O-Glycosidase

1-800-632-7799
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www.neb.com

P0733S

2,000,000 units **40,000,000 U/ml** **Lot: 0011210**

RECOMBINANT **Store at –20°C** **Exp: 10/14**

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

Galβ(1–3)GalNAc-α-*O*-Ser/Thr

B. Core 3

GlcNAcβ(1–3)GalNAc-α-*O*-Ser/Thr

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 Na_2EDTA.

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.

Quality Controls

Glycosidase Assays:
200,000 units of *O*-Glycosidase were incubated with 0.1 mM of flourescently-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(Fucα1-2)Galβ1-4Glc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC ND
Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-AMC ND

(see other side)

CERTIFICATE OF ANALYSIS

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α-N-Acetylgalactosaminidase:
GalNAcα1-3(Fucα1-2)Galβ1-4Glc-AMC ND

α-Fucosidase:
Fucα1-2Galβ1-4Glc-AMC ND
Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glc-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

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

Endo F_1, F_2, H:
Dansylated invertase high mannose. ND

Endo F_2, F_3:
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 H_2O (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, H_2O 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:

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.

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

β-Mannosidase:
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