Enzymatic Assay of TREHALASE
(EC 3.2.1.28)

PRINCIPLE:

Trehalose + H\textsubscript{2}O \xrightarrow{\text{Trehalase}} 2 Glucose

CONDITIONS: T = 37°C, pH = 5.7, A\textsubscript{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 135 mM Citric Acid Buffer, pH 5.7 at 37°C
   (Prepare 100 ml in deionized water using Citric Acid, Free Acid, Monohydrate. Adjust to pH 5.7 at 37°C with 1 M NaOH.)

B. 140 mM D-Trehalose Solution
   (Prepare 10 ml in Reagent A using D(+)Trehalose, Dihydrate.)

C. 500 mM Tris Buffer, pH 7.5 at 37°C
   (Prepare 100 ml in deionized water using Trizma Base. Adjust to pH 7.5 at 37°C with 1 M HCl.)

D. Trehalase Enzyme Solution
   (Immediately before use, prepare a solution containing 0.1 - 0.3 unit/ml of Trehalase in cold Reagent A.)

E. Glucose Determination Vial
   (Use Glucose (HK) 10 Reagent. Dissolve the contents in 10 ml of deionized water.)
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PROCEDURE:

Step 1:

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 A (Citrate Buffer)</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Reagent D (Enzyme Solution)</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 37°C using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B (D-Trehalose)</td>
<td>0.1</td>
<td>------</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and incubate at 37°C for exactly 15 minutes. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent C (Tris Buffer)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Reagent B (D-Trehalose)</td>
<td>------</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Step 2:

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 E (16-10)</td>
<td>3.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Record the initial A_{340nm} for both Test and Blank. Then add:

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Solution</td>
<td>0.1</td>
<td>------</td>
</tr>
<tr>
<td>Blank Solution</td>
<td>------</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A_{340nm} until complete (approximately 5 minutes). Obtain the final A_{340nm} for both the Test and Blank.
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CALCULATIONS:

\[
\Delta A_{340\text{nm}} \text{ Test} = A_{340\text{nm}} \text{ Test Final} - A_{340\text{nm}} \text{ Test Initial}
\]

\[
\Delta A_{340\text{nm}} \text{ Blank} = A_{340\text{nm}} \text{ Blank Final} - A_{340\text{nm}} \text{ Blank Initial}
\]

\[
\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}} \text{ Test} - \Delta A_{340\text{nm}} \text{ Blank})(1.0)(3.1)}{(6.22)(2)(15)(0.1)(0.1)}
\]

6.22 = Millimolar extinction coefficient of \( \beta \)-NADH at 340nm  
2 = Number of Glucose molecules per molecule of Trehalose  
15 = Reaction time (in minutes) of Step 1  
1.0 = Final volume (in milliliters) of Step 1  
3.1 = Final volume (in milliliters) of Step 2  
0.1 = Volume From Step 1 used in Step 2  
0.1 = Volume (in milliliters) of enzyme used  

Units/mg protein = \[
\frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}
\]

UNIT DEFINITION:

One unit will convert 1.0 \( \mu \text{mole} \) of trehalose to 2.0 \( \mu \text{moles} \) of glucose per minute at pH 5.7 at 37°C (liberated glucose determined at pH 7.5).

FINAL ASSAY CONCENTRATION:

In a 0.50 ml reaction mix, the final concentrations are 135 mM citric acid, 28 mM D-trehalose, and 0.01 - 0.03 unit of trehalase.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.