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

Catalog No：E-BC-K1225-M

Specification：96T(38 samples)

Measuring instrument：Microplate reader(260-270 nm)

Detection range：0.008-1 mmol/L

Elabscience® 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@elabscience.com
Website: www.elabscience.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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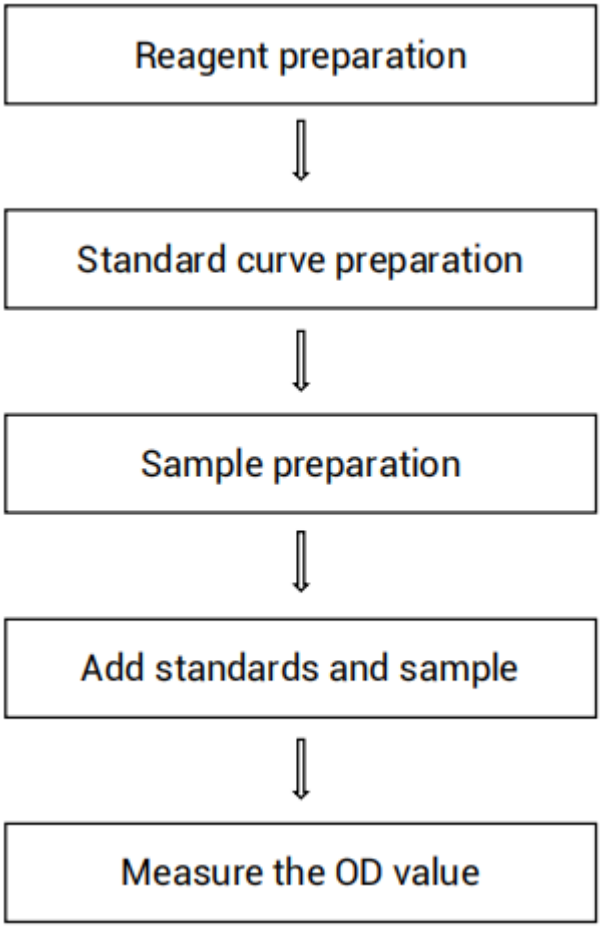
Assay summary



Intended use

The kit is suitable for detecting the content of dehydroascorbic acid (DHA) in animal tissues and plant tissues.

Detection principle

Dehydroascorbic acid (DHA) is the active oxidized form of ascorbic acid (AsA), which reversibly interconverts with the reduced form AsA through redox reactions. In living organisms, DHA and AsA form a oxidation-reduction system which is in dynamic equilibrium, playing roles in mitochondrial accumulation, metabolic coordination, and free radical regulation.

DTT reduces DHA to form ASA, which exhibits a characteristic absorption peak at 265 nm. The DHA content can be calculated by measuring the OD value at 265 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 2 vials	-20°C,12months
Reagent 2	Buffer Solution	18 mL × 1 vial	-20°C, 12 months
Reagent 3	Reducing Agent	Powder × 1 vial	-20°C, 12 months, shading light
Reagent 4	Standard	Powder × 1 vial	-20°C, 12 months, shading light
	UV-Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: All the reagents should be stored according to the table. The reagents from different kits can not be mixed or used interchangeably. For liquid

reagents with small volumes or powders, centrifuge them before use to prevent loss.

Instruments

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

Materials required but not provided

Distilled or deionized water, PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② Reducing Agent Working Solution preparation:
Dissolve one vial of Reducing Agent with 2.2 mL of distilled or deionized water. Mix well to dissolve. Stable for 7 days when stored at -20°C protected from light.

③ 1 mmol/L Standard Solution preparation：
Dissolve one vial of Standard with 5.74 mL of distilled or deionized water. Mix well to dissolve. Stable for 7 days when stored at -20°C protected from light.

④ Standard curve preparation：

Always prepare a fresh set of Standards. Discard Working Standard Dilutions after use.

Dilute 1 mmol/L Standard Solution with distilled or deionized water 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 mol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**1.0**
1 mmol/L Standard (μL)	0	20	40	60	80	120	160	200
distilled or deionized water (μL)	200	180	160	140	120	80	40	0

Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL Extraction Solution with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C. Collect supernatant and keep it on ice for detection and detect within 8 h.

Dilution of sample

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

Sample type	Dilution factor
10% Mouse kidney tissue homogenization	1
10% Mouse liver tissue homogenization	1
10% Mouse spleen tissue homogenization	1
10% Mouse heart tissue homogenization	1
10% Pineapple tissue homogenization	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: Add 20 μL of Standard Solution with different concentrations to the corresponding wells.
A well: Add 20 μL of Extraction Solution to the corresponding wells.
B well: Add 20 μL of Extraction Solution to the corresponding wells.
C well: Add 20 μL of sample to the corresponding wells.
D well: Add 20 μL of sample to the corresponding wells.

② Add 160 μL of Buffer Solution to standard wells, A wells and C wells. Add 180 μL of Buffer Solution to B wells and D wells.

③ Add 20 μL Reducing Agent Working Solution to standard wells, A wells and C wells.

④ Shake the microplate for 5 seconds to ensure complete mixing. Incubate at 25°C for 1 min. Measure the OD value of each well at 265 nm with microplate reader.

Note: For each test, A well and B well need only be tested once.

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 corrected OD value.

3. Plot the standard curve by using corrected 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:

Tissue sample:

$$\text{DHA content (μmol/g wet weight)} = (\Delta A - b) \div a \times V \div m \times f$$

[Note]

ΔA: $(OD_C - OD_D) - (OD_A - OD_B)$

V: The volume of Extraction Solution, mL.

m: The weight of tissue, g

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three pineapple 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 (mmol/L)	0.25	0.50	0.75
%CV	3.1	1.6	1.3

Inter-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.25	0.50	0.75
%CV	2.7	3.6	1.0

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 95%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.25	0.50	0.75
Observed Conc. (mmol/L)	0.24	0.47	0.70
Recovery rate (%)	96	95	94

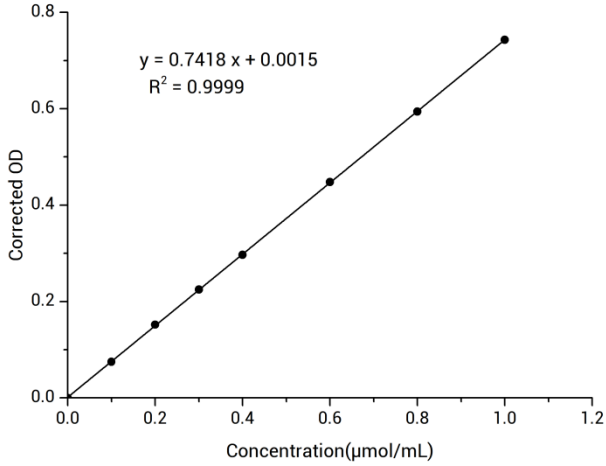
Sensitivity

The analytical sensitivity of the assay is 0.008 mmol/L. 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 (mmol/L)	0	0.1	0.2	0.3	0.4	0.6	0.8	1.0
OD	0.186	0.262	0.337	0.411	0.480	0.638	0.781	0.936
	0.187	0.261	0.340	0.412	0.486	0.630	0.779	0.923
Average OD	0.187	0.262	0.339	0.412	0.483	0.634	0.780	0.930
Corrected OD	0	0.075	0.152	0.225	0.297	0.448	0.594	0.743



Appendix Π Example Analysis

Example analysis：

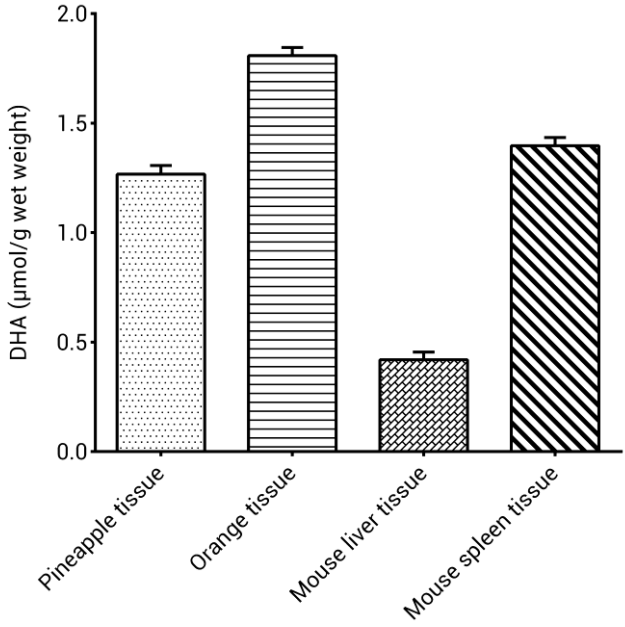
Take 20 μL of 10% pineapple tissue homogenization to the well of microplate. Proceed according to the operating steps. The results are as follows:

standard curve: y = 0.7415 x + 0.0014, the average OD value of the A well is 0.187, the average OD value of the B well is 0.165, the average OD value of the C well is 0.456, the average OD value of the D well is 0.329, ΔA = (0.456 - 0.329)-(0.187 - 0.165) = 0.105, and the calculation result is:

$$\text{DHA content (mmol/L)} = (0.105 - 0.0014) \div 0.7415 \times 0.9 \div 0.1$$

$$= 1.26\ \mu\text{mol/g wet weight}$$

Detect 10% pineapple tissue homogenate, 10% orange tissue homogenate, 10% mouse liver tissue homogenate, 10% mouse spleen tissue homogenate, 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.