecular Weight: 70,000 daltons.

Quality Assurance: No contaminating exoglycosidase or proteolytic activity could be detected.

Quality Controls

Glycosidase Assays: 5,000 units of Endo H were incubated with 0.1 mM of flourescently-labeled oligosaccharides and glycopeptides, in a 10 µl reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

(See other side)

CERTIFICATE OF ANALYSIS

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Specificity:

(Man)n-Man
Man-GlcNAc-GlcNAc–Asn–
x–Man
y

Endo H and Endo H_f cleave only high mannose structures (n = 2–150, x = $(Man)_{1-2}$, y = H) and hybrid structures (n = 2, x and/or y = AcNeu-Gal-GlcNAc)

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Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

(See other side)

CERTIFICATE OF ANALYSIS

No other glycosidase activities were detected (ND) with the following substrates:

β-N-Acetyl-glucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4GlcNAc-AMC ND

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ 1-4Glc-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

β-Mannosidase:
Manβ1-4Manβ1-4Man-AMC ND

Endo F_2, F_3:
Dansylated fibrinogen biantennary. ND

PNGase F:
Fluoresceinated fetuin triantennary. ND

Protease Assay: After incubation of 5,000 units of Endo H_f with 0.2 nmol of a standardized mixture of proteins, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

Notes On Use: Enzymatic activity is not affected by SDS.

To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

References:

1. Maley, F. et al. (1989) *Anal. Biochem.* 180, 195–204.
2. Robbins, P. et al. (1984) *J. Biol. Chem.* 259, 7577–7583.
3. Guan, C and Wong, S., New England Biolabs, Inc., unpublished results.

Companion Product Sold Separately:
RNase B
#P7817S 250 µg

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GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND

α-Galactosidase:
Galα1-3Galβ1-4GlcNAc-AMC ND

α-Neuraminidase:
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ 1-4Glc-AMC ND

α-Mannosidase:
Manα1-3Manβ1-4GlcNAc-AMC
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

β-Xylosidase:
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

β-Mannosidase:
Manβ1-4Manβ1-4Man-AMC ND

Endo F_2, F_3:
Dansylated fibrinogen biantennary. ND

PNGase F:
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