Enzymatic Assay of ISOMALTASE
(EC 3.2.1.10)

PRINCIPLE:

Isomaltose + H_2O $\xrightarrow{\text{Isomaltase}}$ 2 Glucose

Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ Glucose 6-Phosphate

Glucose 6-Phosphate + ß-NADP $\xrightarrow{\text{G-6-P-DH}}$ Gluconate + ß-NADPH

Abbreviations used:
ATP = Adenosine 5'-Triphosphate
G-6-P-DH = Glucose 6-Phosphate Dehydrogenase
ß-NADP = ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form
ß-NADPH = ß-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

CONDITIONS: T = 25°C, pH 6.8, A_{340nm}, Light path = 1 cm

METHOD: Spectrophotometric Stop Rate Determination

REAGENTS:

A. 50 mM Potassium Phosphate Buffer, pH 6.8 at 25°C (Phosphate Buffer)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 6.8 at 25°C with 1 M KOH.)

B. 100 mM Isomaltose Solution (Isomaltose)
(Prepare 10 ml in deionized water using Isomaltose, Sigma Prod. No. I-7253.)

C. 300 mM Triethanolamine HCl Buffer, pH 7.6 at 25°C (TEA Buffer)
(Prepare 100 ml in deionized water using Triethanolamine, Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.6 at 25°C with 1 M NaOH.)

D. 100 mM Magnesium Chloride Solution ($MgCl_2$)
(Prepare 10 ml in deionized water using Magnesium Chloride, 4.9 M Solution, Sigma Stock No. 104-20.)

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REAGENTS: (continued)

E. 16 mM Adenosine 5'-Triphosphate Solution (ATP)
(Prepare 10 ml in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.)

F. 12 mM ß-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized Form, Solution (ß-NADP)
(Prepare 10 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide Phosphate, Sodium Salt, Sigma Prod. No. N-0505.)

G. Hexokinase and Glucose-6-Phosphate Dehydrogenase Enzyme Solution (Hex/G-6-PDH)
(Immediately before use, prepare a solution containing 200 Hexokinase units/ml of Hexokinase and Glucose-6-Phosphate Dehydrogenase, Sigma Prod. No. H-8629 in cold Reagent C.)

H. Isomaltase Enzyme Solution (Isomaltase)
(Immediately before use, prepare a solution containing 0.25 - 0.50 unit/ml of Isomaltase in cold deionized water.)

PROCEDURE:
Step 1:

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

	Test	Blank
Reagent A (Phosphate Buffer)	0.40	0.40
Reagent B (Isomaltose)	0.50	0.50

Mix by swirling and equilibrate to 25°C. Then add:

Reagent H (Isomaltase)	0.10	-------
Deionized Water	-------	0.10

Immediately mix by swirling and incubate at 25°C for exactly 30 minutes. Then place in a boiling water bath for
3 minutes. Cool to room temperature. Then add:

Deionized Water	9.00	9.00

Mix by swirling.

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PROCEDURE: (continued)

Step 2:

Prepare a reaction cocktail by combining the following reagents (in milliliters):

Reagent C (TEA Buffer)	25.00	
Reagent D ($MgCl_2$)	1.00	
Reagent E (ATP)	1.00	
Reagent F (ß-NADP)	1.00	
Reagent G (Hex/G-6-PDH)		0.20

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

	Test	Blank
Reaction Cocktail	2.50	2.50

Equilibrate to 25°C. Then add:

Diluted Test from Step 1	0.50	------
Diluted Blank from Step 1	------	0.50

Mix by inversion and record the increase in A_{340nm} for approximately 5 - 10 minutes until constant using a suitably thermostatted spectrophotometer. Obtain the final ΔA_{340nm} for both the Test and Blank solutions.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}\ \text{Test} - \Delta A_{340nm}\ \text{Blank})(10)(3)(df)}{(2)(6.22)(0.1)(0.5)(30)}$$

10 = Total volume (in milliliters) of assay in Step 1
3 = Total volume (in milliliters) of assay in Step 2
df = Dilution factor
2 = Moles of glucose release per mole of isomaltose
6.22 = Millimolar extinction coefficient of ß-NADPH at 340 nm
0.1 = Volume (in milliliters) of enzyme used in Step 1
0.5 = Volume (in milliliters) of diluted test used in Step 2
30 = Reaction time (in minutes) of Step 1

CALCULATIONS: (continued)

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

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

UNIT DEFINITION:

One unit will convert 1.0 µmole of isomaltose to 2.0 µmoles of D-glucose per minute at pH 6.8 at 25°C (liberated glucose determined at pH 7.6).

FINAL ASSAY CONCENTRATION:

In a 1.00 ml reaction mix, the final concentrations are 20 mM potassium phosphate, 50 mM isomaltose and 0.025 - 0.050 unit isomaltase.

REFERENCE:

Bergmeyer, H.U. (1974) *Methods of Enzymatic Analysis*, Vol. I, 2nd ed., 459-460.

NOTES:

1. This assay is based on the cited reference.

2. Hexokinase Unit Definition: One unit will phosphorylate 1.0 µmole of D-glucose per minute at pH 7.6 at 25°C.

3. Glucose-6-Phosphate Dehydrogenase Unit Definition: One unit will oxidize 1.0 µmole of D-glucose 6-phosphate to 6-phospho-D-gluconate per minute in the presence of NADP at pH 7.4 at 25°C.

4. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.