Ascorbate peroxidase (APX) test kit

(Cat/No.:BC104 Size:50T/48S)

1. Measurement significance

Ascorbate peroxidase (APX) is a highly specific peroxidase that uses ascorbic acid as an electron donor. It is mainly found in plant chloroplasts and cytoplasm. It is a key enzyme for removing H 2 O 2 in chloroplasts and an important antioxidant enzyme for ascorbic acid metabolism. H 2 O 2 is a natural product of the photosynthetic electron transport chain and certain enzyme reactions in plant chloroplasts and is a reactive oxygen species with toxic effects. APX has multiple isozymes, which are mainly divided into two types: one is the chloroplast-type isozyme that is located in the chloroplast and decomposes H 2 O 2 in the chloroplast; the other is the cytoplasmic isozyme that is located in other cellular components outside the chloroplast (located in the cytoplasm, mitochondria, peroxisomes and glyoxysomes, as well as peroxisomes and thylakoid membranes).

2. Determination principle

Ascorbate peroxidase (APX) catalyzes the reaction of ascorbic acid (ASA) and hydrogen peroxide (H 2 O 2) to oxidize ASA into monodehydroascorbic acid (MDASA). As ASA is oxidized, the absorbance value (A 290)at 290nm wavelength in the solution decreases. The activity of ascorbate peroxidase (APX) is calculated based on the decrease in A_{290} per unit time . The amount of ASA oxidized is calculated based on the extinction coefficient of 2.8 (mol/mL)/cm.

3. Self-provided instruments and supplies

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

4. Composition and preparation (The kit is valid for 3 months)

Reagent No.	Reagent name	Reagent loading	Storage conditions
R1	Buffer	100mL×1 bottle	Store at 4 ℃
R2	Substrate powder	1 bottle	Store at 4 ℃

Preparation of substrate solution : Add 5 mL of double distilled water to a bottle of R2 before use to fully dissolve it			
R3	Matrix fluid	5mL×1 bottle	Store at 4 ℃

5. Operation steps

1. Extraction of crude enzyme solution:

reagent 1 at a ratio of tissue mass (g): buffer volume (mL) = 1:9 (e.g., add 0.9 mL R1 buffer to 0.1 g tissue), homogenize in an ice-water bath, centrifuge at 10,000 rpm/min for 10 min, and take the supernatant for testing.

2. Operation table:

Reagents	**Blank tube**	**Determination tube**
Double distilled water (μL)	100	
Sample (μL)		100
R1 (μL)	700	700
R2 (μL)	100	100
R3 (μL)	100	100
Mix quickly, adjust to zero with double distilled water, wavelength 290nm, 1cm optical path quartz cuvette , Determine the light absorbance values A_0 and A_1 at 10s and 130s , $\triangle A=A_0-A_1$		

Note: 1. Preheat double distilled water and reagent 1 at 37 °C for more than 30 minutes before measurement.

2. It is recommended to preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 290nm, and zero it with double distilled water.

3. It is recommended to pour the reaction solution back into the test tube before the second colorimetry (about 120 seconds) , mix it thoroughly and then compare the color (to eliminate the interference of bubbles generated during the reaction).

6. APX activity calculation

(1) Calculated based on sample protein concentration: Unit definition : One activity unit (U) is the amount of protein that catalyzes 1 μmol ASA per minute in each mL of reaction system

$$\text{APX activity (U/mgprot)} = \frac{(\Delta A_{measure} - \Delta A_{blank}) \times 10^6}{\varepsilon \times d} \times \frac{V_{total}}{V_{sample} \times Cpr} \div T$$

ε: molar extinction coefficient (the molar extinction coefficient of ASA at 290nm is 2.8mol/mL/cm);
d: cuvette light path, 1 cm;
10 6 : 1mol=10 6 μmol;
V total : total volume of reaction system, 1 mL;
V sample : sampling volume, 0.1 mL;
T: reaction time, 2 min.
Cpr: protein concentration of the sample to be tested (mgprot/mL), which needs to be determined separately. It is recommended to use our BCA protein quantitative assay kit;

Calculation example : Take 0.1g of rice leaves, add 0.9mL of buffer solution , prepare homogenate supernatant, measure the absorbance of blank tube A0 to be 0.512 , A1 to be 0.508 , ΔA blankto be 0.004 , measure the absorbance of tube A0 to be 0.849 , A1 to be 0.760 , ΔA measure to be 0.089 . The sample protein concentration is measured to be 0.3528mgprot/mL . Substitute the data into the calculation formula:

$$\text{APX } activity\ (\text{U/mgprot}) = \frac{0.089 - 0.004}{2.8 \times 1} \times \frac{1}{0.1 \times 0.3258} \div 2$$

(2) Calculated by sample quality:

Unit definition : 1 μmol ASA catalyzed per gram of tissue per minute in per mL of reaction system is 1 activity unit (U)

$$\text{APX}activity\ (\text{U/g}tissue) = \frac{(\Delta A_{measure} - \Delta A_{blank}) \times 10^6}{\varepsilon \times d} \times \frac{V_{total} \times V_{extract}}{V_{sample} \times W} \div T \times N$$

V extract : total volume of extract, mL;
W: sample fresh weight, g;
N: dilution factor of the sample before testing.

Calculation example : Take 0.1g of tomato leaves, add 0.9mL of buffer solution , prepare homogenate supernatant, measure the absorbance of blank tube A0 to be 0.510 , A1 to be 0.506 , ΔA blankto be 0.004 , measure the absorbance of tube A0 to be 0.800 , A1 to be 0.752 , ΔA measuredto be 0.048 . Substitute the data into the calculation formula:

$$\text{APX}activity\ (\text{U/mgprot}) = \frac{0.048 - 0.004}{2.8 \times 1} \times \frac{1 \times 0.9}{0.1 \times 0.1} \div 2$$

$$= 0.7071 \quad \text{U/g}tissue$$