EZMeta™ Ascorbate Peroxidase (APX) Colorimetric Activity Kit

Cat #: D-AKC3091

Size: 48T / 96T

Storage: Stored at 4°C for 6 months, protected from light

Product Information

Applicable samples: Plant Tissues

Assay Principle

Ascorbate Peroxidase (APX) is one of the important antioxidant enzymes for scavenging reactive oxygen species in plants and one of the key enzymes in ascorbic acid metabolism. APX has a variety of isoenzymes, which are localized in chloroplasts, cytoplasm, mitochondria, peroxides and glyoxylate bodies, as well as on peroxide and thylakoid membranes. APX catalyzes the oxidation of AsA by H2O2 and is a major consumer of AsA in plants. The activity of APX directly affects the content of AsA, and there is a negative correlation between APX and AsA. EZMeta™ Ascorbate Peroxidase (APX) Colorimetric Activity Kit provides a simple assay for the detection of APX activity in biological samples such as plant tissue samples. APX catalyzes the oxidation of AsA by H_2O_2, and the activity of APX was calculated by measuring the oxidation rate of AsA.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	60 mL	120 mL	4°C
Reagent Ⅱ	1	1	4°C, protected from light
Reagent Ⅲ	15 μL	30 μL	4°C, protected from light

Materials Required but Not Supplied

·Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 290 nm

·Incubator, freezing centrifuge

·96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips

·Deionized water

·Homogenizer (for tissue samples)

Reagent Preparation

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent Ⅱ: Prepared before use, 48 T was added with 1.5 mL deionized water, 96 T was added with 3 mL deionized water, fully dissolved. Use within 3 days and store at 4°C, protected from light.

ReagentⅢ: Prepared before use, 48 T was added with 1.5 mL deionized water, 96 T was added with 3 mL deionized water, fully dissolved. Stored at 4°C, protected from light.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for one month.

1. Plant tissue samples: Weigh 0.1 g tissue, add 1 mL Reagent Ⅰ and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 13,000 g for 20 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

Note: If the protein concentration of the sample is need to be determined, it is recommended to use EZMeta™ Ascorbate Peroxidase (APX) Colorimetric Activity Kit to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 290 nm, Ultraviolet spectrophotometer was returned to zero with deionized water.

2. Incubate Reagent Ⅰ at 25°C for 30 min.

3. Add 20 μL sample, 140 μL Reagent Ⅰ, 20 μL Reagent Ⅱ and 20 μL ReagentⅢinto 96-well UV plate or microquartz cuvette, and mix quickly.

4. Measure the absorbance value at 290 nm with a microplate reader, record 10 s absorbance value as A_1 and the absorbance value at 2 min 10 s as A_2, and calculate $\Delta A=A_1-A_2$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 large expected difference samples. Because the enzyme activity is calculated based on the reaction rate, in order to ensure that the reaction time of each sample is as consistent as possible, it is not recommended to test too many samples at the same time. If ΔA is less than 0.001, increase the sample quantity appropriately. If ΔA is greater than 1, the sample can be appropriately diluted with Reagent Ⅰ, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula as below

1. Calculation of APX activity in tissues

(1) Calculated by protein concentration

Active unit definition: 1 nmol AsA consumed per min in 1mg tissue protein reaction system is defined as a unit of enzyme activity.

APX (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(Cpr\times V_{Sample})\div T$**=3,571.43×ΔA÷Cpr**

(2) Calculated by sample fresh weight

Active unit definition: 1 nmol AsA consumed per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

APX (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ Sample}$×W)÷T=**3,571.43×ΔA÷W**

Where: V_{Total}: total reaction volume, 2×10^{-4} L; ε: AsA molar extinction coefficien, 2.8×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; V_{Sample}: sample volume added, 0.02 mL; $V_{Total\ Sample}$: Reagent Ⅰ volume added, 1 mL; T: reaction time, 2 min; Cpr; sample protein concentration, mg/mL; W: sample weight, g.

B. Microquartz cuvette calculation formula

The optical diameter d: 0.5 cm in the above calculation formula can be adjusted to d: 1 cm for calculation.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.