Ascorbate Peroxidase (APX) Activity Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: BC0220

Size: 50T/48S

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 60 mL×1	2-8℃
Powder I	Powder×1	2-8℃
Reagent I	Liquid 40 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:

Refrigerated centrifuge, ultraviolet spectrophotometer, 1 mL quartz cuvette, transferpettor, mortar/ homogenizer, water bath, 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 procedure

1. Preheat spectrophotometer 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. Add reagents with the following list:

Reagent (μL)	Test tube	Blank tube
Sample	100	-
Distilled water	-	100
Reagent I	700	700
Reagent II	100	100
Reagent III	100	100
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

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.

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

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}: Total volume(L), 1000 μL=1×10^{-3} L;

10^6: 1 mol=1×10^6 μmol;

V_S: Supernatant volume(mL), 100 μL=0.1 mL;

T: Reaction time(min), 2 minutes;

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

W: Sample weight, g.

Cpr: Supernatant protein concentration, mg/mL;

Recent Product Citations:

[1] Zhao X, Zhang Y, Ma Y, Zhang L, Jiang Y, Liang H, Wang D. Inhibitory mechanism of low-oxygen-storage treatment in postharvest internal bluing of radish (Raphanus sativus) roots. Food Chem. 2021 Dec 1; 364:130423. doi: 10.1016/j.foodchem.2021.130423. Epub 2021 Jun 19.

PMID: 34198034.

[2] Zhang Y, Hu Y, Wang Z, Lin X, Li Z, Ren Y, Zhao J. The translocase of the inner mitochondrial membrane 22-2 is required for mitochondrial membrane function during Arabidopsis seed development. J Exp Bot. 2023 Aug 17;74(15):4427-4448. doi: 10.1093/jxb/erad141. PMID: 37105529.

[3] Zhang Z, Zhang Y, Yuan L, Zhou F, Gao Y, Kang Z, Li T, Hu X. Exogenous 5-aminolevulinic acid alleviates low-temperature injury by regulating glutathione metabolism and β-alanine metabolism in tomato seedling roots. Ecotoxicol Environ Saf. 2022 Oct 15; 245:114112. doi: 10.1016/j.ecoenv.2022.114112. Epub 2022 Sep 22. PMID: 36155340.

[4] Wang X, Zhang X, Jia P, Luan H, Qi G, Li H, Guo S. Transcriptomics and metabolomics provide insight into the anti-browning mechanism of selenium in freshly cut apples. Front Plant Sci. 2023 May 8;14:1176936. doi: 10.3389/fpls.2023.1176936. PMID: 37223812; PMCID: PMC10200898.

[5] Lin D, Yan R, Xing M, Liao S, Chen J, Gan Z. Fucoidan treatment alleviates chilling injury in cucumber by regulating ROS homeostasis and energy metabolism. Front Plant Sci. 2022 Dec 23; 13:1107687. doi: 10.3389/fpls.2022.1107687. PMID: 36618644; PMCID: PMC9816408.

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.

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 $\Delta A_B=A_1-A_2$ = 0.786-0.776 = 0.01, $\Delta A_T= A_3-A_4$ = 1.649-1.273 = 0.376 with 1ml quartz cuvette, and calculate the enzyme activity according to the sample mass

APX (U/g mass) = 1.79 × (ΔA_T - ΔA_B) × W = 1.79 × (0.376-0.01) × 0.1 = 6.55 U/g mass

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