

## Dehydroascorbic Acid(DHA)Content Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Detection equipment:** Ultraviolet spectrophotometer

**Cat No:**AK0466

**Size:**50T/48S

### Components:

Extract solution:Liquid 60 mL×1, store at room temperature.

Reagent I: Liquid40 mL×1, store at room temperature.

Reagent II: Powder×1, store at -20°C and avoid light; Add 5 mL of distilled water before use, mix thoroughly. Store at -20°C after spacing out.

Standard: Powder×1, store at -20°C and avoid light. Add 5.743 mL of distilled water to dissolve, then take 0.1 mL and put in 0.9 mL of distilled water, mix thoroughly and to be prepared as 0.1 μmol/mL DHA. Store at -20°C after dividing the solution into several parts.

### Description:

AsA is an important indicator of plant cells, the content of AsA, redox state(AsA/DHA) and its synthesis and metabolism-related enzyme activities are related to the response of plants to a series of environmental stress. DHA is a reversible oxidized form of AsA. It forms a redox system with ascorbic acid in the living body and has the function of an electron acceptor.

DTT deoxidize DHA to form AsA. According to the generation rate of AsA, can calculate the content of DHA.

### Technical Specifications

Minimum Detection Limit: 0.0065 μmol/mL

Linear Range : 0.015625- 1 μmol/mL

### Required but not provided

Low temperature centrifuge, ultraviolet spectrophotometer, 1 mL quartz cuvette, adjustable pipette, mortar/homogenizer, ice and distilled water.

### Protocol:

#### I. DHA Extraction:

Add 1 mL extract solution to 0.1 g of sample, fully grind on ice. centrifuge at 16000 g and 4°C for 20 min. Supernatant is ready for test.

#### II. Procedure

1. Preheat ultraviolet spectrophotometer for 30 min, adjust wavelength to 265 nm, set zero with distilled water.
2. Preheat Reagent I at 25°C water bath for 30 min.

3. Standard tube: Add 100  $\mu$ L of standard, 800  $\mu$ L of Reagent I and 100  $\mu$ L of Reagent II to 1 mL quartz cuvette, mix thoroughly and quickly, detect at 265 nm, record the absorbance at 10s and 130s. Record  $A_1, A_2, \Delta A_S = A_2 - A_1$ .

4. Test tube: Add 100  $\mu$ L of supernatant, 800  $\mu$ L of Reagent I and 100  $\mu$ L of Reagent II to 1 mL quartz cuvette, mix thoroughly and quickly, detect at 265 nm, record the absorbance at 10s and 130s. Record  $A_3, A_4, \Delta A_T = A_4 - A_3$ .

### III. Calculation

#### 1. Protein concentration

$$\text{DHA}(\mu\text{mol}/\text{mg prot}) = C_S \times (\Delta A_T \div \Delta A_S) \div \text{Cpr} = 0.1 \times \Delta A_T \div \Delta A_S \div \text{Cpr}$$

#### 2. Sample weight

$$\text{DHA}(\mu\text{mol}/\text{g}) = [C_S \times \Delta A_T \div \Delta A_S \times V_{ST}] \div W = 0.1 \times \Delta A_T \div \Delta A_S \div W$$

$C_S$ : DHA concentration, 0.1  $\mu\text{mol}/\text{mL}$ ;

$V_{ST}$ : Supernatant total volume, 1.0 mL;

Cpr: Supernatant protein concentration, mg/mL;

W: Sample weight, g.

#### Note:

Before the formal experiment, do 1~2 pre-experiments to ensure that the value of  $\Delta A$  is less than 0.4.

#### Experimental instances:

1. Take 0.1g of photinia, add 1mL of extract reagent, homogenate on ice. Centrifuge at 16000g for 20 minutes at 4  $^{\circ}\text{C}$ , take the supernatant, dilute four times, and test according to the measured steps.

Calculate  $\Delta A_T = A_4 - A_3 = 1.207 - 1.19 = 0.017$ ,  $\Delta A_S = A_2 - A_1 = 0.295 - 0.043 = 0.252$ , calculate the enzyme activity according to sample weight:

$$\text{DHA}(\mu\text{mol}/\text{g weight}) = 0.5 \times \Delta A_T \div \Delta A_S \div W \times 4 \text{ (dilution ratio)} = 1.349 \mu\text{mol}/\text{g weight}.$$

#### Related products:

AK0464/AK0463	L-galactose- 1,4-lactone dehydrogenase (Gal LDH) Assay Kit
AK0462/AK0461	Ascorbic Acid Oxidase(AAO) Activity Assay Kit
AK0576/AK0575	Ascorbate Peroxidase (APX) Activity Assay Kit