Endo H

10,000 units 500,000 U/ml Lot: 0161210
RECOMBINANT Store at –20°C Exp: 10/14

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:

(Man)ₙ−Man \downarrow \text{Man-GlcNAc-GlcNAc-Asn}−x−Man

Endo H and Endo Hs cleave only high mannose structures (n = 2–150, x = (Man)₁₋₉ y = H) and hybrid structures (n = 2, x and/or y = AcNeuGal-GlcNAc)

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 Na₂EDTA.

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 H₂O (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, H₂O 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 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)
No other glycosidase activities were detected (ND) with the following substrates:

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

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

**β-Galactosidase:**
Gallβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND

**α-Galactosidase:**
Galα1-3Galβ1-4Gal-AMC ND

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

**α-Mannosidase:**
Manβ1-3Manβ1-4GlcNAc-AMC
Manα1-6Manβ1-6(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<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:**

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