CheKine™ Micro Ascorbic Acid Oxidase (AAO) Activity Assay Kit

Cat #: KTB3093 Size: 48 T/96 T

	Micro Ascorbic Acid Oxidase (AAO) Activity Assay Kit		
REF	**Cat #:** KTB3093	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Ascorbic Acid Oxidase (AAO) is a glycoprotein located in plant cell wall, belonging to the "blue-copper oxidase" family. Ascorbic acid and AAO in the cell wall are closely related to cell wall metabolism and growth. MDHA formed from AsA oxidation by AAO can be reduced by cytochrome b on the plasma membrane, and the transmembrane transport of electrons in this process can promote cell growth. CheKine™ Micro Ascorbic Acid Oxidase (AAO) Activity Assay Kit provides a simple assay for the detection of AAO activity in biological samples such as plant tissues. AAO can directly oxidize AsA, and the activity of AAO can be calculated by measuring the oxidation of AsA.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	10 mL	20 mL	4℃
Reagent Ⅱ	1	1×2	4℃, 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℃.

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

Reagent Ⅱ: Prepared before use, each bottle was added with 5 mL deionized water, fully dissolved. Use within 3 days and store at 4℃, protected from light.

Sample Preparation

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

1. Plant tissue samples: 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 16,000 g for 20 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

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. Because Extraction Buffer contains a certain concentration of protein (about 1 mg/mL), it is necessary to subtract the protein content of Extraction Buffer itself when determining 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℃ for 30 min.
3. Add 20 μL sample, 170 μL Reagent Ⅰ and 10 μL Reagent Ⅱ into 96-well UV plate or microquartz cuvette, and mix quickly.
4. 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 $\Delta A=A_1-A_2$.

Note: (1) In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 large expected difference samples. (2) Because the enzyme activity 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. (3) If ΔA is less than 0.001, increase the sample quantity appropriately. If ΔA 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 calculation formula as below

1. Calculation of AAO activity in tissues

(1) Calculated by protein concentration

Active unit definition: 1 nmol AsA oxidized per min in 1mg tissue protein in 25℃ reaction system is defined as a unit of enzyme activity.

$AAO(U/mg\ prot)=[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(Cpr\times V_{Sample})\div T$**$=184.5\times\Delta A\div Cpr$**

(2) Calculated by sample fresh weight

Active unit definition: 1 nmol AsA oxidized per min in 1 g tissue in 25℃ reaction system is defined as a unit of enzyme activity.

$AAO(U/g\ fresh\ weight)=[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\div V_{Total\ Sample}\times W)\div T$**$=184.5\times\Delta A\div W$**

Where: V_{Total}: total reaction volume, 2×10^{-4} L; ε: AsA molar extinction coefficien at 265 nm, 5.42×10^4 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; V_{Sample}: sample volume added, 0.02 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; T: reaction time, 2 min; Cpr; sample protein concentration, mg/mL; W: sample weight, g.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

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.