Cysteinyl Sulfoxide Lyase（CSL）Activity text Kit

Detection Equipment: Spectrophotometer

Catalog Number: BC5390

Size: 50T/24S

Components:Please carefully check the volume of the reagent and the volume in the bottle before use. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation condition
Extract	Liquid 30 mL×1	2-8℃ storage
Reagent I	Liquid 3.5 mL×1	2-8℃ storage
Reagent II	Powder ×1	2-8℃ storage
Reagent III	Liquid 7 mL×1	2-8℃ storage
Reagent IV	Liquid 7 mL×1	2-8℃ storage
Reagent V	Liquid 35 mL×1	2-8℃ storage
Standard	Liquid 1 mL×1	2-8℃ storage

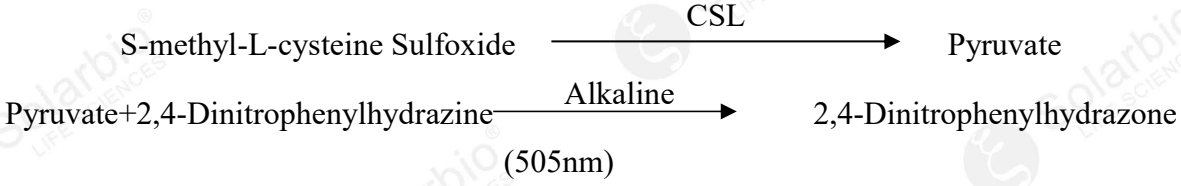
Solution reparation :

1. Reagent II: The reagent is placed in the EP tube in the reagent bottle. Add 4 mL distilled water to dissolve before use. It can be stored for 4 weeks at -20℃ to avoid repeated freezing and thawing.
2. Reagent II working solution: according to the sample volume, the reagent 2 was diluted by 5000 times with distilled water, and used now.
3. Standard: 20μmol/mL sodium pyruvate standard solution.
4. Preparation of 0.625μmol/mL sodium pyruvate standard solution: Take 30μL of 20μmol/mL standard solution in EP tube, add 930μL distilled water to fully dissolve, and prepare 0.625μmol/mL sodium pyruvate standard solution for later use.

Description:

Cysteinyl sulfoxide lyase, referred to as alliinase, also known as alliinase. Cysteyl sulfoxide lyase is found in almost all allium plants, such as garlic, onion, leek, etc. Alliinase is present in the vacuole, and its natural substrate alliin is present in the cytoplasm. Alliinase, in contact with alliine, catalyzes the production of allicin and cis-and trans-ajoene and produces by-products such as pyruvate and ammonia, which are also the main source of the punchy odor of plants such as garlic.

Cysteinyl sulfoxide lyase catalyzes the formation of pyruvate from S-methyl-L-cysteine sulfoxide. Pyruvate can react with 2, 4-dinitrophenylhydrazine to form 2, 4-dinitrophenylhydrazone, which is brownish red under alkaline conditions. CSL enzyme activity was calculated by measuring the change in absorbance at 505nm.



Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorbance value of the sample is not within the

measurement range, it is recommended to dilute or increase the sample size for detection.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water bath, desk centrifuge, transferpettor, 1 mL glass cuvette, mortar/homogenizer, ice and distilled water.

Protocol:

I. Sample processing (The sample size to be tested can be appropriately adjusted, and the specific proportion can be referred to the literature)

Tissue sample: According to the ratio of tissue mass (g) : extraction liquid volume (mL) 1:5-10 (it is recommended to weigh about 0.1g tissue and add 1mL extraction liquid), homogenize in an ice bath, and extract at 4℃ for 30 minutes. After centrifugation at 12000g for 10min at 4 ° C, the supernatant was removed and placed on ice until measured.

Liquid sample: Direct measurement. (If the solution appeared cloudy, the supernatant was centrifuged and then measured.)

II. Measurement Steps

1. Preheat the visible spectrophotometer for more than 30min, adjust the wavelength to 505nm, and zero it with distilled water.
2. Operation table (1.5mL EP tube operation) :

Reagent name（μL）	Test tube	Control tube	Standard tube	Standard blank tube
Sample	100	100	-	-
Standard	-	-	100	-
Reagent I	100	-	-	-
Reagent II working solution	100	100	100	100
Distilled water	-	100	100	200
After mixing, reaction at 37℃ for 30min				
Reagent III	100	100	100	100
Reagent IV	100	100	100	100
After mixing, reaction at 37℃ for 30min				
Reagent V	500	500	500	500

Mix well and leave at room temperature for 10min. Absorbance at a wavelength of 505nm was measured in a 1mL glass cuvette. Recorded as A_{text}, $A_{control}$, $A_{standard}$, $A_{standard\ blank}$. $\Delta A_{text} = A_{text} - A_{contrast}$, $\Delta A_{standard} = A_{standard} - A_{standard\ blank}$. (Standard tube and standard blank tube only need 1-2 times.)

III. Calculations

1. Calculated by sample protein concentration

Unit definition: at 37℃, 1μmol of pyruvate catalyzed per mg of histone per minute is defined as a unit of enzyme activity.

CSL activity (U/mg prot) = ($\Delta A_{text} \times C_{standard} \div \Delta A_{standard}$) $\times V_{sample} \div (V_{sample} \times Cpr) \div T \times F = 0.0208 \times \Delta A_{text} \div \Delta A_{standard} \div Cpr \times F$

2. Calculated by sample quality

Unit definition: 37℃, 1μmol pyruvate catalytic production per g tissue per minute is defined as a unit of enzyme activity.

CSL activity (U/g mass) = ($\Delta A_{text} \times C_{standard} \div \Delta A_{standard}$) $\times V_{sample} \div (V_{sample} \div V_{total\ sample} \times W) \div T \times F = 0.0208 \times \Delta A_{text} \div \Delta A_{standard} \div W \times F$

3.Calculated by volume of serum (plasma)

Definition of a unit: 1μmol pyruvate per minute catalyzed by a liquid such as serum (plasma) per mL was defined as one unit of enzyme activity.

CSL activity (U/mL)= ($\Delta A_{text} \times C_{standard} \div \Delta A_{standard}$) $\times V_{sample} \div V_{sample} \div T \times F = 0.0208 \times \Delta A_{text} \div \Delta A_{standard} \times F$

$C_{standard}$: The concentration of sodium pyruvate standard solution, 0.625μmol/mL; V_{sample}: The sample volume added to the reaction system, 0.02mL; $V_{total\ sample}$: added extraction liquid volume, 1mL; T: Reaction time, 30min; Cpr: Protein concentration, mg/mL; W: Sample quality, g; F: Sample dilution ratio.

Note:

1. If the test result ΔA is > 1, the sample can be diluted with distilled water or the first step reaction time can be shortened; If the light absorption value is small, the sample size can be increased or the reaction time of the first step can be extended to 1h or longer. Change the calculation formula during calculation.
2. For the initial text of the sample, it is recommended to use distilled water for gradient dilution of the sample homogenate to determine the best dilution multiple. It is recommended to directly dilute the sample of onion, mushroom and garlic 4-10 times before exploring the optimal dilution ratio. (Lab Onions were diluted 8 times and garlic 64 times.)

Experimental example:

1. Weigh 0.1233g garlic sample, add the extract liquid for ice bath homogenization, dilute the supernatant 64 times with distilled water, follow the measurement procedure, use 1mL glass cuvette to measure $\Delta A_{text} = A_{text} - A_{control} = 0.6899-0.184 = 0.505$, $\Delta A_{standard} = A_{standard} - A_{standard\ blank} = 0.738-0.026 = 0.712$, put into the formula calculation:
   CSL activity (U/g mass) = $0.0208 \times \Delta A_{text} \div \Delta A_{standard} \div W \times F = 7.658$ U/g mass.
2. Weigh 0.1263g onion sample, add the extraction liquid for ice bath homogenization, dilute the superserum 4 times, follow the measurement procedure, use 1mL glass cuvette to measure $\Delta A_{text} = A_{text} - A_{control} = 0.66-0.411 = 0.249$, $\Delta A_{standard} = A_{standard} - A_{standard\ blank} = 0.738-0.026 = 0.712$, put into the formula calculation:
   CSL activity (U/g mass) = $0.0208 \times \Delta A_{text} \div \Delta A_{standard} \div W \times F = 0.230$ U/g mass.

References:

[1] Su Qianqian, Tang Jing, Zhao Liyan, et al. Effects of nanocomposite packaging on the quality and formaldehyde content of shiitake mushrooms during storage [J]. Food Science, 2015(8):6.

[2] Kumagai H, †Hidetoshi KONO, Sakurai H, et al. Comparison of C-S Lyase in Lentinus edodes and Allium sativum [J]. Bioscience, Biotechnology, and Biochemistry, 2022(66): 2560–2566.