EZMeta™ Ascorbic Acid (AsA) Colorimetric Assay Kit

Cat #: D-AKC3092

Size: 48T / 96T

Storage: Stored at 4°C for 6 months, protected from light

Product Information

Detection range: 6.25-2,000 μmol/L

Applicable samples: Animal and Plant Tissues, Cells, Serum and other fluids

Assay Principle

Ascorbic acid (AsA), also known as vitamin C, is a coenzyme, free radical scavenger, electron community/acceptor, and substrate for oxalate and tartrate biosynthesis. As the most important antioxidant in plant cells, AsA plays an important role in protecting chloroplasts from oxidative damage and is one of the important indicators to measure the quality of crop products. EZMeta™ Ascorbic Acid (AsA) Colorimetric Assay Kit provides a simple test to detect the AsA content in biological samples such as animal and plant tissues, cells, blood serum and other fluids. Ascorbate oxidase (AAO) catalyzes the oxidation of AsA to DHA, and the AsA content can be calculated by measuring the oxidation rate of AsA.

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	10 μL	-20°C, protected from light
Standard	1	4°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.

Working Reagent Ⅱ: Prepared before use. According to the dosage, the mixture was fully mixed according to the volume ratio of Reagent Ⅱ: Reagent Ⅰ is 1:250 and used immediately. Stored at -20°C, protected from light.

Standard: Prepare before use, add 5.679 mL deionized water to fully dissolve, the concentration of AsA is 10 mmol/L. Take 40 µL solution, add 960 µL deionized water, and mix well, that is, 400 µmol/L AsA. The prepared solution can be stored at 4°C, protected from light and used within 3 days. It can also be stored at -20°C and protected from light after aliquoting to 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. Animal tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 20 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Plant tissue: 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 8,000 g for 20 min at room temperature. Use supernatant for assay, and place it on ice to be tested.

3. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 20 min at 4°C. Use supernatant for assay, and place it on ice to

be tested.

4. Serum or other liquid samples: Test directly.

Note: If the protein concentration of the sample is need to be determined, it is recommended to use EZMeta™ Ascorbic Acid (AsA) 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 Working 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 $\Delta A_{Standard}=A_1-A_2$.

4. In 96-well UV plate or microquartz cuvette, 20 μL Sample, 160 μL Reagent Ⅰ, and 20 μL Working 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 $\Delta A_{Test}=A_3-A_4$.

Note: (1) If the number of samples is large, Reagent I and Working Reagent II can be mixed and used according to the volume ratio of 8:1 to make the working solution, which can be used as required by the sample, and it is prohibited to complete the mixture at one time. The order of adding sample is 180 μL working solution, 20 μL Sample (or Standard), and the time began when the Sample (or Standard) was added. (Standard tubes only need to be measured 1-2 times). (2) In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 large expected difference samples. (3) Because the content 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. (4) 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 or microquartz cuvette calculation formula as below

1. Calculated by protein concentration

AsA(nmol/mg prot)=($C_{Standard}$×ΔA_{Test}÷$\Delta A_{Standard}$)÷Cpr=**400×ΔA_{Test}÷$\Delta A_{Standard}$÷Cpr**

2. Calculated by sample fresh weight

AsA(nmol/g fresh weight)=($C_{Standard}$×ΔA_{Test}÷$\Delta A_{Standard}$)×$V_{Total\ Sample}$÷W=**400×ΔA_{Test}÷$\Delta A_{Standard}$÷W**

3. Calculated by cell number

AsA(nmol/10^4 cell)=($C_{Standard}$×ΔA_{Test}÷$\Delta A_{Standard}$)×$V_{Total\ Sample}$÷cell number=**400×ΔA_{Test}÷$\Delta A_{Standard}$÷cell number**

4. Calculated by liquid volume

AsA(nmol/mL)=$C_{Standard}$×ΔA_{Test}÷$\Delta A_{Standard}$=**400×ΔA_{Test}÷$\Delta A_{Standard}$**

$C_{Standard}$: AsA concentration, 400 μmol/L=400 nmol/mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr; sample protein concentration, mg/mL; W: sample weight, g; Cell number: measured in 10^4, ten thousand.

Disclaimer

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