Enzymatic Assay of ß-GALACTOSIDASE (EC 3.2.1.23)

PRINCIPLE:

ONP-ß-D-Galactopyranoside $\xrightarrow{\text{ß-Galactosidase}}$ o-Nitrophenol + ß-D-Galactose

Abbreviations used:
ONP-ß-$_D$-Galactopyranoside = o-Nitrophenyl-ß-$_D$-Galactopyranoside

CONDITIONS: T = 25°C, pH 4.0, A_{410nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 400 mM Citrate Buffer, pH 4.0 at 25°C
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, Prod. No. C-7129. Adjust to pH 4.0 at 25°C with 1 M NaOH.)

B. 10 mM o-Nitrophenyl-ß-$_D$-Galactoside Substrate Solution (ONP-Gal)
(Prepare 5 ml in deionized water using o-Nitrophenyl-ß-$_D$-Galactopyranoside, Prod. No. N-1127.)

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 0.05 - 0.10 units/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 (Citrate Buffer)	0.40	0.40
Reagent B (ONP-Gal)	0.50	0.50

Mix by inversion and equilibrate to 25°C. Monitor the A_{410nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.10	------
Deionized Water	------	0.10

Immediately mix by inversion and incubate for exactly 10 minutes. Then add:

Reagent C (Borate Buffer)	3.00	3.00

Mix and record the ΔA_{410nm} for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{\Delta A_{410nm}\ \text{Test} - \Delta A_{410nm}\ \text{Blank}\ (4.0)}{(10)\ (4.6)\ (\text{mg enzyme/RM})}$$

4.0 = Total volume of assay
10 = Conversion factor for 10 minutes to 1 minute
4.6 = Millimolar extinction coefficient of o-Nitrophenol at pH 9.8
RM = Reaction Mix

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of o-nitrophenyl ß-$_D$-galactoside to o-nitrophenol and $_D$-galactose per minute at pH 4.0 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 1.0 ml reaction mix, the final concentrations are 160 mM citrate buffer, 5.0 mM ONP-Gal and 0.005 - 0.010 units ß-Galactosidase.

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REFERENCE:

(1969) *J. Biol. Chem.* **244**, 2970

NOTES:

1. 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.