O-Glycosidase & Neuraminidase Bundle

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E0540S

Lot: 0011202 **Store at –20°C** **Exp: 2/14**

O-Glyco*s*idase
2,000,000 units
40,000,000 U/ml Lot: 0011110

Neuraminidase
2,000 units
50,000 U/ml Lot: 0131110

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

Neuraminidase is the common name for Acetylneuraminyl hydrolase (Sialidase). This Neuraminidase catalyzes the hydrolysis of α2-3, α2-6, and α2-8 linked N-acetyl-neuraminic acid residues from glycoproteins and oligosaccharides.

Specificity of *O*-Glycosidase:

A. Core 1

Galβ(1–3)GalNAc-α-*O*-Ser/Thr

B. Core 3

GlcNAcβ(1–3)GalNAc-α-*O*-Ser/Thr

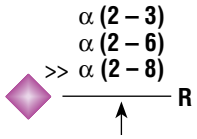
C. Core 7 (1)

GalNAcα(1–6)GalNAc-α-*O*-Ser/Thr

D. Immature Core

GalNAc-α-*O*-Ser/Thr

Specificity of Neuraminidase:



Source: *O*-Glycosidase is cloned from *Enterococcus faecalis* and expressed in *E. coli* (1).

Neuraminidase is cloned from *Clostridium perfringens* (1) and overexpressed in *E. coli* at NEB (2).

Reagents Supplied with Enzymes:
10X Glycoprotein Denaturing Buffer, 10X G7 Reaction Buffer, 10% NP-40

Reaction Conditions:
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.

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

Unit Definition of *O*-Glycosidase: 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 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 is then incubated at 37°C for 1 hour. *O*-linked disaccharide carbohydrates are determined by Morgan and Elson Assay (4). Note: Under denaturing conditions the enzyme activity is increased two-fold. This observation is substrate dependent.

Unit Definition of Neuraminidase: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 5 minutes at 37°C in a total reaction volume of 10 µl.

Specific Activity of *O*-Glycosidase: ~50,000,000 units/mg.

Molecular Weight of *O*-Glycosidase: 147,000 daltons.

Specific Activity of Neuraminidase: ~225,000 units/mg.

Molecular Weight of Neuraminidase: 43,000 daltons.

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

Quality Controls

Glycosidase Assays: 200,000 units of *O*-Glycosidase and 500 units of Neuraminidase 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.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

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

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 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 and 500 units of Neuraminidase 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.

Note: 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.

References:

1. Koutsioulis, D., Landry, D. and Guthrie, E.P. (2008) *Glycobiology* 18, 799–805.
2. Roggentin, P. et al. (1988) *FEBS Lett.* 238, 31–34.
3. Guan, C., unpublished observations.
4. Morgan, W.T.J. and Elson, L.A. (1934) *Biochem. J.* 28, 988–995.

U.S. Publication No. US 2011/0053215