

## 1-Pyrroline-5-Carboxylic Acid Synthetase (P5CS) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Detection equipment:** Spectrophotometer/microplate reader

**Cat No:** AK0064

**Size:** 100T/96S

### Components:

Extract solution I: 110 mL×1, store at 4°C .

Extract solution II: 1.1 mL×1, store at -20°C .

Reagent I: 15 mL×1, store at 4°C .

Reagent II: 5 mL×1, store at 4°C .

Reagent III: Powder×3, store at -20°C . Add 5 mL of distilled water when the solution will be used. Unused reagent can store at -20C for one month after packing.

Reagent IV: Powder×1, store at -20°C . Add 3 mL of distilled water when the solution will be used. Unused reagent can store at -20C for one month after packing.

### Description:

Under different stress conditions such as drought, salinization, heavy metals, and ultraviolet rays, it can induce the accumulation of proline in plants and protect the plants. In higher plants, there are two pathways for proline synthesis, with glutamate and ornithine as precursors. The glutamate pathway is mainly responsible for the accumulation of proline under stress conditions, while the ornithine pathway works mainly under nitrogen-rich conditions and has nothing to do with the accumulation of proline under stress conditions.

1-Pyrroline-5-carboxylic acid synthetase (P5CS) is a key enzyme in the glutamate synthesis pathway of proline. P5CS is a bifunctional enzyme that catalyzes glutamate phosphorylation under the action of NADPH and ATP And glutamic acid  $\gamma$  - semialdehyde reduction, the activity of P5CS can be calculated by measuring the change in absorbance of NADPH at 340 nm.

### Required but not provided

Spectrophotometer/microplate reader, low temperature centrifuge, water-bath, transferpettor, micro quartz cuvette/96 UV well plate, homogenizer, ice, distilled water and EP tubes.

### Protocol:

#### I. Crude enzyme extraction:

The mass of tissue (g): the volume of extract (mL) is 1:5~10(it is suggested to take about 0.1 g of tissue, add 1 mL of Extract solution I and 10  $\mu$ L of Extract solution II), fully grinding on ice. Centrifuge at 8000

rpm for 15 minutes at 4C, take the supernatant and place it on ice for test.

## II. Procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 340 nm, set zero with distilled water.
2. Place Reagent III and Reagent IV on ice for test.
3. Procedure test

Reagent (μL)	Blank tube (B)	Test tube (T)
Distilled water	20	-
Sample	-	20
Reagent I	120	120
Reagent II	20	20
Reagent III	20	20
Reagent IV	20	20

Add reagents to micro quartz cuvette or 96 UV well plate orderly, mix thoroughly. Detect the absorbance at 340 nm at the time of 10 seconds record as A1. Then place dishes with the reaction solution in a 37Cwater bath or incubator for 5 minutes. Take it out and wipe it clean, immediately measure the absorbance at the time of 310 seconds which record as A2.  $\Delta A_T = A_{T1} - A_{T2}$ ,  $\Delta A_B = A_{B1} - A_{B2}$ ,  $\Delta A = \Delta A_T - \Delta A_B$ ,

**Note:**The Blank tube only needs to be measured one or twice.

## III. Calculations of P5CS activity :

1. Protein concentration:

Unit definition: One unit of P5CS activity is defined as the amount of enzyme that per milligram of protein oxidation 1 mmol of NADPH per minute in the reaction system.

$$P5CS (U/mg \text{ prot}) = \Delta A \div \epsilon \div d \times V_{RV} \times 10^9 \div (V_s \times C_{pr}) \div T = 321.54 \times \Delta A \div C_{pr} \div d$$

2. Sample weight:

Unit definition: One unit of P5CS activity is defined as the amount of enzyme that per gram of tissue oxidation 1 mmol of NADPH per minute in the reaction system.

$$P5CS (U/ g \text{ weight}) = \Delta A \div \epsilon \div d \times V_{RV} \times 10^9 \div (W \times V_s \div V_{RV}) \div T = 324.76 \times \Delta A \div W \div d$$

$V_{RV}$ : Total reaction volume, 0.2 mL;

$\epsilon$ : Molar extinction coefficient,  $6.22 \times 10^3 \text{ L/mol/cm}$ ;

$d$ : Micro quartz cuvette light path, 1 cm; 96-well UV plate light path, 0.6 cm;

$V_s$ : Sample volume, 0.02 mL;

$V_E$ : Extract solution volume, 1.01 mL;

$T$ : Reaction time(min), 5 minutes;

$C_{pr}$ : Sample protein concentration, mg/mL;

$W$ : Sample weight, g.

### Note:

1. If  $A1 > 1.2$  or  $\Delta A > 0.5$ , please dilute the sample to appropriate concentration, multiply dilute times in the formular.

2. Try to use fresh samples for testing, please keep on ice during operation.
3.  $\Delta A_B$  generally does not exceed 0.05.

**Experimental Examples:**

1. Take 0.1g of Shamrock sample, Process the sample, diluted 2 times, carry out the determination according to the operation steps. The calculation is:  $\Delta A_t = A_{1t} - A_{2t} = 1.0302 - 0.9842 = 0.046$  ,  $\Delta A_b = A_{1b} - A_{2b} = 0.7431 - 0.7248 = 0.0183$  ,  $\Delta A = \Delta A_t - \Delta A_b = 0.0277$  , calculate the enzyme activity according to sample weight:

1-Pyrroline-5-Carboxylic Acid Synthetase Activity (U/g weight) =  $324.76 \times \Delta A \div W = 179.9$  U/g weight。

**Related Products:**

AK0564/AK0563 Proline(PRO) Content Assay Kit

AK0417/AK0416 Glutamic Acid(Glu) Content Assay Kit

AK0069/AK0068 Nithine- $\delta$ -aminotransferase( $\delta$ -OAT) Activity Assay Kit

AK0600/AK0599 Glutamate Synthase(GOGAT) Activity Assay Kit

AK0423/AK0422 Glutamic-pyruvic Transaminase(GPT) Activity Assay Kit