

O-Glycosidase



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P0733S 003141216121

P0733S



2,000,000 units 40,000,000 U/ml Lot: 0031412

RECOMBINANT Store at -20°C Exp: 12/16

Description: *O*-Glycosidase, also known as Endo- α -*N*-Acetylgalactosaminidase, catalyzes the removal of Core 1 and Core 3 *O*-linked disaccharides from glycoproteins.

Specificity:

A. Core 1



B. Core 3



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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 μ l (1 unit of both *O*-Glycosidase and PNGase F will remove equivalent molar amounts of *O*-linked disaccharides and *N*-linked oligosaccharides, respectively).

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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).

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.

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 μ l reaction for 20 hours at 37°C. The reaction products were analyzed by TLC for digestion of substrate.

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No other glycosidase activities were detected (ND) with the following substrates:

β -*N*-Acetylgalactosaminidase:
GalNAc β 1-4Gal β 1-4Glc-AMC ND

α -*N*-Acetylgalactosaminidase:
GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC ND

Gal β 1-4 (Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND

Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC ND

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal-AMC ND

Gal α 1-6Gal α 1-6Glc α 1-2Fru-AMC ND

(see other side)

CERTIFICATE OF ANALYSIS

No other glycosidase activities were detected (ND) with the following substrates:

β -*N*-Acetylgalactosaminidase:
GalNAc β 1-4Gal β 1-4Glc-AMC ND

α -*N*-Acetylgalactosaminidase:
GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc-AMC ND

α -Fucosidase:
Fuc α 1-2Gal β 1-4Glc-AMC ND

Gal β 1-4 (Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND

Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC ND

α -Galactosidase:
Gal α 1-3Gal β 1-4Gal-AMC ND

Gal α 1-6Gal α 1-6Glc α 1-2Fru-AMC ND

(see other side)

CERTIFICATE OF ANALYSIS

α -Neuraminidase:
Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-AMC ND

α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-AMC ND
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

β -Glucosidase:
Glc β 1-4Glc β 1-4Glc-AMC ND

α -Glucosidase:
Glc α 1-6Glc α 1-4Glc-AMC ND

β -Xylosidase:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

β -Mannosidase:
Man β 1-4Man β 1-4Man-AMC ND

Endo F₁, F₂, H:
Dansylated invertase high mannose. ND

Endo F₂, F₃:
Dansylated fibrinogen biantennary. ND

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α -Mannosidase:
Man α 1-3Man β 1-4GlcNAc-AMC ND
Man α 1-6Man α 1-6(Man α 1-3)Man-AMC ND

β -Glucosidase:
Glc β 1-4Glc β 1-4Glc-AMC ND

α -Glucosidase:
Glc α 1-6Glc α 1-4Glc-AMC ND

β -Xylosidase:
Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl-AMC ND

β -Mannosidase:
Man β 1-4Man β 1-4Man-AMC ND

Endo F₁, F₂, H:
Dansylated invertase high mannose. ND

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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 μ l 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 μ g of glycoprotein, 1 μ l of 10X Glycoprotein Denaturing Buffer and H₂O (if necessary) to make a 10 μ l total reaction volume.
2. Denature glycoprotein by heating reaction at 100°C for 10 minutes.
3. Make a total reaction volume of 20 μ l by adding 2 μ l 10X GlycoBuffer 2, 2 μ l 10% NP40, 2 μ l Neuraminidase, H₂O and 1–5 μ l *O*-Glycosidase.
4. Incubate reaction at 37°C for 1–4 hours.

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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.

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References:

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2. Morgan, W.T.J. and Elson, L.A. (1934) *Biochem. J.* 28, 988–995.



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References:

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