TECHNICAL MANUAL

Ascorbate Peroxidase (APX) Activity Assay Kit

- **SKU CODE:** MAES0424
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Prepare blank and sample tubes
- Add buffer and substrate solutions
- Measure absorbance at 290 nm

2. Intended use

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

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

Measuring instrument: Spectrophotometer (290 nm)

Detection range: 0.071-47 U/g tissue

3. Detection principle

Ascorbate Peroxidase (APX) catalyzes the reaction between ascorbic acid (ASA) and hydrogen peroxide (H2O2), causing ASA to be oxidized to monodehydroascorbic acid (MDASA). The absorbance of the solution at 290 nm decreases as ASA is oxidized. The APX activity can be calculated by detecting the decrease in A290.

4. Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Extracting Solution	60 mL x 1 vial	60 mL x 2 vials	2-8°C, 12 months
Reagent 2	Buffer Solution	40 mL x 1 vial	40 mL x 2 vials	2-8°C, 12 months
Reagent 3	Substrate	Powder x 1 vial	Powder x 2 vials	2-8°C, 12 months, protect from light
Reagent 4	Substrate Solution	12 mL x 1 vial	12 mL x 1 vial	2-8°C, 12 months

5. 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)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate application solution:** Dissolve one vial of substrate with 6 mL of double distilled water and mix thoroughly. Store at 2-8°C for up to 3 days, protected from light.

7. 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°C. ④ Centrifuge at 10,000 x 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): • 10% Epipremnum aureum tissue homogenate: 1 • 10% Carrot tissue homogenate: 1 • 10% Green pepper tissue homogenate: 1 • 10% Mushroom tissue homogenate: 1 Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For dilution of other sample types, please perform a pre-test to confirm the dilution factor.

8. Key points of the assay

1. If the value of A1 is more than 2.0, please dilute the sample and repeat 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 bubbles are produced in the sample tubes, eliminate bubble interference by mixing.

9. Operating steps

1. Preheat the buffer solution at 37°C for 1 hour before detection.

2. Preheat the spectrophotometer for 30 minutes and set the spectrophotometer to zero with double distilled water at 290 nm using a 1 mL 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 thoroughly.
5. Add 0.1 mL of substrate solution (record the time immediately) and mix thoroughly with a vortex mixer.
6. Measure the absorbance at 290 nm at 15 seconds (A1), then incubate the reaction solution at 37°C and measure the absorbance at 135 seconds (A2). ΔA = A1 - A2.

10. Calculation

The sample:

1. Calculate according to protein concentration:

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

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

2. Calculate according to sample weight:

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

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

[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: optical path of quartz cuvette, 1 cm

t: reaction time, 2 min

V_1: total volume of reaction system, 1 mL

V_2: volume of reagent 1 for preparing tissue homogenate

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

f: dilution factor of sample before testing

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

m: weight of sample, g

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

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.

Inter-assay Precision

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

Three samples of high, medium, and low concentrations were tested with 6 replicates each to obtain an average recovery rate of 96%.

Recovery

Parameters	Standard 1	Standard 2	Standard 3
Expected Conc. (U/g tissue)	8.5	26.5	40.5

Parameters	Standard 1	Standard 2	Standard 3
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.

12. Appendix II Example Analysis

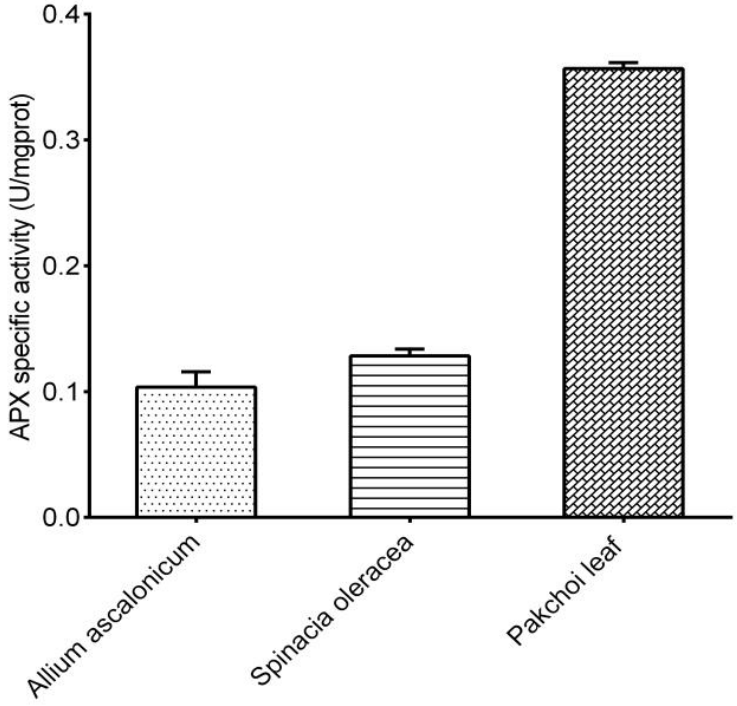
Example analysis:

Weigh 0.10 g of spinach (Spinacia oleracea) and cut into pieces, add 0.9 mL of extracting solution, homogenize the sample, centrifuge at 10,000 x g for 10 minutes at 4°C, take 0.1 mL of supernatant and carry out the assay according to the operating steps. The results are as follows:

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

APX activity (U/mgprot) = (0.314 - 0.012)/(2.8 × 1) × 1/(0.1 × 4.2 × 1) ÷ 2 = 0.128 (U/mgprot)

Detect 10% shallot (Allium ascalonicum) tissue homogenate (protein concentration 2.88 mgprot/mL), 10% spinach (Spinacia oleracea) tissue homogenate (protein concentration 4.20 mgprot/mL), and 10% pakchoi leaf tissue homogenate (protein concentration 6.41 mgprot/mL) according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.