Specificity of Neuraminidase:

D. Immature Core

B. Core 3 glycoproteins and oligosaccharides.

This Neuraminidase is the common name for Acetyl-

removal of Core 1 and Core 3

Endo-

Description:

50,000 U/ml Lot: 0141206

2,000 units

Neuraminidase

Lot: 0011206 Store at –20°C Exp: 6/14

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 Neu5Acx2-3Galβ1-3GlcNAcβ1-4Glcβ1-4Xyl-2-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.

Specificity of O-Glycosidase:

A. Core 1

Galβ1(1–3)GalNAcα-β-O-Ser/Thr

B. Core 3

GlcNAcβ1(1–3)GalNAcα-β-O-Ser/Thr

C. Core 7 (1)

GalNAccα1(1–6)GalNAcα-β-O-Ser/Thr

D. Immature Core

GalNAcα-β-O-Ser/Thr

Specificity of Neuraminidase:

α(2–3)

α(2–6)

α(2–8)

R

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$_2$O (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$_2$O and 1–5 µl O-Glycosidase.

4. Incubate reaction at 37°C for 1–4 hours. This observation is substrate 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 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

α-N-Acetylgalactosaminidase: GalNAcc1-3(Fucα1-2)Galβ1-4Glc-AMC

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

β-Galactosidase: Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC

β-Galactosidase: Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC

β-Galactosidase: Galβ1-3Galβ1-4Glc-AMC

β-Mannosidase: Manβ1-4Manβ1-4Man-AMC

β-Glucosidase: Glcβ1-4Glcβ1-4Glc-AMC

β-Glucosidase: Glcα1-6Glcα1-4Glc-AMC

β-Xylosidase: Xylβ1-4Xylβ1-4Xyl-AMC

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

α-N-Acetylgalactosaminidase: GalNAcc1-3(Fucα1-2)Galβ1-4Glc-AMC

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

β-Galactosidase: Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC

β-Galactosidase: Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC

β-Galactosidase: Galβ1-3Galβ1-4Glc-AMC

β-Mannosidase: Manβ1-4Manβ1-4Man-AMC

β-Glucosidase: Glcβ1-4Glcβ1-4Glc-AMC

β-Glucosidase: Glcα1-6Glcα1-4Glc-AMC

β-Xylosidase: Xylβ1-4Xylβ1-4Xyl-AMC

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:


U.S. Publication No. US 2011/0053215