Dehydroascorbic Acid(DHA)ContentAssay Kit

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

Detection equipment: Spectrophotometer/microplate reader

Cat No:NA0534

Size:100T/96S

Components:

Extract solution: Liquid 100 mL×1, store at room temperature.

Reagent I: Liquid 20 mL×1, store at room temperature.

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

Standard: Powder×1,store at -20℃ and avoid light. Add 5.743 mL of distilled water to dissolve, then take 0.1mL and putin 0.9 mL of distilled water, mix thoroughly and to be prepared as 0.1 μmol/mL DHA. Store at -20℃ 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：0.0016 μmol/mL

Linear Range：0.03125-3 μmol/mL

Required but not provided

Low temperature centrifuge, ultraviolet spectrophotometer/microplate reader, micro quartz cuvette/96 well flat-bottom plate(UV plate), adjustable pipette, mortar/homogenizer, ice and distilled water.

Protocol:

I. DHA Extraction:

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

II. Procedure

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

3. Standard tube: Add 20 μL of standard, 160 μL of Reagent I and 20 μL of Reagent II to micro quartz cuvette/96 well flat-bottom plate(UV plate), 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 20 μL of supernatant, 160 μL of Reagent I and 20 μL of Reagent II to micro quartz cuvette/96 well flat-bottom plate(UV plate), 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

DHA(μmol/mg prot) =$C_S \times (\Delta A_T \div \Delta A_S) \div Cpr = 0.1 \times \Delta A_T \div \Delta A_S \div Cpr$

2. Sample weight

DHA(μmol/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;

Cpr: Supernatant protein concentration, mg/mL;

W: Sample weight, g.

Note:

Before the formal experiment, do 1~2 pre-experiments. If the value of ΔA is greater than 1, it is recommended that the sample be diluted with extract for determination.

Experimental instances：

1. Take 0.1g of photinia, add 1mL of extract reagent, homogenate on ice. Centrifuge at 16000g for 20 minutes at 4℃, take the supernatant, dilute by 4 times and test according to the measured steps. Calculate ΔA_T=A4-A3=1.0792-1.0621=0.0171，ΔA_S=A2-A1=0.4723-0.0746=0.3977, calculate the enzyme activity according to sample weight:

DHA(μmol/g weight) =$1 \times \Delta A_T \div \Delta A_T \div W \times 4$（dilution ratio）=1.720 μmol/g weight.

Related products：

NA0774/NA0533	L-galactose-1,4-lactone dehydrogenase (Gal LDH)Assay Kit
NA0773/NA0532	Ascorbic Acid Oxidase(AAO)Activity Assay Kit
NA0852/NA0610	Ascorbate Peroxidase (APX) Activity Assay Kit