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CheKine™ Micro Cysteinyl Sulfoxide Lyase (CSL) Activity Assay Kit

Cat #: KTB3013 Size: 48 T/48 S 96 T/96 S

	Micro Cysteinyl Sulfoxide Lyase (CSL) Activity Assay Kit		
REF	**Cat #:** KTB3013	LOT	**Lot #:** Refer to product label
	Detection range: 0.05-2 μmol/mL		**Sensitivity:** 0.05 μmol/mL
	Applicable samples: Plant Tissue, Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Cysteinylsulfoxide lyase (CSL), referred to as alliinase, also known as alliinase. Cysteinyl sulfoxide lyase is found in almost all kinds of allium plants, such as garlic, onion, leek, etc. Alliinase is found in the vacuole, and its natural substrate alliinine is present in the cytoplasm; alliinase comes into contact with alliin and catalyzed the production of alliin and cis and trans ahocene and produces by-products such as pyruvate and ammonia, and is also the main source of spicy odors in plants such as garlic. CheKine™ Micro Cysteinyl Sulfoxide Lyase (CSL) Activity Assay Kit can be used to detect biological samples such as plant tissue or liquid samples. In the kit, CSL catalyzed the S-methyl-L-cysteine sulfoxide reaction to produce pyruvate, reacted with 2,4-dinitrobenzene hydrazine, which turned brown-red under alkaline conditions and had characteristic absorption peaks at 510 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	80 mL	80 mL×2	4℃
Reagent Ⅰ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅲ	7.5 mL	15 mL	4℃, protected from light
Reagent Ⅳ	3 mL	6 mL	4℃, protected from light
Reagent Ⅴ	15 mL	30 mL	4℃
Standard	Powder×1 vial	Powder×1 vial	4℃, 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 visible spectrophotometer capable of measuring absorbance at 510 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℃.

Working Reagent I: Prepared before use. Add 1 mL deionized water for 48 T and 2 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 II: Prepared before use. Add 50 mL deionized water for 48 T/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: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

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

Reagent V: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Standard: Prepared before use. Add 1 mL deionized water for each bottle to fully dissolve, that is 20 μmol/mL sodium pyruvate Standard. Equilibrate to room temperature before use; Store at 4℃, protected from light for 1 month. Using 20 μmol/mL sodium pyruvate Standard, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Deionized water (μL)	Concentration (μmol/mL)
Std.1	100 μL of 20 μmol/mL Standard	900	2
Std.2	800 μL of Std.1 (2 μmol/mL)	200	1.6
Std.3	500 μL of Std.2 (1.6 μmol/mL)	500	0.8
Std.4	500 μL of Std.3 (0.8 μmol/mL)	500	0.4
Std.5	500 μL of Std.4 (0.4 μmol/mL)	500	0.2
Std.6	500 μL of Std.5 (0.2 μmol/mL)	500	0.1
Std.7	500 μL of Std.6 (0.1 μmol/mL)	500	0.05
Blank	0	500	0

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.

1. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize or mortar on ice and leaching at 4℃ for 40 min. Centrifuge at 15,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Liquid samples: Test directly. If the solution is turbid, centrifuge at 15,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

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 510 nm. Visible spectrophotometer was returned to zero with deionized water.

2. 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)
Sample	20	20	0	0
Standard	0	0	20	0
Deionized water	0	10	10	30
Working Reagent Ⅰ	10	0	0	0
Working Reagent Ⅱ	10	10	10	10
Mix well, incubate for 20 min at 37℃.				
Reagent Ⅲ	40	40	40	40
Reagent Ⅳ	20	20	20	20
Mix well, incubate for 5 min at 25℃.				
Reagent Ⅴ	100	100	100	100

3. Mix well, incubate for 5 min at 25℃, detect the absorbance at 510 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: (1) 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. (2) In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. During the experiment, garlic bulb were diluted 40 times and leek leaf were diluted 2 times. (3) If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. If ΔA_{Test} is greater than $\Delta A_{Standard}$ of 2 μmol/mL, 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.

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 (μmol/mL).

2. Calculation of the CSL activity

(1) Calculated by protein concentration

Active unit definition: At 37℃, 1 μmol pyruvate is pcatalyzed per min in 1 mg tissue protein reaction system is defined as a unit of enzyme activity.

CSL (U/mg prot)=$(V_{Sample}\times x)\div(V_{Sample}\times Cpr)\div T\times F$**$=0.05x\div Cpr\times F$**

(2) Calculated by fresh weight of samples

Active unit definition: At 37℃, 1 μmol pyruvate is pcatalyzed per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

CSL (U/g fresh weight)=$(V_{Sample}\times x)\div(W\times V_{Sample}\div V_{Total\ sample})\div T\times F$**$=0.05x\div W\times F$**

(3) Calculated by volume of liquid samples

Active unit definition: At 37℃, 1 μmol pyruvate is pcatalyzed per min in 1 mL liquid samples reaction system is defined as a unit of enzyme activity.

CSL (U/mL)=(V_{Sample}×x)÷V_{Sample}÷T×F**=0.05x×F**

V_{Sample}: Added the sample volume, 0.02 mL; $V_{Total\ sample}$: Added the Extraction Buffer volume, 1 mL;T: Reaction time, 20 min; Cpr: sample protein concentration, mg/mL; W: Sample weight, g; F: Dilution multiple of sample.

Typical Data

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

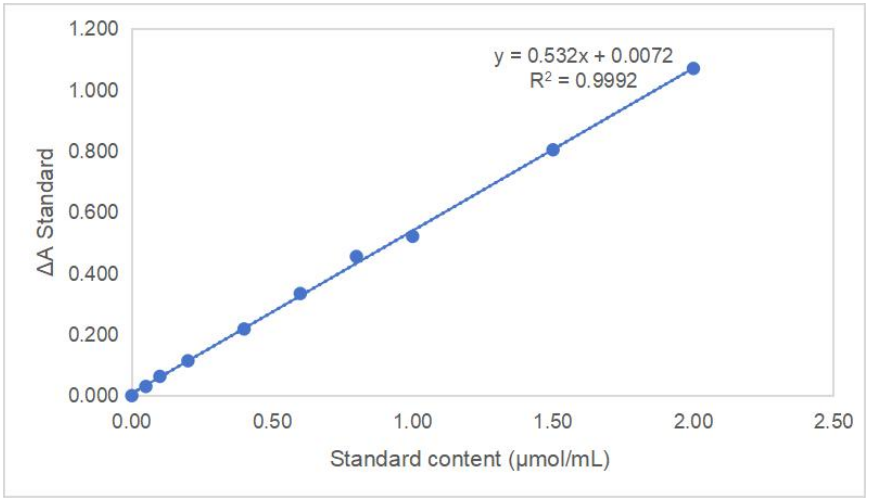

Figure 1. Standard curve of CSL.

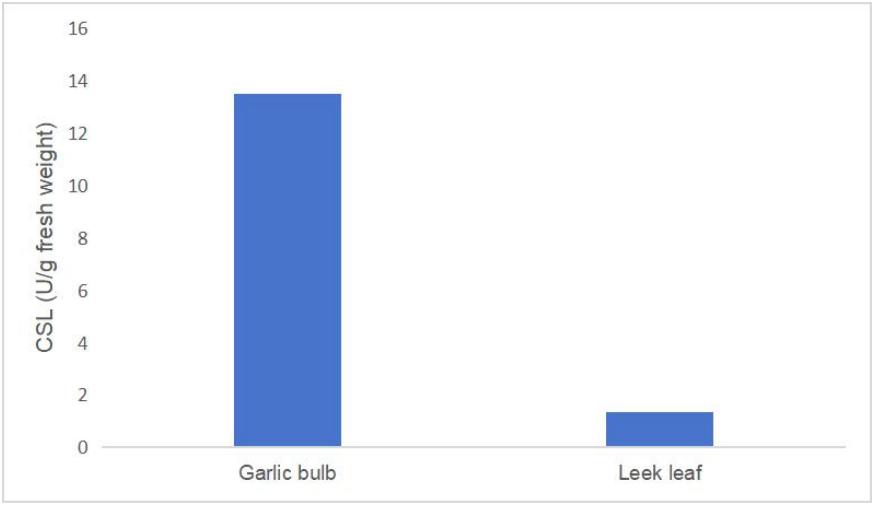

Figure 2. Determination of CSL activity in garlic bulb and leek leaf by this kit.

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.