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CheKine™ Mirco Sucrose Phosphate Synthase (SPS) Activity Assay Kit

Cat #: KTB3130 Size: 48 T/24 S 96 T/48 S

	Mirco Sucrose Phosphate Synthase (SPS) Activity Assay Kit		
REF	**Cat #:** KTB3130	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Sucrose is not only an important photosynthetic product but also the primary substance transported within plants and serves as a form of carbohydrate storage. Sucrose phosphate synthase (SPS, EC 2.4.1.14) uses fructose-6-phosphate as a receptor, and the formed sucrose-6-phosphate is converted into sucrose by sucrose phosphate phosphatase. The SPS-sucrose phosphate phosphatase system is generally considered the main pathway for sucrose synthesis. CheKine™ Mirco Sucrose Phosphate Synthase (SPS) Activity Assay Kit provides a simple, convenient, and rapid method for detecting SPS activity, suitable for plant tissue samples. The principle involves SPS catalyzing the formation of sucrose-6-phosphate from fructose-6-phosphate. Sucrose-6-phosphate reacts with resorcinol to produce a color change, exhibiting a characteristic absorption peak at 480 nm, with the intensity of the color being proportional to the level of enzyme activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60×2 mL	4℃
Reagent Ⅰ	1.8 mL	3.6 mL	-20℃, protected from light
Reagent Ⅱ	1.2 mL	2.4 mL	4℃
Reagent Ⅲ	17 mL	34 mL	4℃
Reagent Ⅳ	5 mL	10 mL	4℃, protected from light
Standard	1 mL	1 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 480 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator, analytical balance, ice maker, freezing centrifuge

- Deionized water
- Dounce homogenizer

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Reagents not used immediately can be aliquoted and stored at -20℃ away from light for up to one month. Avoid repeated freeze-thaw cycles.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅲ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅳ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Note: Reagent Ⅳ is toxic, Extraction Buffer has a pungent odor, so it is recommended to experiment in a fume hood.

Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: 1. It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), Cat #: KTD3002, if it is calculated by protein concentration.

2. The samples extracted by this kit can also be used for the determination of KTB3140, KTB3150 and KTB3110.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 480 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are operated in the 1.5 mL EP Tube):

Reagent	Test Tube (μL)	Control Tube (μL)	Blank Tube (μL)	Standard Tube (μL)
Sample	10	10	0	0
Deionized Water	0	45	55	45
Standard	0	0	0	10
Reagent Ⅰ	45	0	0	0
Mix well and incubate in an accurate 25℃ water bath for 10 min				
Reagent Ⅱ	15	15	15	15
Boil for approximately 10 min in a boiling water bath (make sure the lid is tightly sealed to prevent water loss), then cool				
Reagent Ⅲ	210	210	210	210
Reagent Ⅳ	60	60	60	60
Mix well, boil in a boiling water bath for 30 min, and then cool. Transfer 200 μL to a microglass cuvette or a 96-well plate, and measure the absorbance at 480 nm, recording them as A_{Test}, $A_{Control}$, A_{Blank} and $A_{Standard}$, respectively. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.				

Note: Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for

pre-experiment. The Standard Tube and Blank Tube need to be prepared only 1-2 times, while each Test Tube requires a corresponding Control Tube.If A_{Test} is less than 0.05, increase the sample quantity appropriately. If A_{Test} is greater than 1.0, the sample can be further diluted with Extraction Buffer before proceeding with the experiment, and the final dilution factor should be taken into account in the calculations.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculated by protein concentration

Active unit definition: The amount of enzyme that catalyzes the production of 1 μg of sucrose per min per mg of tissue protein.

SPS (U/mg prot)=$(C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Sample})\div(Cpr\times V_{Sample})\div T$**=$100\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr$**

2. Calculated by sample fresh weight

Active unit definition: The amount of enzyme that catalyzes the production of 1 μg of sucrose per min per g of tissue.

SPS (U/g fresh weight)=$C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Sample})\div(W\div V_{Total\ Sample}\times V_{Sample})\div T$**=$100\times\Delta A_{Test}\div\Delta A_{Standard}\div W$**

Where: $C_{Standard}$: Concentration of Standard; 1,000 μg/mL; V_{Sample}: supernatant volume added to the reaction system, 0.01 mL; T: reaction time, 10 min; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; W: sample fresh weight, g; Cpr: supernatant protein concentration, mg/mL.

Precautions

1. Try to complete the measurement within 30 min.

Typical Data

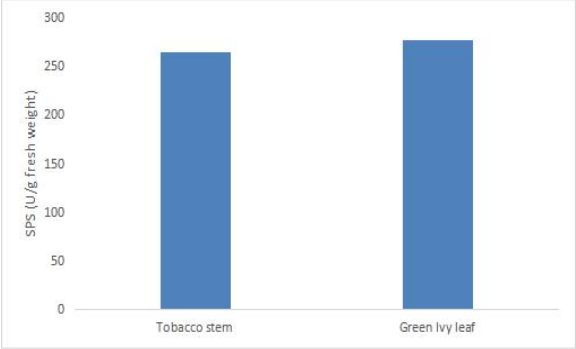

Figure 1. Determination SPS activity in Tobacco stem and Green Ivy laef by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.