

## Product Specification Sheet

**Product Name:** NanH, Exo- $\alpha$ -sialidase (EC 3.2.1.18)

**Catalog Number:** FSB0034

**Lot Number:** 04152014

Description	
<p><b>Neuraminidase H (NanH)</b> is an Exo-<math>\alpha</math>-sialidase derived from <i>Salmonella enteric subsp. enterica</i> serovar Typhimurium str. LT2 and recombinantly expressed in <i>E. coli</i>, NanH cleaves terminal sialic acid from oligosaccharides, glycoproteins, glycolipids, and synthetic substrates and prefers <math>\alpha(2-3)</math> linkages ~250-fold over <math>\alpha(2-6)</math> linkages.</p>	

Product Information	
Quantity	> 10 Units ~ 15 $\mu$ g [NanH]
Purity	>95% by SDS-PAGE
MW	45,672.4 Daltons (Calculated)
Extinction A280	1 mg/ml = 1.245/cm path length (Calculated)
PI	8.72 (Calculated)
Storage	Format: Liquid Storage Buffer: 100 mM acetate pH 5.5, 25 mM NaCl Storage Temperature: 2-8 $^{\circ}$ C Storage Stability: 6 months
Unit Definition	One unit is defined as the amount of enzyme that releases 1 $\mu$ mol of terminal $\alpha$ -sialic acid from 3' sialyl-lactose per minute.
Specific Activity	3' NANA-lactose - 670 $\mu$ mol Units/mg NanH 6' NANA-lactose - 2.5 $\mu$ mol/min mg NanH
Specific Activity Conditions	3'sialyl lactose - 0.5 ng/mL NanJ, 1.5 mM 3'sialyl lactose, 80 $\mu$ 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 - 500 ng/mL NanJ, 1.5 mM 6'sialyl lactose, 80 $\mu$ 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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## Fetuin 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 NanH Standard Solution (dilute 1  $\mu\text{L}$  NanH Solution into 20  $\mu\text{L}$  BSA Storage Buffer)
- (2) Transfer 30  $\mu\text{L}$  1x Fetuin Standard Solution into a fresh PCR tube label: Negative
- (3) Transfer 30  $\mu\text{L}$  2x Fetuin Standard Solution into a fresh PCR tube label: Test
- (4) Add 1  $\mu\text{L}$  30x NanH Standard Solution into the Test solution and mix gently
- (5) Add 1  $\mu\text{L}$  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  $\mu\text{g}$  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

Experimental conditions should be optimized for each sample system.

## References

L. Warren, **The thiobarbituric acid assay of sialic acids.** J. Biol. Chem. **234**, 1971-1975 (1959).

LL Hoyer, P Roggentin, R Schauer, ER Vimr, **Purification and properties of cloned Salmonella typhimurium LT2 sialidase with virus-typical kinetic preference for sialyl alpha 2-3 linkages.** J Biochem. **110**, 462-7 (1991).

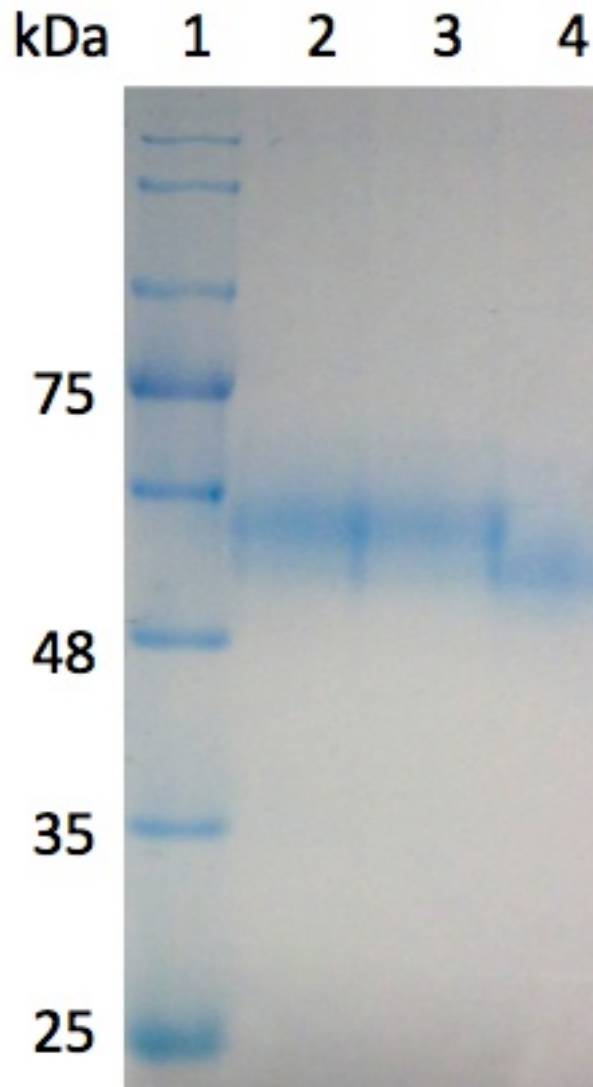
JB Bouwstra, CM Deyl, JF Vliegenthart, **Purification and kinetic properties of sialidase from Clostridium perfringens.** Biol Chem Hoppe Seyler. **368**, 269-75 (1987).

## 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. NanH hydrolysis of Fetuin. Lane 1:** Protein Marker, **Lane 2:** Untreated Fetuin (4  $\mu$ g) **Lane 3:** NanH treated Fetuin (4  $\mu$ g) 5 min, **Lane 4:** NanH treated Fetuin (4  $\mu$ g) 60 min.

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