Enzymatic Assay of ß-GALACTOSIDASE
(EC 3.2.1.23)

PRINCIPLE:

PNP-ß-$_D$-Galactopyranoside $\xrightarrow{\text{ß-Galactosidase}}$ p-Nitrophenol + $_D$-Galactose

Abbreviations used:
PNP-ß-$_D$-Galactopyranoside = p-Nitrophenyl-ß-$_D$-Galactopyranoside

CONDITIONS: T = 25°C, pH = 4.4, A_{400nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 200 mM Sodium Acetate Buffer, with 0.25% (w/v) Bovine Serum Albumin and 100 mM Sodium Chloride, pH 4.4 at 25°C.
(Prepare 100 ml in deionized water using Sodium Acetate Trihydrate, Prod. No. S-8625, Albumin Bovine, Prod. No. A-4503 and Sodium Chloride Prod. No. S-9625. Adjust to pH 4.4 at 25°C with 1 M Acetic Acid.)

B. 10 mM p-Nitrophenyl-ß-$_D$-Galactoside Solution (PNP-Gal)
(Prepare 5 ml in deionized water using p-Nitrophenyl ß-$_D$-Galactopyranoside, Prod. No. N-1252.)

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. ß-Galactosidase Enzyme Solution
(Immediately before use, prepare a solution containing approximately 1.0 unit/ml of ß-Galactosidase in cold deionized water.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Sodium Acetate Buffer)	0.40	0.40
Reagent D (Enzyme Solution)	0.02	------

Mix by inversion and equilibrate to 25°C. Monitor the A_{400nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent B (PNP-Gal)	0.50	0.50

Immediately mix by inversion and incubate at 25° C for exactly 10 minutes. Then add:

Reagent C (Borate Buffer)	3.00	3.00
Reagent D (Enzyme Solution)	------	0.02

Mix and record the A_{400nm} for both the Test and Blank.

CALCULATIONS:

$$\text{units/mg enzyme} = \frac{A_{400nm}\ \text{Test} - A_{400nm}\ \text{Blank}\ (3.92)}{(10)(18.0)(\text{mg enzyme/RM})}$$

3.92 = Total volume of assay
10 = Conversion factor for 10 minutes to 1 minute
18 = Millimolar extinction coefficient of p-Nitrophenol at 400 nm[1]
RM = Reaction Mix

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of p-nitrophenyl ß-D-galactopyranoside to p-nitrophenol and D-galactose per minute at pH 4.4 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 0.92 ml reaction mix, the final concentrations are 87 mM sodium acetate, 5.4 mM p-nitrophenyl-ß-galactopyranoside, 0.11% bovine serum albumin, 43.5 mM sodium chloride, and 0.02 units ß-galactosidase

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REFERENCE:

Distler, J. J., and Jourdian, G. W., (1973) *J. Biol. Chem.* **248**, 6772-6780

NOTES:

1. This enzymatic assay is a modification of the assay described in the cited literature reference.

2. All products 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.