

Product Information

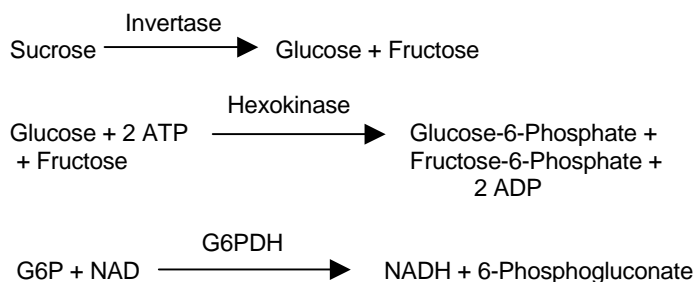
Sucrose Assay Kit

Product Code **SCA-20**

TECHNICAL BULLETIN

Product Description

Enzymes, as analytical tools, have found widespread use in the food, biochemical, and pharmaceutical industries. 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.



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 (G6P) is then oxidized to 6-phosphogluconate in the presence of nicotinamide adenine dinucleotide (NAD) in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH). 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

1. Sucrose Assay Reagent (Product Code S 1299)
Reconstitute the vial with 2 ml of water. After addition of water, stopper the vial and immediately mix several times by inversion. DO NOT SHAKE.

Each vial when reconstituted with 2 ml of water contains approximately 150 units/ml of invertase from baker's yeast.

The dry reagent is stored at 2-8 °C. The 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 reconstituted solution appears turbid.

The 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. The reconstituted reagent may be aliquoted and stored at -20 °C for 6 months.

2. Glucose (HK) Assay Reagent (Product Code G 3293)
Reconstitute the vial with 50 ml of water. After addition of water, stopper the vial and immediately mix several times by inversion. DO NOT SHAKE.

Each vial when reconstituted with 50 ml of water contains 1.5 mM NAD, 1.0 mM ATP, 1.0 unit/ml of hexokinase, and 1.0 unit/ml of glucose-6-phosphate dehydrogenase with sodium benzoate and potassium sorbate as preservatives.

The dry reagent is stored at 2-8 °C. The 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 reconstituted solution appears turbid.

The 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. The reagent is not suitable for use if the absorbance of the freshly reconstituted solution measured at 340 nm versus water as the reference is greater than 0.350.

3. Sucrose Standard (Product Code S 1174) This standard is used as a control to ensure assay reliability. The dry reagent is stable for at least 2 years when stored desiccated at room temperature. Moisture content will vary depending on storage conditions.

Equipment Required but Not Provided

- Spectrophotometer suitable for measuring absorbance at 340 nm
- Cuvets
- Pipettes capable of accurately dispensing 100 μ l to 2 ml

Precautions and Disclaimer

Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions**Sample Preparation**

Liquids: Dilute sample with water to approximately 100-1000 μ g sucrose/ml.

Filter or deproteinize the solution if necessary to clarify. Liquid samples 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 water. The solution may be heated to approximately 60 $^{\circ}$ C to aid extraction. Dilute with water to approximately 100-1000 μ g sucrose/ml. Filter or deproteinize solution if necessary to clarify.

For samples containing a large amount of glucose (ratio of glucose to sucrose is greater than 5:1), the glucose must be removed before assaying for sucrose. To remove glucose from the sample, mix the following in a 10 ml volumetric flask:

- 2.0 ml of 0.3 M triethanolamine with 3 mM MgSO_4 , pH 7.5
- 5.0 ml of sample (100-1000 μ g sucrose/ml)
- 0.1 ml of glucose oxidase/catalase solution (70 units of glucose oxidase, Product Code G 7016 and 15,000 units of catalase, Product Code C 9631)

Bubble air through the solution for 2 hours. Check the pH periodically during this time and neutralize the solution using a dilute NaOH solution, if necessary. Stop the reaction by incubation in a boiling water bath for 15 minutes to inactivate the enzymes. Cool the solution, dilute to 10 ml with water, and mix. Centrifuge solution to clarify, if necessary. Allow for a dilution factor of 2 in the calculations.

Procedure

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

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

Tube	Sucrose Assay Reagent (ml)	Sample Volume (ml)	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 and incubate for 10 minutes at room temperature (18-35 $^{\circ}$ C).
3. Add 2.0 ml of the Glucose Assay Reagent to each tube.
4. Mix and incubate for 15 minutes at room temperature (18-35 $^{\circ}$ C).
5. Measure the absorbance at 340 nm.

Calculations

Total Blank - The total blank takes into account the contribution to the absorbance of the sample, the Glucose Assay Reagent, and the Sucrose Assay Reagent. The absorbance of the Glucose Assay Reagent is subtracted from the sample blank, so that the absorbance of the Glucose Assay Reagent is only counted once in the total absorbance, since it is in both the sample blank and the Sucrose Assay Reagent blank.

$$A_{\text{Total Blank}} = (A_{\text{Sample Blank}} - A_{\text{Glucose Assay Reagent Blank}}) + A_{\text{Sucrose Assay Reagent Blank}}$$

$$\Delta A = A_{\text{Test}} - A_{\text{Total Blank}}$$

Sucrose concentration (mg/ml):

$$\text{mg/ml of sucrose} = \frac{(\Delta A)(TV)(\text{Molecular Weight of Sucrose})(F)}{(\epsilon)(d)(SV)(\text{Conversion Factor for } \mu\text{g to mg})}$$

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

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

A = Absorbance at 340 nm

ϵ = Millimolar Extinction Coefficient for NADH at 340 nm
millimolar⁻¹ cm⁻¹ or (ml/ μ moles)(1/cm)

d = Light path (cm)

TV = Total Assay Volume

SV = Sample Volume

F = Dilution Factor from sample preparation.

References

1. Bergmeyer, H. U., and Bernt, E., Methods of Enzymatic Analysis, Bergmeyer, H.U., eds., Academic Press (New York, NY: 1974), 2nd edition, 1177 -1179.
2. Southgate, D.A.T., Determination of Food Carbohydrates, Applied Science Publishers, London (1976).

CMH/MAM 8/03

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