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Cinnamyl Alcohol Dehydrogenase (CAD) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: AK0107

Size:100T/96S

Components:

Extract solution: Liquid 120 mL×1. Store at 4℃ . Contains insoluble matter, shake well before use.

Reagent I: Powder×1. Store at -20℃ . Dissolve with 5 mL of Reagent III before use. For long term preservation, separate into small tubules and storage at -20 ℃, avoid repeated freezing and thawing.

Reagent II: Liquid×1. Store at 4℃ . Dissolve with 1 mL of absolute ethanol (self-provided reagent) before use. Then transfer it all to a 15 mL brown reagent bottle (provided in the kit) and add 14 mL of absolute ethanol to mix it, store at 4℃ .

Reagent III: Liquid 25 mL×1. Store at 4℃ .

Product Description:

Cinnamyl alcohol dehydrogenase (CAD) is one of the key enzymes in the lignin biosynthetic pathway, which catalyzes the formation of cinnamyl alcohol that corresponds to coumarin, mustard aldehyde and coniferaldehyde. CAD is mostly found in higher plants, yeast, and bacteria, the study of this enzyme can explore the metabolic mechanism of lignin deposition during the development of various biological cells, and provide a basis for reducing the content of fruit stone cells and improving their quality.

CAD catalyzes cinnamaldehyde and NADPH to cinnamyl alcohol and $NADP^+$. In this kit, the activity of CAD is determined by the decrease rate of NADPH at 340 nm.

Reagents and Equipment Required but Not Provided:

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

Procedure:

I. Sample preparation:

1. Tissue

Add 1 mL of Extract solution to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

2. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, after centrifugation, discard supernatant. Suggest add 1 mL of Extract solution to 5 million of bacteria or cells. Use ultrasonication to split bacteria and cells (place on ice, ultrasonic power 20%, working time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 10000 ×g for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice

before testing.

II. Determination procedure:

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

2. Working solution: make working solution as the volume ratio of Reagent I: Reagent II: Reagent III: =1:1:2 (V:V:V), prepare the reagent when it will be use.

3. Add the following reagents

Reagent (μL)	Blank tube (B)	Test tube (T)
Sample	-	20
Distilled water	20	-
Working solution	180	180
Add the above reagents to micro quartz cuvette/96 UV well plate in order, timing after add working solution, mix thoroughly. Detect the absorbance at 340 nm at the time of 10 seconds record as A_{T1} or A_{B1}. Then place dishes with the reaction solution in a 25℃-water bath for 5 minutes. Take it out and wipe it clean, immediately measure the absorbance at the time of 310 seconds which record as A_{T2} or A_{B2} . $\Delta A_T = A_{T1}-A_{T2}$, $\Delta A_B = A_{B1}- A_{B2}$, $\Delta A=\Delta A_T-\Delta A_B$. The blank tube only needs to be tested one or two times.		

III. Calculation:

A. micro quartz cuvette

1. Protein concentration:

Unit definition: One unit of enzyme activity is the amount of enzyme that catalyzes the generation of 1 nmol of NADPH per minute every milligram of protein.

$CAD\ (U/mg\ prot)=[\Delta A\div(\varepsilon\times d)\times10^9\times Vrv]\div(Vs\times Cpr)\div T=321.54\times\Delta A\div Cpr$

2. Sample weight:

Unit definition: One unit of enzyme activity is the amount of enzyme that catalyzes the generation of 1 nmol of NADPH per minute every gram of tissue.

$CAD\ (U/g)=[\Delta A\div(\varepsilon\times d)\times10^9\times Vrv]\div(W\div Ve\times Vs)\div T=321.54\times\Delta A\div W$

3. Bacteria or cultured cells

Unit definition: One unit of enzyme activity is the amount of enzyme that catalyzes the generation of 1 nmol of NADPH per minute every 1 0000 cells or bacteria.

$CAD\ (U/10^4\ cell)=[\Delta A\div(\varepsilon\times d)\times10^9\times Vrv]\div(Vs\div Ve\times500)\div T=0.643\times\Delta A$

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

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume, 2×10^{-4} L;

Vs: Sample volume, 0.02 mL;

Ve: Extract volume, 1 mL;

Cpr: Sample protein concentration (mg/mL);

T: Reaction time, 5 minutes;

W: Sample weight(g);

500: 5 million cells or bacteria;

10^9: 1 mol =10^9 nmol.

B. 96 well UV plate

In the above formula, d- 1cm is changed to d-0.6cm (96 well plate optical diameter) for calculation.

Note:

1. If A_{T1}<0.8, please dilute the sample to appropriate concentration, multiply dilute times in the formula.
2. The blank tube is a detection hole for detecting the quality of each reagent component, and normally that the change of ΔA_B does not exceed 0.04.

Experimental Examples:

1. Take about 0. 1g of clover leaves, add 1mL of extract to homogenize in ice bath, then centrifuge at 10000g, 4℃ for 10min, take the supernatant, follow the determination procedure, calculate ΔAt=A1t-A2t=1.7889- 1.7045=0.0844,ΔAb=A1b-A2b=0.9630-0.9433=0.0197,ΔA=ΔAt-ΔAb=0.0844-0.0197=0.0647, calculated according to the sample quality:

CAD activity（U/g mass）=321.54×ΔA÷W=208.0364 U/g mass。

Related Products:

AK0246/AK0245 Isocitrate Lyase (ICL) Activity Assay Kit

AK0149/AK0148 Acetokinase (ACK) Activity Assay Kit