CheKine™ Mirco Sucrose Synthase-I (SS-I) Activity Assay Kit

Cat #: KTB3140 Size: 48 T/96 T

	Mirco Sucrose Synthase-I (SS-I) Activity Assay Kit		
REF	**Cat #:** KTB3140	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Sucrose is the primary form in which photosynthetic products from sources (such as leaves) are transported to “sink” organs. Sucrose synthase (EC 2.4.1.13) is a reversible enzyme that can catalyze both the synthesis and breakdown of sucrose, making it a key enzyme in sucrose metabolism. Studying the activity of its invertase direction (SS-I) is significant for understanding plant sucrose degradation and starch synthesis. CheKine™ Mirco Sucrose Synthase-I (SS-I) Activity Assay Kit offers a simple, convenient, and rapid approach for detecting sucrose synthase (SS-I) activity, suitable for plant tissue samples. The principle of the kit involves SS-I catalyzing the reaction between sucrose and UDP to produce free fructose and UDPG; the content of reducing sugars is then measured using the 3,5-dinitrosalicylic acid method to reflect the level of enzyme activity.

Materials Supplied and Storage Conditions

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

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- Water bath, analytical balance, ice maker, low-temperature centrifuge
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water

• 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. Store at 4℃.

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

Working Reagent III: Prepared before use. Take one vial of Reagent III and add 1.2 mL of Reagent II to dissolve it completely. Use immediately after preparation.

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

Standard: Prepared before use. Dissolve by adding 1 mL of deionized water to make a 20 mg/mL fructose solution, and reserve for later use. This solution can be kept at 4℃ for up to one weeks.

Standard preparation: Using 20 mg/mL fructose solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume	Deionized Water Volume (μL)	Concentration (mg/mL)
Std.1	160 μL 20 mg/mL Standard	240	8
Std.2	120 μL 20 mg/mL Standard	280	6
Std.3	100 μL 20 mg/mL Standard	300	5
Std.4	80 μL 20 mg/mL Standard	320	4
Std.5	60 μL 20 mg/mL Standard	340	3
Std.6	40 μL 20 mg/mL Standard	360	2
Std.7	20 μL 20 mg/mL Standard	380	1
Std.8	0	500	0（Blank Tube）

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.

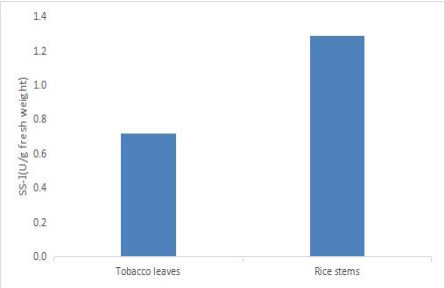
Homogenize the tissue at a ratio of tissue mass (g): Extraction Buffer volume (mL) of 1: 5-10 (it is recommended to weigh approximately 0.1 g of tissue and add 1 mL of Extraction Buffer) in an ice bath. Centrifuge at 8,000 g and 4℃ for 10 min, and retain the supernatant on ice for analysis.

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

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in a 1.5 mL EP tube):

Reagent	Control Tube (μL)	Test Tube (μL)	Standard Tube (μL)
Sample	10	10	0
Standard	0	0	10



Working Reagent III	0	40	0
Reagent I	40	0	40
Mix well, incubate in an accurate 30℃ water bath for 30 min, and then incubate in a 95℃ water bath for 10 min			
Reagent III	50	50	50
Mix well and place in a 95℃ water bath for 5 min (make sure it is tightly covered to prevent water evaporation). Cooling down to room temperature (To avoid splashing that could cause scalding and affect the experimental data, it is recommended to maintain consistent cooling times after each experiment.)			
Deionized Water	400	400	400
Mix well, take 200 μL of the upper layer liquid and transfer it to a micro glass cuvette or a 96-well plate, then measure the absorbance at 540 nm, recording the values as $A_{Control}$, $A_{Test,}$, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.			

Note: The standard curve and blank need to be measured only 1-2 times, while each test tube requires a corresponding control tube. Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA_{Test} is less than 0.1, it is advisable to reduce the dilution ratio or increase the sample volume appropriately. If ΔA_{Test} is greater than 1.2, 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. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation, and bring the ΔA_{Test} into the equation to get the x value (mg/mL).

2. Calculation of SS-Ⅰ activity:

(1) Calculated by protein concentration

Unit Definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μg of reducing sugar per min per mg of tissue protein.

SS-Ⅰ (U/mg prot)=$x\times V_{Total}\div(Cpr\times V_{Sample}\div V_{Total\ Sample})\div T$**=x÷6÷Cpr**

(2) Calculated by sample fresh weight

Unit Definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μg of reducing sugar per min per g of tissue.

SS-Ⅰ (U/g fresh weight)=$x\times V_{Total}\div(W\times V_{Sample}\div V_{Total\ Sample})\div T$**=x÷6÷W**

Where: V_{Total}: total reaction volume, 0.05 mL; V_{Sample}: sample volume added, 0.01 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 30 min; Cpr; sample protein concentration, mg/mL; W: sample weight, g.

Typical Data

Figure 1. Determination SS-Ⅰ activity in Tobacco leaves and Rice stems 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.