Sucrose Phosphate Synthase Microplate Assay Kit

Catalog # AS0039

Detection and Quantification of Sucrose Phosphate 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 phosphate synthase (SPS, EC 2.4.1.14) is the key enzyme of carbon flux into sucrose fixation in plants. It catalyzes the synthesis of sucrose-phosphate from UDP-glucose and fructose-6-phosphate predominantly in the cytosol of sucrose-source leaf tissue.

Fructose-6-phosphateiscatalyzed by sucrose phosphate synthase togeneratesucrose phosphate,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 ml x 4	4 °C
Substrate	Powder x 1	4 °C
Substrate Diluent	3 mlx 1	4 °C
Reaction Buffer	10 ml x 1	4 °C
Stop Solution	1 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 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

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 8000g 4°C for 10minutes, 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 intothe microcentrifuge tubes:

Reagent	Sample	Standard	Blank
Sample	10 µl	--	--
Standard	--	10 µl	--
Distilled water	--	--	10 µl
Substrate	30 µl	30 µl	30 µl
Mix, put it in the oven, 30 °C for 10 minutes.			
Stop Solution	10 µl	10 µl	10 µl
Mix, put them into the boiling water for 10 minutes, then put them on ice.			
Reaction Buffer	100 µl	100 µl	100 µl
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.			

VI. CALCULATION

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

1. According to the protein concentration of sample

$$SPS\ (U/mg) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 200 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$SPS\ (U/g) = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 200 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

$C_{Standard}$: the standard concentration, 4 mg/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;

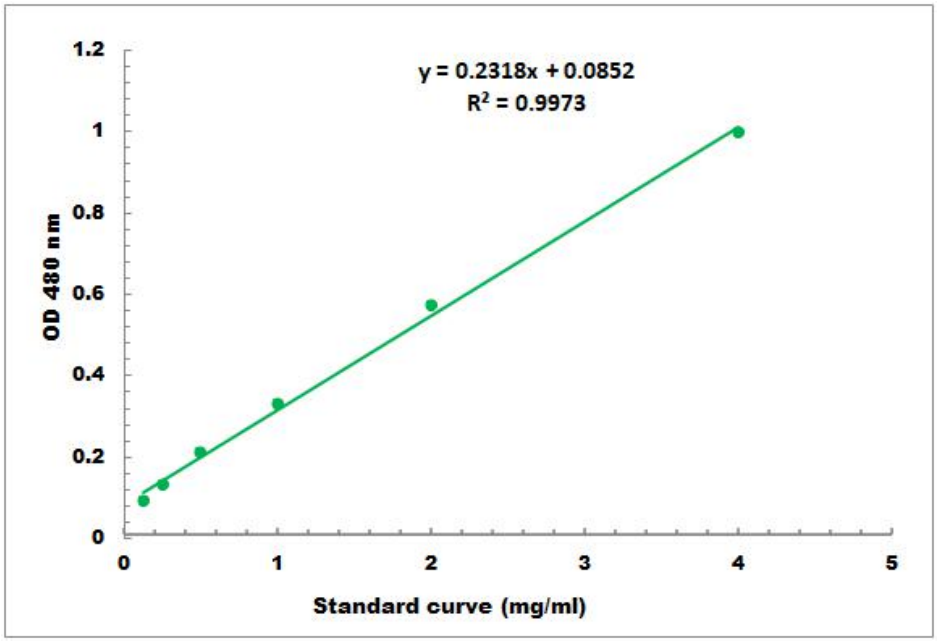
V_{Sample}: the volume of sample, 0.01 ml;

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 μg/ml - 4000 μg/ml

VIII. TECHNICAL SUPPORT

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

VIII. NOTES