Enzymatic Assay of TREHALASE  
(EC 3.2.1.28)

PRINCIPLE:

Trehalose + H$_2$O $\xrightarrow{\text{Trehalase}}$ 2 Glucose

CONDITIONS: $T = 37^\circ$C, $pH = 5.7$, $A_{340nm}$, 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, Sigma Prod. No. C-7129. 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, Sigma Prod. No. T-5251.)

C. 500 mM Tris Buffer, $pH$ 7.5 at 37°C  
(Prepare 100 ml in deionized water using Trizma Base, Sigma Prod. No. T-1503. 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 Sigma Stock No. 16-10, 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:

| Reagent B (D-Trehalose) | 0.1 |

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

| Reagent C (Tris Buffer)  | 0.5  | 0.5   |
| Reagent B (D-Trehalose)  | ------ | 0.1   |

Step 2:

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

<table>
<thead>
<tr>
<th>Reagent E (16-10)</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td></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>Reagent</th>
<th>Test Solution</th>
<th>Blank Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
<td>------</td>
</tr>
<tr>
<td></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:

$\Delta A_{340nm}^{\text{Test}} = A_{340nm}^{\text{Test Final}} - A_{340nm}^{\text{Test Initial}}$

$\Delta A_{340nm}^{\text{Blank}} = A_{340nm}^{\text{Blank Final}} - A_{340nm}^{\text{Blank Initial}}$

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

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

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of trehalose to 2.0 µmoles 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.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

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