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ProductInformation

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of β-GALACTOSIDASE
(EC 3.2.1.23)
Sigma Prod. No. G-3782

PRINCIPLE:

ONP β-D-Galactopyranoside + H_2O $\xrightarrow{\beta\text{-Galactosidase}}$ o-Nitrophenol + β-D-Galactose

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

CONDITIONS: T = 37°C, pH = 7.2, A_{410nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 7.2 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Dibasic, Trihydrate, Sigma Prod. No. P-5504. Adjust to pH 7.2 at 37°C with 100 mM Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379.)

B. 68 mM o-Nitrophenyl β-D-Galactopyranoside Solution (ONP-Gal)
(Prepare 5 ml in Reagent A using o-Nitrophenyl β-D-Galactopyranoside, Sigma Prod. No. N-1127. Warm gently to solubilize, keep at 37°C.)

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

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

E. β-Galactosidase Enzyme Solution
(Immediately before use, prepare a solution containing 2.0 - 4.0 units/ml of β-Galactosidase in cold Reagent A.)

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PROCEDURE:

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

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

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

Reagent B (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 of o-Nitrophenol at 410 nm[1]
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 to o-nitrophenol and D-galactose per minute at pH 7.2 at 37°C.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 93 mM potassium phosphate, 2.3 mM o-nitrophenyl β-D-galactopyranoside, 1.0 mM magnesium chloride, 112 mM 2-mercaptoethanol and 0.20 - 0.40 unit β-galactosidase.

REFERENCE:

Craven, G.R., Steers, E., Jr. and Anfinsen, C.B. (1965) *J. Biol. Chem.* **240**, 2468-2477

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.

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