

**Enzymatic Assay of α -N-ACETYL GALACTOSAMINIDASE
(EC 3.2.1.49)**

PRINCIPLE:

PNP-NAG + H₂O $\xrightarrow{\alpha\text{-N-Acetylgalactosaminidase}}$ NAG + p-Nitrophenol

Abbreviation used:

PNP-NAG = p-Nitrophenyl N-Acetyl- α -D-Galactosaminide

CONDITIONS: T = 37°, pH = 3.65, A_{400nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 100 mM Citrate and 100 mM Sodium Phosphate Buffer, pH 3.65 at 37°C
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, Sigma Prod. No. C-7129, and Sodium Phosphate, Monobasic, Monohydrate, Sigma Prod. No. S-9638. Adjust to pH 3.65 at 37°C with either 1 M NaOH or 1 M HCl.)
- B. 100 mM Citrate and 100 mM Sodium Phosphate Buffer with 0.1% (w/v) Bovine Serum Albumin, pH 3.65 at 37°C
(Enzyme Diluent)
(Prepare 25 ml in Reagent A using Albumin, Bovine, Sigma Prod. No. A-4503.)
- C. 5.0 mM p-Nitrophenyl N-Acetyl- α -D-Galactosaminide (PNP-NAG)
(Prepare 2 ml in Reagent A using p-Nitrophenyl N-Acetyl- α -D-Galactosaminide, Sigma Prod. No. N-4264.)
- D. 200 mM Borate Buffer, pH 9.8 at 25°C (Borate)
(Prepare 25 ml in deionized water using Boric Acid, Sigma Prod. No. B-0252. Adjust to pH 9.8 at 25°C with 1 M NaOH.)
- E. α -N-Acetylgalactosaminidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.02 - 0.05 unit/ml of α -N-Acetylgalactosaminidase in cold Reagent B.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent C (PNP-NAG)	0.10	0.10
Equilibrate to 37°C. Then add:		
Reagent E (Enzyme Solution)	0.05	-----
Immediately mix by inversion and incubate at 37°C for exactly 10 minutes. Then add:		
Reagent D (Borate)	1.00	1.00
Reagent E (Enzyme Solution)	-----	0.05

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:

$$\text{Units/ml enzyme} = \frac{(A_{400\text{nm}} \text{ Test} - A_{400\text{nm}} \text{ Blank})(1.15)(\text{df})}{(18)(10)(0.05)}$$

- 1.15 = Total volume (in milliliters) of assay
- df = Dilution factor
- 18 = Millimolar extinction coefficient¹ of p-Nitrophenol at 400 nm
- 10 = Time (in minutes) of assay per the Unit Definition
- 0.05 = Volume (in milliliter) of enzyme used

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

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

UNIT DEFINITION:

One unit will release 1.0 μ mole of p-nitrophenol from p-nitrophenyl-N-acetyl- α -D-galactosaminide per minute at pH 3.65 at 37°C.

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FINAL ASSAY CONDITION:

In a 0.15 ml reaction mix, the final concentrations are 100 mM citric acid, 100 mM sodium phosphate, 0.03% (w/v) bovine serum albumin, 3.3 mM p-nitrophenyl N-acetyl-a-D-galactosaminide, and 0.001 - 0.0025 unit a-N-acetylgalactosaminidase.

REFERENCE:

Uda, Y., Li, S.-C., and Li, Y.-T. (1977) *Journal of Biological Chemistry* **252**, 5194-5200

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

NOTES:

1. The millimolar extinction coefficient of p-nitrophenol at 400 nm is described in Bessey, O.A., Lowry, O.H., and Brock, M.J. (1946).
2. This assay is based on the assay procedure described in Uda, Y., Li, S.-C., and Li, Y.-T. (1977).
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.