Ascorbate Peroxidase (APX) Activity Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: NA0852

Size: 50T/48S

Components:

Reagent I: Liquid 90 mL×1,store at 4℃.

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

Reagent III: Liquid 5 mL×1, store at 4℃.

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, 1 mL quartz cuvette, transferpettor, mortar/ homogenizer, ice and distilled water.

Protocol:

I. Sample extraction

Fully homogenized the tissue (mass, g) and Reagent I (volume, mL) on ice bath by the ratio of 1:5~10 (It is suggested that 0.1 g of tissue add 1 mL of Reagent I). 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. Blank tube: Add 100 μL of distilled water, 700 μL of preheat Reagent I, 100 μL of Reagent II and 100 μL of Reagent III to 1 mL quartz cuvette. 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 100 μL of supernatant, 700 μL of preheat Reagent I, 100 μL of Reagent II and 100 μL of Reagent III to 1 mL quartz cuvette. 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

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] 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.

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) = $1.79 \times (\Delta A_T - \Delta A_B) \times W = 1.79 \times (0.376-0.01) \times 0.1 = 6.55$ U/g mass

Related Products:

NA0776/NA0535 Ascorbic Acid(AsA) Content Assay Kit

NA0775/NA0534 Dehydroascorbic Acid(DHA) Content Assay Kit

NA0774/NA0533 L-galactose-1,4-lactone Dehydrogenase(Gal LDH) Activity Assay Kit

NA0773/NA0532 Ascorbic Acid Oxidase(AAO) Activity Assay Kit

NA0817/NA0575 Monodehydroascorbate Reductase(MDHAR) Activity Assay Kit