# Cysteinyl Sulfoxide Lyase（CSL）Activity text Kit

**Detection Equipment:** Spectrophotometer/Microplate Reader

**Catalog Number:** BC5395

**Size:** 100T/48S

**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 60 mL×1 | 2-8°C storage |
| Reagent I | Liquid 1.5 mL×1 | 2-8°C storage |
| Reagent II | Powder ×1 | 2-8°C storage |
| Reagent III | Liquid 3 mL×1 | 2-8°C storage |
| Reagent IV | Liquid 3 mL×1 | 2-8°C storage |
| Reagent V | Liquid 12 mL×1 | 2-8°C storage |
| Standard | Liquid 1 mL×1 | 2-8°C storage |

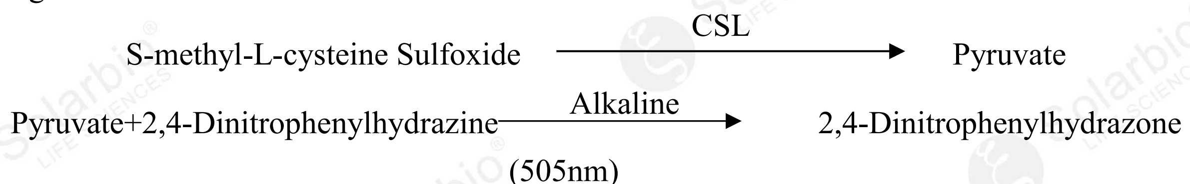
Solution reparation :

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°C 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/microplate reader, desk centrifuge, Water bath / constant temperature incubator ， adjustable pipette, micro glass cuvette/96 well flat-bottom plate, 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℃, 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 (Add the following reagents to 1.5mLEP tube or 96 well flat-bottom plate) :

| Reagent name （μL） | Test tube | Control tube | Standard tube | Standard blank tube |
|---|---|---|---|---|
| Sample | 20 | 20 | - | - |
| Standard | - | - | 20 | - |
| Reagent I | 20 | - | - | - |
| Reagent II working solution | 20 | 20 | 20 | 20 |
| Distilled water | - | 20 | 20 | 40 |
| After mixing, reaction at 37℃ for 30min | | | | |
| Reagent III | 20 | 20 | 20 | 20 |
| Reagent IV | 20 | 20 | 20 | 20 |
| After mixing, reaction at 37℃ for 30min | | | | |
| Reagent V | 100 | 100 | 100 | 100 |

Mix well and leave at room temperature for 10min. The absorbance of each tube was measured at a wavelength of 505nm. 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) = ($\Delta A_{text}$ ×$C_{standard}$ ÷$\Delta A_{standard}$) ×$V_{sample}$ ÷ ($V_{sample}$ ×Cpr) ÷T×F=0.0208×$\Delta A_{text}$ ÷$\Delta A_{standard}$ ÷Cpr ×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) = ($\Delta A_{text}$ ×$C_{standard}$ ÷$\Delta A_{standard}$) ×$V_{sample}$ ÷ ($V_{sample}$ ÷$V_{sample\ total}$ ×W) ÷T×F=0.0208×$\Delta A_{text}$ ÷$\Delta A_{standard}$ ÷W×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)= ($\Delta A_{text}$ ×$C_{standard}$ ÷$\Delta A_{standard}$) ×$V_{sample}$ ÷ $V_{sample}$ ÷ T×F=0.0208×$\Delta A_{text}$ ÷$\Delta A_{standard}$×F

$C_{standard}$: The concentration of sodium pyruvate standard solution, 0.625μmol/mL; $V_{sample}$: The sample volume added to the reaction system, 0.1mL; $V_{total\ sample}$: 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 determination 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 to the ice bath homogenate, dilute the supernatant with distilled water 64 times, according to the text steps, and calculate $\Delta A_{text}$ =$A_{text}$ -$A_{control}$ =0.46-0.113=0.347, $\Delta A_{standard}$ =$A_{standard}$ -$A_{standard\ blank}$ =0.551-0.064=0.487 by using 96-well plate. Put into the formula to calculate:
   CSL activity (U/g mass) = 0.0208×$\Delta A_{text}$ ÷$\Delta A_{standard}$ ÷W×F=7.693 U/g mass.
2. Weigh 0.1263g onion sample, add the extract for ice bath homogenate, dilute the supernatant 4 times, and follow the text steps. Calculate $\Delta A_{test}$ =$A_{test}$ -$A_{control}$ =0.477-0.382=0.095, $\Delta A_{standard}$ =$A_{standard}$ -$A_{standard\ blank}$ =0.551-0.056=0.487 by using 96-well plate. Put into the formula to calculate:
   CSL activity (U/g mass) = 0.0208×$\Delta A_{text}$ ÷$\Delta A_{standard}$ ÷W×F=0.169 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.