Endo H

**Certification of Analysis**

**Specialty:**
\[
(\text{Man})_n \text{Man} \\
\text{Man-GlcNAC-GlcNAC-Asn}\]

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

**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 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.

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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:**
Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-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 bi antennary. ND

**PNGase F:**
Fluoresceinated fetuin tri antennary. 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

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