Ascorbate Peroxidase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1052

(Version 1.2C)

Detection and Quantification of Ascorbate Peroxidase(APX) Activity in Tissue extracts, Cell lysateSamples.

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

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.

Ascorbate Peroxidase ActivityColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring ascorbate peroxidaseactivity levels.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	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 290 nm
2. Distilled water
3. Pipettor, multi-channel 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%, sonicate 3s, interval 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.

IV. REAGENT PREPARATION

Substrate I: Add 13 ml Assay Bufferto dissolve before use.Keep in dark and store at 4 °C for 3-4 days or -20°C for 3-4 weeks after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

VI. 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.		

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

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

1.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(OD_{Sample(310)} - OD_{Sample(10)}) - (OD_{Control(310)} - OD_{Control(10)})}{\varepsilon \times d \times C_{Protein} \times T} \times \frac{V_{Total}}{V_{Sample}} (U/mg)$$

1.2 According to the weight of sample

$$\text{Activity} = \frac{(OD_{Sample(310)} - OD_{Sample(10)}) - (OD_{Control(310)} - OD_{Control(10)})}{\varepsilon \times d \times W \times T} \times \frac{V_{Total} \times V_{Assay}}{V_{Sample}} (U/g)$$

1.3According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(OD_{Sample(310)} - OD_{Sample(10)}) - (OD_{Control(310)} - OD_{Control(10)})}{\varepsilon \times d \times N \times T} \times \frac{V_{Total} \times V_{Assay}}{V_{Sample}} (U/10^4)$$

1.4 According to the volume of sample

$$\text{Activity} = \frac{(OD_{Sample(310)} - OD_{Sample(10)}) - (OD_{Control(310)} - OD_{Control(10)})}{\varepsilon \times d \times T} \times \frac{V_{Total}}{V_{Sample}} (U/mg)$$

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 $\times 10^4$

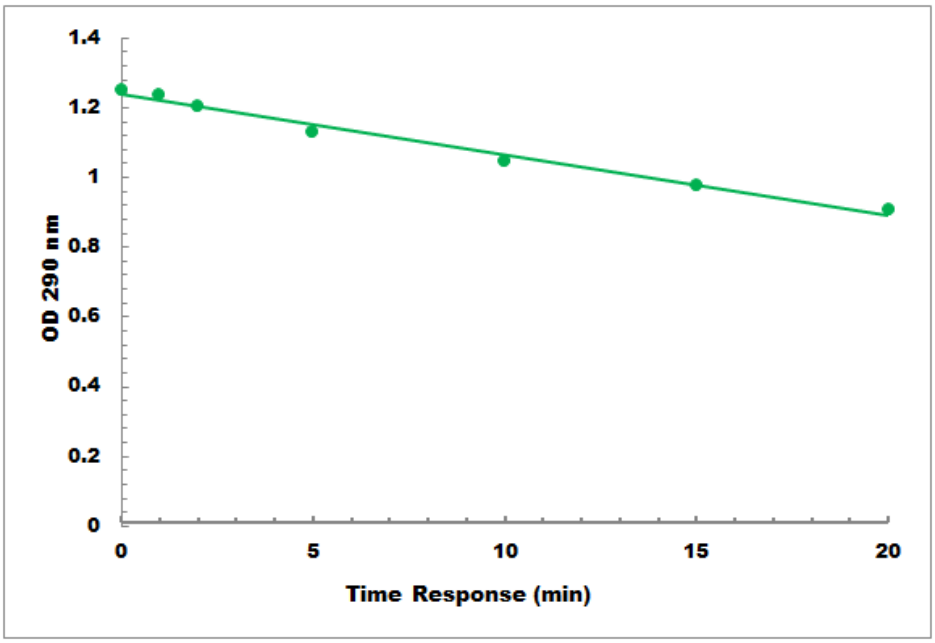
V_{Total}: thetotal volume of all reagents in assay procedure, ml

V_{Sample}: the volume of sample in assay procedure, ml

V_{Assay}: the volume of Assay Buffer II in sample preparation, ml

T: the reaction time, minutes

VIII. TYPICAL DATA



Samples were assayed using the 96-well microplate