Sucrose Microplate Assay Kit

User Manual

Catalog # ASK1036

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

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

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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 is a disaccharide which can be converted into one glucose and one fructose. Fructose can react with resorcinol to generate colored substance, have characteristic absorption peak at 480nm.

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II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer I	1 ml x 1	4 °C
Reaction Buffer II	10 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

Dye Reagent: add 5 ml distilled water to dissolve before use.

Standard: add 2 ml distilled water to dissolve before use, the concentration will be 1 mg/ml.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 480 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

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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.

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V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Standard	Blank
Sample	40 µl	--	--
Standard	--	40 µl	--
Distilled water	--	--	40 µl
Reaction Buffer I	10 µl	10 µl	10 µl
Mix, put them into the boiling water for 10 minutes, then put them on ice.			
Reaction Buffer II	100 µl	100 µl	100µl
Dye Reagent	50 µl	50 µl	50 µl
Mix, them into the boiling water for 5 minutes. Centrifuge and transfer all reagents to the microplate, record absorbance measured at 480 nm.			

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VI. CALCULATION

1. According to the protein concentration of sample

$$\text{Fructose (mg/mg)} = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the standard concentration, 1 mg/ml;

$C_{Protein}$: the protein concentration, mg/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.

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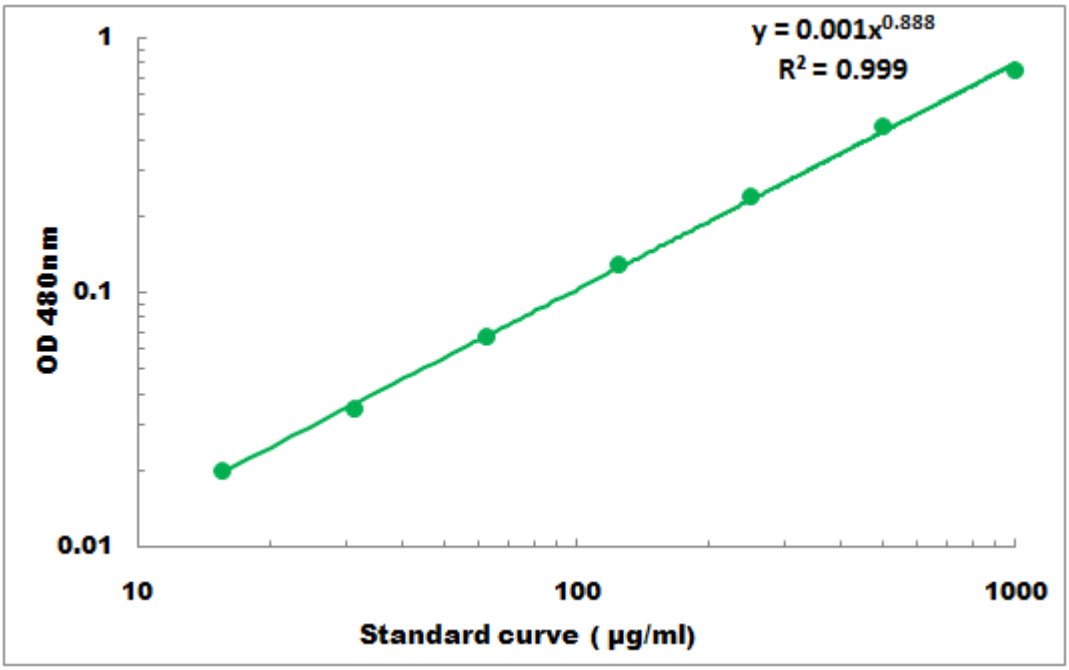
VII. TYPICAL DATA

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



Detection Range: 10 μg/ml - 1000 μg/ml

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