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Ascorbate Peroxidase (APX) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Detection equipment: Spectrophotometer/ Microplate reader

Cat No: AK0575

Size: 100T/96S

Components:

Reagent I: Liquid 120 mL×1, store at 4℃ .

Reagent II: Powder×1, store at 4℃ . Dissolved with 5 mL of distilled water before use.

Reagent III: Liquid 0.5 mL×1, store at 4℃ . Before use, according to the sample volume， dilute Reagent III with distilled water 8 times.

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.

Reagents and Equipment Required but Not Provided：

Refrigerated centrifuge, spectrophotometer/microplate reader, micro quartz cuvette/96 well UV flat-bottom plate, transferpettor, mortar/ homogenizer, ice and distilled water.

Protocol:

I. Sample extraction

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

II. Determination

1. Preheat ultraviolet spectrophotometer or microplate reader for 30 minutes, adjust wavelength to 290 nm, set zero with distilled water.
2. Preheat Reagent I at 25℃ water bath for 30 minutes.
3. **Blank tube:** Add 20 μL of distilled water, 140 μL of preheat Reagent I, 20 μL of Reagent II and 20μL of Reagent III at micro quartz cuvette or 96 well UV flat-bottom plate. Mix thoroughly and timing, measure the absorption values at 10s and 130s at 290 nm, record as A1 and A2 respectively, ΔA_B=A1-A2.
4. **Test tube:** Add 20 μL of supernatant, 140 μL of preheat Reagent I, 20 μL of Reagent II and 20 μL of Reagent III at micro quartz cuvette or 96 well UV flat-bottom plate. Mix thoroughly and timing, measure

the absorption values at 10s and 130s at 290 nm, record as A3 and A4 respectively, ΔA_T=A3-A4.

III. Calculation

A. Micro quartz cuvette

1) 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.

APX(U/mg prot) = $(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RT}\times 10^6\div(Cpr\times V_S)\div T=1.79\times(\Delta A_T-\Delta A_B)\div Cpr$

2)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.

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

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 μL=2×10^{-4} L;

10^6: 1mol=1×10^6 μmol;

W: Sample weight, g;

Cpr: Supernatant protein concentration, mg/mL;

V_S: Supernatant volume(mL), 20 μL=0.02 mL;

V_{ST}: Reagent I volume, 1 mL;

T: Reaction time(min), 2 minutes.

B. 96 well UV flat-bottom plate

1) 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.

APX(U/mg prot) = $(\Delta A_T-\Delta A_B)\div(\varepsilon\times d)\times V_{RT}\times 10^6\div(Cpr\times V_S)\div T=3\times(\Delta A_T-\Delta A_B)\div Cpr$

2) 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.

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

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 μL=2×10^{-4} L;

10^6: 1 mol=1×10^6 μmol;

Cpr: Supernatant protein concentration, mg/mL;

W: Sample weight, g;

V_S: Supernatant volume(mL), 20 μL=0.02 mL;

V_{ST}: Reagent I volume, 1 mL;

T: Reaction time(min), 2 minutes.

Experimental Examples:

1. Take 0.1 g of clover and add 1mL of Reagent Ⅰ for homogenization, take the supernatant, and then operate according to the determination steps. Calculate the $\Delta A_B=A_1-A_2$ = 0.6653-0.6499=0.0154, $\Delta A_T= A_3-A_4$ =1.5311- 1.2553=0.2758 with 1ml quartz cuvette, and calculate the enzyme activity according to the sample mass:

APX (U/g mass) = $1.79 \times (\Delta A_T - \Delta A_B) \times W = 1.79 \times (0.2758-0.0154) \div 0.1 = 4.66$ U/g mass

Recent Product Citations:

[1] Meng C, Quan T Y, Li Z Y, et al. Transcriptome profiling reveals the genetic basis of alkalinity tolerance in wheat[J]. BMC genomics, 2017, 18(1): 24.

[2] Qin Y, Djabou A S M, An F, et al. Proteomic analysis of injured storage roots in cassava (Manihot esculenta Crantz) under postharvest physiological deterioration[J]. PloS one, 2017, 12(3).

[3] Zhang Z, Liu H, Sun C, et al. A C_2H_2 zinc-finger protein OsZFP213 interacts with OsMAPK3 to enhance salt tolerance in rice[J]. Journal of plant physiology, 2018, 229: 100- 110.

[4] Zhao Y, Yu W, Hu X, et al. Physiological and transcriptomic analysis revealed the involvement of crucial factors in heat stress response of Rhododendron hainanense[J]. Gene, 2018, 660: 109- 119.

[5] Djabou A S M, Qin Y, Thaddee B, et al. Effects of Calcium and Magnesium Fertilization on Antioxidant Activities during Cassava Postharvest Physiological Deterioration[J]. Crop Science, 2018, 58(3): 1385- 1392.

References:

[1] Shigeoka S, Nakano Y, Kitaoka S. Metabolism of hydrogen peroxide in Euglena gracilis Z by L-ascorbic acid peroxidase[J]. Biochemical Journal, 1980, 186(1): 377.

[2] Caverzan A, Passaia G, Rosa S B, et al. Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection[J]. Genetics and molecular biology, 2012, 35(4): 1011- 1019.

Related Products:

AK0468/AK0467	Ascorbic Acid (AsA) Content Assay Kit
AK0466/AK0465	Dehydroascorbic Acid (DHA) Content Assay Kit
AK0464/AK0463	L-galactose- 1,4-lactone Dehydrogenase (Gal LDH) Activity Assay Kit
AK0462/AK0461	Ascorbic Acid Oxidase (AAO) Activity Assay Kit
AK0526/AK0525	Monodehydroascorbate Reductase (MDHAR) Activity Assay Kit