

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

**PRINCIPLE:**

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

Abbreviations used:

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

**CONDITIONS:** T = 25°C, pH 3.5,  $A_{410\text{nm}}$ , Light path = 1 cm

**METHOD:** Spectrophotometric Stop Rate Determination

**REAGENTS:**

- A. 400 mM Citrate Buffer, pH 3.5 at 25°C  
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, Sigma Prod. No. C-7129. Adjust to pH 3.5 at 25°C with 1 M NaOH.)
- B. 10 mM o-Nitrophenyl  $\beta$ -D-Galactoside Substrate Solution (ONP-Gal)  
(Prepare 5 ml in deionized water using o-Nitrophenyl  $\beta$ -D-Galactopyranoside, Sigma Prod. No. N-1127.)
- 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.  $\beta$ -Galactosidase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.05-0.10 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 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
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Mix and record the  $\Delta A_{410nm}$  for both the Test and Blank.

**CALCULATIONS:**

$$\text{Units/ml enzyme} = \frac{(\Delta A_{410nm} \text{ Test} - \Delta A_{410nm} \text{ Blank}) (4) (df)}{(10) (4.6) (0.1)}$$

4 = 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 pH 9.8

0.1 = Volume (in milliliters) of enzyme used

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

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

**Enzymatic Assay of  $\beta$ -GALACTOSIDE  
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**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 3.5 at 25°C.

**FINAL ASSAY CONCENTRATION:**

In a 1.00 ml reaction mix, the final concentrations are 160 mM citrate buffer, 5.0 mM o-nitrophenyl  $\beta$ -D-galactoside and 0.005-0.010 unit  $\beta$ -galactosidase.

**REFERENCES:**

Bahl, O.P. and Agrawal, K.M.L. (1969) *J. Biol Chem.* **244**, 2970-2978.

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

**NOTES:**

1. This assay is based on the cited references.
2. 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.**