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

Reagents Supplied with Enzyme:
10X Glycoprotein Denaturing Buffer: 5% SDS, 0.4 M DTT
10X Glycobuffer 3: [0.5 M Sodium Acetate (pH 6.0 @ 25°C)]

Reaction Conditions:
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 Glycobuffer 3, 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 GlycoBuffer 3, 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**: Fucα1-2Galβ1-4Glc-AMC Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND
- **β-Galactosidase**: Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND
- **α-Galactosidase**: Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND
- **α-Neuraminidase**: Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC ND
- **α-Mannosidase**: Manβ1-3Manβ1-4GlcNAc-AMC Manβ1-6Manβ1-6(Manβ1-3)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

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