SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of β-GALACTOSIDASE
(EC 3.2.1.23)
Sigma Prod. No. G-5160
α-Nitrophenyl β-D-Galactopyranoside as Substrate

PRINCIPLE:
ONP β-D-Galactopyranoside + H₂O → α-Nitrophenol + β-D-Galactose

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

CONDITIONS: T = 30°C, pH = 4.5, A₄₁₀nm, 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 α-Nitrophenyl β-D-Galactoside Solution (ONP-Gal)
   (Prepare 10 ml in Reagent C using α-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:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Phos-Cit Buffer)</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Reagent D (ONP-Gal)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

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

- Reagent F (Enzyme Solution) 0.10 -----

Immediately mix by inversion and incubate at 30°C for exactly 10 minutes. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (Borate Buffer)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>------</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

CALCULATIONS:

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

4 = Total volume (in milliliters) of the assay  
10 = Time of assay (in minutes) as per the Unit Definition  
4.6 = Millimolar extinction coefficient of α-Nitrophenol at 410 nm  
0.1 = Volume (in milliliters) of enzyme used

UNIT DEFINITION:

One unit will hydrolyze 1.0 μmole of α-nitrophenyl β-D-galactoside to α-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 α-nitrophenyl β-D-galactopyranoside, and 0.02 - 0.04 unit β-galactosidase.

REFERENCE:


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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