

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

Catalog No: E-BC-K353-M

Specification: 48T(46 samples)/96T(94 samples)

Measuring instrument: Microplate reader (280-300 nm)

Detection range: 0.011-1.47 U/L

Elabscience® Ascorbate Peroxidase (APX)

Colorimetric Activity Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help:

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

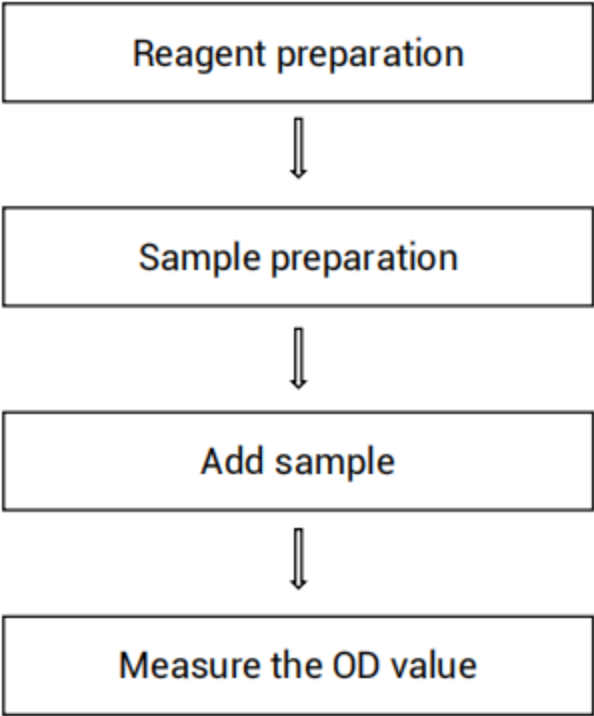
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary	3
Intended use	4
Detection principle	4
Kit components & storage.....	4
Materials prepared by users	5
Reagent preparation.....	5
Sample preparation.....	5
The key points of the assay	6
Operating steps.....	7
Calculation.....	8
Appendix I Performance Characteristics	9
Appendix II Example Analysis	10
Statement	11

Assay summary



Intended use

This kit can be used to measure the ascorbate peroxidase (APX) activity in plant tissue samples.

Detection principle

Ascorbate Peroxidase (APX) can catalyze the reaction between ascorbic acid (ASA) and hydrogen peroxide (H_2O_2), and ASA can be oxidized to monodehydroascorbic acid (MDASA). The absorbance of solution at 290 nm will decline as the oxidation of ASA. The APX activity can be calculated by detecting the decrease of A_{290} .

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Extracting Solution	60 mL × 1 vial	60 mL × 2 vials	2-8°C, 12 months
Reagent 2	Buffer Solution	9 mL × 1 vial	18 mL × 1 vial	2-8°C, 12 months
Reagent 3	Substrate	Powder × 1 vial	Powder × 2 vials	2-8°C, 12 months shading light
Reagent 4	Substrate Solution	1.2 mL × 1 vial	1.2 mL × 2 vials	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	1 pieces	2 pieces	
	Sample Layout Sheet	1 piece	1 piece	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (280-300 nm, optimum wavelength: 290 nm),

Micropipettor, Incubator, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

- ① Equilibrate all reagents to room temperature before use.
- ② The preparation of substrate application solution :
Dissolve a vial of substrate with 3 mL of double distilled water and mix fully. Store at 2~8°C for 3 days protected from light.

Sample preparation

① Sample preparation

Tissue sample:

- ① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).
- ② Wash tissue in cold double distilled water.
- ③ Homogenize 20 mg tissue in 180 μ L extracting solution with a dounce homogenizer at 4°C.
- ④ Centrifuge at 10000 $\times$ g for 10 minutes to remove insoluble material.
Collect supernatant and keep it on ice for detection.
- ⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K168-M).

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% <i>Epipremnum aureum</i> tissue homogenization	1
10% Carrot tissue homogenization	1
10% Green pepper tissue homogenization	1
10% Mushrooms tissue homogenization	1
10% Avocado tissue homogenization	1
10% Pumpkin tissue homogenization	1
10% Chive tissue homogenization	1
10% Spinach tissue homogenization	1
10% Bok choy tissue homogenization	1
10% Oyster mushroom tissue homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

- ① If the value of A_1 is more than 2.0, please dilute the sample and then carry the assay.
- ② The reaction time should be strictly controlled.
- ③ Preheat the buffer solution at 37°C for 1 hour before detection.
- ④ When there are bubbles produced in the sample tubes, the interference of bubbles can be eliminated by mixing.

Operating steps

- ① Preheat the buffer solution at 37°C for 1 h before detection.
- ② Blank well: Add 20 μL of extracting solution to the corresponding wells.
Sample well: Add 20 μL of sample to the corresponding wells.
- ③ Add 140 μL of buffer solution and 20 μL of substrate application solution into each well, mix fully.
- ④ Add 20 μL of substrate solution (record the time immediately) and mix fully with microplate reader for 3 s.
- ⑤ Measure the absorbance at 290 nm at 15 seconds (A_1), then incubate the reaction solution at 37°C for 5 minutes and measure the absorbance of each well (A_2), respectively. $A=A_1-A_2$.

Calculation

The sample:

1. Calculate according to the protein concentration:

Definition: The amount of enzyme of 1 μmol of ASA catalyzed by 1 mg protein in 1 mL reaction system per minute is defined as 1 unit.

$$\text{APX activity (U/mgprot)} = \frac{\Delta A}{\epsilon \times d} \div t \times \frac{V_1}{(V_3 \times C_{pr})} \times f$$

2. Calculate according to the weight of sample:

Definition: The amount of enzyme of 1 μmol of ASA catalyzed by 1 g tissue sample in 1 mL reaction system per minute is defined as 1 unit

$$\text{APX activity (U/g tissue)} = \frac{\Delta A}{\epsilon \times d} \div t \times \frac{V_1 \times V_2}{(V_3 \times m)} \times f$$

[Note]

ΔA : $\Delta A_{\text{sample}} - \Delta A_{\text{blank}}$

ϵ : molar extinction coefficient of ASA at 290 nm, 2.8 mL/($\mu\text{mol} \cdot \text{cm}$).

d: the optical path of 96-well microplate, 0.56 cm.

t: the reaction time, 5 min.

V_1 : the total volume of reaction system, 0.2 mL.

V_2 : the volume of extracting solution for homogenate, 0.18 mL.

V_3 : the volume of sample added to the reaction, 0.02 mL.

f: dilution factor of sample before tested.

C_{pr} : the protein concentration of sample, mgprot/mL.

m: the weight of sample, g

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three 10% pumpkin tissue homogenization samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mgprot)	0.04	0.079	0.112
%CV	6.9	5.7	6.1

Inter-assay Precision

Three 10% pumpkin tissue homogenization samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/mgprot)	0.03	0.28	0.27
%CV	2.6	5.8	9.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (U/mgprot)	0.045	0.079	0.114
Observed Conc. (U/mgprot)	0.044	0.079	0.112
Recovery rate (%)	98	100	99

Sensitivity

The analytical sensitivity of the assay is 0.011 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

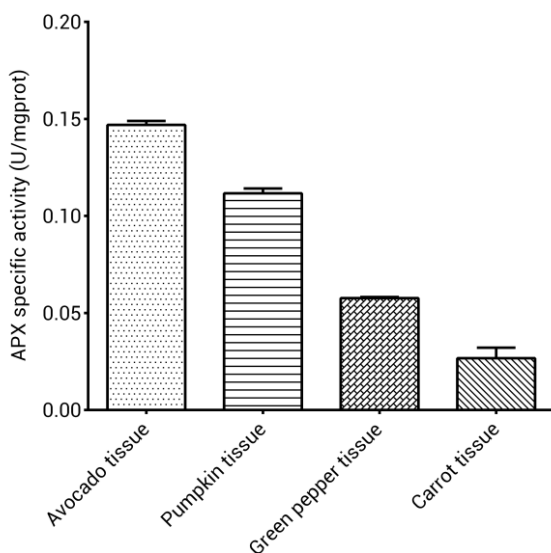
Appendix Π Example Analysis

Example analysis:

Take 20 μL of 10% Green pepper tissue homogenization supernatant to the well. Proceed according to the operation steps. The results are as follows: The ΔA of the sample is 0.104, the ΔA of the blank is 0.0085, the concentration of protein in sample is 2.29 mgprot/mL, and the calculation result is:

$$\text{APX activity (U/mgprot)} = \frac{0.104 - 0.0085}{2.8 \times 0.56} \times \frac{0.2}{0.02} \div 2.29 \div 2 = 0.053 \text{ (U/mgprot)}$$

Detect 10% Avocado tissue homogenate (the concentration of protein in sample is 2.73 mgprot/mL), 10% Pumpkin tissue homogenate (the concentration of protein in sample is 1.69 mgprot/mL), 10% Green pepper tissue homogenate (the concentration of protein in sample is 2.29 mgprot/mL), 10% Carrot tissue homogenate (the concentration of protein in sample is 2.46 mgprot/mL) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.

