Ascorbate Oxidase

Microplate Assay Kit

Cat #: orb390757 (manual)

Detection and Quantification of Ascorbate Oxidase (AAO) Activity in Tissue extracts, Cell lysate Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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INTRODUCTION

Ascorbate oxidase (AAO) is an apoplastic enzyme involved in metabolism of plant ascorbate (AA). Ascorbate (AA) plays a key role in defense against oxidative stress and is particularly abundant in photosynthetic tissues. Over 90% of the ascorbate is localized in the cytoplasm, but a substantial proportion is exported to the apoplast.

The assay is initiated with the enzymatic oxidation of AsA by AAO. AsA can be measured at a colorimetric readout at 265 nm.

KIT COMPONENTS

Component	Volume	Storage
96-Well UV Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	Powder x 1	-20 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Substrate: add 1 ml Reaction Buffer to dissolve before use.

Positive Control: add 1 ml distilled water to dissolve before use, then add 0.5 ml into 0.5 ml distilled water, mix.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 265 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times) ; centrifuged at 16000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 16000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

ASSAY PROCEDURE

Warm the Reaction Buffer to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Positive Control
Reaction Buffer	180 µl	180 µl
Substrate	10 µl	10 µl
Sample	10 µl	--
Positive Control	--	10 µl
Mix, measured at 265 nm and record the absorbance of 10th second and 130th second.		

CALCULATION

Unit Definition: One unit of AAO is the amount of enzyme that will oxidize 1 μmol AsA per minute.

1. According to the protein concentration of sample

$$AAO\ (U/mg) = (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (\varepsilon \times d) \times V_{Total} \times 10^6 / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.308 \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / C_{Protein}$$

2. According to the weight of sample

$$AAO\ (U/g) = (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (\varepsilon \times d) \times V_{Total} \times 10^6 / (W \times V_{Sample} / V_{Asaay}) / T$$

$$= 0.308 \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / W$$

3. According to the quantity of cells or bacteria

$$AAO\ (U/10^4) = (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / (\varepsilon \times d) \times V_{Total} \times 10^6 / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 0.308 \times (OD_{Sample\ (10S)} - OD_{Sample\ (130S))} / N$$

ε: molar extinction coefficient, 5.42×10^4 L/mol/cm;

d: the optical path of 96-Well microplate, 0.6 cm;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

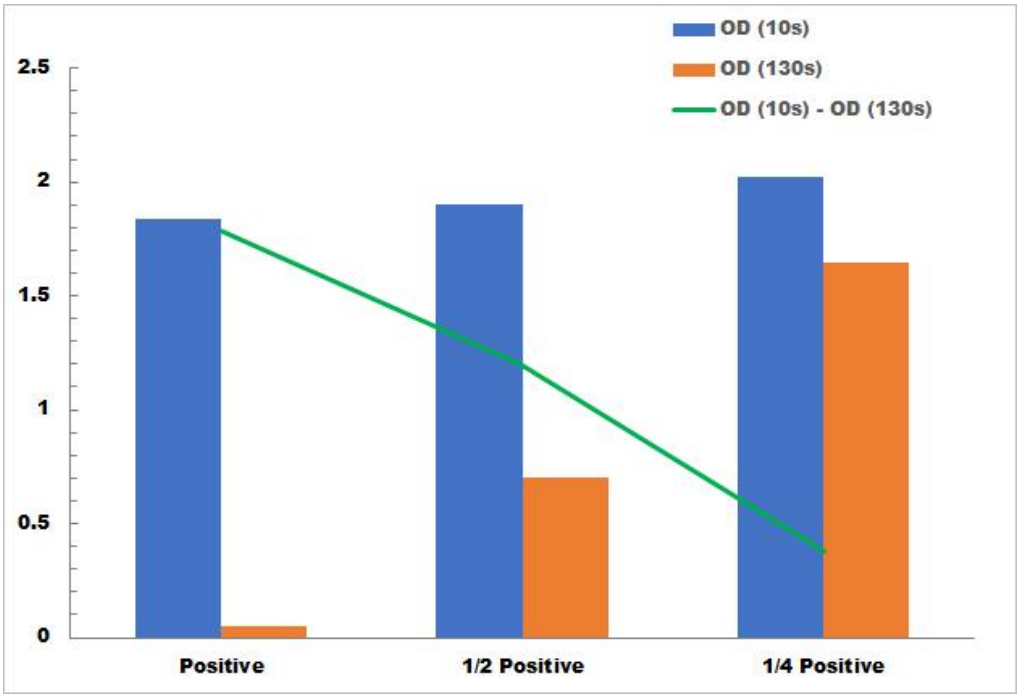
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 2 minutes.

TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Positive Control reaction in 96-well plate assay with decreasing the concentration