Endo H

Recombinant Store at –20°C Exp: 8/16

10,000 units 500,000 U/ml Lot: 0161408

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

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.

Specificity:

\[
\text{(Man)}_n \text{Man} \quad \text{Man-GlcNAc-GlcNAc-Asn-} \\
\text{x-Man} \\
\text{y}
\]

Endo H and Endo Hf cleave only high mannose structures (n = 2–150, x = \(\text{(Man)}_x\), y = \(\text{H}\)) and hybrid structures (n = 2, x and/or y = \(\text{AcNeu-Gal-GlcNAc}\))

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

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 24 hours at 37°C. The reaction products were analyzed by TLC for digestion of oligosaccharides and glycopeptides, in a 10 µl reaction for 20 hours at 37°C. The reaction products were analyzed by SDS-PAGE analysis using Coomassie Blue detection.

Glycosidase Assays: 5,000 units of Endo H were incubated with 0.1 mM of fluorescein-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.

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 fluorescein-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:**
Fucoc1-2Galβ1-4Glc-AMC Galβ1-4
(Fucc1-3)GlcNAcβ1-3Galβ1-4Glc-AMC  ND

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

**α-Galactosidase:**
Galβ1-3Galβ1-3Galβ1-4Glc-AMC  ND

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

**α-Mannosidase:**
Manβ1-3Manβ1-3Manβ1-4Man-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, F₄:
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 Sold Separately:
RNase B  
#P7817S  250 µg