SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of β-GALACTOSIDASE
(EC 3.2.1.23)
Sigma Prod. Nos. G-1875, G-2513, G-5635, G-6008, and G-4155

PRINCIPLE:

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

Abbreviation used:
ONP β-D-Galactopyranoside = o-Nitrophenyl β-D-Galactopyranoside

CONDITIONS: T = 37°C, pH = 7.3, A_{410nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Sodium Phosphate Solution
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751.)

B. 100 mM Sodium Phosphate Buffer, pH 7.3 at 37°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876. Adjust to pH 7.3 at 37°C with Reagent A.)

C. 68 mM o-Nitrophenyl β-D-Galactoside Solution (ONP-Gal)
(Prepare 5 ml in Reagent B using o-Nitrophenyl β-D-Galactopyranoside, Sigma Prod. No. N-1127.)

D. 30 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)

E. 3.36 M 2-Mercaptoethanol Solution (2-ME)
(Prepare 2 ml in deionized water using 2-Mercaptoethanol, Sigma Prod. No. M-6250.)

F. β-Galactosidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 1.0 unit/ml of β-Galactosidase in cold Reagent B.)

Enzymatic Assay of β-GALACTOSIDASE
(EC 3.2.1.23)
Sigma Prod. Nos. G-1875, G-2513, G-5635, G-6008, and G-4155

PROCEDURE:

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

	Test	Blank
Reagent B (Buffer)	2.60	2.70
Reagent D ($MgCl_2$)	0.10	0.10
Reagent E (2-ME)	0.10	0.10
Reagent F (Enzyme Solution)	0.10	------

Mix by inversion and equilibrate to 37°C. Monitor the A_{410nm} until constant, using a suitably thermostatted spectrophotometer.[1] Then add:

Reagent C (ONP-Gal)	0.10	0.10

Immediately mix by inversion and record the increase in A_{410nm} for approximately 5 minutes. Obtain the ΔA_{410nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{410nm}/\text{min Test} - \Delta A_{410nm}/\text{min Blank})(3)(df)}{(3.5)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
3.5 = Millimolar extinction coefficient[2] of o-Nitrophenol at 410 nm
0.1 = Volume (in milliliter) 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 µmole of o-nitrophenyl β-D-galactoside per minute at pH 7.3 at 37°C.

Enzymatic Assay of β-GALACTOSIDASE
(EC 3.2.1.23)
Sigma Prod. Nos. G-1875, G-2513, G-5635, G-6008, and G-4155

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 93 mM sodium phosphate, 2.3 mM o-nitrophenyl β-D-galactoside, 1.0 mM magnesium chloride, 112 mM 2-mercaptoethanol and 0.02 - 0.1 unit β-galactosidase.

REFERENCE:

Craven, G.R., Steers, E., Jr., and Anfinsen, C.B. (1965) *Journal of Biological Chemistry* **240**, 2468-2477

NOTES:

1. The equilibration time must not exceed 3 minutes.

2. The millimolar extinction coefficient was determined experimentally by Sigma Chemical Company.

3. This assay is based on the cited reference.

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.