EZMeta™ Anthocyanidin Reductase (ANR) Colorimetric Activity Kit

Cat #: D-AKC3010

Size: 48T / 96T

Storage: Stored at -20℃ for 6 months

Product Information

Applicable samples: Plant Tissues

Assay Principle

Anthocyanidin reductase (ANR) is a key enzyme in the biosynthesis of procyanidins, catalyzing anthocyanidins to produce cis-flavane-3-alcohols and plays an important role in the synthesis of flavonoids and accumulation of anthocyanins in plants. EZMeta™ Anthocyanidin Reductase (ANR) Colorimetric Activity Kit provides a convenient tool for detection of ANR activity. The principle is that ANR catalyzes the production of flavane-3-alcohol and NADP+ from acetyl anthocyanins and NADPH. NADPH has an absorption peak at 340 nm, but NADP+ does not. The enzyme activity of ANR was calculated by detecting the rate of decrease in absorption at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4°C
Reagent Ⅰ	10 mL	20 mL	4°C
Reagent Ⅱ	1	1	-20°C
Reagent Ⅲ	1	1	-20°C
Reagent Ⅳ	1	1	4°C

Materials Required but Not Supplied

·Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm

·96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips

·Ice maker, refrigerated centrifuge, incubator

·Deionized water, anhydrous ethanol

·Homogenizer

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Shake thoroughly before use. Store at 4°C.

Reagent Ⅰ: Ready to use as supplied. Store at 4°C.

Reagent Ⅱ: Powder. Add 1 mL deionized water for 96 T or 0.5 mL deionized water for 48 T to dissolve before use. This solution can be stored at -20°C after aliquoting to avoid repeated freezing and thawing.

ReagentⅢ: Powder. Add 1 mL 50% ethanol for 96 T or 0.5 mL 50% ethanol for 48 T to dissolve before use. This solution can be stored at -20°C after aliquoting to avoid repeated freezing and thawing.

ReagentⅣ: Powder. Add 1 mL deionized water for 96 T or 0.5 mL deionized water for 48 T to dissolve before use. Store at 4°C.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for one month.

Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

Note: It will be better to quantify the total protein with EZMeta™ Anthocyanidin Reductase (ANR) Colorimetric Activity Kit, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Preheat the incubator to 37°C.

3. Add the following reagents:

Reagent	Test Well (μL)	Control Well (μL)
Reagent Ⅰ	170	170
Reagent Ⅱ	10	10
ReagentⅢ	5	5
Sample	10	0
Mix thoroughly at 37°C for 30 min		
ReagentⅣ	5	5
Sample	0	10

4. Mix thoroughly, detect absorbance of test well and contrast well at 340 nm, named A_{Test}, $A_{Control}$, $\Delta A=A_{Control}-A_{Test}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.001, increase the sample quantity appropriately. If ΔA is greater than 0.4, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula

1. Calculated by protein concentration

Unit definition: 1 nmol NADPH oxidated per min in 1 mg tissue protein reaction system is defined as a unit of enzyme

activity.

ANR (U/mg prot)=($\Delta A_{Test} \div \varepsilon \div d \times V_{Reaction\ Total} \times 10^9$)÷(Cpr×$V_{Sample}$)÷T×n=**214.36×$\Delta A_{Test}$÷Cpr×n**

2. Calculated by fresh weight of samples

Unit definition: 1 nmol NADPH oxidated per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

ANR (U/g)=($\Delta A_{Test} \div \varepsilon \div d \times V_{Reaction\ Total} \times 10^9$)÷(W×$V_{Sample}$÷$V_{Sample\ Total}$)÷T×n=**214.36×$\Delta A_{Test}$÷W×n**

Where: ε: NADPH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; $V_{Reaction\ Total}$: total reaction volume, 200 μL=2×10^{-4} L; 10^9: 1 mol=1×10^9 nmol; Cpr: sample protein concentration, mg/mL; V_{Sample}: sample volume added, 0.01 mL; T: reaction time, 30 min; n: dilution factor; W: sample weight, g; $V_{Sample\ Total}$: Extraction Buffer volume added, 1 mL.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Typical Data

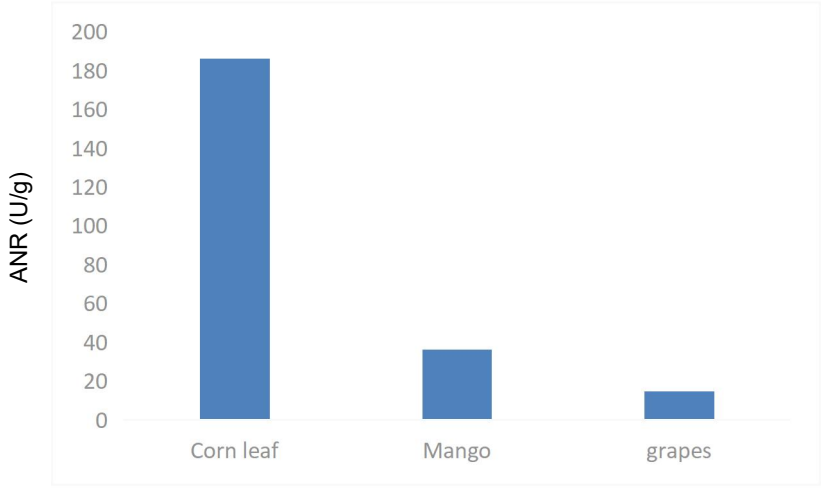

Figure 1. ANR activity in corn leaf, mango and grapes respectively. Assays were performed following kit protocol.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.