4-Coumaric Acid: Coenzyme A Ligase (4CL) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC4225

Size:100T/96S

Components:

Extract solution: 60 mL×2 bottle, stored at 4℃ (suspension, use after mixing).

Reagent I: 15 mL×1 bottle, stored at 4℃.

Reagent II: powder×1 bottle, stored at -20℃. Add 3 mL of distilled water to dissolve before use, and it can be stored at -20℃ after sub packaging to avoid repeated freezing and thawing.

Reagent III: 3 mL×1 bottle, stored at 4℃.

Reagent IV: Powder×1 bottle, stored at -20℃. Before use, dissolve it with 4 mL of distilled water for standby, and it can be stored at -20℃ after sub charging to avoid repeated freezing and thawing.

Reagent V: Powder×1 tube, stored at 4℃. Add 1 mL of ethyl alcohol to dissolve. Dilute 40 times with distilled water for standby, prepare when the solution will be used.

Product Description:

4-Coumaric Acid: Coenzyme A Ligase (4-coumarate: CoA ligase, 4CL) is one of the key enzymes in lignin biosynthesis. It mainly catalyzes cinnamic acid and its hydroxy or methoxy derivatives to produce corresponding coenzyme A esters. These intermediates then enter the pathway of synthesis of phenylpropanoid derivatives. This enzyme mainly exists in higher plants, yeasts and fungi. The study of this enzyme can explore the metabolic mechanism of lignin deposition during the development of various biological cells, and provide the basis for reducing the content of fruit stone cells and improving their quality.

4-coumaric acid and CoA are catalyzed by 4CL to form 4-coumaric acid CoA, which has a characteristic absorption peak at 333 nm. The formation rate of 4-coumaric acid CoA can reflect 4CL activity.

Reagents and Equipment Required but Not Provided

Spectrophotometer/microplate reader, table type low temperature centrifuge, water bath, micro quartz cuvette/96 well flat-bottom (UV) plate, adjustable pipette, mortar/homogenizer, EP tube, ethyl alcohol, ice and distilled water.

Procedure

I. Extraction of crude enzyme solution:

1. Bacteria/cultured cells:

First collect bacteria/cells into the centrifuge tube, and then discard the supernatant. The number of bacteria/cells (10^4): the volume of the extract solution (mL) is 500-1000:1 (it is recommended to

add 1 mL of the extract solution to 5 million bacteria/cells), ultrasound breaks bacteria/cells (ice bath, power 20% or 200W, ultrasound 3 s, interval 10 s, repeat 30 times). Centrifuge at 8000 ×g for 10 minutes at 4℃, take the supernatant and put it on ice for testing.

2. Tissue: the proportion of tissue mass (g): the volume of extract (mL) of 1:5~10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of extract solution), homogenate at ice. After centrifuge at 8000×g for 10 minutes at 4℃, the supernatant is taken and placed on ice for testing.

II. Test procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 min, adjust the wavelength to 333 nm, and adjust to zero with the distilled water.

2. Preparation of working solution: prepare the working solution according to the volume ratio of Reagent II: Reagent III: Reagent IV: Reagent V=1:1:1:1, which is now in use.

3. Operation table: carry out the following operations in 0.5 mL EP tube:

Reagent Name (μL)	Test Tube (A_T)	Blank Tube (A_B)
Sample	20	-
Distilled water	-	20
Working solution	80	80
Reagent I	100	100
Measure the initial value A1 at 333 nm after fully mixing, measure the absorbance A2 again after reaction at 37℃ for 1 hour, calculate the $\Delta A_T = A2_T - A1_T$, $\Delta A_B = A2_B - A1_B$, $\Delta A = \Delta A_T - \Delta A_B$.		

III. Calculation of 4CL:

1. Calculation by micro quartz cuvette

(1) Calculated by tissue protein concentration:

Definition of enzyme activity: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol of 4-coumaric acid CoA per minute per milligram of tissue protein.

4CL (U/mg prot)=$[\Delta A \times V_{RT} \div (\varepsilon \times d) \times 10^9] \div (V_S \times Cpr) \div T = 476.19 \times \Delta A \div Cpr$.

(2) Calculated by the quality of tissue samples:

Definition of enzyme activity: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol of 4-coumaric acid CoA per minute per gram of tissue.

4CL activity (U/g fresh weight) =$[\Delta A \times V_{RT} \div (\varepsilon \times d) \times 10^9] \div (W \times V_S \div V_{ST}) \div T = 476.19 \times \Delta A \div W$

(3) By bacterial/cell density:

Definition of enzyme activity: One unit of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol of 4-coumaric acid CoA per minute per 10000 bacterial/cell.

4CL activity (U/10^4 cell)=$[\Delta A \times V_{RT} \div (\varepsilon \times d) \times 10^9] \div (500 \times V_S \div V_{ST}) \div T = 0.9124 \times \Delta A$

V_{RT}: Total volume of reaction system, 2×10^{-4} L;

ε: Molar extinction coefficient of 4-coumaric acid coenzyme A, 2.1×10^4 L/mol/cm;

d: Light diameter of cuvette, 1 cm;

V_S: Added sample volume, 0.02 mL;

V_{ST}: Added extract volume, 1 mL;

T: Reaction time, 1 hour;

Cpr: Sample protein concentration, mg/mL;

W: Sample mass, g;

500: Total bacteria/cells, 5 million.

2. Calculated by 96 well (UV) plate

Change the d=1 cm in the above formula to 0.6 cm (the optical diameter of 96 well plate) for calculation.

Note:

1. If ΔA is greater than 0.5, dilute the crude enzyme solution with distilled water and conduct the determination.
2. It is suggested that too many samples should not be determined at one time to avoid too much time delay of enzymatic reaction.
3. The blank tube is a test tube for testing the quality of each reagent component. Under the normal conditions, the change does not exceed 0.01.

Experimental examples:

1. Take 0.1 g of shepherd's purse and add 1 mL of Extract solution for sample processing. After centrifugation to take the supernatant, proceed according to the determination procedure. After determination with micro quartz cuvette, calculate $\Delta A_T=A2_T-A1_T=0.7425-0.5851=0.1574$, $\Delta A_B=A2_B-A1_B=0$, $\Delta A=\Delta A_T-\Delta A_B=0.1574$. The enzyme activity is calculated according to the sample mass.

4CL activity (U/g fresh weight) =476.49×ΔA÷W=749.52 U/g fresh weight.

Related products:

BC2010/BC2015	Glycollic Oxidase Activity Assay Kit
BC4170/BC4175	Cinnamyl Alcohol dehydrogenase Dehydrogenase(CAD) Activity Assay Kit
BC4180/BC4185	Shikimic acid Dehydrogenase(SD) Activity Assay Kit
BC4200/BC4205	Lignin Activity Assay Kit