

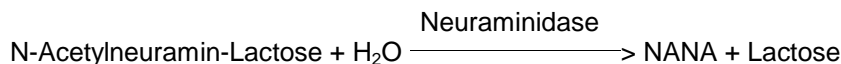


Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of NEURAMINIDASE¹ (EC 3.2.1.18) N-Acetylneuramin-Lactose as Substrate

PRINCIPLE:



Abbreviation:

NANA = N-Acetylneuraminic Acid

CONDITIONS: T = 37°C, pH = 5.0, A_{550nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 100 mM Sodium Acetate Buffer with 2 mM Calcium Chloride, pH 5.0 at 37°C²
(Prepare 100 ml in deionized water using Sodium Acetate Anhydrous, Sigma Prod. No. S-8750 and Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881. Adjust to pH 5.0 at 37°C with 1 M HCl.)
- B. 0.085% (w/v) N-Acetylneuramin-Lactose (2→3) isomer Solution (NAN-Lactose)³
(Prepare 5 ml in Reagent A using N-Acetylneuramin-Lactose, Sigma Prod. No. A-3307.)
- C. 0.2% (w/v) Bovine Serum Albumin Solution (BSA)
(Prepare 100 ml in deionized water using Albumin, Bovine, Sigma Prod. No. A-6003.)
- D. 5% (w/v) Phosphotungstic Acid Solution (PT)
(Prepare 100 ml in 2.5 M HCl using Phosphotungstic Acid Free Acid, Sigma Prod. No. P-4006.)
- E. Neuraminidase Enzyme Solution
(Immediately before use, prepare a solution containing approximately 0.02 - 0.04 unit/ml of Neuraminidase in cold Reagent C.)
- F. 9 M Phosphoric Acid Solution
(Prepare 20 ml in deionized water using Phosphoric Acid, Sigma Prod. No. P-6560.)

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REAGENTS: (continued)

- G. 200 mM Sodium m-Periodate Solution (Per)
(Prepare 10 ml in Reagent F using Sodium m-Periodate, Sigma Prod. No. S-1878.)
- H. 10% (w/v) Sodium m-Arsenite with 50 mM Sulfuric Acid and 500 mM Sodium Sulfate Solution (Ars)
(Prepare 100 ml in deionized water using Sodium m-Arsenite, Sigma Prod. No. S-7400, Sulfuric Acid, Sigma Prod. No. S-1526, and Sodium Sulfate, Anhydrous, Sigma Prod. No. S-9627.)
- I. 0.6% (w/v) Thiobarbituric Acid and 500 mM Sodium Sulfate Solution (TBA)
(Prepare 100 ml in deionized water using 2-Thiobarbituric Acid, Sigma Prod No. T-5500, and Sodium Sulfate, Anhydrous, Sigma Prod. No. S-9627.)
- J. 5% (v/v) HCl in Butanol Solution (But)
(Prepare by combining 5 ml of concentrated Hydrochloric Acid, Sigma Prod. No. H-7020 and 95 ml of n-Butanol, Sigma Stock No. BT-105.)
- K. 0.16 mM N-Acetylneuraminic Acid Standard Solution (NANA)
(Prepare 10 ml in deionized water using N-Acetylneuraminic Acid, Sigma Prod. No. A-2751.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.20	0.20
Reagent B (NAN-Lactose)	0.20	0.20

Mix by inversion and equilibrate to 37°C. Then add:

Reagent E (Enzyme Solution)	0.10	-----
Reagent C (BSA)	-----	0.10

Mix by inversion immediately and incubate at 37°C for exactly 10 minutes. Then add:

Reagent D (PT)	0.50	0.50
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Mix by inversion immediately and centrifuge for 3 minutes.

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COLORIMETRIC ASSAY:

Standard Curve:

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Blank</u>
Reagent K (NANA)	0.05	0.10	0.20	0.30	0.00
Deionized water	0.45	0.40	0.30	0.20	0.50
Reagent D (PT)	0.50	0.50	0.50	0.50	0.50

Mix by inversion and centrifuge for 3 minutes.

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Test Blank</u>	<u>Std1</u>	<u>Std2</u>	<u>Std3</u>	<u>Std4</u>	<u>Std Blank</u>
Test Supernatant	0.50	----	----	----	----	----	----
Test Blank Supernatant	----	0.50	----	----	----	----	----
Std 1 Supernatant	----	----	0.50	----	----	----	----
Std 2 Supernatant	----	----	----	0.50	----	----	----
Std 3 Supernatant	----	----	----	----	0.50	----	----
Std 4 Supernatant	----	----	----	----	----	0.50	----
Std Blank Supernatant	----	----	----	----	----	----	0.50
Reagent G (Per)	0.10	0.10	0.10	0.10	0.10	0.10	0.10

Mix by inversion and incubate for 20 minutes at 25°C. Then add:

Reagent H (Ars)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
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Vortex until the yellow-brown color disappears. Then add:

Reagent I (TBA)	3.00	3.00	3.00	3.00	3.00	3.00	3.00
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Transfer to a boiling water bath and incubate for 15 minutes. Transfer to a cold water bath for 5 minutes. Then add:

Reagent J (But)	4.60	4.60	4.60	4.60	4.60	4.60	4.60
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Shake or vortex the solution until most of the pink color has been extracted from the lower layer. Centrifuge for 2-3 minutes. Transfer the upper layer to suitable cuvettes and record the A_{550nm} for Test, Test Blank, Standard, and Standard Blank.

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CALCULATIONS:

Standard Curve:

$$\Delta A_{550\text{nm}} \text{ Standard} = A_{550\text{nm}} \text{ Standard} - A_{550\text{nm}} \text{ Standard blank}$$

Plot the $\Delta A_{550\text{nm}}$ Standard vs $\mu\text{moles n-Acetylneuraminic acid}$.

Sample Determination:

$$\Delta A_{550\text{nm}} \text{ Test} = A_{550\text{nm}} \text{ Test} - A_{550\text{nm}} \text{ Test Blank}$$

Determine the $\mu\text{moles of n-Acetylneuraminic Acid (NANA)}$ liberated using the Standard curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of NANA liberated}) (\text{df})}{(0.1) (10)}$$

df = Dilution factor

0.1 = Volume (in milliliter) of enzyme used

10 = Time (in minutes) of the assay as per the Unit Definition

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will liberate 1.0 μmole of N-acetylneuraminic acid per minute at pH 5.0 at 37°C using NAN-lactose as substrate.

FINAL ASSAY CONCENTRATIONS:

In a 0.50 ml reaction mix, the final concentrations are 80 mM sodium acetate, 1.6 mM calcium chloride, 0.034% (w/v) n-acetylneuramin-lactose (2→3 isomer), 0.04% (w/v) bovine serum albumin, and 0.002 - 0.004 unit neuraminidase.

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REFERENCES:

Schneir, M.L. and Rafelson, M.E., Jr. (1966) *Biochim. Biophys. Acta.* **130**, 1-11

Cassidy, J.T., Jourdian, G.W. and Roseman, S. (1965) *J. Biol. Chem.* **240**, 3501-3506

Warren, L. (1959) *J. Biol. Chem.* **234**, 1971-1975

NOTES:

1. Do not use this assay for Neuraminidase from Newcastle disease virus, Sigma Prod. No. N-5146, and Neuraminidase from *Streptococcus* sp., Sigma Prod. No. N-5271.
2. Only Neuraminidase from *Vibrio cholera* requires calcium ions. However, the presence of calcium ions does not interfere with the activity of Neuraminidases from other sources.
3. This assay system is operating at less than the K_m value for the substrate. It is important that the concentration of the 2 \rightarrow 3 isomer is 0.085% (w/v).
4. Final volumes should be 0.50 ml for all test solutions performed.
5. This assay is based on the cited references.
6. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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