Sucrose Phosphorylase

Microplate Assay Kit

Cat #: orb707336 (manual)

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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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 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 340 nm.

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
Standard	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Substrate: add 18 ml Diluent to dissolve before use.

Enzyme: add 1 ml Diluent to dissolve before use.

Standard: add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

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.

ASSAY PROCEDURE

Warm all regents to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 µl	--
Distilled water	--	--	200 µl
Substrate	180 µl	--	--
Enzyme	10 µl	--	--
Sample	10 µl	--	--
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.			

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.

CALCULATION

Unit Definition: One unit of Sucrose Phosphorylase activity is defined as the enzyme produces 1 nmol of NADPH per minute.

1. According to the protein concentration of sample

$$SP\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 4000 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$SP\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$SP\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$SP\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 4000 \times (OD_{Sample\ (130S)} - OD_{Sample\ (10S)}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 400 nmol/ml;

$V_{Standard}$: the volume of standard, 200 μl = 0.2 ml;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

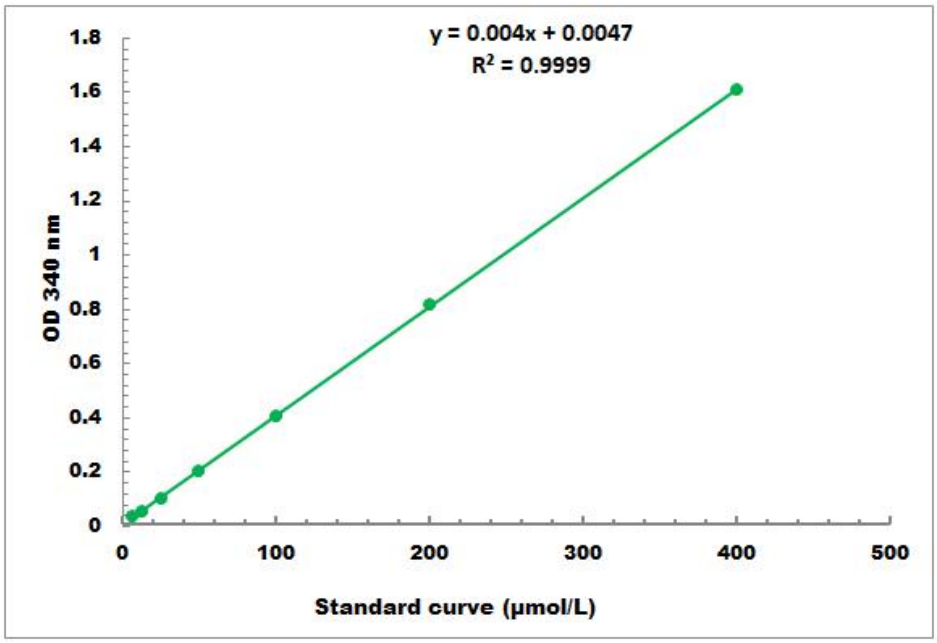
V_{Sample}: the volume of sample, 0.01 ml;

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

T: the reaction time, 2 minutes.

TYPICAL DATA

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



Detection Range: 4 µmol/L - 400 µmol/L

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