EZMeta™ Dehydroascorbic Acid (DHA) Colorimetric Assay Kit

Cat #: D-AKC3090

Size: 96T

Storage: Stored at -20℃ for 6 months, protected from light

Product Information

Applicable samples: Plant Tissues

Assay Principle

AsA is an important physiological index of plant cells. Changes in AsA content, REDOX status (AsA/DHA ratio), and the activities of enzymes related to its synthesis and metabolism are involved in plant responses to a series of environmental stresses. DHA is a reversibly oxidized form of AsA. In vivo, DHA, together with ascorbic acid, forms a REDOX system and acts as an electron acceptor. EZMeta™ Dehydroascorbic Acid (DHA) Colorimetric Assay Kit provides a simple assay to measure DHA activity in biological samples such as plant tissues. DTT reduced DHA to produce AsA, and the DHA content could be calculated by measuring the rate of AsA formation in the system.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	96 T	
Extraction Buffer	100 mL	4°C
Reagent Ⅰ	20 mL	4°C
Reagent Ⅱ	1	-20°C, protected from light
Standard	1	-20°C, protected from light

Materials Required but Not Supplied

·Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 265 nm

·Incubator, freezing centrifuge

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

·Deionized water

·Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent Ⅱ: Prepared before use, add 5 mL deionized water to fully dissolve. Stored aliquots at -20°C, protected from light, and avoid repeated freezing and thawing.

Standard: Prepare before use, add 5.743 mL deionized water to fully dissolve, the concentration of DHA is 1 μmol/mL. Stored aliquots at -20°C, protected from light, and avoid repeated freezing and thawing.

Sample Preparation

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

1. Plant tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 16,000 g for 20 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

Note: If the protein concentration of the sample is need to be determined, it is recommended to use EZMeta™ Dehydroascorbic Acid (DHA) Colorimetric Assay Kit to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 265 nm, Ultraviolet spectrophotometer was returned to zero with deionized water.

2. Incubate Reagent Ⅰ at 25°C for 30 min.

3. In 96-well UV plate or microquartz cuvette, 20 μL Standard, 160 μL Reagent Ⅰ, and 20 μL Reagent Ⅱ were added into the standard tube, mix quickly. Measure the absorbance value at 265 nm with a microplate reader, record 10 s absorbance value as A_1 and the absorbance value at 2 min 10 s as A_2, and calculate $\Delta A_{Standard}=A_2-A_1$.

4. In 96-well UV plate or microquartz cuvette, 20 μL Sample, 160 μL Reagent Ⅰ, and 20 μL Reagent Ⅱ were added into the test tube, mix quickly. Measure the absorbance value at 265 nm with a microplate reader, record 10 s absorbance value as A_3 and the absorbance value at 2 min 10 s as A_4, and calculate $\Delta A_{Test}=A_4-A_3$.

Note: (1) In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 large expected difference samples. (2) Because the enzyme activity is calculated based on the reaction rate, in order to ensure that the reaction time of each sample is as consistent as possible, it is not recommended to test too many samples at the same time. (3) If ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1, 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 as below

1. Calculated by protein concentration

DHA(nmol/mg prot)=$(C_{Standard}\times\Delta A_{Test}\div\Delta A_{Standard})\div Cpr$=**$1{,}000\times\Delta A_{Test}\div\Delta A_{Standard}\div Cpr$**

2. Calculated by sample fresh weight

DHA(nmol/g fresh weight)=($C_{Standard}$×ΔA_{Test}÷$\Delta A_{Standard}$)×$V_{Total\ Sample}$÷W=**1,000×ΔA_{Test}÷$\Delta A_{Standard}$÷W**

$C_{Standard}$: DHA concentration, 1 μmol/mL=1,000 nmol/mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr; sample protein concentration, mg/mL; W: sample weight, g.

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.

Disclaimer

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