

## Anthocyanin Reductase Activity Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Cat No:** NA0351

**Size:**50T/24S

### Components:

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

Reagent I: 50mL×1. Storage at 4°C.

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

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

Reagent IV: 2mL×1. Storage at 4°C.

### 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°C 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°C 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°C 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),  $\Delta A = A(C) - A(T) = A_2 - A_1$ .

### 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.

$$\text{ANR (U/mg prot)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{rv} \div (V_s \times C_{pr}) \div T = 107.18 \times \Delta A \div C_{pr}$$

#### 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.

$$\text{ANR (U/g)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{rv} \div (W \div V_{sv} \times V_s) \div T = 107.18 \times \Delta A \div W$$

#### 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<sup>4</sup> cells or bacteria in the reaction system per min.

$$\text{ANR (U/10}^4\text{cell)} = \Delta A \div (\epsilon \times d) \times 10^9 \times V_{rv} \div (500 \div V_{sv} \times V_s) \div T = 0.2144 \times \Delta A$$

$V_{rv}$ : total reaction volume, 1 mL;

$\epsilon$ : NADPH molar extinction coefficient,  $6.22 \times 10^3$  L/mol/cm;

$d$ : light path of cuvette, 1 cm;

$V_s$ : supernatant volume (mL), 0.05 mL;

$C_{pr}$ : sample protein concentration (mg/mL);

$T$ : Reaction time (min), 30 min;

$W$ : Sample weight(g);

$V_{sv}$ : Extraction volume, 1 mL;

500: 5 million cells.

$10^9$ : unit conversion coefficient,  $1 \text{ mol} = 10^9 \text{ nmol}$

### Note:

1. Dilute react mixture with reagent 1 or decrease sample volume if  $\Delta A > 0.4$  or  $A(C) > 1$ . Increase react time (45min or 60min) and sample volume if  $\Delta A$  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,  $\Delta A = A_c - A_t = 0.922 - 0.813 = 0.109$ , calculate the enzyme based on the sample weight:

$$\text{ANR Activity (U/g weight)} = 107.18 \times \Delta A \div W = 107.18 \times 0.109 \div 0.1 = 116.83 \text{ U/g weight.}$$

### Related Products:

NA0763/NA0522 Uric Acid(UA) Content Assay Kit

NA0765/NA0524 Plant Total Phenol Content Assay Kit

