Cinnamate-4-hydroxylase (C4H) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/microplate reader

Cat No: BC4085

Size: 100T/96S

Components:

Extract solution: Liquid 100 mL×1, store at 4℃;

Reagent I: Liquid 20 mL×1, store at 4℃;

Reagent II: Powder×2, store at 4℃. Add 2 mL ethanol **(self-provided reagent)** before use. Fully dissolved. The unused reagent can be stored 4 weeks in 4℃.

Reagent III: Powder×2, store at 4℃. Add 2 mL distilled water when the solution will be used. Mix thoroughly. The unused reagent can be stored 4 weeks in -20℃.

Product Description:

C4H is also called trans cinnamic acid-4-monooxygenase. It is an enzyme that catalyzes cinnamic acid to form coffee bean and coumaric acid. C4H mainly exists in higher plants, yeasts and fungi. It is a key enzyme in the process of lignin synthesis.

C4H catalyzes the cinnamic acid and NADPH to form 4-coumarite and NADP. The decrease rate of NADPH at 340nm can reflect the activity of C4H.

Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, water-bath, transferpettor, micro quartz cuvette/96 well flat-bottom UV plate, mortar/homogenizer, ethanol, ice and distilled water.

Protocol

I. Preparation:

1. Tissue:

Add 1 mL of extract reagent to 0.1 g of tissue. Homogenate on ice. Centrifuge at 12000 g 4℃ for 15 minutes. Take the supernatant on ice for test.

2. Cells or bacterial

Collect bacteria or cells into the centrifuge tube. Discard the supernatant after centrifugation. It is suggested to take about 5 million bacteria/cell and add 1 mL extract reagent. Bacteria/cell is split by ultrasonication (power 20%, ultrasonic 3s, interval 10s, repeat for 30 times). Centrifuge at 12000 g 4℃ for 15 minutes. Take the supernatant on ice for test.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 340 nm, set the counter to zero with distilled water.

2. Operation table: add the following reagents to the micro quartz cuvette/96 well UV plate

Reagent Name (μL)	Test tube (A_T)

Reagent I	140
Reagent II	20
Reagent III	20
Sample	20

Mix thoroughly. The absorbance at 340nm for 10s is recorded as A1. Then put it into a 37℃-water bath or 37℃-incubator for 3 min. (If the microplate reader has temperature control function, adjust the temperature to 37℃). Then take it out and wipe it out quickly. Measure the absorbance at 190s, and record it as A2. ΔA=A1-A2.

III. C4H Calculation:

a. Micro quartz cuvette

1. Protein concentration:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the consumption of 1 nmol NADPH per minute every mg tissue protein in the reaction system.

C4H (U/mg prot) =[ΔA÷(ε×d)×V_{RT}]÷(V_S×Cpr)÷T= 535.91×ΔA÷Cpr

2. Sample weight:

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the consumption of 1 nmol NADPH per minute every gram tissue weight in the reaction system.

C4H (U/g weight) =[ΔA÷(ε×d)×V_{RT}]÷(W÷V_E×V_S)÷T=535.91×ΔA÷W

3. Cells or bacterial

Unit definition: One unit of enzyme is defined as the amount of enzyme that catalyzes the consumption of 1 nmol NADPH per minute every 10^4 cells or bacterial in the reaction system.

C4H (U/10^4 cell) =[ΔA÷(ε×d)×V_{RT}]÷(500×V_S÷V_E)÷T=1.072×ΔA

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

d: Light path of cuvette, 1 cm;

V_{RT}: Total reaction volume, 0.0002 L;

V_S: Sample volume, 0.02 mL;

V_E: Extract solution volume of cells, 1 mL;

500: Cells or germ, 5 million;

T: Reaction time, 3 minutes;

Cpr: Protein concentration, mg/mL;

b. 96 well UV plate

The optical diameter d=1 cm of the cuvette in the above formula is changed to 0.6 cm of the 96 well UV plate.

Note:

1. When ΔA is greater than 0.4, it is recommended to dilute the sample with extraction solution. then measure it. When ΔA is too small, it is recommended to increase the enzymatic reaction time (5 min or 10 min) or add the volume of sample to determine.

Experimental Examples:

1. Take 0.1g of soybeans (germinated) and add 1mL extract to homogenize and grind, take the supernatant and operate according to the measurement procedure, measure by the micro quartz cuvette and calculate ΔA=A1-A2=1.1227-0.9854=0.1373, calculate the enzyme based on the sample weight:

C4H Activity（U/g weight）=535.91×ΔA÷W=535.91×0.1373÷0.1=735.80 U/g weight。

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