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Ascorbate Peroxidase (APX) Assay Kit

BC5505-02 (100T/96S)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Ascorbate Peroxidase (APX) is an important antioxidase of plant scavenging reactive oxygen, also is one key enzyme of ascorbic acid metabolism. APX has a variety of isozymes located in chloroplast, cytoplasm, mitochondria, peroxides and glyoxylate, peroxisome and thylakoid membrane respectively. APX is the main consumer of plant AsA, which catalyzes the oxidation of AsA by H_2O_2 . The activity of APX directly affects the content of ASA, and there is a negative correlation between APX and ASA.

APX catalyzes the oxidation of ASA by H_2O_2 . In this kit, the activity of APX is calculate by the oxidize rate of AsA.

Kit components

Reagent	Volume	Storage
Extraction Reagent	110mL	2-8°C
Powder I	Powder x 1	2-8°C
Reagent I	15mL	2-8°C
Reagent II	Powder x 2	2-8°C
Reagent III	0.25mL	2-8°C
96 well UV plate	1 nos	2-8°C

Solution Preparation:

- 1. Extract solution:** Before use, pour the powder I into the extraction solution, the solution is suspension, shake well and use it. It can be stored for 12 weeks at 2-8°C
- 2. Reagent II:** Before use, add 10 mL distilled water to dissolve thoroughly; It can be stored at 2-8°C for 1 week.
- 3. Reagent III:** Centrifuge before use. Before use. take an appropriate amount of reagent according to the sample size and dilute it 100 times with distilled water.

Reagents and Equipment Required but Not Provided

Refrigerated centrifuge, ultraviolet spectrophotometer, 1 mL quartz cuvette, transferpettor, mortar/homogenizer, water bath, ice and distilled water.

Operation Procedures

I. Sample extraction

Add 1 mL of Reagent I to 0.1 g of sample. Grind thoroughly on ice. Centrifuge at 13000 ×g for 20minutes at 4°C, take the supernatant on ice for test.

II. Determination procedure

1. Preheat ultraviolet spectrophotometer or microplate reader for 30 minutes, adjust wavelength to 290 nm, spectrophotometer set zero with distilled water.
2. Preheat Reagent I at 25°C water bath for 30 minutes.
3. Add reagents with the following list:

Reagent (μL)	Test tube	Blank tube
Sample	20	-
Distilled water	-	20
Reagent I	140	140
Reagent II	20	20
Reagent III	20	20

Mix thoroughly and timing, measure the absorption values at 10s and 130s at 290nm, record as A1,A3 and A2, A4 respectively, $\Delta A_T = A1-A2$, $\Delta A_B = A3-A4$.

Calculation

A. Micro quartz cuvette

I. Calculate by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of ASA in the reaction system per minute every milligram protein.

$$\text{APX activity (U/mg prot)} = (\Delta A_T - \Delta A_B) + (\epsilon \times d) \times V_{RT} \times 10^6 \div (C_{pr} \times V_S) \div T = 1.79 \times (\Delta A_T - \Delta A_B) \div C_{pr}$$

II. Calculate by fresh sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of ASA in the reaction system per minute every gram tissue sample.

$$\text{APX activity (U/g weight)} = (\Delta A_T - \Delta A_B) + (\epsilon \times d) \times V_{RT} \times 10^6 \div (V_S \div V_{ST} \times W) \div T = 1.79 \times (\Delta A_T - \Delta A_B) \div W$$

ε: Molar absorption coefficient of AsA at 290 nm, 2.8×10^3 L/mol/cm;

d: Cuvette light path(cm), 1 cm;

V_{RT} : Reaction total volume(L), $200 \mu\text{L} = 2 \times 10^{-4}$ L;

10^6 : $1 \text{ mol} = 1 \times 10^6 \mu\text{mol}$;

W: Sample weight, g;

C_{pr} : Supernatant protein concentration, mg/mL;

V_S : Supernatant volume(mL), $20 \mu\text{L} = 0.02$ mL;

V_{ST} : Added Extraction reagent volume, 1 mL;

T: Reaction time(min), 2 minutes.

B. 96 well UV flat-bottom plate

I. Calculate by sample protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of ASA in the reaction system per minute every milligram protein.

$$\text{APX activity (U/mg prot)} = (\Delta A_T - \Delta A_B) + (\epsilon \times d) \times V_{RT} \times 10^6 \div (C_{pr} \times V_S) \div T = 3 \times (\Delta A_T - \Delta A_B) \div C_{pr}$$

II. Calculate by fresh sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1 μmol of ASA in the reaction system per minute every gram tissue sample.

$$\text{APX activity (U/g weight)} = (\Delta A_T - \Delta A_B) + (\epsilon \times d) \times V_{RT} \times 10^6 \div (V_S \div V_{ST} \times W) \div T = 3 \times (\Delta A_T - \Delta A_B) \div W$$

ε: Molar absorption coefficient of AsA at 290 nm, 2.8×10^3 L/mol/cm;

d: 96 well plate light path (cm), 0.6 cm;

V_{RT} : Reaction total volume(L), $200 \mu\text{L} = 2 \times 10^{-4}$ L;

10^6 : $1 \text{ mol} = 1 \times 10^6 \mu\text{mol}$;

W: Sample weight, g;

C_{pr} : Supernatant protein concentration, mg/mL;

V_S : Supernatant volume(mL), $20 \mu\text{L} = 0.02$ mL;

V_{ST} : Added Extraction reagent volume, 1 mL;

T: Reaction time(min), 2 minutes