



Product Information

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of β -GALACTOSIDASE

(EC 3.2.1.23)

Sigma Prod. No. G-5160

o-Nitrophenyl β -D-Galactopyranoside as Substrate

PRINCIPLE:

ONP β -D-Galactopyranoside + H₂O $\xrightarrow{\beta\text{-Galactosidase}}$ o-Nitrophenol + β -D-Galactose

Abbreviation used:

ONP β -D-Galactopyranoside = o-Nitrophenyl β -D-Galactopyranoside

CONDITIONS: T = 30°C, pH = 4.5, A_{410nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

- A. 10 mM Citric Acid Solution
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Anhydrous, Sigma Prod. No. C-0759.)
- B. 20 mM Sodium Phosphate Solution
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876.)
- C. 20 mM Phosphate-Citrate Buffer, pH 4.5 at 30°C (Phos-Cit Buffer)
(Prepare 100 ml using Reagent B. Adjust to pH 4.5 at 30°C with Reagent A.)
- D. 10 mM o-Nitrophenyl β -D-Galactoside Solution (ONP-Gal)
(Prepare 10 ml in Reagent C using o-Nitrophenyl β -D-Galactopyranoside, Sigma Prod. No. N-1127.)
- E. 200 mM Borate Buffer, pH 9.8 at 30°C
(Prepare 100 ml in deionized water using Boric Acid, Sigma Prod. No. B-0252. Adjust to pH 9.8 at 30°C with 1 M NaOH.)
- F. β -Galactosidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 0.4 unit/ml of β -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 C (Phos-Cit Buffer)	0.40	0.40
Reagent D (ONP-Gal)	0.50	0.50

Mix by inversion and equilibrate to 30°C, using a suitable spectrophotometer. Then add:

Reagent F (Enzyme Solution)	0.10	-----
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Immediately mix by inversion and incubate at 30°C for exactly 10 minutes. Then add:

Reagent E (Borate Buffer)	3.00	3.00
Reagent F (Enzyme Solution)	-----	0.10

Mix by inversion and record the $A_{410\text{nm}}$ for both the Test and Blank.

CALCULATIONS:

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

4 = Total volume (in milliliters) of the assay

df = Dilution factor

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

4.6 = Millimolar extinction coefficient of o-Nitrophenol at 410 nm

0.1 = Volume (in milliliters) of enzyme used

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of o-nitrophenyl β -D-galactoside to o-nitrophenol and D-galactose per minute at pH 4.5 at 30°C.

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FINAL ASSAY CONCENTRATIONS:

In a 1.00 ml reaction mix, the final concentrations are 18 mM sodium phosphate, 5 mM o-nitrophenyl β -D-galactopyranoside, and 0.02 - 0.04 unit β -galactosidase.

REFERENCE:

Bahl, O.P. and Agrawal, K.M.L (1969) *Journal of Biological Chemistry* **244**, 2970-2978.

Borooah, S., Leaback, D.H. and Walker, P.G. (1961) *Biochemical Journal* **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.

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