**O-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 GlycoBuffer 2
- 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 GlycoBuffer 2. 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).

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

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

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

**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.

**Non-Denaturing Unit Definition Assay:** No other glycosidase activities were detected (ND) with the following substrates:

- **β-N-Acetylgalactosaminidase:** GalNAcβ1-4Galβ1-4Glc-AMC ND
- **α-N-Acetylgalactosaminidase:** GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND
- **β-Fucosidase:** Fucα1-2Galβ1-4Glc-AMC ND
- **Galβ1-3Galα1-4Galβ1-4Glc-AMC ND**
- **Galβ1-4GalNAcα1-3Galβ1-4Glc-AMC ND**
- **α-Galactosidase:** Galβ1-3Galα1-4Galβ1-4Glc-AMC ND
- **Galβ1-4GalNAcα1-3Galβ1-4Glc-AMC ND**
- **β-Galactosidase:** Galα1-3Galβ1-4Gal-AMC ND
- **Galα1-6Galα1-6Glccα1-2Fru-AMC ND**

**Molecular Weight:** 53,000,000 units/mg.

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

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- **β-Fucosidase:** Fucα1-2Galβ1-4Glc-AMC ND
- **Galβ1-3Galα1-4Galβ1-4Glc-AMC ND**
- **Galβ1-4GalNAcα1-3Galβ1-4Glc-AMC ND**
- **α-Galactosidase:** Galβ1-3Galα1-4Galβ1-4Glc-AMC ND
- **Galβ1-4GalNAcα1-3Galβ1-4Glc-AMC ND**
- **β-Galactosidase:** Galα1-3Galβ1-4Gal-AMC ND
- **Galα1-6Galα1-6Glccα1-2Fru-AMC ND**

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**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.

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**References:**