

Product Specification Sheet

Product Name: Exo- α -sialidase from *Clostridium perfringens* (EC 3.2.1.18)

Catalog Number: FSB0001

Lot Number: 07C22

Description	
<p>Neuraminidase is the small Exo-α-sialidases derived from <i>Clostridium perfringens</i>. Recombinantly expressed in <i>E. coli</i>, this neuraminidase hydrolyses terminal sialic acid linkages from oligosaccharides, glycoproteins, glycolipids, and homopolymers of N-acetylneuraminic acid and synthetic substrates. $\alpha(2-3) > \alpha(2-6) \gg \alpha(2-8)$</p>	

Product Information	
Quantity	>10 Units
Purity	>95% by SDS-PAGE
Concentration	2230 U/mL
Molecular Weight	42.56 kDa
A280	1 mg/ml = 1.94/cm path length
PI	5.99
Storage	Format: Liquid Buffer: 100 mM acetate pH 5.5, 25 mM NaCl Temperature: 2-8 °C Stability: 1 year
Unit Definition	One unit is defined as the amount of enzyme that releases 1 μ mol of terminal α -sialic acid from 3' sialyl-lactose per minute.
Specific Activity	3' NANA-lactose - 520 μ mol Units/mg Neuraminidase 6' NANA-lactose - 85 μ mol/min mg Neuraminidase
Specific Activity Conditions	3'sialyl lactose - 5 ng/mL Neuraminidase, 1.5 mM 3'sialyl lactose, 80 μ L, 0.2 mg/mL BSA, 20 mM sodium acetate pH 5.5, 25 mM NaCl, 37 C. Initial activity was measured in 20 min time intervals from 0 to 140 min using our Thiobarbituric Acid Assay Kit (FSB3002). 6' sialyl lactose - 50 ng/mL Neuraminidase 1.5 mM 6'sialyl lactose, 80 μ L, 0.2 mg/mL BSA, 20 mM sodium acetate pH 5.5, 25 mM NaCl, 37 C. Initial activity was measured in 20 min time intervals from 0 to 140 min using our Thiobarbituric Acid Assay Kit.

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Activity Assay

Removing the negatively charged sialic acids from a glycoprotein raises its charge enabling a simple SDS-PAGE assay for activity detection as follows:

Procedure

- (1) Prepare the following solutions:
 - a) 1x BSA Storage Buffer: (BSA 0.4 mg/mL, 20 mM acetate pH 5.5, 25 mM NaCl) ($\leq 100 \mu\text{L}$)
 - b) 1x Fetuin Standard Solution (Fetuin 2 mg/ml, 20 mM acetate pH 5.5, 25 mM NaCl) ($\leq 100 \mu\text{L}$)
 - c) 30x Neuraminidase Standard Solution (dilute 1 μL Neuraminidase Solution into 20 μL BSA Storage Buffer)
- (2) Transfer 30 μL 1x Fetuin Standard Solution into a fresh PCR tube label: Negative
- (3) Transfer 30 μL 2x Fetuin Standard Solution into a fresh PCR tube label: Test
- (4) Add 1 μL 30x Neuraminidase Standard Solution into the Test solution and mix gently
- (5) Add 1 μL 1x BSA Storage Buffer to the PCR tube marked Negative and mix gently
- (6) Cap both PCR tubes and incubate at 37 C for 1 hour
- (7) Stop the reaction by boiling for 10 min
- (8) Cool, centrifuge the PCR tubes to collect samples
- (9) Aliquot for SDS PAGE (~4 μg Fetuin per lane for Coomassie blue staining)
- (10) Run SDS PAGE gel with protein standard markers for analysis of desialylation
- (11) Compare the resulting bands to determine the extent of desialylation
- (12) Analysis can be expedited using ImageJ software (free from NIH)
- (13) Multiple time points, or substrate, or enzyme concentrations can be studied if desired

References

- L. Warren, **The thiobarbituric acid assay of sialic acids**. J. Biol. Chem. **234**, 1971-1975 (1959).
- JT Cassidy, GW Jourdian, S Roseman, **The sialic acids. VI. Purification and properties of sialidase from Clostridium perfringens**. J Biol Chem. **240**, 3501-6 (1965).
- M Chiarezza, D Lyras, SJ Pidot, M Flores-Díaz, MM Awad, CL Kennedy, LM Cordner, T Phumoonna, R Poon, ML Hughes, JJ Emmins, A Alape-Girón, JI Rood, **The NanI and NanJ Sialidases of Clostridium perfringens Are Not Essential for Virulence**. Infect Immun. **77**, 4421-8 (2009).

Related Products

Catalog Number	Product Name
FSB3002	Thiobarbituric Acid Assay Kit
FSB2001	3' Sialyl-lactose
FSB2002	6' Sialyl-lactose

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Figures

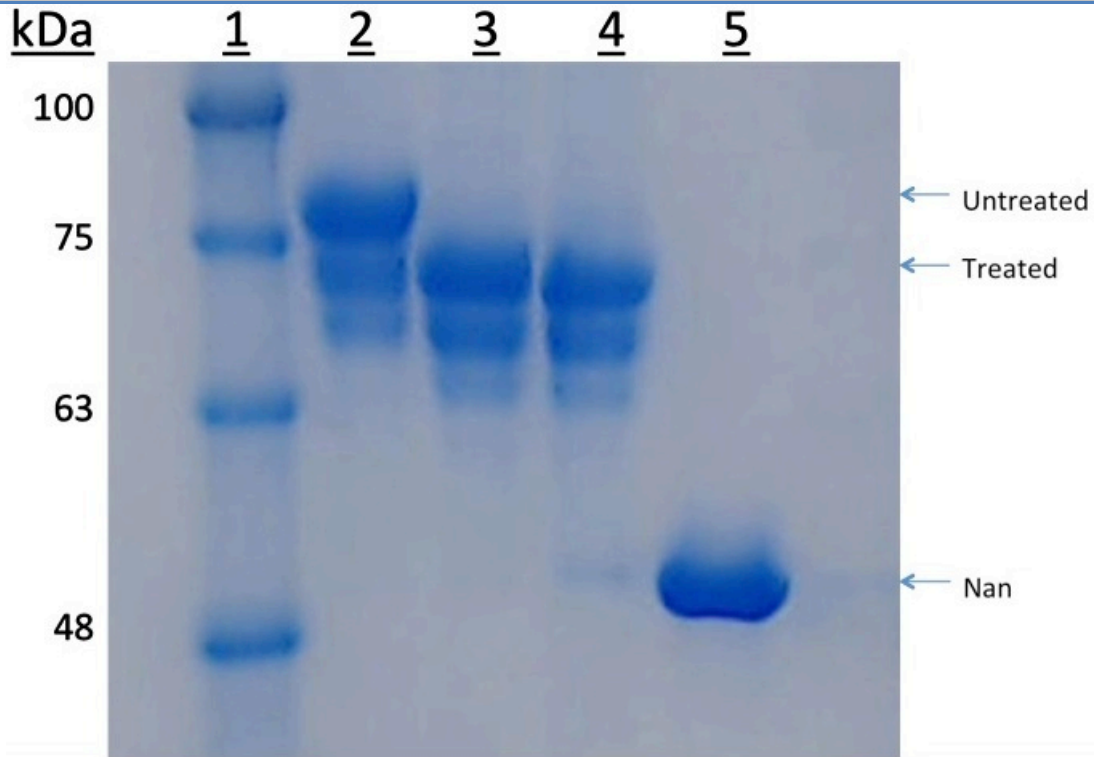


Figure 1. Test of Neuraminidase hydrolysis of Fetuin. Lane 1: Protein Marker, Lane 2: Untreated Fetuin (4 μ g) Lane 3: Neuraminidase treated Fetuin (4 μ g) 5 min, Lane 4: Neuraminidase treated Fetuin (4 μ g) 60 min, Lane 5: Neuraminidase (3 μ g).

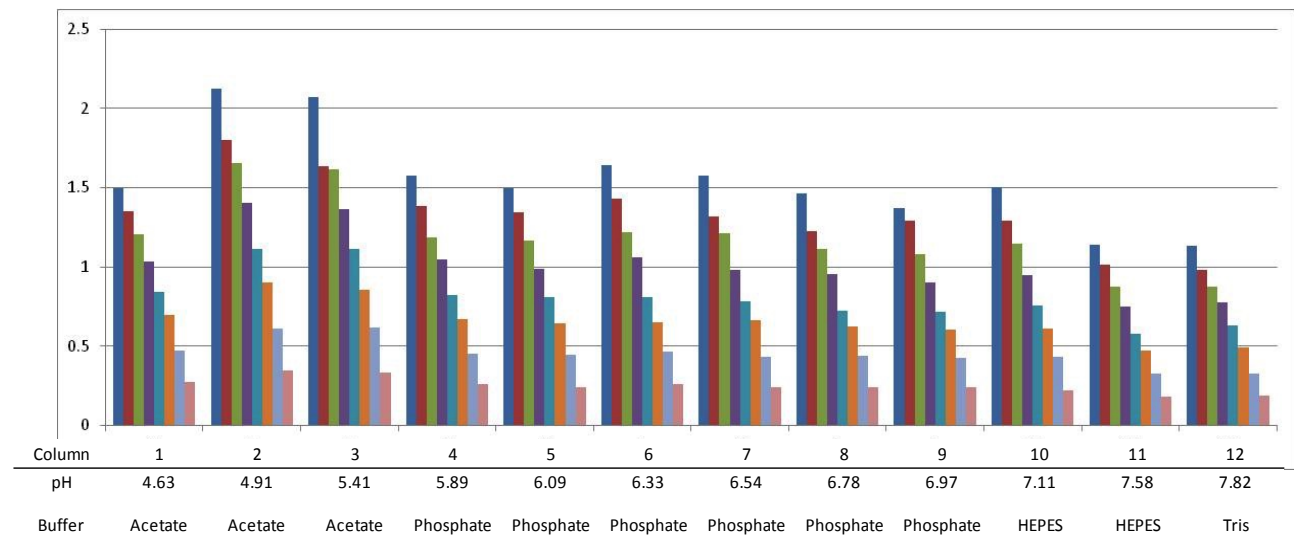


Figure 2. Buffer test of Neuraminidase hydrolysis of Fetuin in a thiobarbituric acid assay. Linear variation of Fetuin concentration (2 mg/mL to 0.25 mg/mL) Row A-H

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