

O-Glycosidase & Neuraminidase Bundle



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E0540S 004150417041

E0540S



Lot: 0041504 Store at -20°C Exp: 4/17

O-Glycosidase

2,000,000 units
40,000,000 U/ml Lot: 0011206

Neuraminidase

2,000 units
50,000 U/ml Lot: 0141206

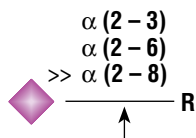
Description: O-Glycosidase, also known as Endo- α -N-Acetylgalactosaminidase, catalyzes the removal of Core 1 and Core 3 O-linked disaccharides from glycoproteins.

Neuraminidase is the common name for Acetylneuraminyl hydrolase (Sialidase). This Neuraminidase catalyzes the hydrolysis of α 2-3, α 2-6, and α 2-8 linked N-acetylneuraminic acid residues from glycoproteins and oligosaccharides.

Specificity of O-Glycosidase:

- A. Core 1
 $\text{Gal}\beta(1-3)\text{GalNAc-}\alpha\text{-O-Ser/Thr}$
- B. Core 3
 $\text{GlcNAc}\beta(1-3)\text{GalNAc-}\alpha\text{-O-Ser/Thr}$
- C. Core 7 (1)
 $\text{GalNAc}\alpha(1-6)\text{GalNAc-}\alpha\text{-O-Ser/Thr}$
- D. Immature Core
 $\text{GalNAc-}\alpha\text{-O-Ser/Thr}$

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 GlycoBuffer 2, 10% NP-40

Reaction Conditions:

Typical reaction conditions are as follows:

- 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).
- Denature glycoprotein by heating reaction at 100°C for 10 minutes.
- Make a total reaction volume of 20 μ l by adding 2 μ l 10X GlycoBuffer 2, 2 μ l 10% NP-40, 2 μ l Neuraminidase, H₂O and 1–5 μ l O-Glycosidase.
- 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 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).

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 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 Neu5Ac α 2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc-7-amino-4-methyl-coumarin (AMC), in 5 minutes at 37°C in a total reaction volume of 10 μ l.

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 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.

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:
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:
Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc-AMC ND
Fuc α 1-2Gal 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

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

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 μ l 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:

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



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