EnzyChrom™ Sucrose Assay Kit (ESUC-100)

Quantitative Colorimetric Sucrose Determination at 565nm

DESCRIPTION

SUCROSE ($C_{12}H_{22}O_{11}$) is a disaccharide of glucose and fructose with an α-1,2-glycosidic linkage. It is the most common food sweetener and the most important sugar in plants. In mammals, sucrose is readily digested in the stomach into glucose and fructose, which are rapidly absorbed into the bloodstream in the small intestine. Simple, direct and high-throughput assays for measuring sucrose concentrations find wide applications. BioAssay Systems' improved assay uses invertase to digest sucrose into fructose and glucose. The resulting fructose is then quantified using our fructose assay reagent. The measured color intensity at 565nm is directly proportional to the sucrose concentration in the sample.

KEY FEATURES

No interference by glucose.

Use 20 µL samples. Linear detection range: 17 to 2000 µM sucrose.

APPLICATIONS

Assays: sucrose in biological samples (e.g. serum, plasma, urine, saliva, milk, culture medium), food, juice, beverage and other agricultural products.

Drug Discovery/Pharmacology: effects of drugs on sucrose metabolism.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Invertase:**	120 µL
PMS Solution:	1.5 mL	**Enzyme:**	Dried
MTT Solution:	1.5 mL	**Enzyme Buffer:**	150 µL
Standard:	400 µL 40 mM Sucrose		

Storage conditions. The kit is shipped on ice. Store all components at -20°C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

ASSAY PROCEDURE

Note: (1) The following substances interfere and should be avoided in sample preparation: ascorbic acid, SDS (>0.2%), sodium azide, NP-40 (>1%) and Tween-20 (>1%). (2) This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to standard and samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Reagent Preparation: Reconstitute Enzyme by adding 120 µL Enzyme Buffer to the Enzyme tube. Make sure Enzyme is fully dissolved by pipetting up and down. Store reconstituted Enzyme at -20°C and use within 2 months.

Sample treatment: liquid samples such as serum, plasma and fruit juices can be assayed directly. Milk samples should be cleared by mixing 600 µL milk with 100 µL 6 N HCl. Centrifuge 5 min at 14,000 rpm. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL 6 N NaOH. The neutralized supernatant is ready for assay (dilution factor n = 1.36).

1. Equilibrate all components to room temperature. Briefly centrifuge the tubes before opening. Keep thawed tubes on ice during assay.
2. *Standards*: mix 12 µL 40 mM Standard with 228 µL dH_2O (final 2000 µM). Dilute standard in dH_2O as follows.

No	2000 µM STD + H_2O	Vol (µL)	Sucrose (µM)
1	100 µL + 0 µL	100	2000
2	60 µL + 40 µL	100	1200
3	30 µL + 70 µL	100	600
4	0 µL +100 µL	100	0

Transfer 20 µL diluted standards into separate wells of a clear flat-bottom 96-well plate.

Samples: transfer 20 µL of each sample into separate wells of the plate.

Note: if a sample is known to contain fructose, prepare an extra sample blank well with 20 µL of the sample.

3. Color r*eaction*. Prepare enough Working Reagent by mixing, for each reaction well, 56 µL Assay Buffer, 1 µL Invertase, 1 µL Enzyme, 14 µL PMS Solution and 14 µL MTT Solution. Add 80 µL Working Reagent to each well.

Note: for the sample that contains fructose, prepare a blank control reagent with no Invertase (i.e., 56 µL Assay Buffer, 1 µL Enzyme, 14 µL PMS Solution and 14 µL MTT Solution). Add 80 µL of the control Reagent to each Sample Blank well.

Immediately tap plate to mix. Incubate 60 min in the dark at room temperature.

4. Read optical density at 565nm (520-600nm).

Note: If the calculated sucrose concentration of a sample is higher than 2000 µM, dilute sample in water and repeat the assay. Multiply result by the dilution factor n.

CALCULATION

Subtract blank value (water, #4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the sucrose concentration of Sample,

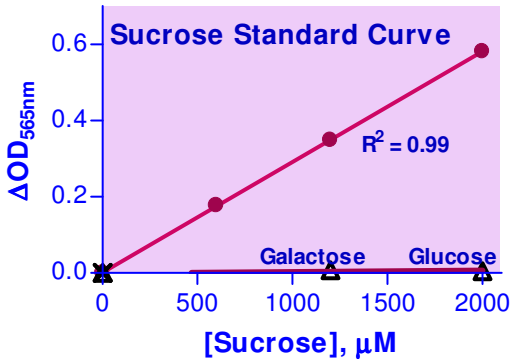
$$[\text{Sucrose}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

OD_{SAMPLE}, OD_{BLANK} are optical density values of the sample and H_2O Blank (or Sample Blank if sample contains fructose), respectively. n is the sample dilution factor.

Conversions: 1 mM sucrose equals 34.2 mg/dL, 0.034% or 342 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom uncoated 96-well plates, optical density plate reader.



PUBLICATIONS

1. Zhu, M. et al. (2020). Identification and gene mapping of the starch accumulation and premature leaf senescence mutant ossac4 in rice. Journal of Integrative Agriculture, 19(9): 2150-2164.
2. Chen, X et al. (2018). Identification and gene fine mapping of starch accumulation and early senescent leaf mutant esl10 in rice. Crop Science, 58(1), 204-217.
3. Huang, J. et al. (2018). Gene mapping of starch accumulation and premature leaf senescence in the ossac3 mutant of rice. Euphytica, 214(10), 177.