Ascorbic Acid (AsA) Content Assay Kit

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

Detection equipment: Spectrophotometer

Cat No:BC1230

Size:50T/48S

Components:

Reagent I: Liquid 50mL×1, store at 4℃.

Reagent II: Liquid50mL×1, store at 4℃.

Reagent III: Liquid25μL×1, store at 4℃; The liquid is placed in the EP tube in the reagent bottle. Before use, according to the dosage and the volume ratio of Reagent III to Reagent II is 1:250, and the mixture is ready to use.

Standard: Powder×1,store at 4℃ and avoid light. Add 5.679mL of distilled water before use, mix thoroughly.Add0.96mLof distilled water to0.04mL of Standard, mix thoroughly and to be prepared as 400μmol/L AsA.

Description:

AsA is also called Vitamin C.AsAis the substrate of coenzyme, free radical scavenger, electron copolymer/receptor, biosynthesis of oxalate and tartrate. As the most important antioxidant in plant cells,AsAhas important function in protecting chloroplast from oxidizing. It is also one of the important indexes to measure the quality of crop products.

Ascorbate oxidase (AAO)catalyzeAsAto form DHA. According to detect the oxidize rate of AsA, can calculate the content of AsA.

Technical Specifications

Minimum Detection Limit：34.725 μmol/L

Linear Range：50-1400 μmol/L

Required but not provided

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

Protocol:

I. AsA Extraction:

1. Tissue

Accordance the ratio oftissue(g) : Reagent I volume (mL)=1: 5~10, (add 1 mL Reagent I to 0.1 g tissue). Homogenate on ice. centrifuge at 8000 g and 4℃ for 20 min. Supernatant is ready for test.

2. Bacteria or cells

Accordance the ratio ofcells amount (10^4) : Reagent I volume (mL)=500~1000: 1,(add 1 mL Reagent I to 5 million cells). Ultrasonic on ice bath to smash cells, (powder 300w, ultrasonic 3s, interval 7s for 3 min). centrifuge at 8000 g and 4℃ for 20 min. Supernatant is ready for test.

3. **Serum:** Directly detect.

II. Determination procedure

1. Preheat ultraviolet spectrophotometer for 30 min, adjust wavelength to 265 nm, set zero with distilled water.
2. Preheat Reagent II at 25℃ water bathfor 30 min.
3. Standard tube: Add 100μLof standard, 800μLof Reagent II and 100μLof Reagent III, mix thoroughly, detect at 265nm, record the absorbance A1 at 30s and A2 at 150s, ΔA_S= A1-A2.
4. Test tube: Add 100 μL of supernatant, 800 μL of Reagent II and 100 μL of Reagent III, mix thoroughly, detect at 265 nm, record the absorbance A3 at 30s and A4 at 150s, ΔA_T= A3-A4.

Note: Standard tube just test once or twice.

III. Calculation

1. Protein concentration

   AsA (nmol/mg prot) =$[C_S\times \Delta A_T \div \Delta A_S \times V_{SR}] \div (Cpr\times V_{SR})=400\times \Delta A_T \div \Delta A_S \div Cpr$

2. Sample weight

   AsA(nmol/g) =$[C_S\times \Delta A_T \div \Delta A_S \times V_{SR}] \div (W\times V_{SR} \div V_{ST}) =400\times \Delta A_T \div \Delta A_S \div W$

3. Cells amount

   AsA(nmol/10^4cell) =$[C_S\times \Delta A_T \div \Delta A_S \times V_{SR}] \div$ (Cellamount× $V_{SR} \div V_{ST}$)

   $=400\times \Delta A_T \div \Delta A_S \div$Cell amount

4. Liquid volume

   AsA (nmol/mL) =$[C_S\times \Delta A_T \div \Delta A_S \times V_{SR}] \div V_{SR}=400\times \Delta A_T \div \Delta A_S$

C_S: Standard solution concentration, 400 μmol/L;

V_{ST}: Supernatant total volume, 1.0 mL=0.001 L;

V_{SR}: Supernatant volume in reaction solution, 0.1 mL;

Cpr: Protein content, mg/mL;

W: Sample weight, g.

Cell amount: 10^4

Note:

1. If the initial absorbance value of the sample is greater than 1.3, it is recommended that the sample be diluted with Reagent I and determined.
2. Prepare Reagent III and Standardwhen the solution will be used. Store at 4℃, use up within 3 days.

Experimental instances：

1. Take 0.1g of hawthorn fruit, add 1mL of Reagent I, homogenate on ice. Centrifuge at 8000g for 20 minutes at 4℃, take the supernatant, and test according to the measured steps.

CalculateΔA_T=A3-A4=1.140-0.763=0.377， ΔA_S=A1-A2=0.343-0.012=0.331, calculate the enzyme activity according to sample weight:

AsA(nmol/g weight) =400×ΔA_T÷ΔA_S÷W =4556 nmol/g weight.

Recent Product citations

[1] Yawen Ji,Panpan Zhang,Yixiao Xing,et al. Effect of 1α,25-dihydroxyvitamin D3 on the osteogenic differentiation of human periodontal ligament stem cells and the underlying regulatory mechanism. International Journal of Molecular Medicine. October 2018;(IF2.928)

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