SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of TREHALASE
(EC 3.2.1.28)

PRINCIPLE:

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

CONDITIONS: \( T = 37 \, ^\circ\text{C}, \, \text{pH} = 5.7, \, A_{340nm}, \, \text{Light path} = 1 \, \text{cm} \)

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, Product 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, Product No. T 5251.)

C. 500 mM Tris Buffer, pH 7.5 at 37 °C
   (Prepare 100 ml in deionized water using Trizma Base, Product 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 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:

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<th>Test</th>
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| Reagent A (Citrate Buffer) | 0.3 | 0.3 |
| Reagent D (Enzyme Solution) | 0.1 | 0.1 |

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:

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| Reagent E (16-10) | 3.0 | 3.0 |

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:

| Test Solution | 0.1 | ----- |
| Blank Solution | ----- | 0.1 |

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:

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

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

\[ (\Delta A_{340 \text{nm}} \text{ Test} - \Delta A_{340 \text{nm}} \text{ Blank})(1.0)(3.1) \]

Units/ml enzyme =

\[ \frac{(6.22)(2)(15)(0.1)(0.1)}{1} \]

6.22 = Millimolar extinction coefficient of \( \beta \)-NADH at 340 nm
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 =

mg protein/ml enzyme

UNIT DEFINITION:

One unit will convert 1.0 \( \mu \)mole of trehalose to 2.0 \( \mu \)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.
2. Where Product or Stock numbers are specified, equivalent reagents may be substituted.

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