

Dehydroascorbic Acid(DHA)ContentAssay Kit

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

Detection equipment: Ultraviolet spectrophotometer

Cat No: BC1240

Size:50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8°C
Reagent I	Liquid 40 mL×1	2-8°C
Reagent II	Powder×1	-20°C
Standard	Powder×1	-20°C

Solution Preparation:

1. Reagent II: Add 5 mL of distilled water before use, mix thoroughly. Store at -20°C after spacing out.
2. Standard: 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 μL of standard, 800 μL of Reagent I and 100 μL of Reagent II to 1 mL quartz cuvette, mix thoroughly and quickly, detect at 265 nm, record the absorbance at 10s and 130s. Record A₁, A₂, ΔA_S=A₂-A₁.
4. Test tube: Add 100 μL of supernatant, 800 μL of Reagent I and 100 μL of Reagent II to 1 mL quartz cuvette, mix thoroughly and quickly, detect at 265 nm, record the absorbance at 10s and 130s. Record A₃, A₄, ΔA_T=A₄-A₃.

III. Calculation

1. Protein concentration

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

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 μmol/mL;

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

C_{pr}: 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 ΔA 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°C, take the supernatant, dilute four times, and test according to the measured steps. Calculate ΔA_T= A₄-A₃=1.207-1.19=0.017, Δ A_S=A₂-A₁=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:

BC1250/BC1255	L-galactose-1,4-lactone dehydrogenase (Gal LDH) Assay Kit
BC1260/BC1265	Ascorbic Acid Oxidase(AAO) Activity Assay Kit
BC0220/BC0225	Ascorbate Peroxidase (APX) Activity Assay Kit