

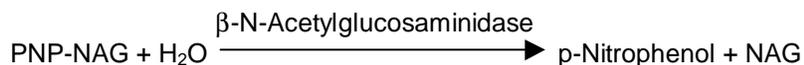


SIGMA QUALITY CONTROL TEST PROCEDURE

Product Information

Enzymatic Assay of β -N-ACETYGLUCOSAMINDASE Product No. A-7708

PRINCIPLE:



Abbreviations used:

PNP-NAG = p-Nitrophenyl N-Acetyl- β -D-Glucosaminide

NAG = N-Acetyl- β -D-Glucosamine

CONDITIONS: T = 30°C, pH = 5.0, $A_{400\text{nm}}$, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 100 mM Citrate Buffer with 200 mM Sodium Chloride and 0.02% (w/v) Albumin, pH 5.0 at 30°C
(Prepare 100 ml in deionized water using Citric Acid, Monohydrate, Sigma Prod. No. C-7129, Sodium Chloride, Sigma Prod. No. S-9625, and Albumin, Bovine Serum, Sigma Prod. No. A-4503. Adjust to pH 5.0 at 30°C with 1 M NaOH.)
- B. 10 mM p-Nitrophenyl N-Acetyl- β -D-Glucosaminide Solution (PNP-NAG)
(Prepare 5 ml in deionized water using p-Nitrophenyl N-Acetyl- β -D-Glucosaminide, Sigma Prod. No. N-9376.)
- C. 200 mM Borate Buffer, pH 9.8 at 30°C
(Prepare 100 ml in deionized water using Boric Acid, Sigma Prod. No. B-0252. Adjust to pH 9.8 at 30°C with 1 M NaOH.)
- D. β -N-Acetylglucosaminidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.1 units/ml of β -N-Acetylglucosaminidase in cold Reagent A.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	0.40	0.40
Reagent B (Substrate)	0.50	0.50

Mix by inversion and equilibrate to 30°C. Then add:

Reagent D (Enzyme Solution)	0.10	-----
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Mix by inversion and incubate for exactly 10 minutes at 30°C. Then add:

Reagent C (Borate Buffer)	3.00	3.00
Reagent D (Enzyme Solution)	0.10	-----

Mix by inversion and transfer to suitable cuvettes. Record the A_{400nm} for both Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{400nm}/\text{min Test} - \Delta A_{400nm}/\text{min Blank}) (4)}{(10) (18) (0.1)}$$

4 = Total volume (in milliliters) of solution

10 = Time of assay (Unit definition)

18 = Millimolar extinction coefficient of p-Nitrophenol at 400 nm

0.1 = Volume (in milliliters) of enzyme used

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

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

UNIT DEFINITION:

One unit will hydrolyze 1.0 μ mole of p-nitrophenyl N-acetyl- β -D-glucosaminide to p-nitrophenol and N-acetyl- β -D-glucosaminide per minute at pH 5.0 at 30°C.

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FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 50 mM citrate buffer, 0.01% (w/v) albumin, 100 mM sodium chloride, 5 mM p-nitrophenyl N-acetyl- β -D-glucosaminide, and 0.01 units β -N-Acetylglucosaminidase.

REFERENCES:

Lewy, G.a., and Conchie, J. (1966) Methods in Enzymology VIII, 577-584.

Bessey, O. A. et al. (1946) Journal of Biological Chemistry 164, 321.

NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.
2. The following enzyme impurities are measured by using the following substrates substituted into the above procedure as Reagent B.

β -N-Acetylgalactosaminidase	Substrate = N-9003 at 2 mM in dH ₂ O
β -Galactosidase	Substrate = N-1252 at 10 mM in dH ₂ O
α -Glucosidase	Substrate = N-1377 at 10 mM in dH ₂ O
α -L-Fucosidase	Substrate = N-3628 at 10 mM in dH ₂ O
α -Mannosidase	Substrate = N-2127 at 10 mM in dH ₂ O

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