Ascorbate Peroxidase (APX) Activity Assay Kit

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

Detection equipment: Spectrophotometer/ Microplate reader

Cat No: BC0225

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Powder I	Powder×1	2-8℃
Reagent I	Liquid 15 mL×1	2-8℃
Reagent II	Powder×2	2-8℃
Reagent III	Liquid 0.25 mL×1	2-8℃

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℃.

2. Reagent II: Before use, add 10 mL distilled water to dissolve thoroughly; It can be stored at 2-8℃ for 1 week. (Due to the poor stability of the reagent, give one more bottle).

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.

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:

Spectrophotometer/microplate reader, centrifuge, micro quartz cuvette/96 well UV flat-bottom plate, transferpettor, water bath /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, spectrophotometer set zero with distilled water.
2. Preheat Reagent I at 25℃ 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 290 nm, record as A1, A3 and A2, A4 respectively, ΔA_T=A1-A2, ΔA_B=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 activity(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 activity(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=1×10^6μmol;

W: Sample weight, g;

Cpr: Supernatant protein concentration, mg/mL;

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

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

T: Reaction time(min), 2 minutes.

Experimental Examples:

1. Take 0.1 g of clover and add 1mL of Extraction reagent for homogenization, take the supernatant, and then operate according to the determination steps. Calculate the $\Delta A_B=A_3-A_4$ = 0.5453-0.5287=0.0166, $\Delta A_T= A_1-A_2$ =0.7097-0.6137=0.096 with 1ml quartz cuvette, and calculate the enzyme activity according to the sample mass:

APX activity(U/g mass) = $1.79\times(\Delta A_T-\Delta A_B)\times W$ = $1.79\times(0.096-0.0166)\div0.1$=1.421 U/g mass.

Recent Product Citations:

[1] Zhao H, Qian R, Liang X, Ou Y, Sun C, Lin X. Indium induces nitro-oxidative stress in roots of wheat (Triticum aestivum). J Hazard Mater. 2022 Apr 15;428:128260. doi: 10.1016/j.jhazmat.2022.128260. Epub 2022 Jan 12. PMID: 35038664.

[2] Yan YF, Wu TL, Du SS, Wu ZR, Hu YM, Zhang ZJ, Zhao WB, Yang CJ, Liu YQ. The Antifungal Mechanism of Isoxanthohumol from Humulus lupulus Linn. Int J Mol Sci. 2021 Oct 7;22(19):10853. doi: 10.3390/ijms221910853. PMID: 34639194; PMCID: PMC8509189.

[3] Zou Y, Cao S, Zhao B, Sun Z, Liu L, Ji M. Increase in glutathione S-transferase activity and antioxidant damage ability drive resistance to bensulfuron-methyl in Sagittaria trifolia. Plant Physiol Biochem. 2022 Nov 1;190:240-247. doi: 10.1016/j.plaphy.2022.09.007. Epub 2022 Sep 15. PMID: 36148723.

[4] Yuan WJ, He ZY, Zhang SP, Zheng YP, Zhang XQ, He SQ, He YX, Li Y. Comparative transcriptomics provides insights into the pathogenic immune response of brown leaf spots in weeping forsythia. Tree Physiol. 2023 Sep 6;43(9):1641-1652. doi: 10.1093/treephys/tpad060.

PMID: 37171622.

[5] Zhang C, Zhou P, Mei J, Xie J. Effects of Different Pre-Cooling Methods on the Shelf Life and Quality of Sweet Corn (Zea mays L.). Plants (Basel). 2023 Jun 19;12(12):2370. doi: 10.3390/plants12122370. PMID: 37375995; PMCID: PMC10303236.

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:

BC1230/BC1235	Ascorbic Acid (AsA) Content Assay Kit
BC1240/BC1245	Dehydroascorbic Acid (DHA) Content Assay Kit
BC1250/BC1255	L-galactose-1,4-lactone Dehydrogenase (Gal LDH) Activity Assay Kit
BC1260/BC1265	Ascorbic Acid Oxidase (AAO) Activity Assay Kit
BC0650/BC0655	Monodehydroascorbate Reductase (MDHAR) Activity Assay Kit

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