



Sucrose Phosphate Synthase Assay Kit

Sucrose Phosphate Synthase Assay Kit is a detection kit for the quantification of Sucrose Phosphate Synthase Activity in tissue extracts and cell lysate.

Catalog number: ARG82023

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Sucrose-phosphate synthase (EC 2.4.1.14) is a plant enzyme involved in sucrose biosynthesis. Specifically, this enzyme catalyzes the transfer of a hexosyl group from uridine diphosphate glucose (UDP-glucose) to D-fructose 6-phosphate to form UDP and D-sucrose-6-phosphate. This reversible step acts as the key regulatory control point in sucrose biosynthesis, and is an excellent example of various key enzyme regulation strategies such as allosteric control and reversible phosphorylation.

This enzyme participates in starch and sucrose metabolism. [Provide by Wikipedia: Sucrose phosphate synthase]

PRINCIPLE OF THE ASSAY

This Sucrose Phosphate Synthase Assay Kit is a simple colorimetric assay that measures the amount of Sucrose phosphate synthase activity in tissue extracts and cell lysate. The assay is based on the enzyme driven reaction. 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 O.D. 480 nm. The concentration of sucrose phosphate synthase in the samples is then determined by comparing the O.D. 480 nm absorbance of samples to the standard curve.

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MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well Microplate	1 plate	RT
Assay Buffer	4 x 30 mL (ready to use)	4°C
Substrate (lyophilized)	1 vial	4°C
Substrate Diluent	3 mL (ready to use)	4°C
Reaction Buffer	10 mL	4°C
Stop Solution	1 mL	4°C
Dye Reagent (lyophilized)	1 vial	4°C
Standards (lyophilized)	1 vial	4°C
Technical Manual	1 ea	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 480 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

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SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue samples: Weigh out 0.1 g tissue, homogenize with 1 mL of Assay Buffer on ice, centrifuged at 8,000 x g for 10 minutes at 4°C. Take the supernatant into a new centrifuge tube and keep it on ice for detection.

Liquid samples: detect it directly, or dilute with Assay Buffer.

REAGENT PREPARATION

- **Substrate:** Add 3 mL of Substrate Diluent to dissolve before use.
- **Dye Reagent:** Add 5 mL of distilled water to dissolve before use.
- **Standards:** Add 1 mL of distilled water to dissolve before use. The concentration will be 4 mg/mL. Use the 4 mg/mL Standards to prepare a series of diluted standards according to the Table below.

Standard tube	Final Standard conc. (mg/mL)	distilled water (µL)	Volume of 4 mg/mL Standards (µL)
S1	4	0	500
S2	2	250	250 of S1
S3	1	250	250 of S2
S4	0.5	250	250 of S3
S5	0.25	250	250 of S4
S6	0.125	250	250 of S5

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ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Add following reagents into the microcentrifuge tubes:			
Reagent	Sample	Standards	Blank
Sample	10 μ L		
Standards		10 μ L	
Distilled water			10 μ L
Substrate	30 μ L	30 μ L	30 μ L
Mix well and incubate for 10 minutes at 30°C			
Stop Solution	10 μ L	10 μ L	10 μ L
Mix well and incubate for 10 minutes in the boiling water bath , then put them in ice.			
Reaction Buffer	100 μ L	100 μ L	100 μ L
Dye Reagent	50 μ L	50 μ L	50 μ L
Mix well, and incubate for 5 minutes in the boiling water bath . Centrifuge and transfer all reagents to the 96 Well Microplate . Read the absorbance at O.D. 480 nm .			

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Control, Blank and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Unit Definition: One unit of SPS activity is defined as the enzyme generates 1 µg of sucrose per minute.

5. According to the weight of sample:

SPS (U/g)

$$= \left\{ \left[(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \right] / (V_{\text{Sample}} \times W / V_{\text{Assay}}) \right\} / T$$

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

6. According to the protein concentration of sample:

SPS (U/mg)

$$= \left\{ \left[(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}}) / (OD_{\text{Standard}} - OD_{\text{Blank}}) \right] / (V_{\text{Sample}} \times C_{\text{Protein}}) \right\} / T$$

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

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Note:

W: the weight of sample, g;

C_{Standard}: the concentration of standard, 4 mg/mL = 4000 µg/mL;

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

V_{Assay}: the volume of Assay Buffer, 1 mL;

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

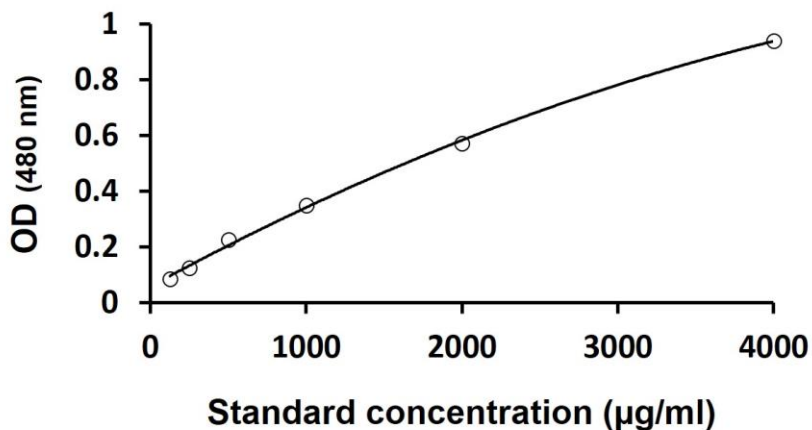
V_{standard}: the volume of Standard, 0.01 mL;

T: the reaction time, 10 minutes.

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EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Sucrose Phosphate Synthase Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

100 µg/mL