Ascorbic Acid Oxidase (AAO) Activity Assay Kit

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

Detection equipment: Spectrophotometer/microplate reader

Cat No: BC1265

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 20 mL×1	2-8℃
Reagent II	Powder×1	2-8℃

Solution Preparation:

1. Reagent II: Dissolve thoroughly with 10 mL distilled water before use.

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/microplate reader, micro quartz cuvette/96 well flat-bottom plate (UV plate), 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℃ for 20 min. Supernatant is ready for test.

II. Procedure

1. Preheat ultraviolet spectrophotometer/microplate reader for 30 min, adjust wavelength to 265 nm and set zero with distilled water.
2. Preheat Reagent I at 25℃ water bath for 30 min.
3. Add reagents to micro quartz cuvette/96 well UV plates according to the following table.

	Distilled water	Supernatant	Reagent I	Reagent II

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com

Blank tube (B)	20		170	10
Test tube (T)		20		

Mix thoroughly, detect the absorbance of 265 nm at 10s and 130s at 265 nm, record A1, A2. A1 subtract A2, obtain ΔA_B, ΔA_T.

III. Calculation

A. Micro quartz cuvette

1) Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol AsA at 25℃ per minute every milligram protein.

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

2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol AsA at 25℃ per minute every gram sample.

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

ε: The molar absorption coefficient of AsA at 265 nm, 5.42×10^4 L/mol/cm;

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

V_{RT}: Reaction total volume(L), 200 μL=2×10^{-4} L;

10^6: 1 mol=1×10^6 μmol;

Cpr: Supernatant protein concentration(mg/mL); Need do another test. Suggest PC0020, BCA Protein Assay Kit;

W: Sample weight, g;

V_S: Supernatant volume(mL), 20 μL=0.02 mL;

V_{ST}: Extraction solution volume, 1 mL;

T: Reaction time(min), 2 min.

B. 96 well flat-bottom plate (UV plate)

1) Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol AsA at 25℃ per minute every milligram protein.

$AAO(U/mg\ prot) = (\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RT}\times 10^6\div(Cpr\times V_S)\div T=0.1538\times(\Delta A_T-\Delta A_B) \div Cpr$

2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol AsA at 25℃ per minute every gram sample.

$AAO(U/g\ weight) = (\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RT}\times 10^6\div(V_S\div V_{ST}\times W)\div T=0.1538\times(\Delta A_T-\Delta A_B) \div W$

ε: The molar absorption coefficient of AsA at 265 nm, 5.42×10^4 L/mol/cm;

d: 96 well plate cuvette light path(cm), 0.6 cm;

V_{RT}: Reaction total volume(L), 200 μL=2×10^{-4} L;

10^6: 1 mol=1×10^6 μmol;

Cpr: Supernatant protein concentration(mg/mL); Need do another test. Suggest use the BCA Protein Test Assay Kit by ours;

W: Sample weight, g;

V_S: Supernatant volume(mL), 20 μL=0.02 mL;

V_{ST}: Extraction solution volume, 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:

BC1230/BC1235	Ascorbic Acid (AsA) Content Assay Kit
BC1240/BC1245	Dehydroascorbic Acid (DHA) Assay Kit
BC0650/BC0655	Monodehydroascorbate Reductase(MDHAR) Activity Assay Kit

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com