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CheKine™ Micro Sucrose Synthetase (SS) Activity Assay Kit

Cat #: KTB3110 Size: 48 T/96 T

	Micro Sucrose Synthetase (SS) Activity Assay Kit		
REF	**Cat #:** KTB3110	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Sucrose is the primary form by which photosynthetic products from sources such as leaves are transported to “sink” organs. Sucrose synthase (EC 2.4.1.13) is a bidirectional enzyme that catalyzes both sucrose synthesis and its breakdown, making it one of the key enzymes in sucrose metabolism. The study of the synthetic direction activity of SS-Ⅱ is of significant importance for understanding plant sucrose synthesis. CheKine™ Micro Sucrose Synthetase (SS) Activity Assay Kit enables the detection of sucrose synthase activity in plant tissues. The assay principle relies on SS-Ⅱ catalyzing a reaction between free fructose and the glucose donor UDPG to form sucrose. Subsequently, the formed sucrose reacts with resorcinol, causing a color change. This color change exhibits a characteristic absorption peak at 480 nm. The intensity of the color is directly proportional to the enzyme activity, allowing for the quantification of sucrose synthase activity within the sample.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	1.25 mL	2.5 mL	-20℃
Reagent Ⅱ	5 mL	10 mL	4℃
Reagent Ⅲ	1 mL	2 mL	4℃
Reagent Ⅳ	12.5 mL	25 mL	4℃
Reagent Ⅴ	3 mL	6 mL	4℃, protected from light

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
- Water bath, ice maker, centrifuge

• Deionized water
• Homogenizer (for tissue samples)

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. Store at -20℃.
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℃.
Reagent Ⅴ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Sample Preparation

Note: It is recommended to use fresh samples. If the experiment is not conducted immediately, the samples can be stored at -80℃ for 1 month. The temperature and time of thawing should be controlled during the determination. When thawing at room temperature, the sample should be thawed within 4 h.
Weigh 0.1 g tissue , add 1 mL Extraction Buffer, homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Take the supernatant and place it on the ice for testing.
Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine catalog number: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

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. Sample measurement. (The following operations are operated in a 1.5 mL EP tube)

Regent	Test Tube (μL)	Control Tube (μL)	Standard Tube (μL)	Blank Tube (μL)
Sample	10	10	0	0
Deionized water	0	45	45	55
Reagent Ⅰ	0	0	10	0
Reagent Ⅱ	45	0	0	0
Mix well, take an accurate water bath at 25℃ for 10 min				
Reagent Ⅲ	15	15	15	15
Boil in a water bath for approximately 10 min (with the lid tightly closed to prevent water loss), then cool down				
Reagent Ⅳ	210	210	210	210
Reagent Ⅴ	60	60	60	60
Mix well and incubate in boiling water bath for 30 min. After cooling, transfer 200 μL of the mixture to a microglass cuvette or a 96-well plate. Measure the absorbance of each tube at 480 nm, recording the values as A_{Test}, $A_{Control}$, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, and $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.				

Note: Only one standard tube and one blank tube need to be prepared. For each test tube, an individual control tube should be set up. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the ΔA_{Test} is less than 0.01, the sample size can be appropriately increased. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or

decrease the sample quantity appropriately.

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:

Unit definition: One unit of enzyme activity is the amount of enzyme that catalyzes the production of 1 μg of sucrose per min per mg of tissue protein.

$(C_{Standard}\times V_{sample}\times \Delta A_{Test}\div \Delta A_{Standard})\div(V_{sample}\times Cpr)\div T=\mathbf{100\times \Delta A_{Test}\div \Delta A_{Standard}\div Cpr}$

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

(2) Calculation according to the weight of the sample:

Unit definition: One unit of enzyme activity is the amount of enzyme that catalyzes the production of 1 μg of sucrose per min per g tissue.

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

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

Precautions

1. It is recommended to complete the measurement within 30 min.

Typical Data

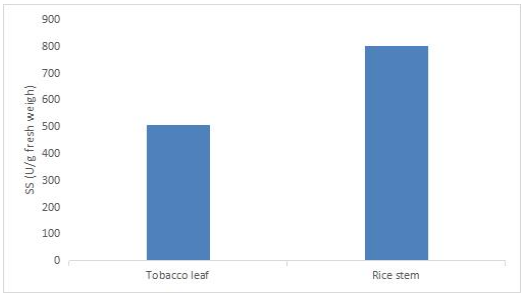

Figure 1. SS activity in Tobacco leaf and Rice stem was detected with this kit

Recommended Products

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

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.