Ascorbate Peroxidase Microplate Assay Kit

Catalog # AS0052

Detection and Quantification of Ascorbate Peroxidase Activity in Tissue extracts, Cell lysate Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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I. INTRODUCTION

Ascorbate peroxidase is a hydrogen peroxide-scavenging enzyme that is specific to plants and algae and is indispensable to protect chloroplasts and other cell constituents from damage by hydrogen peroxide and hydroxyl radicals produced from it.

The assay is initiated with the enzymatic oxidation of AsA by APX. AsA can be measured at a colorimetric readout at 290 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well UV Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Substrate I	Powderx 1	4 °C
Substrate II	5 mlx 1	4 °C
Technical Manual	1 Manual	

Note:

Substrate I: add 13 ml Assay Bufferto dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 290 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay buffer for 5 ×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s,repeat 30 times); centrifuged at 13000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 13000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Warm theSubstrate I to room temperature before use.

Add following reagents intothe microplate:

Reagent	Sample	Blank
Sample	20 µl	--
Distilled water	--	20 µl
SubstrateI	130 µl	130 µl
SubstrateII	50 µl	50 µl
Mix,measured at 290 nm and recordthe absorbance of 10thsecond and 310th second.		

VI. CALCULATION

Unit Definition: One unit of APX is the amount of enzyme that will oxidize 1μmolAsA per minute.

1. According to the protein concentration of sample

$$APX\ (U/mg) = [(OD_{Sample(10S)} - OD_{Sample(310S)}) - (OD_{Blank(10S)} - OD_{Blank(310S)})] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times C_{Protein}) / T$$

$$= 1.19 \times [(OD_{Sample(10S)} - OD_{Sample(310S)}) - (OD_{Blank(10S)} - OD_{Blank(310S)})] / C_{Protein}$$

2. According to the weight of sample

$$APX\ (U/g) = [(OD_{Sample(10S)} - OD_{Sample(310S)}) - (OD_{Blank(10S)} - OD_{Blank(310S)})] / (\varepsilon \times d) \times V_{Total} / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 1.19 \times [(OD_{Sample(10S)} - OD_{Sample(310S)}) - (OD_{Blank(10S)} - OD_{Blank(310S)})] / W$$

3.According to the quantity of cells or bacteria

$$APX\ (U/10^4) = [(OD_{Sample(10S)} - OD_{Sample(310S)}) - (OD_{Blank(10S)} - OD_{Blank(310S)})] / (\varepsilon \times d) \times V_{Total} / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 1.19 \times [(OD_{Sample(10S)} - OD_{Sample(310S)}) - (OD_{Blank(10S)} - OD_{Blank(310S)})] / N$$

ε: molar extinction coefficient,2.8×10^3L/mol/cm = 2.8ml/μmol/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 $\times 10^4$;

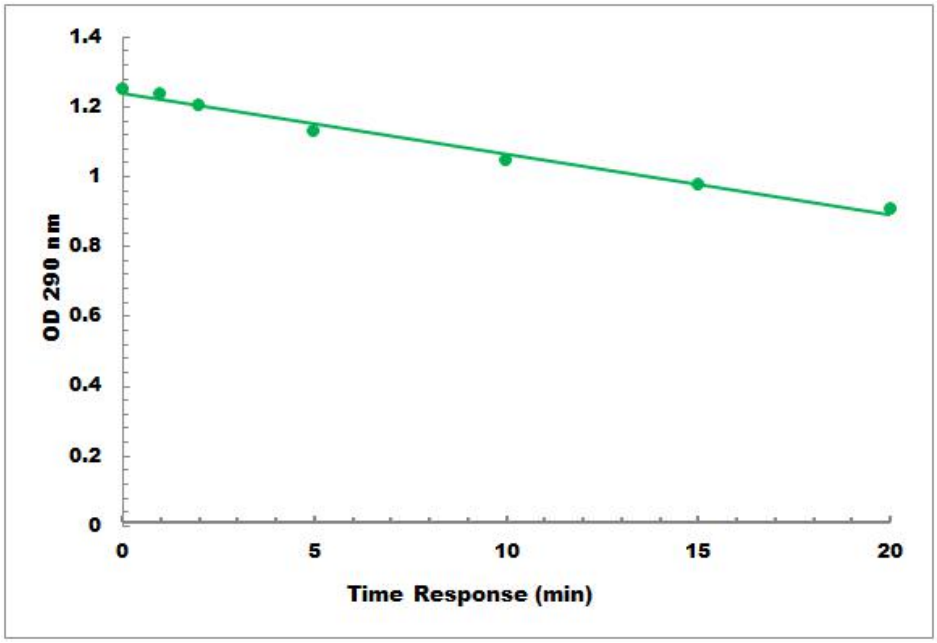
V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

V_{Sample}: the volume of sample, 0.02 ml;

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

T: the reaction time, 5 minutes.

VII. TYPICAL DATA



Samples were assayed using the 96-well microplate.

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES