

# Endo H



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



P0702S 016130515051

## P0702S

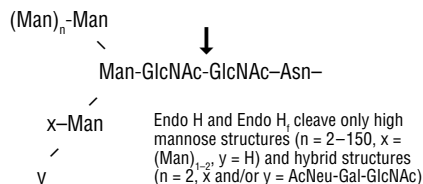


10,000 units 500,000 U/ml Lot: 0161305

RECOMBINANT Store at -20°C Exp: 5/15

**Description:** Endoglycosidase H is a recombinant glycosidase which cleaves the chitobiose core of high mannose and some hybrid oligosaccharides from N-linked glycoproteins (1)

### Specificity:



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

### Applications:

- Removal of carbohydrate residues from proteins

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 5 mM Na<sub>2</sub>EDTA.

### 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:

- Combine 1–20 µg of glycoprotein, 1 µl of 10X Glycoprotein Denaturing Buffer and H<sub>2</sub>O (if necessary) to make a 10 µl total reaction volume.
- Denature glycoprotein by heating reaction at 100°C for 10 minutes.
- Make a total reaction volume of 20 µl by adding 2 µl of 10X G5 Reaction Buffer, H<sub>2</sub>O and 1–5 µl Endo H.
- 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

are added and the reaction mix is incubated for 1 hour at 37°C. Separation of reaction products is visualized by SDS-PAGE.

**Specific Activity:** ~ 915,000 units/mg

**Molecular Weight:** 29,000 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 fluorescently-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

# Endo H



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



P0702S 016130515051

## P0702S

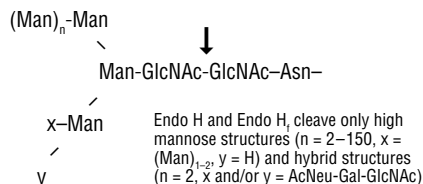


10,000 units 500,000 U/ml Lot: 0161305

RECOMBINANT Store at -20°C Exp: 5/15

**Description:** Endoglycosidase H is a recombinant glycosidase which cleaves the chitobiose core of high mannose and some hybrid oligosaccharides from N-linked glycoproteins (1)

### Specificity:



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

### Applications:

- Removal of carbohydrate residues from proteins

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 5 mM Na<sub>2</sub>EDTA.

### 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:

- Combine 1–20 µg of glycoprotein, 1 µl of 10X Glycoprotein Denaturing Buffer and H<sub>2</sub>O (if necessary) to make a 10 µl total reaction volume.
- Denature glycoprotein by heating reaction at 100°C for 10 minutes.
- Make a total reaction volume of 20 µl by adding 2 µl of 10X G5 Reaction Buffer, H<sub>2</sub>O and 1–5 µl Endo H.
- 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

are added and the reaction mix is incubated for 1 hour at 37°C. Separation of reaction products is visualized by SDS-PAGE.

**Specific Activity:** ~ 915,000 units/mg

**Molecular Weight:** 29,000 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 fluorescently-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

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:**  
Galα1-3Galβ1-4Galα1-3Gal-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

Page 2 (P0702)

**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:**  
Galα1-3Galβ1-4Galα1-3Gal-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

Page 2 (P0702)

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

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

**Endo F<sub>2</sub>, F<sub>3</sub>:**  
Dansylated fibrinogen biantennary. ND

**PNGase F:**  
Fluoresceinated fetuin triantennary. ND

**Protease Assay:** After incubation of 5,000 units of Endo H 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.

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

**Endo F<sub>2</sub>, F<sub>3</sub>:**  
Dansylated fibrinogen biantennary. ND

**PNGase F:**  
Fluoresceinated fetuin triantennary. ND

**Protease Assay:** After incubation of 5,000 units of Endo H 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:**  
RNase B (NEB #P7817S)

#### 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:**  
RNase B (NEB #P7817S)