CheKine™ Micro Sucrose Content Assay Kit

Cat #: KTB3100 Size: 48 T/48 S 96 T/96 S

	Micro Sucrose Content Assay Kit		
REF	**Cat #:** KTB3100	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Sucrose is the primary product of photosynthesis in plants and serves as the main form for sugar transport and storage. Hence, determining the sucrose content is of great significance for understanding plant sugar metabolism. Moreover, sucrose content is one of the key quality control parameters for various products such as beverages, honey, dried fruits, confectionery, and dairy products. CheKine™ Micro Sucrose Content Assay Kit is designed to quantify sucrose levels in plant samples. The principle involves initially heating the sample with an alkali to degrade reducing sugars. Subsequently, under acidic conditions, sucrose is hydrolyzed into glucose and fructose, where fructose further reacts with resorcinol to produce a colored substance. This colored product exhibits a characteristic absorption peak at 480 nm, which is then measured for quantification.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4℃
Reagent Ⅰ (Standard)	1 mL	2 mL	4℃
Reagent Ⅱ	1 mL	2 mL	4℃
Reagent Ⅲ	12 mL	24 mL	4℃
Reagent Ⅳ	4 mL	8 mL	4℃, protected from light
Reagent Ⅴ	Powder×1 vial (0.25 g)	Powder×1 vial (0.5 g)	RT

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
- Water bath, ice maker, centrifuge, analytical balance

• 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 4℃.
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. it has pungent odor and toxicity, it is recommended to conduct experiments in the fume hood.
Reagent Ⅴ: Ready to use as supplied. Store at RT.

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.
Plant Tissues: Weigh 0.1 to 0.2 g of the sample and crush it at room temperature. Add 0.5 mL of Extraction Buffer, grind adequately, and quickly transfer the mixture to a centrifuge tube. Place the tube in a water bath set at 80℃ for 10 min, agitating it 3 to 5 times during this period. After cooling, centrifuge at 8,000 g for 10 min at RT, then collect the supernatant. To the supernatant, add 2 mL of Reagent Ⅴ, subject it to decolorization at 80℃for 30 min. Afterwards, introduce an additional 0.5 mL of Extraction Buffer, allow it to cool, and once again centrifuge at 4,000 g for 10 min at RT. Finally, collect the supernatant for further analysis.
Note: The Extraction Buffer contains components that denature proteins; therefore, if protein concentration calculations are required, proteins must be re-extracted using a different lysis buffer for accurate determination. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: 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	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Sample	0	0	25
Reagent Ⅰ (Standard)	0	25	0
Deionized water	25	0	0
Reagent Ⅱ	15	15	15
Mix well and boil in a water bath for approximately 5 min with the lid securely tightened to prevent water evaporation			
Reagent Ⅲ	175	175	175
Reagent Ⅳ	50	50	50
Mix thoroughly and then incubate in a boiling water bath for 30 min. After cooling, transfer 200 μL of the mixture to a micro glass cuvette or a 96-well plate, and measure the absorbance at 480 nm, recording the values as A_{Blank}, $A_{Standard}$ and A_{Test}. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, and $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.			

Note: Only one standard tube and one blank tube need to be prepared. In order to guarantee the accuracy of

experimental results, need to do a pre-experiment with 2-3 samples. If the A_{Test} is less than 0.05, the sample size can be appropriately increased. If A_{Test} is greater than 2.0, 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:

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

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

Sucrose(mg/g fresh weight)=$((C_{Standard}\times V_{Sample})\times \Delta A_{Test}\div \Delta A_{Standard}\div (V_{Sample}\times W\div V_{Sample\ Total})$**=$\Delta A_{Test}\div \Delta A_{Standard}\div W$**

Where: $C_{Standard}$: the concentration of the standard tube, 1 mg/mL; V_{Sample}: the volume of the sample in the reaction system, 0.025 mL; $V_{Sample\ Total}$: The volume of Extraction Buffer added, 1 mL; Cpr: protein concentration, mg/mL; W: fresh weight of the sample, g.

Typical Data

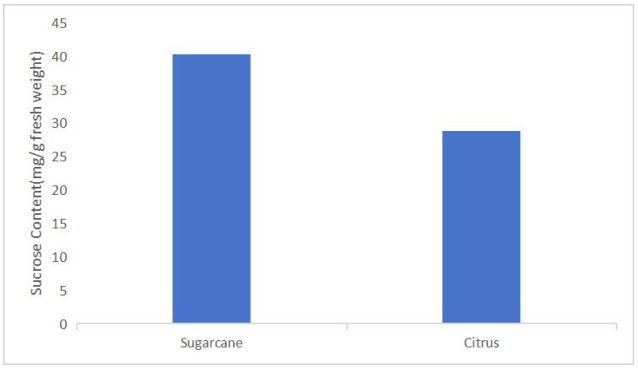

Figure 1. Sucrose content in Sugarcane and Citrus was detected with this kit

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. For your safety and health, please wear a lab coat and disposable gloves.