Enzymatic Assay of α-MANNOSIDASE
(EC 3.2.1.24)

PRINCIPLE:
p-Nitrophenyl-α-D-Mannoside + H2O α-Mannosidase → D-Mannose + p-Nitrophenol

CONDITIONS: T = 25°C, pH = 4.5, A405nm, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 100 mM Citrate Buffer, pH 4.5 at 25°C
(Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate, Prod. No. C-7129. Adjust to pH 4.5 at 25°C with 1 M NaOH.)

B. 10 mM p-Nitrophenyl α-D-Mannoside Solution (PNP-Man)
(Prepare 5 ml in deionized water using p-Nitrophenyl α-D-Mannopyranoside, Prod. No. N-2127.)

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

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>Deionized Water</td>
<td>------</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent A (Buffer)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent B (PNP-Man)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

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PROCEDURE:  (continued)

Mix by inversion and equilibrate to \( 25^\circ \text{C} \). Then add:

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

Immediately mix by inversion and incubate at \( 25^\circ \text{C} \) for exactly 5 minutes in a suitable spectrophotometer. Then add:

| Reagent C (Borate) | 2.00 | 2.00 |

Mix by inversion and record the \( A_{405 \text{nm}} \) for both the Test and Blank.

CALCULATIONS:

\[
\text{Units/mg enzyme} = \frac{(\Delta A_{405 \text{nm}}/\text{min Test} - \Delta A_{405 \text{nm}}/\text{min Blank})}{(5 \times 18.5 \times \text{mg enzyme/RM})}
\]

3.1 = Total volume (in milliliters) of Solution
5 = Time of assay (Unit definition)
18.5 = Millimolar extinction coefficient of p-Nitrophenol at 405 nm
RM = Reaction Mix

UNIT DEFINITION:

One unit will hydrolyze 1.0 \( \mu \text{mole} \) of p-nitrophenyl \( \alpha \)-\( \text{D} \)-mannoside to p-nitrophenol and \( \text{D} \)-mannose per minute at \( \text{pH} 4.5 \) at \( 25^\circ \text{C} \).

FINAL ASSAY CONCENTRATION:

In a 1.10 ml reaction mix, the final concentrations are 45 mM citric acid, 4.5 mM p-nitrophenyl \( \alpha \)-\( \text{D} \)-mannoside and 0.005 – 0.01 unit \( \alpha \)-mannosidase.

REFERENCES:


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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.