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

Ascorbate peroxidase (APX) Assay Kit

Catalog No.: BC00197

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Ascorbate peroxidase (APX) Assay Kit
Detection Method	Colorimetric
Sample Type	Plant
Assay Type	Quantitative
Detection Instrument	Ultraviolet Spectrophotometer (290 nm)

Product Introduction

Ascorbate Peroxidase (APX) is a highly specific peroxidase that uses ascorbic acid as an electron donor. It is mainly distributed in plant chloroplasts and cytoplasm, serving as a key enzyme for scavenging H_2O_2 in chloroplasts and an important antioxidant enzyme involved in ascorbate metabolism.

Hydrogen peroxide (H_2O_2) is a natural product of the photosynthetic electron transport chain and certain enzymatic reactions in plant chloroplasts, and it belongs to toxic reactive oxygen species. APX possesses multiple isoenzymes, which are mainly divided into two major types: one is the chloroplastic isoenzyme localized in chloroplasts and responsible for decomposing H_2O_2 within chloroplasts; the other is the cytosolic isoenzyme located in cellular compartments outside chloroplasts (localized in the cytoplasm, mitochondria, peroxisomes, glyoxysomes, as well as peroxisomal and thylakoid membranes).

Principle

Ascorbate peroxidase (APX) catalyzes the reaction between ascorbic acid (ASA) and hydrogen peroxide (H_2O_2), oxidizing ASA into monodehydroascorbic acid (MDASA).

With the oxidation of ASA, the absorbance at 290 nm (A_{290}) in the solution decreases. The activity of ascorbate peroxidase (APX) is calculated according to the reduction value of A_{290} per unit time. The oxidation amount of ASA is calculated using an extinction coefficient of 2.8 (mol/mL)/cm.

Components

No.	Components	Size	Notes
Reagent 1	Buffer	100 mL	Store at 4°C
Reagent 2	Substrate powder	1 vial	Store at 4°C
Reagent 3	Substrate solution	5 mL	Store at 4°C

Substrate solution preparation: Before use, add 5 mL of double distilled water to one bottle of Reagent 2 and dissolve thoroughly.

Storage

The reagents can be stored at 4°C for up to 3 months with stability.

Preparation

Low-temperature centrifuge, ultraviolet spectrophotometer, 1 cm optical path quartz cuvette, pipette, mortar, ice and distilled water.

- **Sample handling**

Add Reagent 1 at a ratio of tissue mass (g) to buffer volume (mL) = 1:9 (e.g., add 0.9 mL of R1 buffer to 0.1 g tissue). Homogenize in an ice-water bath, centrifuge at 10000 rpm for 10 minutes, and collect the supernatant for subsequent determination.

Operation process

1. Preheat double distilled water and Reagent 1 at 37°C for more than 30 minutes.
2. Preheat the spectrophotometer for over 30 minutes, adjust the wavelength to 290 nm, and zero the instrument with double distilled water.

	Blank tube	Assay tube
Distilled water (μL)	100	
Sample (μL)		100
Reagent 1 (μL)	700	700
Substrate solution (μL)	100	100
Reagent 3 (μL)	100	100
Mix rapidly, zero the instrument with double distilled water. Set the wavelength to 290 nm and use a 1 cm optical path quartz cuvette. Determine the absorbance values A_0 at 10 s and A_1 at 130 s, then calculate $\triangle A = A_0 - A_1$.		

Note: Before the second colorimetric measurement (at about 120 seconds), it is recommended to pour the reaction solution back into the test tube, mix thoroughly, and then proceed with the colorimetric detection to eliminate interference from bubbles generated during the reaction.

Calculation

1. Calculated based on sample protein concentration

   Definition: One unit (U) of enzyme activity is defined as the amount of enzyme that catalyzes the oxidation of 1 μmol ASA per milligram of protein per minute in each milliliter of the reaction system.

$$\text{APX Activity (U/mg)} = \frac{\Delta A_{\mathbf{Assay}} - \Delta A_{\mathbf{Blank}}}{\varepsilon \times d} \times \frac{V_{Total}}{V_{Sample} \times Cpr} \div T$$

2. Calculated based on sample fresh weight

   Definition: One unit (U) of activity is defined as the amount of enzyme that catalyzes the oxidation of 1 μmol ASA per gram of tissue per minute in 1 mL reaction system.

$$\text{APX Activity (U/g)} = \frac{\Delta A_{\mathbf{Assay}} - \Delta A_{\mathbf{Blank}}}{\varepsilon \times d} \times \frac{V_{Total} \times V_{Extract}}{V_{Sample} \times W} \div T \times N$$

Annotation:

V_{Total}: Total volume of enzymatic reaction, 1 mL

V_{Sample}: Sample dosage, 0.1 mL

T: Reaction time, 2 min

Cpr: Sample protein concentration, mg/mL, shall be determined separately

ε: Molar extinction coefficient (the molar extinction coefficient of ASA at 290 nm is 2.8 μmol/mL/cm)

d: Optical path of cuvette, 1 cm

$V_{Extract}$: Total volume of extraction solution, mL

W: Fresh weight of sample, g

N: Dilution multiple of sample before assay

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.