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CheKine™ Micro Granule-Bound Starch Synthase (GBSS) Activity Assay Kit

Cat #: KTB1373 Size: 48 T/96 T

	Micro Granule-Bound Starch Synthase (GBSS) Activity Assay Kit		
REF	**Cat #:** KTB1373	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Granule-bound starch synthase (GBSS, EC2.4.1.21) exists in the starch body in a bound state, catalyze the starch chain elongation reaction, and is mainly responsible for the synthesis of amylopectin. CheKine™ Micro Granule-Bound Starch Synthase (GBSS) Activity Assay Kit can detect plant tissues. In this kit, GBSS catalyzes the reaction between ADPG and starch primers (glucan), transfers glucose molecules to starch primers, and generates ADP at the same time. Furthermore, the addition of pyruvate kinase, hexokinase and glucose 6-phosphate dehydrogenase in the reaction system catalyzed the reduction of $NADP^+$ to NADPH in turn, where the amount of NADPH generated was proportional to the amount of ADP generated in the previous reaction. GBSS activity could be calculated by measuring the increase of NADPH at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	100 mL	100 mL×2	4℃
Reagent Ⅰ	20 mL	40 mL	4℃
Reagent Ⅱ A	1	1	4℃, protected from light
Reagent Ⅱ B	1	1	4℃, protected from light
Reagent Ⅱ C	1	1	-20℃, protected from light
Reagent Ⅲ	1	1	-20℃, protected from light
Reagent Ⅳ	1	1	-20℃, protected from light
Reagent Ⅴ	25 μL	50 μL	-20℃, protected from light
Reagent Ⅵ	25 μL	50 μL	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV microplate or microquartz cuvette, precision pipettes, disposable pipette tips
- Water bath, cryogenic centrifuge,1.5 mL EP tube
- Deionized water
- Mortar or homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Note: The Extraction Buffer has a pungent odor, so it is recommended to experiment in a fume hood.
Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.
Working Reagent II: Prepared before use. Add 7 mL Reagent Ⅰ for 48 T and 14 mL Reagent Ⅰ for 96 T to Reagent Ⅱ A to fully dissolve. Heat slowly, gradually heat up to boil to dissolve it, cool it, mix with Reagent Ⅱ B and Reagent Ⅱ C and dissolve it for use. The remaining reagent can also be stored at -20℃ and protected from light for 2 weeks after aliquoting to avoid repeated freezing and thawing.
Reagent III: Prepared before use. Add 4 mL Reagent Ⅰ for 48 T and 8 mL Reagent Ⅰ for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.
Reagent IV: Prepared before use. Add 5 mL Reagent Ⅰ for 48 T and 10 mL Reagent Ⅰ for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.
Reagent V: Prepared before use. Add 0.225 mL deionized water for 48 T and 0.45 mL deionized water for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.
Reagent VI: Prepared before use. Add 0.225 mL deionized water for 48 T and 0.45 mL deionized water for 96 T to fully dissolve. The remaining reagent can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

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: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Discard the supernatant, add 1 mL Extraction Buffer to the precipitation and mix thoroughly, 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 it is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Well (μL)
Sample	75
Working Reagent Ⅱ	135

Mix well, hold at 30℃ for 20 min, place in boiling water bath for 1 min (cover tightly to prevent water loss), and cool in ice bath	
Reagent III	75
Mix well, hold at 30℃ for 30 min, place in boiling water bath for 1 min (cover tightly to prevent water loss), and cool in ice bath. Centrifuge at 10,000 g for 10 min at 4℃, take the supernatant. The following operations are operated in the 96-well UV microplate or microquartz cuvette:	
Supernatant	150
Reagent IV	100
Reagent V	5
Reagent VI	5

3. Mix thoroughly, measure the absorbance value A_1 at 10 s at 340 nm, and the absorbance value A_2 at 130 s. Finally calculate $\Delta A=A_2-A_1$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.02, increase the sample quantity appropriately. If ΔA is greater than 0.6, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. If ΔA is negative, the sample does not contain GBSS or is degraded.

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.

Calculation of GBSS activity:

A. 96-well UV plates calculation formula as below

(1) Calculated by protein concentration

Active unit definition: The production of 1 nmol of NAPDH per milligram of protein per min was defined as one unit of enzyme activity.

GBSS (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\times Cpr)\div T\times 1.9$**$=1059\times\Delta A\div Cpr$**

(2) Calculated by fresh weight of samples

Active unit definition: The production of 1 nmol of NAPDH per gram tissue per min was defined as one unit of enzyme activity.

GBSS (U/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\times V_{Sample}\div V_{Total\ sample})\div T\times 1.9$**$=1059\times\Delta A\div W$**

V_{Total}: total reaction volume, 2.6×10^{-4} L; ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol /cm; d: the light path of the 96-well UV plate, 0.5 cm; V_{Sample}: sample volume added, 0.075 mL; $V_{Total\ sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 2 min; 1.9: dilution ratio; Cpr: sample protein concentration, mg/mL; W: weight of sample, g.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

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

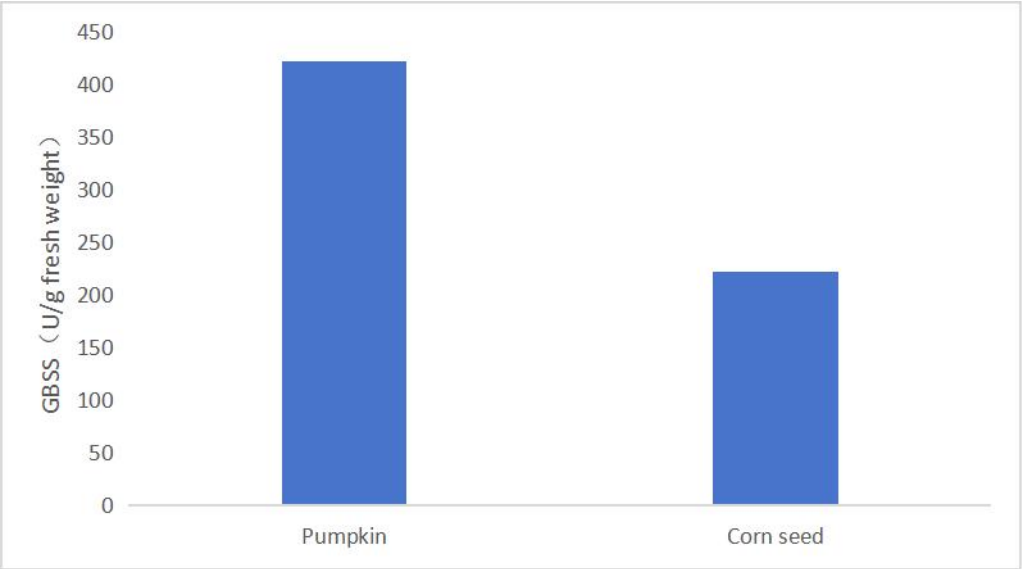

Figure 1. Determination GBSS activity in pumpkin and corn seed by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB1371	CheKine™ Micro Starch Activity Assay Kit
KTB1372	CheKine™ Micro Soluble Starch Synthase (SSS) Activity Assay Kit
KTB1390	CheKine™ Micro Starch Branching Enzyme(SBE) Activity Assay Kit

Disclaimer

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