Sucrose Phosphate Synthase

Microplate Assay Kit

User Manual

Catalog # CAK1039

(Version 1.1C)

Detection and Quantification of Sucrose Phosphate Synthase (SPS) Activity in Tissue extracts, Cell lysate Samples.

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

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-phosphate is catalyzed by sucrose phosphate synthase to generate sucrose 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 ml x 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
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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, multi-channel 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 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.			

Note:

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

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

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

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;

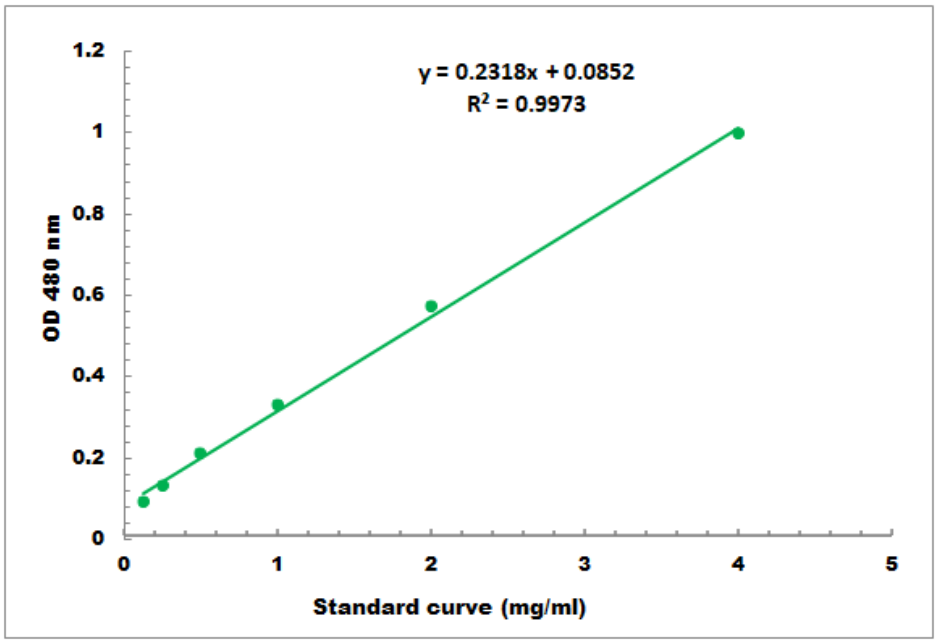
V_{Sample}: the volume of sample, 0.01 ml;

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.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES