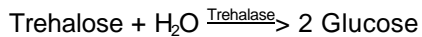


Enzymatic Assay of TREHALASE (EC 3.2.1.28)

PRINCIPLE:



CONDITIONS: T = 37°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.)

**Enzymatic Assay of TREHALASE
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PROCEDURE:

Step 1:

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

	<u>Test</u>	<u>Blank</u>
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	-----
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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:

	<u>Test</u>	<u>Blank</u>
Reagent E (16-10)	3.0	3.0

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

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

Immediately mix by inversion and record the increase in $A_{340\text{nm}}$ until complete (approximately 5 minutes). Obtain the final $A_{340\text{nm}}$ 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 β -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 μ mole of trehalose to 2.0 μ 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:

Dahlqvist, A. (1968) *Analytical Biochemistry* **22**, 99-107

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.