Anthocyanin Reductase Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC4090

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		

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), △A=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) = $\Delta A \div (\varepsilon \times d) \times 10^9 \times Vrv \div (Vs \times Cpr) \div T = 107.18 \times \Delta A \div Cpr$

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) $= \Delta A \div (\varepsilon \times d) \times 10^9 \times Vrv \div (W \div Vsv \times Vs) \div T = 107.18 \times \Delta A \div W$

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) $= \Delta A \div (\varepsilon \times d) \times 10^9 \times Vrv \div (500 \div Vsv \times Vs) \div T = 0.2144 \times \Delta A$

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 △A>0.4 or A(C)>1. Increase react time (45min or 60min) and sample volume if △A 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。

Related Products:

BC1360/BC1365 Uric Acid(UA) Content Assay Kit

BC1340/BC1345 Plant Total Phenol Content Assay Kit

BC1330/BC1335 Plant Flavonoids Content Assay Kit