SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of β-N-ACETYLGLUCOSAMINIDASE (EC 3.2.1.30) from Jack Beans Sigma Prod. No. A-2264

PRINCIPLE:

PNP-NAG + H_2O $\xrightarrow{\text{β-N-Acetylglucosaminidase}}$ p-Nitrophenol + NAG

Abbreviations:
PNP-NAG = p-Nitrophenyl N-Acetyl-β-D-Glucosaminide
NAG = N-Acetyl-β-D-Glucosamine

CONDITIONS: T = 25°C, pH = 5.0, A_{400nm}, 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) Bovine Serum Albumin, pH 5.0 at 25°C
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, Sigma Prod. No. C-7129, Sodium Chloride, Sigma Prod. No. S-9625, and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 5.0 at 25°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 25°C
(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. β-N-Acetylglucosaminidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.05 - 0.1 unit/ml of β-N-Acetylglucosaminidase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
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	------

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_{400nm} for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

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

4 = Total volume (in milliliters) of Assay
10 = Time of assay (in minutes) as per the Unit Definition
18 = Millimolar extinction coefficient of p-Nitrophenol at 400 nm[1]
0.1 = Volume (in milliliter) of enzyme used

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

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

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UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of p-nitrophenyl N-acetyl-β-D-glucosaminide to p-nitrophenol and N-acetyl-β-D-glucosamine per minute at pH 5.0 at 25°C.

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) bovine serum albumin, 5 mM p-nitrophenyl N-acetyl-β-D-glucosaminide and 0.005 - 0.01 unit β-N-acetylglucosaminidase.

REFERENCES:

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

Li, S.-C. and Li, Y.-T. (1970) *Journal of Biological Chemistry* **245**, 5153-5160

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

NOTES:

1. The millimolar extinction coefficient is described in Bessey, O.A. et al. (1946).

2. This assay is based on the cited references.