(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Sucrose Phosphate Synthase (SPS) Activity Assay Kit

Catalog No.: BC00146

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Sucrose Phosphate Synthase (SPS) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Tissues, fluids
Assay Type	Quantitative
Detection Instrument	Microplate reader (optimal detection wavelength 480 nm)

Product Introduction

Sucrose phosphate synthase (SPS) primarily synthesizes sucrose in green photosynthetic organs, while its activity is very low in storage organs. However, experiments have shown that an inefficient cycle exists in the sucrose flow within the sucrose pool, where sucrose is simultaneously synthesized and broken down, at which point SPS activity is very high. Therefore, the role of SPS in sink tissues should not be underestimated. In both photosynthetic and non-photosynthetic tissues, SPS activity is regulated by metabolites and reversible protein phosphorylation.

Principle

SPS catalyzes the formation of sucrose-6-phosphate from fructose, and the content of the substance generated by the reaction of sucrose-6-phosphate with resorcinol is determined by measuring the absorbance at 480 nm, thereby allowing the calculation of SPS activity.

Components

Components	Size	Storage
Extract	30 mL	2~8℃
Reagent 1	2.1 mL	-20℃ or less
Reagent 2	1 mL	2~8℃

Reagent 3	20 mL	2~8℃
Reagent 4	2 tubes of powder	2~8℃
Preparation of reagent 4 application solution: Add 4 mL of distilled water to one vial of powder immediately before use, dissolve, and use within one week.		
Standard products	1 tube of powder	2~8℃
Consumables 1	Microplate (96 wells) 1 plate	RT
Consumables 2	Plate Sealer 2 pieces	RT

Storage

The kit has a shelf life of 6 months

Preparation before the experiment

Sample processing

1. Tissue preparation: Rinse the plant tissue thoroughly with physiological saline, blot dry with filter paper, and weigh accurately. Add extraction buffer at a ratio of weight (g): volume (ml) = 1:9 (1:2 for samples with high water content). Homogenize in an ice-water bath to prepare a 10% homogenate. Centrifuge at 4000 rpm for 10 minutes, and collect the supernatant for analysis. (For protein concentration determination, use a protein quantification kit BC00006.)
2. Liquid: Measure directly. If turbid, centrifuge at 4000 rpm for 2 minutes and collect the supernatant for testing.

Reagent kit preparation

Standard curve: Add 1 mL of distilled water to the standard powder to prepare a 10 mg/mL standard solution. Then dilute with distilled water to concentrations of 1, 2, 4, 6, 8, and 10 mg/mL. Add the solutions sequentially: 20 μL standard + 40 μL distilled water + 10 μL reagent 2 + 200 μL reagent 3 + 60 μL reagent 4 working solution. Mix well, incubate at 95℃ for 20 min, cool under running water, and take a 200 μL sample of the reaction solution and read the value at 480 nm

using a Microplate reader.

Operation process

Operation table: (Operating in an orifice plate)

Reagents(μL)	Control tube	Test tube
Distilled water	--	40
Sample	20	20
Reagent 1	40	--
Mix well and incubate in a 37°C water bath for 20 minutes.		
Reagent 2	10	10
Mix well, incubate in a 95 °C water bath for 10 minutes, then remove and cool under running water.		
Reagent 3	200	200
Reagent 4 Application Solution	60	60
Mix well, incubate in a 95℃ water bath for 20 min, then remove and cool under running water. Pipette 200 μL of the reaction solution into a 96-well plate (avoid aspirating air bubbles). Read the OD value of each well at 480 nm using a microplate reader. $\Delta A = A_{test} - A_{control}$		

Note: 1. It is best to take 1-2 sample supernatants for preliminary testing before conducting the formal experiment in order to determine the optimal sampling concentration.

2. If ΔA is small (or hovers around zero), the sample volume can be increased (e.g., if 30 μL is added, the volume of reagent added should be reduced accordingly), or the sample concentration can be increased (e.g., 0.2 g is added to 1 mL of extraction solution for extraction), or the reaction time at 37℃ can be extended (e.g., 40 min; the corresponding variables should be substituted into the calculation formula for calculation). If the sample ΔA value is greater than 0.6, the sample supernatant needs to be diluted before measurement.

3. A control tube should be set up for each sample.

Result calculation

1. Calculated based on sample protein concentration

Unit definition: One enzyme activity unit is defined as the conversion of 1 milligram of tissue protein into 1 μg of sucrose per minute.

$$\text{SPS activity}\ (\mu g/min/mg\ prot) = \frac{\triangle A + 0.009}{0.004} \div T \div Cpr \times V_{sample}$$

2. Calculated based on sample fresh weight

Unit definition: One unit of enzyme activity is defined as the conversion of 1 μg of fructose per minute of substrate into 1 gram of tissue.

$$\text{SPS activity}\ (\mu g/min/g) = \frac{\triangle A + 0.009}{0.004} \div T \div (\frac{W}{V_{sample\ total}} \times V_{sample})$$

Annotation

V_{sample} : Add 0.02 mL of sample volume;

$V_{sample\ total}$: The total volume of extract added during pretreatment , in mL;

T : Reaction time , 20 min ;

Cpr : Tissue homogenate protein concentration , mgprot/mL (prot refers to protein);

W : Sample mass , g;

Precautions

This product is for research use only and must not be used for clinical diagnosis. Do not take it.