

**Enzymatic Assay of  $\beta$ -N-ACETYLGLUCOSAMINIDASE<sup>1</sup>**  
**(EC 3.2.1.30)**

**PRINCIPLE:**

PNP-NAG  $\xrightarrow{\beta\text{-N-Acetylglucosaminidase}}$  p-Nitrophenol + NAG

Abbreviations:

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

NAG = N-Acetyl- $\beta$ -D-Glucosamine

**CONDITIONS:** T = 25°C, pH = 4.25, A<sub>405nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

- A. 100 mM Citrate Buffer with 200 mM Sodium Chloride and 0.02% Albumin, pH 4.25 at 25°C  
(Prepare 100 ml in deionized water using Citric Acid, Monohydrate, Prod. No. C-7129, Sodium Chloride, Prod. No. S-9625, and Albumin, Bovine Serum, Prod. No. A-4503. Adjust to pH 4.25 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, Prod. No. N-9376.)
- C. 200 mM Borate Buffer, pH 9.8 at 25°C  
(Prepare 100 ml in deionized water using Boric Acid, 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 0.06 - 0.12 units/ml of  $\beta$ -N-Acetylglucosaminidase in cold deionized water.)

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**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Deionized Water	-----	0.10
Reagent A (Buffer)	0.40	0.40
Reagent B (PNP-NAG)	0.50	0.50

Mix and equilibrate to 25°C. Then add:

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

Reagent C (Borate)	3.00	3.00
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Mix and transfer to suitable cuvettes. Record the  $A_{400\text{nm}}$  for both the Test and Blank using a suitable spectrophotometer.

**CALCULATIONS:**

$$\text{Units/mg enzyme} = \frac{(\Delta A_{405\text{nm}} \text{ Test} - \Delta A_{405\text{nm}} \text{ Blank}) (4)}{(10) (18) (\text{mg enzyme/RM})}$$

4 = Total volume (in milliliters) of Solution

10 = Time of assay (Unit definition)

18 = Millimolar extinction coefficient of p-Nitrophenol at 405 nm

RM = Reaction Mix (total volume = 1 ml)

**UNIT DEFINITION:**

One unit will hydrolyze 1.0  $\mu$ mole of p-nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide to p-nitrophenol and N-acetyl- $\beta$ -D-glucosamine per minute at pH 4.25 at 25°C.

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**FINAL ASSAY CONCENTRATION:**

In a 1 ml reaction mix, the final concentrations are 45 mM citric acid, 90 mM sodium chloride, 0.01% BSA, 4.5 mM p-nitrophenyl-N-Acetyl- $\beta$ -D-glucosaminide and 0.006 - 0.012 units  $\beta$ -N-acetylglucosamidase.

**REFERENCES:**

Levy, G.A., and Conchie, J. (1966) *Methods in Enzymology* **VIII**, 571-584.

**NOTES:**

1. Not to be used for Prod. Nos. A-7053 and A-7640.
2. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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