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CheKine™ Micro ADP-Glucose Pyrophosphorylase (AGP) Activity Assay Kit

Cat #: KTB1345 Size: 48 T/48 S 96 T/96 S

	Micro ADP-Glucose Pyrophosphorylase (AGP) Activity Assay Kit		
REF	**Cat #:** KTB1345	LOT	**Lot #:** Refer to product label
	Applicable sample: Plant tissues		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

ADP-glucose pyrophosphorylase (AGP, EC2.7.7.21) mainly exists in plants, glucose-1-phosphoric acid catalytic reaction with ATP generation of starch synthesis precursor ADPG directly, is the main limiting step of plant starch biosynthesis. CheKine™ Micro ADP-Glucose Pyrophosphorylase (AGP) Activity Assay Kit can detect plant tissues. In this kit, the reverse reaction catalyzed by AGP generates G1P, and the hexose phosphate mutase and glucose 6-phosphate dehydrogenase added to the reaction system catalyze 6-phosphogluconic acid and NADPH in turn. The AGP activity can be calculated by measuring the increase rate of NADPH at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4℃
Reagent Ⅰ	10 mL	20 mL	4℃
Reagent Ⅱ A	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅱ B	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅳ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅴ	Powder×1 vial	Powder×1 vial	-20℃, protected from light

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 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. Transfer Reagent II B to Reagent II A bottle, add 5 mL deionized water for 48 T and 10 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 III: Prepared before use. Add 0.8 mL deionized water for 48 T and 1.6 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 IV: Prepared before use. Add 0.8 mL deionized water for 48 T and 1.6 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 V: Prepared before use. Add 0.8 mL deionized water for 48 T and 1.6 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.
Working Reagent: For each well, prepare 35 μL Working Reagent. According to the ratio of Reagent III: Reagent IV: Reagent V=1: 1: 1. Working Reagent is freshly prepared.

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℃. Use supernatant for assay, and place it on ice to be tested.
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 KTB1372.

Assay Procedure

1. Preheat the microplate reader or ultraviole spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. Preheat Reagent I at 30℃ for 10 min.
3. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Well (μL)
Sample	10
Reagent I	50
Working Reagent II	80
Mix well, hold at 30℃ for 15 min, place in boiling water bath for 1 min (cover tightly to prevent water loss), and cool in ice bath. Centrifuge at 4,000 g for 5 min at 4℃, take the supernatant. The following operations are operated in the 96-well UV microplate or microquartz cuvette:	

Supernatant	80
Cooled Reagent Ⅰ	85
Working Reagent	35

4. 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 AGP 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 AGP 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.

AGP (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\times Cpr)\div T\times 1.75$**=5,627×ΔA÷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.

AGP (U/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\times V_{Sample}\div V_{Total\ sample})\div T\times 1.75$**=5,627×ΔA÷W**

V_{Total}: total reaction volume, 2×10^{-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, 0.01 mL; $V_{Total\ sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 2 min; 1.75: 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.

Precautions

1. In the operation of 96-well UV microplate or microquartz cuvette, it is recommended to use cooled Reagent Ⅰ to room temperature, otherwise it will cause a high reading background.

Typical Data

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

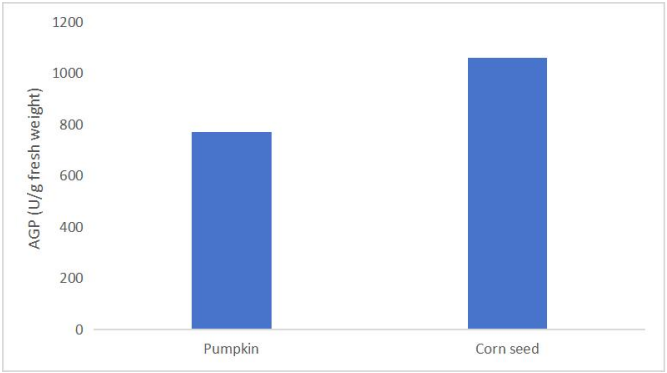

Figure 1. Determination AGP activity in pumpkin and corn seed by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB1371	CheKine™ Micro Starch Activity Assay Kit
KTB1372	CheKine™ Micro Soluble Starch Synthase (SSS) Activity Assay Kit
KTB1373	CheKine™ Micro Granule-Bound Starch Synthase (GBSS) 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. For your safety and health, please wear a lab coat and disposable gloves.