



## CheKine™ Micro Ascorbic Acid (AsA) Assay Kit

Cat #: KTB3092

Size: 96 T

	<b>Micro Ascorbic Acid (AsA) Assay Kit</b>		
<b>REF</b>	<b>Cat #:</b> KTB3092	<b>LOT</b>	<b>Lot #:</b> Refer to product label
	<b>Applicable samples:</b> Animal and Plant Tissues, Cells, Serum and other fluids		<b>Detection range:</b> 6.25-2,000 µmol/L
	<b>Storage:</b> Stored at -20°C for 6 months, protected from light		

### Assay Principle

Ascorbic acid (AsA), also known as vitamin C, is a coenzyme, free radical scavenger, electron community/acceptor, and substrate for oxalate and tartrate biosynthesis. As the most important antioxidant in plant cells, AsA plays an important role in protecting chloroplasts from oxidative damage and is one of the important indicators to measure the quality of crop products. CheKine™ Micro Ascorbic Acid (AsA) Assay Kit provides a simple test to detect the AsA content in biological samples such as animal and plant tissues, cells, blood serum and other fluids. Ascorbate oxidase (AAO) catalyzes the oxidation of AsA to DHA, and the AsA content can be calculated by measuring the oxidation rate of AsA.

### Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	96 T	
Extraction Buffer	100 mL	4°C
Reagent I	20 mL	4°C
Reagent II	10 µL	-20°C, protected from light
Standard	1	4°C, protected from light

### Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 265 nm
- Incubator, freezing centrifuge
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer (for tissue samples)

## Reagent Preparation

**Extraction Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

**Reagent I :** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

**Working Reagent II :** Prepared before use. According to the dosage, the mixture was fully mixed according to the volume ratio of Reagent II : Reagent I is 1:250 and used immediately. Stored at -20°C, protected from light.

**Standard:** Prepare before use, add 5.679 mL deionized water to fully dissolve, the concentration of AsA is 10 mmol/L. Take 40 µL solution, add 960 µL deionized water, and mix well, that is, 400 µmol/L AsA. The prepared solution can be stored at 4°C, protected from light and used within 3 days. It can also be stored at -20°C and protected from light after aliquoting to avoid repeated freezing and thawing.

## Sample Preparation

**Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for one month.**

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 20 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Plant tissue: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 20 min at room temperature. Use supernatant for assay, and place it on ice to be tested.

3. Cells: Collect  $5 \times 10^6$  cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 20 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

4. Serum or other liquid samples: Test directly.

**Note: If the protein concentration of the sample is need to be determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.**

## Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 265 nm, Ultraviolet spectrophotometer was returned to zero with deionized water.

2. Incubate Reagent I at 25°C for 30 min.

3. In 96-well UV plate or microquartz cuvette, 20 µL Standard, 160 µL Reagent I, and 20 µL Working Reagent II were added into the standard tube, mix quickly. Measure the absorbance value at 265 nm with a microplate reader, record 10 s absorbance value as  $A_1$  and the absorbance value at 2 min 10 s as  $A_2$ , and calculate  $\Delta A_{\text{Standard}} = A_1 - A_2$ .

4. In 96-well UV plate or microquartz cuvette, 20 µL Sample, 160 µL Reagent I, and 20 µL Working Reagent II were added into the test tube, mix quickly. Measure the absorbance value at 265 nm with a microplate reader, record 10 s absorbance value as  $A_3$  and the absorbance value at 2 min 10 s as  $A_4$ , and calculate  $\Delta A_{\text{Test}} = A_3 - A_4$ .

**Note: (1) If the number of samples is large, Reagent I and Working Reagent II can be mixed and used according to the volume ratio of 8:1 to make the working solution, which can be used as required by the sample, and it is prohibited to complete the mixture at one time. The order of adding sample is 180 µL working solution, 20 µL Sample (or Standard), and the time began when the Sample (or Standard) was added. (Standard tubes only need to be measured 1-2 times). (2) In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 large expected difference samples. (3) Because the content is calculated based on the reaction rate, in order to ensure that the reaction time of each sample is as consistent as possible, it is not recommended to test too many samples at the same time. (4) If  $\Delta A_{\text{Test}}$  is less than 0.001, increase the sample quantity appropriately. If  $\Delta A_{\text{Test}}$  is greater than 1, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.**

## Data Analysis

**Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.**

A. 96-well UV plates or microquartz cuvette calculation formula as below

1. Calculated by protein concentration

$$\text{AsA}(\text{nmol/mg prot}) = (\text{C}_{\text{Standard}} \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}}) \div \text{Cpr} = \mathbf{400 \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}} \div \text{Cpr}}$$

2. Calculated by sample fresh weight

$$\text{AsA}(\text{nmol/g fresh weight}) = (\text{C}_{\text{Standard}} \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}}) \times V_{\text{Total Sample}} \div W = \mathbf{400 \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}} \div W}$$

3. Calculated by cell number

$$\text{AsA}(\text{nmol}/10^4 \text{ cell}) = (\text{C}_{\text{Standard}} \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}}) \times V_{\text{Total Sample}} \div \text{cell number} = \mathbf{400 \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}} \div \text{cell number}}$$

4. Calculated by liquid volume

$$\text{AsA}(\text{nmol/mL}) = \text{C}_{\text{Standard}} \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}} = \mathbf{400 \times \Delta A_{\text{Test}} \div \Delta A_{\text{Standard}}}$$

$\text{C}_{\text{Standard}}$ : AsA concentration, 400  $\mu\text{mol/L}$  = 400 nmol/mL;  $V_{\text{Total Sample}}$ : Extraction Buffer volume added, 1 mL; Cpr; sample protein concentration, mg/mL; W: sample weight, g; Cell number: measured in  $10^4$ , ten thousand.

## Recommended Products

Catalog No.	Product Name
KTB3090	CheKine™ Micro Dehydroascorbic Acid (DHA) Assay Kit
KTB3091	CheKine™ Micro Ascorbate Peroxidase (APX) Activity Assay Kit

## Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.