Enzymatic Assay of β-GALACTOSIDASE
(EC 3.2.1.23)

PRINCIPLE:

\[
\beta\text{-Galactosidase} \\
ONP \beta\text{-D-Galactopyranoside} \rightarrow \text{o-Nitrophenol + } \beta\text{-D-Galactose}
\]

Abbreviation used:
ONP \beta\text{-D-Galactopyranoside} = \text{o-Nitrophenyl } \beta\text{-D-Galactopyranoside}

CONDITIONS:  \( T = 37^\circ C, \ pH = 7.3, \ A_{410nm} \), 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.)

B.  100 mM Sodium Phosphate Buffer, pH 7.3 at 37°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous.
Adjust to pH 7.3 at 37°C with Reagent A.)

C.  68 mM \text{o-Nitrophenyl } \beta\text{-D-Galactoside Solution (ONP-Gal)}
(Prepare 5 ml in Reagent B using \text{o-Nitrophenyl } \beta\text{-D-Galactopyranoside.)}

D.  30 mM Magnesium Chloride Solution (MgCl₂)
(Prepare 10 ml in deionized water using Magnesium Chloride, Hexahydrate.)

E.  3.36 M 2-Mercaptoethanol Solution (2-ME)
(Prepare 2 ml in deionized water using 2-Mercaptoethanol.)

F.  \beta\text{-Galactosidase Enzyme Solution}
(Immediately before use, prepare a solution containing 0.2 - 1.0 unit/ml of \beta\text{-Galactosidase in cold Reagent B.)}
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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 B (Buffer)</td>
<td>2.60</td>
<td>2.70</td>
</tr>
<tr>
<td>Reagent D (MgCl₂)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent E (2-ME)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent F (Enzyme Solution)</td>
<td>0.10</td>
<td>-----</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
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<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (ONP-Gal)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

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

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay
df = Dilution factor
3.5 = Millimolar extinction coefficient² 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-β-galactoside per minute at pH 7.3 at 37°C.
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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:


NOTES:

1. The equilibration time must not exceed 3 minutes.
2. The millimolar extinction coefficient was determined experimentally.
3. This assay is based on the cited reference.
4. Where our Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of our quality control procedure contact our Technical Service Department.