PRODUCT INFORMATION & MANUAL

Ascorbate peroxidase/APX Activity Assay Kit (Colorimetric)

NBP3-25864

For research use only.

Not for diagnostic or therapeutic procedures.

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Ascorbate peroxidase/APX Activity Assay Kit (Colorimetric)

Catalog No: NBP3-25864

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 0.071 U/g tissue

Detection range: 0.071-47 U/g tissue

Average intra-assay CV (%): 4.8

Average inter-assay CV (%): 6.4

Average recovery rate (%): 96

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲Background

Ascorbate Peroxidase (APX) is composed of three subunits of cytoplasm, peroxisome and chloroplast isozymes. APX is unique to plants and algae, and is necessary to protect chloroplasts and other cellular components from damage by hydrogen peroxide and hydroxyl radicals. APX is the core component of ascorbic acid-glutathione cycle, which is the main hydrogen peroxide detoxification system of plant cells under abiotic stress.

▲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	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	2- 8℃ , 12 months
Reagent 2	Buffer Solution	40 mL × 2 vials	2- 8℃ , 12 months
Reagent 3	Substrate	Powder × 2 vials	2- 8℃ , 12 months, shading light
Reagent 4	Substrate Solution	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.			

▲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. 01M, pH 7.4)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. If the value of A1 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 reagent 2 at 37℃ for 1 hour before detection.
4. When there are bubbles produced in the sample tubes, the interference of bubbles can be eliminated by mixing.

Pre-assay preparation

▲ Reagent preparation

Preparation of reagent 3 application solution

Dissolve 1 vial of reagent 3 with 6 mL of double distilled water and mix fully. The prepared solution can be store at 2-8℃ with shading light for 3 days.

▲ Sample preparation

Tissue homogenate

Take 0.02-1g fresh tissue to wash with double distilled water. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of reagent 1 (2-8 ℃) (mL): the weight of the tissue (g) =10:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4℃ . Take the supernatant to preserve it on ice for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.071-47 U/g tissue).

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).

Assay protocol

Detailed operating steps

(1) Preheat the reagent 2 at 37℃ for 1 hour 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 reagent 1 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 reagent 2 and 0.1 mL of reagent 3 application solution into each tube, mix fully.

(5) Add 0.1 mL of reagent 4 (record the time immediately) and mix fully with a vortex mixer.

(6) Measure the absorbance at 290 nm at 15 second (A1), then incubate the reaction solution at 37℃ and measure the absorbance at 135 second (A2), respectively. △A = A1-A2.

Summary operation table

	Blank tube	Sample tube
Reagent 1 (mL)	0.1	
Sample (mL)		0.1
Reagent 2 (mL)	0.7	0.7
Reagent 3 application solution (mL)	0.1	0.1
Reagent 4 (mL)	0.1	0.1
Record the time immediately when adding the reagent 4 and mix fully. Measure the absorbance at 290 nm at 15 second (A_1), then incubate the reaction solution at 37℃ and measure the absorbance at 135 second (A_2), respectively. $\triangle A=A_1-A_2$.		

▲Calculation

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.

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

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

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 wet weight of sample, g.

Appendix I Data

▲Example analysis

Weight 0.10 g of spinacia oleracea and cut into pieces, add 0.9 mL of reagent 1, homogenized the sample, centrifuge at 10000 g for 10 min at 4℃ , take 0.1 mL of supernatant and carry the assay according to the operation table.

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:

$$\text{APX activity(U/mgprot)} = \frac{0.314-0.012}{2.8\times1} \times \frac{1}{0.1\times4.2} \times 1 \div 2$$

$$= 0.128 \text{ (U/mgprot)}$$

Appendix II References

1. Najami N, Janda T, Barriah W, et al. Ascorbate peroxidase gene family in tomato: its identification and characterization. Molecular Genetics & Genomics, 2008, 279(2): 171-182.
2. Asada K. Ascorbate peroxidase – a hydrogen peroxide–scavenging enzyme in plants. Physiologia Plantarum, 1992, 85(2): 235-241.
3. Shigeoka S, Ishikawa T, Tamoi M, et al. Regulation and function of ascorbate peroxidase isoenzymes. Journal of Experimental Botany, 2002, 53(372): 1305-1319.
4. Sofo A, Scopa A, Nuzzaci M, Vitti A. Ascorbate Peroxidase and Catalase Activities and Their Genetic Regulation in Plants Subjected to Drought and Salinity Stresses. International Journal of Molecular Sciences, 2015, 16(6): 13561-13578.