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

Note: Take two or three different samples for prediction before test.
Detection equipment: Spectrophotometer
Cat No: BC4420
Size: 50T/48S

Components:
Extract solution I: 60 mL×1, store at 4℃.
Extract solution II: 0.6 mL×1, store at -20℃.
Reagent I: 40 mL×1, store at 4℃.
Reagent II: 6 mL×1, store at 4℃.
Reagent III: Powder×2, store at -20℃. Add 2.5 mL of distilled water when the solution will be used. Unused reagent can store at -20℃ for one week after packing.
Reagent IV: Powder×2, store at -20℃. Add 3 mL of distilled water when the solution will be used. Unused reagent can store at -20℃ for one week 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 γ - 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, low temperature centrifuge, water-bath, transferpettor, 1 mL quartz cuvette, 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 μL of Extract solution II), fully grinding on ice. Centrifuge at 8000 rpm for 15 minutes at 4℃, 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	100	-
Sample	-	100
Reagent I	600	600
Reagent II	100	100
Reagent III	100	100
Reagent IV	100	100
Add reagents to 1 mL micro quartz cuvette 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 37 ℃ water 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 prot）$=\Delta A\div\varepsilon\div d\times V_{RV}\times10^9\div(Vs\times Cpr)\div T=321.54\times\Delta A\div Cpr$

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 weight）$=\Delta A\div\varepsilon\div d\times V_{RV}\times10^9\div(W\times Vs\div V_E)\div T=324.76\times\Delta A\div W$

V_{RV}: Total reaction volume, 1 mL;

ε: Molar extinction coefficient, 6.22×10^3L/mol/cm;

d: Cuvette light diameter(cm), 1 cm;

Vs: Sample volume, 0.1 mL;

V_E: Extract solution volume, 1.01 mL;

T: Reaction time(min), 5 minutes;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g.

Note:

1. If A1>1.2 or ΔA>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. ΔA_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: ΔAt= A1t-A2t=0.923-0.895=0.028，ΔAb=A1b-A2b=0.667-0.659=0.008， ΔA=ΔAt-ΔAb=0.02，， calculate the enzyme activity according to sample weight:

1-Pyrroline-5-Carboxylic Acid Synthetase Activity（U/g weight）=324.76×ΔA÷W×diluted times=129.9 U/g weight。

Related Products:

BC0290/BC0295 Proline(PRO) Content Assay Kit
BC1580/BC1585 Glutamic Acid(Glu) Content Assay Kit
BC4400/BC4405 Nithine-δ-aminotransferase(δ-OAT) Activity Assay Kit
BC0070/BC0075 Glutamate Synthase(GOGAT) Activity Assay Kit
BC1550/BC1555 Glutamic-pyruvic Transaminase(GPT) Activity Assay Kit