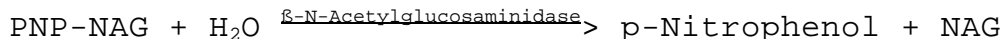


**Enzymatic Assay of β -N-ACETYLGLUCOSAMINIDASE
(EC 3.2.1.30)**

PRINCIPLE:



Abbreviations:

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

NAG = N-Acetyl- β -D-Glucosamine

CONDITIONS: T = 37°C, pH = 4.8, $A_{405\text{nm}}$, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 100 mM Citrate Buffer
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, Sigma Prod. No. C-7129.)
- B. 200 mM Sodium Phosphate Buffer
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876.)
- C. Citrate Phosphate Buffer, pH 4.8 at 37°C (Cit-Phos)
(Prepare by adjusting the pH of Reagent A with Reagent B.)
- D. 2.0 mM p-Nitrophenyl N-Acetyl- β -D-Glucosaminide Solution with 0.1% (w/v) Bovine Serum Albumin (PNP-NAG)
(Prepare 10 ml in Reagent C using p-Nitrophenyl N-Acetyl- β -D-Glucosaminide, Sigma Prod. No. N-9376 and Albumin, Bovine, Sigma Prod. No. A-4503.)
- E. 1 M Sodium Carbonate Solution (Na_2CO_3)
(Prepare 100 ml in deionized water using Sodium Carbonate, Anhydrous, Sigma Prod. No. S-2127.)
- F. β -N-Acetylglucosaminidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.02 - 0.04 unit/ml of β -N-Acetylglucosaminidase in cold Reagent C.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent C (Cit-Phos)	0.50	0.50
Reagent D (PNP-NAG)	0.50	0.50

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

Reagent F (Enzyme Solution)	0.10	----
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Mix by swirling and incubate for exactly 5 minutes at 37°C. Then add:

Reagent E (Na ₂ CO ₃)	2.00	2.00
Reagent F (Enzyme Solution)	----	0.10

Mix by swirling and transfer to suitable cuvettes. Record the A_{405nm} for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(A_{405\text{nm}} \text{ Test} - A_{405\text{nm}} \text{ Blank}) (3.1) (\text{df})}{(5) (18.5) (0.1)}$$

3.1 = Total volume (in milliliters) of assay

df = Dilution factor

5 = Time of assay (in minutes)

18.5 = Millimolar extinction coefficient of p-Nitrophenol

at 405nm¹

0.1 = Volume (in milliliter) of enzyme used

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

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-glucosamine per minute at pH 4.8 at 37°C.

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FINAL ASSAY CONCENTRATION:

In a 1.10 ml reaction mix, the final concentrations are 0.91 mM p-nitrophenyl N-acetyl- β -D-glucosaminide, 0.05% (w/v) bovine serum albumin and 0.002 - 0.004 unit of β -N-acetylglucosaminidase. The final concentrations of citric acid and sodium phosphate varies.

REFERENCES:

Bowers, Jr., G.N., McComb, R.B., Christensen, R.G., Jr., and Schaffer, R. (1980) *Clin. Chem.* **26**, 724-729

Borooah, J., Leaback, D.H. and Walker, P.G. (1961) *Biochem. J.* **78**, 106-110

NOTES:

1. The extinction coefficient is described in Bowers, Jr., G.N. et al. (1980).
2. This assay is based on the cited references.
3. 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.