



Sucrose Phosphorylase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1140

(Version 1.5C)

Detection and Quantification of Sucrose

Phosphorylase Activity in Tissue extracts, Cell lysate, Cell culture
media and Other biological fluids Samples.

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

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I. INTRODUCTION

Sucrose phosphorylase (EC 2.4.1.7) is an important enzyme in the metabolism of sucrose and regulation of other metabolic intermediates. Sucrose phosphorylase is in the class of hexosyltransferases. More specifically it has been placed in the retaining glycoside hydrolases family although it catalyzes a transglycosidation rather than hydrolysis. Sucrose phosphorylase catalyzes the conversion of sucrose to D-fructose and α -D-glucose-1-phosphate. It has been shown in multiple experiments that the enzyme catalyzes this conversion by a double displacement mechanism.

Sucrose Phosphorylase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining sucrose phosphorylase activity in various samples. Sucrose phosphorylase activity is determined by NADPH formation rate. The reaction products can be measured at a colorimetric readout at 450 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Diluent	20 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Enzyme	Powder x 1	-20 °C
Dye Reagent A	Powder x 1	4 °C
Dye Reagent B	1 ml x 1	4 °C
Standard	Powder x 1	-20 °C
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 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 2 mmol/L of standard stock solution, store at 4 °C for 1 day or -20°C for 3 days after reconstitution. Then dilute to 400 µmol/L standard top solution by adding 0.2 ml stock solution into 0.8 ml distilled water. 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 400/200/100/50/25/12.5/6.25 µmol/L.

Substrate: Add 12 ml Diluent to dissolve before use. Keep in dark for immediate use or store at -20°C for 2-3 weeks after reconstitution.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Diluent to dissolve before use. Store at -80°C for 1 month after reconstitution.

Dye Reagent A: Briefly centrifuge prior to opening. Add 5 ml distilled water to dissolve before use, mix, store at 4°C for 1 month after reconstitution.

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

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4°C for 15 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Add 0.9 ml Assay Buffer into 0.1 ml liquid sample, centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Control	Standard	Blank
Substrate	120 μ l	120 μ l	--	--
Enzyme	10 μ l	10 μ l	--	--
Sample	10 μ l	--	--	--
Distilled water	--	10 μ l	40 μ l	140 μ l
Mix and incubate at 30°C for 5 minutes.				
Standard	--	--	100 μ l	--
Dye Reagent A	50 μ l	50 μ l	50 μ l	50 μ l
Dye Reagent B	10 μ l	10 μ l	10 μ l	10 μ l
Mix and incubate at room temperature for 2 minutes, record absorbance measured at 450 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 Sucrose Phosphorylase activity is defined as the enzyme produces 1 μmol of NADPH per minute.

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

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

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times V / (V + V_{\text{Assay}}) \times T} (\text{U/mL})$$

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

N: the quantity of total cell or bacteria sample, 10^4

C_{Standard} : the concentration of standard, $\mu\text{mol/mL}$

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

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

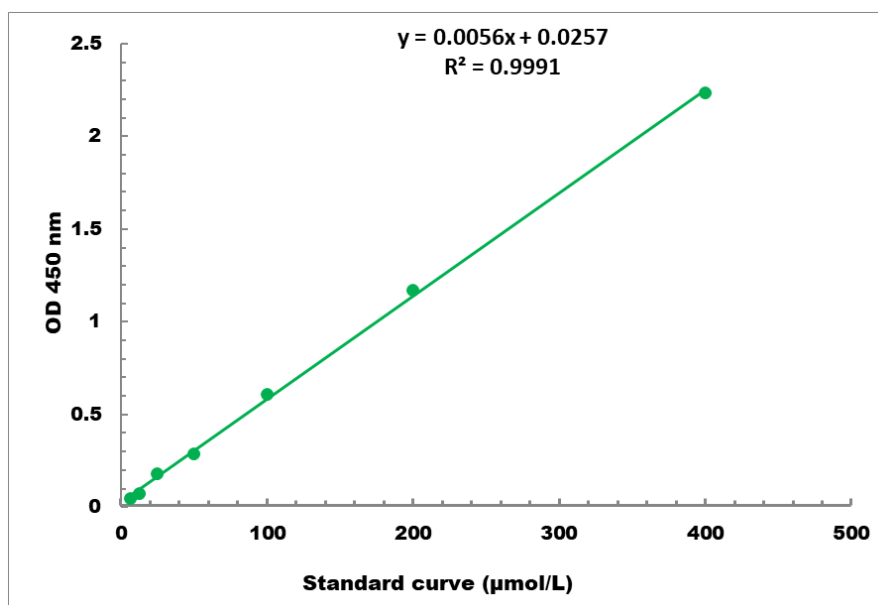
V_{Assay} : the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

V: the volume of sample in sample preparation, ml

VIII. TYPICAL DATA

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



Detection Range: 4μmol/L - 400 μmol/L