



CheKine™ Anthocyanidin reductase (ANR) Activity Colorimetric Assay Kit

Cat #: KTB3010

Size: 48 T/96 T

	Anthocyanidin reductase (ANR) Activity Colorimetric Assay Kit		
REF	Cat #: KTB3010	LOT	Lot #: Refer to product label
	Applicable samples: Plant Tissues		
	Storage: Stored at -20°C for 6 months		

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. CheKine™ Anthocyanidin reductase (ANR) Activity Colorimetric Assay 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 capable of measuring absorbance at 340 nm
- Incubator, Ice Maker, Refrigerated Centrifuge
- 96-well UV microplate, Precision Pipettes, Disposable Pipette Tips
- Deionized Water
- Anhydrous ethanol
- Dounce homogenizer (for Tissue Samples)

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.

Reagent III: 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.

Reagent IV: 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.

Assay Procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 340 nm.
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
Reagent III	5	5
Sample	10	0
Mix thoroughly at 37°C for 30 min		
Reagent IV	5	5
Sample	0	10

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

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.

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.

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

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.

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

Where: ϵ : NADPH molar extinction coefficient, 6.22×10^3 L/mol/cm; d : 96 well plate diameter, 0.5 cm; $V_{\text{Reaction Total}}$: total reaction volume, $200 \mu\text{L} = 2 \times 10^{-4}$ L; 10^9 : $1 \text{ mol} = 1 \times 10^9 \text{ nmol}$; C_{pr} : 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; $V_{\text{Sample Total}}$: Extraction Buffer volume added, 1 mL.

Typical Data

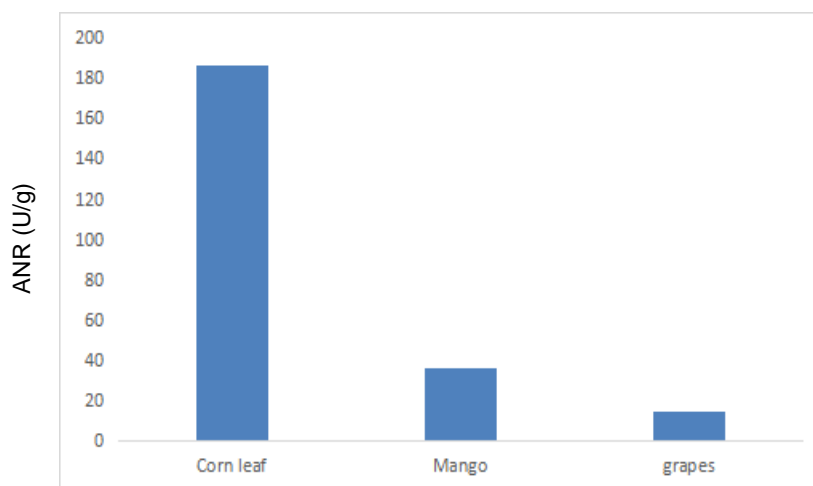


Figure. ANR Activity in Corn leaf, Mango and Grapes respectively. Assays were performed following kit protocol.

Recommended Products

Catalog No.	Product Name
KTB1541	CheKine™ Tannin Colorimetric Assay Kit
KTB1542	CheKine™ Tannase (TAN) Activity Colorimetric Assay Kit

Disclaimer

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