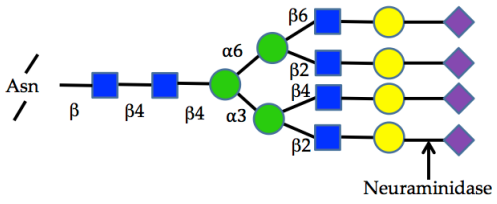
Product Specification Sheet

Product Name: Exo-α-sialidase from *Clostridium perfringens* (EC 3.2.1.18)
Catalog Number: FSB0001
Lot Number: 12162012

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

Product Information	
Quantity	>10 Units ~ 20 µg
Purity	>95% by SDS-PAGE
Molecular Weight	48.4 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: 6 months
Unit Definition	One unit is defined as the amount of enzyme that releases 1 µmol of terminal α-sialic acid from 3' sialyl-lactose per minute.
Specific Activity	3' NANA-lactose - 520 µmol Units/mg Neuraminidase 6' NANA-lactose - 85 µmol/min mg Neuraminidase
Specific Activity Conditions	3'sialyl lactose - 5 ng/mL Neuraminidase, 1.5 mM 3'sialyl lactose, 80 µL, 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 µL, 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) (≤ 100 µL)

b) 1x Fetuin Standard Solution (Fetuin 2 mg/ml, 20 mM acetate pH 5.5, 25 mM NaCl) (≤ 100 µL)

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 desialyation

(11) Compare the resulting bands to determine the extent of desialyation

(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

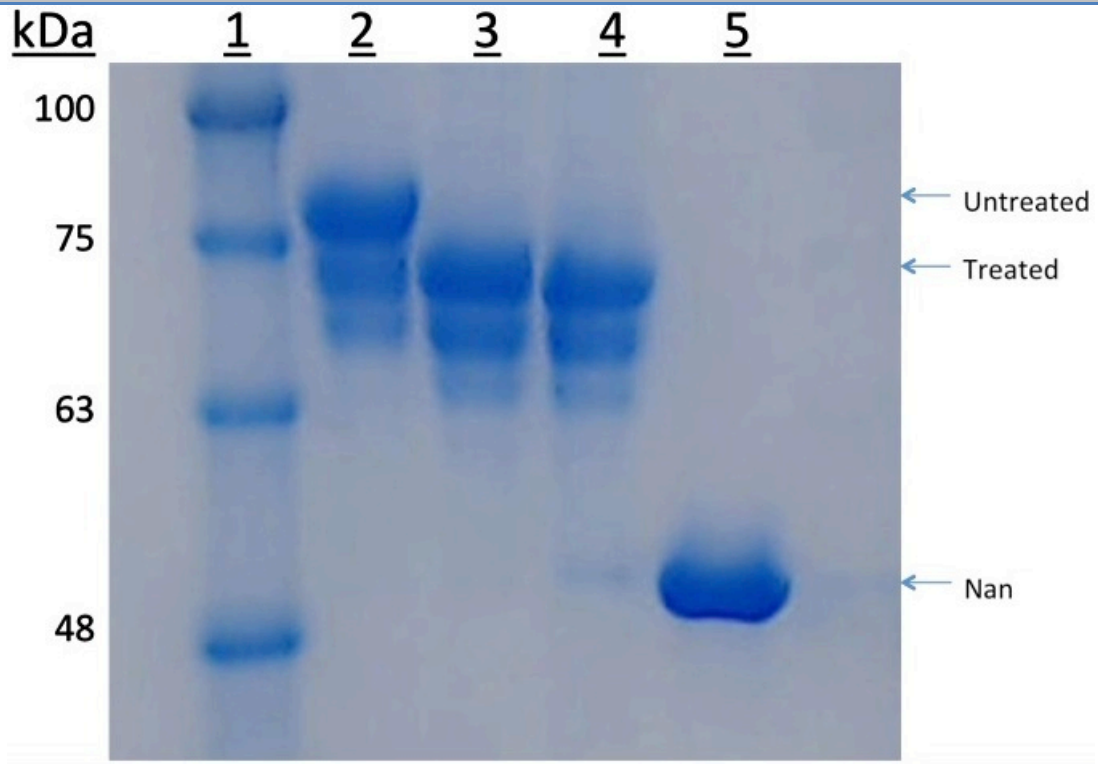

Figure 1. Test of Neuraminidase hydrolysis of Fetuin. Lane 1: Protein Marker, **Lane 2:** Untreated Fetuin (4 µg) **Lane 3:** Neuraminidase treated Fetuin (4 µg) 5 min, **Lane 4:** Neuraminidase treated Fetuin (4 µg) 60 min, **Lane 5:** Neuraminidase (3 µg).

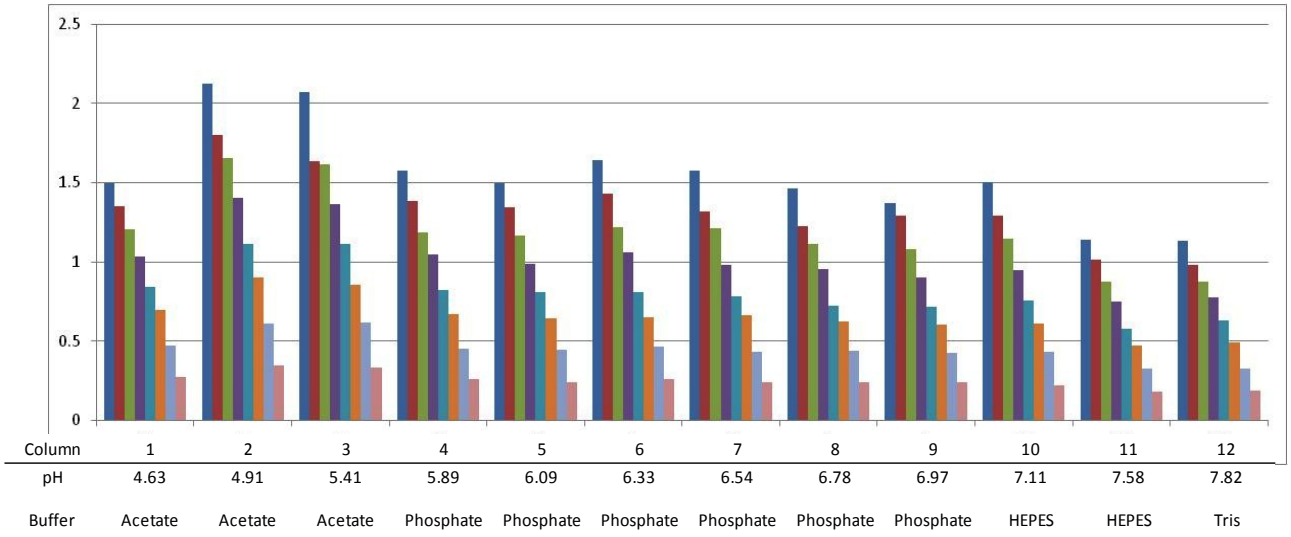

Figure 2. Buffer test of Neuraminidase hydrolysis of Fetuin in a thiobarbituric acid assay. Linear variation of Fetuin concentration (2 mg/mL to 0.25 mg/mL) Row A-H

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