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CheKine™ Micro Starch Debranching Enzyme (SBE) Activity Assay Kit

Cat #: KTB3034 Size: 48 T/48 S 96 T/96 S

	Micro Starch Debranching Enzyme (SBE) Activity Assay Kit		
REF	**Cat #:** KTB3034	LOT	**Lot #:** Refer to product label
	Detection range: 0.05-0.8 mg/mL		**Sensitivity:** 0.05 mg/mL
	Applicable sample: Animal and Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Starch debranching enzyme (SBE) specifically cleaves the α-1,6-glycosidic bonds of amylopectin, producing linear glucose chains, and plays a crucial role in adjusting the chain length of amylopectin molecules. CheKine™ Micro Starch Debranching Enzyme (SBE) Activity Assay Kitprovides a simple, convenient, and rapid method for detecting SBE activity, suitable for plant and animal tissue samples. The principle involves using the 3,5-dinitrosalicylic acid (DNS) method to measure the reducing sugars generated by SBE-catalyzed amylopectin hydrolysis. The SBE activity is calculated based on the change in reducing sugar content.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	15 mL	30 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃
Reagent Ⅲ	12 mL	24 mL	4℃
Reagent Ⅳ	40 mL	80 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 540 nm
- 96-well microplate or microglass cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube, 2 mL EP tube
- Water bath, low-temperature centrifuge

• Deionized water

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 the 48 T kit, add 10 mL of deionized water to Reagent Ⅱ; for the 96 T kit, add 20 mL of deionized water to Reagent Ⅱ and dissolve completely before use. Unused reagents can be stored at 4℃ for up to two weeks.

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 has certain irritation, so personal protection is recommended during use.

Standard: Prepared before use. Add 1 mL of deionized water and dissolve completely to prepare a 10 mg/mL glucose standard solution. Unused reagents should be stored at 4℃ for up to two weeks.

1 mg/mL Glucose Standard Preparation: Dilute 200 μL of the 10 mg/mL glucose standard with 1,800 μL deionized water to obtain a 1 mg/mL glucose standard. Further dilute the 1 mg/mL standard as follows:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (mg/mL)
Std.1	400 μL of 1 mg/mL Standard	100	0.8
Std.2	300 μL of 1 mg/mL Standard	200	0.6
Std.3	250 μL of 1 mg/mL Standard	250	0.5
Std.4	200 μL of 1 mg/mL Standard	300	0.4
Std.5	150 μL of 1 mg/mL Standard	350	0.3
Std.6	100 μL of 1 mg/mL Standard	400	0.2
Std.7	50 μL of 1 mg/mL Standard	450	0.1
Std.8	25 μL of 1 mg/mL Standard	475	0.05

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

Sample Preparation

Note: Fresh samples are recommended. If not used immediately, store at -80℃ for up to one month.

Weigh approximately 0.1 g of tissue and homogenize in 1 mL Extraction Buffer on ice. Centrifuge at 15,000 g, 4℃ for 10 min. Collect the supernatant and keep on ice for assay.

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. Heat-inactivate an aliquot of the sample supernatant at 95℃ for 5 min (seal tightly to prevent evaporation). Cool to room temperature and centrifuge at 8,000 g, 4℃ for 5 min. Collect the supernatant for assay.
3. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Tube (μL)	Control Tube (μL)	Standard Tube (μL)	Blank Tube (μL)
Sample supernatant	100	0	0	0
Heat-inactivated sample	0	100	0	0
Reagent Ⅰ	0	100	0	0

Working Reagent II	100	0	0	0
Mix well, incubate at 37℃ for 2 h				
Reagent III	100	100	0	0
Standard	0	0	300	0
Deionized water	0	0	0	300
Reagent IV	300	300	300	300
Reagent III (μL)	215	215	215	215

4. Mix well, heat at 95℃ for 5 min (seal tightly), cool under running water, and centrifuge at 8,000 g, 4℃ for 5 min. Transfer 200 μL to a 96-well plate or micro glass cuvette, and record the absorbance at 540 nm. The Test Well is marked as A_{Test}, the Control Well is marked as $A_{Control}$, the Standard Well is marked as $A_{Standard}$, and the Blank Well is recorded as A_{Blank}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Standard Tube and Blank Tube The blank and standard tubes need only be run 1–2 times. Each test sample requires a control. If ΔA_{Test} is less than 0.05 mg/mL of the $\Delta A_{Standard}$, the sample amount can be appropriately increased. If ΔA_{Test} exceeds 0.8 mg/mL of the $\Delta A_{Standard}$, the supernatant can be further diluted with deionized water. Multiply the final result by the dilution factor, or reduce the amount of sample used for extraction.

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 and obtain the standard equation. The determination of ΔA_{Test} is substituted into the equation to get x (mg/mL).

2. Calculation of the SBE activity

Active unit definition: One unit of SBE activity is defined as the amount of enzyme that produces 1 mg of glucose per gram of tissue per hour.

SBE (U/fresh weight)=$x\times V_{total}\div(W\times V_{sample}\div V_{total\ sample})\div T\times F$**$=1.5\times x\div W\times F$**

Where: V_{total}: Total reaction volume, 0.3 mL; V_{sample}: Sample volume added, 0.1 mL; $V_{total\ sample}$: Extraction Buffer volume, 1 mL; T: reaction time, 2 h; W: weight of sample, g; F: dilution multiple.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

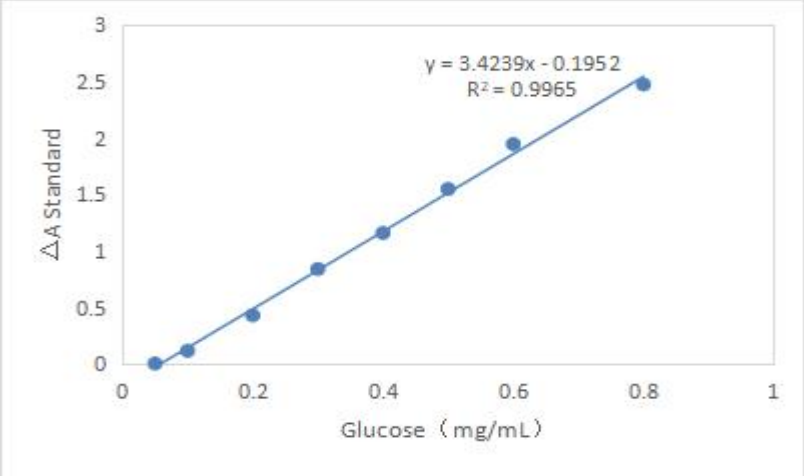

Figure 1. Standard curve of glucose

Examples:

Take 0.105 g of Arabidopsis, analyze according to the above steps, use 96-well plate to calculate ΔA_{Test}=0.092-0.082=0.01, x=0.06. The activity calculated according to the fresh weight of the sample is as follows:

S-SC (U/fresh weight)=1.5×0.06÷0.105=0.857 U/g.

Recommended Products

Catalog No.	Product Name
KTB1371	CheKine™ Micro Starch Assay Kit
KTB1370	CheKine™ Micro α-Amylase Activity 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.