CheKine™ Mirco Sucrase Activity Assay Kit

Cat #: KTB3150 Size: 48 T/24 S 96 T/48 S

	Mirco Sucrase Activity Assay Kit		
REF	**Cat #:** KTB3150	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Sucrase (EC 3.2.1.26) is one of the key enzymes for carbohydrate digestion and absorption, capable of hydrolyzing sucrose into monosaccharides that can be absorbed by the body. CheKine™ Mirco Sucrase Activity Assay Kit offers a simple, convenient, and rapid approach for assessing sucrase activity, which is suitable for plant tissue samples. The principle is that 3,5-dinitrosalicylic acid, when heated with reducing sugars, is reduced to a brown-red amino compound, and within a certain range, the quantity of reducing sugars is proportional to the color intensity of the reaction solution. This method is easy to perform, quick, and has minimal interference from impurities.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	1.2 mL	2.4 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃
Reagent Ⅲ	2.4 mL	4.8 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	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 520 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Water bath, analytical balance, ice maker, low-temperature centrifuge
- 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℃.

Working Reagent II: Prepared before use. For a 48 T, add 1 mL of deionized water, and for a 96 T, add 2 mL of deionized water, mixing until fully dissolved. Unused reagent can be stored at 4℃ for up to one week.

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

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

Standard: Prepared before use. Dissolve by adding 1 mL of deionized water to make a 10 mg/mL glucose solution, and reserve for later use. This solution can be kept at 4℃ for up to two weeks.

Standard preparation: Using 10 mg/mL glucose solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume	Deionized Water Volume (μL)	Concentration (mg/mL)
Std.1	100 μL 10 mg/mL Standard	400	2
Std.2	75 μL 10 mg/mL Standard	425	1.5
Std.3	50 μL 10 mg/mL Standard	450	1
Std.4	40 μL 10 mg/mL Standard	460	0.8
Std.5	30 μL 10 mg/mL Standard	470	0.6
Std.6	20 μL 10 mg/mL Standard	480	0.4
Std.7	10 μL 10 mg/mL Standard	490	0.2
Std.8	0	500	0 (Blank Tube)

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

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.

Plant tissues: 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: 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 KTB3130, KTB3140 and KTB3110.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 520 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)
Reagent I	15	15	15

Deionized Water	15	0	0
Sample	30	30	0
Standard	0	0	30
Working Reagent II	0	15	15
Mix well and incubate in an accurate 25℃ water bath for 10 min			
Reagent III	30	30	30
Mix well and place in a 95℃ water bath for 10 min (make sure it is tightly covered to prevent water evaporation). Cooling down to room temperature.			
Deionized Water	210	210	210
Mix well, take 200 μL of the upper layer liquid and transfer it to a microglass cuvette or a 96-well plate, then measure the absorbance at 520 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: (1) 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 0.02, 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. (2) If multiple samples are to be tested, ensure that the cooling times are consistent.

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 sucrose activity:

(1) Calculated by protein concentration

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

Sucrose (U/mg prot)=$(1,000\times x\times V_1)\div(Cpr\times V_1\div V_2)\div T$**$=100\times x\div Cpr$**

(2) Calculated by sample fresh weight

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

Sucrose (U/g fresh weight)=$(1,000\times x\times V_1)\div(W\times V_1\div V_2)\div T$**$=100\times x\div W$**

Where: 1,000: 1 mg/mL=1,000 μg/mL; V_1: volume of sample added to the reaction system, 0.03 mL; V_2: volume of Extraction Buffer added, 1 mL; T: reaction time, 10 min; Cpr; sample protein concentration, mg/mL; W: sample weight, g.

Typical Data

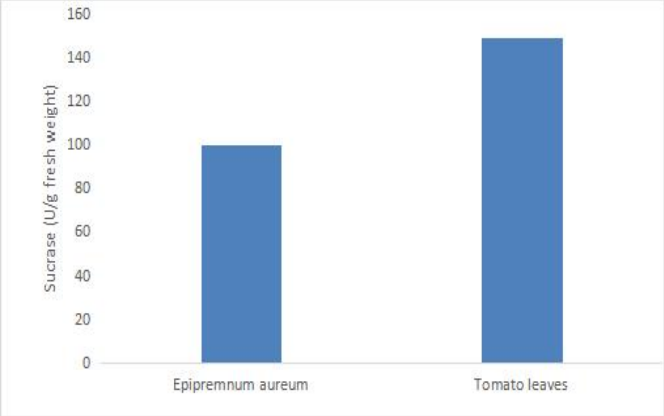

Figure 1. Determination sucrose activity in Epipremnum aureum and Tomato leaves by this assay kit

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.