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

Cat #: KTB1390 Size: 48 T/96 T

	Micro Starch Branching Enzyme (SBE) Activity Assay Kit		
REF	**Cat #:** KTB1390	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissues, Bacteria		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Starch Branching Enzyme (SBE, EC 2.4.1.18) mainly exists in plants. It is a key enzyme involved in the synthesis of amylopectin and catalyzes the transformation of amylose into amylopectin. The determination of starch branching enzyme (SBE) activity is of great significance in the study of starch biosynthesis, selection of high-quality crop varieties and quality genetic improvement. CheKine™ Micro Starch Branching Enzyme (SBE) Activity Assay Kit provides a convenient tool for detection of SBE Activity. The principle is that the combination of amylose and iodine has a maximum absorption peak detected at 660 nm. SBE reduces the content of amylose, thereby reducing the absorption value at 660 nm, which can reflect the activity of SBE. The enzyme activity of SBE was calculated by detecting the percentage decrease in absorbance within a certain period of time.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Assay Buffer	10 mL	20 mL	4℃
Substrate	1	1	4℃
Chromogen	1 mL	2 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 660 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, water bath, ice maker, incubator
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Substrate: Add 6 mL deionized water for 96 T or 3 mL deionized water for 48 T before use, then shake upside down several times and heat to dissolve. This solution can be stored at 4℃. If there is precipitation, it can be heated at 70℃ to dissolve.

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

Sample Preparation

Note: Fresh samples are recommended. If not assayed immediately, samples can be stored at -80℃ for one month.

1. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 15,000 g for 15 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2. Bacteria: Collect 5×10^6 bacteria into the centrifuge tube, wash bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 15, 000 g for 15 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), Cat #: KTD3002, if the content 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 660 nm, visible spectrophotometer was returned to zero with deionized water. Preheat the incubator to 37℃.

2. Add the following reagents respectively into each EP tube:

Reagent	control tube (μL)	Test tube (μL)
Sample of Boiled for 1 min	65	0
Sample	0	65
Assay Buffer	85	85
Substrate	25	25
Mix well, incubate at 37℃ for 20 min, put it in a boiling water bath for 5 min to terminate the reaction (cover tightly to prevent water loss), and cool down to room temperature.		
Deionized Water	115	115
Chromogen	10	10

3. Mix well and let stand at room temperature for 5 min, take out 200 μL to a 96-well plate or microglass cuvette. Then reading the values at 660 nm, recorded as $A_{Control}$, A_{Test}, respectively.

Note: A control tube is required for each test tube. The different samples can be added to different control tubes, and boiled for 1 min. If there is precipitation in Substrate, be sure to dissolve it in a 70℃ water bath before use.

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.

A. 96-well plates calculation formula

1. Calculated by fresh weight of samples

Unit definition: Expressed by the percentage decrease in absorbance at a wavelength of 660 nm. 0.5% iodine blue value decreased per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

SBE (U/g)=$(A_{Control}-A_{Test})\div A_{Control}\times V_{Reaction\ Total}\times100\%\div0.5\%\div(W\div V_{Extraction}\times V_{Sample})\div T$**=46.15×$(A_{Control}-A_{Test})\div A_{Control}\div W$**

2. Calculated by protein concentration

Unit definition: Expressed by the percentage decrease in absorbance at a wavelength of 660 nm. 0.5% iodine blue value decreased per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

SBE (U/mg prot)=($A_{Control}$-A_{Test})÷$A_{Control}$×$V_{Reaction\ Total}$×100%÷0.5%÷(Cpr×V_{Sample})÷T=**46.15×($A_{Control}$-A_{Test})÷$A_{Control}$÷Cpr**

3. Calculated by bacteria number

Unit definition: Expressed by the percentage decrease in absorbance at a wavelength of 660 nm. 0.5% iodine blue value decreased per min in 10^4 bacteria reaction system is defined as a unit of enzyme activity.

SBE ($U/10^4$)=($A_{Control}$-A_{Test})÷$A_{Control}$×$V_{Reaction\ Total}$×100%÷0.5%÷(Total number of bacteria÷$V_{Extraction}$×V_{Sample})÷T

=46.15×($A_{Control}$-A_{Test})÷$A_{Control}$÷500

B. Microglass cuvette calculation formula

1. Calculated by fresh weight of samples

Unit definition: Expressed by the percentage decrease in absorbance at a wavelength of 660nm. 1% iodine blue value decreased per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

SBE (U/g)=($A_{Control}$-A_{Test})÷$A_{Control}$×$V_{Reaction\ Total}$×100%÷1%÷(W÷$V_{Extraction}$×V_{Sample})÷T=**23.075×($A_{Control}$-A_{Test})÷$A_{Control}$÷W**

2. Calculated by protein concentration

Unit definition: Expressed by the percentage decrease in absorbance at a wavelength of 660 nm. 1% iodine blue value decreased per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

SBE (U/mg prot)=($A_{Control}$-A_{Test})÷$A_{Control}$×$V_{Reaction\ Total}$×100%÷1%÷(Cpr×V_{Sample})÷T=**23.075×($A_{Control}$-A_{Test})÷$A_{Control}$÷Cpr**

3. Calculated by bacteria number

Unit definition: Expressed by the percentage decrease in absorbance at a wavelength of 660 nm. 1% iodine blue value decreased per min in 10^4 bacteria reaction system is defined as a unit of enzyme activity.

SBE ($U/10^4$)=($A_{Control}$-A_{Test})÷$A_{Control}$×$V_{Reaction\ Total}$×100%÷1%÷(Total number of bacteria÷$V_{Extraction}$×V_{Sample})÷T

=23.075×($A_{Control}$-A_{Test})÷$A_{Control}$÷500

Where: $V_{Reaction\ Total}$: total reaction volume, 0.3 mL; W: sample weight, g; $V_{Extraction}$: Extraction Buffer volume added, 1 mL; V_{sample}: sample volume added, 0.065 mL; T: reaction time, 20 min; Cpr: sample protein concentration, mg/mL; 500: Total number of cells, $5×10^6$.

Typical Data

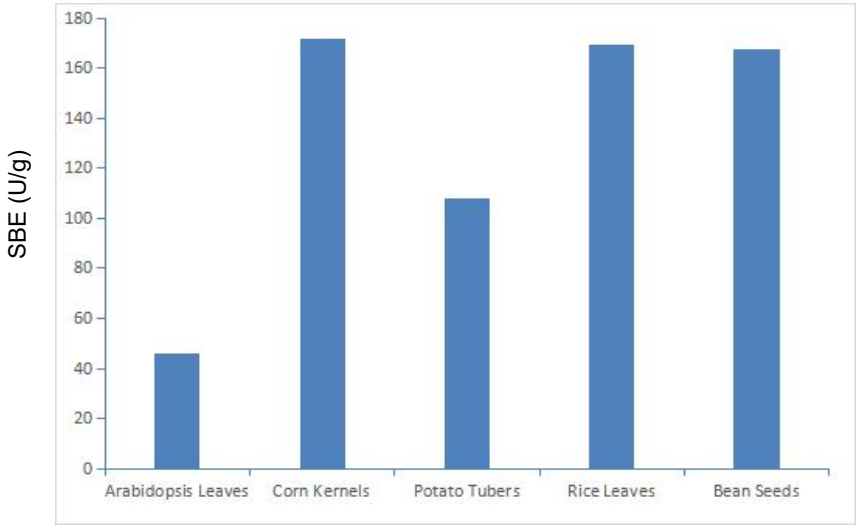

Figure 1. SBE activity in arabidopsis leaves, corn kernels, potato tubers, rce leaves, bean seeds respectively. Assays were performed following kit protocol.

Recommended Products

Catalog No.	Product Name
KTB1370	CheKine™ Micro α-Amylase Activity Assay Kit
KTB1380	CheKine™ Micro β-Amylase Activity Assay Kit

Disclaimer

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