Endo H

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

**Applications:**
- Removal of carbohydrate residues from proteins

**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.**

**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 fluorescencently-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.
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(Fucoc1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

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

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

**α-Neuraminidase:**
Neu5Acβ2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-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 F2, F3:**
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

New England Biolabs® is a registered trademark of New England Biolabs, Inc.