

**Enzymatic Assay of  $\beta$ -N-ACETYLGLUCOSAMINIDASE<sup>1</sup>**  
**(EC 3.2.1.52)**

**PRINCIPLE:**

PNP-NAG + H<sub>2</sub>O  $\xrightarrow{\beta\text{-N-Acetylglucosaminidase}}$  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>400nm</sub>, 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 4.25 at 25°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 4.25 at 25°C with 1 M NaOH.)
- B. 10 mM p-Nitrophenyl N-Acetyl- $\beta$ -D-Glucosaminide Solution<sup>2</sup> (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 0.05 - 0.1 unit/ml of  $\beta$ -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 (PNP-NAG)	0.50	0.50

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

Reagent D (Enzyme Solution)	0.10	-----
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Mix by inversion and incubate for exactly 10 minutes at 25°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  $A_{400\text{nm}}$  for both the Test and Blank using a suitable spectrophotometer.

**CALCULATIONS:**

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

df = Dilution factor

4 = Total volume (in milliliters) of assay

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- $\beta$ -D-glucosamine per minute at pH 4.25 at 25°C.

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**FINAL ASSAY CONCENTRATION:**

In a 1.00 ml reaction mix, the final concentrations are  
50 mM citric acid, 100 mM sodium chloride, 0.01% (w/v)  
BSA,  
5 mM p-nitrophenyl N-acetyl- $\beta$ -D-glucosaminide and  
0.005 - 0.01 unit  $\beta$ -N-acetylglucosaminidase.

**REFERENCES:**

Bessey, O.A., Lowry, O.H., and Brock, M.J. (1946) *Journal of Biological Chemistry* **164**, 321-329

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

**NOTES:**

1. This assay is not to be used to assay  $\beta$ -N-Acetylglucosaminidase, Sigma Prod. Nos. A-3189, A-1005, and A-2264.
2. Stir gently, with heat in order to solubilize.
3. This assay is based on the cited reference.
4. 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.**