

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Ascorbate Oxidase (AAO) Activity Assay Kit

Catalog No.: BC00157

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale)	order@enkilife.com
✉ Email (Techsupport)	techsupport@enkilife.com
☎ Tel:	0086-27-87002838
🌐 Website:	www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Ascorbate Oxidase (AAO) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Tissues
Assay Type	Quantitative
Detection Instrument	Ultraviolet spectrophotometer (optimal detection wavelength 265 nm)

Product Introduction

AAO is a glycoprotein located in the plant cell wall and belongs to the "copper oxidase" family.

Ascorbic acid and AAO within the cell wall are closely related to cell wall metabolism and growth.

MDHA, formed by the oxidation of AsA by AAO, can be reduced by cytochrome b on the plasma membrane; the transmembrane transport of electrons in this process promotes cell growth.

Principle

AAO can directly oxidize AsA, and the activity of AAO can be calculated by measuring the amount of AsA oxidized.

Components

Components	Size	Storage
Reagent 1	Liquid, 50mL × 1 bottle	2~8°C
Reagent 2	Liquid, 60mL × 1 bottle	2~8°C
Reagent 3	Powder × 2 tubes	2~8°C
	Add 2mL of distilled water and shake well to dissolve before use.	

Storage

The kit has a shelf life of 6 months.

Preparation before the experiment

Sample processing

Tissue sample preparation: Homogenize the tissue sample in an ice bath at a ratio of 1:5~10 (tissue mass (g): reagent volume (mL) (it is recommended to weigh approximately 0.1g of tissue and add 1mL of reagent). Centrifuge at 16000g, 4°C for 10min, and collect the supernatant on ice for analysis.

Operation process

1. Preheat reagent 2 in a water bath at 25°C for 30 minutes.
2. Operating steps;

Reagents(μL)	Blank tube	Test tube
Double-distilled water	100	--
Crude enzyme solution of sample	--	100
Reagent 2 (pre-warmed)	850	850
Reagent 3	50	50
A ₁₀ at 10 s and A ₁₃₀ at 130 s using a 1 mL quartz cuvette at a wavelength of 265 nm, zeroed with double-distilled water. Calculate: $\Delta A = A_{10} - A_{130}$		

Result calculation

1. Calculated based on sample protein concentration

Unit definition :At 25°C, one unit (1 U) is defined as the amount of enzyme that oxidizes 1 nmol of AsA per minute per milligram of protein.

$$\begin{aligned}\text{AAO activity} \\ (\text{U/mg prot}) &= \frac{\Delta A_{\text{test}} - \Delta A_{\text{blank}}}{\epsilon \times d} \times V_{\text{reaction total}} \times 10^9 \div (\text{Cpr} \times V_{\text{sample}}) \div T \\ &= 92.4 \times (\Delta A_{\text{test}} - \Delta A_{\text{blank}}) \div \text{Cpr}\end{aligned}$$

2. Calculated by sample quality

Unit definition: At 25°C, one unit (1 U) is defined as the amount of enzyme that oxidizes 1 nmol of AsA per minute per gram of sample.

$$\begin{aligned}\text{AAO activity} \\ (\text{U/g}) &= \frac{\Delta A_{\text{test}} - \Delta A_{\text{blank}}}{\epsilon \times d} \times V_{\text{reaction total}} \times 10^9 \div (W \times V_{\text{sample}} \div V_{\text{sample total}}) \div T \\ &= 92.4 \times (\Delta A_{\text{test}} - \Delta A_{\text{blank}}) \div W\end{aligned}$$

Annotation

ϵ : The molar absorptivity of AsA at 265 nm is 5.42×10^4 L/mol/cm.

d: Cuvette path length (cm), 1 cm;

$V_{\text{reaction total}}$: Total volume of the reaction system, $1000 \mu\text{L} = 1 \times 10^{-3}\text{L}$

$V_{\text{sample total}}$: 1 mL of the extracted solution added;

V_{sample} : Volume of supernatant added to the reaction system (mL), $100 \mu\text{L} = 0.1\text{mL}$;

10^6 : $1 \text{ mol} = 1 \times 10^6 \mu\text{mol}$

T: Catalytic reaction time, 2 min;

Cpr: Protein concentration in the supernatant (mg/mL), which needs to be determined separately. It is recommended to use our company's BCA protein content assay kit (BC00006).

W: Sample mass, g;

Precautions

This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.