

# Ascorbic Acid Oxidase (AAO) Activity Assay Kit

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

**Detection equipment:** Ultraviolet spectrophotometer

**Cat No:** NA0773

**Size:** 50T/48S

## Components:

Extract solution: Liquid 60 mL×1, store at 4°C.

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

Reagent II: Powder×1, store at 4°C. Add 10 mL of distilled water before use. Mix thoroughly.

## Description:

AAO is a glycoprotein that located in plant cell wall, belong to the "blue copper oxidase" family. Ascorbic acid and AAO in the cell wall are closely related to cell wall metabolism and growth. AAO catalyzes the oxidation of AsA to MDHA, which can be reduced by cytochrome b on the plasma membrane. The transmembrane transport of electrons in this process can promote cell growth.

AAO can directly oxidize AsA. The activity of AAO can be calculated by measuring the oxidation amount of AsA.

## Required but not provided

Low temperature centrifuge, ultraviolet spectrophotometer, 1 mL quartz cuvette, adjustable pipette, mortar, ice and distilled water.

## Protocol:

### I. Sample Extraction:

Add 1 mL of Extract solution to 0.1 g of sample, fully grind on ice. centrifuge at 11000 g and 4°C for 20 min. Supernatant is ready for test.

### II. Determination procedure

1. Preheat ultraviolet spectrophotometer for 30 min, adjust wavelength to 265 nm and set zero with distilled water.
2. Preheat Reagent I at 25°C water bath for 30 min.
3. Add reagents to 1 mL quartz cuvette according to the following table.

	Distilled water	Supernatant	Reagent I	Reagent II
Blank tube (B)	100		850	50
Test tube (T)		100		

Mix thoroughly, detect the absorbance of 265 nm at 10s and 130s, record A<sub>1</sub>, A<sub>2</sub>. A<sub>1</sub> subtract A<sub>2</sub>, obtain  $\Delta A_B$ ,  $\Delta A_T$ .

### III. Calculation

### Use 1 mL quartz cuvette test calculation as follows

#### 1) Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1  $\mu\text{mol}$  AsA at 25°C per minute every milligram protein.

25°C each milligram protein per minute oxidize 1  $\mu\text{mol}$  AsA define as 1 U.

$$\text{AAO(U/mg prot)} = (\Delta A_T - \Delta A_B) \div (\epsilon \times d) \times V_{RT} \times 10^6 \div (\text{Cpr} \times V_S) \div T \\ = 0.0923 \times (\Delta A_T - \Delta A_B) \div \text{Cpr}$$

#### 2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1  $\mu\text{mol}$  AsA at 25°C per minute every gram sample

$$\text{AAO(U/g weight)} = (\Delta A_T - \Delta A_B) \div (\epsilon \times d) \times V_{RT} \times 10^6 \div (V_S \div V_{ST} \times W) \div T \\ = 0.0923 \times (\Delta A_T - \Delta A_B) \div W$$

$\epsilon$ : The molar absorption coefficient of AsA at 265 nm,  $5.42 \times 10^4$  L/mol/cm;

$d$ : Cuvette light path(cm), 1 cm;

$V_{RT}$ : Reaction total volume (L),  $1000 \mu\text{L} = 1 \times 10^{-3}$  L;

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

$\text{Cpr}$ : Supernatant protein concentration(mg/mL); Need do another test. Suggest use PC0020, BCA Protein Assay Kit;

$V_S$ : Supernatant volume (mL),  $100 \mu\text{L} = 0.1$  mL;

$T$ : Reaction time(min), 2 min.

#### Note:

Because Reagent I contains proteins(1mg/ml), it's necessary to minus the extract solution protein concentration during calculating sample protein concentration.

#### Related Products:

NA0776/NA0535 Ascorbic Acid (AsA) Content Assay Kit

NA0775/NA0534 Dehydroascorbic Acid (DHA) Assay Kit

NA0817/NA0575 Monodehydroascorbate Reductase(MDHAR) Activity Assay Kit