**Glycosidase**

**Source:** Cloned from *Enterococcus faecalis* and expressed in *E. coli* (1).

**Applications:**
- Removal of Core 1 & Core 3 O-linked disaccharide glycans from glycoproteins

Supplied in: 50 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C) and 1 mM Na₂EDTA.

**Reagents Supplied with Enzyme:**
- 10X Glycoprotein Denaturing Buffer
- 10X G7 Reaction Buffer
- 10% NP-40

**Unit Definition:** One unit is defined as the amount of enzyme required to remove 0.68 nmol of O-linked disaccharide from 5 mg of neuraminidase digested, non-denatured fetuin (2) in 1 hour at 37°C in a total reaction volume of 100 µl (1 unit of both O-Glycosidase and PNGase F will remove equivalent molar amounts of O-linked disaccharides and N-linked oligosaccharides, respectively).

**Non-Denaturing Unit Definition Assay:** Two fold serial dilutions of O-Glycosidase are added to a reaction mixture of 5 mg of neuraminidase digested fetuin with 1X G7 Reaction Buffer. The reaction mix is then incubated at 37°C for 1 hour. O-linked disaccharide carbohydrates are determined by the Morgan and Elson Assay (2).

**Note:** Under denaturing conditions the enzyme activity is increased two-fold. This observation is substrate dependent.

**Specific Activity:** 53,000,000 units/mg.

**Molecular Weight:** 147,000 daltons.

**Quality Assurance:** No contaminating exoglycosidase or proteolytic activity could be detected.

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**Quality Controls**

**Glycosidase Assays:**
200,000 units of O-Glycosidase 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.

No other glycosidase activities were detected (ND) with the following substrates:
- β-N-Acetylgalactosaminidase: GalNAcβ1-4Galβ1-4Glc-AMC ND
- α-N-Acetylgalactosaminidase: GalNAcα1-3(Fucx1-2)Galβ1-4Glc-AMC ND
- α-Fucosidase: Fucx1-2Galβ1-4Glc-AMC ND
- Galβ1-4 (Fucx1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

(see other side)
**β-Galactosidase:**
Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC ND
Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC ND

**α-Galactosidase:**
Galα1-3Galβ1-4Gal-AMC ND
Galα1-6Galα1-6Glcα1-2Fru-AMC ND

**α-Mannosidase:**
Manα1-3Manβ1-4GlcNAc-AMC ND
Manα1-6Manα1-6(Manα1-3)Man-AMC ND

**β-Glucosidase:**
Glcβ1-4Glcβ1-4Glc-AMC ND

**α-Glucosidase:**
Glcα1-6Glcα1-4Glc-AMC ND

**β-Xylosidase:**
Xylβ1-4Xylβ1-4Xylβ1-4Xyl-AMC ND

**β-Mannosidase:**
Manβ1-4Manβ1-4Man-AMC ND

**Endo F1, F2, H:**
Dansylated invertase high mannosae. ND

**Endo F1, F2:**
Dansylated fibrinogen biantennary. ND

**PNGase F:**
Fluoresceinated fetuin triantennary. ND

**Protease Assay:**
After incubation of 1,400,000 units of O-Glycosidase with 0.2 nmol of a standard mixture of proteins in a 20 µl reaction, for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

**Protocol:**
Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate. Typical reaction conditions are as follows:

1. Combine 10–20 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 10X G7 Reaction Buffer, 2 µl 10% NP40, 2 µl PNGase F, H2O and 1–5 µl O-Glycosidase.
4. Incubate reaction at 37°C for 1–4 hours.

**Notes on Use:**
Since O-Glycosidase is inhibited by SDS, it is essential to have NP-40 in the reaction mixture. It is not known why this non-ionic detergent counteracts the SDS inhibition at the present time. Double digest with Endo H must have NP-40 present (NP-40 does not inhibit Endo H).

To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

Recommended storage temperature is –20°C.

**Heat Inactivation:**
65°C for 10 minutes.

**References:**