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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

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

PRINCIPLE:

N-Acetylneuramin-Lactose + H_2O $\xrightarrow{\text{Neuraminidase}}$ NANA + Lactose

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°C
(Prepare 100 ml in deionized water using Sodium Acetate Anhydrous, Product No. S 8750 and Calcium Chloride, Dihydrate, Product 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)[1]
(Prepare 5 ml in Reagent A using N-Acetylneuramin-Lactose, Product No. A 3307.)

C. 0.2% (w/v) Bovine Serum Albumin Solution (BSA)
(Prepare 100 ml in deionized water using Albumin, Bovine, Product No. A 6003.)

D. 5% (w/v) Phosphotungstic Acid Solution (PT)
(Prepare 100 ml in 2.5 M HCl using Phosphotungstic Acid Free Acid, Product No. P 4006.)

REAGENTS: (continued)

E. Neuraminidase Insoluble Enzyme Solution
(Immediately before use, mix by swirling and place 2 - 4 ml of suspension on a Whatman #50 filter paper and remove the suspension medium and soluble Neuraminidase by vacuum filtration. Stop the vacuum filtration before the gel cake becomes dry or begins to crack. It should be moist. Wash the gel cake with 10 volumes of deionized water, and again stop the vacuum filtration before the gel becomes dry or begins to crack. Resuspend 0.9 g of the washed gel cake with 1 ml of deionized water. Mix by swirling to form a homogenous gel slurry. Then dilute to 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, Product No. P 6560.)

G. 200 mM Sodium m-Periodate Solution (Per)
(Prepare 10 ml in Reagent F using Sodium m-Periodate, Product 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, Product No. S 7400, Sulfuric Acid, Product No. S 1526, and Sodium Sulfate, Anhydrous, Product 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, Product No. T 5500, and Sodium Sulfate, Anhydrous, Product No. S 9627.)

J. 5% (v/v) HCl in Butanol Solution (But)
(Prepare by combining 5 ml of concentrated Hydrochloric Acid, Product No. H 7020 and 95 ml of n-Butanol, Product Code BT-105.)

K. 0.16 mM N-Acetylneuraminic Acid Standard Solution (NANA)
(Prepare 10 ml in deionized water using N-Acetylneuraminic Acid, Product No. A 2751.)

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PROCEDURE:

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

	Test	Blank
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

Mix by inversion immediately and centrifuge for 3 minutes.

COLORIMETRIC ASSAY:

Standard Curve:

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

	Std 1	Std 2	Std 3	Std 4	Blank
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.

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COLORIMETRIC ASSAY: (Continued)

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

	Test	Test Blank	Std1	Std2	Std3	Std4	Std Blank
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

COLORIMETRIC ASSAY:
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

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

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

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:

ΔA_{550nm} Standard = A_{550nm} Standard - A_{550nm} Standard blank

Plot the ΔA_{550nm} Standard vs ìmoles n-Acetylneuraminic acid.

Sample Determination:

ΔA_{550nm} Test = A_{550nm} Test - A_{550nm} Test Blank

Determine the ìmoles 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

Units/ml of Packed Gel = Units/ml enzyme x 2

CALCULATIONS:

$$\text{Units/g agarose} = \frac{(\text{units/ml enzyme})(1000)}{15.7}$$

1000 = Conversion of mg to g
15.7 = mg agarose per ml of suspension

UNIT DEFINITION:

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

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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.

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. This assay system is operating at less than the K_m value for the substrate. It is important that the concentration of the 2→3 isomer is 0.085% (w/v).

2. Final volumes should be 0.50 ml for all test solutions performed.

3. This assay is based on the cited references.

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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