Endo H

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

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

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

**Reaction Conditions:**
- Typical reaction conditions 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 to 100°C for 10 minutes.
  3. Make a total reaction volume of 20 µl by adding 2 µl of 10X GS Reaction Buffer, H2O and 1–5 µl Endo H.
  4. Incubate reaction at 37°C for 1 hour.

**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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**Endo Hf**

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

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

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

**Reaction Conditions:**
- Typical reaction conditions 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 to 100°C for 10 minutes.
  3. Make a total reaction volume of 20 µl by adding 2 µl of 10X GS Reaction Buffer, H2O and 1–5 µl Endo H.
  4. Incubate reaction at 37°C for 1 hour.

**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 Hf 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.
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:**
Galα1-3Galβ1-4Galα1-3Galβ1-4Glc-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 F₄, F₅:
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)