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

Catalog No：E-BC-K353-S

Specification：50Assays(48 samples)/100Assays (96 samples)

Measuring instrument：Spectrophotometer（290 nm）

Detection range：0.071-47 U/g tissue

Elabscience® Ascorbate Peroxidase (APX) 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.

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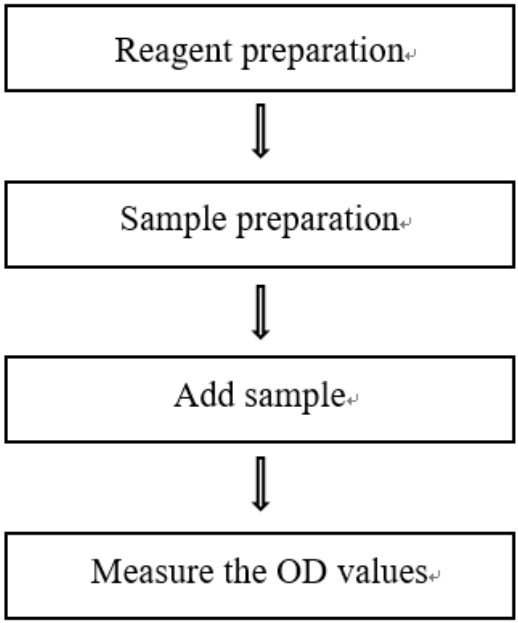
Assay summary



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 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Extracting Solution	60 mL × 1 vial	60 mL × 2 vials	2-8 ℃, 12 months
Reagent 2	Buffer Solution	40 mL × 1 vial	40 mL × 2 vials	2-8 ℃, 12 months
Reagent 3	Substrate	Powder × 1 vial	Powder × 2 vials	2-8 ℃, 12 months shading light
Reagent 4	Substrate Solution	12 mL ×1 vial	12 mL ×1 vial	2-8 ℃, 12 months

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:

Spectrophotometer (290 nm), Vortex mixer, Micropipettor, Water bath, 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 6 mL of double distilled water and mix fully. Store at 2~8 ℃ 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 μL extracting solution with a dounce homogenizer at 4 ℃.

④ Centrifuge at 10000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
10% Epipremnum aureum tissue homogenization	1
10% Carrot tissue homogenization	1
10% Green pepper tissue homogenization	1
10% Mushrooms 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 Preheat the spectrophotometer for 30 min and set the spectrophotometer to zero with double distilled water at 290 nm with 1 mL slit cuvette.

③ Blank tube: Add 0.1 mL of extracting solution into a 2 mL EP tube.
Sample tube: Add 0.1 mL of sample into a 2 mL EP tube.

④ Add 0.7 mL of buffer solution and 0.1 mL of substrate application solution into each tube, mix fully.

⑤ Add 0.1 mL of substrate solution (record the time immediately) and mix fully with a vortex mixer.

⑥ Measure the absorbance at 290 nm at 15 second (A_1), then incubate the reaction solution at 37°C and measure the absorbance at 135 second (A_2), respectively. $\triangle A=A_1-A_2$.

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.

$$\text{APX activity (U/mgprot)} = \frac{\triangle A}{\varepsilon \times 1} \div t \times \frac{V_1}{(V_3 \times Cpr)} \times f$$

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

$$\text{APX activity (U/g tissue)} = \frac{\triangle A}{\varepsilon \times 1} \div t \times \frac{V_1 \times V_2}{(V_3 \times m)} \times f$$

[Note]

ΔA: ΔA_{sample}-ΔA_{blank}

ε: molar extinction coefficient of ASA at 290 nm with 1 cm diameter cuvette, 2.8 mL/(μmol cm).

1: the optical path of quartz cuvette, 1 cm.

t: the reaction time, 2 min.

V_1: the total volume of reaction system, 1 mL.

V_2: the volume of reagent 1 for preparing tissue homogenate.

V_3: the volume of sample added to the reaction, 0.1 mL.

f: dilution factor of sample before tested.

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

m: the weight of sample, g

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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/g tissue)	1.50	18.40	32.80
%CV	5.2	4.7	4.5

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/g tissue)	1.50	18.40	32.80
%CV	5.7	6.7	6.8

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 96%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (U/g tissue)	8.5	26.5	40.5
Observed Conc. (U/g tissue)	8.4	24.4	39.3
Recovery rate (%)	99	92	97

Sensitivity

The analytical sensitivity of the assay is 0.071 U/g tissue. 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 II Example Analysis

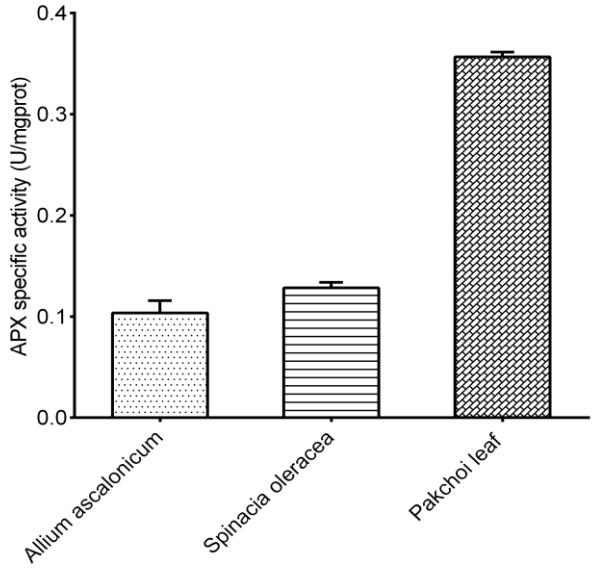
Example analysis:

Weight 0.10 g of spinacia oleracea and cut into pieces, add 0.9 mL of extracting solution, homogenized the sample, centrifuge at 10000 g for 10 min at 4℃, take 0.1 mL of supernatant carry the assay according to the operation steps. The results are as follows:

The △A of the sample is 0.314, the △A of the blank is 0.012, the concentration of protein in sample is 4.20 mgprot/mL, and the calculation result is:

$$\frac{\text{APX activity}}{\text{(U/mgprot)}} = \frac{0.314 - 0.012}{2.8 \times 1} \times \frac{1}{0.1 \times 4.2} \times 1 \div 2 = 0.128 \text{ (U/mgprot)}$$

Detect 10% allium ascalonicum tissue homogenate (the concentration of protein in sample is 2.88 mgprot/mL), 10% spinacia oleracea tissue homogenate (the concentration of protein in sample is 4.20 mgprot/mL), 10% pakchoi leaf tissue homogenate (the concentration of protein in sample is 6.41 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.