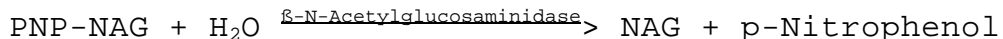


**Enzymatic Assay of  $\beta$ -N-ACETYLGLUCOSAMINIDASE  
(EC 3.2.1.52)**

**PRINCIPLE:**



Abbreviations:

PNP-NAG = p-Nitrophenyl N-Acetyl- $\beta$ -D-Glucosaminide

NAG = N-Acetyl-D-Glucosamine

**CONDITIONS:** T = 25°C, pH = 4.0, A<sub>400nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

- A. 100 mM Citrate Buffer with 0.02% (w/v) Bovine Serum Albumin and 200 mM Sodium Chloride, pH 4.0 at 25°C (Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, Sigma Prod. No. C-7129, Albumin, Bovine, Sigma Prod. No. A-4503, and Sodium Chloride, Sigma Prod. No. S-9625. Adjust to pH 4.0 at 25°C with 1 M NaOH.)
- B. 10 mM p-Nitrophenyl N-Acetyl- $\beta$ -D-Glucosaminide Solution (PNP-NAG) (Prepare 5 ml in deionized water using p-Nitrophenyl N-Acetyl- $\beta$ -D-Glucosaminide, Sigma Prod. No. N-9376.)
- C. 200 mM Borate Buffer, pH 9.8 at 25°C (Borate) (Prepare 100 ml in deionized water using Boric Acid, Sigma Prod. No. B-0252. Adjust to pH 9.8 at 25°C with 1 M NaOH.)
- D.  $\beta$ -N-Acetylglucosaminidase Enzyme Solution (Immediately before use, prepare a solution containing 0.1 - 0.2 unit/ml of  $\beta$ -N-Acetylglucosaminidase in cold Reagent A.)

**Enzymatic Assay of  $\beta$ -N-ACETYLGLUCOSAMINIDASE  
(EC 3.2.1.30)**

**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 (PNP-NAG)	0.50	0.50

Mix by swirling and equilibrate to 25°C. Then add:

Reagent D (Enzyme Solution)	0.10	-----
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Immediately mix by swirling and incubate at 25°C for exactly 10 minutes. Then add:

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

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

**CALCULATIONS:**

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

4 = Total volume (in milliliters) of assay

df = Dilution factor

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

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

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  $\mu$ mole of p-nitrophenyl N-acetyl- $\beta$ -D-glucosaminide to p-nitrophenol and N-acetyl-D-glucosamine per minute at pH 4.0 at 25°C.

**Enzymatic Assay of  $\beta$ -N-ACETYLGLUCOSAMINIDASE  
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**FINAL ASSAY CONCENTRATION:**

In a 1.00 ml reaction mix, the final concentrations are 40 mM citric acid, 80 mM sodium chloride, 0.008% (w/v) bovine serum albumin, 5 mM p-nitrophenyl N-acetyl- $\beta$ -D-glucosaminide and 0.01 - 0.02 unit  $\beta$ -N-acetylglucosaminidase.

**REFERENCES:**

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

Bessey, O.A. et al., (1946) *Journal of Biological Chemistry* **164**, 321-329

**NOTES:**

1. This assay is based on the cited references.
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.**