Technical Manual

Ascorbate Peroxidase (APX) Activity Assay Kit

- Catalogue Code: MAES0182
- Size: 100 Assays
- Research Use Only

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0.071-47 U/g tissue

Sensitivity:

0.071 U/g tissue

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use

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

4. 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}.

5. Kit Components & Storage

Item	Specification	Storage
Extracting Solution	60 mL × 2 vials	2-8°C, 6 months
Buffer Solution	40 mL × 2 vials	2-8°C, 6 months
Substrate	Lyophilized × 2 vials	2-8°C, 6 months, avoid direct sunlight
Substrate Solution	12 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Water bath
- Incubato
- Centrifuge
- Spectrophotometer (290 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. If the value of A_1 is more than 2.0, 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

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **substrate application solution:** Dissolve 1 vial of substrate with 6 mL of double distilled water and mix fully. The prepared solution can be store at 2-8°C and by avoiding direct sunlight for 3 days.

8. Sample Preparation

Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of extracting solution (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 290 nm

10. Operation Steps

1. Preheat the buffer solution at 37°C 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 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. $\triangle A=A_1-A_2$.

Operation Table

	Blank tube	Sample tube
Extracting solution (mL)	0.1	
Sample (mL)		0.1
Buffer solution (mL)	0.7	0.7
Substrate application solution (mL)	0.1	0.1
Substrate solution (mL)	0.1	0.1
Record the time immediately when adding the reagent 4 and mix fully with a vortex mixer. 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. $\triangle A=A_1-A_2$.		

11. Calculations

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.

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

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.

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

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 extracting solution 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.

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12. Performance Characteristics

Detection Range	0.071-47 U/g tissue
Sensitivity	0.071 U/g tissue
Average recovery rate (%)	96
Average inter-assay CV (%)	6.4
Average intra-assay CV (%)	4.8

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°C, 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:

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

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

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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