

## Ascorbic Acid (AsA) Content Assay Kit

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

**Detection equipment:** Spectrophotometer

**Cat No:**AK0468

**Size:**50T/48S

### Components:

**Reagent I:**Liquid 50mL×1, store at 4°C .

**Reagent II:** Liquid50mL×1, store at 4°C .

**Reagent III:** Liquid25 $\mu$ L×1, store at 4°C; 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°C 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 400 $\mu$ mol/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  $\mu$ mol/L

Linear Range : 50- 1400  $\mu$ mol/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°C for 20 min. Supernatant is ready for test.

##### 2. Bacteria or cells

Accordance the ratio of cells 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°C 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°C water bath for 30 min.

3. Standard tube: Add 100 $\mu$ L of standard, 800 $\mu$ L of Reagent II and 100 $\mu$ L of Reagent III, mix thoroughly, detect at 265nm, record the absorbance A1 at 30s and A2 at 150s,  $\Delta A_S = A1 - A2$ .

4. Test tube: Add 100  $\mu$ L of supernatant, 800  $\mu$ L of Reagent II and 100  $\mu$ L of Reagent III, mix thoroughly, detect at 265 nm, record the absorbance A3 at 30s and A4 at 150s,  $\Delta A_T = A3 - A4$ .

**Note:** Standard tube just test once or twice.

## III. Calculation

1. Protein concentration

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

2. Sample weight

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

3. Cells amount

$$AsA \text{ (nmol/}10^4 \text{ cell)} = [C_S \times \Delta A_T \div \Delta A_S \times V_{SR}] \div (\text{Cell amount} \times V_{SR} \div V_{ST}) \\ = 400 \times \Delta A_T \div \Delta A_S \div \text{Cell amount}$$

4. Liquid volume

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

$C_S$  : Standard solution concentration, 400  $\mu$ mol/L;

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

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

$C_{pr}$ : 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 Standard when the solution will be used. Store at 4°C, 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 °C, take the supernatant, and test according to the measured steps.

Calculate  $\Delta A_T = A3 - A4 = 1.140 - 0.763 = 0.377$ ,  $\Delta A_S = A1 - A2 = 0.343 - 0.012 = 0.331$ , calculate the enzyme activity according to sample weight:

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$AsA(nmol/g \text{ weight}) = 400 \times \Delta A_T \div \Delta A_S \div W = 4556 \text{ nmol/g weight.}$

### **Recent Product citations**

[1] Yawen Ji, Panpan Zhang, Yixiao Xing, et al. Effect of  $1\alpha,25$ -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