TECHNICAL MANUAL

Dehydroascorbic Acid Colorimetric Assay Kit

- **SKU CODE:** MAES0527
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure absorbance values

2. Intended use

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

3. Detection principle

Ascorbic acid (AsA) is an important physiological marker in plant cells. Changes in its content, redox status (AsA/DHA ratio), and the activities of enzymes related to its synthesis and metabolism are involved in plant responses to various environmental stresses. Dehydroascorbic acid (DHA) is a reversibly 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 based on DTT reducing DHA to produce AsA. The DHA content can be calculated by measuring the formation rate of AsA in the system at 265 nm.

4. 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, protected from light
	UV-Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

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

6. Reagent preparation

1. Equilibrate all reagents to 25 °C before use. Buffer solution should be preheated in a water bath at 25 °C for 30 min.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 5 mL of double distilled water, mix well to dissolve. Aliquot and store at -20 °C for 1 month, protected from light.
3. **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 fresh. Aliquot and store at -20 °C for 10 days, protected from light.
4. **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 to serial concentrations. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 μmol/mL.

7. 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 μL extraction solution with a dounce homogenizer at 4 °C.

3. Centrifuge at 16,000×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 (MAES0126).

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 perform a pretest to confirm the dilution factor.

8. Operating steps

1. Standard well: Add 20 µL of standard solution with different concentrations to the corresponding wells. Sample well: Add 20 µL of sample to the corresponding wells.
2. Add 160 µL of buffer solution to each well.
3. Add 20 µL of substrate working solution to each well.
4. Mix thoroughly with microplate reader for 3 s. Measure the OD value of each well at 265 nm with microplate reader immediately, record as A1. Incubate at 25 °C for 2 min, protected from light. Mix thoroughly with microplate reader for 3 s. Measure the OD value of each well at 265 nm with microplate reader immediately, record as A2.

9. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absolute OD value.

3. Plot the standard curve using absolute OD value of standards and corresponding 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):

DHA content (µmol/g wet weight) = (ΔA_265 - b) ÷ a ÷ m × V × f

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

DHA content (µmol/mg_{prot}) = (ΔA_265 - b) ÷ a ÷ C_{pr} × f

[Note]:

ΔA_265: ΔA_Sample - ΔA_Blank

m: The weight of the sample, g

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

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

f: Dilution factor of sample before test

10. Appendix I Performance Characteristics

Parameters:

Intra-assay Precision

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

Inter-assay Precision

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

Recovery

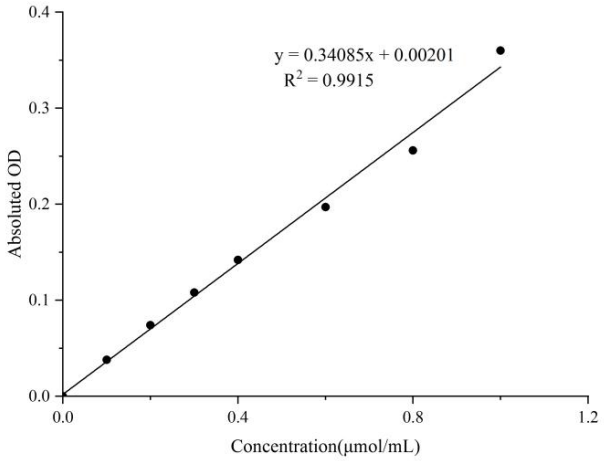
Three samples of high, middle, and low concentration were tested with 6 parallel measurements for each concentration to obtain an average recovery rate of 99.3%.

Sensitivity

The analytical sensitivity of the assay is 0.0016 μmol/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.

Standard curve data

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), the standard curve and data are provided below for reference only.



11. Appendix II Example Analysis

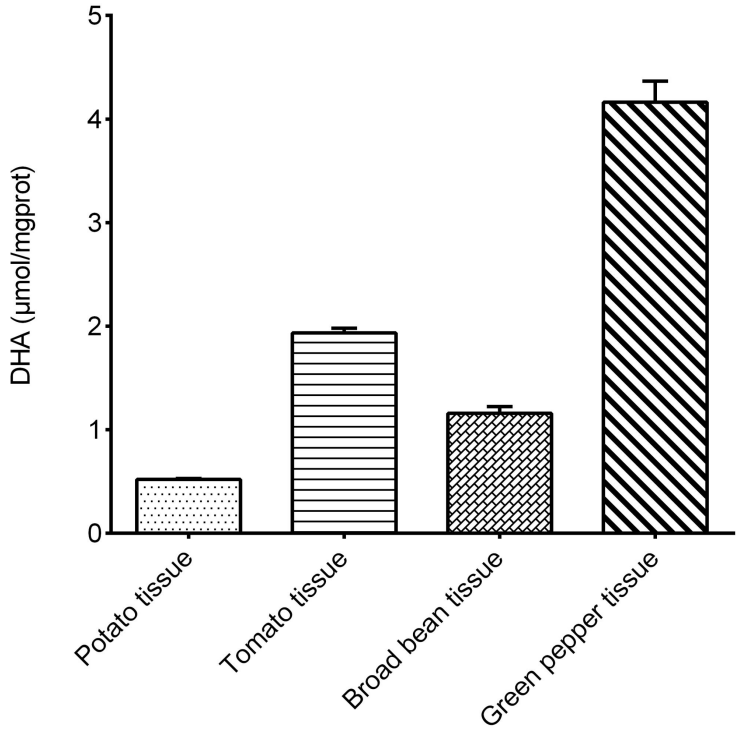
Example analysis:

Take 20 μL of 10% tomato tissue homogenate and perform 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 mg_{prot}/mL, and the calculation result is:

DHA (μmol/mg_{prot}) = (0.013 - 0.00201) ÷ 0.34085 ÷ 0.017 = 1.90 μmol/mg_{prot}

Detection of 10% potato tissue (protein concentration: 0.037 mg_{prot}/mL), 10% tomato tissue (protein concentration: 0.017 mg_{prot}/mL), 10% broad bean tissue (protein concentration: 0.036 mg_{prot}/mL), 10% green pepper tissue (protein concentration: 0.031 mg_{prot}/mL), according to the protocol, the results are as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.