



Sucrose Synthase Microplate Assay Kit

Catalog # AS0038

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

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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I. INTRODUCTION

Sucrose synthase (SUS; EC 2.4.1.13) plays important roles in sugar metabolism and abiotic stress response. SUS catalyze the synthesis of free fructose and glucose in plants.

SUS catalyze the UDPG reaction of free fructose and glucose to generate sucrose, and then react with resorcinol present a color change,have acharacteristic absorption peak at 480nm.The intensity of the product color, measured at 480 nm, is proportionate to the enzyme activity in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Substrate	Powder x 1	4 °C
Substrate Diluent	3 mlx 1	4 °C
Reaction Buffer	10 mlx 1	4 °C
Stop Solution	1 mlx 1	4 °C
Dye Reagent	Powderx 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 1 ml distilled water to dissolve before use, the concentration will be 4 mg/ml.

Substrate: add 3 ml Substrate Diluent to dissolve before use.

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
8. Ice

IV. SAMPLE PREPARATION

1. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For liquid samples

Detect it directly, or dilute with Assay Buffer.

V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Standard	Blank
Sample	10 μ l	--	--
Standard	--	10 μ l	--
Distilled water	--	--	10 μ l
Substrate	30 μ l	30 μ l	30 μ l
Mix, put it in the oven, 30°C for 10 minutes.			
Stop Solution	10 μ l	10 μ l	10 μ l
Mix, put them into the boiling water for 10 minutes, then put them on ice.			
Reaction Buffer	100 μ l	100 μ l	100 μ l
Dye Reagent	50 μ l	50 μ l	50 μ l
Mix, put them into the boiling water for 5 minutes. Centrifuge and transfer all reagents to the microplate, record absorbance measured at 480 nm.			

VI. CALCULATION

Unit Definition: One unit of SUS activity is defined as the enzyme generates 1 µg of sucrose per minute.

1. According to the protein concentration of sample

$$\text{SUS (U/mg)} = \frac{C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times (V_{\text{Sample}} \times C_{\text{Protein}})} \times \frac{1}{T}$$
$$= 200 \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})} \times \frac{1}{C_{\text{Protein}}}$$

2. According to the weight of sample

$$\text{SUS (U/g)} = \frac{C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times (V_{\text{Sample}} \times W)} \times \frac{V_{\text{Assay}}}{T}$$
$$= 200 \times \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})} \times \frac{1}{W}$$

C_{Standard} : the standard concentration, 4mg/ml = 4000 µg/ml;

C_{Protein} : the protein concentration, mg/ml;

W : the weight of sample, g;

V_{Standard} : the volume of standard, 0.01 ml;

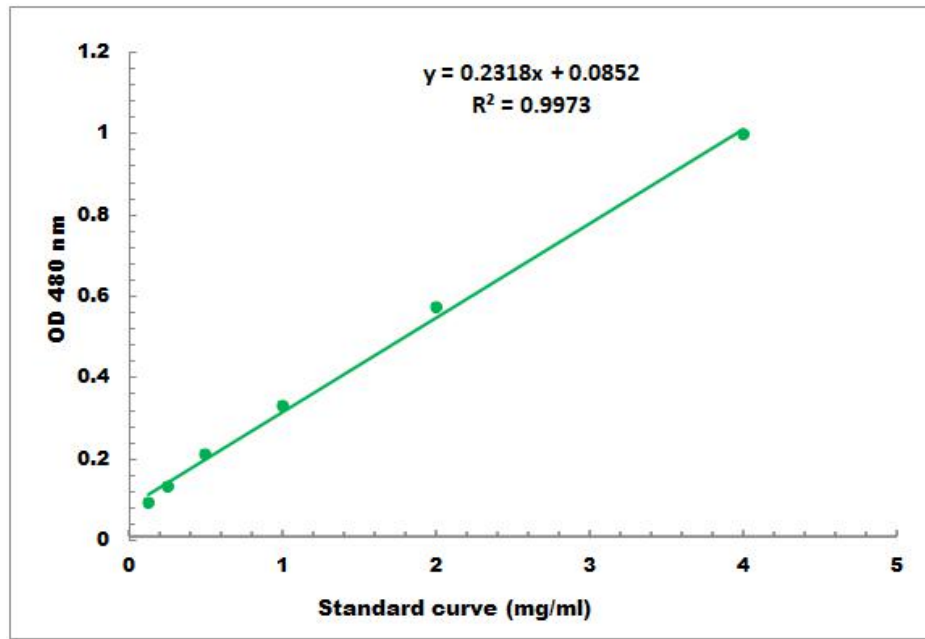
V_{Sample} : the volume of sample, 0.01 ml;

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

T : the reaction time, 10 minutes.

VII. TYPICAL DATA

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



Detection Range: 100 μ g/ml -4000 μ g/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES