

## EnzyChrom™ Sucrose Assay Kit (ESUC-100)

### Quantitative Colorimetric Sucrose Determination at 565nm

#### DESCRIPTION

**SUCROSE** (C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>) 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)

<b>Assay Buffer:</b> 10 mL	<b>Invertase:</b> 120 μL
<b>PMS Solution:</b> 1.5 mL	<b>Enzyme:</b> Dried
<b>MTT Solution:</b> 1.5 mL	<b>Enzyme Buffer:</b> 150 μL
<b>Standard:</b> 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<sub>2</sub>O (final 2000 μM). Dilute standard in dH<sub>2</sub>O as follows.

No	2000 μM STD + H <sub>2</sub> O	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 reaction.** 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,

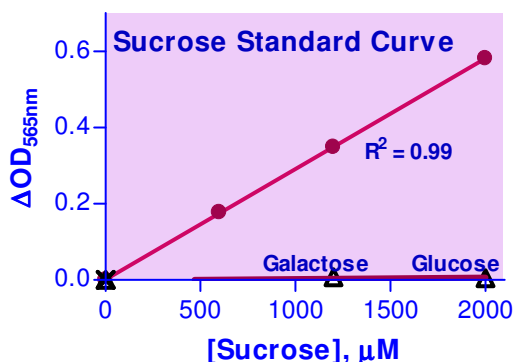
$$[\text{Sucrose}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M})$$

OD<sub>SAMPLE</sub>, OD<sub>BLANK</sub> are optical density values of the sample and H<sub>2</sub>O 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.



#### LITERATURE

1. Harrison, C.S. and Faler, K.T. (1957). Analysis of the chemical sucrose determination of extracellular fluid volume using C<sup>14</sup>-labeled sucrose. *Am. J. Physiol.* 188:568-570.
2. Holmes, E.W. (1997). Coupled enzymatic assay for the determination of sucrose. *Anal. Biochem.* 244: 103-109.
3. Young, M.K. and Prudden, J.F. (1954). Simultaneous determination of sucrose and inulin in biologic fluids. *J. Lab. Clin. Med.* 44:160-165.