3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-576
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

SIGMA QUALITY CONTROL TEST PROCEDURE

Enzymatic Assay of TREHALASE (EC 3.2.1.28)

PRINCIPLE:

Trehalose + H_2O $\xrightarrow{\text{Trehalase}}$ > 2 Glucose

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, 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.)

Enzymatic Assay of TREHALASE
(EC 3.2.1.28)

PROCEDURE:

Step 1:

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

	Test	Blank
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:

	Test	Blank
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.

Enzymatic Assay of TREHALASE
(EC 3.2.1.28)

CALCULATIONS:

ΔA_{340nm} Test = A_{340nm} Test Final - A_{340nm} Test Initial

ΔA_{340nm} Blank = A_{340nm} Blank Final - A_{340nm} 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 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 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:

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

NOTES:

1. This assay is based on the cited reference.
2. Where Product or Stock numbers are specified, equivalent reagents may be substituted.