



Sucrose Phosphate Synthase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1039

(Version 1.2C)

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.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	4
IV. REAGENT PREPARATION.....	5
V. SAMPLE PREPARATION.....	6
VI. ASSAY PROCEDURE.....	7
VII. CALCULATION.....	8
VIII.TYPICAL DATA.....	9

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 a characteristic 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
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 4 mg/ml of standard top solution. Store at 4 °C for 1 month or -20°C for 6-7 months after reconstitution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 4/2/1/0.5/0.25/0.125/0.0625 mg/ml.

Dye Reagent: Add 5 ml distilled water to dissolve before use. Keep in dark for immediate use or store at -20°C for 1-2 weeks after reconstitution.

Substrate: Add 3 ml Substrate Diluent to dissolve before use. Keep in dark for immediate use.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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.

VI. 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, them into the boiling water for 5 minutes. Centrifuge and transfer all reagents to the microplate, record absorbance measured at 480 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

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

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) - \text{Intercept}}{\text{Slope} \times T} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

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

2.3 According to the weight of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein}: the protein concentration of sample, mg/mL

W: the weight of total sample, g

C_{Standard}: the concentration of standard, µg/mL

V_{Standard}: the volume of standard in assay procedure, mL

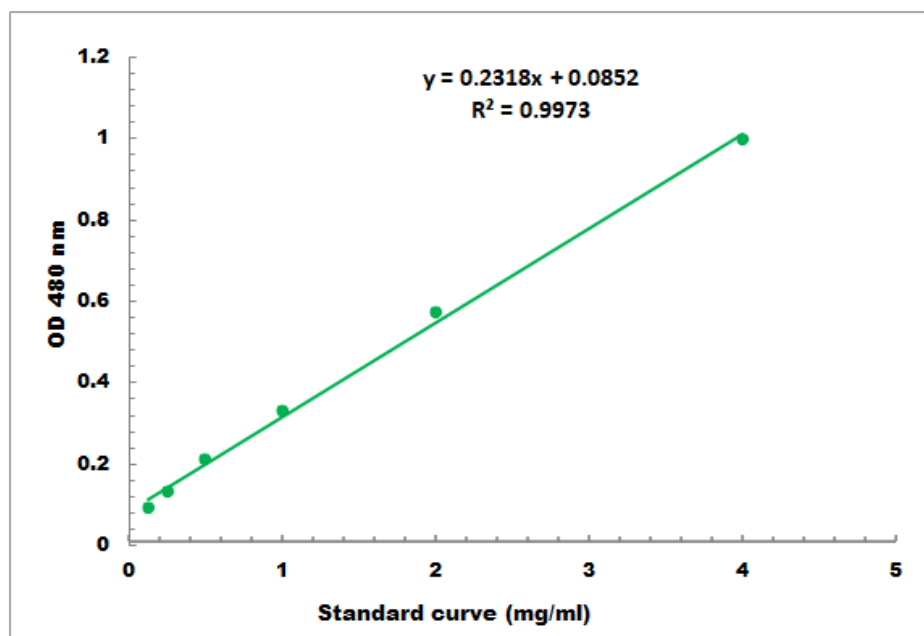
V_{Sample}: the volume of sample in assay procedure, mL

V_{Assay}: the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. 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