

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

Catalog No: E-BC-K864-M

Specification: 96T(80 samples)

Measuring instrument: Microplate reader(260-270nm)

Detection range: 0.03-1.00 $\mu\text{mol/mL}$

Elabsience[®] Dehydroascorbic Acid (DHA) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help:

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabsience.com

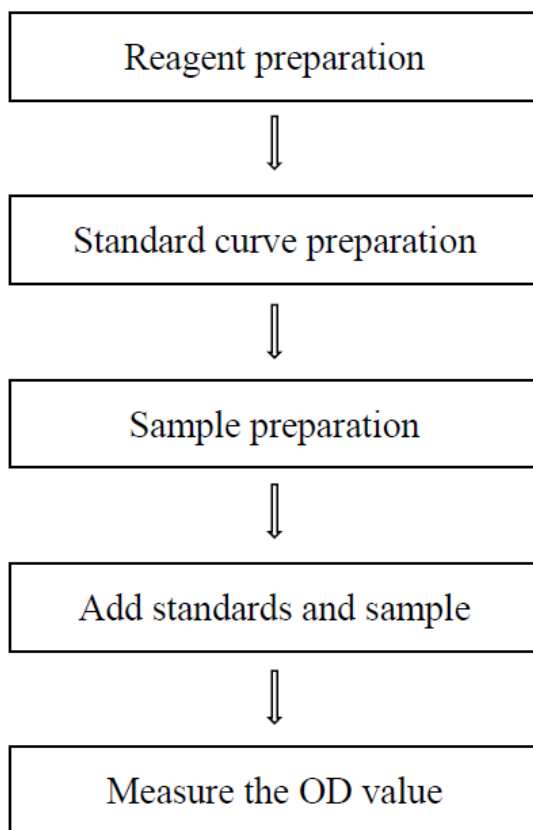
Website: www.elabsience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Assay summary



Intended use

This kit can be used to measure dehydroascorbic acid (DHA) content in plant tissue samples.

Detection principle

Ascorbic acid (AsA) is an important physiological index of plant cells. The changes of its content, redox status (AsA/DHA ratio), and the activities of enzymes related to its synthesis and metabolism are involved in the response of plants to a series of environmental stresses. Dehydroascorbic acid (DHA) is a reversible oxidized form of AsA. In vivo, dehydroascorbic acid (DHA) and ascorbic acid form a redox system and act as an electron acceptor.

The detection principle of this kit is that DTT reduces DHA to produce AsA, and the DHA content can be calculated by measuring the formation rate of AsA in the system at 265nm.

Kit components & storage

| Item | Component | Size (96 T) | Storage |
|-----------|---------------------|-----------------|-------------------------------------|
| Reagent 1 | Extraction Solution | 110 mL × 1 vial | 2-8 °C, 12 months |
| Reagent 2 | Buffer Solution | 20 mL × 1 vial | 2-8 °C, 12 months |
| Reagent 3 | Substrate | Powder × 1 vial | 2-8 °C, 12 months |
| Reagent 4 | Standard | Powder × 1 vial | 2-8 °C, 12 months, shading light |
| | UV-Microplate | 96 wells | No requirement |
| | Plate Sealer | 2 pieces | |

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain

sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (260-270 nm, optimum wavelength: 265 nm)

Reagent preparation

① Equilibrate all the reagents to 25 °C before use, buffer solution was preheated in a water bath at 25 °C for 30 min.

② The preparation of substrate working solution:

Dissolve one vial of substrate with 5 mL of double distilled water, mix well to dissolve. Aliquoted storage at -20 °C for 1 month protected from light.

③ The preparation of 1 µmol/mL standard solution:

Dissolve one vial of standard with 5.743 mL of double distilled water, mix well to dissolve. The 1 µmol/mL standard solution should be prepared on spot. Aliquoted storage at -20 °C for 10 days protected from light.

④ The preparation of standard curve:

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 µmol/mL standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 µmol/L. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|------------------------------------|----------|------------|------------|------------|------------|------------|------------|------------|
| Concentration (µmol/mL) | 0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.6 | 0.8 | 1.0 |
| 1 µmol/mL standard (µL) | 0 | 20 | 40 | 60 | 80 | 120 | 160 | 200 |
| Double distilled water (µL) | 200 | 180 | 160 | 140 | 120 | 80 | 40 | 0 |

Sample preparation

① Sample preparation:

Tissue samples:

- ① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).
- ② Homogenize 20 mg tissue in 180 μ L extraction solution with a dounce homogenizer at 4 $^{\circ}$ C.
- ③ Centrifuge at 16000 \times g for 20 min to remove insoluble material. Collect supernatant and keep it on ice for detection.
- ④ Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

| Sample type | Dilution factor |
|--|-----------------|
| 10% Photinia serrulata tissue homogenate | 2-4 |
| 10% Green pepper tissue homogenate | 1 |
| 10% Potato tissue homogenate | 1 |

Note: The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

- ① Standard well: Take 20 μL of standard solution with different concentration to the corresponding well.

Sample well: Take 20 μL of sample to the corresponding well.

- ② Add 160 μL of buffer solution to each well.
- ③ Add 20 μL of substrate working solution to each well.
- ④ Mix fully with microplate reader for 3 s. Measure the OD value of each well at 265 nm with microplate reader immediately, as A_1 . Incubate at 25 $^{\circ}\text{C}$ for 2 min protected from light. Mix fully with microplate reader for 3 s. Measure the OD value of each well at 265 nm with microplate reader immediately, as A_2 .

Calculation

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absolved OD value.
3. Plot the standard curve by using absolved OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve ($y = ax + b$) with graph software (or EXCEL).

The sample:

1. Tissue sample (Calculate for sample weight):

$$\begin{array}{c} \text{DHA content} \\ (\mu\text{mol/g wet weight}) \end{array} = (\Delta A_{265} - b) \div a \div m \times V \times f$$

2. Tissue sample (Calculate for sample protein concentration):

$$\begin{array}{c} \text{DHA content} \\ (\mu\text{mol/mgprot}) \end{array} = (\Delta A_{265} - b) \div a \div C_{pr} \times f$$

[Note]:

$$\Delta A_{265}: \Delta A_{\text{Sample}} - \Delta A_{\text{Blank}}$$

m: The weight of the sample, g

V: The volume of the extraction solution during homogenation, mL

C_{pr}: Concentration of protein in sample, mgprot/mL

f: Dilution factor of sample before test

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three potato tissue samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|----------------------------|----------|----------|----------|
| Mean($\mu\text{mol/mL}$) | 0.25 | 0.50 | 0.75 |
| %CV | 4.2 | 3.5 | 2.5 |

Inter-assay Precision

Three potato tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|-----------------------------|----------|----------|----------|
| Mean ($\mu\text{mol/mL}$) | 0.25 | 0.50 | 0.75 |
| %CV | 5.6 | 4.2 | 3.5 |

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99.3%.

| | Sample 1 | Sample 2 | Sample 3 |
|---------------------------------------|----------|----------|----------|
| Expected Conc. ($\mu\text{mol/mL}$) | 0.25 | 0.50 | 0.75 |
| Observed Conc. ($\mu\text{mol/mL}$) | 0.24 | 0.50 | 0.77 |
| Recovery rate (%) | 96.0 | 100.0 | 102.0 |

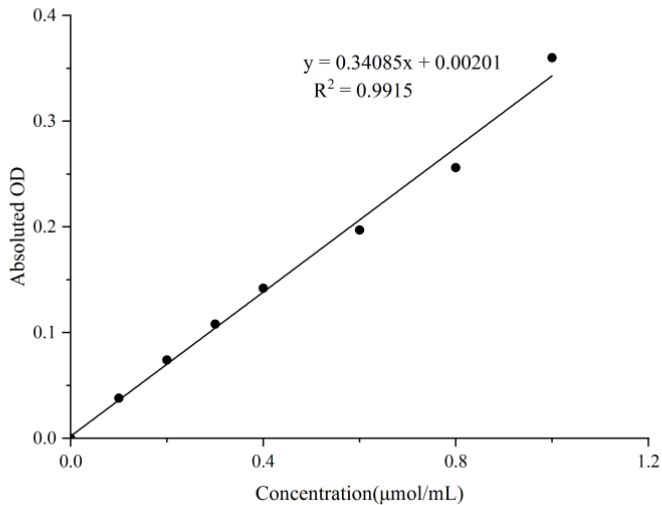
Sensitivity

The analytical sensitivity of the assay is $0.0016 \mu\text{mol/mL}$. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.

| Concentration (μmol/L) | 0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.6 | 0.8 | 1.0. |
|---|-------|-------|-------|-------|-------|-------|-------|-------|
| A ₁ | 0.071 | 0.084 | 0.097 | 0.110 | 0.121 | 0.142 | 0.170 | 0.138 |
| | 0.071 | 0.083 | 0.097 | 0.109 | 0.120 | 0.141 | 0.171 | 0.141 |
| Average A ₁ | 0.071 | 0.084 | 0.097 | 0.110 | 0.120 | 0.142 | 0.170 | 0.140 |
| A ₂ | 0.071 | 0.121 | 0.171 | 0.217 | 0.262 | 0.342 | 0.427 | 0.500 |
| | 0.071 | 0.122 | 0.171 | 0.218 | 0.262 | 0.336 | 0.424 | 0.501 |
| Average A ₂ | 0.071 | 0.122 | 0.171 | 0.218 | 0.262 | 0.339 | 0.426 | 0.500 |
| Average A ₂ - A ₁ | 0 | 0.038 | 0.074 | 0.108 | 0.142 | 0.197 | 0.256 | 0.360 |
| Absluted OD | 0 | 0.038 | 0.074 | 0.108 | 0.142 | 0.197 | 0.256 | 0.360 |



Appendix II Example Analysis

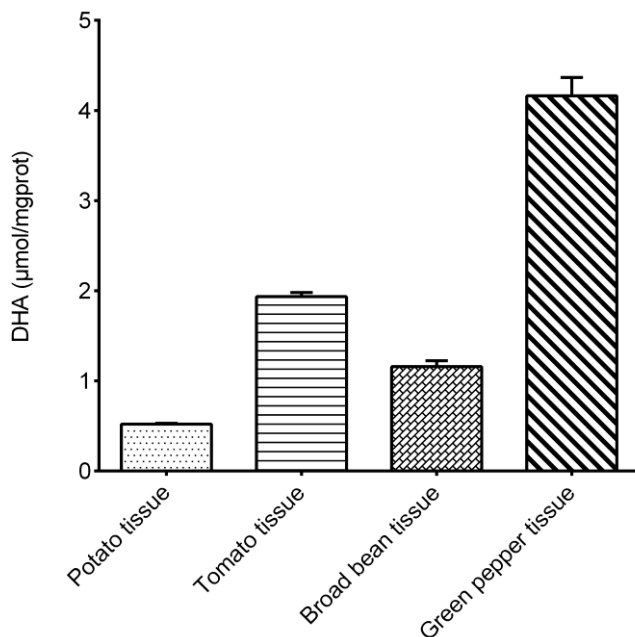
Example analysis:

Take 20 μL of 10% tomato tissue homogenate and carry the assay according to the operation steps. The results are as follows:

standard curve: $y = 0.34085x + 0.00201$, the ΔA value of the sample is 0.013, the ΔA value of the blank is 0.000, the concentration of protein is 0.017 mgprot/mL , and the calculation result is:

$$\text{DHA } (\mu\text{mol/mgprot}) = (0.013 - 0.00201) \div 0.34085 \div 0.017 = 1.90 \mu\text{mol/mgprot}$$

Detect 10% potato tissue(the concentration of protein is 0.037 mgprot/mL), 10% tomato tissue(the concentration of protein is 0.017 mgprot/mL), 10% broad bean tissue(the concentration of protein is 0.036 mgprot/mL), 10% green pepper tissue(the concentration of protein is 0.031 mgprot/mL), according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.