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CheKine™ Micro Pyrrolidine-5-Carboxylate Synthase (P5CS) Activity Assay Kit

Cat #: KTB3014 Size: 48 T/48 S 96 T/96 S

	Micro Pyrrolidine-5-Carboxylate Synthase (P5CS) Activity Assay Kit		
REF	**Cat #:** KTB3014	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissue		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Proline is an important osmotic adjustment substance in plants to adapt to adversity stress. Proline metabolism in higher plants is divided into two synthetic pathways: glutamic acid (Glu) and ornithine (Orn) due to different initial substrates. Pyrroline -5-carboxylate synthase (P5CS is a key enzyme in proline synthesis from glutamic acid, which plays a key role in plants' adaptation to adversity stress. CheKine™ Micro Pyrrolidine-5-Carboxylate Synthase (P5CS) Activity Assay Kit can be used to detect biological samples such as plant tissue. In the kit, P5CS catalyzes the decomposition of ATP to produce ADP and inorganic phosphorus in the process of glutamic acid producing P5C. The activity of P5CS can be determined by measuring the amount of inorganic phosphorus produced per unit time by ammonium molybdate colorimetry.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃, protected from light
Reagent Ⅰ	12 mL	24 mL	4℃, protected from light
Reagent Ⅱ	12 mL	24 mL	4℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×2 vials	4℃, protected from light
Reagent Ⅳ	Powder×1 vial	Powder×2 vials	4℃, protected from light
Reagent Ⅴ	10 mL	20 mL	4℃
Standard	1 mL	1 mL	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 660 nm

- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.
Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.
Reagent II: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.
Working Reagent III: Prepared before use. Add 10 mL deionized water to each bottle to dissolve thoroughly. The remaining reagent can also be stored at 4℃ and protected from light for 1 week.
Working Reagent IV: Prepared before use. Add 10 mL deionized water to each bottle to dissolve thoroughlyit in a water bath at 60℃. The remaining reagent can also be stored at 4℃ and protected from light for 1 week.
Reagent V: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.
Working Reagent: Prepared before use. According to the ratio of deionized water: Working Reagent III: Working Reagent IV: Reagent V=2: 1: 1: 1. Working Reagent is freshly prepared. The prepared Working Reagent should be light yellow. If it is colorless, the reagent will be ineffective. If it is blue, it will be phosphorus pollution.
Standard: Ready to use as supplied; 10 μmol/mL standard phosphorus solution. Equilibrate to room temperature before use; Store at 4℃.
0.5 μmol/mL standard phosphorus solution: Prepare 0.5 μmol/mL standard phosphorus solution by diluting 50 μL 10 μmol/mL standard phosphorus solution into 950 μL deionized water. Using 0.5 μmol/mL standard phosphorus solution subsequent detection.
Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

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 or mortar on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay.
Note: 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 660 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Enzymatic reaction measurement. (The following operations are operated in 96-well plate or microglass cuvette **in turn**)

Reagent	Test Tube (μL)	Control Tube (μL)
Sample	100	0
Reagent I	100	0
Mix well and react accurately at 37℃ for 10 min.		
Reagent II	100	100
Reagent I	0	100

Sample	0	100

Mix well, centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay.

3. Sample measurement. (The following operations are operated in 96-well plate or microglass cuvette)

Reagent	Test Well (μL)	Control Well (μL)	Standard Well (μL)	Blank Well (μL)
Supernatant	20	20	0	0
Deionized water	0	0	0	20
Standard	0	0	20	0
Working Reagent	200	200	200	200

4. Mix well, let stand at 25℃ for 30 min. detect the absorbance at 660 nm. The Blank Well is recorded as A_{Blank}, the Standard Well is marked as $A_{Standard}$, the Control Well is marked as $A_{Control}$, and the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Standard Well and Blank Well only need to be done once or twice, Each Test Well needs to be provided with a Control Well. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.0, 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.

Calculation of the P5CS activity

(1) Calculated by protein concentration

Active unit definition: 1 nmol of p-nitrophenol is produced per h in 1mg tissue protein reaction system is defined as a unit of enzyme activity.

$P5CS\ (U/mg\ prot)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total}\div(V_{Sample}\times Cpr)\div T=\mathbf{9\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr}$

(2) Calculated by fresh weight of samples

Active unit definition: 1 nmol of p-nitrophenol is produced per h in 1 g tissue reaction system is defined as a unit of enzyme activity.

$P5CS\ (U/g\ fresh\ weight)=C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard}\times V_{Total}\div(V_{Sample}\times W)\div T=\mathbf{9\times\Delta A_{Test}\div\Delta A_{Standard}\div W}$

$C_{Standard}$: Standard concentration, 0.5 μmol/mL; V_{Sample}: Added the sample volume, 0.1 mL; V_{Total}: Enzymatic reaction volume, 0.3 mL;T: Reaction time, 10 min=1/6 h; Cpr: sample protein concentration, mg/mL; W: Sample weight, g.

Typical Data

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

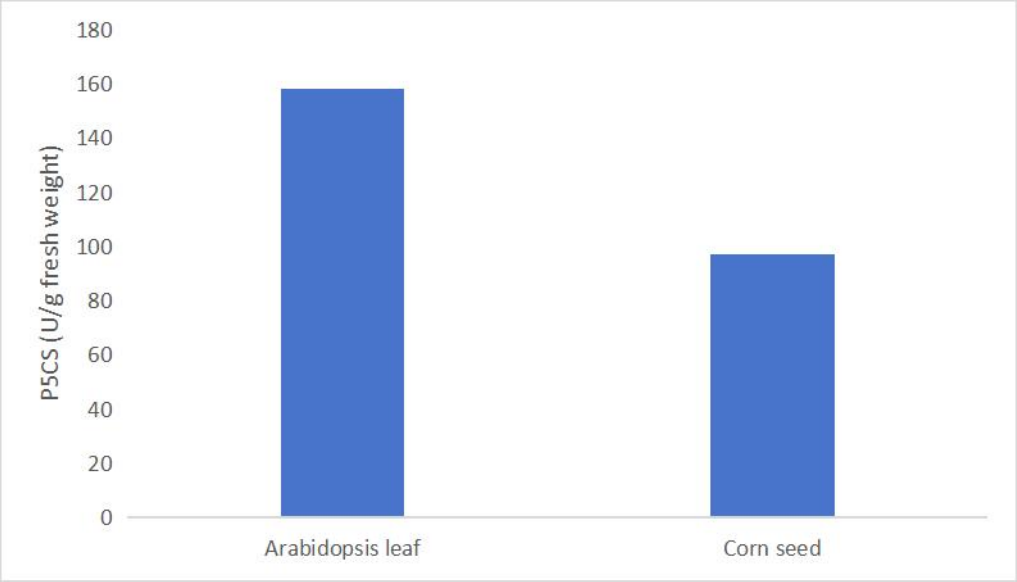

Figure 1. Determination of P5CS activity in arabidopsis leaf and corn seed by this kit.

Recommended Products

Catalog No.	Product Name
KTB1150	CheKine™ Micro Peroxidase (POD) Activity Assay Kit
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Content 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.