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Ascorbic Acid (AsA) Content Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Cat No: AK0467

Size: 100T/96S

Components:

Reagent I: Liquid 100 mL×1, store at 4℃ .

Reagent II: Liquid 20 mL×1, store at 4℃ .

Reagent III: Liquid10μL ×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 5.679 mL of distilled water before use, mix thoroughly. Add 0.96 mLof distilled water to 0.04 mLof Standard, mix thoroughly, be prepared as 400 μmol/L AsA.

Description:

AsA is also called Vitamin C.AsA is the substrate of coenzyme, free radical scavenger, electron copolymer/receptor, biosynthesis of oxalate and tartrate. As the most important antioxidant in plant cells,AsA has 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) catalyze AsA to form DHA. According to detect the oxidize rate of AsA, can calculate the content of AsA.

Technical Specifications

Minimum Detection Limit：0.7539 μmol/L

Linear Range ：6.25- 1400 μmol/L

Required but not provided:

Mortar, ice, low temperature centrifuge, ultravioletspectrophotometer/microplate reader, micro quartz cuvette/96 well flat-bottom plate (UV plate), adjustable pipette and distilled water.

Protocol:

I. AsA extraction:

1. **Tissue:**

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

2. **Bacteria or cells:**

Accordance the ratioof cells amount (10^4): Reagent I volume (mL)=500~1000: 1, (add 1 mL of Reagent I

to 5 million cells). Ultrasonic on ice bath to smash cells, (power 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/microplate reader for 30 min, adjust wavelength to 265 nm, set zero with distilled water.

2. Preheat Reagent II at 25℃ water bath for 30 min.

3. Standard tube: Add 20 μL of standard, 160 μL ofReagent II and 20 μL of Reagent III to micro quartz cuvette or 96 well flat-bottom plate(UV), mix thoroughly, detect at 265 nm, record the accordance A1 for 30s and A2 for 150s. ΔA_S=A1-A2.

4. Test tube: Add 20 μL of supernatant, 160 μL of Reagent II and 20 μL of Reagent III to micro quartz cuvette or 96 well plate (UV), mix thoroughly, detect at 265 nm, record the accordance A3 for 30s and A4 for 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) =$[C_S \times \Delta A_T \div \Delta A_{SA} \times V_{SR}] \div (W \times V_{SR} \div V_{ST}) = 400 \times \Delta A_T \div \Delta A_S \div W$
3. Cells amount:
   AsA(nmol/10^4cell) =$[C_S \times \Delta A_T \div \Delta A_S \times V_{SR}] \div (\text{cell amount} \times V_{SR} \div V_{RT})$
   $=400 \times \Delta A_T \div \Delta A_S \div \text{cell amount}$
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, 0.02 mL;
Cpr: protein concentration, mg/mL;
W: Sample weight, g
Cell amount: A unit of measurement based on 10^4

Note:

1. If the initial absorbance value of the sample is greater than 1.4, 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. Calculate Δ A_T=A3-

A4=1.0228-0.6627=0.3601， ΔA_S=A1-A2=0.3660-0.0090=0.3570, calculate the enzyme activity according to sample weight:

AsA(nmol/g weight) =400× $\Delta A_T \div \Delta A_T \div$ W =4035 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：

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