Enzymatic Assay of β-MANNOSIDASE
(EC 3.2.1.25)

PRINCIPLE:

\[ \text{p-Nitrophenyl} \ \beta-D-\text{Mannoside} + \text{H}_2\text{O} \xrightarrow{\beta-\text{Mannosidase}} \text{D-Mannose} + \text{p-Nitrophenol} \]

CONDITIONS:  \( T = 25^\circ \text{C} \),  \( \text{pH} = 4.0 \),  \( A_{400\text{nm}} \),  \text{Light path} = 1 \text{ cm} 

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 100 mM Citrate Buffer, pH 4.0 at 25°C
   (Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, Prod. No. C-7129. Adjust to pH 4.0 at 25°C with 1 M NaOH.)

B. 10 mM p-Nitrophenyl β-D-Mannoside Solution (PNP-β-Man)
   (Prepare 5 ml in deionized water using p-Nitrophenyl β-D-Mannopyranoside, Prod. No. N-1268.)

C. 200 mM Borate Buffer, pH 9.8 at 25°C
   (Prepare 100 ml in deionized water using Boric Acid, Prod. No. B-0252. Adjust to pH 9.8 at 25°C with 1 M NaOH.)

D. β-Mannosidase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.05 – 0.1 unit/ml of β-Mannosidase in cold deionized water.)

PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.10</td>
<td>------</td>
</tr>
</tbody>
</table>

Mix by swirling and equilibrate to 25°C. Then add:
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PROCEDURE:  (continued)

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
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<tbody>
<tr>
<td>Reagent B (PNP-β-Man)</td>
<td>0.50</td>
<td>0.50</td>
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</table>

Immediately mix by swirling and incubate at 25°C for exactly 10 minutes. Then add:

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<tbody>
<tr>
<td>Reagent C (Borate)</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>----</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by swirling and transfer to suitable cuvettes. Record the A₄₀₀nm for both the Test and Blank using a suitable spectrophotometer.

CALCULATIONS:

\[
\text{Units/mg enzyme} = \frac{(A_{400\text{nm}}/\text{min Test} - A_{400\text{nm}}/\text{min Blank}) \times (4)}{(10) \times (18) \times (\text{mg enzyme/RM})}
\]

4 = Total volume (in milliliters) of Solution
10 = Time of assay (in minutes) as per the Unit Definition
18 = Millimolar extinction coefficient of p-Nitrophenol at 400 nm
RM = Reaction Mix

UNIT DEFINITION:

One unit will hydrolyze 1.0 µmole of p-nitrophenyl β-D-mannopyranoside to p-nitrophenol and D-mannopyranoside per minute at pH 4.0 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 40 mM citric acid, 5.0 mM p-nitrophenyl β-D-mannoside and 0.005 - 0.01 unit β-mannosidase.
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REFERENCES:


NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

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