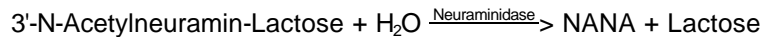


Enzymatic Assay of NEURAMINIDASE (EC 3.2.1.18)

PRINCIPLE:



Abbreviation used:

NANA = N-Acetylneuraminic Acid

CONDITIONS: T = 37°C, pH 6.5, A_{549nm}, Light path = 1 cm

METHOD: Colorimetric

REAGENTS:

- A. 150 mM Sodium Acetate Buffer with 450 mM Sodium Chloride and 27 mM Calcium Chloride, pH 6.5 at 37°C
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625, Sodium Chloride, Sigma Prod. No. S-9625, and Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881. Adjust to pH 6.5 at 37°C with 1 M HCl.)
- B. 5.0 mM 3'-N-Acetylneuramin-Lactose Solution (NAN-Lactose)
(Prepare 2 ml in Reagent A using 3'-N-Acetylneuramin-Lactose, Sodium Salt, Sigma Prod. No. A-8681. **Prepare Fresh.**)
- C. 9 M Phosphoric Acid Solution
(Prepare 20 ml in deionized water using Phosphoric Acid, Sigma Prod. No. P-6560.)
- D. 250 mM Sodium m-Periodate Solution (Per)
(Prepare 10 ml in Reagent C using Sodium m-Periodate, Sigma Prod. No. S-1878.)
- E. 550 mM Sodium m-Arsenite and 500 mM Sodium Sulfate Solution (Ars)
(Prepare 100 ml in deionized water using Sodium m-Arsenite, Sigma Prod. No. S-7400, and Sodium Sulfate, Anhydrous, Sigma Prod. No. S-9627.)

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REAGENTS: (continued)

- F. 56 mM 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. In order for the solution to become clear, adjust the pH between 7.5 and 8.0 with either 1 M HCl or 1 M NaOH.)
- G. 0.13 mM N-Acetylneuraminic Acid Standard solution (NANA Std)
(Prepare 10 ml in deionized water using N-Acetylneuraminic Acid, Sigma Prod. No. A-2388.)
- H. 0.1% (w/v) Bovine Serum Albumin Solution (BSA)
(Prepare 10 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503.)
- I. Cyclohexanone (Cyl Hex)
(Use Cyclohexanone, Sigma Prod. No. C-8390, undiluted.)
- J. Neuraminidase Enzyme Solution
(Immediately before use, prepare a solution containing approximately 0.01 - 0.02 unit/ml of Neuraminidase in Reagent H.)

PROCEDURE:

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

	<u>Test</u>	<u>Test Blank</u>	<u>Std 1</u>	<u>Std 2</u>	<u>Std 3</u>	<u>Std 4</u>	<u>Std 5</u>	<u>Std Blank</u>
Reagent A (Buffer)	0.076	0.076	0.076	0.076	0.076	0.076	0.076	0.076
Reagent B (NAN-Lactose)0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	
Reagent G (NANA Std)	---	---	0.025	0.050	0.075	0.100	0.125	---
Deionized Water	---	---	0.100	0.075	0.050	0.025	---	0.125

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

Reagent J (Enz Soln)	0.125	---	---	---	---	---	---	---
Reagent H (BSA)	---	0.125	---	---	---	---	---	---

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

Reagent D (Per)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
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PROCEDURE: (continued)

Mix by inversion and incubate at 25°C for 30 minutes. Then add:

Reagent E (Ars)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
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Immediately mix by vortexing. Continue to agitate for 2-3 minutes. Then add:

Reagent F (TBA)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
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Mix by vortexing and incubate in a boiling water bath for 15 minutes. Cool on ice to room temperature. Then add:

Reagent I (Cyl Hex)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
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Mix by vortexing. Centrifuge for 2 minutes. Transfer the upper layer to suitable cuvettes and record the $A_{549\text{nm}}$ for Test, Test Blank, Standards, and Standard Blank.

CALCULATIONS:

Standard Curve:

$$\Delta A_{549\text{nm}} \text{ Standard} = A_{549\text{nm}} \text{ Standard} - A_{549\text{nm}} \text{ Standard Blank}$$

Plot the $\Delta A_{549\text{nm}} \text{ Standard}$ vs μmoles of N-Acetylneuraminic Acid.

Sample Determination:

$$\Delta A_{549\text{nm}} \text{ Test} = A_{549\text{nm}} \text{ Test} - A_{549\text{nm}} \text{ Test Blank}$$

Determine the μmoles of n-Acetylneuraminic Acid (NANA) liberated using the Standard Curve.

$$\text{Units/ml enzyme} = \frac{(\mu\text{moles of NANA liberated})(\text{df})}{(0.125)(10)}$$

df = Dilution factor

0.125 = Volume (in milliliter) of enzyme used

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

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CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

One unit will liberate 1.0 μ mole of N-acetylneuraminic acid per minute at pH 6.5 at 37°C using 3'-N-Acetylneuramin-lactose as the substrate.

FINAL ASSAY CONCENTRATION:

In a 0.251 ml reaction mix, the final concentrations are 150 mM sodium acetate, 450 mM sodium chloride, 27 mM calcium chloride, 1.0 mM 3'-N-acetylneuramin-lactose, 0.05% (w/v) bovine serum albumin, and 0.0013-0.003 unit neuraminidase.

REFERENCE:

Warren, L. (1959) *Journal of Biological Chemistry* **234**, 1971-1975

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.