

O-Glycosidase



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P0733S 001130215021

P0733S



2,000,000 units 40,000,000 U/ml Lot: 0011302
RECOMBINANT Store at -20°C Exp: 2/15

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 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 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 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 μ l 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:
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

(see other side)

CERTIFICATE OF ANALYSIS

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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

(see other side)

CERTIFICATE OF ANALYSIS

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-Neuraminidase: Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-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

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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
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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

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β-Mannosidase: Manβ1-4Manβ1-4Man-AMC	ND
Endo F₁, F₂, H: Dansylated invertase high mannose.	ND
Endo F₂, F₃: 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.

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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 Neuraminidase, H₂O and 1–5 μl *O*-Glycosidase.
4. Incubate reaction at 37°C for 1–4 hours.

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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.

References:

1. Koutsioulis, D., Landry, D. and Guthrie, E.P. (2008) *Glycobiology* 18, 799–805.
2. Morgan, W.T.J. and Elson, L.A. (1934) *Biochem. J.* 28, 988–995.

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