ProductInformation

SUCROSE ASSAY KIT
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INTRODUCTION

Enzymes, as analytical tools, have found widespread use in the food, biochemical and pharmaceutical industry. Enzymatic methods are specific, reproducible, sensitive, rapid and therefore ideal for analytical purposes. Due to the high specificity and sensitivity of enzymes, quantitative assays may be done on crude materials with little or no sample preparation.

This kit is for the quantitative, enzymatic determination of sucrose in food and other materials.

PRINCIPLE

$$\text{Sucrose} \xrightarrow{\text{Invertase}} \text{Glucose} + \text{Fructose}$$

$$\text{Glucose} + \text{Fructose} + 2\,\text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-Phosphate} + \text{Fructose-6-Phosphate} + 2\,\text{ADP}$$

$$\text{Glucose-6-Phosphate} + \text{NAD} \xrightarrow{\text{Glucose-6-Phosphate Dehydrogenase}} \text{6-Phosphogluconate} + \text{NADH}$$

Sucrose is hydrolyzed to glucose and fructose by invertase. Glucose and fructose are phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase. Glucose-6-phosphate (G-6-P) is then oxidized to 6-phosphogluconate in the presence of nicotinamide adenine dinucleotide (NAD) in a reaction catalyzed by glucose-6-phosphate dehydrogenase. During this oxidation, an equimolar amount of NAD is reduced to NADH. The consequent increase in absorbance at 340 nm is directly proportional to sucrose concentration.

REAGENTS

Refer to Material Safety Data Sheets for updated risk, hazard or safety information.

1. **Sucrose Assay Reagent** (Sigma Product No. S 1299)
   Reconstitute reagent vial with 2 ml of deionized water. Stopper vial and immediately mix several times by inversion. DO NOT SHAKE.

   Each vial when reconstituted with 2 ml of deionized water contains approximately 150 U/ml Invertase (Baker's Yeast).

   The dry reagent is stored at 2-8 °C. Reconstituted reagent is stable, in the absence of visible microbial growth for 7 days at 18-26 °C and for 2 weeks at 2-8 °C. Reconstituted reagent may be aliquoted and stored at –20 °C for 6 months (do not re-freeze).

   Reagent should be discarded if the vial exhibits caking due to possible moisture penetration, if the vial contents do not dissolve completely upon reconstitution or if the solution appears turbid.

2. **Glucose (HK) Assay Reagent** (Sigma Product No. G 3161)
Reconstitute reagent vial with 50 ml of deionized water. Stopper vial and immediately mix several times by inversion. DO NOT SHAKE.

Each vial when reconstituted with 50 ml of deionized water contains 1.5 mM NAD, 1.0 mM ATP, 1.0 U/ml Hexokinase and 1.0 U/ml of Glucose-6-Phosphate Dehydrogenase, and Sodium Azide, 0.05%, as preservative.

The dry reagent is stored at 2-8 °C. Reconstituted reagent is stable, in the absence of visible microbial growth, for 7 days at 18-26 °C and for 4 weeks at 2-8 °C.

Reagent is not suitable for use if the absorbance of the freshly reconstituted solution measured at 340 nm vs water as reference is greater than 0.350. Reagent should be discarded if the vial exhibits caking due to possible moisture penetration, if the vial contents do not dissolve completely upon reconstitution or if the solution appears turbid.

3. **Sucrose Standard** (Sigma Product No. S 1174)
Used as a control to ensure assay reliability. Dry reagent is stable for at least 2 years when stored desiccated at room temperature.

APPARATUS

1. Spectrophotometer suitable for measuring absorbance at 340 nm.
2. Cuvets
3. Pipets capable of accurately dispensing 100 µl to 2 ml.

SAMPLE PREPARATION

Liquids Dilute sample with deionized water to approximately 100 - 1000 µg sucrose/ml.

Filter or deproteinize solution if necessary to clarify. Solutions that are strongly colored and that have a low sucrose concentration should be decolorized. Carbonated or fermented products must be degassed.

Solids Weigh out sample to nearest 0.1 mg. Extract sample with deionized water. The solution may be heated (up to about 60 °C) to aid extraction. Dilute with deionized water to approximately 100 - 1000 µg sucrose/ml. Filter or deproteinize solution if necessary to clarify.

Note: If the sample contains a large amount of glucose (ratio of glucose to sucrose is greater than 5 to 1), the glucose must be removed before assaying for sucrose[1].

In a 10 ml volumetric flask, mix:
2.0 ml 0.3 M Triethanolamine - 3 mM $MgSO_4$, pH 7.5
5.0 ml Sample (100 to 1000 µg sucrose/ml)
0.1 ml Glucose Oxidase/Catalase solution
(70 units Glucose Oxidase, Sigma Product Number G7016 and 15000 units Catalase, Sigma Product Number C 9631)

Bubble air through the solution for 2 hours. Check pH periodically during this time and neutralize the solution using dilute NaOH if necessary.

Incubate solution in a boiling water bath for 15 minutes to inactivate enzymes.

Cool solution, dilute to the 10 ml mark with deionized water and mix. Centrifuge solution to clarify, if necessary. Allow for a dilution factor of 2 in the calculations.

DETERMINATION

Dilute sample solution to an approximate sucrose concentration of 100 - 1000 µg/ml. Repeat assay and vary the sample volume if necessary to give a ΔA_{340} between 0.03 and 1.6.

1. Pipet the following solutions into the appropriately marked test tubes.

Tube	Sucrose Assay Reagent (ml)	Sample Volume (ml)	Deionized Water (ml)
Sucrose Assay Reagent Blank	0.1	---	0.1
Sample Blank	---	0.1	0.1
Glucose Assay Reagent Blank	---	---	0.2
Test	0.1	0.1	---

2. Mix tubes and incubate for 10 minutes at room temperature (18-35 °C).

3. Add 2.0 ml of Glucose Assay Reagent to each tube.

4. Mix tubes and incubate for 15 minutes at room temperature (18-35 °C).

5. Measure the absorbance at 340 nm.

CALCULATIONS

$A_{\text{TOTAL BLANK}} = (A_{\text{SAMPLE BLANK}} - A_{\text{GLUCOSE ASSAY REAGENT BLANK}}) + A_{\text{SUCROSE ASSAY REAGENT BLANK}}$

$\Delta A = A_{TEST} - A_{TOTAL\ BLANK}$

$$\text{MG SUCROSE} = \frac{(\Delta A)(TV)(\text{Molecular Weight of Sucrose})(F)}{(\varepsilon)\ (d)\ (SV)\ (\text{Conversion Factor for µg to mg})}$$

$$= \frac{(\Delta A)(2.2)(342.3)(F)}{(6.22)(1)(0.1)(1000)}$$

$$= (\Delta A)(F)(1.21)$$

A = Absorbance at 340 nm

d = Light path (cm)

SV = Sample Volume

ε = Millimolar Extinction Coefficient for NADH at 340 nm

TV = Total Assay Volume

F = Dilution Factor from sample preparation

REFERENCES

1. Bergmeyer, H. U. and Bernt, E., Methods Enzym. Anal., Editor: Bergmeyer, Hans Ulrich. Publisher: Academic Press, New York. (2nd Ed.) 1177 -1179 (1974).

2. Southgate, D.A.T., Determination of Food Carbohydrates, Applied Science Publishers, London (1976).