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Anthocyanin Reductase Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: AK0352

Size:50T/24S

Components:

Extract solution: 30mL×1. Storage at 4℃, shake thoroughly before use.

Reagent I: 50mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at 4℃, dissolve thoroughly with 3 ml of distilled water before use.

Reagent III: Powder×1. Storage at -20℃ , dissolve thoroughly with 1 ml of distilled water and 1 ml of alcohol before use. It can be stored at -20℃ after dispensing to avoid repeated freezing and thawing.

Reagent IV: 2mL×1. Storage at 4℃.

Product Description:

Anthocyanin reductase (ANR) is a key enzyme in the biosynthesis pathway of procyanidins, which converts anthocyanins into the cis-flavan-3-alcohol. It plays an important role in plants regulation.

ANR converts cyanidin chloride to flavane-3-alcohol under the action of NADPH. The activity of ANR can be reflected by measuring the reduction rate of NADPH at 340nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, low temperature centrifuge, adjustable transferpettor, water bath, 1ml quartz cuvette, mortar, ice, alcohol and distilled water.

Sample preparation:

1. Tissue: Add 1 ml of extract solution into 0. 1g of tissue, fully grinding on ice. centrifuge at 12000rpm 4℃ for 15min, supernatant on ice is used for test.
2. Cells or microbial sample: collect cells or microbial sample to centrifuge and remove supernatant. Suggested 5 million with 1mL of extract solution, split bacteria and cells with ultrasonication (power 20%, work time 3s, interval 10s, for 30 times), centrifuge at 12000rpm 4℃ for 15min, supernatant on ice is used for test.

Procedure:

1. Preheat spectrophotometer for 30min, adjust the wavelength to 340 nm, set the counter to zero with distilled water.
2. Add the following reagents to 1ml quartz cuvette:

Reagent name	Test tube (T)	Contrast tube (C)
Reagent I (μL)	850	850
Reagent II (μL)	50	50
Reagent III (μL)	25	25
Sample (μL)	50	-
Mix thoroughly at 37℃ for 30 min		

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Reagent IV (μL)	25	25
Sample (μL)	-	50

Mix thoroughly, detect absorbance of test tube and contrast tube at 340nm, named A(T), A(C), ΔA=A(C)-A(T)=A2-A1.

Calculation:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the decreasing of 1 nmol of NADPH per minutes every milligram tissue protein in the reaction system.

ANR (U/mg prot) = ΔA÷(ε×d)×10^9 ×Vrv÷(Vs×Cpr)÷T =107. 18×ΔA÷Cpr

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the decreasing of 1 nmol of NADPH per min every gram tissue in the reaction system.

ANR(U/g) =ΔA÷(ε×d)×10^9 ×Vrv÷(W÷Vsv×Vs)÷T=107. 18×ΔA÷W

3. Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the decreasing of 1 nmol of NADPH every 10^4 cells or bacteria in the r eaction system per min.

ANR(U/10^4cell) =ΔA÷(ε×d)×10^9 ×Vrv÷(500÷Vsv×Vs)÷T=0.2144×ΔA

Vrv: total reaction volume,1 mL;

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

d: light path of cuvette, 1cm;

Vs: supernatant volume (mL), 0.05 mL;

Cpr: sample protein concentration (mg/mL);

T: Reaction time (min), 30 min;

W: Sample weight(g);

Vsv: Extraction volume, 1 mL;

500: 5 million cells.

10^9 : unit conversion coefficient, 1mol = 10^9nmol

Note:

1. Dilute react mixture with reagent 1 or decrease sample volume if ΔA>0.4 or A(C)>1. Increase react time (45min or 60min) and sample volume if ΔA is too low.
2. After adding reagent 4, the determination should be completed within 15 minutes.
3. Detect sample concentrate separately.

Experimental Examples:

1. Take 0. 1g of apple and add 1mL extract to homogenize and grind, take the supernatant and operate according to the measurement procedure, ΔA=Ac-At=0.922-0.813=0. 109 , calculate the enzyme based on the sample weight:

ANR Activity（U/g weight）= 107. 18×ΔA÷W=107. 18×0. 109÷0. 1=116.83 U/g weight。

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