Sucrose Microplate Assay Kit

User Manual

Catalog # CAK1036

(Version 2.2D)

Detection and Quantification of Sucrose Content in Tissue extracts, Cell lysate Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

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.

Sucrose Microplate Assay Kit provides a convenient means to measure sucrose concentration in biological samples. In the assay, sucrase cleaves sucrose, resulting in the formation of fructose and glucose. The enzyme catalysed reaction products can be measured at a colorimetric readout at 540 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Enzyme	Powder x 1	-20 °C
Reaction Buffer	12 ml x 1	4 °C
Dye Reagent	20 ml x 1	4 °C
Standard	Powder x 1	4 °C
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Note:

Enzyme: add 1 ml reaction buffer to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use, the concentration will be 10 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 540 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, put it in water bath of 80 °C for 10 minutes, centrifuged at 4,000g at room temperature for 10 minutes, take the supernatant into a new centrifuge tube.

2. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank
Sample	40 µl	40 µl	--	--
Standard	--	--	40 µl	--
Distilled water	--	--	--	40 µl
Reaction Buffer	50 µl	60 µl	50 µl	50 µl
Enzyme	10 µl	--	10 µl	10 µl
Mix, put it in the oven, 37 °C for 20 minutes.				
Dye Reagent	100 µl	100 µl	100 µl	100 µl
Mix, put it into the convection oven, 90 °C for 10 minutes, record absorbance measured at 540nm.				

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

$$\text{Fructose (μmol/ml)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 10 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

2. According to the weight of sample

$$\text{Fructose (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 10 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the concentration of Standard, 10 mmol/L = 10 μmol/ml;

W: the weight of sample, g;

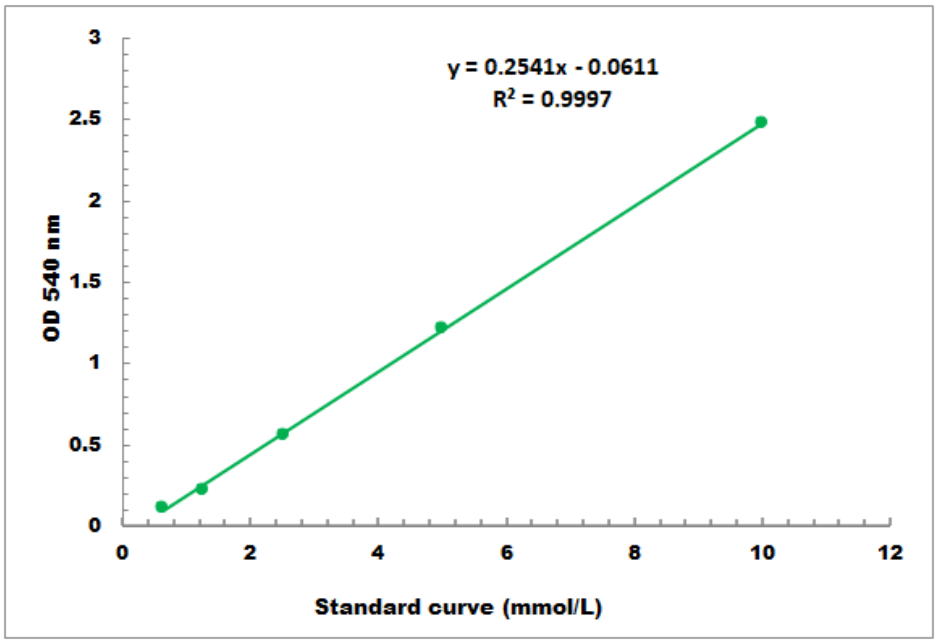
$V_{Standard}$: the volume of standard, 0.04 ml;

V_{Sample}: the volume of sample, 0.04 ml;

V_{Assay}: the volume of Assay buffer, 1 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.5 mmol/L - 10 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES