

**Enzymatic Assay of  $\beta$ -GALACTOSIDASE  
(EC 3.2.1.23)**

**PRINCIPLE:**

ONP  $\beta$ -D-Galactopyranoside + H<sub>2</sub>O  $\xrightarrow{\beta\text{-Galactosidase}}$  o-Nitrophenol +  $\beta$ -D-Galactose

Abbreviations used:

ONP  $\beta$ -D-Galactopyranoside = o-Nitrophenyl  $\beta$ -D-Galactopyranoside

**CONDITIONS:** T = 37°C, pH 6.0, A<sub>405nm</sub>, Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

- A. 100 mM Sodium Acetate Buffer, pH 6.0 at 37°C  
(Prepare 100 ml in deionized water using Sodium Acetate, Trihydrate, Sigma Prod. No. S-8625. Adjust to pH 6.0 at 37°C with 1 M Acetic Acid.)
- B. 2.0 mM o-Nitrophenyl  $\beta$ -D-Galactoside with 0.01% (w/v) Bovine Serum Albumin, pH 6.0 (Substrate Solution)  
(Prepare 100 ml in Reagent A using o-Nitrophenyl  $\beta$ -D-Galactopyranoside, Sigma Prod. No. N-1127 and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 6.0 at 30°C, if necessary, with 1 M Acetic Acid or 1 M NaOH.)
- C. 1000 mM Sodium Carbonate Solution (Na<sub>2</sub>CO<sub>3</sub>)  
(Prepare 100 ml in deionized water using Sodium Carbonate, Anhydrous, Sigma Prod. No. S-2127.)
- D.  $\beta$ -Galactosidase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.02 - 0.04 unit/ml of  $\beta$ -Galactosidase in cold deionized water.)

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**PROCEDURE:**

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

	<u>Test</u>	<u>Blank</u>
Reagent B (Substrate solution)	0.50	0.50
Deionized Water	0.30	0.30

Mix by inversion and equilibrate to 37°C. Then add:

Reagent D (Enzyme Solution)	0.20	-----
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Immediately mix by inversion and incubate for exactly 10 minutes. Then add:

Reagent C (Na <sub>2</sub> CO <sub>3</sub> )	4.00	4.00
Reagent D (Enzyme Solution)	-----	0.20

Mix by inversion and record the A<sub>405nm</sub> for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(A_{405\text{nm}} \text{ Test} - A_{405\text{nm}} \text{ Blank}) (5) (df)}{(10) (4.6) (0.2)}$$

5 = Total volume (in milliliters) of assay

df = Dilution factor

10 = Time of assay (in minutes) as per the Unit Definition

4.6 = Millimolar extinction coefficient of o-Nitrophenol  
at 405 nm<sup>1</sup>

0.2 = Volume (in milliliters) 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 hydrolyze 1.0  $\mu$ mole of o-nitrophenyl  $\beta$ -D-galactoside to o-nitrophenol and D-galactose per minute at pH 6.0 at 37°C.

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**FINAL ASSAY CONCENTRATION:**

In a 1.00 ml reaction mix, the final concentrations are 50 mM sodium acetate, 0.005% (w/v) bovine serum albumin, 1.0 mM o-nitrophenyl  $\beta$ -D-galactopyranoside and 0.004 - 0.008 unit  $\beta$ -galactosidase.

**REFERENCE:**

Kuby, S.A. and Lardy, H.S. (1953) *Journal of the American Chemical Society* **75**, 890-896.

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

**NOTES:**

1. This value has been experimentally determined by Sigma.
2. This assay is based on the cited reference.
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.**